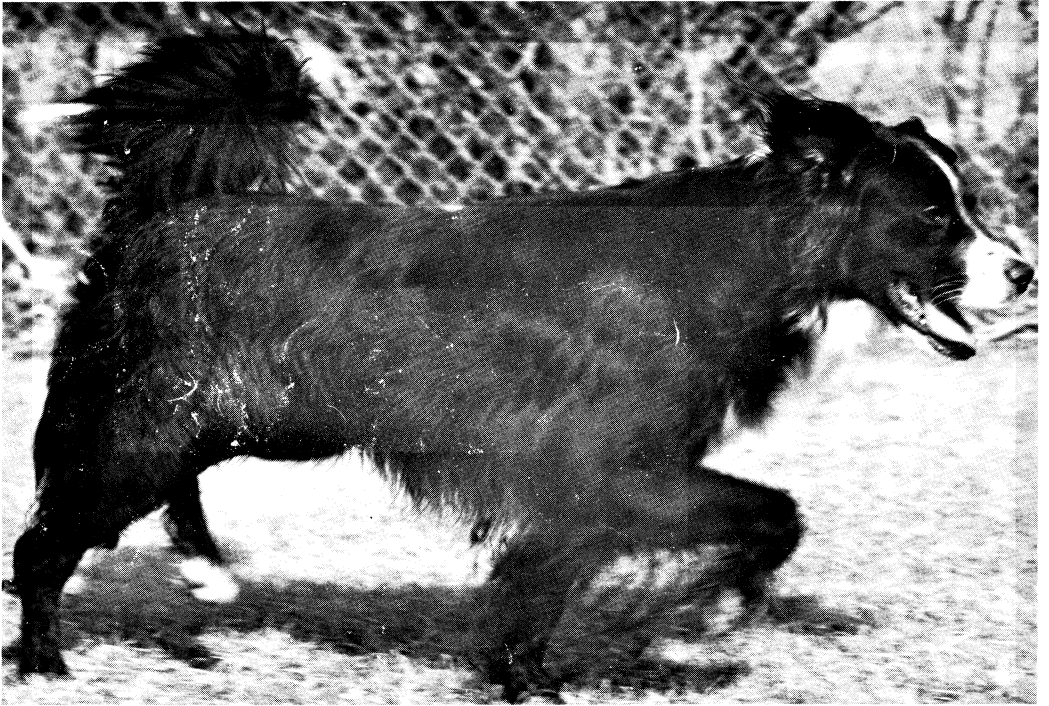


Experience in HEPATIC TRANSPLANTATION

THOMAS E. STARZL, M.D.

WITH THE ASSISTANCE OF
CHARLES W. PUTNAM, M.D.

Thomas
Stark



Recipient of an orthotopic liver homograft, 5 years later. The operation was on March 23, 1964.

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THOMAS E. STARZL, Ph.D., M.D.

PROFESSOR OF SURGERY,

UNIVERSITY OF COLORADO SCHOOL OF MEDICINE:

CHIEF, SURGICAL SERVICE,

VETERANS ADMINISTRATION HOSPITAL, DENVER, COLORADO

with the assistance of

CHARLES W. PUTNAM, M.D.

INTERN IN SURGERY, UNIVERSITY OF COLORADO SCHOOL OF MEDICINE,
DENVER, COLORADO

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to

Tim, Becky, and Tommy

CONTRIBUTING AUTHORS

J. ANTONIO ALDRETE, M.D.

Associate Professor of Anesthesiology, University of Colorado School of Medicine; Chief Anesthesiologist, Veterans Administration Hospital, Denver, Colorado

CARL G. GROTH, M.D.

Formerly, International Exchange Fellow, University of Colorado School of Medicine; Assistant Professor of Surgery, Karolinska Institutet, Stockholm, Sweden

NOBORU KASHIWAGI, M.D.

Research Fellow in Transplantation, University of Colorado School of Medicine, Denver, Colorado

K. A. PORTER, M.D., D.Sc.

Chairman, Department of Pathology, St. Mary's Hospital and Medical School, London, England

PAUL I. TERASAKI, Ph.D.

Professor of Surgery, University of California, Los Angeles, California

MEDICAL ART—JEAN McCONNELL

GRAPHIC ART—RUTH R. OGDEN

PHOTOGRAPHIC ART—JACK FASON, B.A., M.A., F.B.P.A.

ERNEST O. ANDERSON

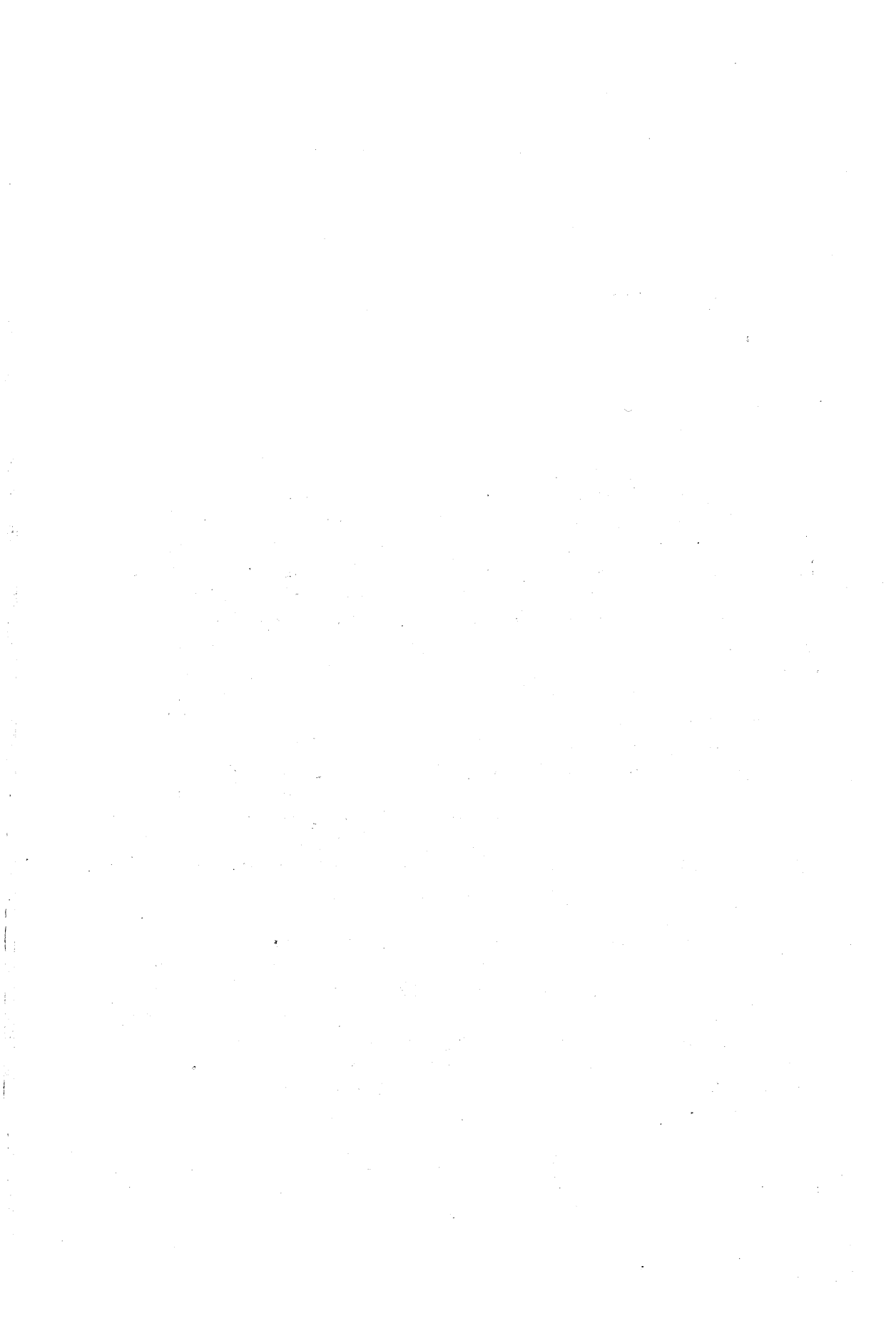
FOREWORD

This book about transplantation of the liver recounts and details experience with the first attempts at this kind of operation in humans. A number of the patients have been helped by these efforts. Furthermore, progress toward the ultimate goal of general applicability is amply documented. However, the text is more than a summary and progress report. In clear relief against the background of clinical experience is shown the confluence of modern biology and medicine ranging from old and new anatomy to genetics, immunology, pharmacology, chemistry, microbiology, and more. As such, we have an outstanding example of what surgery is about and where it is going.

The most distinct impression left with the reader is that of the intertwining of an extensive laboratory program with the clinical endeavor. At first, the results with the latter trials were totally disheartening. Instead of causing defeat and retreat, the consequence of the early failures was to stimulate the re-examination of the apparent problems in more detail and to evolve solutions. The course has been back and forth from the clinic to the laboratory with complete dissolution of the artificial barrier between "basic" and "clinical" sciences.

In this work can be discerned a model that deserves study of how to advance medicine and surgery against the grudging opposition posed by the unknown. Central to the effort has been the attitude that laboratories are to help people and, more specifically, patients. In relating the fascinating story of liver transplantation, Dr. Starzl has written about a small group of patients, a larger number of participating researchers, and the accomplishments of a vigorous segment of the scientific community.

WILLIAM R. WADDELL, M.D.
Professor and Chairman,
Department of Surgery
University of Colorado School of Medicine



PREFACE

Five years ago a monograph entitled *Experience in Renal Transplantation*,¹ published by the W. B. Saunders Company described an extensive clinical trial with that form of treatment at the University of Colorado. It was conceded that kidney transplantation was still an experimental undertaking, but it was also evident that the majority of the patients in the reported series had received real benefit. In the ensuing years renal transplantation has become increasingly accepted as a service even though it has continued to provide a fertile area for clinical investigation.

In the foregoing publication a chapter was devoted to speculation about the effect that such research would have in promoting the transplantation of other vital organs. It was suggested that improvements achieved with the kidney in surgical technique, organ storage, histocompatibility typing, and immunosuppression could be generally applied. Furthermore, there was no reason to think that several important phenomena seen in the early postoperative period after renal homotransplantation would not also pertain with other organs. These included the reversibility of rejection and an adaptive change in the host-graft relationship which often permitted a late relaxation in the requisite intensity of chronic immunosuppression.

Now there are clear signs that homotransplantation of the liver will be a valuable means in the future of treating patients who have an otherwise hopeless prognosis from hepatic disease. As with the kidney, the early efforts to provide patients with new liver tissue were beset with difficulties and tragedies. Problems were encountered which were not anticipated from previous animal studies, often necessitating a return to the laboratory for long intervals.

Eventually a much more complete view emerged of the requirements for clinical hepatic transplantation, as can be appreciated from the fact that four patients in our series have lived for at least one year after complete removal and replacement of their diseased livers. In reviewing this progress, the usual practice of devoting a section to historical background will be omitted. The first description of transplantation of the whole canine liver by Welch² was published

only 14 years ago. This and almost all other articles on the subject are still of current interest and will be frequently alluded to in subsequent chapters.

A comment may be in order concerning the organization and focus of the text. There are two general approaches to transplantation of the liver which provide the basis for division of the book into several of its component parts. With the first method, the host liver is removed and replaced with a homograft (orthotopic homotransplantation). The alternative technique is the insertion of an extra liver (auxiliary homotransplantation) at an ectopic site.

Both procedures were developed in dogs, and much of the most incisive information about them was obtained from animal experimentation. However, this book was not designed to recapitulate such laboratory research except as a means of better understanding the small but pathfinding experience with human liver transplantation. Instead, the primary objective was to systematically develop a clinical point of view about liver transplantation.

The number of cases with which to do this is still small, and individual patients are alluded to repeatedly in different parts of the book. In order to facilitate their identification, they have been designated with sequential code numbers. Those who received orthotopic and auxiliary liver homografts are referred to as OT and AT, respectively. A summary table of the salient features of each case is in the up-to-date appendix to which the reader can refer from time to time for purposes of continuity.

The emphasis on the clinical material is in no way a denigration of the importance of past or future investigations in animals. In our own laboratories, research on liver transplantation has occupied our almost continuous attention for more than 10 years, first in Chicago and later in Denver. It is doubtful if even a small fraction of the work actually carried out would have been possible without the continuous, unselfish, and imaginative efforts during most of this time of Mr. Paul Taylor, the present administrative chief of our research laboratories.

The program was also sustained by many other gifted collaborators, among whom were several surgeons whose contributions were so noteworthy as to require special mention. The first was Dr. Harry A. Kaupp, Jr., who was a resident in surgery at Northwestern University in Chicago from 1959 to 1962 and who is now practicing in Allentown, Pennsylvania. Next was Dr. Thomas L. Marchioro at the University of Colorado. He is currently an Associate Professor of Surgery at the University of Washington, Seattle. In the summer of 1966, Dr. Lawrence Brettschneider, a Commander in the United States Navy, was placed on detached service at the University of Colorado, where he made his major research objective the development of a technique of long-term liver preservation. Dr. Carl-Gustav Groth, a fellow from the Serafimerlasarettet Hospital, Stockholm, cared for the first five patients who achieved extended survival in 1967 and early 1968; his uncanny clinical judgment made possible the diagnosis and treatment of a number of life threatening complications. Since September, 1967, Dr. Israel Penn, an Associate Professor of Surgery, has shouldered a heavy responsibility in the program, as have many fellows including Drs. Herve Blanchard (Montreal), Peter Bell (Glasgow), Robert McGuire

(New York City), John Homatas (Athens), Reginaldo Picache (Manila), and Geoffrey Giles (Leeds).

Finally, no undertaking of the magnitude of the establishment of an organ transplantation center could succeed without broad institutional, federal, and community support. This has been given to us from the beginning by the Veterans Administration, the University of Colorado, and the United States Public Health Service, and by the practicing physicians of Denver.

THOMAS E. STARZL

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1. Starzl, T. E.: *Experience in Renal Transplantation*. Philadelphia, W. B. Saunders Company, 1964.
2. Welch, C. S.: A note on transplantation of the whole liver in dogs. *Transpl. Bull.* 2:54, 1955.

Note About Liver Chemistries

The liver function tests depicted in the graphs and described in the text were not all done in the same laboratory. For example, the bloods from the small children were examined with micro-techniques, whereas larger aliquots of the specimens from the adults were usually submitted to standard analysis. The results of most of the determinations were not markedly different with the variant methods. However, the alkaline phosphatase values were expressed in several ways which are summarized below.

<i>Units</i>	<i>Normal Range</i>
Bessey-Lowry	1 to 3
Bodansky	3 to 5
International (micro)	55 to 260*

*Range slightly variable according to exact age.

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IN MEMORIAM



ANDRES ZAHLER MAYANZ, M.D.
(1943-1968)

On October 16, 1968, two anesthesiologists, Dr. Antonio Aldrete and Dr. Andres Zahler, were driving from Denver to Leadville, Colorado, to carry out some studies in high altitude respiratory physiology. En route, the car went out of control and fell into a mountain pass. Dr. Zahler suffered a lethal brain injury from which he died on October 24, 1968. His parents, Mr. and Mrs. Jorge Zahler, who are both lawyers, had been told by their son that he wished to be an organ donor in the unlikely eventuality of such a circumstance. Accordingly, his liver and a kidney were removed after death and transplanted.

“Andy” Zahler was born in Chile on March 20, 1943. He received his early education and medical school training in Santiago and then came to Denver for an internship and a residency in anesthesiology. He was happy in Colorado. In addition to his general duties in patient care, he became involved in clinical and laboratory research and with the activities of the transplantation team. He was a shy and modest young man who distinguished himself by his industry and intelligence, but above all by his kindness and gentleness.

It is doubtful if anyone who knew Andy Zahler could or would ever want to forget him. He was convinced of the goodness of people and he understood their pain in a way not common in 25 year old men. The act of trying to help others at the time of his death was perfectly consistent with his beliefs about life.

PART I

DEMAND, SUPPLY, AND SELECTION

Chapter One

CANDIDACY

The potential usefulness of liver transplantation can be appreciated from the survey compiled from the Vital Statistics for the United States⁵⁰ by Couch.⁷ An estimated 15,000 persons between the ages of five and 60 died of liver disease in 1963; the diagnosis was cirrhosis in more than 90 per cent. Deaths from liver disease, excluding those caused by biliary atresia, were also studied in England and Wales by Terblanche and Riddell.⁴⁷ The incidence per capita was almost the same as in the United States, although alcoholic cirrhosis was less frequently recorded.

Admittedly, only a fraction of these patients could have been considered for treatment with liver transplantation since acceptance for this kind of therapy requires fulfillment of several criteria. As with renal transplantation, a highly desirable feature is freedom from disease in organs other than that to be transplanted. Thus, patients with extrahepatic metastases from hepatomas or those with secondary involvement of the liver from an uncontrolled primary malignant lesion would be excluded.

There are a number of additional potential contraindications. For example, children with biliary atresia often have other severe congenital anomalies.²⁵ In adults, the presence of atherosclerosis in coronary, cerebral, or other vessels may be a factor, particularly in older patients, although the incidence and severity of occlusive vascular disease may be lower in people with chronic liver disease than in the general population.^{8, 14} Many of the alcoholic cirrhotics seen by us have also had pulmonary disease as a result of years of self-abuse and excessive smoking. In addition, the behavior of the alcoholic may be so unreliable that the necessarily strict postoperative medical regimen could not be enforced.

The extent of metabolic derangement already present in patients with chronic liver failure will be an important consideration, at least for the time being. The patient with terminal renal disease can be rescued from a moribund state with intensive dialysis and kept in good condition for weeks or months (or even years) until the arrival of a satisfactory renal homograft. For the victim of end stage liver disease, such delaying maneuvers are usually impossible since effective means of resuscitation and interval maintenance are not yet available.

A host of derivative complications such as gastrointestinal ulceration, bleeding diathesis, renal failure, coma, and hypoglycemia may cause death before the only operation which might hold any hope of survival can be attempted.

Finally, existing extrahepatic infection is a contraindication to operation. Even under optimal circumstances, there is considerable risk of sepsis with the immunosuppressive drugs currently used to prevent homograft rejection. If a focus of infection cannot be eradicated prior to institution of therapy with these agents, there is little hope that this can be achieved afterward. This is a particularly limiting factor in patients dying of hepatic cirrhosis. In many such cases, indwelling urinary catheters, which were placed with the development of the hepatorenal syndrome, have already caused urinary tract sepsis by the time of the first evaluation for transplantation. Moreover, the majority of such potential recipients who have bled or passed into coma are found to have radiographic or clinical evidence of hypostatic or aspiration pneumonitis.

HEPATIC MALIGNANCY

In the non-neoplastic disorders, the objective of transplantation is to provide more adequate liver function. Removal of the patient's diseased organ is not, at least in concept, an obligatory component of the operation.

Primary Liver Carcinoma

Consequently, the unequivocal indication for the operation of liver replacement (orthotopic homotransplantation) was originally considered to be primary hepatic malignancy which could not be treated with conventional techniques of subtotal liver resection. Eleven patients with this diagnosis were in the series at the University of Colorado^{35-38, 41-43} and three others have been reported by Calne.^{5,6}

Of our 11 patients with hepatoma, there were four who had pre-existing cirrhosis (OT 2, 4, 5, and 15) and seven who did not (OT 3, 6, 8, 14, 17, 23, and 25). In every case, the diagnosis of malignancy had been made by biopsy at another hospital. There were 10 hepatic cell carcinomas and one cholangiocarcinoma. Useful information about the extent of the lesions and the effect upon them of prior irradiation or chemotherapy was obtained with liver scans (Figs. 1 and 2).

Tumor metastasis as a contraindication to transplantation was investigated with chest x-rays, bone surveys, and bone marrow examinations; these studies were usually carried out by the referring physician, as outlined in Chapter Four. A final decision for or against hepatic replacement was not made until a cadaveric liver became available and definitive exploration was carried out. In many cases, the effort was abandoned when tumor was found outside the liver. In spite of these precautions, one of our patients (OT4) was found at autopsy, one week after operation, to have a metastasis in a lumbar vertebra. Others have developed recurrent tumor long after transplantation (Chapter Seventeen).

There were two male patients who benefited from consideration of hepatic

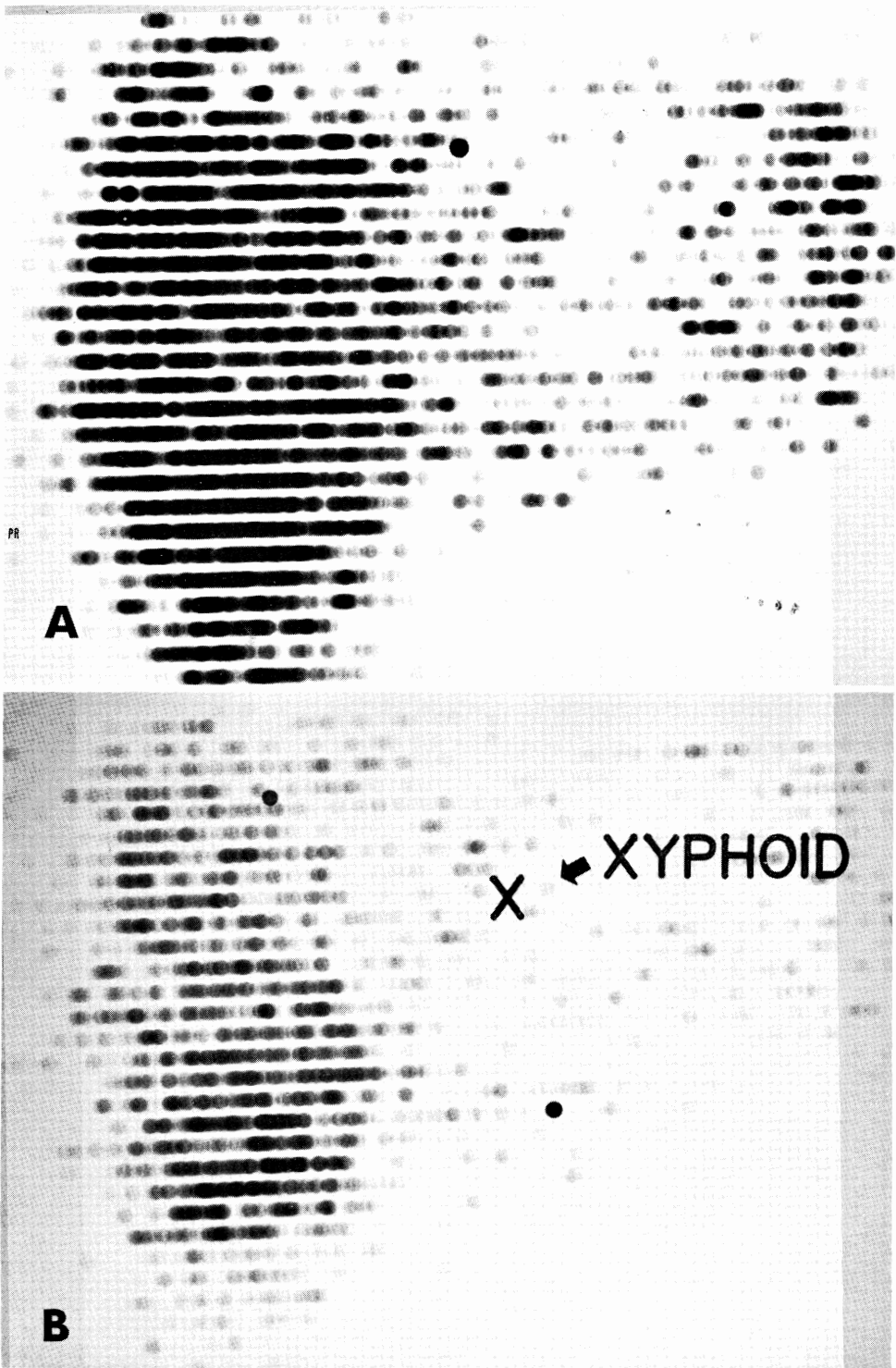


Figure 1. Growth of a hepatoma in patient OT 8. A. Liver scan in February, 1967, using ^{198}Au . The child was then 13 months old. Most of the tumor was in the left lobe but smaller growths in the right lobe precluded resection. B. A repeat study three months later showing only a small remnant of functional liver tissue. The excised organ weighed 1185 gm. (By permission of *Ann. Surg.* 168:392, 1968.)

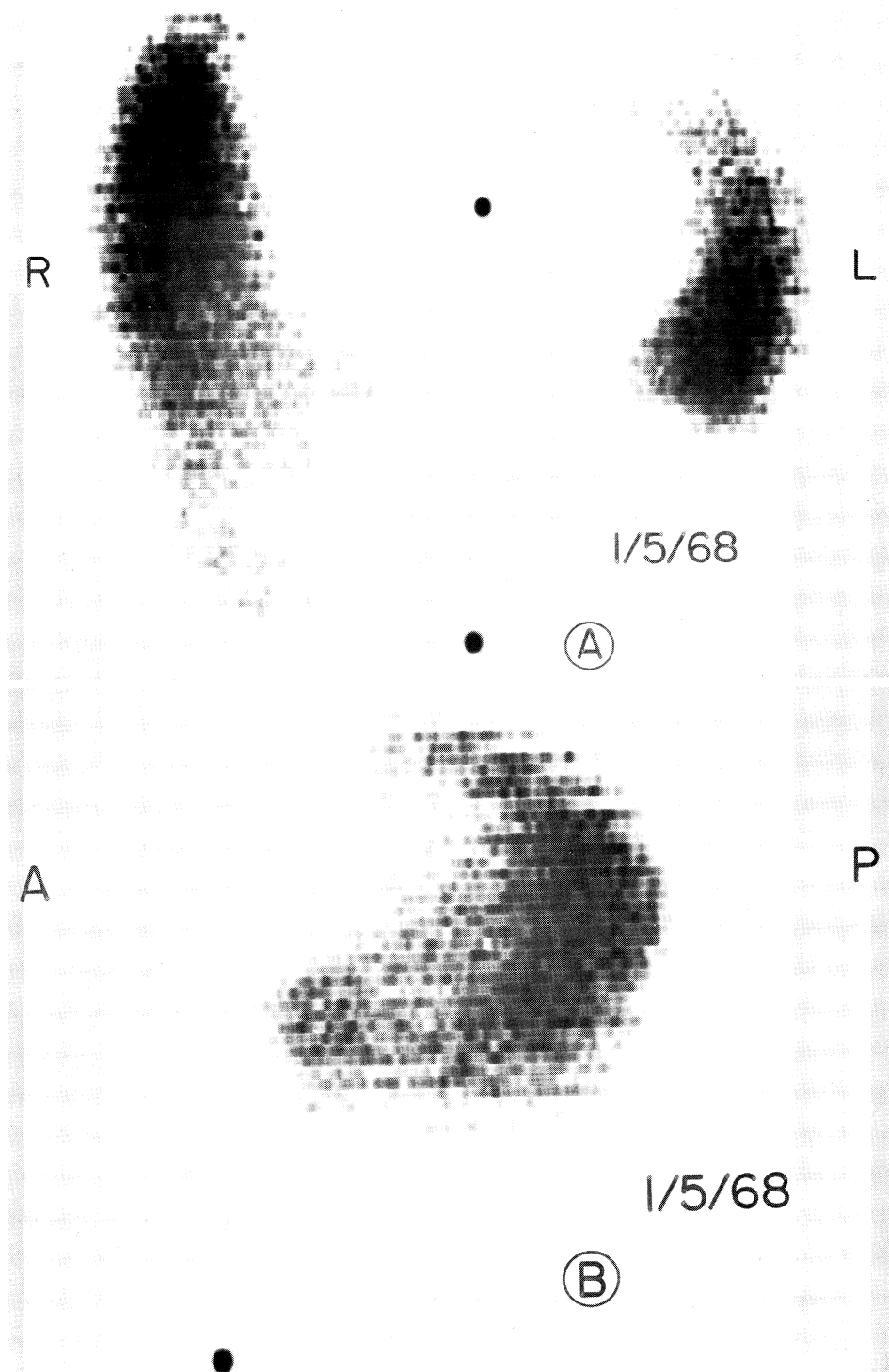


Figure 2. Liver scan with ^{99m}Tc of a 16 year old girl whose diagnosis was hepatoma. Note the central location of the principal mass. The determination of inoperability for subtotal hepatic resection was made in October, 1967, in another city; a feeding gastrostomy was performed because of severe anorexia. The liver scan was obtained on January 5, 1968. Orthotopic homotransplantation was carried out on March 17, 1968, at which time there was tumor invasion of the main hepatic veins below the line of suprahepatic vena caval transection. A, posteroanterior scan; B, lateral scan.

transplantation, although the procedure was not performed. In one, a small cholangiocarcinoma was located at the bifurcation of the common hepatic duct. Although a cadaveric liver was available, it was decided to attempt a Longmire procedure²¹ as has been described by Waddell³¹ for the treatment of a central hilar malignancy. There was a prompt fall of serum bilirubin from 58 mg per cent to normal (Fig. 3). This case was reported with a two and a half year follow-up since it then represented the longest known survival after such a palliative operation.⁴⁴ The patient is still alive after more than four years.

The other man was found at operation to have a liver cell carcinoma which could be removed by resection of two and a half liver segments as well as a portion of the abdominal wall. He recovered promptly but died with widespread metastases 27 months later.

It remains to be determined whether orthotopic liver transplantation will be an effective method for treating primary carcinoma of either the liver cells (hepatoma) or intrahepatic bile ducts (cholangiocarcinoma). The natural history of both diseases is grim if partial hepatectomy cannot be carried out, the average survival being only about three to four months in large collected series.^{3, 11, 33} Total hepatectomy with liver replacement extends the criteria of local resecta-

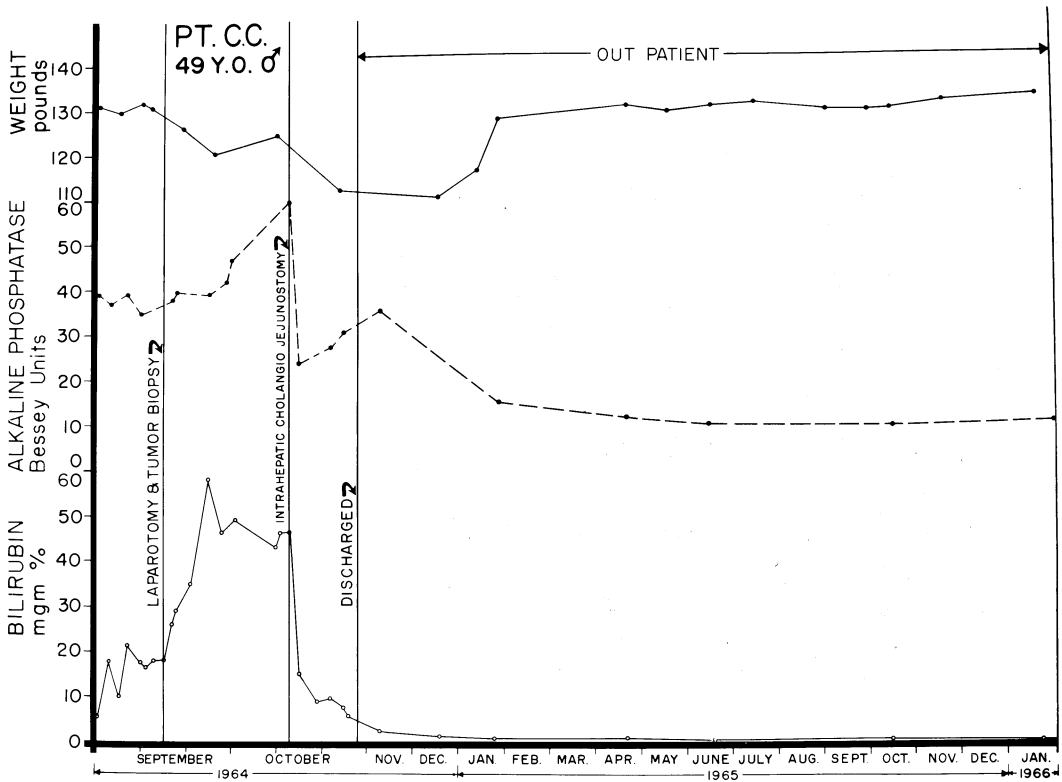


Figure 3. Course of a patient who was explored on October 13, 1964, for consideration of orthotopic liver transplantation. Although a potential cadaveric donor was near death in the same hospital, it was decided to perform a palliative Longmire procedure instead (intrahepatic cholangiojejunostomy). The hyperbilirubinemia was promptly relieved. There has been slow growth of the original sclerosing adenocarcinoma at the junction of the right and left common ducts. However, the patient is still alive more than four years later. He now has carcinomatosis. (By permission of *Amer. J. Surg.* 114: 722, 1967.)

bility but it does not insure against extrahepatic spread and, in fact, immunosuppressive therapy could conceivably accelerate metastatic growth (see Chapter Seventeen).

Liver Metastases

The differentiation of primary hepatoma from liver metastases can be extremely difficult, even with adequate biopsy material for histologic examination. A potentially discriminating biochemical test was reported by Tatarinov⁴⁶ and several later investigators^{2, 13, 17, 49} who found an unusual protein of the alpha₁ globulin class in the serum of 50 to 80 per cent of patients with liver cell carcinoma. It was speculated that the undifferentiated hepatoma cells synthesized this primitive serum protein, which is not normally found except in the fetus or newborn. False positive tests with hepatic metastases have been documented in only a few patients, of whom all were infants or children with embryonal primary malignant lesions.¹³

There have only been two examples of liver transplantation for metastatic liver disease. The diagnosis was known in Demirleau's case,⁹ but in Moore's the preoperative impression of hepatoma had to be revised when a small primary carcinoma of the sigmoid colon was found at autopsy 11 days after transplantation.²⁴

It is possible that occasional patients with metastatic liver disease could be benefited by orthotopic liver transplantation although, for reasons to be discussed in Chapter Seventeen, this is unlikely. Presumably, the conditions would be the development of isolated hepatic metastases long after resection of the primary neoplasm. We have seen patients die with tumor detectable only in the liver, years after resection of a melanoma of the eye, or after complete local control of carcinomas or carcinoids of the intestinal tract.

NON-NEOPLASTIC DISEASE

The central issues in transplantations in patients with non-neoplastic liver disease are if and when to proceed, whether to use an auxiliary or an orthotopic homograft, and what the life expectancy might be without this heroic form of treatment.

Congenital Biliary Atresia

The decisions are relatively easy if the diagnosis is extrahepatic biliary atresia, a disease described by Potts²⁸ as having been responsible for the "darkest chapter in pediatric surgery." The course is inexorable if, as is usually the case, there are no ducts available for biliary reconstruction. Under these circumstances, survival is uncommon for more than two and essentially unheard of for as long as four or five years.¹⁹ Moreover, the last months of life are an agony

to both parents and child because of itching, ascites, hemorrhages, recurrent respiratory infections, and, finally (in the event of a merciful providence), coma. It is not hard to justify the decision for transplantation once serious deterioration has begun (Fig. 4). In our series there have been 11 children with this diagnosis treated with orthotopic transplantation (OT 1, 7, 9, 10, 11, 12, 13, 16, 18, 21, 24) and one more (AT 3) in whom an auxiliary procedure was attempted.

In considering the proper form of treatment, there are factors favoring the orthotopic in preference to the auxiliary operation aside from the fact that the latter procedure has special physiologic disadvantages, as will be discussed in detail in Chapter Twenty-one. There are hepato- and splenomegaly, which may be so marked as to cause respiratory embarrassment in the later stages of the disease. This mechanical handicap is eliminated by the removal of both organs in the course of orthotopic homotransplantation. The livers in the patients with biliary atresia treated by us have weighed 415 to 885 gm (compared to an expected normal of 200 to 250 gm) and the spleens were 93 to 415 gm (normal at age two, approximately 35 gm.)

With the alternative procedure of auxiliary transplantation, there is little room in the overcrowded abdomen for an additional large organ. This was illustrated in Absolon's recipient¹ by the necessity for performing splenectomy and left nephrectomy before the incision could be closed, and by one of our pediatric patients^{12, 40} in whom the vascular supply of the graft was compressed when the abdominal wall was reapproximated (AT 3), leading to loss of the organ (see Chapter Twenty-two).

If the diagnosis is *intrahepatic atresia*, a distinction that can usually be made with biopsy, a doubly cautious attitude is necessary in recommending transplantation. Most children with this intrahepatic defect are dead by the age of five years, but survival for 10 years or longer has been recorded.^{18, 22, 45} Only one of our patients had intrahepatic atresia (OT 19).

Post-necrotic and Alcoholic Cirrhosis

Until now, there have been few reported attempts at liver transplantation in cases of cirrhosis; our experience for this indication has been limited to one orthotopic (OT 22) and three auxiliary procedures (AT 1, 2, and 4). There were probably two reasons for the paucity of cases. At least in the past, death was imminent, usually as the consequence of recent or ongoing variceal hemorrhage, when most such candidates were first considered. Cadaveric donors seldom become available on such short notice, and many prospective recipients have died in our institutions and elsewhere while awaiting a hepatic homograft.

Secondly, the fate of better risk patients with less advanced disease cannot be predicted with the absolute certainty which is possible with the diagnosis of liver carcinoma or extrahepatic biliary atresia. Most physicians with experience in liver disease can recall one or more deeply jaundiced, ascitic, and semicomatose cirrhotics who unexpectedly recovered completely enough to be discharged from the hospital. The possibility of a remission, however remote, has been responsible for many retrospectively regretted decisions against transplantation.

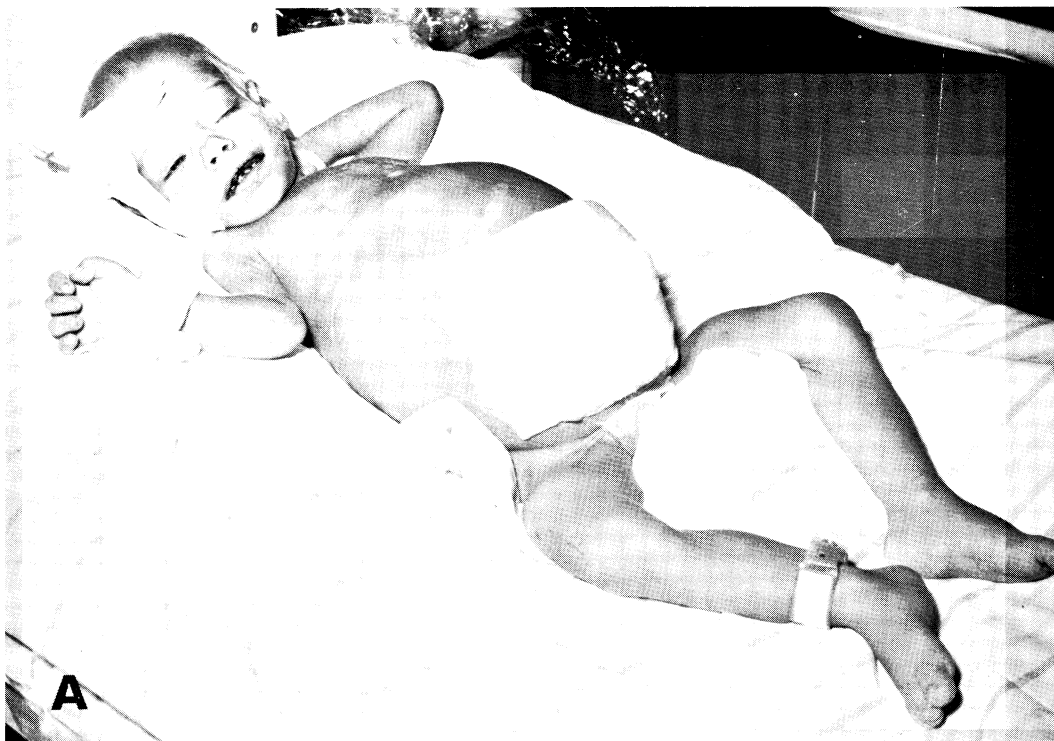


Figure 4. One of the patients (OT 12) with biliary atresia who received an orthotopic liver homograft. *A.* The infant was 16 months old and was admitted to the hospital in a moribund state. The pad on the lower abdomen covers a peritoneal fistula, which had developed some weeks previously after a paracentesis. *B.* The early convalescence after transplantation was very rapid. This picture was taken one month postoperatively. The child died three and a half months post-transplantation of septic infarction of the right lobe of the homograft (Chapter Fifteen).

The foregoing factors of both predictably high operative risk in cirrhotic patients and uncertainty about the natural course of the disease have also influenced judgment about the choice of procedure in the direction of auxiliary transplantation, which could be a potentially less difficult operation and one which does not involve the sacrifice of any residual host liver function. Whether these are sufficient reasons to select a method of therapy which has yielded inferior results in experimental animals as well as in patients (Chapters Twenty-one and Twenty-two) is open to the kind of serious question which can be answered only with more experience.

Now that the possibility of obtaining extended survival after liver transplantation has been proved, it may become possible to relax the indications for intervention, particularly in cases of postnecrotic cirrhosis in which the maximum value of abstinence from alcohol has usually already been realized. An ideal prospect might be someone whose physical condition is relentlessly deteriorating; who has progressive jaundice, hypoproteinemia, prothrombin depression, and other worsening indices of liver malfunction; and whose mental competence is failing. Such a patient is known to have little chance of surviving even if continuously hospitalized.³²

Having made a decision for transplantation, it would probably be best to make the final choice of procedure contingent upon the circumstances of organ supply. For example, if a histocompatible (Chapter Three) liver of an infant or child became available, it could be placed as an auxiliary organ by one of the techniques described in Chapter Twenty-two; the problem of adding a cumbersome intra-abdominal mass would be minimized. On the other hand, an adult liver might be placed orthotopically if it were thought that the operation could be tolerated. If not, an attempt at auxiliary transplantation might be justifiable in spite of its drawbacks.

Other Chronic Diseases

Cirrhosis and liver failure resulting from less common diseases should also be amenable to treatment under the same guidelines as in the preceding section. Examples might include primary biliary (Hanot's) cirrhosis, Wilson's hepatolenticular degeneration, irreparable biliary duct injury, and hemachromatosis. To our knowledge, liver transplantation for these diseases has not yet been performed. One of our recipients of an orthotopic homograft (OT 20) had an unusual disorder. She apparently had neonatal hepatitis and later postnecrotic cirrhosis. At the time of transplantation, she was eight years old. The biliary drainage system was patent but the intrahepatic ducts had marked cystic changes (Fig. 5).

Acute Liver Failure

The role, if any, of transplantation in the treatment of fulminating viral or toxic hepatitis will eventually have to be defined by clinical trial. There seems little doubt that patients dying of acute hepatic failure can be helped with the

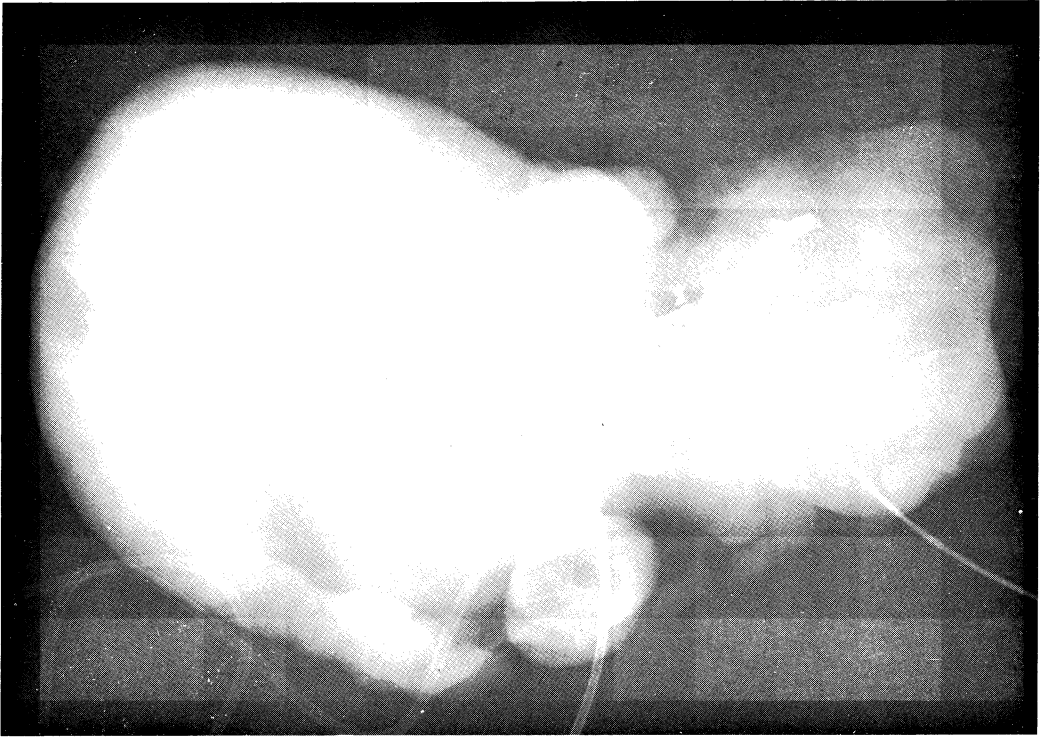


Figure 5. A cholangiogram of the liver removed from patient OT 20. Note the widespread saccular cholangiectasis.

kind of extracorporeal liver,^{10, 26, 39} exchange transfusion,^{30, 48} or cross circulation^{4, 15, 31} procedures that are used for only a few hours. The improvement is, of course, ordinarily too short-lived to influence the ultimate outcome.

What is needed is assistance for intervals long enough to permit healing and regeneration of the gravely damaged host liver. Auxiliary homografts might permit this, subject to several potentially important reservations. First, in infectious hepatitis it will have to be established that the viremia which may be present, but which has not been consistently or convincingly demonstrated during the period of rapidly developing jaundice,²⁹ will not lead to acute destruction of the homograft. Second, the use of immunosuppressive agents to protect the homograft would be predicted to adversely affect the host's immunologic defense against the invading microorganisms (see Chapter Seventeen).

With either infectious hepatitis or the more favorable situation of acute yellow atrophy resulting from drugs or other toxic agents, another factor may conceivably defeat the ultimate purpose of eventually re-establishing dependence upon the acutely diseased organ. It is highly likely for reasons discussed in Chapter Twenty-one that a well functioning auxiliary homograft will inhibit to some extent the recovery of the injured host liver.

Metabolic Diseases

Several recent studies^{16, 23, 41} have provided strong evidence that liver homografts retain their metabolic specificity after transfer to a new host (Chapter

Eighteen). A practical implication of these demonstrations is that certain liver-based metabolic disorders might become treatable with such a procedure or even with auxiliary transplantation. The concept has been conclusively tested by Kuster et al.²⁰ Using mongrel canine donors, they were able to cure the gout naturally present in Dalmatian recipients. Conversely, the transplantation of Dalmatian livers conferred the defect in uric acid metabolism upon mongrel recipients.

Clinical liver transplantation has not yet been performed for this kind of objective, and if it is attempted in the future it will be for extremely limited indications. Lists have been compiled of many liver-based inborn errors of metabolism which hypothetically should be curable by hepatic transplantation.^{27, 34} However, most of the resulting diseases cause neither serious morbidity nor mortality. In others, the crippling or lethal complications can be controlled or prevented by dietary restriction, enzyme replacement, specific medication, or other forms of conservative therapy. None of these disorders could be considered at present to be sufficiently grave and untreatable to warrant recommendation of hepatic transplantation with its high early risk and unknown long term dangers.

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Chapter Two

THE LIVER DONOR

Some of the practical problems of cadaveric organ supply have often introduced a regrettable but unavoidable impersonal note into the preoperative relationship of the transplant physicians and surgeons to the prospective recipients. Someone in need of a liver homograft may be entered on a candidacy list for hepatic transplantation, but this is no assurance that treatment will ever become a reality or even that the patient will be seen by the staff at the transplantation center. Whether the undertaking will become possible depends upon the procurement of a liver which is of the proper size and antigenic structure and which has not been so badly damaged that it cannot support life in its new host. Only after the arrival and study of a potential donor can a designation be made of the appropriate recipient.

The governing role of unpredictable donor availability in determining which recipient can be treated, and at what time, will continue to be a major barrier to the full utilization of cadaveric organs of all varieties until techniques of organ banking can be perfected. Then, as now, however, a vital factor will be the procurement of organs which have not already been irreversibly damaged during the agonal phase of donor life. Errors in judgment about donor suitability were often made, particularly early in our experience with liver transplantation, and were probably at least partly responsible for many of the failures.

DONOR SELECTION

Several hours are usually required to study the donor, to make a discriminating choice of a recipient, and to ready the equipment to be used for organ preservation. The criteria for accepting a donor are accordingly somewhat restrictive. Someone who is lifeless on arrival at the hospital or who dies unexpectedly within the institution and cannot be temporarily resuscitated is usually excluded. A good donor would be a young person who has had a grave brain injury due either to trauma or neurological disease, but in whom an adequate cardiopulmonary state can be at least temporarily maintained with ventilator support and, if necessary, with cardiotonic and vasopressor drugs. In many such patients,

it is evident from the outset that the damage to the central nervous system is incompatible with recovery.

Cooperative action by the medical community at large is necessary if organs are to be procured under these circumstances. Often, the condition of the prospective donor is so patently hopeless at the time of initial examination or after varying periods of observation that attempts at reanimation or continued mechanical support would be a cruelty to the patient and relatives if there were not another objective than the extension of life in a vegetative state. In Denver many physicians have assumed the responsibility of short term resuscitation in this kind of situation rather than stopping treatment, have talked frankly to the family about the reasons, and have then contacted the transplantation team. In such instances, permission for postmortem removal of the organs was obtained at some time before death. There have been no adverse legal consequences or known psychologic complications.

The investigations which follow have an ordered sequence. Blood is immediately drawn for a battery of standard liver function tests. In addition, examinations are started which will be crucial for the selection of a recipient. The red cell type is determined at once so that prospective recipients can be narrowed to those who are acceptable by the rules of blood group compatibility, which have been well worked out in renal transplantation^{6,9} (Table 1).

A comment is in order about donor-recipient combinations in which there is compatibility but not conformity of ABO groups; examples would be a transplantation from an O donor to an A, B, or AB recipient, or from an A or B donor to an AB recipient. Several years ago there was some uneasiness that the reticuloendothelial system of the graft might react under these circumstances with the isoantigens of recipient red cells and cause red cell destruction.¹⁰ This anxiety has proved to be groundless. There have now been four cases in our experience with O to A (OT 4), O to B (OT 19), or A to AB (OT 15 and 23) transfers. A significant hemolysis did not occur. One of the recipients (OT 4) died

Table 1. Direction of Acceptable Tissue Transfer When the Donor and Recipient Have Different ABO Red Cell Types*

O to non-O	Safe
Rh— to Rh+	Safe
Rh+ to Rh—	Relatively safe
A to non-A	Dangerous
B to non-B	Dangerous
AB to non-AB	Dangerous

*O is universal donor; AB is universal recipient.

after six and a half days, but the other three survived for many months (Chapter Seventeen).

In addition to red cell typing, study of lymphocyte antigens is begun immediately in order to establish the histocompatibility profile as described in Chapter Three. For donors the necessary tests are first performed in Denver using antisera provided by Dr. Paul Terasaki of Los Angeles, and if time permits, confirmatory examinations are later carried out in the California laboratory. The analysis in Denver requires two to four hours. As soon as the results are available, it is possible to compare the antigen characteristics of the potential donor with those of a pool of red-cell compatible recipients for whom the same information has been previously obtained by Terasaki. A final recipient choice can then be made on the basis of this biologic criterion.

Some thought must also be given to the technical soundness of the proposed procedure, a consideration which in cases of nonmalignant liver disease may determine whether an orthotopic or auxiliary operation should be done. For example, the availability of a well matched child's liver for an adult cirrhotic would dictate its use as an auxiliary organ, whereas an adult sized homograft for the same patient would most likely be placed in the orthotopic position. The ages and weights of all donors and recipients in our series of both kinds of operations are given in Chapter Twenty-four. The ultimate decision about the mechanical feasibility of the undertaking was a highly individual one, based on careful physical examination of both the donor and the recipient. One of our female patients (OT 14), who was 16 years old, weighed 49 kg and received the liver of a 27 year old man who weighed 82 kg. There was no problem of size disparity because of the hepatomegaly which a hepatoma had caused in her excised organ. There were several other examples of age and weight disproportions in our cases (Chapter Twenty-four). The most dangerous combinations seemed to be those in which the donors were larger than the recipients (see Chapters Nine and Ten).

The role of donor age in determining the quality of the organ is probably extremely unpredictable. It may be that the liver is less consistently affected than the kidney, lungs, or heart by the degenerative changes of senescence. This possibility was suggested by the autopsy investigation of Morgan and Feldman¹ in which 62 of 74 livers from patients over 75 years of age were said to be normal; no lesions of the arterial blood supply were found. In a clinical study, Cohen and his associates² reported that more than three fourths of patients who were more than 65 years old had essentially normal liver function tests.

At the Denver Veterans Administration Hospital a postmortem study was made by Dr. George Peters³ of the livers of 30 consecutive older patients. The criteria for inclusion in his study group were: age exceeding 50 years (50 to 89; average, 65.5), freedom from tuberculosis or neoplasia, and absence of known extrahepatic biliary tract abnormalities or primary hepatic disease. A manifestation of generalized atherosclerosis was the cause of death in half the patients. The next most common primary diagnosis was pulmonary emphysema.

Fifteen of the 30 patients in Peters' series had no atherosclerosis whatever in the celiac axis or any of its branches (Table 2), and in 80 per cent the hepatic

Table 2. Incidence of Atherosclerosis of the Celiac Axis or its Branches in 30 Consecutive Autopsies of Patients Who Were 50 to 89 Years Old (Average, 65.5)*

AREA OF ATHEROSCLEROSIS	NO. OF AUTOPSIES
No involvement	15
Celiac axis only	3
Splenic artery only	5
Celiac axis and splenic artery	1
Gastroduodenal and splenic arteries	1
Hepatic artery and/or lobar branches	5
TOTAL	30

*The study was carried out by Dr. George N. Peters, Resident in Surgery, VA Hospital, Denver, Colorado.

artery was completely spared. The other 20 per cent had grossly visible atherosclerotic plaques in the common, proper, or lobar hepatic arteries, but these were neither circumferential nor occlusive in any case. Almost all the livers appeared to be in good condition by gross examination. However, the parenchyma had minor histologic abnormalities in the majority of the specimens.

Early in our experience several donors were accepted who were beyond 45 years (55 to 79; average, 69). None of the recipients (OT 2 to 6 and AT 1) lived more than three and a half weeks. In all there were histologic changes in the homografts recovered at autopsy which were thought to have predated transplantation. More recently, the less hazardous policy of using only donors less than 45 years old has been followed, and in fact no liver has been taken from anyone older than 27 years since Case OT 7.

An accurate picture of the quality of the donor liver may be difficult to obtain during the terminal phases of life. The family can usually provide a history of the presence or absence of previous liver disease, drinking habits, and pre-existing nonhepatic disorders. The results of hepatic function tests are vital to the assessment. The most reliance is placed on determinations of serum bilirubin, alkaline phosphatase, SGOT, SGPT, and total proteins, and upon measurement of the prothrombin time.

However, the liver chemistries often have abnormalities which do not preclude proceeding if these are acute and due to a failing circulation. A good example was the 82 kg donor alluded to previously who provided a liver for the 16 year old recipient. The man had been in good health until 10 hours before death when he sustained a fatal gunshot wound of the head while playing Russian roulette with a small caliber pistol. Six hours after the accident, while life was being maintained with a mechanical ventilator and infusion of vaso-

pressor drugs, the prothrombin time was depressed to 30 per cent, the SGOT was 200 SF units (normal 32), and the serum bilirubin was 1.8 mg per cent. Three hours later, and one hour before death, the prothrombin time was further depressed to 7 per cent. Nevertheless, the first prothrombin determination in the recipient after a seven hour preservation and orthotopic transplantation was 50 per cent. In this case, the young age of the donor and the shortness of the interval between injury and death were highly favorable factors.

THE EVENTS OF DEATH

It is evident from the previous comments that serious damage to the vital organs can occur during the terminal stages of life. The magnitude of injury is probably proportional to the degree and duration of terminal circulatory failure. The extent of hepatic injury can be tracked with serial liver function tests. It can also be indirectly estimated with some accuracy by close attention to urinary output; if this remains adequate it is highly likely that the other viscera, including the liver, are also being well perfused. All organs are apt to be seriously or irreparably damaged in a patient who has been in agonal condition and anuric for several days or possibly even for several hours.

The extraordinary efforts which must be made to prevent circulatory depression in the face of a hopeless prognosis usually require explanation to the relatives who by this time appreciate the fact that there is no hope for recovery. This is especially true if cardiac massage becomes necessary in a patient who has an isoelectric electroencephalogram, fixed dilated pupils and no reflex initiation of spontaneous respiration; one of the donors in our series (OT 9) had 18 separate episodes of cardiac arrest and most had more than one.

Until very recently the ultimate definition of death in our institutions was cessation of heart beat in a patient who also had been shown to have a central nervous system injury which was incompatible with life. Consequently, a final cardiac massage usually triggered the subsequent activity which will be described in Chapters Five and Six. However, adherence to the condition of cardiac asystole was not always easy. The reason was that cessation of the heart beat of several donors occurred outside the operating room area and at a time when the preparations for the recipient operation had not yet been completed. Under these circumstances vigorous resuscitatory measures were taken, with temporary restoration of a spontaneous cardiac rhythm. In some of these cases, the donors were subsequently removed to the operating room, where respiratory support was discontinued by the service responsible for their terminal care. In others, the initial stages of the hepatectomy were carried out with the family's knowledge and permission in the presence of an intact circulation. With either set of conditions, reliance was ultimately placed on rapidly instituted procedures to halt or slow normothermic ischemic injury after effective circulation had ceased.

More recently there has been acceptance at the University of Colorado of the concept of brain death as it was first outlined, defended, and applied at the Univer-

sity of Louvain by Alexandre.¹ Initially the fear that the quality of terminal care provided for the donor might be thereby lessened caused us to speak out against the pronouncement of death in the presence of a heart beat.⁷⁻⁸ Our later experience, using the criteria outlined by Alexandre¹ and those similar ones of the Harvard University Committee,³ has convinced us that such anxieties were unfounded.

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Chapter Three

HISTOCOMPATIBILITY TYPING

by Paul I. Terasaki, Ph.D.

That histocompatibility will influence the outcome of liver transplantation in man cannot be disputed, for genetically determined transplantation antigens have been shown to be of importance with every other organ and in every species thus far studied. The principal question to be answered is to what *degree* histocompatibility antigens will be of importance when their influence is modified by currently available means of immunosuppression.

A precise answer to this question cannot now be formulated because of deficiencies of the tissue typing methodology currently being used to identify donor-recipient incompatibilities. Although a large amount of information has recently been gathered and collated, it must be recognized that typing of human cells is essentially an effort of the last five years and one that is still highly developmental. It is not known, for example, how many significant histocompatibility antigens have yet to be discovered or even what are the relative strengths of those antigens which are already well recognized.

In this chapter the material for discussion will be divided into three parts. The first will concern factors which could lead to typing errors or misinterpretation. Second, correlations will be described between tissue matching and the outcome after human renal transplantation. Finally, the use of histocompatibility analysis will be discussed as it has been applied for clinical liver transplantation.

SOURCES OF TYPING ERRORS

There are several ways of assessing histocompatibility differences between donors and recipients. The ones with the most promise of clinical applicability are thought to be those which attempt to detect antigens in peripheral lymphocytes. The reagents used for this purpose are human isoimmune antisera obtained from persons who have accidentally or deliberately been sensitized to white cell antigens. The cytolysis of test lymphocytes by such antisera implies

the presence of the same or a similar antigen as that which originally sensitized the serum donor, and failure of such a reaction implies the absence of the antigen. The assumption inherent in the method is that the analysis of the peripheral lymphocytes will faithfully reflect the histocompatibility composition of all tissues within that individual. For the most part, this appears to be true, although certain histocompatibility antigens may be lacking on lymphocytes but present on different organs.

The results of the antigen studies in the prospective donor and recipient are used for the matching. When the lymphocytes of both members of a pair react the same to a group of antisera which are thought to measure a given histocompatibility determinant, *conformity* of that antigen is said to be present. The absence of an antigen in a donor which is present in a recipient is defined as *compatibility*. When an antigen is found on the donor lymphocytes but not on those of the recipient, a *mismatch* exists. For any given case, conformity of antigens would be preferable; compatibility would be the next most satisfactory condition; and the least desirable would be an overt mismatch. In practice, there are limitations to the accuracy and completeness with which the state of matching can be assessed at the present time.

Reproducibility

Even within the same laboratory, the results of antigen analysis for a given patient may be variable from day to day. The incidence of nonreproducibility with leukoagglutination techniques is reported to be in the range of 10 to 20 per cent; it is less than 5 per cent using the lymphocyte cytotoxicity test.⁸

False Values

The incidence of false negative and false positive reactions is not known, although it will not be surprising if it is shown several years from now to have been high in the currently studied cases. Detection of this kind of error is possible by combined serologic and genetic studies. It is well known that red cell ABO typing had many technical errors even 20 years after its discovery.³⁰ Type O and type AB children should not result from matings of O \times AB parents, though their occurrence was said in different series to be as high as 26.3 per cent, until the publication of the triallelic hypothesis of Bernstein in 1926.

Similar genetic analysis has already aided in the identification of false reactions, particularly when testing was carried out within family groups. At the Third Histocompatibility Workshop in Torino, Italy, it was found that the antigenic specificities of the HL-A histocompatibility system could be fitted into a hypothesis of a single chromosome locus with almost no exceptions;² furthermore, the alleles tended to be divisible into two distinct subloci. Some of the individual antisera gave conflicting answers, but the overall results indicated essentially no recombinants in all the 11 families studied.¹ With this kind of

information, it was possible to focus immediate attention upon tests which did not conform to a rational pattern.

False negative reactions in which a cell possesses an antigen but fails to react have been described as "ANAP" (agglutination negative, absorption positive) by van Rood²⁸ and "CYNAP" (cytotoxicity negative, absorption positive) by Ceppellini.¹ Along these lines, reactions which are negative but become positive with longer incubation could be called "CYNIT" (cytotoxicity negative, insufficient time), as we have described.⁸

False positive findings could result from the use of partially damaged cells or impure cell suspensions, or from other defects in the methodology.

Incomplete Knowledge of Antigens

Only a portion of the significant histocompatibility determinants have so far been identified. The consequent degree of incompleteness of antigen analysis with the currently available techniques is not known. However, within each of the two main subloci of the HL-A system which have been proposed by several authors,^{3, 6, 15, 25} the number of missing alleles may be estimated by adding together the known gene frequencies. Singal's data (Table 3), which include a new allele (Te 12) plus four others at the first LA sublocus and a total of five alleles^{17, 18} at the second sublocus, suggest that 24 and 48 per cent of each of the two chromosomal regions are not accounted for by the methods used today.¹⁸ Thus, two or more additional antigens probably exist at the first site and three or more at the second sublocus. In non-Caucasian races even larger discrepancies in gene frequency remain unresolved (Table 3).

A third "4a-4b" sublocus has been proposed,^{7, 29} although the validity of these antigens has been questioned by Kissmeyer-Nielsen.⁶ Van Rood's original analyses of gene frequencies²⁹ left no room for additional alleles in this area of the chromosome. However, the sum of the 4a and 4b gene frequencies (Te 3 and Te 7) in our laboratories was so low that several more alleles could be postulated.

In addition to undisclosed antigens of the HL-A system, presumably weaker alleles at other sites within the same or different chromosomes will undoubtedly be revealed as the serologic and genetic analyses are refined. In the meanwhile, the larger the proportion of unknown to known specificities, the poorer the correlation will be between typing and the controllability of rejection after human organ transplantation. Any dissociation between the tissue matching and the clinical results would be predicted to be greater in nonrelated than in intra-family pairs. If a good conformity of the known histocompatibility determinants exists between consanguineous donors and recipients, the matching of many unmeasured antigens may be assumed, since the unknown HL-A factors are probably linked closely to the known ones. Moreover, non-HL-A histocompatibility antigens determined by other loci would tend to be compatible by chance, more among related than nonrelated donors.

An important point to be noted is that unknown antigens can account only for the poor outcome of "matched" transplants and do not explain "mismatched"

Table 3. Phenotype Frequency of HL-A Antigens in a Random Population*

ANTIGEN	CAUCASIAN	NEGRO	ORIENTAL
First sublocus			
HL-A1	29.0	15.6	0
HL-A2	45.2	34.4	43.4
HL-A3	29.8	18.9	21.2
Te 4	19.8	23.8	53.5
Te 12	14.7	33.6	44.4
Second sublocus			
HL-A5	21.4	33.6	49.5
HL-A7	27.8	33.6	9.1
HL-A8	19.4	4.1	2.0
Te 9	19.1	13.1	17.2
Te 10	10.7	6.7	40.4

*Gene frequencies may be derived from these figures by the formula $G = 1 - \sqrt{f}$; f = frequency of individuals negative for the determinant. Figures are percentages.

grafts which appear to succeed. The number of cases in the latter category is apt to increase in the future. As new antigens are uncovered, the currently matched grafts may fall into the incompatible category, whereas mismatched grafts can only be changed to the category of those with a greater degree of incompatibility.

Antigenic Strength

Until more data become available about the relative strengths of the different antigens, it will not be possible to prognosticate the outcome accurately in the presence of incompatibilities of one or more of these determinants. It has been established that no single mismatch can cause uniform failure.^{16, 24} The largest reported series with an isolated specificity of mismatch has been of the HL-A2 (Mac) antigen. There were 26 recipients of renal homografts;²⁴ seven of the 26 had uncontrollable rejection leading to loss of the transplant or death. Eleven of the remaining 19 had excellent courses and 10 of these had been surviving for more than two years. The other eight patients had either shorter or less satisfactory courses but were still alive.²⁴

These observations suggest that the HL-A2 antigen did not always play an equivalent role in the severity of the immunologic repudiation. The variability could presumably be explained by the effect of other frequently unanalyzable factors, including differences in host responsiveness, the presence or absence of undetected antigens, and the efficiency with which immunosuppression could be delivered. The influence of six other HL-A antigens was also recently studied after 147 parent to offspring and 107 sibling renal transplantations. None of the determinants was found to be of unique potency.¹⁶ In fact, if all the specificities were pooled so that each one was considered to be roughly equivalent to the others and the cases were simply codified as mismatched or matched, then the results were almost exactly as found with an even cruder classification in the first study of kidney patients in 1964 done on the Denver series of transplantations.^{20, 27}

The failure in our laboratories^{10, 16, 26, 27} and in those of Payne,¹² van Rood,²⁹ Dausset,⁴ and Amos²¹ to find a single antigen of overriding significance suggests that the major histocompatibility determinants are all of intermediate strength. Eventually, a relative risk factor for each one must be determined.²² It will not be surprising if the most unacceptable situations will involve mismatches with certain special combinations of two or more antigen incompatibilities.

CORRELATION OF HISTOCOMPATIBILITY WITH OUTCOME AFTER RENAL TRANSPLANTATION

It is now generally conceded that the extreme view can no longer be supported that the HL-A antigens are not histocompatibility determinants. However, in view of the many limitations just cited, the question must be raised about the advisability of using typing as an important criterion for donor selection.

The justification for this continued practice comes from the fact that recipients of HL-A compatible kidneys have generally fared better clinically than those who have not had this advantage.^{4, 10, 12, 16, 19, 20, 26, 27, 29} Not only has their survival been at a higher rate, but they have also had less histopathologic damage to the homografts^{13, 14} as well as better renal function.⁹ The most striking benefits of compatibility have seemed to accrue at a considerable time after transplantation. Thus, the risk from early homograft repudiation was often not closely correlated with the degree of incompatibility. However, with the passage of time, a divergence of results became more clear between the matched and mismatched cases.

In the foregoing studies the association of a good or bad match with a favorable or poor clinical result, respectively, was most clear after sibling transplantations. The correlation was less complete with parent to offspring combinations. In the latter group there was a incongruously large number of good results in the face of adverse matching or, conversely, some poor results despite apparently satisfactory antigen pairing.¹⁶ The most likely explanation was the genetic basis discussed in the preceding section. It will be recalled that, according to current concepts, a parental donor would always automatically have at least half of the

HL-A antigens in common with any offspring. In contrast, the siblings from such a union could theoretically share all the antigens with 25 per cent of their mates, half the antigens with another 50 per cent, and none at all with the remaining 25 per cent. Thus, the spectrum with intersibling transplantation could range from an essentially perfect match to one equivalent to that obtainable by random pairing of nonrelated individuals.

As might also be predicted, the poorest correlation of all between typing analysis and clinical outcome has been in nonrelated transplantations. Nevertheless, there has been some evidence, albeit as yet equivocal, that donor selection on the basis of typing may be of value even under these circumstances.^{11, 23}

HISTOCOMPATIBILITY TESTING IN LIVER TRANSPLANTATION

The logistics of obtaining antigen profiles for patients in widely separated parts of the country are discussed in Chapters Two and Four. Recipient typing has generally been carried out with blood that was shipped to Los Angeles. The donors were studied in Denver and, when time permitted, in Los Angeles as well. The matching of the donor with the most compatible recipient was facilitated by intercity telephone conferences. The efforts at prospective matching were begun in 1966 and have continued for all cases to the present time.

The difficulty of obtaining conformity or compatibility of all measured antigens can be appreciated by a glance at Table 4. In this analysis 12 histocompatibility groups were taken into consideration. There were 38 prospective liver recipients of blood type O who were studied in relation to 13 potential hepatic donors of the same erythrocyte group; there was a total of 494 possible donor-recipient combinations.

It can be seen (Table 4) that conformity of match (grade A) was present in only 0.2 per cent of these donor-recipient pairs and that phenotypic compatibility (grade B) pertained in only 5.1 per cent. In most instances, one (20.9 per cent grade C) or more (73.9 per cent grade D) HL-A groups were incompatible. Not all the recipients were entered simultaneously into the pool of Table 4. In addition, no more than one of the donors was ever available at any given time. Consequently, the actual number of theoretically possible matches was a tiny fraction of the proposed 494. Furthermore, the actual selectivity was even further limited by other considerations. These included the clinical condition of the donors and the recipients as well as their sizes and ages. The need for a massive pool, as has been advocated for renal transplantation,²³ is evident.

Detection of Preformed Antibodies

The adverse effect of pre-existing antibodies upon a transplanted organ for which they have an avidity will be discussed in Chapters Fourteen and Nineteen. In our laboratory preformed antibodies have been considered to be present if the patient's serum caused cytolysis of the lymphocytes of more than four members

Table 4. Matches Obtained Between 13 Prospective Donors and a Pool of 38 Prospective Recipients*

RECIP. NAME	ANTIGENS	1,3,7,11	1,3,5	1,3,6	2,4,5,6,11	2,3,7	2,4	2,4,6,10	1,3,8	2,5	1,6,8,10	2	2,5,9	4,6,7	DONORS
Bel	12,13	D	D	D	D	D	D	D	D	D	D	C	D	D	
Ben	1,2,5	D	C	D	D	D	C	D	D	B	D	B	C	D	
Ble	2,4,9	D	D	D	D	D	B	D	D	C	D	B	C	D	
Bro	2,3,5	D	C	D	D	C	C	D	D	B	D	B	C	D	
Bul	2,6,7,12	D	D	D	D	C	C	D	D	C	D	B	D	C	
Cal	1,7,8	D	D	D	D	D	D	D	C	D	D	C	D	D	
Col	8	D	D	D	D	D	D	D	D	D	D	C	D	D	
Cun	1,4,5,11	D	C	D	D	D	C	D	D	C	D	C	D	D	
Dor	2,5,10,11	D	D	D	D	D	C	D	D	B	D	B	C	D	
Dos	1,8,10,12	D	D	D	D	D	D	D	C	D	C	C	D	D	
Fol	1,8,11	D	D	D	D	D	D	D	C	D	D	C	D	D	
Gal	5,12	D	D	D	D	D	D	D	D	C	D	C	D	D	
Gar	3,6,7,8,11	C	D	C	D	C	D	D	C	D	D	C	D	C	
Gar	3,5,7,11,12	C	C	D	D	C	D	D	D	C	D	C	D	D	
Gon	1,5,9	D	C	D	D	D	D	D	D	C	D	C	C	D	
Gri	3,8,12	D	D	D	D	D	D	D	C	D	D	C	D	D	
Har	1,9	D	D	D	D	D	D	D	D	D	D	C	D	D	
Hic	2,3,5,11	D	C	D	D	C	C	D	D	B	D	B	C	D	
Joh	2,3,5	D	C	D	D	C	C	D	D	B	D	B	C	D	
Ken	1,7	D	D	D	D	D	D	D	D	D	D	C	D	D	
Kil	3,6,12	D	D	C	D	D	D	D	D	D	D	C	D	D	
Lab	4,10	D	D	D	D	D	C	D	D	D	D	C	D	D	
Lov	2,6,7,8	D	D	D	D	D	C	D	D	C	D	B	D	C	

Table 4. Matches Obtained Between 13 Prospective Donors and a Pool of 38 Prospective Recipients* (*Continued*)

RECIP. NAME	ANTIGENS	1,3,7,11	1,3,5	1,3,6	2,4,5,6,11	2,3,7	2,4	2,4,6,10	1,3,8	2,5	1,6,8,10	2	2,5,9	4,6,7	DONORS
Mad	4,12	D	D	D	D	D	C	D	D	D	D	C	D	D	
May	1,3,5,6	D	B	B	D	D	D	B	C	C	D	C	D	D	
Mar	2,4	D	D	D	D	D	A	D	D	C	D	B	D	D	
Min	3,7,9,12	D	D	D	D	C	D	D	D	D	D	C	D	D	
Pic	3	D	D	D	D	D	D	D	D	D	D	C	D	D	
Roy	8,9	D	D	D	D	D	D	D	D	D	D	C	D	D	
She	1,2,8,9	D	D	D	D	D	C	D	C	C	D	B	C	D	
Sol	2,6,10,11	D	D	D	D	D	C	C	D	C	D	B	D	D	
Sta	2,6,7,9,11	D	D	D	D	C	C	D	D	C	D	B	C	C	
Sto	1,7,8	D	D	D	D	D	D	D	C	D	D	C	D	D	
Thu	2,6	D	D	D	D	D	C	D	D	C	D	B	D	D	
Tru	2,5,11	D	D	D	D	D	C	D	D	B	D	B	C	D	
Wah	1,2,5	D	C	D	D	D	C	D	D	B	D	B	C	D	
You	3,5,6,8	D	C	C	D	D	D	D	C	C	D	C	D	D	
Zaw	1,6,8,9,12	D	D	C	D	D	D	D	C	D	D	C	D	D	

*All donors and recipients were red cell type O. Matching grades were assigned by the following criteria: A = identical; B = compatible (donor having no antigens foreign to the recipient); C = incompatible for 1 group; D = incompatible for 2 or more groups. The potential matches totaled 494%, of which the following percentages fell into each grade: A = 0.2%; B = 5.1%; C = 20.9%; and D = 73.9%.

of a panel of approximately 40 normal blood donors. Of 113 prospective liver recipients there were only 12 (10.6 per cent) who had such evidence of presensitization. The incidence was less than half of that seen in uremic patients under consideration for renal transplantation.¹¹

There are two reasonable explanations for the smaller number in the hepatic cases. First, the frequency with which these patients had been transfused was lower. In addition, there were fewer parous females among the candidates for liver transplantation. It could be expected that the risk from immediate rejection would be correspondingly reduced.

In kidney transplantation, a very heavy risk of "hyperacute rejection" must be accepted if preformed cytotoxins in a recipient can be demonstrated to react against the lymphocytes of the renal donor.¹¹ So far there have been no hepatic transplantations in the Denver series in which the recipient had a known positive crossmatch with the donor (Table 5). None of the organs which did not function well at the outset were thought to have failed because of an accelerated rejection.

Quality of Antigen Matching

The mismatches found between the recent liver recipients and their donors are shown in Table 5. Only three patients were judged to be completely compatible for all the major groups being tested at the times the operations were performed. Unfortunately, one of the recipients (OT 18) died after four days of a hepatic artery thrombosis. The fate of another one (OT 8), an 18 month old child, will be presented in detail in Chapters Fifteen and Seventeen. Here it will only be said that the magnitude of rejection did not seem to be particularly great, either early or late after transplantation.

The third patient (OT 16) was thought to have an even better donor. In this case there was complete conformity of all nine antigen groups tested, including the six internationally accepted ones of the HL-A system that are listed in Table 3. Nevertheless, an irreversible rejection developed within two weeks, necessitating retransplantation 68 days after the first operation. The patient was mismatched with the second liver donor in two determinants (HL-A5 and HL-A6). This homograft also underwent vigorous rejection but it has continued to support life for more than nine months.

In all the other cases mismatches were present of one to three antigen groups. The number of observations in patients who survived the immediate effects of operation has been too small to permit meaningful correlative studies between the degree of compatibility and the ultimate clinical outcome. The difficulties of correlation were further compounded by the fact that the intensity of immunosuppression was very much less in some of the earlier cases as compared to the subsequent ones (see differences in Chapters Fourteen and Fifteen).

Consequently, no statement can be made on the basis of these data at the present time, supporting a clear association between the results of any of the antigen studies and the clinical events. In Chapter Twenty it will also be noted that a decisive relation between donor-recipient compatibility and the histopathologic findings in the homografts studied to date has yet to be established.

This does not mean that histocompatibility studies will not be used as grounds for recipient selection in future attempts at liver transplantation; the converse is true. It has become apparent that the validation of tissue typing principles and the improvement of the methodologic details for antigen analysis cannot be easily achieved by studying the extraordinarily complex cases of hepatic and cardiac transplantation. For the perfection of typing techniques, continued dependence will be necessary on the simpler experimental model of

Table 5. Histocompatibility Data for All Liver Transplantations since May 21, 1967

RECIPIENT	CROSS MATCH	HL-A GROUP MISMATCHES	SUMMARY GRADE	SURVIVAL (Days) ^o		REMARKS
				LIVING	DEAD	
OT 7	—	HL-A2,HL-A5	D		10	Died of unrelieved hepatic insufficiency.
OT 8	—	None	B		400	Died of carcinomatosis.
OT 9	Negative	HL-A2	C		133	Had septic hepatic infarction†; died of liver failure.
OT 10	—	HL-A7,±HL-A6	C		186	Had septic hepatic infarction†; died of liver failure.
OT 11	Negative	HL-A2	C		61	Died of septic hepatic infarction.†
OT 12	—	HL-A6, HL-A7, Te 11	D		105	Died of septic hepatic infarction.†
OT 13	—	HL-A8, Te 11	D	416		Indolent late rejection starting after 6 months.
OT 14	—	HL-A2	C	380		Indolent late rejection starting in third month.
OT 15	Negative	Te 9	C		339	Died of recurrent malignancy. See Chapter 17 for details.
OT 16 ^a	Negative	None	A	—	—	Homograft replaced after 68 days.
OT 16 ^b	Negative	HL-A6,±HL-A5	C	310 [‡]		Indolent late rejection.
OT 17	—	HL-A1,HL-A8	D		35	Died of pneumonia.
OT 18	—	None	B		4	Died of hepatic arterial thrombosis.
OT 19	—	HL-A2,HL-A3,HL-A7	D	254		Normal liver function.
OT 20	Negative	HL-A1, HL-A2, HL-A8	D		1/2	Hepatic artery occlusion.
OT 21	—	HL-A1, Te 4, Te 9	D		1/2	Portal vein thrombosis.
OT 22	Negative	Te 12	C		10	Extrahepatic biliary obstruction; infection.
OT 23	Negative	HL-A6	C		143	Died of recurrent malignancy
OT 24	Negative	HL-A1	C		11	Died of unrelieved hepatic insufficiency.
OT 25	Negative	HL-A7	C		39	Died bile fistula
AT 4	Negative	Te 4	C		24	Died of unrelieved hepatic insufficiency.

^oAs of April 1, 1969.
[†]Septic hepatic infarction and its relation to rejection are discussed in Chapter Fifteen.
[‡]The total survival of 310 days includes 68 days after the first transplantation and 243 after the second.

clinical renal transplantation. The lessons learned from these efforts can then be generally applied, as has already been done, to the transplantation of other organs.

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Chapter Four

RECIPIENT SELECTION AND PREPARATION

As mentioned in Chapter Two, the availability of a suitable cadaveric organ is the central issue in determining whether a liver transplantation of one kind or other can be performed for any given patient. The degree to which the liver is thought to have been injured by the events of the donor death is one critical factor in the decision to proceed. In all recent cases another consideration has been to attempt before operation to demonstrate a reasonable histocompatibility match between the donor and the recipient (Chapter Three). The logistical problems posed by these requirements have been partly ameliorated by the creation of an intercity pool of collaborating physicians who care for the prospective recipients until shortly before the transplantation.

LONG DISTANCE EVALUATION

All the initial steps are carried out by mail or telephone. Much of the necessary information can be obtained by asking the referring physician to provide copies of all operative notes, hospital discharge summaries, and pathologic reports and to fill out a short form which contains the following specific inquiries:

Hepatic Recipient Protocol

Patient's name	Clinical condition	Total protein and albumin/ globulin ratio
Date of birth	(a) Mental	Prothrombin time
Sex	(b) Respiratory	Other associated disorders
Height	(c) Cardiovascular	Terasaki antigens
Weight	(d) Ascites	Parents' name
Pathologic diagnosis	(e) Renal	Parents' address
Previous operations	(f) Developmental	Parents' telephone number
Blood type	status	

The only special effort which is involved is the drawing of a venous blood sample to be analyzed by Dr. Paul Terasaki of Los Angeles for the presence or absence of lymphocyte antigen groups (see Chapter Three). The results of the latter examination are returned from Terasaki's laboratory within a few days and entered on the summary sheet in the patient's file. There is little more to do except to wait for a potential donor to appear who has the proper ABO blood type, the approximately correct size, and a reasonably compatible profile of lymphocyte antigens. These conditions may never be met, in which case the transplant candidate usually does not come to Denver. However, if a possible donor is found, the prospective recipient is flown from his home city to Colorado as quickly as possible. The times from notification to arrival in Denver have ranged from 20 minutes to 12 hours.

The system has worked remarkably well as the result of the skill and judgment of the family physicians who have accepted the responsibility of coordinating these efforts in different cities. These men have often carried out extra diagnostic studies to establish the operability in cases of liver malignancy, or in benign disease to rule out abnormalities of extrahepatic organ systems. Moreover, they have provided careful medical management, including paracenteses and thoracenteses during the waiting period, a task made less futile by the possibility of eventually obtaining a new liver. Thus far, we have yet to be surprised to meet patients for the first time who had either less or more advanced illnesses than would have been expected from their physicians' appraisals.

Unfortunately, victims of liver disease who are valid candidates for hepatic transplantation cannot wait long for the foregoing appropriate donor circumstances. On several occasions, such patients have died a few hours before a phone call was placed to ask that they come to Colorado. More than 95 per cent of those on the waiting list have succumbed before anything could be done.

The number of these tragedies will surely be reduced as more centers undertake liver transplantation. It should then be possible to exchange patient information so that a candidate could be on call from several clinics without any preconceived bias about where he would eventually go. An alternative solution to that of sending patients to the organ source would be to ship cadaveric livers as needed from one city to another. This is not yet practical because of the limitations of hepatic preservation techniques.

EMERGENCY EVALUATION

The advantage of the foregoing approach is balanced by the very limited evaluation which is feasible at the time of admission for transplantation. In some of our cases, the recipient arrived after the donor had already died and the liver had been removed and placed in a preservation unit (Chapter Five). In others, only a few minutes were available for examination of the patient and the collection of samples before it was necessary to move to the operating room. After a preliminary physical examination, the situation required the mobiliza-

tion of a team of specialists who, with the aid of a continuously present member of the transplantation team, focused their attention upon a number of specific questions about the state of the heart, lungs, kidneys, and central nervous system, as well as that of the diseased liver.

Special attention was paid to the cardiorespiratory system, particularly in children with biliary atresia. In these cases, the presence of enlarged livers and spleens with or without ascites usually caused elevation of the diaphragms and compression of the lungs. In addition, all the patients with this diagnosis had significant pulmonary arteriovenous shunting, an abnormality which was detected with arterial blood gas (pO_2 , pCO_2 , and pH) studies^{4,5} and which rapidly disappeared after successful orthotopic hepatic transplantation.⁶ The chest x-rays of these children had abnormally prominent hilar shadows (Fig. 6); fluoroscopy usually revealed sluggish but otherwise normal movement of the diaphragms.

In most cases a small catheter was placed into the trachea and used to collect samples for bacteriologic and virus cultures. Cultures were also taken from the blood, stool, skin, and sputum. The results were never available until after the transplantation but they then became indispensable (Chapter Sixteen) for the planning of postoperative antibiotic therapy.

It was also often necessary to proceed without knowing the results of other hematologic or biochemical examinations. However, priority was given in the laboratory to the hematocrit, white cell count, prothrombin time, blood urea nitrogen, serum electrolytes, plasma glucose, and blood pH since each of these determinations was used to guide some aspect of the intraoperative care. Extra blood, plasma, and urine from both the donor and recipient were stored for less urgently needed routine or research studies to be carried out later. A collection was always made for confirmatory restudy by Terasaki of the lymphocyte antigens.

Finally, the blood bank was informed of the impending procedure so that fresh blood could be drawn for transfusion and for the preparation of platelet concentrates. It has been shown that abrupt thrombocytopenia may follow the revascularization of liver homografts in dogs or humans²; in some cases this has required prompt therapy.

THERAPY

Virtually all liver recipients are poor risks for a major operation, and many of those with hepatic failure from benign disease may appear at first evaluation to be hopeless. Symptomatic relief may be obtained by the performance of paracentesis or thoracentesis. Unfortunately, there is probably little of real value that can be done to reduce the consequent operative hazards short of providing liver tissue. It has been suggested^{1,3} that one way of doing this before the definitive procedure would be with hepatic support, using an extracorporeal heterologous liver.

Such an approach has not yet been tried. One reason is that even patients

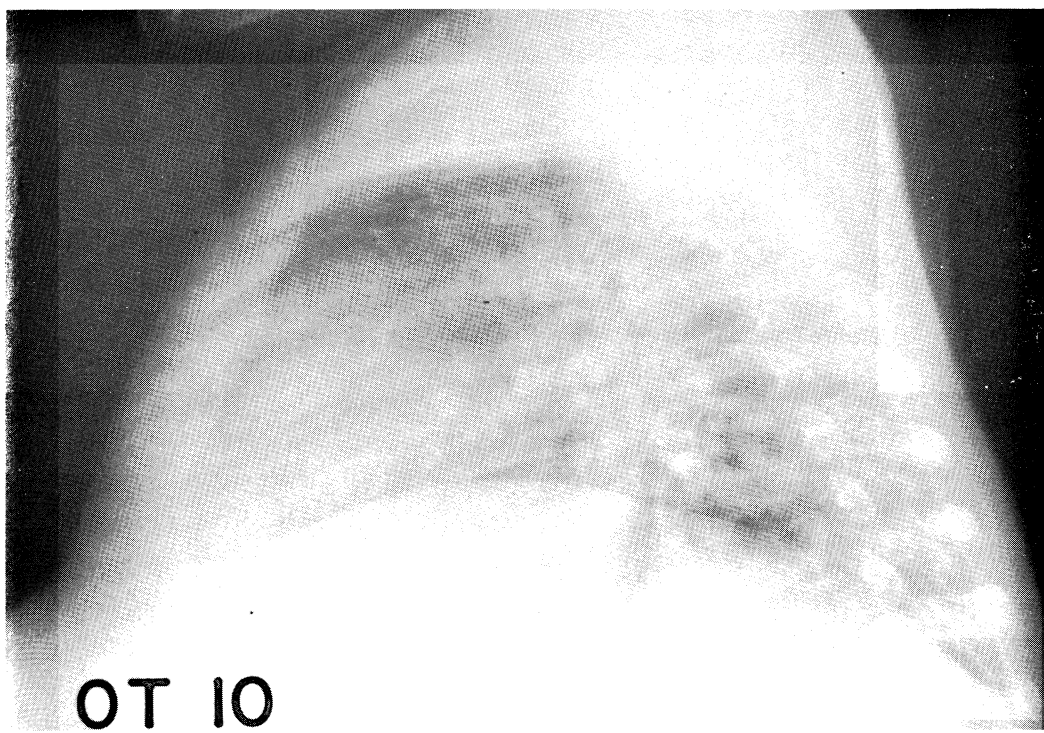
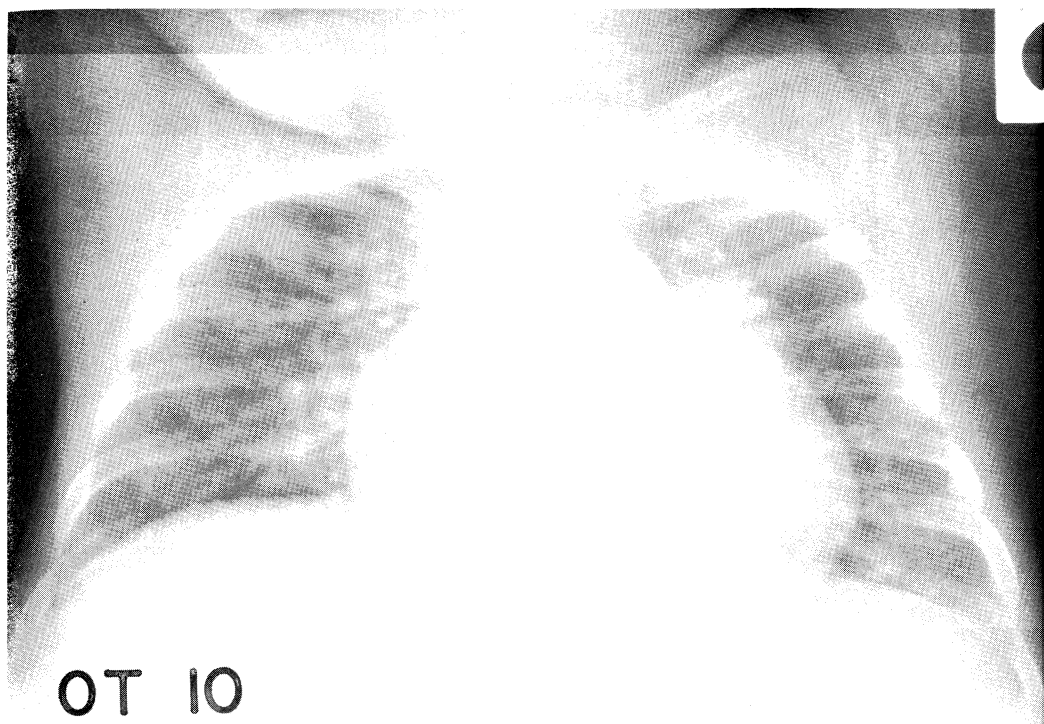


Figure 6. The chest x-ray of patient OT 10 just before orthotopic transplantation. Note the very prominent pulmonary vascular markings resulting from arteriovenous shunting. The osteoporotic changes in the bones and the elevated diaphragm seen here are also typical findings in the child with extrahepatic biliary atresia.

near death from complications of hepatic disease can apparently be brought through the transplantation procedure with almost immediate improvement, providing the graft functions properly. A good example was patient OT 12 in our series (Fig. 4A, Chapter One), who was admitted in terminal condition with a hepatorenal syndrome (BUN 70), a continuously draining peritoneal fistula from the site of previous paracentesis, and major hemorrhage from esophageal varices which ceased immediately after insertion of the new liver. She returned from the operating room in much better condition than she entered it, had normal renal function one day later, and was eating within 48 hours. The ascitic leak ceased spontaneously a few days later.

It is possible that one reason for the paradoxical ability of these moribund recipients to survive such major surgery is the troublesome operative bleeding which is almost invariably encountered and the consequent necessity for major blood replacement. The intraoperative exchange transfusions usually given are of the magnitude of those which have been reported by Trey, Burns, and Saunders of Capetown⁷ to be of benefit in acute liver insufficiency.

Although little can be done for the liver failure, secondary effects on the kidneys can be effectively treated. One of our patients who was provided with an auxiliary hepatic homograft (AT 4) had developed the anuria of an advanced hepatorenal syndrome with a serum sodium of 104 mEq/liter, a potassium of 6.2 mEq/liter, a chloride of 70 mEq/liter and a CO₂ of 9 mEq/liter. His blood urea nitrogen and creatinine were 140 and 15 mg per cent, respectively. The electrolyte disequilibrium was corrected by a four hour hemodialysis, after which he tolerated a prolonged transplantation procedure without difficulty (Chapter Twenty-two).

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PART II

THE CADAVERIC LIVER

Chapter Five

DONOR HEPATECTOMY AND LIVER PRESERVATION

The whole liver can be subjected to severe ischemia under normothermic conditions with the expectation of prompt recovery, but only if the insult is of very short duration. It has already been emphasized (Chapter Two) that part or all of this slender margin of safety may be used up during the terminal hours or days of donor life if there is a protracted premortem period of ineffective circulation. After death, further tissue injury is inevitable, but this can be minimized in various ways.

THE "LIVING CADAVER"

With the increasing acceptance of irreversible brain injury as sufficient justification for the pronouncement of death (Chapter Two), one of the most serious problems in liver transplantation will be alleviated. If donor hepatectomy can be carried out in the presence of an intact and effective circulation, the interval of normothermic ischemic injury should be virtually eliminated. Under these circumstances, either the donor or the organ, or preferably both, can be cooled at some time prior to the completion of the hepatectomy by use of the simple techniques that were first employed in canine experiments (see later).

If there is time, it may be desirable to administer to the donor one of the drugs which have been said to reduce ischemic injury by stabilizing cell membranes and thereby preventing the disruption of lysosome envelopes. The agents to which this effect has been ascribed include magnesium sulfate, sodium fluoride, adrenochrome, adrenocortical steroids, and chlorpromazine.^{12, 14, 18, 35, 36}

EMERGENCY POSTMORTEM MEASURES

Our experience with liver procurement under the optimal conditions of a continuing donor circulation has been relatively limited. In the orthotopic

series, for example, brain death (see Chapter Two) was accepted as a basis for organ removal only in Cases OT 19 onward. Virtually all the donors before this time also had cessation of heart beat.

If cardiac arrest occurs, some perfusion of the liver can be restored by the immediate institution of cardiac massage. This was carried out in most of our early cases, but usually only until effective cooling could be accomplished by one of the methods described later. The use of external massage has the specific danger that the liver may be lacerated by the forceful compression of the xiphisternum.²⁹ Minor parenchymal lacerations were found in several of the organs used as homografts in our series. In one (OT 16), there were large bruises in both lobes at the time of donor hepatectomy, the one on the left side being extensive enough to be visible on the first post-transplantation liver scans (Fig. 7).

A much more decisive step than these crude attempts to re-establish a circulation is to cool the liver. The extreme sensitivity of hepatic tissue to normothermic ischemia was learned by Goodrich and Welch¹⁵ with the earliest attempts at transplantation of the canine liver. In their laboratory experiments, in which the timing of donor and recipient operations could be precisely coordinated and the hepatic blood supply maintained virtually until the moment of

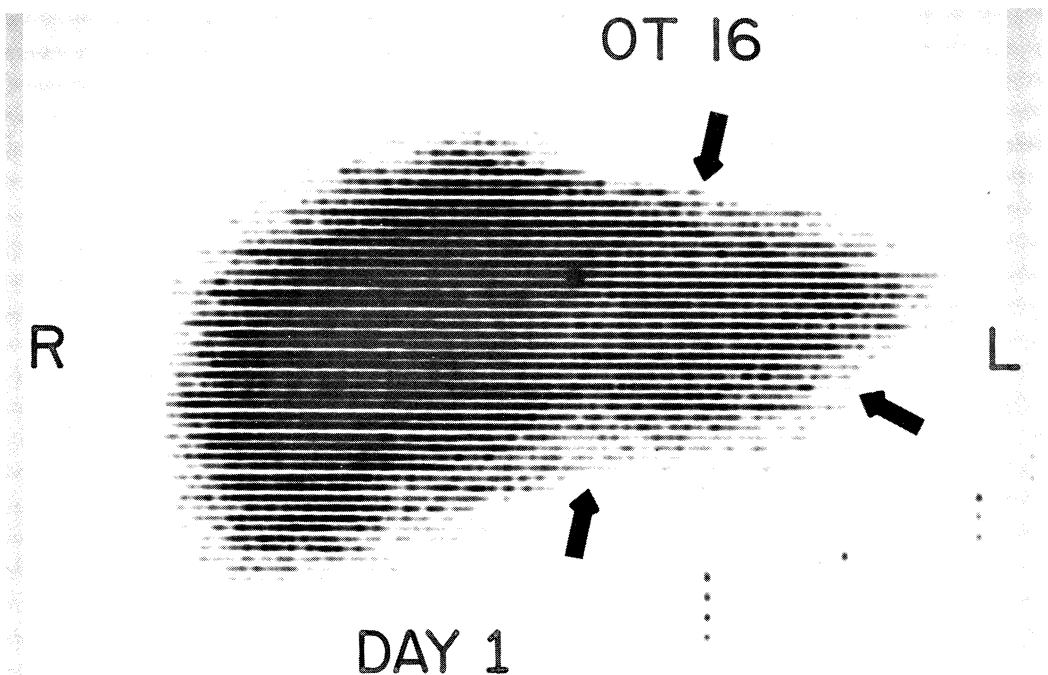


Figure 7. A liver scan obtained in patient OT 16, one day after orthotopic homotransplantation. The donor had had many cardiac arrests, which had been treated with closed chest massage. At the time of donor hepatectomy, large and small bruises were noted in the right and left liver lobes, respectively. It was decided to use the damaged organ. Note that the right lobe appears normal but that there is an area of decreased isotope uptake on the left (arrows). This abnormality persisted for many weeks.

homograft excision, livers removed at room temperatures became unsuitable for auxiliary transplantation within 20 or 30 minutes.

It was later found that the permissible time of ischemia was extended when the dog livers were cooled, first by the induction of total body hypothermia in the live animal and then by intraportal infusion of the excised liver with a chilled (4° C) electrolyte solution at the moment of sacrifice.^{23, 31} The cooled organs could support life if revascularized as orthotopic homografts in recipient dogs within two hours. After longer intervals there was a high incidence of acute hepatic insufficiency, including a hemorrhagic diathesis and a rapidly lethal syndrome termed "outflow block" to which the dog liver is far more susceptible than the analogous pig or human organ. When the latter complication of ischemia developed, the homograft became engorged.³¹ The inability of the blood to escape from the homograft probably was due to constriction of the small intraparenchymal hepatic veins studied by Deysach¹⁰ and Thomas and Essex.³³

The early studies^{23, 31} and the subsequent ones of van Wyk,³⁴ Ono,²⁶ Fonkalsrud,¹³ and Schalm²⁷ and their associates provided ample practical proof that the injury which follows the cessation of hepatic circulation is a graded one which can be considerably slowed by the immediate institution of hypothermia. A quantitative delineation of the protective effect of cooling was added by the biochemical analyses of Sicular and Moore.²⁸ They showed that the ability of dog liver slices to oxidize glucose was lost very rapidly under normothermic conditions but, with immediate refrigeration, the same biochemical reaction remained relatively intact for a number of hours.

Infusion Technique

The objective of rapidly cooling human cadaveric livers can be accomplished with a modification of the simple technique of intraportal infusion used in dogs. It is convenient to enter the splanchnic venous circulation through the superior mesenteric vein since this vessel can be easily and rapidly cannulated at the base of the transverse mesocolon (Fig. 8). There is another advantage. The required dissection, which must often be carried out in haste, is kept away from the structures of the portal triad, which can subsequently be skeletonized more deliberately and with less risk of injury.

During intraportal infusion of one liter of a lactated Ringer's solution at 4° C, the core temperatures of dog livers fall to about 15° C within two or three minutes. Rewarming at room temperature occurs slowly,³¹ making it unnecessary to resort to other chilling devices during the actual transplantation (Fig. 9).

The constituents of the buffered infusate presently used clinically are shown in Figure 8. The low molecular weight dextran is included in the hope that its oncotic properties will reduce edema. As the cold solution passes into the liver, incisions are made in either the suprahepatic or infrahepatic inferior vena cava to permit egress of the fluid after it has passed through the sinusoidal bed. The gallbladder is opened at the same time and bile vigorously flushed out (Fig. 8). In the dog, failure to observe the latter precaution may lead

*2°C Balanced Electrolyte Solution
with 50 mgm procaine, 100 mgm
heparin and 25 gm low molecular
weight dextran per liter*

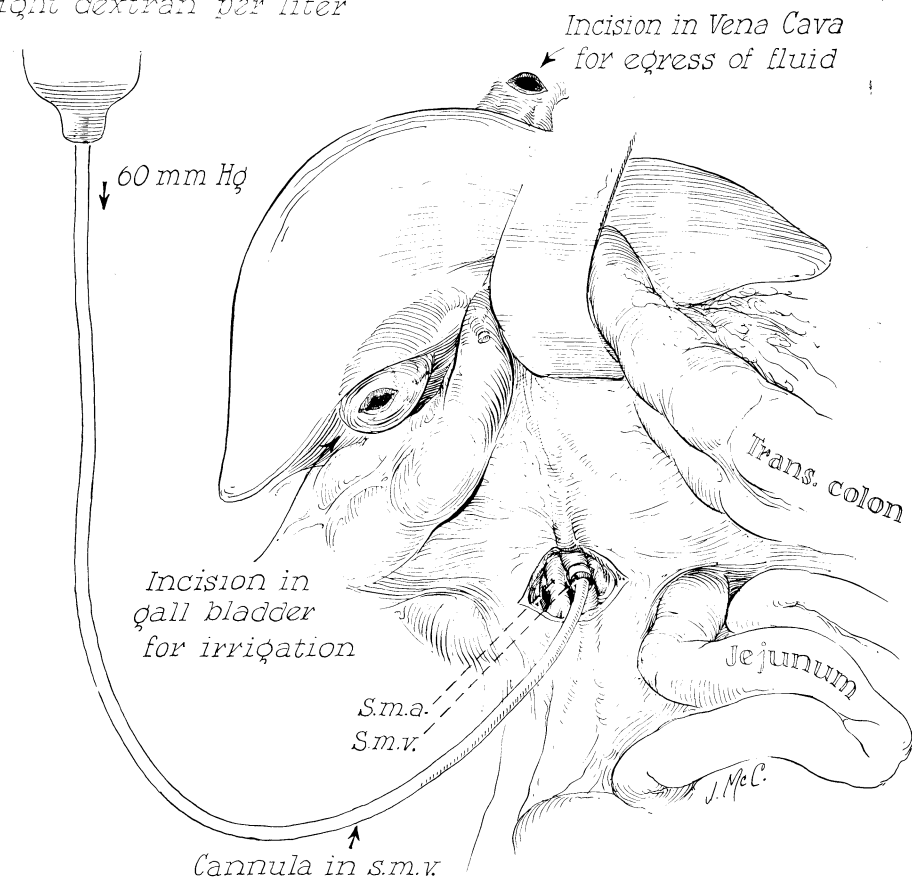


Figure 8. Core cooling of a cadaveric liver by intraportal infusion of a cold solution. The technique can be used as an emergency measure if the donor circulation has ceased. Alternatively, it can be used as the last step before hepatectomy if death has been certified on the basis of irreversible brain injury in a patient who still has a heart beat. The cannula is placed into the readily accessible superior mesenteric vein. This vessel is far enough away from the portal triad so that the portal vein, which will ultimately be used for anastomosis, is not in danger of injury. Egress of the perfusion fluid is provided by the venotomy in the suprahepatic inferior vena cava. Bile is washed from the gallbladder through the cholecystotomy. (By permission of *Ann. Surg.* 168:392, 1968.)

to autolysis and later sloughing of the mucosa. As soon as these steps have been completed, removal of the cooled liver is begun.

Several cadaveric homografts which were protected solely by this kind of hypothermic perfusion provided good early function (OT 9 and 18; AT 2) although one (OT 6) did not; Calne,⁸ Birtch,² and Najarian²⁴ have also obtained satisfactory short term preservation in the same way. The greatest disadvantage of the method when used alone is the time limitation, estimated at two to three hours, within which revascularization should be completed in the human recipient. Consequently, hypothermic infusion has most commonly been used as the first stage in more extended preservation by the ex vivo perfusion tech-

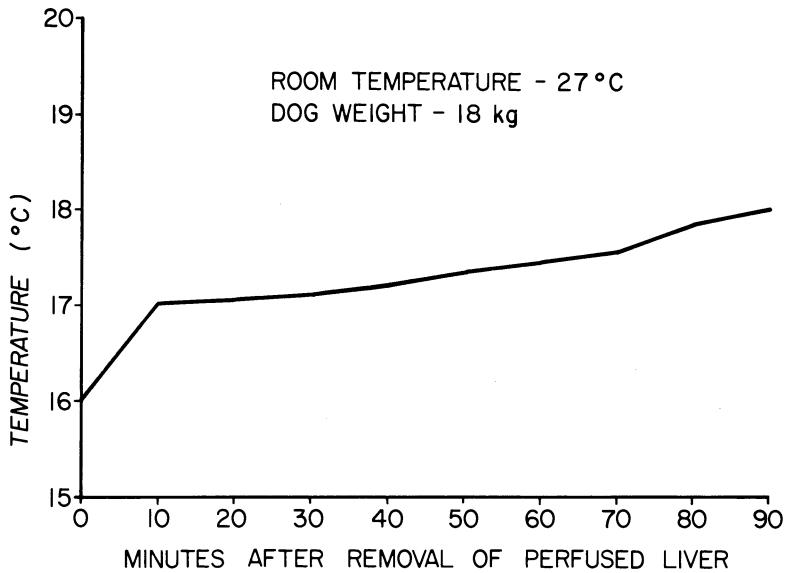


Figure 9. Rewarming curve of the interior of a dog liver which had been cooled to 16° C by intra-portal infusion of 1 liter of chilled (4° C) lactated Ringer's solution. Note the very slow rise in the core temperature. (By permission of Surg. Gynec. Obstet. 111:733, 1960.)

nique to be described later, or as the last step before removal of the organ in cases in which donor life was said to have terminated on the basis of brain death.

Cadaveric Perfusion

An alternative and, in many respects, a more complete measure in cases in which there is no circulation is that of cadaveric perfusion with a heart lung machine.^{19, 20, 32} As quickly as possible after cardiac arrest, cannulas are inserted into the aorta and abdominal vena cava via the femoral vessels. Circulation is instituted through a bubble oxygenator and pump system which has been primed with a cold heparin-containing electrolyte or glucose solution and which has a heat exchanger incorporated into the circuit. The whole cadaver can be thereby simultaneously perfused and cooled, or the perfusion can be confined to the lower half of the body if the thoracic aorta is cross clamped (Fig. 10). The method has been used for donors from two to 73 years of age.

The flow rates used during cadaveric perfusion in adults are usually started at about 35 ml/kg/min. The resultant arterial blood pressure is very low, ordinarily less than 20 mm Hg; efforts to drive this up with greater perfusion are usually unavailing and may be harmful. Marchioro^{19, 20} found that to increase the blood pressure to 50 to 70 mm Hg in dog cadavers, flow rates per kg of as much as 600 ml per minute were required, often resulting in acute swelling or even rupture of the visceral organs.

In spite of the low flows, cooling of the human cadaver is quite rapid (Fig. 11), the core temperature usually falling to 15 or 20° C within 15 to 30 minutes.

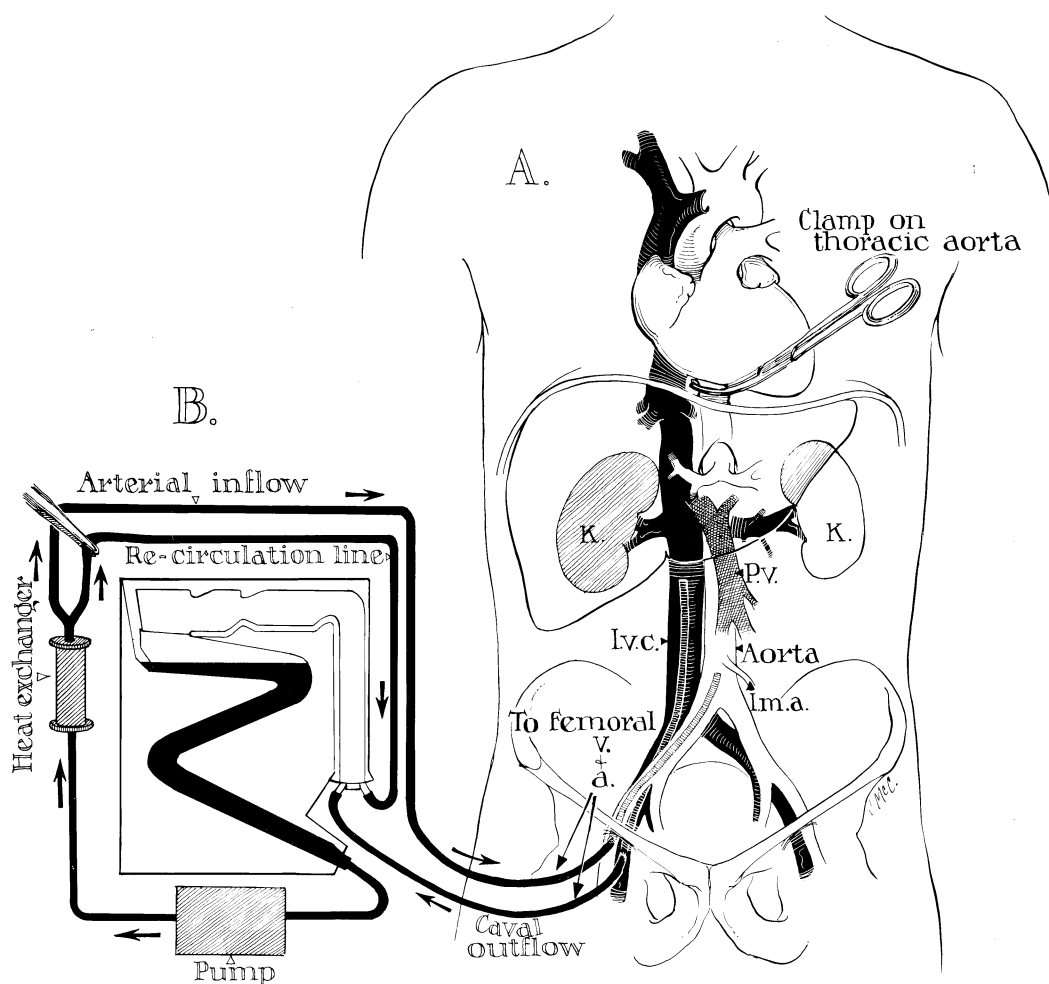


Figure 10. Technique of extracorporeal perfusion with a heart-lung machine. Catheters are inserted via the femoral vessels into the aorta and vena cava as soon as possible after death. The extracorporeal circuit is primed with a glucose or electrolyte solution to which procaine and heparin are added. The cadaver is thus anticoagulated with the first surge of the pump. Temperature control is provided by the heat exchanger. Cross clamping the thoracic aorta limits perfusion to the lower part of the body.

With deepening hypothermia, the perfusion rate is reduced even further, sometimes to as little as 5 to 10 ml/kg. At this stage the best clue that there is overperfusion is the gross finding of venous hypertension, particularly of the portal vein. If appropriate flow adjustments are not made, dissection of the portal triad becomes extremely bloody and difficult.

The technique of cadaveric perfusion as a sole method of liver preservation was studied in dogs by Marchioro,^{19, 20} who instituted the artificial circulation from a few moments to 22 minutes after death from a massive overdose of pentobarbital. A protective effect was demonstrable. Livers removed more than eight hours after donor sacrifice and transplanted as orthotopic homografts supported recipient life for short intervals. However, all the animals had severe

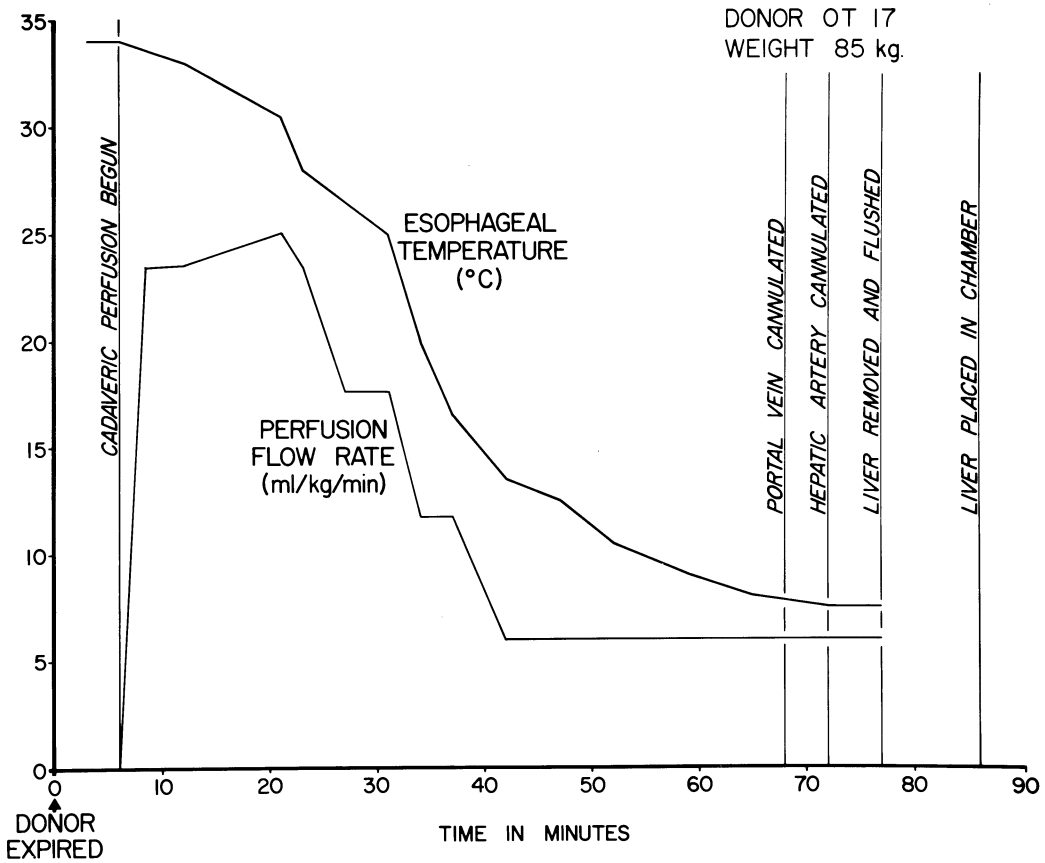


Figure 11. Temperature and flow changes during extracorporeal perfusion of an 85 kg adult male donor who died of an acute brain injury. When the esophageal temperature begins to fall rapidly, the perfusion rates are always reduced. In this case (OT 17), 86 minutes were required to remove the liver, to wash it free of blood and to place it in the preservation chamber. The homograft was ultimately revascularized in the recipient six hours and 40 minutes after donor death. It functioned adequately.

hepatic injury and died within five days. Mikaeloff and Kestens and their associates²² used a similar principle, in which hypothermic perfusion in situ was limited to the isolated dog liver and was instituted at the moment of death rather than at some time afterward as in Marchioro's experiments. Their method permitted good preservation for as long as six and a half hours. Some of the canine recipients of these organs, which were placed as orthotopic homografts, survived for weeks or months.

In several of our early clinical trials (OT 1 to 5 and AT 1), whole cadaver perfusion was employed as the definitive technique of preservation. The livers suffered variable ischemic injury as judged by their function in the recipient after postmortem intervals of 152 to 420 minutes. In these cases, which will be considered in detail in the next chapter, the poor quality of the homografts was almost certainly due to unwise donor selection rather than to any inherent deficiency of the hypothermic cadaveric perfusion. More recently, the same method has been used as a temporary protective device in both adult (OT 14,

15, 17, 22, and 23) and child (OT 16, 19, 20, 21, and 24) donors and it is now thought to be the preferred technique of preservation to be used until the liver can be removed from the cadaver. At this time the organ can be transplanted at once if the recipient is ready or, alternatively, it can be placed in the kind of conservation unit described later in this chapter.

In most of the recent cases in which irreversible brain injury was used as the final criterion of death, the donors had some circulation when dissection of the liver was begun. Almost invariably there was deterioration of the cardiodynamic situation while manipulation continued. As soon as this was observed, the cardiocirculatory bypass was started and maintained until completion of the hepatectomy.

DONOR HEPATECTOMY

Extirpation of the liver can be completed within 15 or 20 minutes if necessary. However, about an hour is required if the major hilar contents are to be carefully dissected, if all potential bleeding sites are to be ligated, and if long vascular cuffs are to be developed for later anastomosis. In donors who have no heart beat, the use of cadaver perfusion, as described previously, allows time for the more deliberate approach.

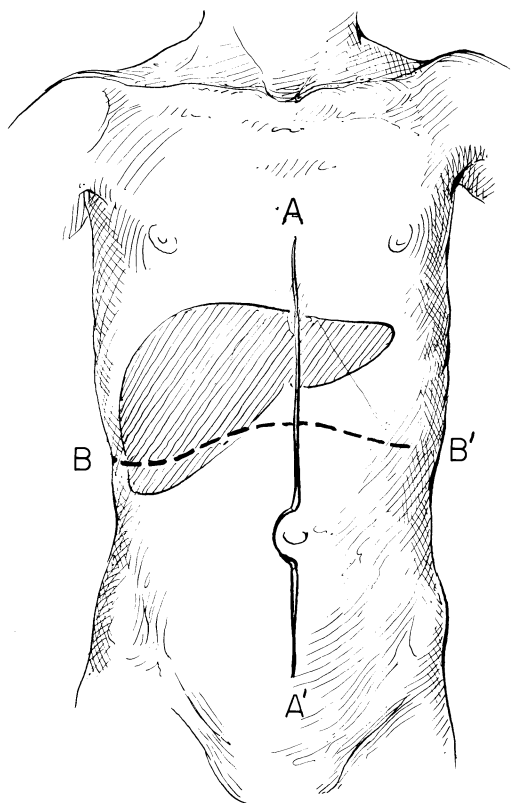


Figure 12. Total abdominal incision (A) used to expose and remove the liver from a cadaveric donor. The lateral extensions (B) are usually made. In addition, one or both hemithoraces can be opened.

Preliminary Steps

Good exposure is provided with a long midline abdominal incision with or without extensions laterally (Fig. 12) or into one or both hemithoraces. The ligaments attaching the liver to the diaphragm are incised, leaving as much length as possible with the specimen to facilitate later resuture to the same structures in the recipient. The falciform and left triangular ligaments are cut first, beginning peripherally where their two component leaves are fused (Fig. 13), and continuing centrally to where the leaves separate, enclosing a portion of the raw area of the liver. The left main hepatic and left phrenic veins can then be seen entering the suprahepatic vena cava (Fig. 14). These tributaries must not be injured.

The Vena Cava

Next, the anterior leaf of the right triangular and coronary ligaments is incised (Fig. 15) and the major raw area on the right is entered, as the liver is retracted up and toward the left (Fig. 16). This plane should be developed with

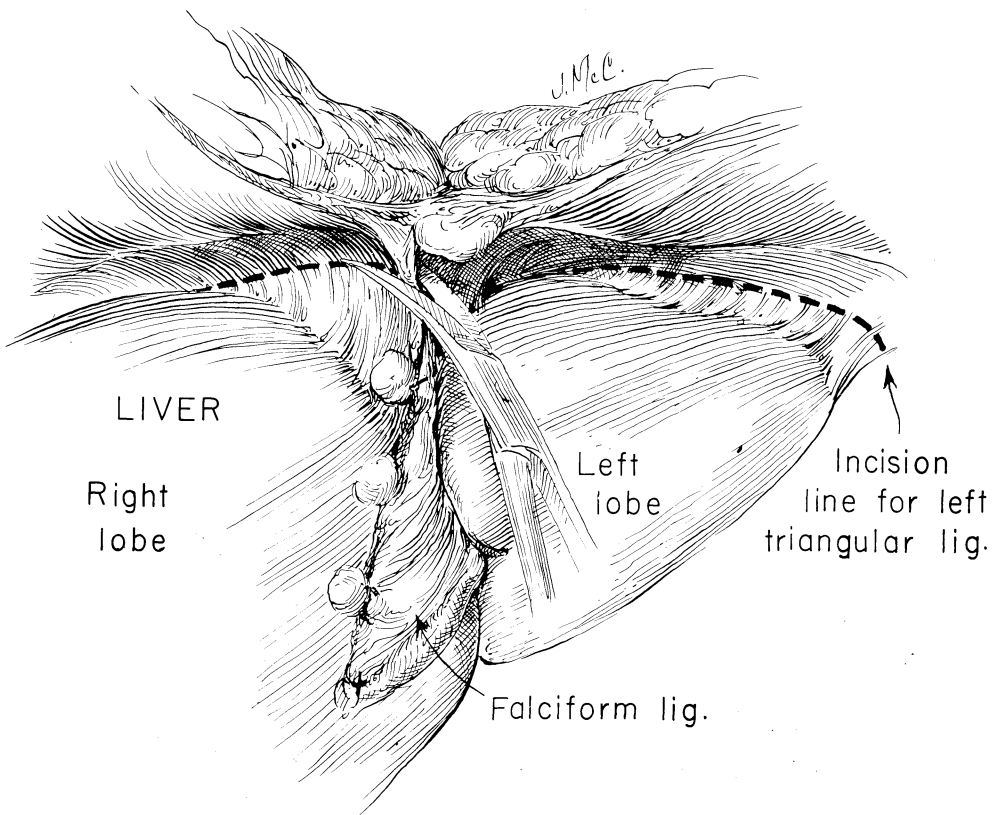
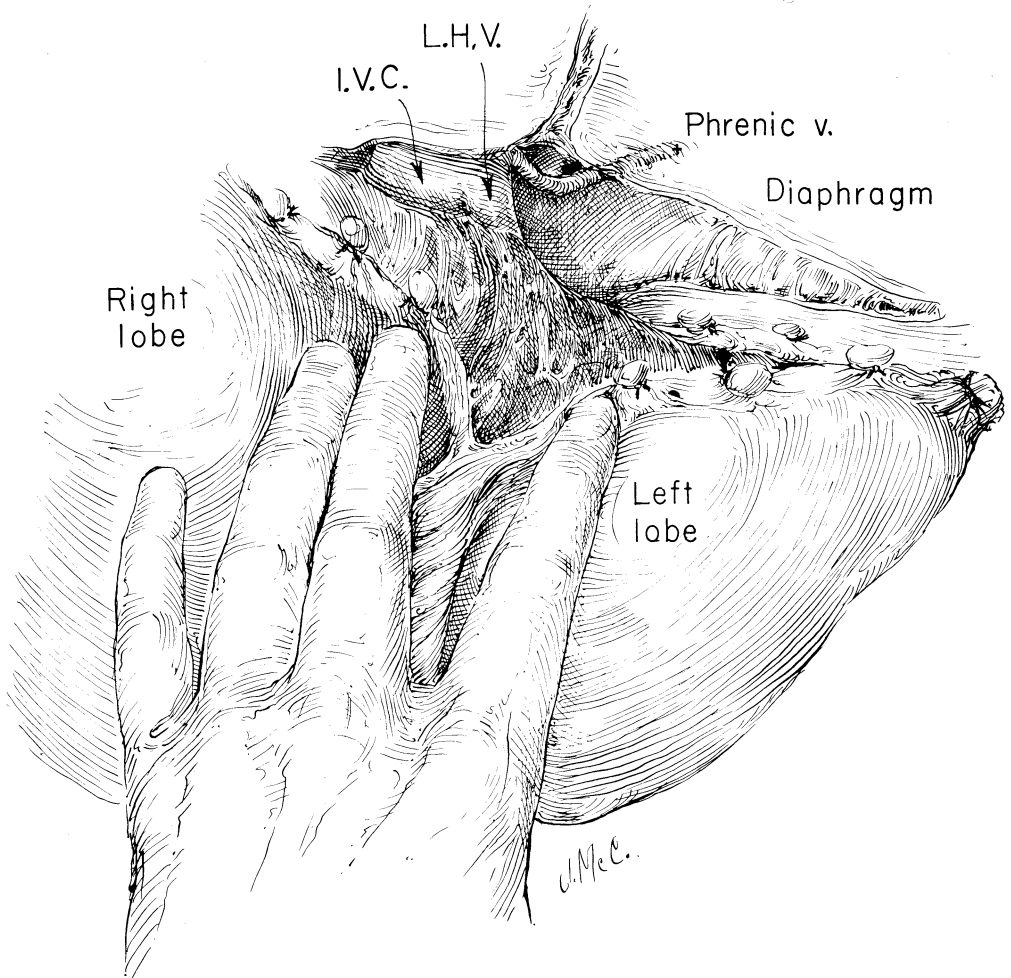


Figure 13. Initial steps in hepatic homograft removal. Note that the ligaments are incised as far away from the liver as possible so that they can be later resutured to the companion structures in the recipient.



✓ *Figure 14.* Exposure and initial dissection of the suprahepatic vena cava and its tributaries. This is done by entering the raw area formed by divergence of the leaves of the falciform and triangular ligaments. A short segment of the left hepatic vein (L.H.V.) is usually seen first.

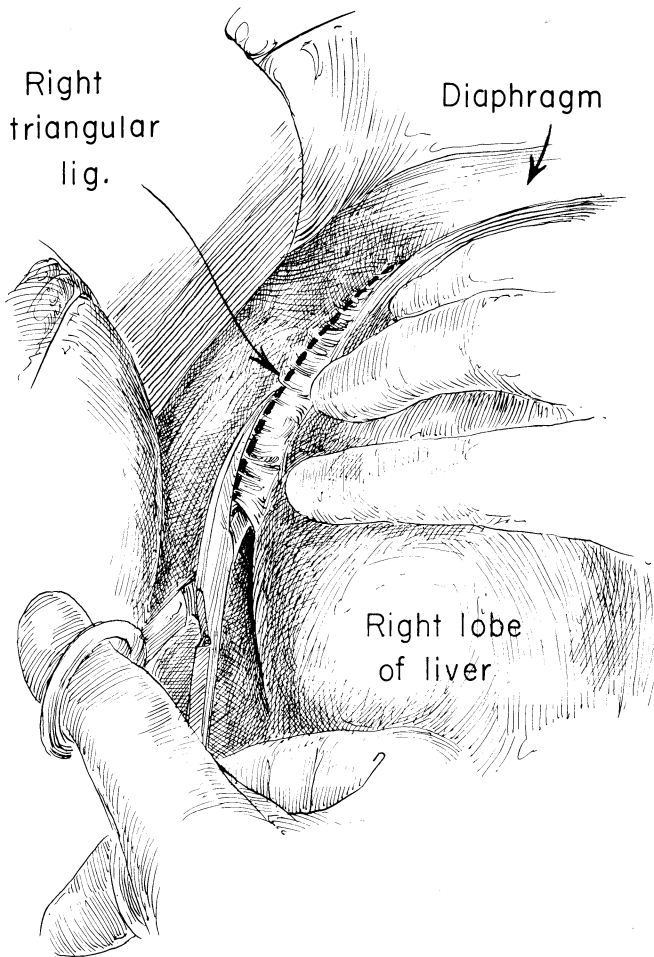
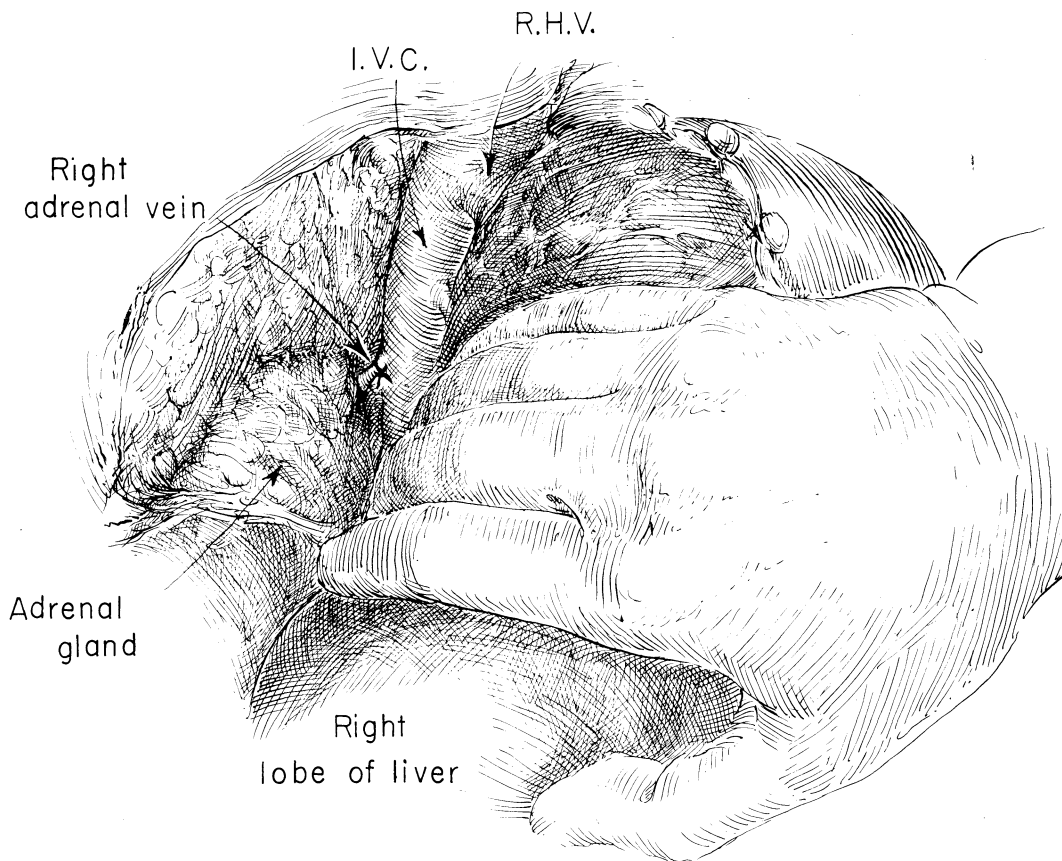


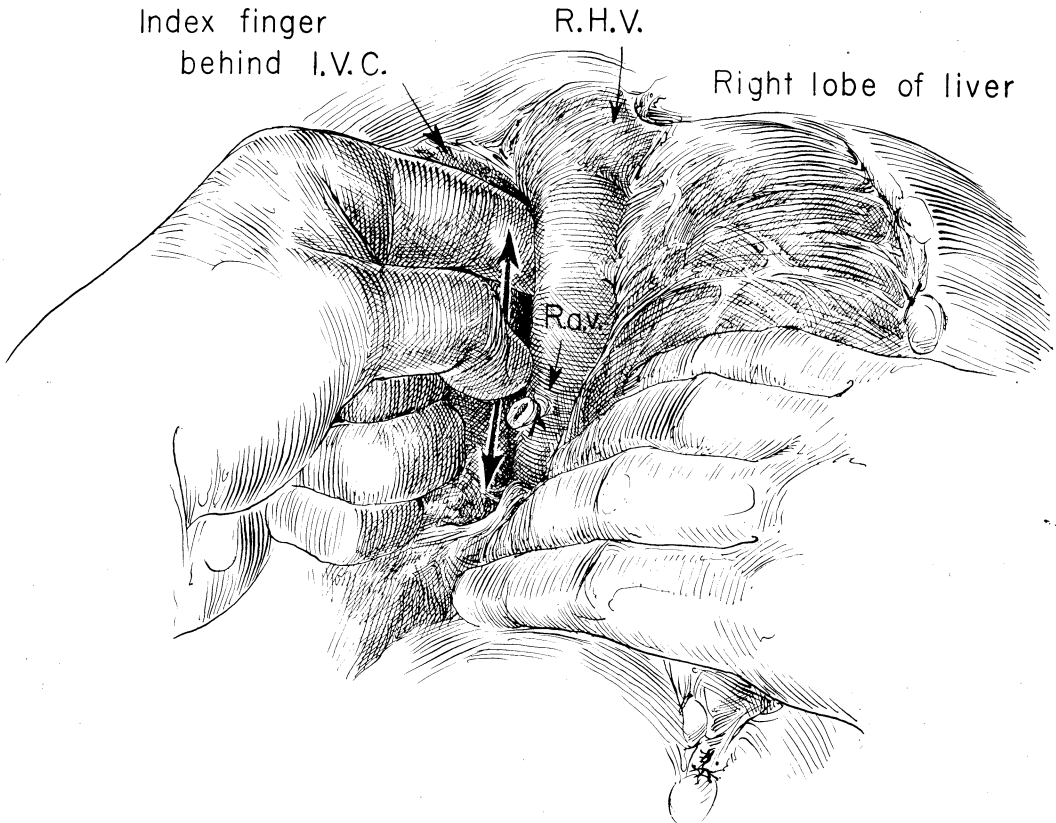
Figure 15. Incision of the right triangular ligament and the anterior leaf of the right coronary ligament.



✓ *Figure 16.* Retraction of the liver to the left. The bare area of the right hepatic lobe has been opened, exposing the adrenal gland. The right adrenal vein is ligated and divided. This is usually the only posterior tributary to the retrohepatic vena cava. At this stage of the dissection the right hepatic vein (R.H.V.) can be identified.

sharp dissection; otherwise, injury to the hepatic capsule is apt to occur. With each scissors cut the right lobe can be more completely elevated until the right adrenal gland is fully displayed, as well as the short right adrenal vein, which is ligated and divided (Fig. 16). Since the right adrenal vein is ordinarily the only posterior tributary to the retrohepatic vena cava, it is usually possible after dividing it to freely pass a finger behind the liver and vena cava all the way from the diaphragm to the level of the renal veins (Fig. 17). If anomalous small venous branches to the vena cava are encountered with this maneuver, they are also sacrificed.

Attention is then returned to the inferior vena cava which is between the dome of the liver and the diaphragm. This short segment of vessel is actually a confluence of the right and left (and often middle) hepatic veins with the vena cava (Fig. 18, inset). The resulting cloaca is used for anastomosis in the recipient. The eventual task is made much easier with each millimeter of added length that is retained with the specimen. Extra tissue can be obtained anteriorly and posteriorly by dissecting off the diaphragmatic reflection and laterally by ligating and dividing one or two phrenic veins on each side (Fig. 18).



✓ Figure 17. Sweeping behind the retrohepatic vena cava with a dissecting finger. This should be possible from the diaphragm to the renal veins. If resistance is encountered, it usually indicates the presence of extra branches which must be ligated and divided. R.a.v. — ligated right adrenal vein.

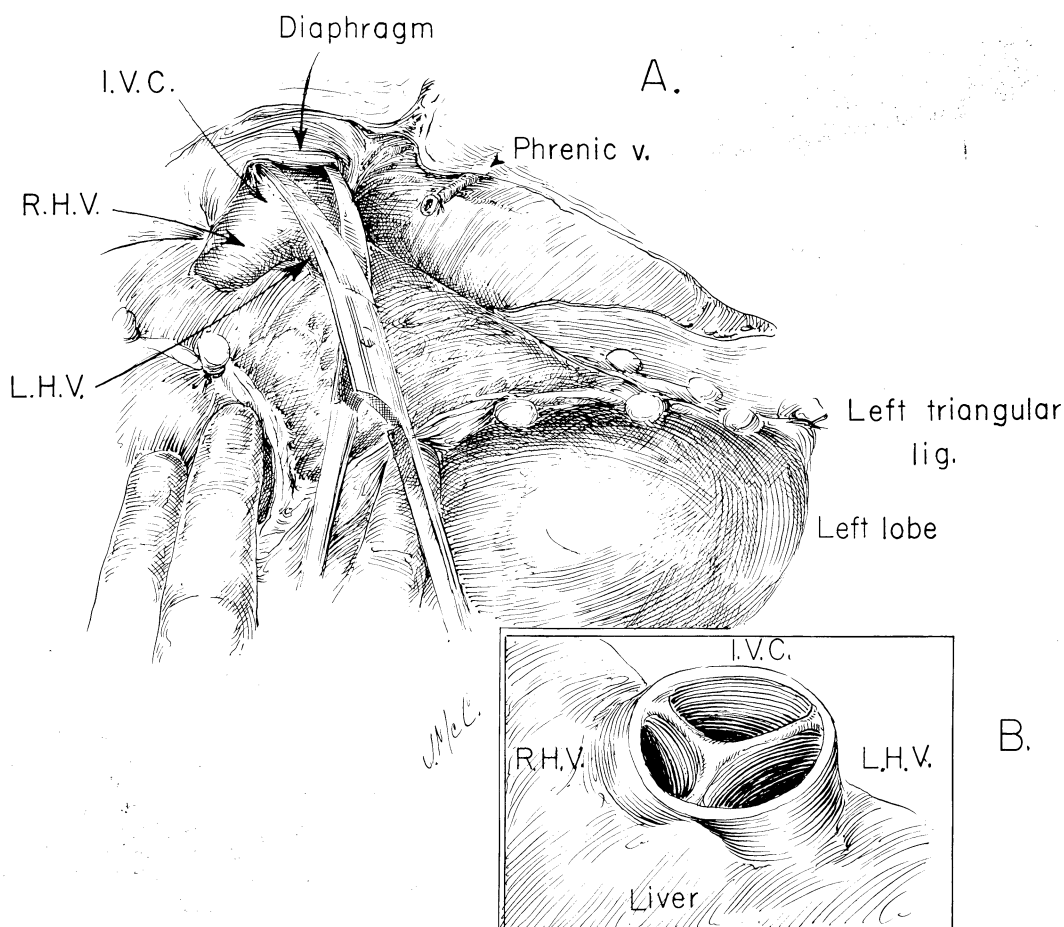


Figure 18. Dissection above the liver. *A.* Development of suprahepatic vena caval cuff. At this stage it is desirable to ligate and divide one or more phrenic veins on each side. The latter step is not mandatory but it allows the mobilization of a longer segment for subsequent anastomosis. Extra length can also be obtained by dissecting off the diaphragmatic reflection, as is being shown. *B.* Cross sectional appearance of the venous confluence above the liver as it is seen from above. The cloaca is formed by the junction of the right and left hepatic veins with the inferior vena cava.

The Portal Triad

Continuous downward traction is maintained on the duodenum. Dissection is kept as inferior as possible. The common bile duct is first transected low in its course. The incised upper end should be inspected to make sure that a single lumen is present; it can then be permanently ligated if biliary reconstruction is to be with cholecystoduodenostomy. If this is the plan, an additional safeguard is to introduce fluid into the gallbladder to make sure it distends the common duct. Failure to observe one or both of these precautions in the presence of undiagnosed anomalies of the homograft extrahepatic ducts has led to total biliary obstruction (Chapter Nine).

The right gastric and gastroduodenal branches of the common hepatic artery are next identified, doubly ligated, and divided (Fig. 19A). It is then pos-

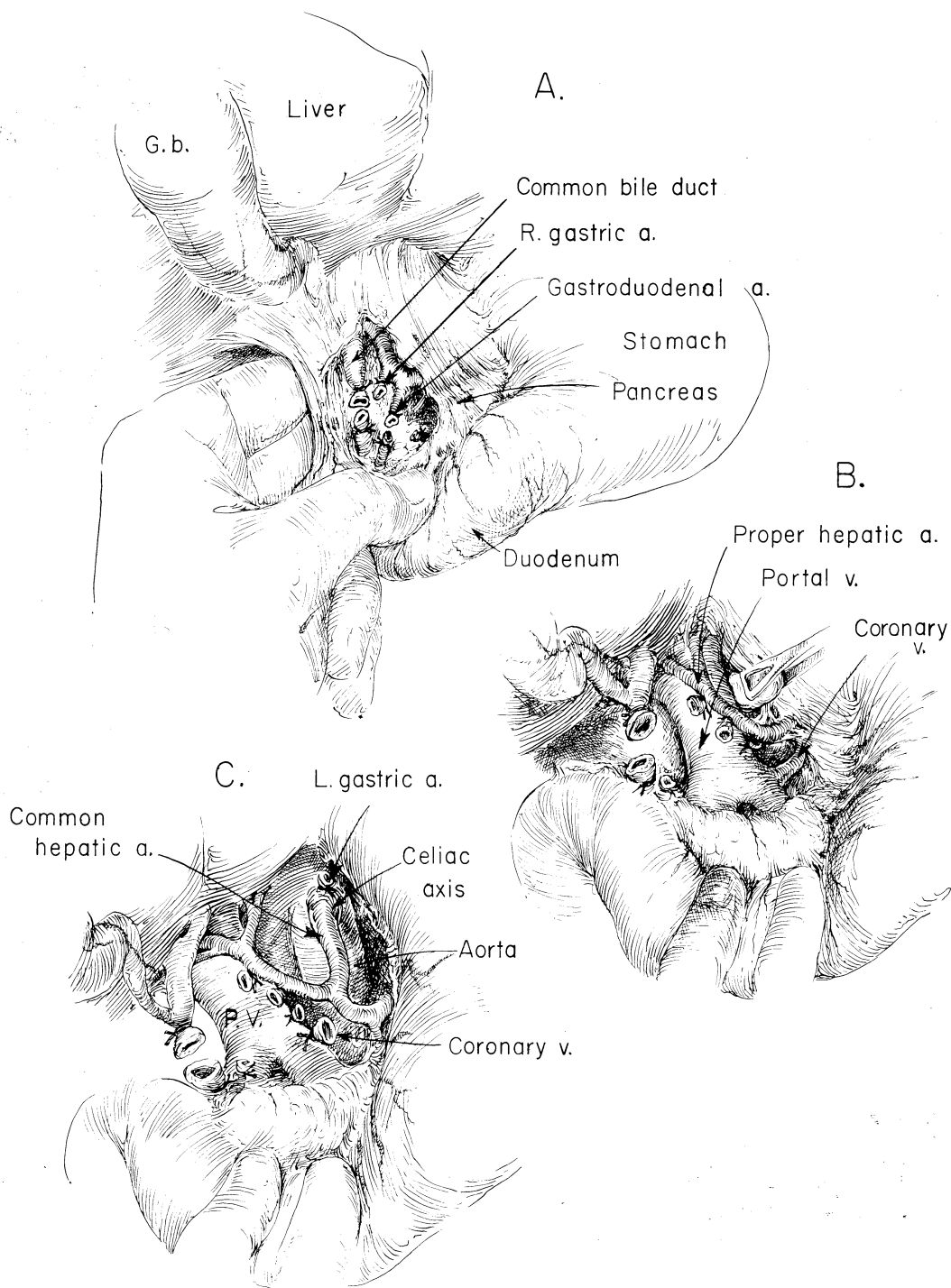


Figure 19. Dissection of the portal triad. **A.** The common duct and the gastroduodenal and **right** gastric arteries are tied off and divided. Before ligation of the common duct, it should be determined that it communicates freely with the gallbladder via the cystic duct (see text). If anomalies are present, failure to observe these precautions may lead to accidental bile duct obstruction (see Chapters Eight and Nine). **B.** The hepatic artery has been mobilized far enough so that the anterior surface of the portal vein is uncovered. The coronary vein entering the left side of the portal trunk is almost always found; this tributary is ligated and divided. **C.** The portal vein has been freed and the celiac axis mobilized. The splenic artery has not yet been ligated and divided. When the liver is removed, all the celiac axis is usually retained with the specimen and, in children, it may be advisable to include a segment of aorta as well (see Figure 20).

sible with blunt dissection to approach the portal vein anteriorly just where it emerges from beneath the neck of the pancreas (Fig. 19B). In the exposed segment of the portal vein, the left gastric (coronary) vein invariably enters on the left lateral aspect (Fig. 19B). This tributary is freed, doubly ligated, and cut in order to prevent its subsequent injury. One or two other variable branches often have to be similarly dealt with, after which the main portal trunk is encircled. At this stage, the tissues posterior to the portal triad are carefully examined. If an anomalous right hepatic artery emanating from the superior mesenteric artery is present it will usually be in this location and can be traced back to its origin.

Thus far in the dissection no deliberate effort will have been made to isolate the main hepatic artery. However, it will usually have been seen in the process of ligating its right gastric and gastroduodenal branches. If not, it now can be easily found to run almost directly left and at a right angle to the axis of the portal triad (Fig. 19).

Having identified the major structures, the rest of the dissection is simple since all that is necessary is to ligate and divide the intervening tissues, always taking care to stay inferior to the common hepatic artery. As the latter vessel is liberated toward its origin, the left gastric and splenic arteries are ligated and divided. In adults (Fig. 19C), enough celiac axis can be freed from within the lesser omental sac to receive the perfusion cannula used for the interim preservation to be described later and have plenty left for anastomosis in the recipient.

Aortic Dissection

When it is planned to perfuse the liver of an infant or child, it is frequently desirable to remove a piece of aorta in continuity with the celiac axis. This can best be done by lifting the spleen and left transverse colon into the wound (Fig. 20), opening the plane between the pancreas and left kidney, dividing the left renal vein, and incising the crura of the diaphragm. In the usual case the exposed aortic branches are ligated and cut except for the celiac axis, permitting the doubly transected aorta to be drawn into the lesser omental sac. When part of the hepatic arterial supply originates from the superior mesenteric artery, the latter vessel can also be retained with the specimen so that rearterialization can later be accomplished with a single aorto-aortic anastomosis (Chapter Eight, Fig. 52C). After removal of the specimen the cannula used for arterial perfusion is tied into one end of the aortic segment and the other end is ligated.

Final Steps

If a perfusion catheter has not already been placed in the superior mesenteric vein this is now inserted below the transverse mesocolon (Fig. 8). The neck of the pancreas is cut across, the splenic vein and other venous tributaries

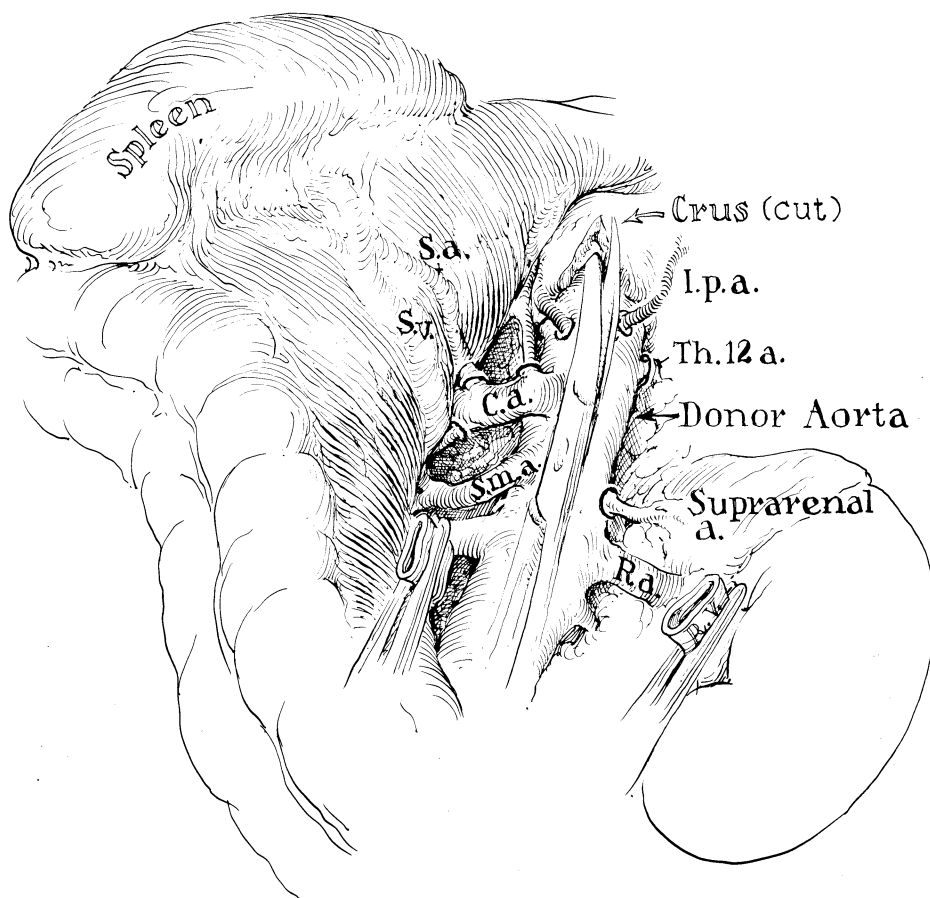


Figure 20. Mobilization of the aorta during donor hepatectomy. Rapid identification and ligation of the branches is facilitated if variable traction is applied to the distal aorta. During the initial dissection, all the aortic branches except the celiac axis and superior mesenteric artery are ligated and divided; the latter vessel is cut only after it has been shown not to give rise to an anomalous hepatic arterial branch. A segment of aorta is ordinarily removed only when the donor is an infant. (By permission of *Ann. Surg.* 168:392, 1968.)

to the portal system at this level are ligated and divided, and the mesenteric vein and its indwelling cannula are completely freed and delivered through the transverse mesocolon. The liver is now ready for removal except for incision of the avascular upper part of the gastrohepatic ligament and the vena cava above and below the liver.

In the transection of the suprahepatic vena cava, all possible length is retained. Efforts at complete transection with one movement of a scissors are very apt to cause serious distortion, as well as sacrifice of posterior wall length. It is safest to first make a small incision in the presenting anterior surface of the vessel (Fig. 21A), into which one blade of the scissors is inserted. The cut is extended to include the anterior half of the circumference. The posterior wall is then incised from within the vessel (Fig. 21B). Next the infrahepatic vena cava is cut across just above the renal veins. The organ can be immediately transplanted if the recipient is ready or, alternatively, transferred to a preservation

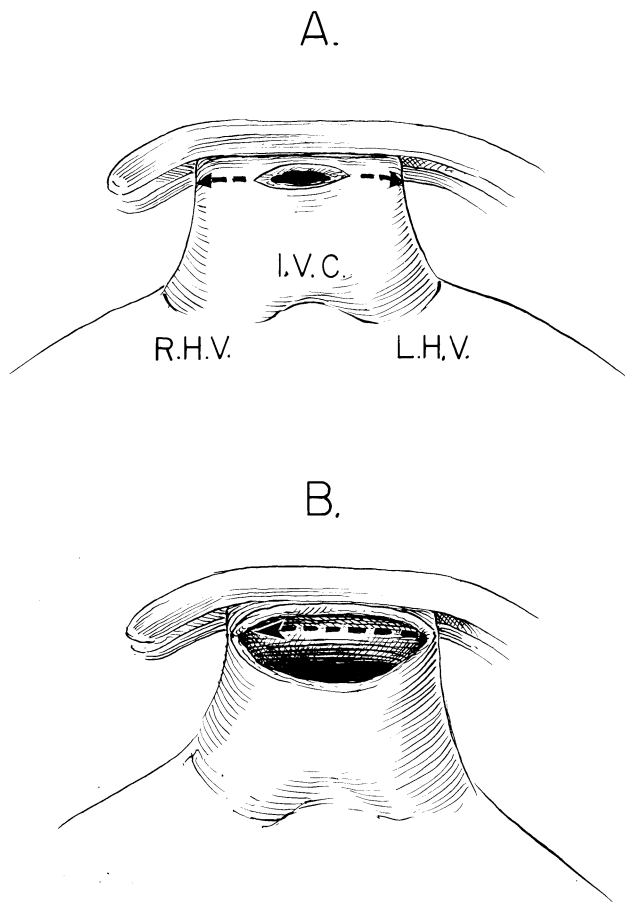


Figure 21. Formation of the supra-hepatic cuff. A. An incision is made in the anterior wall as high as possible. B. This is carried around to the back wall, taking pains to retain maximum length. The internal appearance of the transected specimen is shown in Figure 18B.

apparatus if there is to be a significant delay before the anastomoses can be started.

EX VIVO PERFUSION

If extended preservation is required, the cooled liver is again washed free of blood after its removal by a final infusion of both the portal and arterial systems with the cold solution shown in Figure 8. The fluid used is a balanced electrolyte solution to which low molecular weight dextran is added and of which the pH is adjusted to 7.45. The bloodless organ is then transferred to a preservation chamber.

Until now there has been only one experimental technique for preserving the excised canine liver which has permitted the successful use of the organs as orthotopic homografts after much longer than two hours. The method was reported by Brettschneider et al in 1967³ and was promptly applied clinically as will be described later.

In some ways the bottleneck posed for a long time by the inability to develop effective means of hepatic preservation was surprising since ex vivo

techniques had been used to study hepatic physiology for almost four decades, at first by use of very crude means of perfusion. With attention to the control of pH, temperature, and other physiologic variables and with perfusion of both arterial and portal venous systems, later investigators were able to demonstrate viability and several kinds of function of canine,¹⁷ porcine,¹¹ and bovine⁹ livers for many hours or days after their removal; the literature on this subject has been summarized by O'Donnell and Schiff.²⁵ The ability of these organs to extract metabolites from the perfusate or even to carry on complicated functions of synthesis was deceptively encouraging in view of the later difficulties encountered with the more stringent system of testing by transplantation. It eventually became clear that the retention of measurable function by an isolated perfused liver was no indication of its capacity to support life.

The Preservation Unit

The technique of ex vivo perfusion developed by Brettschneider et al^{3, 4} employs a combination of hypothermia, hyperbaric oxygenation, and perfusion with diluted blood. The design of the unit* permits gas sterilization of all the interior parts with ethylene oxide (Fig. 22) and continuous refrigeration at 4° C. One of these chambers is kept ready for use in each of the three major hospitals of the University of Colorado complex.

When it is known that a liver may soon become available, a unit of blood is drawn from a volunteer who has the same ABO type and who is compatible by direct crossmatching with the prospective cadaveric donor. The homologous blood is added to an equal volume of balanced electrolyte solution which contains 10 mg per cent heparin, 5 gm per cent low molecular weight dextran, 150 mg per cent glucose, 0.2 mEq per 100 ml magnesium sulfate and 5 mg per cent procaine. The mixture, which is buffered to pH 7.4, is slowly recirculated during the waiting period to ensure oxygenation and cooling to about 4° C. The diluted blood passes through pumps that are externally located. Inside the chamber, transit is through glass wool filters, a simple baffle oxygenator, and a reservoir. When the homograft arrives, it is interposed in the system by placing it in a receptacle and connecting the portal and arterial cannulas to the perfusion nozzles (Fig. 22). Outflow from the organ is by gravity drainage.

Flow

The blood flow delivered to the human homografts was 6 to 12 ml/gm liver tissue per hour, divided in a 4:1 portal venous/hepatic arterial ratio. The pumps were preset at approximately the correct rate by estimating the liver to be 2.7 per cent of the body weight of the donor. Thus, a 70 kg man would be predicted to have a 1890 gm liver, which would require perfusion at 11.34-22.68 liters/hour or 189-378 ml/minute. The final decision to use a greater or smaller

*Bethlehem Corporation, Bethlehem, Pennsylvania.

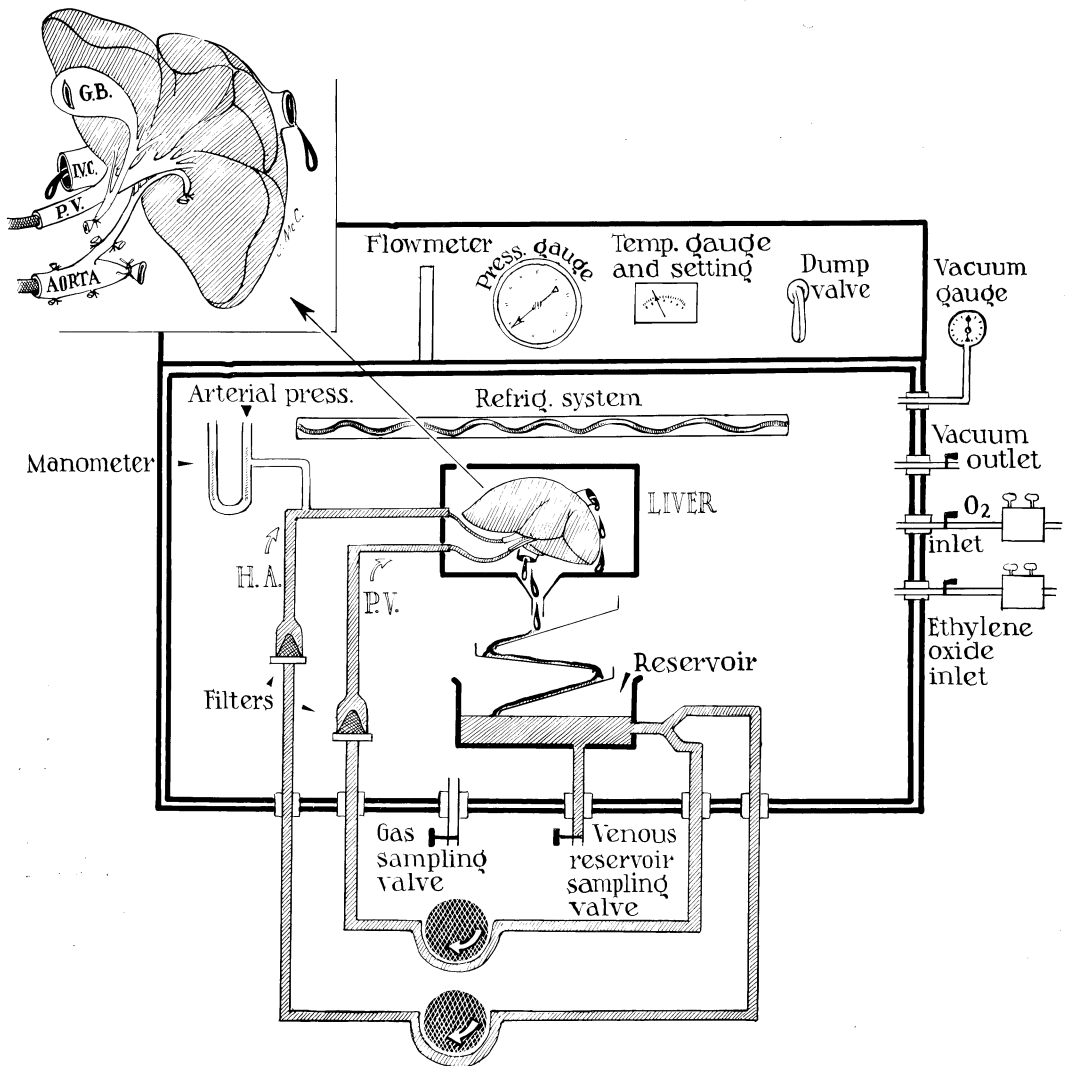


Figure 22. Preservation unit. The perfusion pumps are located outside the hyperbaric chamber; the organ receptacle, the oxygenator, and the venous reservoir are inside. The various chamber inlets permit sampling of the perfusate, gas sterilization, and oxygen delivery and removal. The temperature is electronically controlled. (By permission of Surg. Gynec. Obstet. 126:263, 1968.)

flow was based upon the gross appearance of the organ. The slightest suggestion of swelling was an indication to perfuse more slowly.

The conditions of optimum flow were worked out by Brettschneider^{3, 4} with canine livers and applied directly to human organs. In the dog experiments it was possible to quickly ruin homografts with overperfusion. On the other hand, a reduction of perfusion to 3 ml/gm tissue/hour also had an adverse effect upon the quality of the organs as judged by their later performance after orthotopic transplantation.

Compression and Decompression

Oxygen compression of 40 pounds per square inch pressure (PSIG) was reached within 20 minutes after insertion of the human organs into the cold chamber and institution of perfusion. At the end of residence in the chamber and in preparation for delivery of the livers to the recipient room, graded decompression was begun and completed in 26 to 46 minutes. In dogs it has been noted that gas emboli may develop within hepatic grafts preserved for 10 to 15 hours if decompression is carried out in less than 30 minutes.^{3, 4} The human livers, which were kept in the hyperbaric chamber for shorter periods (one to four hours), did not develop this complication.

Final Washing

Before transfer of the homografts to the recipient room, both arterial and venous systems are again flushed with the balanced electrolyte solution as shown in Figure 8. This step may be unnecessary, but it has practical advantages. First, the bloodless homograft is easier to work with as the various anastomoses are performed. Second, it would be possible to easily detect air emboli in the event of too rapid decompression. Finally, it may be desirable to rid the graft of blood which has been recirculated in an extracorporeal system for some hours, especially because of the finding of Joseph¹⁶ that the effluent of preserved canine livers often contains a vasodepressor substance that can cause shock or even sudden death.

Limitations of the Method

The effectiveness of the foregoing technique of ex vivo preservation was studied precisely in dogs with use of livers which had sustained little or no injury at the time of their insertion into the preservation unit.^{3, 4} This was possible because the organs had a good blood supply until the moment of their removal and because they were immediately cooled thereafter.

Under such circumstances, the livers always provided life sustaining function when transplanted to mongrel recipients as orthotopic homografts fol-

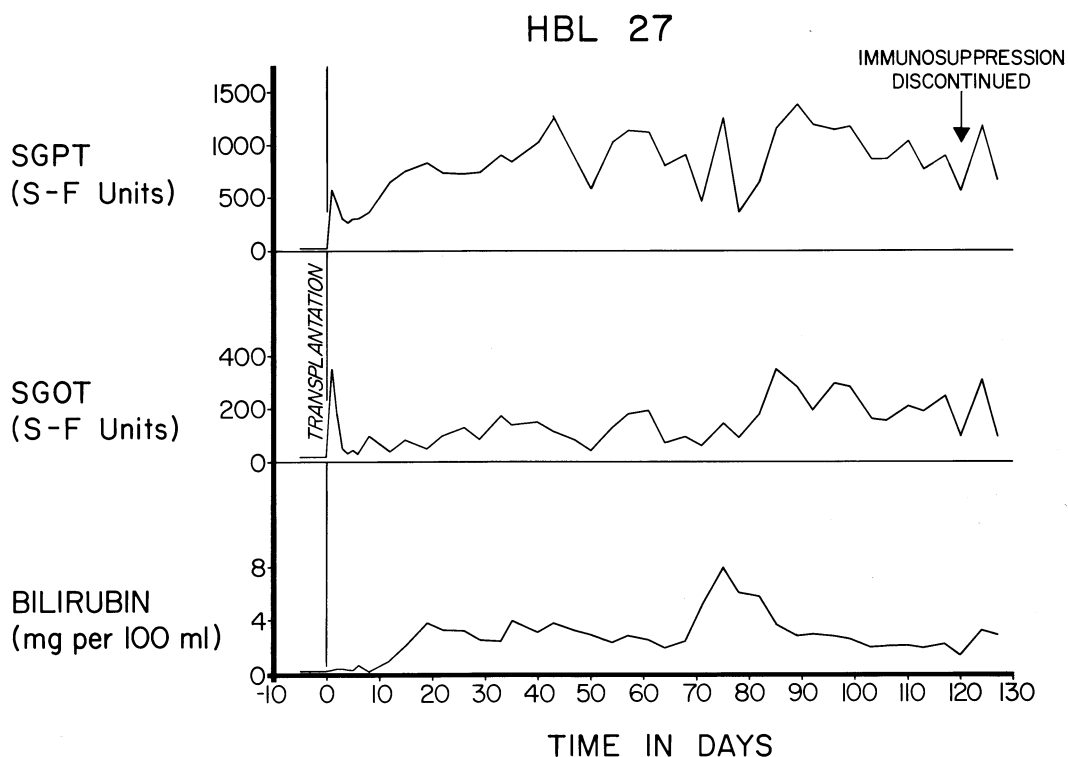


Figure 23. The course of a dog which received an orthotopic liver homograft that had been preserved for more than 24 hours. The sudden early rises in serum transaminases were due to ischemic injury. The animal did not become jaundiced for 10 days and then developed chronic low grade hyperbilirubinemia. Immunosuppression was discontinued after four months. Death from hepatic failure followed two weeks later. (By permission of Appleton-Century-Crofts, Inc., 1968.)

lowing eight to 12 hours residence in the chamber. After 24 hours two of five preserved organs had been so badly injured that the recipients did not survive operation; in the three other one-day experiments, survival was eight to 128 days (Fig. 23) before eventual death from rejection. All efforts to extend the conservation time to two days in either dogs or pigs with this or modified techniques have thus far failed.^{4, 6, 21}

Since cadaveric human livers do not begin their postmortem interval in a comparably uninjured state, it is not possible to say if the results of the canine investigations are completely applicable in a clinical setting. It is clear, however, that conservation of human livers in a highly satisfactory condition is possible for at least seven or eight hours since this has now been accomplished on several occasions.⁷

Efforts at Simplification of Liver Preservation

In canine experiments, systematic variations from the method used clinically were evaluated in order to determine which details of technique were important and which were nonessential. The quality of the homografts was

found to deteriorate with reduction or elimination of perfusion, the omission of hyperbaric oxygenation or hypothermia, or elimination of blood from the perfusate.⁴

The need for homologous blood in the perfusing fluid was examined with special care in canine studies because of the associated inconvenience and potential dangers of a blood system. Unfortunately, it was found that the use of several substitute acellular solutions did not provide as good results. These included low molecular weight dextran perfusates,⁴ diluted or full strength plasma,^{5, 30} undiluted plasma from which the lipoprotein flocculate had been removed⁵ as described by Belzer,¹ and hemoglobin solutions.⁵

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Chapter Six

CONSEQUENCES OF HEPATIC ISCHEMIA

In almost all the first unsuccessful attempts at liver transplantation, poor initial function of seriously damaged homografts played an important or decisive role in the early fatal outcome. Even in the later cases in which extended survival was finally achieved, some element of hepatic malfunction in the early postoperative period jeopardized convalescence to a variable degree at a time before rejection had begun. This was a reflection of the combined injury incurred in the terminal phases of donor life, during the normothermic post-mortem "dead time," and in the subsequent interval when active efforts were being made to cool and preserve the liver.

An analysis of the effect of these nonimmunologic factors upon the behavior of liver homografts and their new hosts can most accurately be done with cases of orthotopic transplantation since, after this operation, the life of the recipient is both immediately and completely dependent on the transplanted liver. Consequently, the following remarks will be based on observations made in dogs and patients after liver replacement. Undoubtedly the conclusions apply to auxiliary homografts which, however, will not be mentioned further here (see Chapter Twenty-two) since their function cannot be so precisely followed.

THE INJURED LIVER IN DOGS

With paired organs such as the kidney, the degree to which ischemic tissue damage adversely influences either short or long term function can be investigated in the absence of an immunologic barrier since autografts can be used in testing. For livers, this approach is not feasible inasmuch as the test organ cannot be removed, leaving the host in an anhepatic state for some hours, and then returned with much hope of survival even with a perfectly preserved organ; the problem is not dissimilar to that posed by the evaluation of heart preservation by cardiac autotransplantation. Fortunately, very clear information has come from canine experiments with orthotopic hepatic homotransplantation.

Minor Damage

In Chapters Eleven and Twelve the postoperative course will be described which can be expected with orthotopic transplantations performed in dogs and pigs under ideal conditions. In such experiments livers are removed from cooled living donors, chilled further by immediate intraportal infusion of a cold solution, and revascularized in the recipient as quickly as possible. The interval of hypothermic ischemia is seldom much more than an hour.

Abnormalities in the hepatic function of the recipient animal are trivial during the first few postoperative days, or may even be undetectable. There is no intraoperative bleeding diathesis or hypoglycemia. The animals awaken promptly from anesthesia, and can often eat by the following morning. Protein synthesis is normal or supernormal for at least several days. Commonly, the only detectable abnormalities in the liver function tests are minor and quickly reversible increases in the serum transaminases (Fig. 24). The animals usually remain in good health until the onset of rejection, which almost never is evident until the third or fourth day in untreated dogs; if immunosuppression is provided, many such animals live for a long time (Chapter Twelve). Comparably perfect organs can probably never be expected in clinical liver transplantation.

Massive Injury

At the other end of the spectrum is liver damage of such magnitude that survival through the operation is not possible. As mentioned in the preceding chapter, the recipient dogs die within a few hours of acute hepatic insufficiency (including hypoglycemia), uncontrollable bleeding, and "outflow block."

Moderate or Serious Injury

Between the extremes there is a syndrome of hepatic damage which is of the utmost importance since many or most of its elements have been seen in virtually every case of clinical liver transplantation. Its features were most clearly delineated during efforts to develop useful methods of organ conservation²⁻⁴ when all gradations of liver injury were caused, depending on the effectiveness of the method being tested and the duration of its use. The most precise information came from the studies of Brettschneider,^{1, 2} with ex vivo preservation for eight to 24 hours.

Many of the recipients of these preserved organs were able to survive operation. However, hemorrhage was a difficult problem, even after conservation for as short a period as eight hours under conditions which yielded good enough results to ultimately warrant clinical application. Typically, bleeding could be controlled only with extraordinary mechanical efforts at hemostasis,

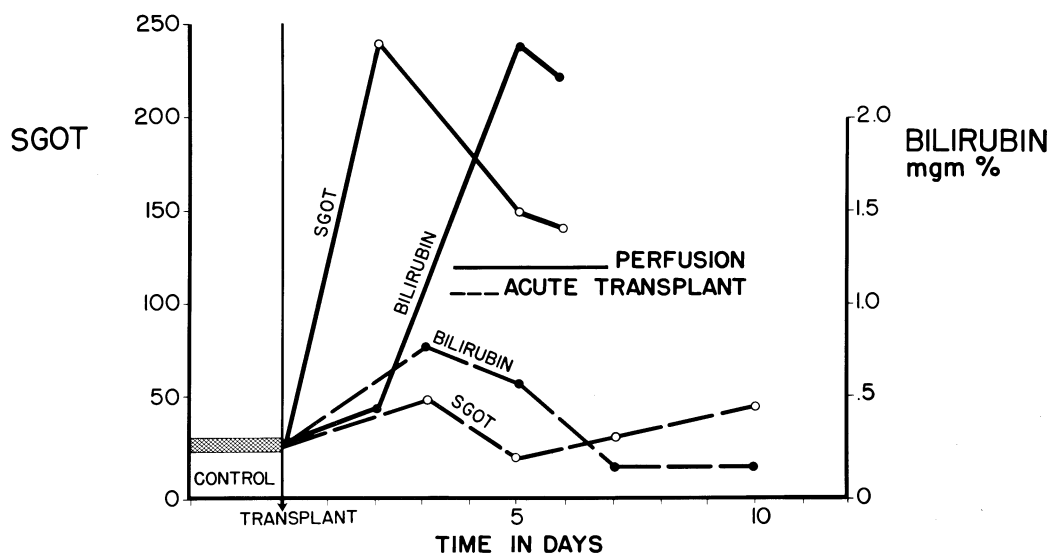


Figure 24. Patterns of hepatic injury in two dogs following orthotopic homotransplantation. Note that there were almost no changes of SGOT and bilirubin in the recipient of an immediately cooled and transplanted organ; the hypothermic ischemia was 55 minutes. In contrast, acute rises of SGOT and serum bilirubin occurred after transplantation of a liver obtained from a cadaveric canine donor. In the latter experiment, the donor dog was killed with an overdose of anesthesia and total body hypothermic perfusion was started five minutes later.¹ The time from death to homograft revascularization in the recipient was 140 minutes. Postoperatively, both dogs were treated with azathioprine. (By permission of Surgery 54:900, 1963.)

multiple blood transfusions, and often the administration of clot-promoting agents such as epsilon aminocaproic acid (EACA) and protamine sulfate. The responsible clotting defect⁵ has been shown to be a complex disorder (Chapter Ten) and one which is rapidly reversible providing the extent of liver damage is not too great.

In such experiments the control of bleeding was only the first step in care. Intensive therapy was usually required with electrolyte solutions or plasma, apparently because of the development of a third fluid space not only within the abdomen where ascites formed and fluid was sequestered in the intestine in proportion to the degree of liver injury, but also in the peripheral tissues where edema was commonly noted. At this time and during the ensuing two or three days there were sharp falls in the concentrations of plasma proteins (Fig. 25).

An interesting study was performed by Kashiwagi on a number of these animals.³ When damaged homografts were transplanted, he often found striking changes in the serum protein constituents in that discrete fractions were found to have completely disappeared. The result was the development of a "stripped tree" appearance of the recipient serum as viewed with immunoelectrophoresis (Fig. 26). The abnormalities were totally reversible if the hepatic injury was moderate; when it was severe, the missing immuno-electrophoretic bands tended to return, but never completely to normal. Moreover, the latter animals were never able to eat and died in spite of treatment with immunosuppression within a week or 10 days as the consequence of wasting, infection, or

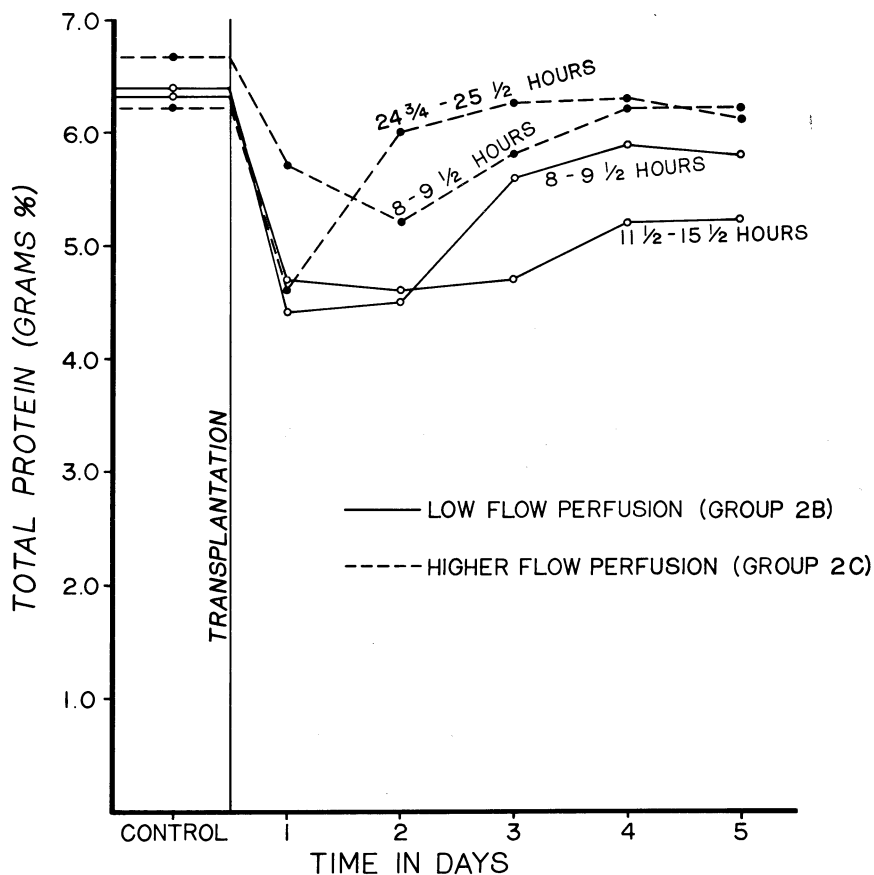


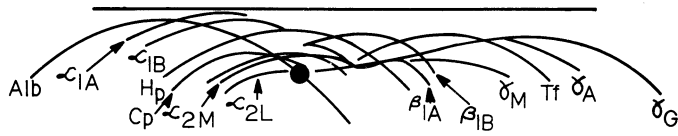
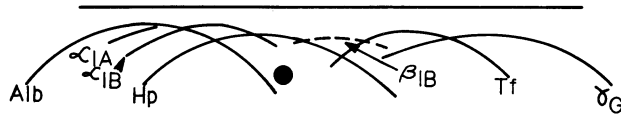
Figure 25. The circulating plasma proteins in canine recipients of orthotopically placed preserved livers. In all series of experiments there was a prompt decrease in plasma protein concentration. The hypoproteinemia was more profound and sustained in the experimental groups in which low flow liver perfusion (3 ml/100 gm hepatic tissue per hour) was used during conservation than when the flow rate was doubled. (By permission of Surg. Gynec. Obstet. 126:263, 1968.)

other complications. In such experiments the aftereffects of ischemia in combination with an unanalyzable further immunologic injury to the homografts apparently precluded more than temporary survival. At autopsy, the predominant histologic features were those of healing parenchymal necrosis rather than rejection.

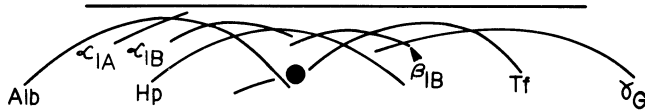
It is of interest that the acute disturbances of protein metabolism were not necessarily accompanied by comparably severe abnormalities in all other measures of liver function. In this kind of unsatisfactory preservation experiment, early hyperbilirubinemia was common (Fig. 24), but a surprising number of the animals died without ever becoming jaundiced. Transient increases in transaminases were invariably seen after operation. In most, there was some evidence of multiple gastrointestinal ulcerations by the time of autopsy, and often these acute lesions were the immediate cause of death by virtue of their perforation or hemorrhage.

These various observations had an important influence on the standards

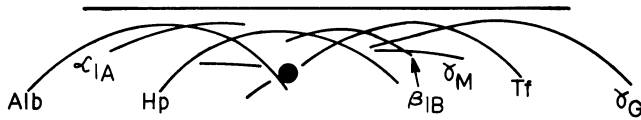
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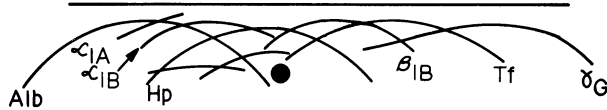
DAY 2



DAY 3



DAY 4



DAY 5

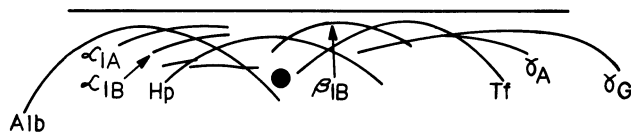


Figure 26. Changes seen by immunoelectrophoresis in the serum protein of a dog which received a damaged liver homograft. Note the early postoperative disappearance of several precipitation bands including ceruloplasmin (C_p), α_{2m} , α_{2l} , $\beta_{1\lambda}$, γ_{λ} , and γ_{λ} . These abnormalities were totally reversible if the hepatic injury was moderate: if the damage was severe, as in this case, the electrophoretic bands tended to return, but never completely to normal. (By permission of Surgery 63:247, 1968.)

imposed upon the method of ex vivo preservation finally accepted for clinical use in many of our later cases. The ability of a conserved organ to support life during and for a few days after operation was not enough. It was necessary to demonstrate long term survival in canine recipients treated with immunosuppression. This was achieved in our laboratories by Brettschneider using livers that had been kept in good condition for eight or 10 hours by the technique described in Chapter Five, with about the same consistency as was possible with immediately cooled and transplanted homografts.² Furthermore, chronic survival was obtained in some experiments in which the homografts were stored for more than 24 hours before transplantation (Fig. 23, Chapter 5).

HOMOGRAFT INJURY IN MAN

In the clinical orthotopic liver transplantations (Table 6), there were examples of hopelessly, severely, moderately, and minimally damaged homografts. As a group, the worst organs were those which were used early in our experience. The donors in the later transplantations were far more carefully selected. They were younger, had shorter terminal illnesses, and maintained more effective circulation until almost the moment of death. Moreover, the warm ischemia times of the organs were very short. Finally, the development of an effective technique for more extended preservation (Chapter Five) made it possible to keep some of the excised livers in good condition for several hours until the recipient operation had been brought to the appropriate stage.

Hopeless Injury

One recipient (OT 1), a 36 month old child with biliary atresia, failed to survive operation. The donor in this case was a three year old boy who had died during the attempted extirpation of a tumor of the diencephalon. External and internal cardiac massage were carried out for 45 minutes before death was pronounced. Fifteen minutes later extracorporeal cadaveric perfusion and cooling were instituted and continued for almost four hours; the liver was finally revascularized in the recipient patient 420 minutes after the cardiac arrest.

A hemorrhagic diathesis ensued, leading to death in four hours. Massive fibrinolysis was demonstrated,^{6,7} which was not controlled by treatment with EACA, fresh blood, and fibrinogen (Chapter Ten). At autopsy, the liver was almost entirely necrotic (Chapter Twenty).

Massive Injury

In two other cases (OT 6 and 7), there was probably never any hope for recovery in view of the widespread hepatic necrosis found histologically in the

autopsy specimens seven and 10 days later, respectively. The older of these patients (OT 6), whose diagnosis was hepatoma, was 29 years old. He received the liver of a 73 year old man who had died 10 hours after suffering a massive stroke. The donor was in agonal condition with no detectable blood pressure for nine hours before death. The liver was cooled by intraportal infusion after a warm ischemic period of four minutes and was revascularized 148 minutes later (Table 6). Within less than an hour, a hemorrhagic diathesis was manifest, necessitating transfusion with 12,500 ml fresh blood during the 14 hour operation. Hemostasis was improved but not completely obtained after 0.9 gm/kg EACA and 16 gm human fibrinogen were given intravenously. At re-exploration 48 hours later three liters of blood were evacuated and residual diffuse bleeding was controlled mechanically. Four days after transplantation, reoperation again became necessary to suture ligate an arterial bleeder in the anastomotic line of the cholecystoduodenostomy; it was not clear if this was due to a technical error or acute peptic ulceration. Transfusions in the seven post-operative days totalled 22,000 ml.

After transplantation, the patient was alert for one and a half days, but then became mentally obtunded and, finally, comatose. Serial determinations of liver chemistries showed grave derangements from the beginning. The serum bilirubin rose from 0.7 to 8.7 mg per cent within 48 hours and continued to increase until the time of death after seven days (Fig. 27); the conjugated component was always less than half the total. The typical acute rise and fall of the serum transaminases were observed (Fig. 27). The Quick prothrombin time fell to 20 per cent and remained fixed at about that level. Plasma fibrinogen ranged from 140 to 190 mg per cent. Plasma albumin levels were maintained with frequent infusions of concentrated human albumin, totalling 325 gm in the seven postoperative days. That hypoglycemia was not observed may have been due to constant glucose infusion. Interpretation of any of the liver chemistry determinations was made inexact by the multiple transfusions of fresh blood given daily.

In this case the development of a large third fluid space was evident from the beginning. Ascites accumulated rapidly at the same time as anasarca was diagnosed. Hyperaldosteronism was apparently not responsible since urine sodium concentrations were never lower than 40 and were usually 80 to 105 mEq/liter; urinary output ranged from 1265 to 2020 ml/day until the last day of life. During the first two days, intravenous therapy with noncolloid solutions was limited to 1200 ml over the total output. Later, as much as 6000 ml in excess of the measurable loss was required to prevent hypotension. Ultimately, the BUN (Fig. 27) and creatinine began to increase, the serum sodium rose to 160 mEq/liter, fluid retention became so extreme that the facial features of the patient were scarcely recognizable, and he died with massive pulmonary edema. At autopsy, other findings included bilateral pleural effusions, massive ascites, and acute renal tubular necrosis. The septic complications in this case and the pathologic changes in the homograft are considered separately in Chapters Sixteen and Twenty.

The other patient (OT 7) was an 11 month old boy with biliary atresia. The

OT 6

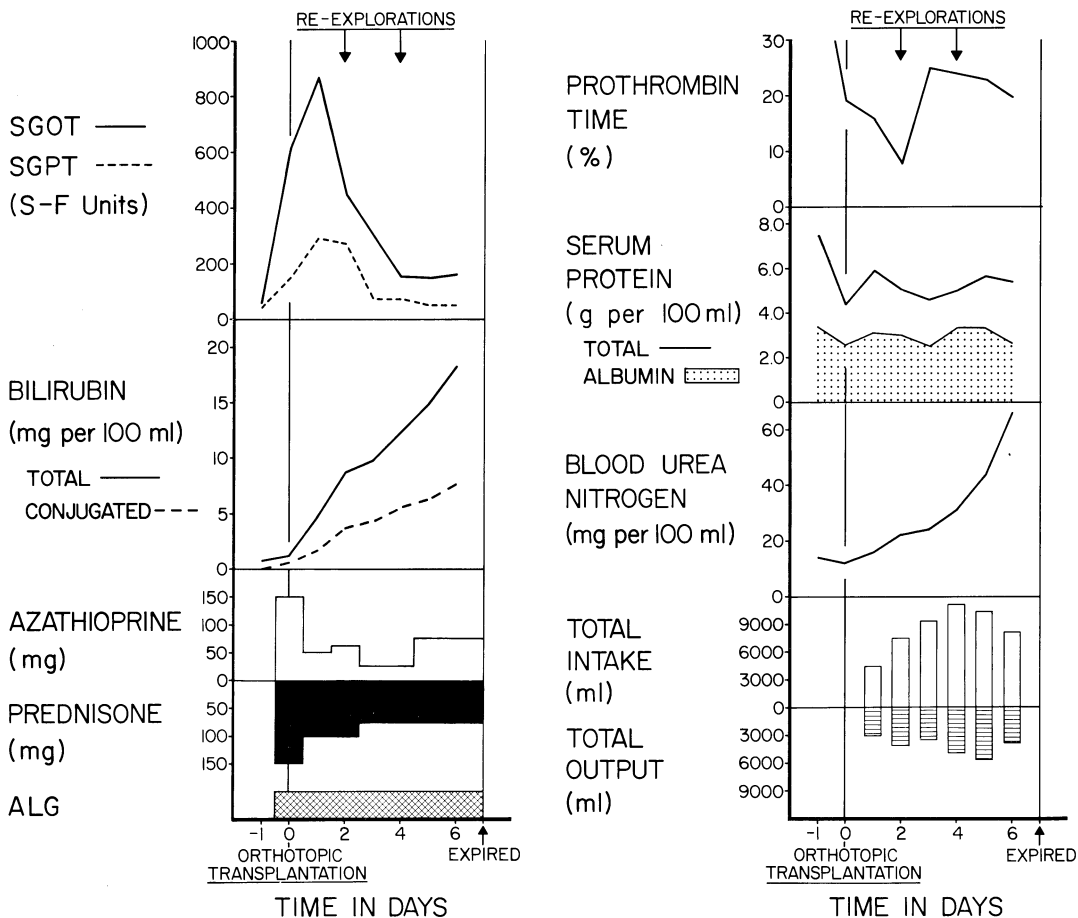


Figure 27. The clinical course of a patient (OT 6) who received a massively damaged homograft. The two re-explorations were for the control of hemorrhage. Note the manifold evidence of poor liver function. The output figures are the total of urine volume plus gastric aspirate and stool. The intake is the total of electrolyte solutions and colloids, including blood.

liver was from a 12 month old girl with a diagnosis of microcephaly who died 48 hours after aspirating a feeding and having a cardiac arrest at a domiciliary home. The donor was in agonal condition for the last 14 hours of life with no detectable blood pressure. Liver cooling by infusion through the superior mesenteric vein was started 14 minutes after death. The hepatectomy was completed in 60 minutes, and the liver then perfused for 240 minutes in the hyperbaric preservation chamber. The time from death to revascularization in the recipient was 371 minutes. Complete hemostasis was obtained within one and a quarter hours. Blood transfusion to the 5 kg recipient was 300 ml. Bleeding did not subsequently occur although all the liver-based clotting factors fell to less than 10 per cent of normal within one day (Fig. 69, Chapter Ten).

Immediately after operation the serum bilirubin, which had been 10.9 mg

per cent, was 4.7 mg per cent. The change was apparently due to exchange transfusion during operation since the bilirubin then began an inexorable rise (Fig. 28). The postoperative increases in SGOT (to 6700 units) and SGPT (maximum 1280 units) were the greatest in our experience with the exception of a case (OT 18) in which there was hepatic artery thrombosis (Chapter Nine). Maintenance of total serum proteins required the infusion of 85 gm of albumin during the 10 days of postoperative life.

Urine output was from 115 to 300 ml/day, with a sodium concentration of 5 to 50 mEq/liter. Azotemia did not develop. Intravenous fluids were designed to keep the child relatively dry with minimal weight gain (Fig. 28). Nevertheless, ascites, which eventually prompted repeated paracenteses, became evident at the end of the first week. The patient became comatose and hypotensive and

OT 7

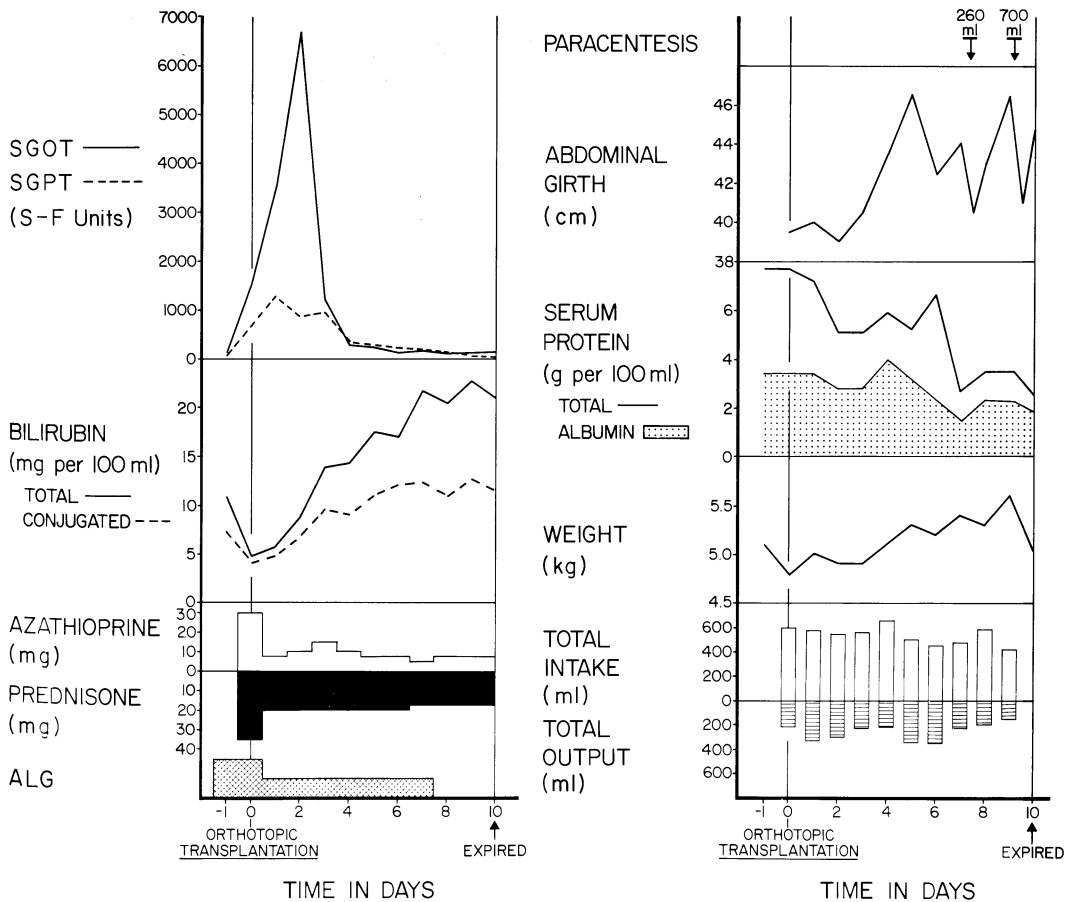


Figure 28. The course of an 11 month old child (OT 7) with biliary atresia after orthotopic transplantation of an unsatisfactory liver. The hepatic function tests were markedly abnormal from the beginning. Fluid retention became a progressively severe problem. At autopsy the homograft had massive necrosis.

Table 6. Information about the Liver Donors and the Methods and Intervals of Preservation in 25 Consecutive Orthotopic Transplantations

OT NUMBER	DONOR AGE	DONOR DIAGNOSIS	DURATION TERMINAL ILLNESS	AGONAL PERIOD OF POOR CIRCULATION	EMERGENCY MEASURES	NORMO- THERMIC ISCHEMIA	TIME FROM		SATIS- FACTORY ORGAN	RECIPIENT INTRAOPERATIVE BLOOD TRANSFUSION
							DONOR DEATH TO REVAS- CULARIZATION	HYPERBARIC CHAMBER		
1	3 years	Cardiac arrest during craniotomy	1 1/2 days	— — —	Cardiac massage Heart-lung perfus.	60 min.	420 minutes	Not used	No	8000 ml
2	55 years	Terminal brain tumor	6 weeks	6 hours	Cardiac massage Heart-lung perfus.	5 min.	152 minutes	Not used	No	2500 ml
3	69 years	Stroke	1 day	8 hours	Cardiac massage Heart-lung perfus.	6 min.	192 minutes	Not used	No	4500 ml
4	73 years	Myocardial infarct.	4 days	6 hours	Cardiac massage Heart-lung perfus.	4 min.	176 minutes	Not used	No	1500 ml
5	64 years	GSW Head (suicide)	5 days	12 hours	Cardiac massage Heart-lung perfus.	4 min.	164 minutes	Not used	No	9000 ml
6	73 years	Stroke	10 hours	9 hours	Cardiac massage Infusion	4 min.	152 minutes	Not used	No	12500 ml
7	1 year	Aspiration (microcephaly)	2 days	14 hours	Cardiac massage Infusion	14 min.	371 minutes	240 minutes	No	300 ml
8	18 months	Aspiration (microcephaly)	4 days	30 minutes	Cardiac massage Infusion	8 min.	311 minutes	146 minutes	Yes	350 ml
9	4 years	Drowning	3 days	60 minutes	Cardiac massage Infusion	0 *	282 minutes	Not used	Yes	500 ml
10	18 months	Acute CNS injury	10 days	30 minutes	Cardiac massage Infusion	6 min.	271 minutes	122 minutes	Yes	500 ml
11	20 months	Krabbe's disease	3 days	15 minutes	Cardiac massage Infusion	7 min.	252 minutes	78 minutes	Yes	1000 ml
12	14 months	Werdnig-Hoffman's disease	4 days	2 hours	Cardiac massage Infusion	10 min.	320 minutes	113 minutes	Yes	1100 ml

13	33 months	Meningitis	10 days	30 minutes	Cardiac massage Infusion	4 min.	323 minutes	61 minutes	Yes	500 ml
14	27 years	GSW Head (suicide)	10 hours	90 minutes	Cardiac massage Heart-lung perfus.	7 min.	451 minutes	217 minutes	Yes	4500 ml
15	20 years	Acute CNS injury	12 hours	2 hours	Cardiac massage Heart-lung perfus.	3 min.	380 minutes	187 minutes	Yes	9000 ml
16 ^A	3 years	Cardiac arrest (laryngotracheitis)	8 days	4 hours	Cardiac massage Heart-lung perfus.	2 min.	245 minutes	79 minutes	Yes	1300 ml
16 ^B	7 years	Acute CNS injury	3 days	10 minutes	Cardiac massage Heart-lung perfus.	0*	179 minutes	Not used	Yes	780 ml
17	22 years	Acute CNS injury	8 hours	2 hours	Cardiac massage Heart-lung perfus.	9 min.	400 minutes	183 minutes	Yes	4800 ml
18	21 months	Acute CNS injury	4 days	15 minutes	Cardiac massage Infusion	0*	250 minutes	Not used	Yes [†]	400 ml
19	10 years	Acute CNS injury	26 hours	30 minutes	Brain death conditions [†] Heart-lung perfus.	0	266 minutes	93 minutes	Yes	1190 ml
20	10 years	Encephalitis	8 days	0 minutes	Brain death conditions [†] Heart-lung perfus.	0	170 minutes	Not used	Yes [†]	4250 ml
21	5 years	Acute CNS injury	3 days	0 minutes	Brain death conditions [†] Heart-lung perfus.	0	294 minutes	167 minutes	Yes [†]	2530 ml
22	25 years	Acute CNS injury	8 days	0 minutes	Brain death conditions [†] Heart-lung perfus.	0	216 minutes	Not used	Yes [†]	19000 ml
23	6 years	Acute CNS injury	8 days	0 minutes	Brain death conditions [†] Heart-lung perfus.	0	206 minutes	Not used	Yes	9500 ml
24	2 years	Brain tumor	6 weeks	0 minutes	Brain death conditions [†] Infusion	0	354 minutes	179 minutes	Yes [†]	5800 ml

*Hypothermia previously instituted for treatment of CNS injury.

[†]In these cases, the times were started with the abdominal incision for hepatectomy.

[‡]Although the organs were thought to be satisfactory, the patients died early in the postoperative period because of occlusive vascular accidents involving either the portal vein or hepatic artery of the homograft or, in one case, because of the accidental production of a complete biliary obstruction.

died 10 days after transplantation. During life, there were three bouts of hypoglycemia, all occurring after the temporary accidental cessation of intravenous glucose infusion. The plasma blood sugars at these times were 5, 27, and 58 mg per cent.

At autopsy the vessels entering and leaving the liver were patent. However, there were large grossly necrotic portions of the liver, especially near the surface. The jejunum had sloughing mucosa, although gastrointestinal hemorrhage had not occurred. The cause of death was acute liver failure. Histologically there was evidence in the homograft of both ischemic damage and severe acute rejection (Chapter Twenty).

Serious Injury

The homografts of four patients (OT 2 to 5) had early changes in liver chemistries as severe as those described in the previous section. However, at least some of the abnormalities had already reached a maximum and were receding at the time of death 22, 7½, 6½, and 23 days post-transplantation, respectively.

The normothermic "dead times" (Table 6) in the four cases were 5, 6, 4, and 4 minutes, respectively, after which extracorporeal cadaveric perfusion was instituted for 75, 104, 89, and 77 minutes, as described in Chapter Five. The total times from donor deaths to revascularization of the homografts in the recipients were 152, 192, 176, and 164 minutes.

Paradoxically, the longest surviving patient (OT 5) was the one with the least satisfactory early function. She had a hepatoma and was not jaundiced before operation. Within four days afterward, her serum bilirubin had risen to 45 mg per cent. By this time her prothrombin time had declined to 20 per cent. Total plasma proteins and the constituent fractions, including albumin and fibrinogen, had fallen drastically (Fig. 29).

A week later the jaundice slowly began to subside. She began to eat. Although ascites developed, there still appeared to be hope for recovery. On the 17th postoperative day she exhibited sudden signs of peritonitis and died a week later. At autopsy the choledochocholedochostomy was found to be disrupted as a result of necrosis of the homograft common duct. Terminally, she had pronounced fluid retention as well as a major gastrointestinal hemorrhage.

The other three patients survived for 6½ (OT 4), 7½ (OT 3), and 22 days (OT 2), and died of multiple causes. Striking increases in serum transaminases had occurred shortly after operation followed by a transient and reversible jaundice (Fig. 30). The direct or contributory cause of death in each instance was multiple pulmonary embolization. The three patients also had terminal gastrointestinal hemorrhage; two had sepsis. In one (OT 2) the abdomen was completely free of ascites at the time of autopsy.

The pathologic features of all four homografts are described in Chapter Twenty. There was little evidence of homograft rejection in the specimens. The predominant histologic findings were those of fresh or healing ischemic damage.

OT 5

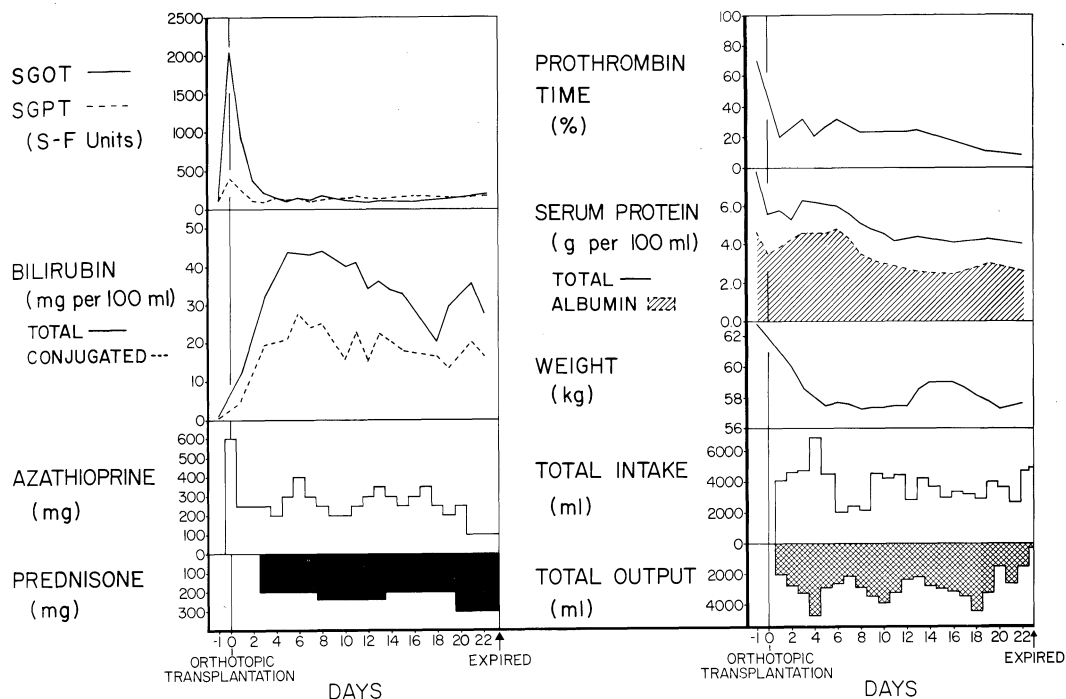


Figure 29. The course of a patient (OT 5) who received a homograft that had been seriously injured by ischemia. In spite of the very poor initial hepatic function, the recipient remained in good condition until the development of biliary peritonitis on the seventeenth postoperative day. Survival was for 23 days.

Moderate or Minimal Injury

Whereas all the patients treated with orthotopic homotransplantation up to May of 1967 received badly damaged livers, each of the subsequent recipients (OT 8 to 24) was given an initially satisfactory organ. Five of the patients died within the first two postoperative weeks, but the failures were due to technical complications (OT 18, 20, 21, 22, 24).

The livers in all the later cases were obtained from donors who had had relatively brief terminal diseases and in whom an effective circulation was maintained until shortly before death (Table 6). Liver cooling was instituted within less than five minutes after the cessation of an adequate circulation by one of the two methods described in the preceding chapter. After cooling and extirpation, the organs were immediately transplanted or they were maintained in the hyperbaric preservation unit. The times from donor death to revascularization in the recipient ranged from 170 to 451 minutes (Table 6). In the cases in which the technique for extended preservation was used the recipient operation was not begun until the homograft had already been removed and evaluated while it was being perfused in the cold hyperbaric chamber.

As in the earlier cases, control of hemorrhage was often difficult, as can be

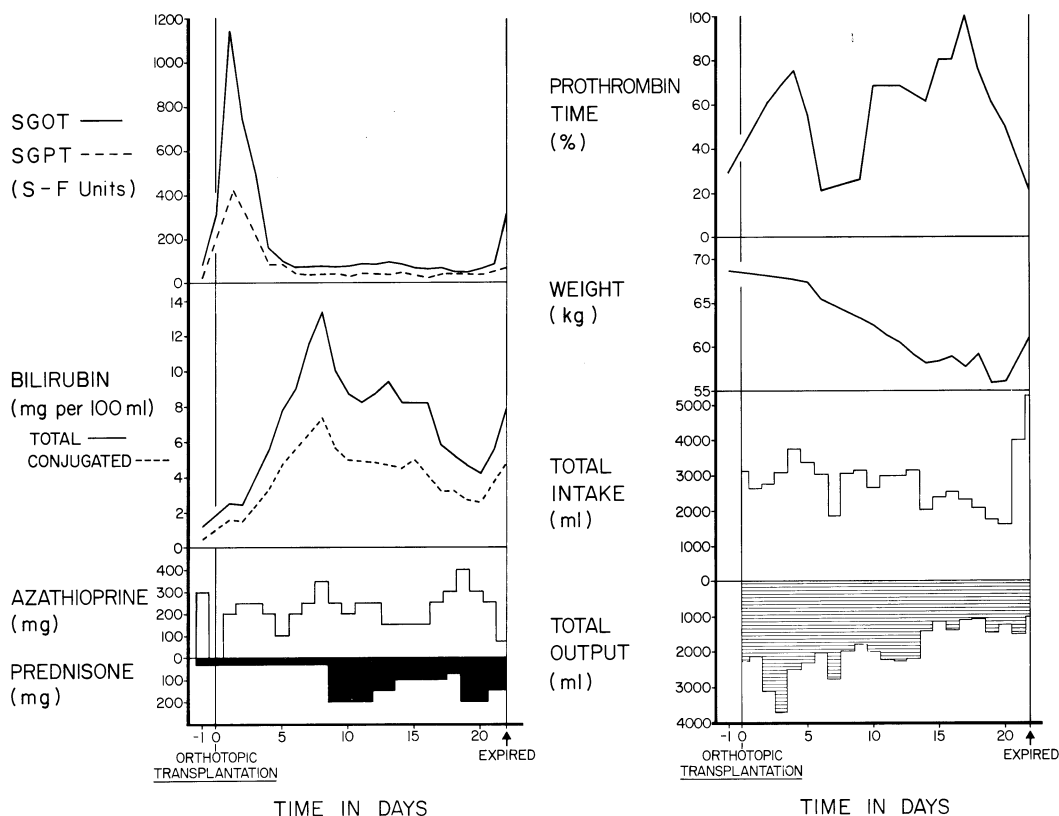


Figure 30. The course of the first patient (OT 2) who survived the operation of orthotopic liver transplantation. The indication for the procedure was hepatoma. The donor had a lingering terminal course caused by a glioblastoma multiforme. After death cardiopulmonary bypass was instituted (see text). The homograft apparently suffered a serious ischemic injury, but one that was reversible. The immediate cause of death of the recipient was massive pulmonary embolization. At autopsy the homograft appeared grossly to be in good condition; ascites was not present.

appreciated by reviewing the magnitudes of intraoperative transfusions required (Table 6). However, hemostasis was obtained in all but two of the recipients (OT 8 and OT 17) without the administration of thrombogenic agents.

On the day following operation there were always sharp rises in the transaminases which quickly returned toward normal (Figs. 31 and 32); these were generally of the same magnitude as in the more seriously injured grafts described in the preceding section. In contrast, however, there was effective bilirubin clearance. Recipients who were jaundiced before operation had immediate improvement which was sustained until the subsequent onset of rejection (Fig. 32). Those patients without pre-existing hyperbilirubinemia maintained low values (Fig. 31) for at least several days.

The recipients who did not suffer technical complications convalesced surprisingly rapidly and after two or three days usually did not appear to be ill.

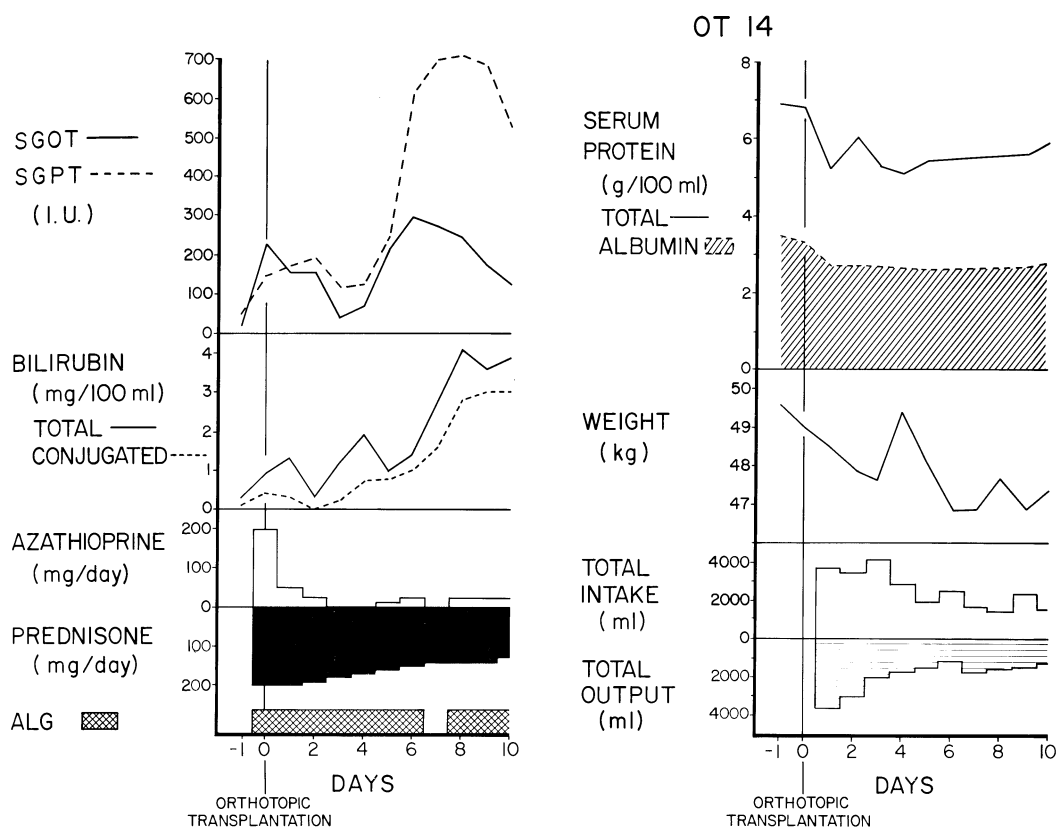


Figure 31. The course of a 16 year old girl (OT 14) who received the liver of a 27 year old adult male. The indication for operation was hepatoma. After the donor had a cardiac arrest, cardiopulmonary bypass was instituted while hepatectomy was carried out. The liver was then preserved by perfusion in a cold hyperbaric oxygen chamber. The time from donor death to revascularization of the liver in the recipient was more than seven and a half hours. Adequate initial function was obtained and continued until the onset of rejection on the sixth postoperative day. Despite the acute hypoproteinemia, there was little tendency to fluid accumulation. The patient is still alive more than a year after the transplantation.

Nevertheless, other measures of liver function were always demonstrably abnormal in one or more ways. In every case a decline in serum protein concentration was noted similar to that described in the patients who received less adequately preserved organs. The falls were sometimes extreme (Fig. 32). Concomitant fluid retention including ascites was variable at this time. There were also depressions of the liver-based clotting factors (Chapter Ten). These changes were relatively transient. Within a few days a brisk diuresis ensued, and the temporarily depressed protein fractions returned toward normal and were usually very well maintained even after the onset of subsequent rejection. The presence of less than perfect hepatic function during the initial stages of convalescence governed a number of specific details of postoperative management. These will be mentioned in Chapter Nine.

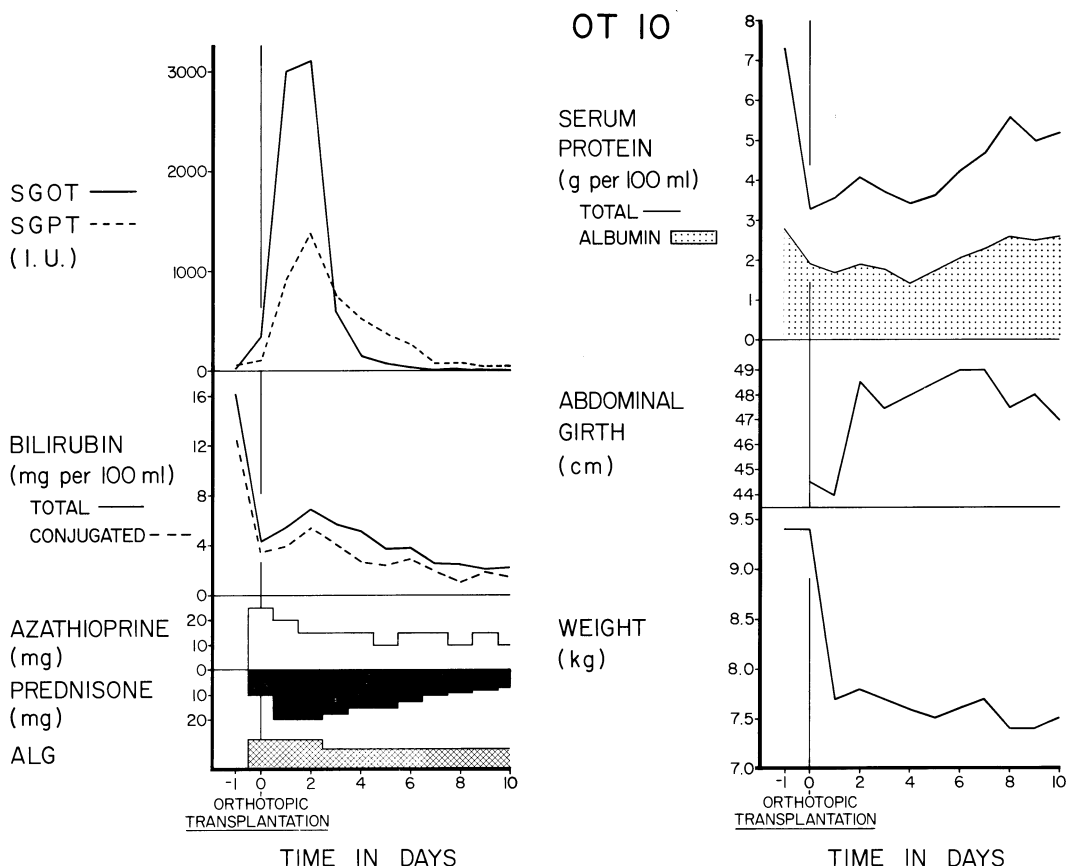


Figure 32. The early course of patient OT 10 after orthotopic hepatic transplantation. The liver was cooled by intraportal infusions six minutes after death was pronounced on the basis of cardiac arrest. The organ was then excised and placed in a hyperbaric conservation unit. The time from death to homograft revascularization was 271 minutes. Although the liver sustained a highly significant ischemic injury, it provided adequate initial function and cleared the pre-existing hyperbilirubinemia. Nevertheless, there was very pronounced hypoproteinemia. Ascites developed which resolved as the serum protein values subsequently improved. The child developed partial gangrene of the right liver lobe one month after operation (see Chapter Fifteen) and eventually died more than six months after the transplantation.

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PART III

ORTHOTOPIC TRANSPLANTATION

Chapter Seven

ANESTHESIA AND INTRAOPERATIVE CARE

Pages 83 - 111

by J. Antonio Aldrete, M.S., M.D.

The use of anesthesia for liver transplantation is complicated for reasons other than the fact that the operations tend to be long and difficult and attended with major blood loss. First, all commonly used anesthetics are at least partially metabolized by the liver; moreover, even those given by inhalation have intermittently been suspected of modifying hepatic blood flow or producing liver injury. Second, hemodynamic changes are caused during the orthotopic operations since the inferior vena cava and the portal vein must temporarily be cross clamped during the anhepatic phase. Finally, the procedure causes major derangements in a number of metabolic processes, including those of blood sugar control and acid-base balance. In this chapter, the foregoing problems will first be considered individually; then the actual anesthetic methods and the techniques of early postoperative care will be described.

THE EFFECT OF THE LIVER ON ANESTHESIA

The role of the liver in the detoxification of anesthetic and related drugs has been summarized in the recent review by Greene.⁵⁸ At least some of the very detailed information about the metabolic pathways of the various agents is derived from laboratory experiments that may not be applicable to humans. This is because enzyme representation and activity may be radically different from one species to another. The problem is not different from that discussed in Chapter Thirteen in relation to the toxicity of azathioprine in dogs as compared to humans.

Even within a species, there may be great variability in the efficiency with which anesthetic agents are eliminated. One of the factors with immediate significance in liver transplantation is that of age, since many potential recipi-

ents are infants or very small children. Moreover, one of the most available sources of homografts could be the population of infants born with fatal congenital defects. A liver from just such a donor was used in one of our adult patients as an auxiliary organ.

In Chapter Twenty-two the limitations of homograft function which might be anticipated with physiologically immature organs will be discussed as they would be predicted to be expressed in defective bilirubin excretion. This is one detail of fetal and newborn hepatic function that has been well studied. Uridine diphosphogalactose (UDPG) dehydrogenase and glucuronyl transferase enzymes, upon which bilirubin metabolism is partially dependent, are deficient at birth.⁴³

The extent to which newborn or young livers cannot be expected for similar reasons to normally detoxify a wide variety of drugs used in the practice of anesthesia can only be speculated upon at the moment in the face of insufficient information on this important subject. It is of interest that at least one anesthetic agent, phenobarbital, can actually help to eliminate bilirubin at this age, apparently by stimulation of the quantitatively subnormal hepatic microsomal enzymes.¹⁵¹

A host of other factors, independent of age, can introduce intraspecies variations in the rate of anesthetic metabolism. These include the degree of pre-existing hepatic or renal disease, the route of administration, total cardiac output or regional blood flow, body temperature, and acid-base balance.

Intravenous Anesthetics

There are few parenterally administered anesthetics in common use today that are not transformed in the liver in the course of their elimination. Consequently, these agents must be used with exceptional caution in cases of liver transplantation.

Barbiturates. In older pharmacology texts correlations were often made between the duration of action of the various barbiturates and the organ systems responsible for their degradation or excretion. Thus, detoxification of the short acting barbiturates was declared hepatic dependent, whereas drugs with a long effect were said to be predominantly eliminated by the kidney. In actuality the duration of action depends primarily on the distribution and binding of the drug, rather than upon its degradation.^{93, 113} In the long run most barbiturates are either destroyed in the liver or conjugated there en route to their ultimate metabolic pathways. Presumably, hepatic microsomal enzymes play the crucial role; this has been particularly well documented with thiopental, hexobarbital and pentobarbital.⁵²

The practical importance of this seemingly academic distinction is that poor liver function will predictably enhance the pharmacologic effect of any of the barbiturate drugs. This is a well accepted clinical generalization. It has also been proved in animals subjected to liver injury by means of chloroform inhalation,¹⁰⁹ hepatic dearterialization,⁹⁵ and other techniques.²⁹

Narcotics. The same precautions pertain with the use of many narcotics. For example, one of the detoxification pathways of the mild and relatively safe analgesic codeine, is by demethylation in the liver and conversion into morphine.³ The N-demethylating enzyme is microsomal and requires nicotinamide adenine dinucleotide phosphate (NADPH) and oxygen; the O-demethylating enzyme has a different cellular origin. The end product conjugates with glucuronic acid and is excreted in the bile.⁵ Some reabsorption occurs, but the majority of the glucuronide is eliminated in the urine.²

Liver metabolism also plays an important role in the detoxification of papaverine, heroin, meperidine hydrochloride (Demerol), methadone, methyldihydromorphinone (Metopon), fentanyl (Sublimaze), and the narcotic antagonist nalorphine (Nalline).

Other drugs. Two very short acting and apparently nontoxic agents will be mentioned here because of their potentially great value in permitting short procedures to be carried out in transplant recipients. One, CI-581*, has already been employed by us on a number of occasions. An intramuscular or intravenous injection renders the patient almost immediately lightly unconscious. The swallowing, respiratory, gag, and cough reflexes are completely retained. The effect lasts for about 10 minutes, long enough to carry out short procedures or complicated dressings. The metabolic pathways of this agent are not known, although its oxidation and demethylation probably occur in the liver.³⁰ Nevertheless, we have not observed any unusual prolongation of effect in our transplant recipients.

We have not had experience with the other drug, propanidid (Epontol), which has been receiving widespread trials in Europe.³³ The unique advantage of its intravenous use is that the conversion from an anesthetized to a fully awake state can be made within a few minutes after discontinuation of administration. The means by which propanidid is detoxified have not yet been fully clarified, although one mechanism is apparently the splitting of its side chains by hepatic esterase.³⁸

Inhalation Anesthetics

With the exception of trichloroethylene (Trilene), all gaseous and volatile agents used as anesthetics were considered until recently to be biologically inert. In 1964, Van Dyke, Chenoweth and Van Poznak¹⁴² indicated that other volatile anesthetics also undergo biotransformation. The other inhalation agents which have either been proved or suspected to undergo chemical reactions include the hydrocarbons, cyclopropane and ethylene, the ethers, and the halogenated hydrocarbons.³¹ Both the liver and the kidney appear to participate in the process.

For example, the inhalation of radioactive diethyl ether has been noted to lead to urinary excretion of the specific carbon label. With similar techniques bromine and chlorine isotopes can be found in the urine after the administra-

*2-Orthochlorophenyl, 2 methylamino cyclohexanone HCl or Ketalar.

tion of chloroform, trichloroethylene, halothane, and trichloroethanol.¹⁴¹ It has been assumed that all these anesthetics undergo an enzymatic cleavage in hepatocytes before the degradation products are cleared by the kidney, emphasizing again the primordial role that the liver plays with almost all anesthetic agents.

Muscle Relaxants

It is highly desirable to have adequate muscle relaxation during liver transplantation, especially to facilitate the demanding dissections and the vascular anastomoses that must be carried out at certain phases of the procedure. The problem is how to achieve this without paying the penalty of a later prolonged apnea. In planning the administration of paralyzing drugs, it is necessary to have some idea of the mechanism of their removal. With all the agents under discussion, there are two main routes of elimination, renal and hepatic. These organ systems contribute in variable proportions to the disposition of the different compounds.

Depolarizing agents. Succinylcholine, which acts by depolarizing striated muscle fibers, is hydrolyzed in the serum by pseudocholinesterase (serum cholinesterase), an enzyme which is synthesized and released by the liver. The hydrolysis takes place in two phases. First, succinylcholine is rapidly broken into succinylmonocholine and choline. Then, in a slower reaction, succinylmonocholine is further degraded into choline and succinic acid.⁵¹

Defective inactivation of succinylcholine would be anticipated if serum cholinesterase levels were lowered for any reason. The anticipated association of an increased duration of action with depression of the enzyme has been noted with severe chronic liver disease,⁵⁰ after the administration of AB-132 for cancer chemotherapy,¹⁴⁶ during organic phosphate intoxication,¹⁵² in neonates,⁸⁵ in women at term pregnancy,¹²⁸ and after the indiscriminate use of a variety of common drugs.^{103, 107, 114}

Decamethonium, a muscle relaxant which is generally used in Great Britain and some of the Commonwealth Nations, is thought to be metabolically inert. It is mostly excreted by the kidney unchanged.¹⁰⁴

Nondepolarizing Agents. Whereas succinylcholine is exclusively dependent upon a liver-produced enzyme for detoxification, the nondepolarizing agents have alternative pathways of elimination. Kalow⁷³ has described three phases in the metabolism of tubocurarine. First, there is passage into the interstitial space. Then, part of the drug is excreted unchanged in the urine, while the other major fraction passes into muscle cells. Finally, the intracellular portion is destroyed by enzyme action.

Cohen and his associates³⁴ showed in normal dogs that almost 90 per cent of a dose of tubocurarine was recoverable in the urine; the rest appeared in the bile. However, the biliary component was increased from less than 10 to almost 40 per cent by the simple expedient of ligating the renal vessels. Moreover, Churchill-Davidson et al³² provided evidence that the same kind of physiologic adjustment probably applies in humans, by demonstrating a prompt recovery from paralyzing doses of tubocurarine in anephric patients. A reasonable conclusion is that curare is ordinarily not significantly processed through the liver,

but that this organ can promptly assume an increased burden of excretion in the face of renal failure.

Gallamine (Flaxedil) is one of the most useful muscle relaxants in a case of liver transplantation which is not complicated by pre-existing kidney damage. There is much evidence that its elimination is almost entirely dependent on adequate renal function.⁹⁷

Local Anesthetics

A discussion on the metabolic fate of these substances must begin by stating that the route of administration and the rate of absorption are more important in determining toxicity than the mechanism of degradation. In a text on hepatic transplantation it is equally important to note at the outset that the detoxification of all the locally used anesthetics is liver dependent in one way or another. In one class of agents, which includes procaine, tetracaine, chlorprocaine and piperocaine, the breakdown is by the same hydrolytic process described earlier for succinylcholine.⁵¹ It follows that patients with deficient pseudocholinesterase as a result of liver failure should be given these substances with caution.

The same applies to a second category of drugs that includes lidocaine, mepivacaine, prilocaine, and dibucaine. These drugs are all thought to be degraded primarily in the liver by hepatic microsomes. The agent among those listed which has been most extensively studied is lidocaine.¹³⁷ It is of interest that a case of central nervous system irritation has been reported in a cirrhotic patient receiving conventional doses of intravenous lidocaine for treatment of cardiac arrhythmias.¹²⁶

Miscellaneous Agents

Chloral hydrate,⁹⁴ tribromoethanol (Avertin),¹⁹ and glutethimide (Doriden)¹⁴⁹ depend almost entirely upon the liver for detoxification; the commonly used tranquilizers do so less completely.²⁸

It would serve no purpose to make a longer list to demonstrate that very few drugs used in anesthesia do not have at least a partial hepatic pathway of metabolism. The way in which this fact influenced the anesthetic techniques for recipients of liver homografts will be discussed later.

THE EFFECT OF ANESTHESIA ON THE LIVER

The planning of anesthesia for hepatic transplantation must take into account the fact that two livers are involved in the course of a single operation. The first is the recipient organ which is almost always the site of chronic disease. The second is a homograft which is invariably injured to some extent as the result of acute ischemia.

Many of the things that can be done to avoid the aggravation of the resulting potentially dangerous situation have little to do with the wise choice or proper administration of specific anesthetic agents. An example would be

the scrupulous maintenance of an adequate blood volume. When hemorrhage occurs, many investigators have reported very sharp declines in hepatic arterial and especially in portal venous blood flow.^{54, 123} The changes, which are usually ascribed to splanchnic vasoconstriction, are rapidly reversed by giving blood transfusions.

Even a low blood pressure in the presence of normal blood volume may have a deleterious effect. Histopathologic evidence of liver injury has been reported after attempts to produce "controlled hypotension" with total spinal anesthesia⁸⁹ or the administration of ganglionic blocking agents.^{121, 125} The value of hypothermia in preventing the adverse effects either of hypovolemia or hypotension or both¹² will not be discussed here since it was taken up in connection with organ preservation in Chapter Five.

Both hypoxia^{56, 117, 129} and hypercarbia^{63, 117, 129} have been demonstrated in well-controlled animal experiments to be capable of causing severe liver injury. Fisher et al⁴⁷ showed in dogs that significant decreases occurred in hepatic arterial flow when arterial saturations of less than 50 per cent were induced by the breathing of hypoxic gas mixtures. Under similar conditions Leevy et al⁸³ were able to document the development of a hepatic oxygen debt.

The safety with which anesthetic drugs can be administered, if other conditions are optimal, has been particularly well documented by the investigations by Burdette, Stevens and Gröschel.²⁷ They studied histologic sections, hippuric acid clearance, ammonia-transfer enzyme activity, and oxygen consumption in liver slices taken from normal patients and from patients with pre-existing liver disease. There were no changes with the use of various premedications or anesthetic agents.

Nevertheless, numerous drugs are capable of producing hepatic injury. Among these it is important to distinguish between the agents that cause a direct injury to the liver and those which are apparently responsible for delayed sensitization reactions.¹⁵³

The anesthetics which are "directly" hepatotoxic inflict an injury upon the cellular membranes of the hepatocytes, some of which undergo necrosis. The damage is most heavily concentrated in the centrilobular or periportal zones and is not accompanied by prominent leukocyte infiltration. Fat accumulation is common in the cells which do not die, probably as a consequence of the block of triglyceride transport. The clinical features are quite diagnostic. The injury occurs in essentially all the individuals of the species tested, develops within 24 to 48 hours, is dose related, and is frequently associated with renal failure.⁷⁶ Classic examples of hepatotoxic agents are carbon tetrachloride and phosphorus.

In contrast, the drugs which cause liver injury by a process of sensitization have a highly unpredictable effect from individual to individual and do not necessarily cause injury in proportion to the quantities given. The areas of liver necrosis, which are usually less extensive, have the hallmarks of an inflammatory response. An even more characteristic lesion is intrahepatic cholestasis. A toxic reaction essentially never occurs with the first dose. Finally, many of the clinical manifestations such as arthralgias, skin rashes, and eosinophilia are highly suggestive of an immunologic reaction.⁷⁷

Intravenous Anesthetics

In man there is little reason to fear that conventional doses of barbiturates have a deleterious effect. Hyperglycemia has been reported during thiopental narcosis, presumably because of accelerated hepatic glycogenolysis.⁶⁵

In animals overdoses of various barbiturates can produce transient depression of liver function, including the ability to excrete bromsulphalein^{17, 145} or to clear endogenous bilirubin. With thiopental the sensitivity to a given dose is increased after starvation.⁸¹ Another kind of alteration was shown by Bloxam,¹⁴ who found that pentobarbital and thiopental inhibited the synthesis of amino acids by perfused rat livers.

Inhalation Anesthetics

The first detailed case report of a fatal outcome after the administration of an anesthetic was written by Guthrie in 1894.⁵⁹ A four year old boy died 30 hours after a chloroform anesthetic that had lasted one hour. At autopsy there were findings of acute liver necrosis accompanied by intense fatty degeneration. Since then innumerable reports have appeared indicting first one agent and then another as the cause for this kind of complication. The supporting evidence has often been scanty. In the following remarks attention will be directed only to the most widely used inhalation anesthetics or to those which have been most suspected of causing hepatic injury.

Allegedly Dangerous Agents. It is not surprising that chloroform is hepatotoxic in view of its structural similarity to carbon tetrachloride. There is no point in reviewing the large and generally unfavorable literature concerning liver complications after the administration of this agent, other than to note in passing that a few groups¹⁰⁸ still believe it to be safe when given properly. In spite of the latter claims, chloroform has been noted to impair oxygen utilization by isolated perfused livers.¹³² In tissue culture it can readily damage hepatocytes, especially if the nutrient medium is deprived of amino acids.³⁷

The other halogenated hydrocarbons, halothane (Fluothane) and methoxyflurane (Penthane), produce similar but far less severe changes in laboratory animals.³⁶ In general, the liver injury is aggravated by other adverse circumstances such as dietary deficiencies, infection, pregnancy, arterial hypotension, hypoxia, and hypercarbia. The damage can be minimized or avoided by the coincident use of hypothermia, high oxygen tensions, amino acid infusion, reserpine, and sympathetic blockade.⁸⁸

Extrapolation to clinical anesthesia of the aforementioned animal experiments with halothane and methoxyflurane has resulted in large scale controversy. With both agents there have been reports of necrosis of the human liver,^{21, 25, 42, 67, 78, 144} more frequently with halothane probably because it has been used far more extensively. As a result of these publications neither anesthetic was administered to the liver transplant recipients.

Avoidance of these inhalation anesthetics will probably be continued for

future cases. Nevertheless, this precaution may be largely without scientific basis. Cases of massive liver necrosis attributable to halothane anesthesia are probably rare; an incidence of only one in 500,000 halothane anesthetics has been reported.¹⁴⁸ The same general conclusion was reached recently by a study group of the National Academy of Sciences.¹³⁶ The evidence has been that most well documented instances of liver injury following the use of halothane may be through a mechanism of repeated exposure to this agent and immunologic sensitization.^{77, 140, 148}

Reputedly Safe Agents. Trifluorethylvinyl ether (fluoroxene, Fluoromar) has been in clinical use since 1956.³⁹ Liver function tests have apparently been unchanged during and after the use of this anesthetic.^{39, 120} No cases of sensitization have been reported. The agent has other advantages which will be discussed later.

Of the other commonly used ethers, only ethyl vinyl ether (Vinamar) has been thought to be almost completely free of liver toxicity.^{40, 41} Diethyl ether and divinyl ether (Vinethene) have both been reported to cause hepatic malfunction or structural injury in experimental animals^{18, 56, 82, 102, 134} as well as in patients;^{35, 46, 87, 102, 112} divinyl ether has been particularly notorious in this respect.

Because the hydrocarbon cyclopropane has long been thought to be almost completely free of hepatotoxicity, it is one of the agents of choice for patients with liver disease. In the few reported cases of liver necrosis with the use of this agent,¹³⁰ other etiologic factors have almost always been identifiable. These have included massive hemorrhage, metabolic disequilibrium, and other medications. Cyclopropane has not been given to our liver recipients, since its administration would preclude the use of electrocautery.

Nitrous oxide is also considered innocuous to the liver, providing adequate oxygenation is maintained during its use.⁹⁸

METABOLIC ALTERATIONS

During orthotopic liver transplantation all patients must pass through a totally anhepatic state which is well tolerated for short intervals. Then, the liverless state is ended more or less rapidly according to the quality of function provided by the newly arrived homograft.

The profound metabolic changes that may occur during this intraoperative period could instinctively be anticipated with no other information than that concerned with the hemodynamics of the normal human liver. The adult organ is perfused with almost one-third of the total cardiac output, the flows per minute through the hepatic artery and portal vein being 300 ml and 1200 ml of blood, respectively. The oxygen extracted from these sources is approximately 60 ml/minute, or about 20 per cent of that used by the entire body. Both the flow and the oxygen consumption are altered by a number of influences. They are increased by hyperthyroidism,⁹⁶ hyperthermia, and shivering.⁸³ They are decreased by hypothermia.⁵³

Acid-Base and Electrolytes

The effects of total hepatectomy on serum electrolytes was studied in dogs by Fisher et al.⁴⁸ Sodium, potassium, and chloride concentrations were altered very little by this procedure. However, there were progressive drops in the carbon dioxide content of the blood. The authors suggested that the latter change was secondary to hyperventilation, which in turn was a physiologic compensation for the accumulation of pyruvic, acetic, hydroxybutyric, acetoacetic, uric, and other organic acids.

This sequence of events can be produced short of hepatectomy by interruption of the vascular supply of the canine liver. Resulting pH falls have been noted within the hepatic parenchyma⁷ or in the peripheral blood⁷⁰ after occlusion of either the hepatic arterial or portal venous supply; the most extreme changes were produced with complete devascularization.

There is the possibility that partial hepatic devascularization could result in a vicious cycle in which the remaining circulation is impaired even more. Eisman and his associates⁴⁴ and Kestens, Farrelly and McDermott⁷⁴ have demonstrated in experiments with isolated perfused livers that acidosis increases hepatic vascular resistance.

In view of the foregoing information, the finding of acidosis in the liver homograft recipients was not a surprise. As will be described later, the situation was corrected intraoperatively at frequent intervals; here it will only be mentioned that such therapy could entail a theoretical risk. The rapid administration of alkaline solutions to fetal rhesus monkeys¹ and infants⁵⁵ has resulted in fatal liver injury.

Modifications in hepatic blood flow may affect other serum electrolyte concentrations. Prompt hyperkalemia has been the most consistent change reported in dogs following portal vein occlusion.¹⁰ The alteration was apparently not exclusively due to the resultant intestinal injury since the same thing has been noted following Eck fistula.⁶ It will be seen later that similar potassium shifts have been characteristic in human recipients of orthotopic livers.

Carbohydrate Metabolism

It has been known for almost 50 years that glucose infusions prolong life in animals subjected to total hepatectomy.^{15, 91, 92, 145} The obvious benefit of such therapy is to prevent the rapid and fatal hypoglycemia which otherwise develops. There are probably more subtle implications. For example, glucose infusions have been shown to decrease the severity of the acidosis in anhepatic dogs⁹⁰ and to reduce the rate of accumulation of plasma amino acids.⁴⁹

The provision of glucose treatment and the maintenance of normal or hyperglycemic blood levels does not long prevent the development of cerebral manifestations in liverless dogs. Vang, Weiss and Drapanas¹⁴³ have published data indicating that one possible explanation may be the progressive inability of the brain cells to metabolize glucose. It is not known how many other tissues

eventually lose this or other of their intrinsic metabolic capabilities. In hepatectomized pigs Norman et al¹⁰⁰ reported decreases in oxygen and ATP concentrations of 32 and 63 per cent, respectively, suggesting a marked general depression of oxidative phosphorylation.

When discussing the management of hypoglycemia, the mistake should not be made of equating this problem solely with the anhepatic state. With hypoxemia Hannon et al⁶¹ have produced low blood sugar values in normal animals. Their explanation was that the ability of the liver mitochondria to produce high-energy phosphate bonds was depressed. In human recipients of hepatic homografts, life-threatening hypoglycemia has been observed in the early post-transplantation period, in spite of the fact that other measures of hepatic function seemed adequate.

Other Considerations

Absent or poor liver function could profoundly affect the physiology of a wide spectrum of hormones since many of these are known to be degraded through hepatic pathways. Two specific examples will be given because of their practical implications. It must be assumed that a variety of sympathomimetic amines undergo metabolic changes in the liver since large amounts of monoamine oxidase (MAO) and catechol O-methyltransferase (COMT) are present in this organ.⁴ Failure of the process of efficient amine inactivation could promote depletion of the homograft's glycogen stores by glycogenolysis.

Moreover, the inability under these circumstances to detoxify administered vasoactive substances could predispose to hypertension in the postoperative period. It will be documented in Chapter Nine that persistently elevated blood pressures have been observed in recipients of orthotopic liver homografts and that these patients have had an elevated urinary concentration of vasopressor substances.

VENOUS STAGNATION

During orthotopic transplantation it is necessary to cross clamp the portal vein and inferior vena cava (see Chapter Eight). In normal dogs it is mandatory that the distal venous pools, particularly that of the portal system, be effectively decompressed during the anhepatic phase. Failure to do this leads to death on the operating table or shortly afterward from a syndrome of irreversible shock.^{20, 45, 69, 133} The essential explanation for the lethal events is the sequestration of blood volume in the splanchnic bed,^{20, 69, 111} coupled with an injury to the capillary bed inflicted by the acute venous hypertension.¹¹⁰

In other animals, including the pigeon¹¹ and turkey,¹⁶ portal vein occlusion is far better tolerated, apparently because naturally occurring collaterals are more available to return the blood to the right heart. The innocuousness of portal vein ligation in birds is explained by the existence of large portal-sys-

temic communications through the venous system of Jacobson.¹⁶ Dogs can tolerate slow portal occlusion if the vessel compression is gradual enough to permit collateral pathways to open up.^{22, 122}

As will be mentioned later, it was eventually found that patients subjected to orthotopic transplantation did not usually become seriously hypotensive during crossclamping of both the portal vein and inferior vena cava. It was thought that the extensive venous collaterals caused by the hepatic disease had made this detail of the operation safer than would have normally been the case. This hypothesis has been confirmed in the laboratory by Picache et al.¹⁰⁶ They submitted dogs to common duct ligation. About two months later, after venous collaterals had formed, orthotopic liver transplantation was performed without provision for decompressing bypasses. The venous occlusion was well tolerated without the dramatic adverse effects invariably seen in normal animals.

In our own animal experience the danger from hypotension has been essentially terminated with revascularization of an adequately preserved homograft. However, Joseph et al.^{71, 72} observed reduced blood pressures after this time, especially just following the restoration of hepatic blood flow. They suggested that the transplanted organ might contain a vasodepressor material (VDM) similar to that described by Schorr et al.¹²³ Joseph's recommendation was that the initial circulation of the homograft be flushed through a venotomy and discarded. We have not observed this precaution.

CLINICAL ANESTHESIA

Although it has already been amply emphasized (Chapters One and Four) that all candidates for liver transplantation are poor risks for anesthesia, some of the reasons will be briefly recapitulated. First, there are usually pre-existing abnormalities of respiratory function. The changes which have been most thoroughly documented are arterial desaturation and hyperventilation, with the secondary development of a compensated respiratory alkalosis and a reduced buffer base. The most plausible explanations for one or more of these findings have been based upon the presence of a variety of shunts which apparently divert considerable volumes of blood from the pulmonary artery^{60, 119} or from portal-systemic collaterals⁹ directly into the pulmonary veins. Additional arteriovenous anastomoses have been described within⁸ or outside the liver.^{68, 150} With the multiplicity of widely distributed shunts, it is not surprising that the cardiac output of cirrhotic patients is usually increased.⁶⁸

Other authors^{62, 75} have suggested that biochemical factors may play a role in the respiratory and cardiodynamic changes. Keys and Snell⁷⁵ observed a decreased affinity of hemoglobin for oxygen in cirrhotic patients, although this finding could not be confirmed by Rodman et al.¹¹⁶ Elevated ammonia levels have been said to stimulate the medullary respiratory centers.¹¹⁵ Kontos et al.⁸⁰ have postulated that vasoactive substances may be present as a result of chronic liver failure and that these may contribute to any of the foregoing alterations.

The list of other physiologic derangements consequent to liver insufficiency is a long one and will not be presented here. However, it can be assumed that most patients with chronic hepatic disease will come to the operating room with an increased plasma volume,^{86, 99, 105} hyperaldosteronism,^{101, 138} and a reduction in the red cell mass.⁶⁴ The anemia is probably partly due to an increased rate of erythrocyte destruction,⁶⁶ although abnormal maturation of red cells may be a contributory factor.¹³

The presence of ascites can pose a serious problem, since the amount of intra-abdominal fluid is often very large. Of necessity, this is abruptly removed at the time of transplantation. The effect of massive but gradual paracentesis in cirrhotic patients has been studied by Knauer and Lowe.⁷⁹ When quantities less than one liter were aspirated, the cardiac output and stroke volume increased. When this amount was exceeded, both values were markedly decreased, presumably because of fluid shifts and a resultant fall in the circulating blood volume.

The Importance of Monitoring

During the actual operation additional physiologic and metabolic derangements are predictably superimposed upon those already present. Control and adequate study of the resulting situation is heavily dependent upon accurate moment-to-moment monitoring of a number of measurements. Provision for this must be assured before the final draping of the patient is begun. The devices used to follow the intraoperative course in all our recent patients have included the following: (1) bipolar electrocardiogram, (2) esophageal stethoscope, (3) rectal thermistor, (4) bladder catheter, (5) venous catheter in the superior vena cava, (6) standard blood pressure cuff, and (7) radial arterial catheter.

The general value of all these methods for increasing the safety of intraoperative management during a variety of complicated procedures has been well established.¹¹⁸ This will not be detailed here, except to draw attention to the special importance of having access to frequent arterial samples. In the course of a hepatic transplantation, it is essential to be able to obtain serial measurements of the blood gases and pH, blood sugar, and electrolytes. Moreover, several determinations of clotting factors are highly desirable (see Chapter Ten). Finally, samples can easily be removed for more esoteric research studies.

At the same time as the monitoring equipment is being attached, a large caliber intravenous catheter should be inserted in the arm or neck. No infusions are used in the lower extremities since inferior vena caval cross clamping will eventually be necessary. A final precaution as equipment is being made ready is to place the patient on a temperature control blanket. At first it was suspected that hyperthermia would be a significant problem after revascularization of the large foreign organ. In actuality the converse proved to be true; hypothermia was very regularly seen. The blanket permitted control of this complication. In addition, heating coils were used in all the later cases through which to run the blood and other intravenous fluids.

Anesthetic Techniques

The haste with which anesthesia must be started for hepatic transplantation was considered in Chapter Four; the degree of urgency was dictated by the events of donor death. At times it was necessary to rush the recipient to the operating room before medical evaluation could be completed or even shortly after the ingestion of a meal. A number of precautionary measures were taken to reduce the consequent hazards in the course of induction.

Standard doses of atropine sulfate were given intravenously after the patient's arrival in the operating room; this was the only preoperative medication. A nasogastric catheter was then immediately passed and irrigated. If food was detected within the stomach, it was removed as completely as possible. However, it was not assumed that this objective had been fully met. Instead, an induction technique was used which promptly ensured an adequate airway and which would prevent aspiration. The safest approach was to carry out tracheal

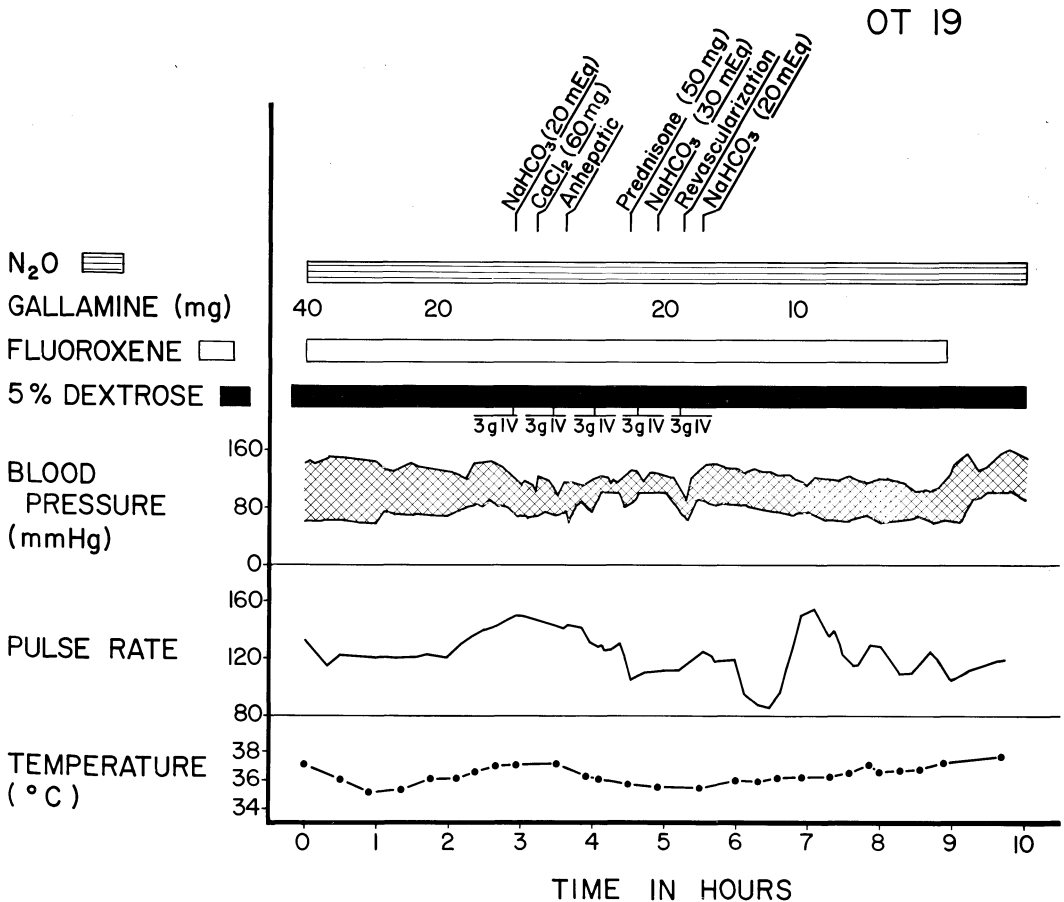


Figure 33. The anesthetic record of a recipient of an orthotopic liver homograft (OT 19). The indication for operation was intrahepatic biliary atresia. An endotracheal tube was placed before the induction of anesthesia. Note the transient but minor fall in blood pressure during the venous occlusions of the anhepatic phase. The patient recovered very promptly from anesthesia. He is still alive and in good condition 11 months post-transplantation.

intubation in the awake state and begin anesthesia only after a complete respiratory seal had been obtained; this was very easily done in infants (Fig. 33). Alternatively, “crash intubation” was elected in some adults (Fig. 34). With this approach the patient was oxygenated for five minutes; then intravenous sodium thiopental and succinylcholine were given rapidly and the endotracheal tube inserted at once, while cricoid pressure was applied.¹²⁷

During the ensuing few minutes the definitive plane of anesthesia was achieved and permission was given for the surgical scrub to begin. In all patients the principal anesthetic was fluoroxene (Fluoromar), which was added to a nitrous oxide-oxygen mixture (66:33 per cent ratio). The rationale for the choice of these agents in terms of their lack of hepatotoxicity was made clear in the first part of this chapter. Another most important consideration was that the combination employed has been shown to be nonexplosive, providing the fluoroxene concentration is kept below 4.4 per cent.⁵⁷ This made possible the

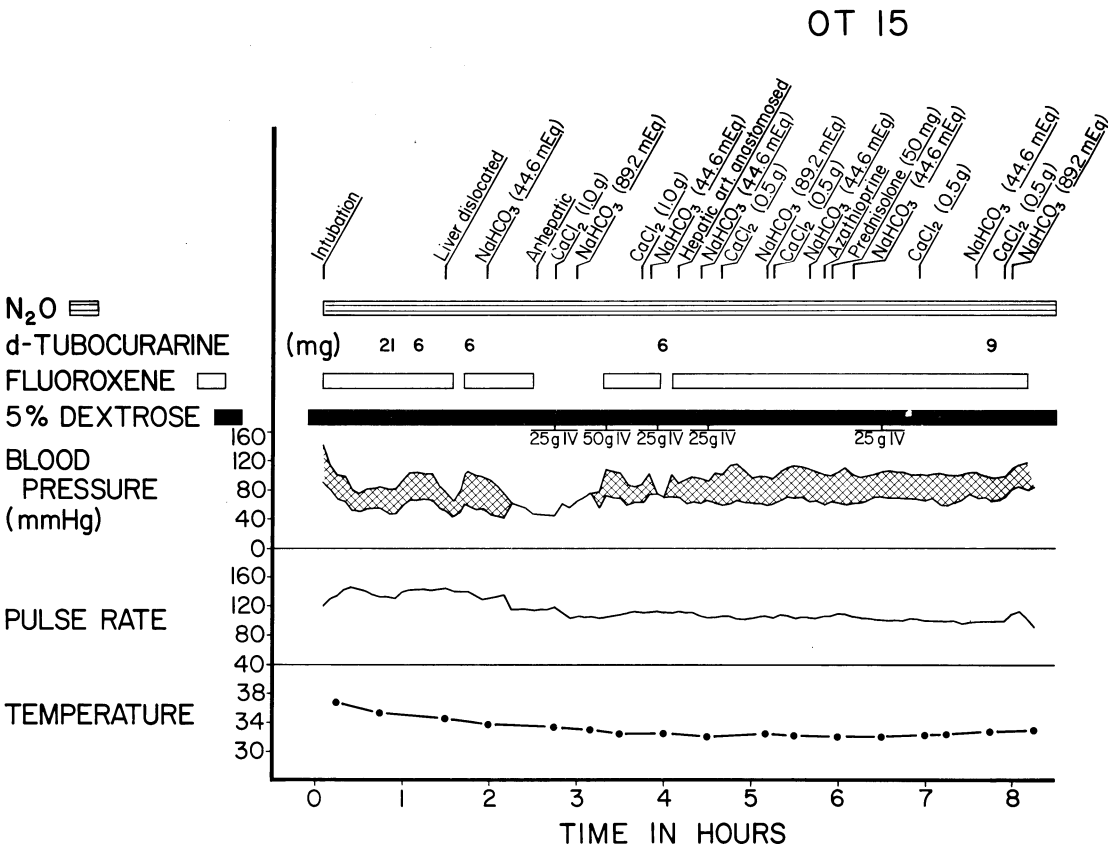


Figure 34. The intraoperative management of a 44 year old man (OT 15) who received liver replacement for the treatment of a very large hepatoma. Severe cirrhosis was also present. “Crash” endotracheal intubation was carried out as described in the text with the aid of a single dose of sodium thiopental (not indicated in the graph). Hemorrhage during removal of the diseased liver was massive and could not be controlled until the organ was removed. Note the progressive decline in blood pressure before this time and the inability to define a systolic diastolic gradient during the anhepatic phase. The only anesthetic continued during the liverless interval was nitrous oxide. The patient recovered promptly from the operation. He eventually developed metastases from which he died 339 days post-transplantation.

use of the electrocautery. A final advantage was the ease and rapidity with which anesthesia could be lightened or deepened according to the circumstances of the moment (Figs. 33 to 35). It was especially important to have this capability during the anhepatic phase or at times of sudden hemorrhage.

Throughout the operation ventilation was assisted or controlled. When indicated to provide adequate exposure and an immobile operating field, nondepolarizing muscle relaxants were administered (Figs. 33 to 35). This was most commonly necessary at the time of the anhepatic phase, during which interval the inhalation anesthetics were either sharply reduced or stopped altogether. The adjustment was made at this time not only for the objective of obtaining better relaxation as the vascular anastomosis was performed, but also to minimize the hypotension which was sometimes induced by the venous occlusions during that period.

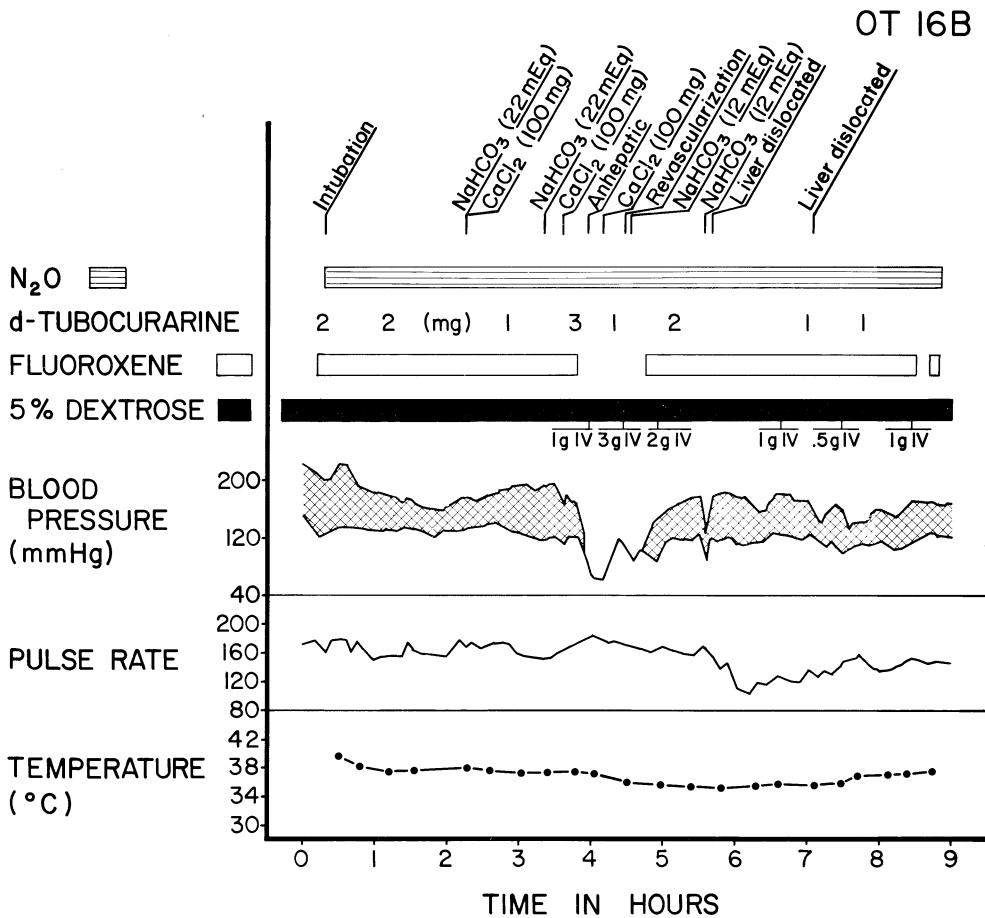


Figure 35. The anesthesia record during orthotopic retransplantation of patient OT 16, 68 days after provision of a first homograft. When the portal vein and inferior vena cava were cross clamped, there was a very severe hypotensive episode. Because of this, the flow through the vena cava was restored before completion of the portal and hepatic arterial anastomoses in contrast to the usual practice of finishing all the vascular connections before exposing the new organ to the host blood. After revascularization it was necessary to dislocate the liver temporarily in order to close a defect in the retrohepatic vena cava. Note the resulting abrupt but transient decline in blood pressure.

Intravenous Therapy

It is obvious that the actual anesthetic techniques used during liver transplantation were not unusually complex (Figs. 33 to 35). The other aspects of intraoperative care were by no means so simple. The main problems were to maintain an adequate blood volume, to repeatedly correct acid-base abnormalities, to prevent hypoglycemia, and to administer other drugs as needed.

At the outset of operation, children were given 5 per cent dextrose in 0.25 per cent sodium chloride solution. Adults were started on 5 per cent dextrose in lactated Ringer's solution (pH approximately 6.8). The exact volumes infused were varied according to the state of hydration of the patient, the amount of ascites removed upon abdominal entry, and the physiologic indices of urine flow and central venous and arterial pressures.

Well before the beginning of the anhepatic phase, the quantities of glucose were increased by adding 25 per cent dextrose in water to the infusion (Figs. 33 to 35); the amounts were calculated to supply 0.5 gm/kg/hour. Blood glucose measurements were thereafter obtained every 15 minutes. The glucose input was modified according to the results of these measures, in an attempt to maintain a blood concentration of 150 to 350 mg per cent. On one occasion, the level rose to 1500 mg per cent within a few minutes; when the infusion rate was lowered, it fell just as quickly.

After revascularization of the homograft, the amounts of dextrose given were slowly reduced to 0.2 gm/kg/hour. At no time was the glucose therapy deliberately stopped either during operation or for many hours or even days afterward. In two patients the intravenous noncolloid solutions were accidentally discontinued briefly in the process of transfer to the intensive therapy unit. Very severe hypoglycemia resulted, with convulsions, unconsciousness, and transient respiratory arrest. Emergency therapy with 50 per cent glucose in water was required.

The magnitude of blood replacement that was necessary has been documented in Chapter Six. The estimation of the adequacy of blood volume was made difficult for special reasons. For one thing, an unknown quantity of blood was removed with the extirpated host liver and an equally immeasurable quantity was required to fill the new organ at the time of its revascularization. Moreover, the extent of fluid shifts consequent to the removal of ascitic accumulations could not be identified. Finally, the venous crossclamping during the anhepatic phase undoubtedly led to sequestration, which could not be quantitated.

Some of the hazards of massive transfusion were minimized or circumvented by the use of fresh blood. In this way the elevated concentrations of potassium, phosphates, ammonia, and lactic acid found in stored blood were avoided. However, special precautions were necessary because of the large volumes of citrate preservative which had to be given. Each unit of ACD blood contains 0.6 gm of citric acid, equivalent to 8.6 mM of hydrogen ion.²³ The consequent acidosis is ordinarily quickly buffered by plasma bicarbonate, hemoglobin, and proteins. Meanwhile, the organic acids are promptly metabolized

by the liver. The latter counterregulatory mechanism is either impaired or totally lost for long periods during hepatic transplantation. Consequently, treatment with bicarbonate was necessary not only to compensate for the ACD but in order to neutralize endogenous acid products as well.

The exact quantities of alkalinizing agents that were required varied from case to case (Figs. 33 to 35) and were adjusted according to the results of repeated blood gas, pH, and bicarbonate determinations. With each set of data the base excess¹²⁴ was calculated and the necessary changes made. The general formula that was followed until this information became available was to administer 2.5 mEq/kg/hour sodium bicarbonate, beginning as soon as significant manipulation of the host liver was started and continuing for at least an hour after revascularization of the homograft. To this basic regimen 10 mEq of sodium bicarbonate were added for each unit of ACD blood.

Prevention of acidosis reduces the hazard of citrate intoxication.^{24, 26} However, ACD blood can produce direct myocardial depression even with perfect acid-base control. This effect is more or less completely counteracted by the provision of exogenous calcium.^{131, 147} Consequently the adult liver recipients were intermittently given 300 to 400 mg of calcium in the chloride or gluconate form. Smaller doses were administered to children.

The calcium administration was especially helpful in the anhepatic phase (Figs. 33 to 35). On several occasions during the liverless intervals of venous crossclamping there was a progressive decline in the blood pressure. This was halted or reversed by the administration of calcium. The same kind of observation has been made in pigs by Terblanche et al.¹³⁹

The other intravenous therapy needed during the operation will not be considered here. In all cases azathioprine and prednisolone are begun during or shortly after homograft revascularization in the dose schedules described elsewhere in the text. Antibiotics are invariably given by intermittent pulses (see Chapter Sixteen). In the event of dire need platelet concentrates, EACA, and fibrinogen may be required (Chapter Ten).

Postoperative Care

The care in the first few hours in the intensive therapy unit was not particularly different from that during the actual operation. All the monitoring procedures mentioned previously were continued. At intervals which were progressively closer together, ventilation support was temporarily discontinued. On these occasions blood gas studies were obtained, the volume of respiratory exchange assessed, and the advisability of withholding further respiratory support considered. In most instances the ventilatory assistance was continued for several hours. Even after adequate air exchange was evident, the endotracheal tube was usually not removed until the patient was obviously objecting to its presence. Both before and after extubation, tracheobronchial suctioning was assiduously carried out. An approximately equal mixture of oxygen and air was

passed through a humidifier and delivered to the area of the face. The infusion of noncolloidal and colloidal fluids was continued at a generally reduced rate; in a few cases, human albumin was given for intravascular volume expansion. Particular attention was paid to ensuring a continuous glucose source. Electrolyte determinations every few hours were used to guide other aspects of fluid therapy.

Most of the pediatric patients left the operating room slightly hypothermic, usually around 35°C (Figs. 33 to 35). During the time they were still on the mechanical ventilator, rewarming to 37°C was accomplished. In almost every case, a rebound increase in the temperature occurred, which ordinarily stopped rising at 38 or 39°C.

Complications

The principal difficulties during the transplantation were for the most part those of the procedure rather than the anesthesia. There were instances when adequate circulating blood volume could not be maintained in the face of hemorrhage or cross clamping of the great veins. Moreover, the blood sugar level and the acid-base balance could not always be kept at the optimum physiologic state. Data on these problems will be presented in the next section.

The operations were often extremely long. The protracted presence of endotracheal tubes probably accounted for the laryngeal and subglottic lesions in two patients (OT 11 and 14) as described in Chapter Nine. The tracheal granuloma in patient OT 14 might have been avoided if the endotracheal cuff had been more frequently deflated.

In some of the earlier patients the mistake was made of attempting to extubate too soon in the postoperative period (AT 1 and 2; OT 2 and 10). Emergency replacement of the airway was necessary. An explanation for the necessity of prolonged ventilatory support in some of the recipients was never completely determined. However, the duration of surgery, the very large incisions, the pre-existing debilitation and the common presence of mild hypothermia, as well as the variable malfunction of the newly placed homograft, could all have played a role. In addition, it is conceivable for reasons mentioned earlier in this chapter that some of the intravenous medications, including the muscle relaxants, could have had a more enduring action than normal.

CLINICAL OBSERVATIONS

This chapter has dealt so far with the principles upon which the anesthetic management during liver transplantation was based and with the actual techniques applied for the human recipients. Now some additional observations will be presented which relate to several more or less unique problems encountered in this kind of procedure.

The Effect of Venous Occlusions on Blood Pressure

The difficulties of evaluating the need for blood replacement as well as the practical problem in some cases of keeping up with the hemorrhage were described earlier. One of the useful guidelines for transfusion therapy was provided by serial hematocrit changes (Figs. 36 and 37) when these measurements were considered in relation to the arterial and central venous pressures and to the urine output. In spite of all efforts the completeness of blood replacement was undoubtedly highly variable when the interval of venous cross clamping was reached. In some cases, the bleeding could not be controlled until the diseased liver was finally removed; in others, the recipients were in a very stable condition at the beginning of the anhepatic phase.

With occlusion of the suprarenal inferior vena cava and the portal vein, a further decline in blood pressure invariably developed, although this was usually not severe. The magnitude of the change in each instance is shown in Table 7. The most serious depression was during the second transplantation of Patient OT 16 (Fig. 35). On the first occasion extensive venous collaterals had been found; there were no major cardiodynamic alterations during the anhepatic phase. The second time, 68 days later, the collaterals had partially involuted. When the vascular structures entering and leaving the rejecting homograft were occluded, the blood pressure fell almost 100 mm Hg. In this case, as well

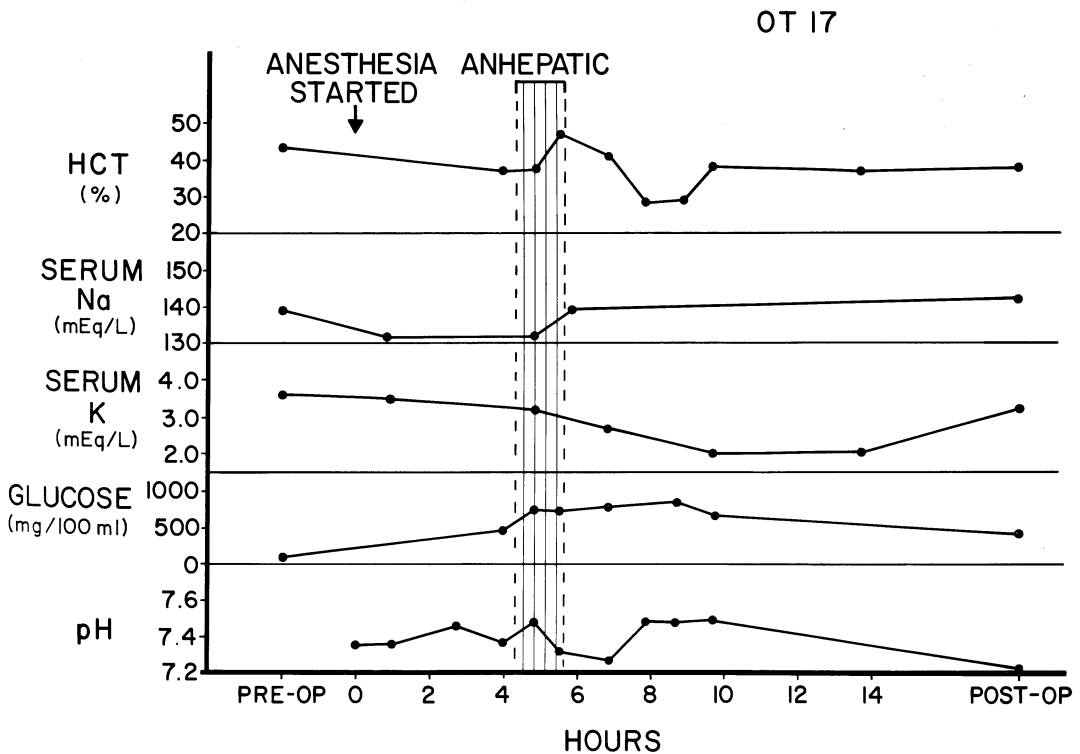


Figure 36. Metabolic changes in the recipient of an orthotopic liver homograft (OT 17). An over-correction was made in the planning of the glucose infusion. Note the very striking hypokalemia after revascularization of the homograft.

OT 20

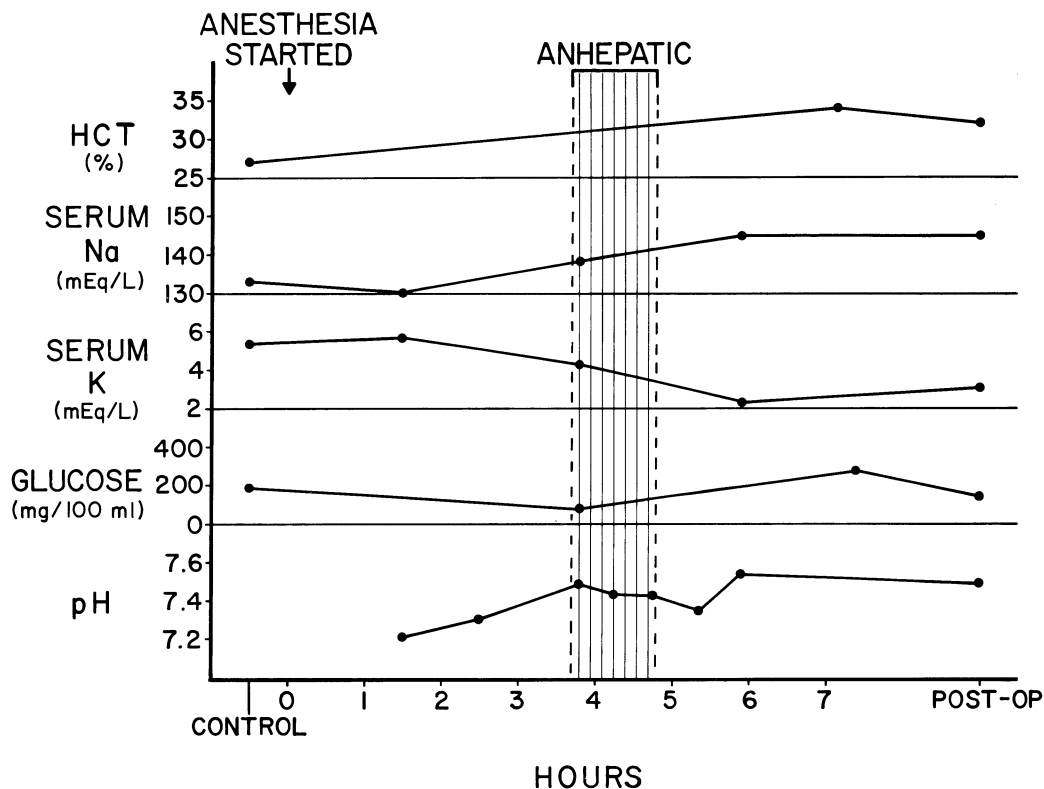


Figure 37. Physiologic measurements in patient OT 20. Before and after the anhepatic phase there was a slow rise in the serum sodium concentration, probably secondary to bicarbonate therapy. Eventually a slight overcorrection of the acidosis was achieved.

as in all the others, an improvement was immediately seen with reopening of the vessels (Figs. 33 to 35; Table 7). A consistent correlation could not be established between the extent of gross collateral formation and the tolerance to venous occlusion as judged by the degree of hypotension.

However, it was our impression that the four patients who had hepatomas without concomitant cirrhosis (OT 8, 14, 17 and 23) were placed at the greatest risk. Their intestines became definitely congested, a change which was not obvious in most of the other cases. When this was seen a request was made that the blood flow be restored through the reconstructed vena cava immediately, rather than wait for the remaining vascular anastomoses to be completed. In this way the duration of stagnation of one of the obstructed venous systems was reduced by 20 to 60 minutes.

Acid-Base and Electrolyte Changes

The final extent of the metabolic acidosis which would have developed in these patients could not be measured since prophylactic therapy was always

Table 7. Changes in the Systolic Blood Pressure (mm Hg) Caused by Simultaneous Cross Clamping of the Portal Vein and Inferior Vena Cava*

PATIENT	PRESSURES WITH CROSS CLAMPING		
	<i>Before</i>	<i>During</i>	<i>After</i>
OT 7	115	110	110
OT 8	90	65	85
OT 9	130	110	120
OT 10	80	65	105
OT 11	70	60	70
OT 12	90	75	85
OT 13	95	90	90
OT 14	105	90	115
OT 15	60	45	75
OT 16 ^A	170	160	160
OT 16 ^B	170	70	130
OT 17	95	90	115
OT 18	125	100	115
OT 19	100	85	105
OT 20	135	105	150
OT 21	100	60	80
OT 22	105	65	95
OT 23	100	60	85
OT 24	80	60	95

*The control measurements were all five minutes before the venous occlusions. The blood pressure declines during clamping were the maximums observed. The final values were obtained 15 minutes after homograft revascularization.

provided, particularly before, during, and just after the anhepatic stage (Figs. 33 to 35). In spite of treatment, and although the patient was continuously hyperventilated throughout, it was usually found that there had been an under-correction (Fig. 36). Additional quantities of bicarbonate were then given according to the calculated base deficit. There was only one instance in which too much alkalinization led to a transient but moderately severe alkalosis. The pH in this recipient (OT 18) rose to 7.68, along with a temporary hypernatremia of 166 mEq/liter.

The administration of large quantities of sodium bicarbonate was apparently made safer by the invariably pre-existing hyponatremia; this may have been the consequence of secondary hyperaldosteronism as discussed earlier. With the progress of the operation and the repetitive correction of alkalosis with the sodium buffer, serum sodium concentrations tended to become progressively increased toward normal (Figs. 36 and 37).

The only other consistent change was in the serum potassium concentration. The values were within normal limits in all the recipients at the beginning of the procedure. The concentrations remained about the same or were slightly elevated until the homograft was revascularized. From this time onward there invariably was a steady decline (Figs. 36 and 37) which usually lasted for several hours. The lowest potassium recorded in any patient was 1.8 mEq/liter.

The reason for the potassium "leak" can only be speculated upon. It may have been related to the large amounts of sodium bicarbonate, which were always given in the preceding hour or two.⁸¹ A much more plausible explanation would be that the potassium was suddenly taken up by the homograft. It has been shown that the liver liberates potassium after conditions of ischemia.^{6, 10, 135} Presumably, an increased uptake by the liver cells to replace the deficit could account for the apparent loss of potassium from the serum.

Blood Cholinesterase

Plasma and red cell cholinesterase levels were measured in five recipients of orthotopic homografts. The results are summarized in Figure 38. The plasma cholinesterase (pseudocholinesterase), which is synthesized mostly by the liver, was discussed earlier in connection with its role in detoxification of various drugs, including succinylcholine. In all five recipients the preoperative values were abnormal. In four of the patients the diagnosis was biliary atresia, but in the fifth (OT 17) it was primary hepatoma. The last patient had no pre-existing serious abnormalities of liver function.

During operation the abnormally low plasma cholinesterase levels rose before the onset of the anhepatic period (Fig. 38), probably as the result of blood transfusions being given. Afterward the values did not decline as transfusions were diminished and discontinued. It was suspected that the late maintenance of the higher concentrations was due to synthesis and release of cholinesterase by the new organs. Whether this conclusion was correct remains to be determined from a greater number of observations.

CHANGE OF CHOLINESTERASE DURING LIVER TRANSPLANTATION

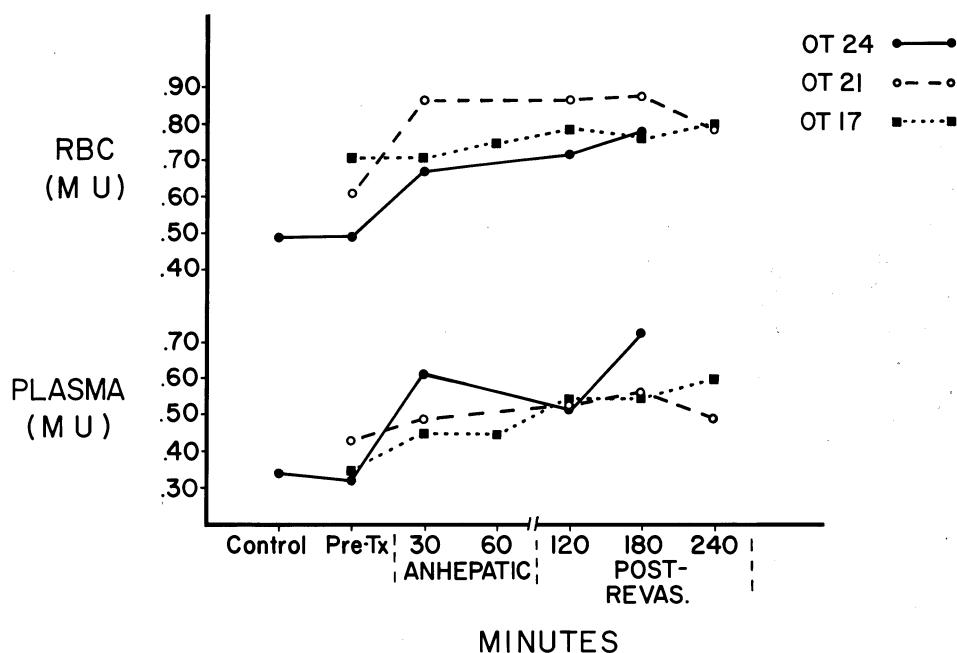


Figure 38. Intraoperative changes in red cell and plasma cholinesterase. See text for discussion.

The results of red cell cholinesterase determinations neither supported nor contravened this idea. The red cell values were initially normal, a finding which was not surprising since erythrocyte cholinesterase is not dependent on liver function. However, small and sustained rises occurred during the procedures, which had not yet returned to the control status at the end of the observation periods (Fig. 38).

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Chapter Eight

THE RECIPIENT OPERATION IN MAN

The first known efforts at experimental orthotopic transplantation of the liver were made by Dr. Jack Cannon of Los Angeles.⁵ With the expectation that the liver played an important role in rejection, he apparently hoped that a hepatic homograft might suffer a different fate from that of other transplanted tissues since it presumably would not contribute to its own repudiation. Details of the procedure or of the animal species in which the experiments were done were not given. Cannon referred to "several successful operations" but without survival of the recipients.

In June, 1958, a well organized program of orthotopic transplantation of the canine liver was undertaken at the Peter Bent Brigham Hospital under the direction of Dr. Francis D. Moore.^{12, 13} Later that summer our own first experiments were begun in the same species at Northwestern University in Chicago. There, a new method for one stage total hepatectomy in dogs had been developed¹⁹ and, in the course of this work, the concept of replacing the excised organ^{9, 25} was first envisioned. The methods independently developed by workers in the two laboratories were not compared until the meeting of the American College of Surgeons at Atlantic City in the autumn of 1959. The absence of communication during the initial year was reflected in a number of differences in the techniques used by the Boston group^{10, 31} and our own.^{1, 26} However, the objectives of the methods were identical in every important respect.

It is unlikely that anyone would wish to attempt a clinical liver transplantation without personally recapitulating in the laboratory at least some of the earlier experience in dogs or, alternatively, in pigs (see Chapter Eleven). The surgical techniques in both species have become well standardized.^{1, 2, 10, 15, 26, 31} However, the descriptions in this chapter will be confined to the details of orthotopic hepatic transplantation as it has been carried out in humans. Various aspects of the surgical technique in man have been reported,^{18, 20, 21, 23, 24, 27, 29, 30} but never in one publication.

RECIPIENT HEPATECTOMY

The Incision

The first orthotopic transplantations in humans²⁹ were performed through thoracoabdominal wounds (Fig. 39C). The view was excellent both for the hepatectomy and insertion of the homograft. However, it is now thought that the advantage of the extra exposure was more than offset by the effort of making and closing the thoracic extension and by the possible introduction of an increased morbidity. In the last 20 patients long transverse abdominal incisions of one kind or other have been used (Fig. 39A and B); if additional room had been needed, entry into the chest could easily have been accomplished (Fig. 39B).

In every case in our series, with a single exception, one or more earlier explorations had been carried out at other institutions. Whenever possible the old scar was reopened and made part of the incision for the transplantation; this was always feasible in the cases of biliary atresia. Two of the adult patients with hepatoma (OT 15 and 17) had short and infected vertical incisions from operations that had been carried out 10 days and three months earlier, respectively. The old wounds were excised and exposure obtained by making fresh transverse incisions (Fig. 39A) across the first operative site. Primary healing was obtained.

Determination of Operability

The order in which dissection is carried out is influenced by the disease for which transplantation is being done. In biliary atresia, it is attempted first to skeletonize the structures of the portal triad. If a dilated duct were found which communicated with the intrahepatic biliary tree, a reconstructive procedure would be strongly considered in preference to transplantation but, to date, this circumstance has not been encountered.

Preliminary dissection of the portal triad may also be indicated in hepatic malignancy if the tumor is located centrally or in the hilar region. On several occasions an evaluation of inoperability was quickly reached when extrahepatic nodal metastases were found in this area. Often, however, it is the convex surface of the liver which requires the closest inspection to rule out invasion of the diaphragm or to be sure that tumor has not extended into the thorax via the hepatic veins and suprahepatic inferior vena cava. In some cases it may be necessary to skeletonize all the major structures entering and leaving the liver before reaching a decision to proceed.

Portal Hypertension

Eighteen of our 25 patients treated with orthotopic homotransplantations had portal hypertension and extensive high pressure venous collaterals. Exsan-

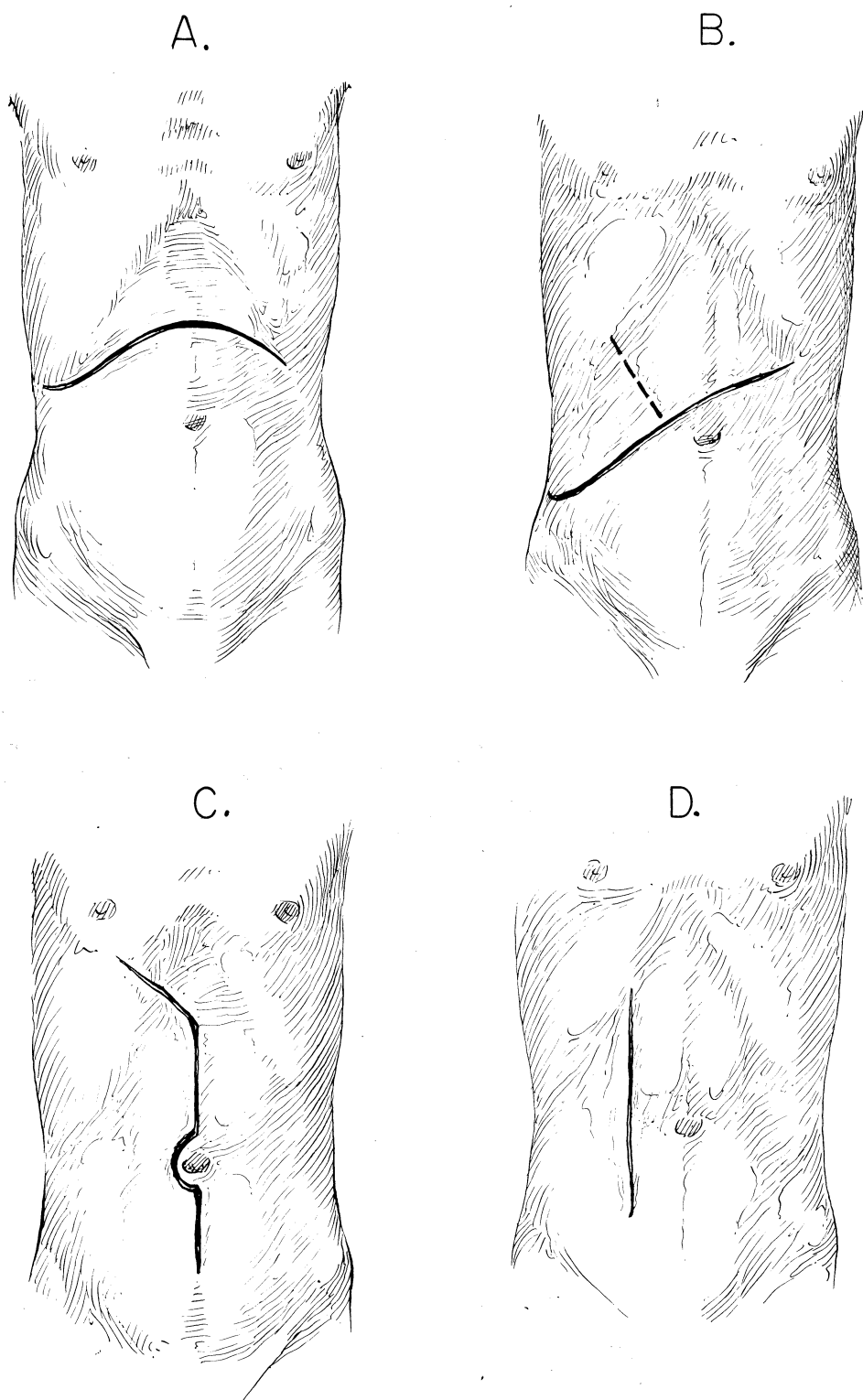


Figure 39. Incisions used for orthotopic liver transplantation. The preferred approaches are shown in A and B; with either of these wounds, an extension into the right thorax can easily be made. Vertical incisions (C and D) have not been used in recent cases.

guinating hemorrhage from these vessels must be prevented. If transplantation has been decided upon, a time-saving maneuver is to ligate the thin walled veins on one side and to cauterize them on the expendable liver side which will be removed. For the latter purpose, a high setting (60 megacycles) is required, great enough to create a spark across a gap of several millimeters from the cautery tip. With this technique the tissue is burned as with an actual cautery. In some instances where dissection is close to the hepatic capsule, this may be the only way to stop the bleeding from the liver.

In spite of these efforts, it was not possible in any of our patients with portal hypertension to obtain a completely dry wound prior to completion of hepatectomy. Fortunately, decompression of the splanchnic system through the homograft resulted in most cases in prompt cessation of the diffuse venous bleeding.

Dissection of the Portal Triad

Access to the portal structures may be hampered not only by the presence of venous collaterals, but also by adhesions from previous operations. A combination of these factors makes a particularly difficult and dangerous situation in children with biliary atresia. All such patients have had earlier explorations in order to rule out the feasibility of duct reconstruction and multiple loops of bowel are always found to be adherent to the liver or gastrohepatic ligament. Sometimes these are stuck so firmly that they can be removed only with the cautery knife and at great risk of their instrumental perforation. Moreover, the cicatricial distortion or foreshortening of the portal triad can make difficult even its identification. It may then be necessary to find the anterior surface of the infrahepatic inferior vena cava and redevelop the obliterated foramen of Winslow. Alternatively, it may be possible to achieve this objective from the left side through the lesser omental sac.

Once the portal triad has been encircled (Fig. 40A), its three constituent structures can be freed with relative safety providing their triangular spatial relationship is appreciated as shown schematically in Figure 40B. Long enough segments of the vessels to permit subsequent anastomosis are developed. This may require ligation and division of the right gastric and gastroduodenal branches of the hepatic artery (Fig. 41). Sacrifice of these vessels has another advantage. As soon as they are cut, it is much easier to see and clean off the portal vein just where it emerges from behind the neck of the pancreas (Fig. 41). Branches of the portal vein also have to be ligated and severed; of these, the coronary (left gastric) vein which enters on the left side and posterolaterally a few millimeters above the pancreas is the most constant (Fig. 42). Control of hemorrhage after accidental avulsion of this vessel with blunt dissection may be exceptionally difficult.

If it is planned to eventually perform choledochocholedochostomy for biliary drainage (an option not available in biliary atresia), the distal common duct and its surrounding adventitia and vessels are left long. Otherwise, it is doubly ligated close to the duodenum (Fig. 42).

(Text continued on page 119.)

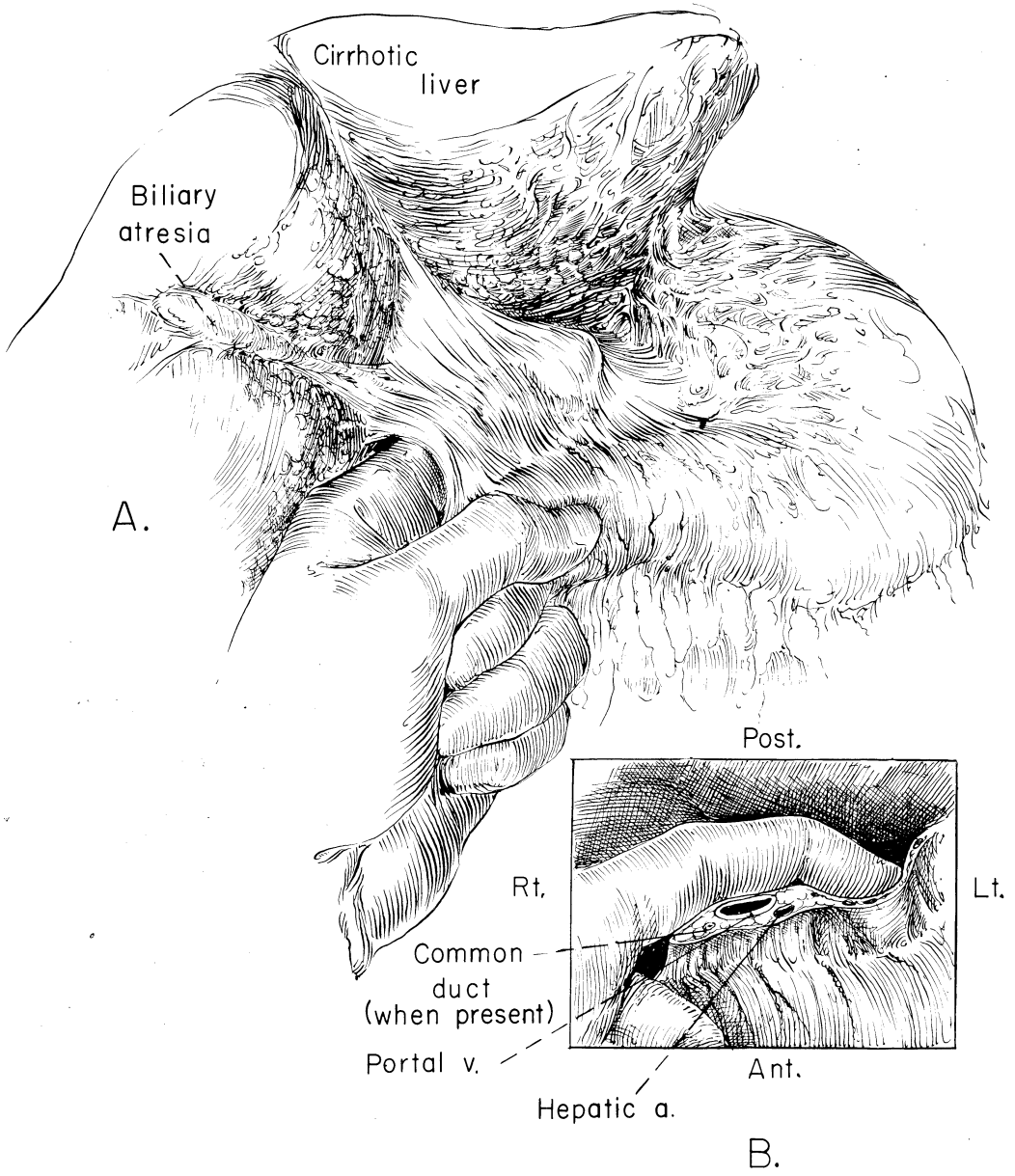


Figure 40. A. Encirclement of the portal structures preparatory to their individual dissection. This can be done either from the right side as indicated or from the left through the lesser omental sac. B. Spatial relationships of the components of the portal triad.

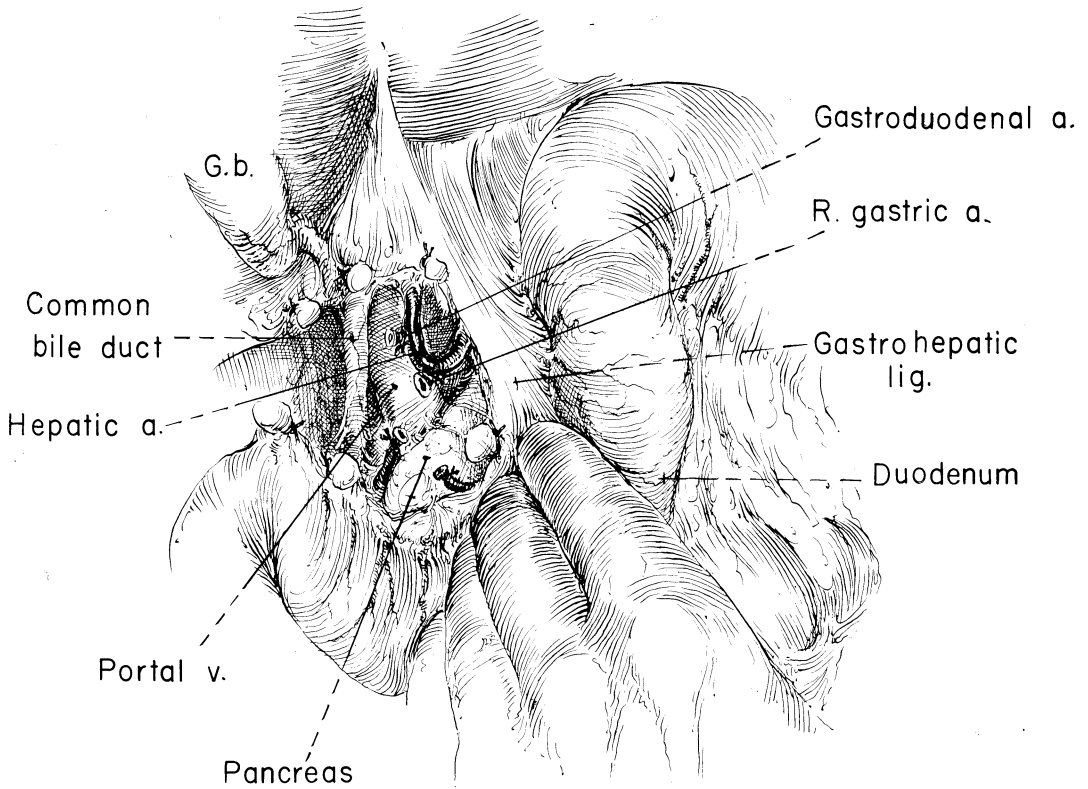


Figure 41. The appearance of the hilum after ligation and division of the right gastric and gastroduodenal arteries. Note the access that is provided to the anterior surface of the portal vein.

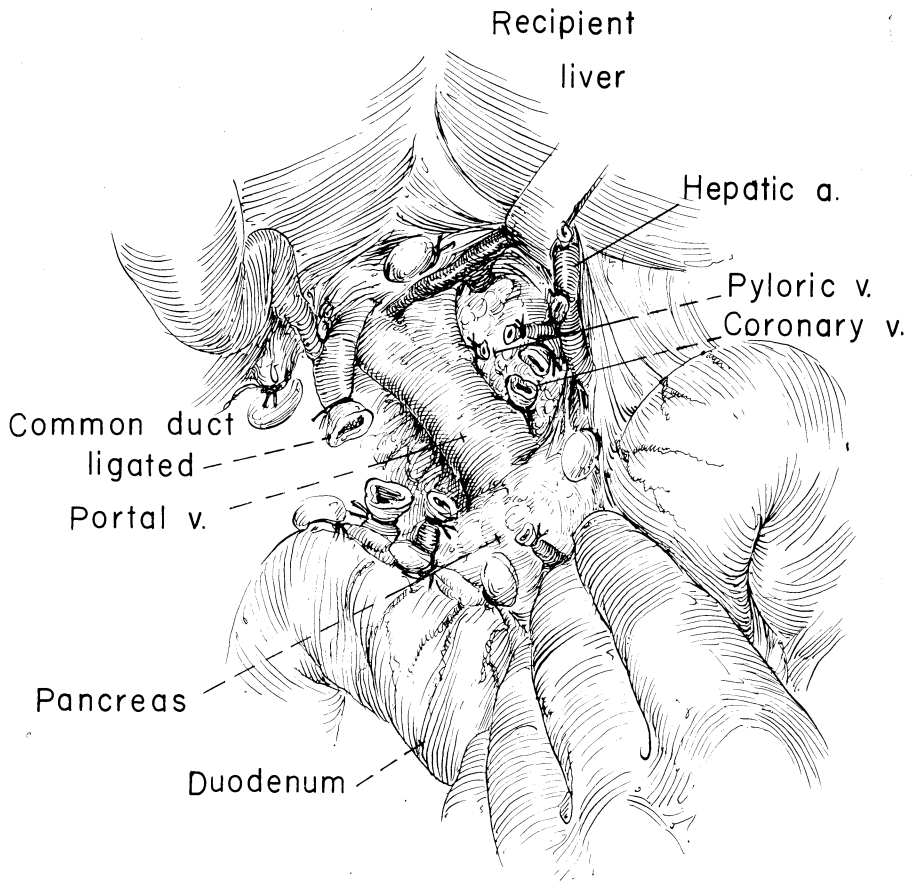


Figure 42. The common duct and several tributaries of the portal vein have been doubly ligated and divided.

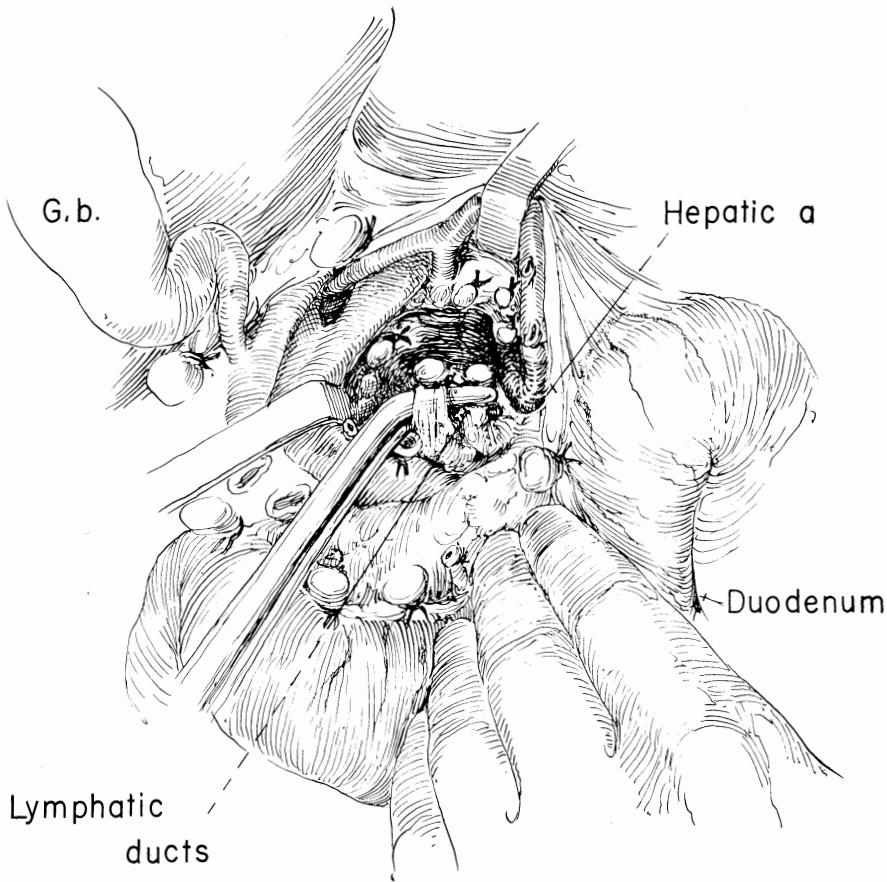


Figure 43. Ligation and division of tissue between the major hilar structures.

As the major structures of the triad are skeletonized, lymphatics, nerves of the hepatic plexus, and areolar tissue are severed (Fig. 43). In order to prevent later lymph leaks, all such intervening bits of tissue must be tied.

Completion of Mobilization and Removal

The steps in the remainder of the hepatic liberation are almost identical to those already described for donor hepatectomy (Chapter Five). The falciform ligament is incised up to the suprahepatic vena cava, taking care to leave enough ligamentous tissue to permit its later suture to the same structure of the homograft. After the left triangular ligament is cut, about half of the circumference of the vena cava and the left main hepatic vein can be seen between the liver and the diaphragm (Fig. 14, Chapter Five).

Mobilization of the right and posterior parts of the liver is more difficult. The first steps are to incise the anterior leaf of the right triangular and coronary ligaments, to retract the liver toward the left, and to enter an extensive raw area to which the right adrenal gland contributes a portion of the floor. The

plane is opened with sharp and blunt dissection until the retrohepatic vena cava is seen.

Behind the liver the vena cava usually has only a single tributary, the right adrenal vein, which must be ligated and divided (Fig. 16, Chapter Five); although this is ordinarily well tolerated, two of our patients (OT 7 and 17) developed venous infarction of the gland (Chapter Nine). At this stage the remaining tissue attaching the retrohepatic vena cava to the posterior body wall is detached, making sure that any unexpected venous branches are ligated and divided. It should now be possible to pass a finger behind the vena cava from the diaphragm to the renal veins without meeting an obstruction, just as was described in the donor operation (Fig. 17, Chapter Five).

Additional work is usually necessary, however, to make the short expanse of suprahepatic vena cava ready for subsequent anastomosis. Even at best, the length which can be made available is limited, but additional mobility is attainable if the diaphragmatic veins that enter the segment are ligated and divided as already described in Chapter Five. There may be only two large phrenic tributaries, one on each side, but more commonly two or three additional smaller vessels must also be similarly disposed of. By so doing, room is made for later application of a vascular clamp which can then be placed without grasping a portion of adjacent diaphragm. If the latter maneuver be-

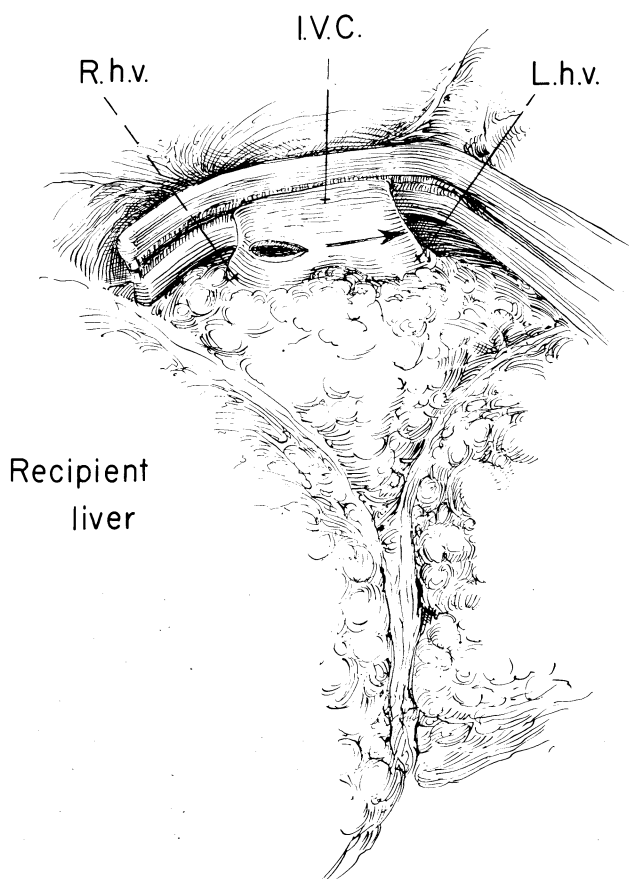


Figure 44. Transection of the suprahepatic inferior vena cava. Note that the line of incision is kept as close to the liver as possible in order to retain the maximum vessel length for subsequent anastomosis. R.h.v. = right hepatic vein; L.h.v. = left hepatic vein; I.V.C. = inferior vena cava.

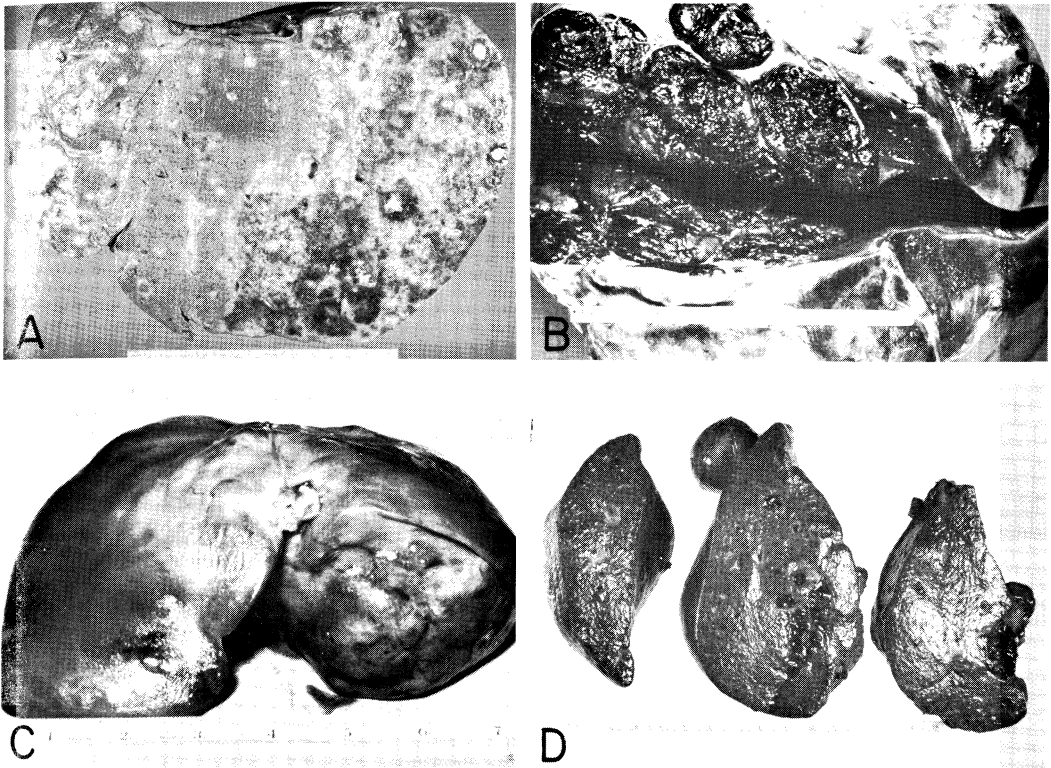


Figure 45. Diseased recipient livers removed at the time of orthotopic hepatic transplantation. *A.* Patient OT 4. The diagnosis in this 52 year old man was hepatoma superimposed upon cirrhosis. The specimen weighed 2400 gm. *B.* Patient OT 5. The hepatoma and liver excised from a 29 year old woman weighed 10 kg. *C.* Patient OT 8. The specimen in this 19 month old girl weighed 1165 gm. The diagnosis was hepatoma. *D.* Patient OT 10. A 655 gm liver removed from a 13 month old girl with extrahepatic biliary atresia.

comes necessary it introduces the risk of crushing the right phrenic nerve and causing paralysis of the right diaphragm (Chapter Nine). It is worth noting that the foregoing extensive manipulations, including dislocation and even twisting of the liver, have had unexpectedly minor cardiocirculatory effects, probably for the reasons to be discussed in the next section (see also Chapter Seven).

After the vessels entering and leaving the liver are skeletonized, they are individually occluded with noncrushing clamps and divided. As much length of these structures as possible is left with the patient. Particular care must be taken in transecting the vena caval cuff at the diaphragm. The vascular clamp is placed as superiorly as is feasible without drawing a piece of the contiguous diaphragm into the bite. A venotomy is then made in either the main right or left hepatic vein. With one blade of the scissors in the lumen and the other outside, the cloaca formed by the confluence of the vena cava and hepatic veins is incised close to the liver around the entire 360 degrees (Fig. 44). The flap developed for eventual circumferential suture is only a few millimeters long. After cutting the infrahepatic vena cava, the portal vein, and the hepatic artery, the specimen is removed (Fig. 45).

The Bypass Problem

In at least one important way an erroneous conclusion was reached from animal experimentation about the technical requisites for successful human liver transplantation. With removal of the host liver it is necessary to temporarily cross clamp the great veins which drain the intestine and lower half of the body (Fig. 46). It was soon learned in the experimental laboratory that dogs subjected to orthotopic hepatic transplantation regularly died of shock if the portal vein and inferior vena cava were not decompressed during this anhepatic interval. Even animals which did not die during operation developed irreparable damage to the small bowel and a resulting hemorrhagic enteritis that caused death from delayed irreversible shock. Moore¹³ dealt with the problem by inserting external bypasses into the two venous systems so that the blood could be rerouted to the jugular veins; premature clotting in the temporary prostheses was prevented with systemic heparinization.

An alternative solution was used in our laboratories.²⁵ The two venous systems of the dog were first placed into communication by performing a side-to-side anastomosis between the partially occluded inferior vena cava and the superior mesenteric vein. Then a single vena caval-jugular bypass permitted decompression of both venous pools (Fig. 47). Heparin was not given. After the

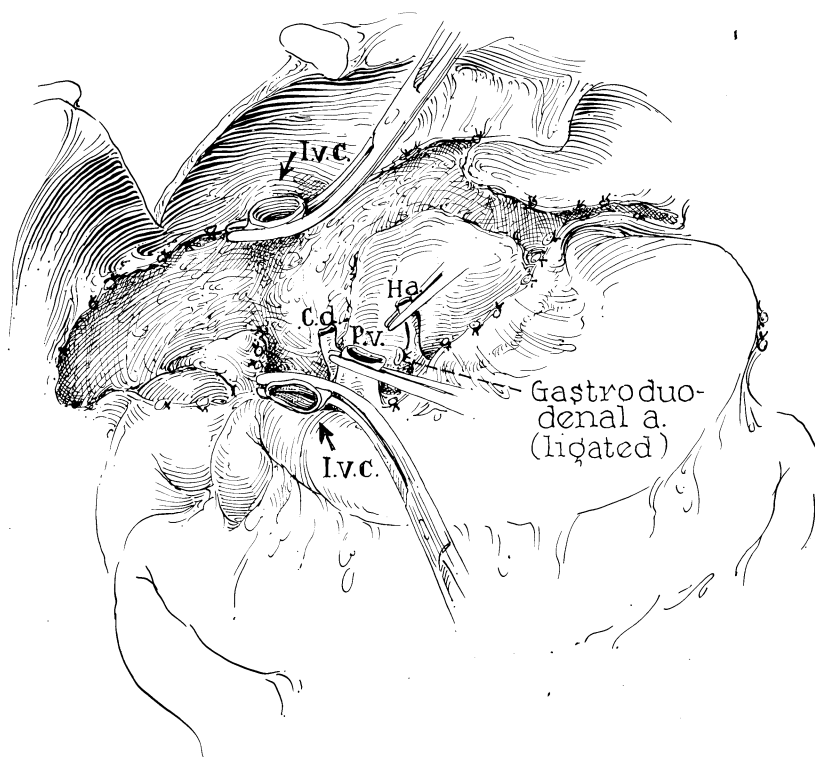


Figure 46. The operative field after removal of the diseased recipient liver. In the illustration an incision in the diaphragm is shown as part of a thoracoabdominal exposure. In all more recent cases, the chest has not been entered. C.d. = common duct; H.a. = hepatic artery; I.V.C. = inferior Vena cava; P.v. = portal vein. (By permission of Surg. Gynec. Obstet. 117:659, 1963.)

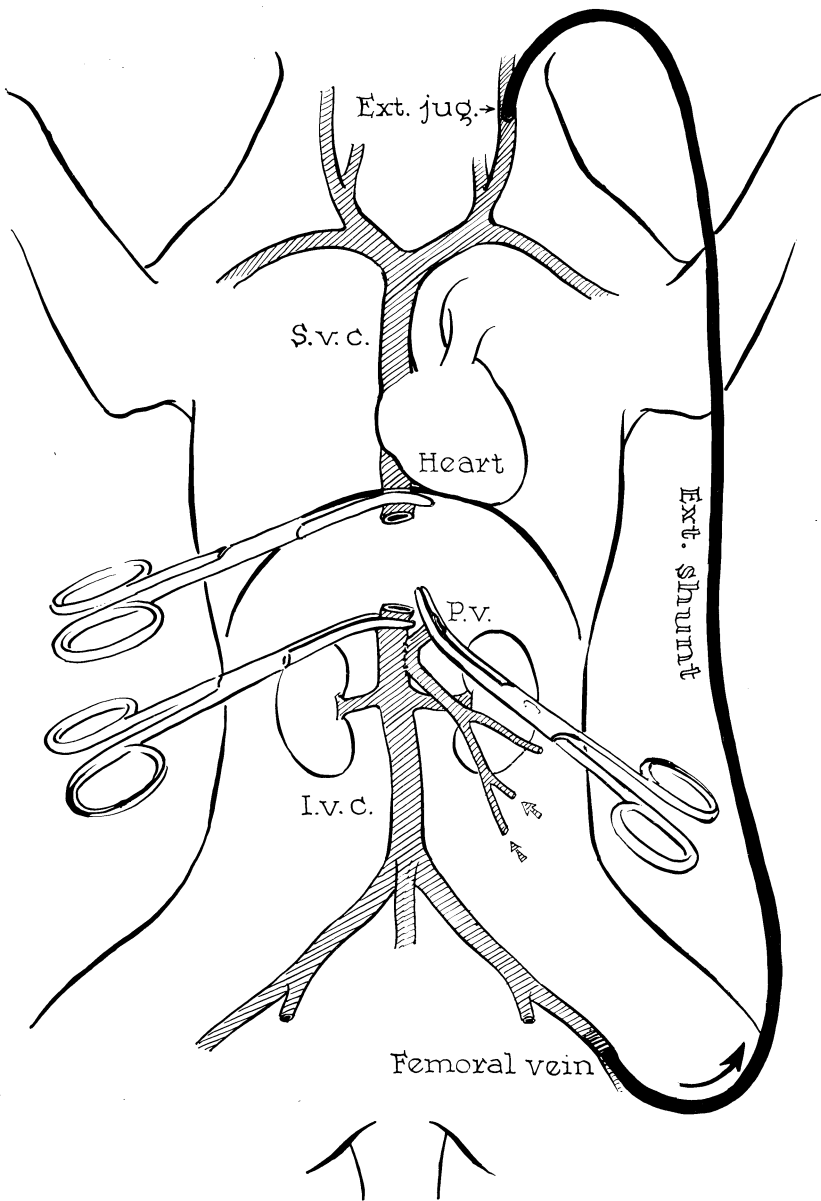


Figure 47. Method used in dogs for decompression of inferior vena caval and splanchnic systems during removal of recipient liver and replacement with a homograft. Note that a preliminary portacaval shunt has been placed. By means of this temporary anastomosis the two venous systems are connected, allowing their decompression with a single external bypass. (By permission of Surg. Gynec. Obstet. 111:733, 1960.)

homograft was in place, the caval-mesenteric shunt was taken down and the resulting defects closed by vascular suture.

The absolute necessity for effective venous decompression in animals was unfortunately extrapolated to be a mandatory technical condition in the first trials of orthotopic liver transplantation in man. In our first five patients as well as those of Moore¹¹ and Demirleau,⁷ plastic tubes were used to bypass the obstruction of either the vena cava or the portal vein, or both (Fig. 48).

The details of the bypass techniques will not be described since it has since been learned that such measures are not needed. In the last 22 orthotopic transplantations they have not been used. Simultaneous cross clamping of the inferior vena cava and portal vein in these patients was for 30 to 95 minutes. The consequent hemodynamic changes were tabulated in Chapter Seven. A slight duskiness of the intestine developed in some patients, but this immediately disappeared when blood flow was restored through the reconstructed venous channels.

The demonstration that bypasses are unnecessary in human recipients of orthotopic homografts was a significant advance since there were serious complications from the techniques used for venous decompression, as will be mentioned in Chapters Nine and Ten. The fact that acute physiologic alterations

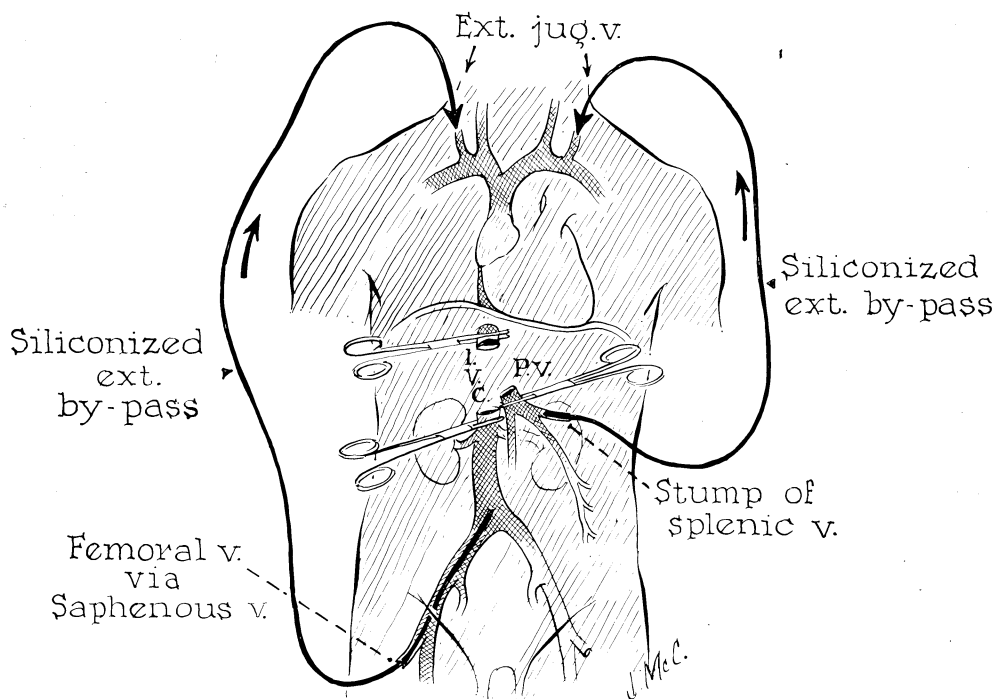


Figure 48. One system of venous bypassing which was used in the early cases of clinical liver transplantation. It has since been learned that these provisions for venous decompression are not necessary in humans. The technique used in our early experience is shown because it helps to explain the pulmonary emboli which were the immediate or contributory causes of death in the first three patients who survived orthotopic transplantation (see Chapter Ten). The clots in two of the patients originated at or near the site of insertion of the femoral catheter. (By permission of Surg. Gynec. Obstet. 117:659. 1963.)

caused by obstructing the two great veins were so minimal in patients probably is partly explained by an inherently richer network of potential collaterals in man by which blood can be rerouted back to the right heart.⁶ An additional decisive factor is presumably the increase in the size and ramifications of such collaterals as the consequence of liver disease. The latter factor has been proved by Picache et al¹⁶ to be important in canine experiments, as was discussed in Chapter Seven.

Deviations in Hepatectomy Technique

The portal hilum was so scarred in two of the children with biliary atresia that it was impossible to liberate the separate structures; in one child, efforts to do so resulted in laceration of the hepatic artery. In both instances the liver could be removed only after a vascular clamp was placed just above the head of the pancreas across the entire triad and the gastrohepatic ligament was cut across. The portal vein and hepatic artery could then be identified in the cross sectional view (Fig. 49) and freed for a long enough distance to permit performance of the anastomoses. Hemorrhage from the intervening tissue was controlled with suture ligatures placed both before and after the eventual release of the clamp.

A life-threatening technical error was made in another patient (OT 6) when a large right phrenic vein was avulsed from the side of the suprahepatic vena cava. The rent quickly extended through the diaphragm, and air was observed to be passing into the lumen. A large noncrushing Potts clamp designed for use in the gastrointestinal tract was applied to a generous rim of adjacent diaphragm, and the retracted vena cava was eventually retrieved for anastomosis.

HOMOGRAFT INSERTION

In principle, the organ placement could hardly be more straightforward since it involves simply the reconstruction of the major vascular channels entering and leaving the liver and the creation of a passage for bile drainage.

Suprahepatic Vena Cava

This is the most difficult and dangerous of the vascular anastomoses, not only because the lengths of host and homograft vessel which are available for sewing are very short. In addition, the presence of the new liver makes it difficult to improve exposure by retraction or other manipulations. Finally, the fact that the suture line, particularly its posterior portion, cannot easily be re-exposed for control of hemorrhage at any subsequent time makes it mandatory that a perfect result be obtained. This must be achieved by fashioning the back

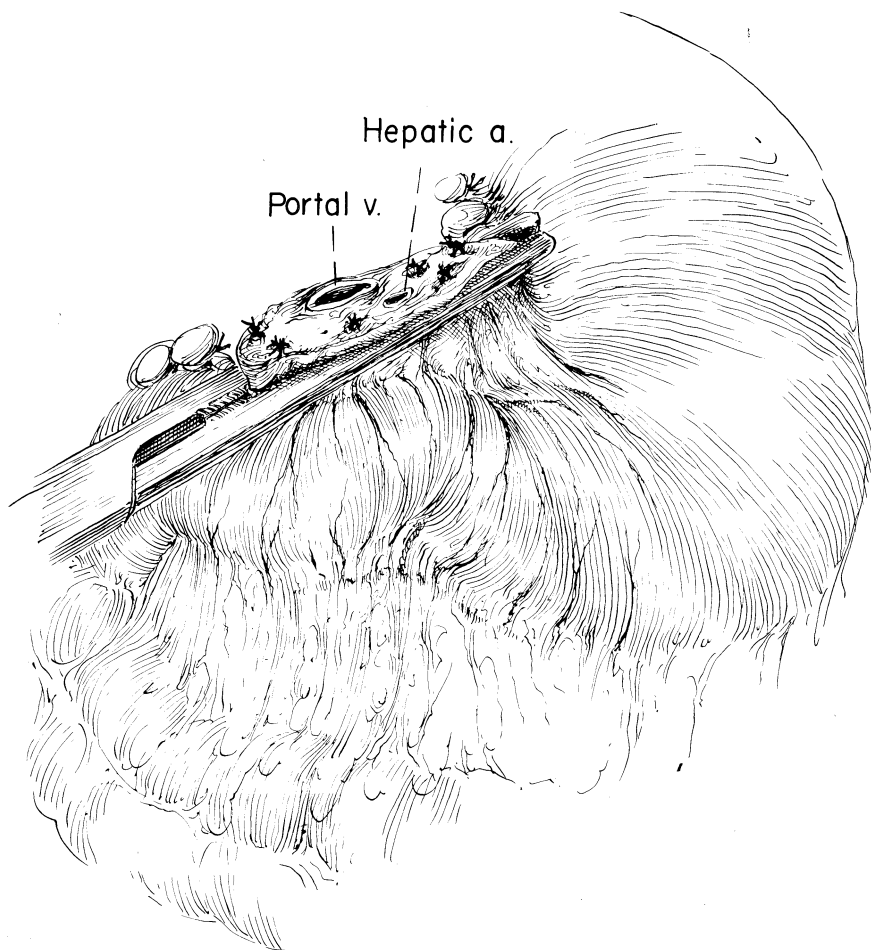


Figure 49. Incision en masse of the portal triad. This maneuver has been necessary on two occasions when the individual structures could not be dissected free. After the transection, the portal vein and hepatic artery could be liberated enough to permit the vascular anastomoses to be performed. See text for discussion.

portion of the anastomosis from within the lumen. For this purpose we have used continuous over and over suturing.^{22, 29} The technique will now be described in detail since it can often be used to advantage in other situations in organ transplantation where there are similar limitations of vessel length and exposure.

The principle of the method,²² as demonstrated in Figure 50A for a side-to-side anastomosis, is the immediate formation of intraluminal shoulders in both vessels to be joined. First, sutures are placed in the extremities (Fig. 50A-1) of the anastomosis. The swaged needle is passed into the posterior part of the lumen of one of the vessels 1 or 2 mm from the line of incision (Fig. 50A-2). A firm bite of the other vessel is then taken, making sure that the entry and exit sites of the needle pass through the intima at some distance from the cut edge (Fig. 50A-3). The full thickness of the wall is included. If the thread is pulled tight, a mound of protruding tissue presents which makes easy the similar

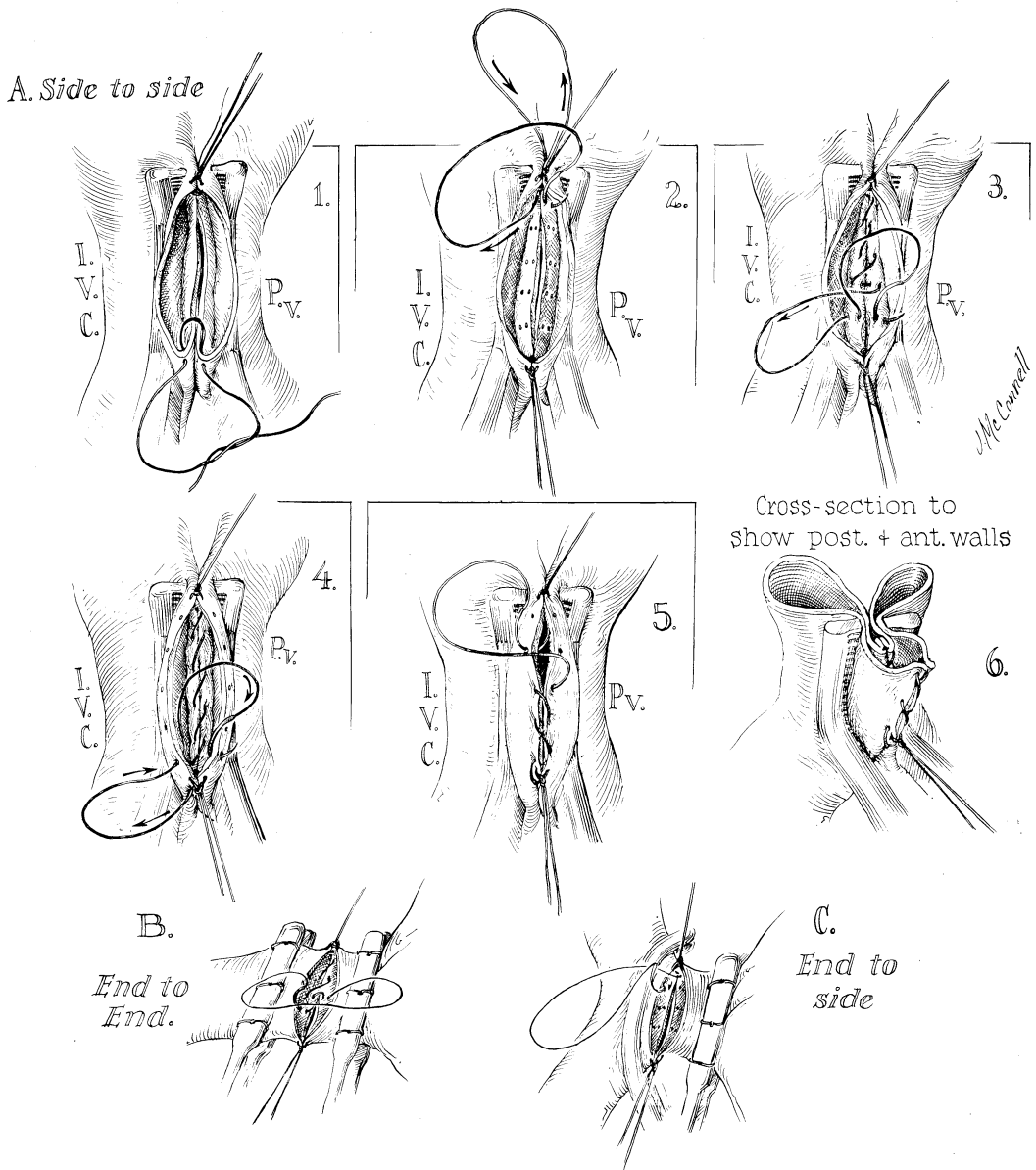


Figure 50. The principle of the intraluminal suturing technique used in liver transplantation. A. The application of the method for a side-to-side anastomosis. B. An end-to-end anastomosis such as that employed for the suprahepatic vena caval, infrahepatic vena caval, and portal venous reconstructions. C. End-to-side anastomosis. (By permission of Surg. Gynec. Obstet. 127:125, 1968.)

placement of subsequent sutures (Fig. 50A-4). When the opposite end of the posterior anastomotic line is reached, the needle is passed outside (Fig. 50A-4) and the anterior row is completed with an everting over and over suture (Fig. 50A-5). The steps are almost exactly the same for an end-to-end (Fig. 50B) or end-to-side anastomosis (Fig. 50C).

The degree of eversion obtained in the back wall is the same as if the sewing were done from the outside (Fig. 50A-6). Consequently, the amount of intraluminal suture material is not increased. There are other advantages. A perfect intimal coaptation can be assured. If the orifices of tributaries to the anastomosed vessels are identified near the suture line, they can be incorporated into the shoulder, thereby circumventing potentially lethal para-anastomotic leaks. Immediate tightening of each stitch facilitates placement of the next bite, making it unnecessary to grasp or manipulate the vessel wall with forceps.

For adults and children, respectively, 4-0 and 5-0 silk are used for the suprahepatic anastomosis. In most cases the two orifices are of different size, necessitating constant correction as individual bites of tissue are taken. There is ordinarily no need to have more than the single layer closure around the entire circumference (Fig. 50B). In some cases, however, it may be desirable to reinforce the back wall. This can be accomplished by a second intraluminal layer which everts the first (Fig. 51).

Infrahepatic Vena Cava

In spite of the facts that an extra length of subhepatic vena cava is retained with the homograft and that the host vena cava is also left long, tailoring is usually not required (Fig. 52). The technique of anastomosis is identical to that described above, including intraluminal suture of the back wall.

Portal Vein

Excess lengths of both host and homograft portal vein are usually available. Both vessels must ordinarily be re-cut so that there is approximation without

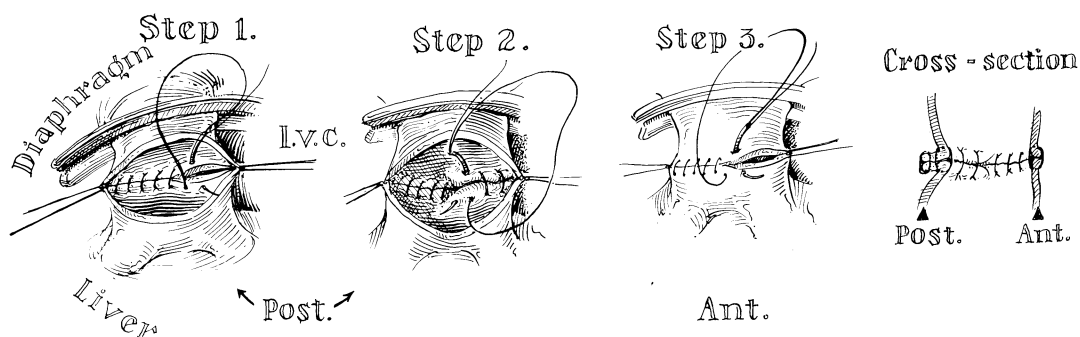


Figure 51. An alternative technique for performance of the suprahepatic vena caval anastomosis. With this method the posterior row is fashioned in two layers from the inside of the lumen. In recent cases this added precaution has seldom been taken. Note that the cuff of the homograft vessel is actually the confluence of the hepatic veins and the vena cava. (By permission of Surg. Gynec. Obstet. 117:659, 1963.)

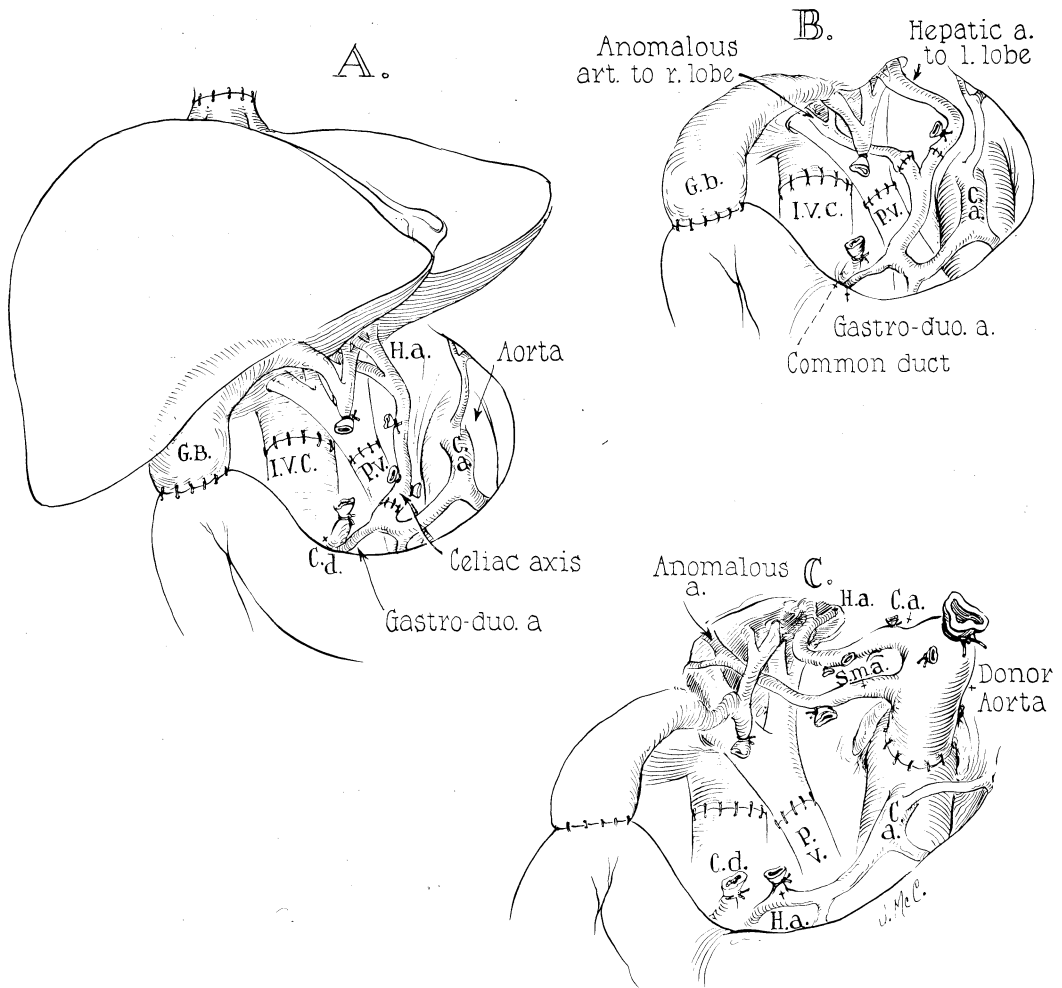


Figure 52. Recipient operations. In most recent cases cholecystoduodenostomy has been performed for biliary drainage as shown. C.a. = celiac axis; C.d. = common duct; G.B. = gallbladder; H.a. = hepatic artery; I.V.C. = inferior vena cava; P.V. = porta vein; S.m.a. = superior mesenteric artery. A. The kind of arterial anastomosis most commonly used. The homograft celiac axis or common hepatic artery is attached to the proper or common hepatic artery of the recipient. B. Arterial reconstruction of two homograft arteries. The vessels are individually anastomosed to the branches of the recipient proper hepatic artery. This technique was used in patients OT 4 and 9. C. Anastomosis of the homograft aorta to the recipient aorta (patient OT 12). The variation was used in this 16 month old recipient because of the double arterial supply to the transplanted liver. (By permission of *Ann. Surg.* 168:392, 1968.)

tension (Fig. 52). In one patient sufficient tissue was not excised; the reconstructed vessel was kinked, necessitating reanastomosis.

Because of the foregoing requirement of perfect length, there is not usually enough room to turn the vessels for external suturing of the back wall. The continuous intraluminal technique mentioned earlier is applied, employing 6-0 vascular silk.

Hepatic Artery

The usual technique of rearterialization is by anastomosis of the homograft common hepatic artery or celiac axis to the recipient common or proper hepatic artery (Fig. 52A). In humans the decision for or against ligation and division of the host gastroduodenal and right gastric arteries is one of convenience since there is no attendant risk of devascularizing the pancreatic head or duodenum as is the case in dogs. A standard continuous arterial suture technique is used for the anastomosis.

The reconstruction is easy in adults inasmuch as the vessels are large, being about the same size and consistency as renal arteries; 6-0 silk is used. In contrast, the hepatic arteries in young children are both small and fragile, the internal diameter at one to two years often being 1 or 2 mm. In such cases the use of the graft celiac axis is usually advisable. This can be connected without difficulty to the recipient common hepatic artery, which is always larger than normal in patients with biliary atresia. The anastomosis is usually made proximal to the right gastric and gastroduodenal branches (Fig. 52A). The suturing is greatly facilitated in infant vessels by the use of continuous 7-0 or 8-0 arterial silk.

Homograft Vascular Anomalies

Some of the most serious technical problems with orthotopic liver transplantation were caused by abnormalities or anomalies of the blood vessels, either of the recipient or of the transplanted liver. Of the first 26 orthotopic hepatic homografts used at the University of Colorado, five had two arteries each (Table 8). In three patients the vessel to all or part of the right lobe came from the superior mesenteric artery. In a fourth the right hepatic artery originated from the aorta. The other liver had an aberrant left lobar branch from the left gastric artery.

Several different techniques of reconstruction were used. In one adult (OT 4) and one child (OT 9), the individual vessels were anastomosed to the right and left branches of the proper hepatic artery (Fig. 52B). In the infant the tiny right branch thrombosed within 48 hours, causing regional hepatic gangrene (Chapter Fifteen). In another child, aged 16 months (OT 12), both homograft vessels were removed from the donor in continuity with the parent trunks and the aorta. The distal end of the homograft aorta was then anastomosed to the side of the upper abdominal aorta of the recipient, proximal to the celiac axis

Table 8. Abnormal Blood Vessels Either of the Recipient or the Homograft Encountered in 25 Consecutive Human Cases of Orthotopic Liver Transplantation at the University of Colorado

	RECIPIENT	HOMOGRAFT	TOTAL
Double hepatic artery	3	5	8
Single hepatic artery from SMA*	1	0	1
Hypoplastic portal vein	1	0	1
Anteduodenal portal vein	1	0	1
Hypoplastic inferior vena cava	1	0	1
Absent inferior vena cava	1	0	1
TOTAL	8†	5‡	13

*SMA = superior mesenteric artery.

†The eight variations were observed in five recipients; there were three in one patient, two in a second, and one each in the three others. Death from technical complications resulted in three of the five patients (see text).

‡In four of the five patients a satisfactory result was obtained. In the fifth (OT 9), the smaller right hepatic artery thrombosed within 48 postoperative hours.

(Fig. 52C). Sufficient exposure could be obtained only after incision of the covering diaphragmatic crura. The proximal end of the graft aorta was ligated. A satisfactory result was obtained.

The other homografts with two arteries were used for adults. In one patient (OT 22) the larger left hepatic branch was removed in continuity with the celiac axis which, in turn, was anastomosed to the common hepatic artery of the host (Fig. 53). After blood flow was restored through this channel to most of the liver, the smaller right hepatic artery, which had originated from the superior mesenteric artery (Fig. 53A), was attached to the long splenic stump of the homograft celiac axis (Fig. 53B). Complete hepatic rearterialization was accomplished.

In the final patient (OT 4) a single arterial anastomosis of the graft celiac axis would have been feasible since a small left hepatic artery originated from the left gastric artery (Fig. 54A). Unfortunately the anomalous vessel was injured during donor hepatectomy. The accident was recognized and a repair carried out by an end-to-end anastomosis (Fig. 54B).

Variants of the Recipient Vasculature

These anomalies proved to be exceptionally dangerous. There were eight abnormal situations (Table 8) of which five were found in two patients. One child (OT 24) had an absent retrohepatic inferior vena cava, a single hepatic

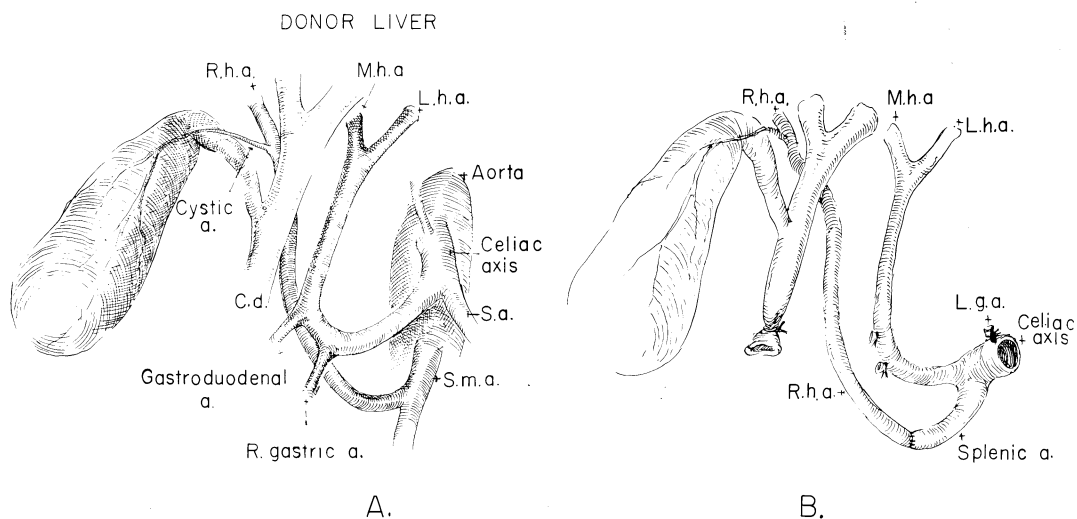


Figure 53. Method of vascular reconstruction used in patient OT 22 because of the presence of two homograft arteries. **A.** The anomalous arterial supply. **B.** The homograft celiac axis was anastomosed to the common hepatic artery of the recipient. After blood flow was restored through the larger left branch, the homograft splenic artery was attached to the small right hepatic branch. C.d. = common duct; L.g.a. = left gastric artery; L.h.a. = left hepatic artery; M.h.a. = middle hepatic artery; R.h.a. = right hepatic artery; S.a. = splenic artery; S.m.a. = superior mesenteric artery.

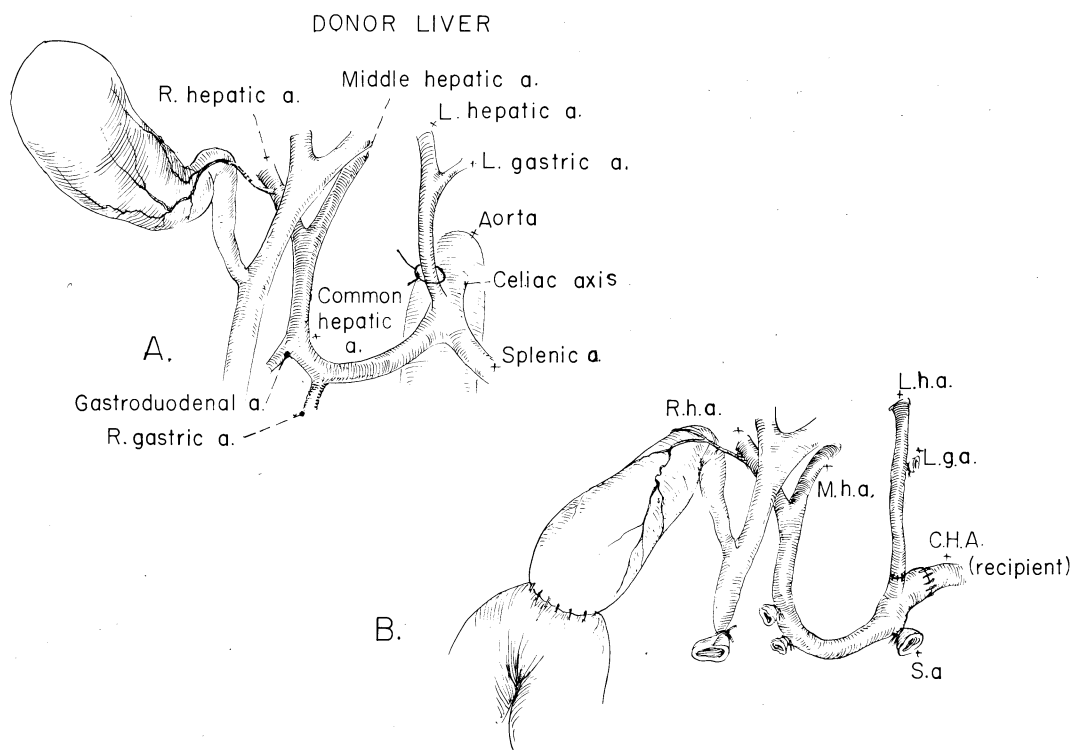


Figure 54. Arterial anomaly in patient OT 4. **A.** A small left hepatic artery arose from the left gastric artery. The variation was not recognized and the left gastric artery was ligated. **B.** An arterial supply to the lateral segment of the left lobe was restored as shown.

artery that arose from the superior mesenteric artery and passed anterior to the duodenum, and a portal vein that was also in front of the duodenum (Fig. 55A). The reconstructive procedure that was carried out involved mobilizing and rerouting the portal vein and hepatic artery. In so doing, the first portion of the duodenum was devascularized and had to be resected (Fig. 55B). Postoperatively the liver functioned poorly and the patient died after 11 days. At autopsy the homograft vessels were patent. However, the tortuous reconstructed hepatic artery was of tiny caliber (Fig. 56). It was suspected that poor hepatic blood flow had been obtained. The impression was supported by the finding of massive necrosis of the homograft (Chapter Twenty). In addition, the head of the pancreas was the site of fat necrosis and other findings of pancreatitis.

Another pediatric patient (OT 21), aged two years, had two diminutive hepatic arteries and a hypoplastic portal vein supplying her diseased liver (Fig. 57B). Because of the small size of the recipient arteries, an attempt was initially made to anastomose the homograft celiac axis to the host aorta. It was noted that the hepatic artery was compressed by the overhanging liver; consequently, the transplant hepatic artery was eventually connected to the larger of the two recipient vessels (Fig. 57B). Systemic heparinization was carried out. The arterial supply remained open, but the patient died 12 hours later of a portal vein thrombosis.

A similar arterial problem led to the death of another patient (OT 20). In this patient the celiac axis was also anastomosed to the recipient aorta because neither of the host arteries was of large caliber. Compression of the homograft blood supply by the caudate lobe of the transplanted liver apparently occurred (Fig. 57A). The patient died of acute hepatic insufficiency 15 hours later. The liver had undergone massive necrosis.

In two other patients the presence of a double hepatic artery (OT 3) or a hypoplastic inferior vena cava (OT 10) was not associated with unusual technical complications. In the first patient the larger host hepatic artery was of adequate caliber for anastomosis. In the second, the tiny host suprarenal vena cava (about 2 mm diameter) was anastomosed to the infrahepatic vena cava of the homograft. The vena caval orifice of the recipient at the diaphragm was of normal size, permitting the suprahepatic anastomosis to be performed in the standard way.

The Question of Size Discrepancy

Ideally, liver homografts would be obtained from donors of approximately the same size and age as the recipients. However, it has become clear that highly significant disparities do not preclude success. In Table 9 the body weight ratios are summarized for 26 consecutive orthotopic homotransplantations. There were 17 instances in which the donors and recipients were about physically equivalent, seven in which the donors were distinctly larger, and two in which the donors were very small. The most oversized organ was used for patient OT 16B, a two year old infant who weighed 11 kg; the homograft was

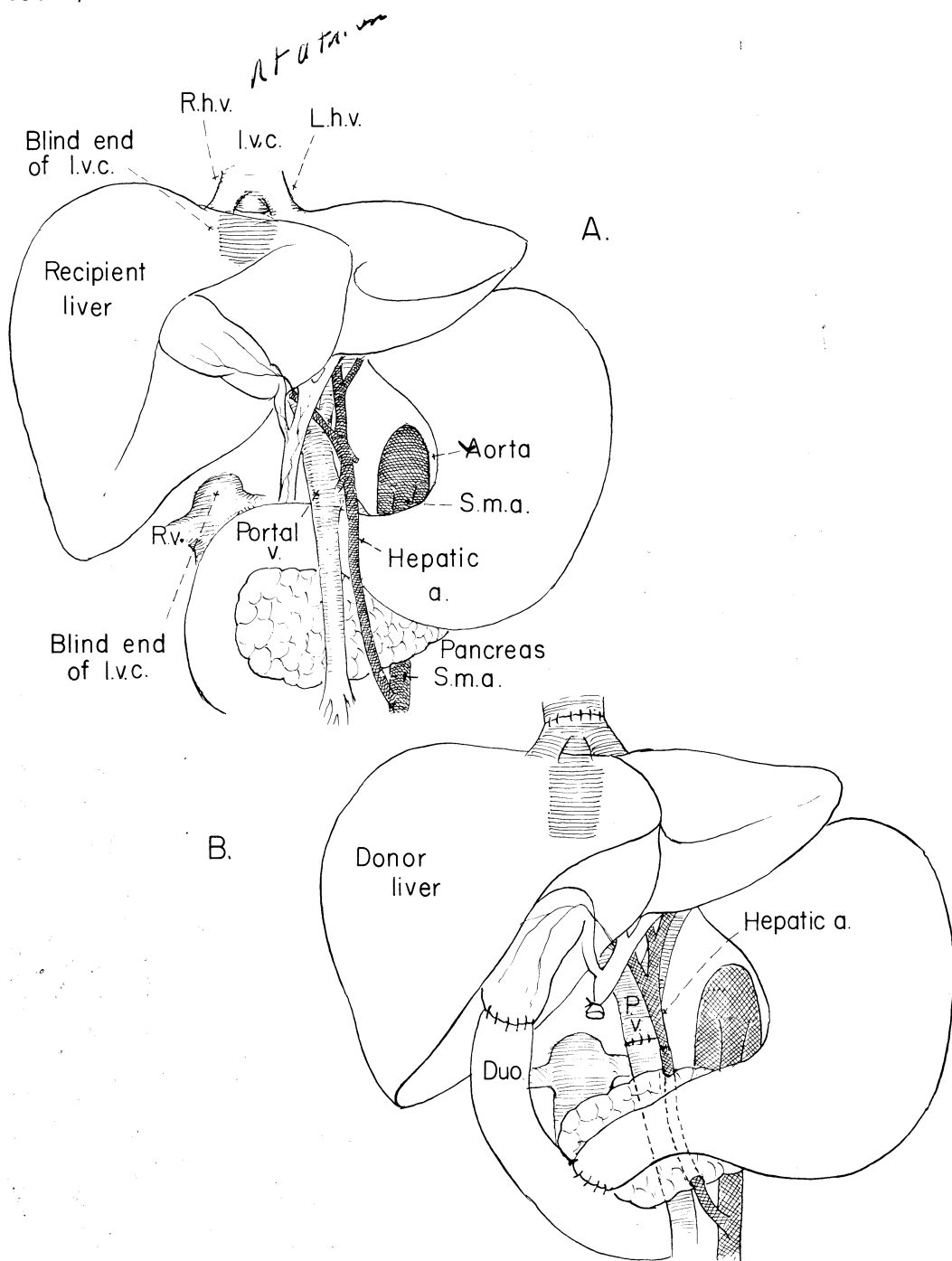
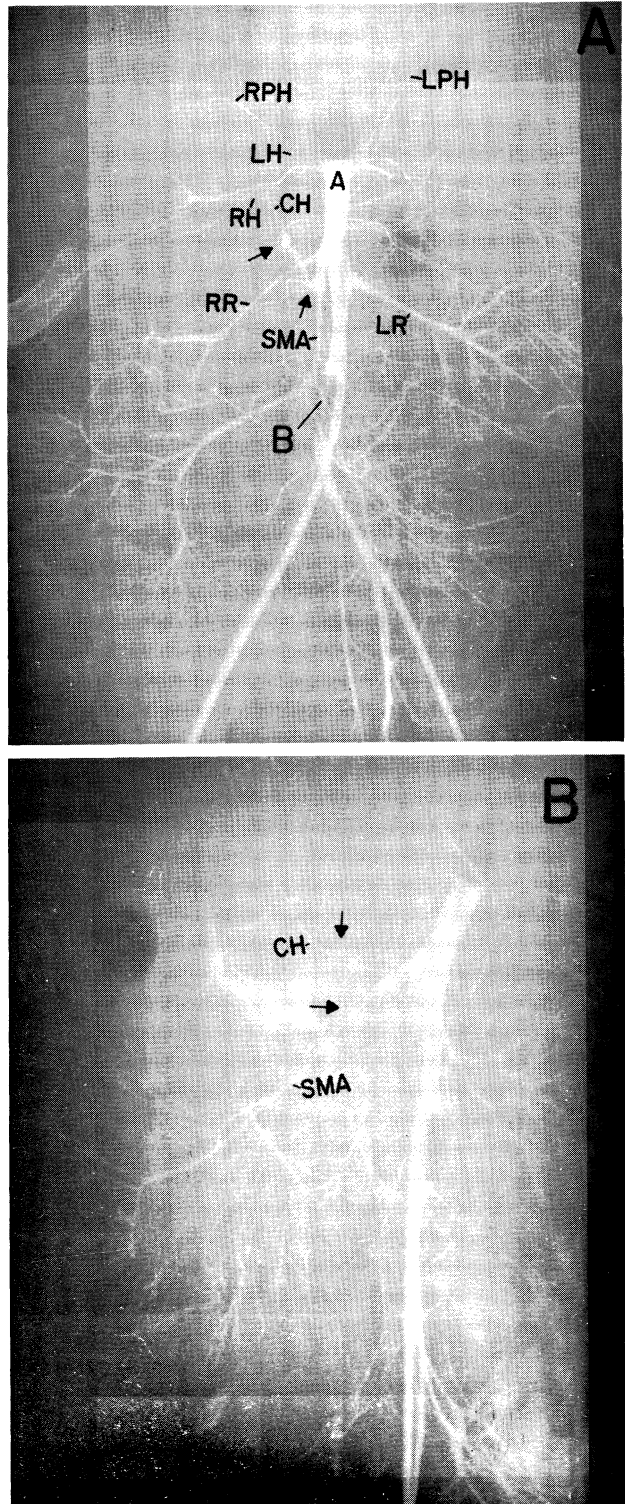


Figure 55. Multiple anomalies of recipient OT 24. The consequent technical problems of orthotopic transplantation are described in the text. I.v.c. = inferior vena cava; L.h.v. = left hepatic vein; P.v. = portal vein; R.h.v. = right hepatic vein; R.v. = renal vein; S.m.a. = superior mesenteric artery. A, The recipient anatomy. B, The technique of reconstruction (see text).

Figure 56. Autopsy aortogram in the patient (OT 24) whose multiple congenital anomalies are depicted in Figure 55. There was no celiac axis in the host and the common hepatic artery originated from the superior mesenteric artery. *A.* Anteroposterior view. The origin of the recipient hepatic artery is indicated by the lower arrow. Its anastomosis to the common hepatic artery of the homograft is at the upper arrow. Note the extremely small size of the reconstructed arterial channel, as well as the sharp angulation at the take off of the host hepatic artery. *B.* Lateral view. The upper and lower arrows indicate the same points as in *A.* *A* = aorta, *B* = catheter balloon, *CH* = common hepatic artery of graft, *LH* = left hepatic artery of graft, *LPH* = left phrenic artery, *LR* = left renal artery, *RH* = right hepatic artery of graft, *RPH* = right phrenic artery, *RR* = right renal artery, *SMA* = superior mesenteric artery.



obtained from a seven year old child whose weight was 25 kg. The most extreme opposite example was in Case OT 23, in which a 15 year old boy with a hepatoma had a 5500 gm liver replaced with the organ of a six year old 27 kg donor. The weight of the homograft was estimated to be 750 gm. Calne has recently reported a similar experience.⁴

At first glance the data compiled in Table 9 would suggest that the acceptance of excessively large donors was attended by an exorbitant risk. In actuality, this may not be true. Although prompt vascular calamities occurred in three of the seven cases in this category, two of the three accidents were probably attributable (Fig. 57) to the recipient anomalies described in the preceding section.

Table 9. Donor-recipient Weight Disparities in 26 Consecutive Orthotopic Liver Transplantations in 25 Recipients*

	NUMBER	SATISFACTORY REVASCULARIZATION	VASCULAR CALAMITY
Weights about equal	17	16	1†
Donor larger (1.5—2.3:1 ratio)	7	4	3‡
Donor smaller (< 2:3 ratio)	2	2	0

*Patient OT 16 received two homografts. The actual ages and weights for each patient are given in Chapter Twenty-four.

†The recipient was the one with multiple congenital anomalies (Fig. 55). At autopsy the vessels were patent but it was suspected that an effective blood flow had not been restored to the homograft (see text).

‡In two of these three patients there were anomalies of the recipient vessels (Fig. 57), which probably played the key role in precipitating the vascular calamities (see text).

Biliary Drainage

In four of our first five patients, a two-layer choledochocholedochostomy was performed, the inner row being with interrupted 6-0 chromic catgut sutures. A T-tube was placed through a stab wound in the recipient common duct and the upper limb passed proximally through the anastomosis (Fig. 58). The homograft gallbladder was removed. It was hoped to obtain a physiologically normal reconstruction. This method cannot, of course, be used in biliary atresia.

More recently, Calne^{3, 4} has also employed choledochocholedochostomy or, alternatively, cholecystocholedochostomy to effect bile drainage. The benefit of preserving function of the sphincter of Oddi for the prevention of subsequent cholangitis is probably a significant one, as will later be suggested in discussing infectious complications (Chapters Fifteen and Sixteen).

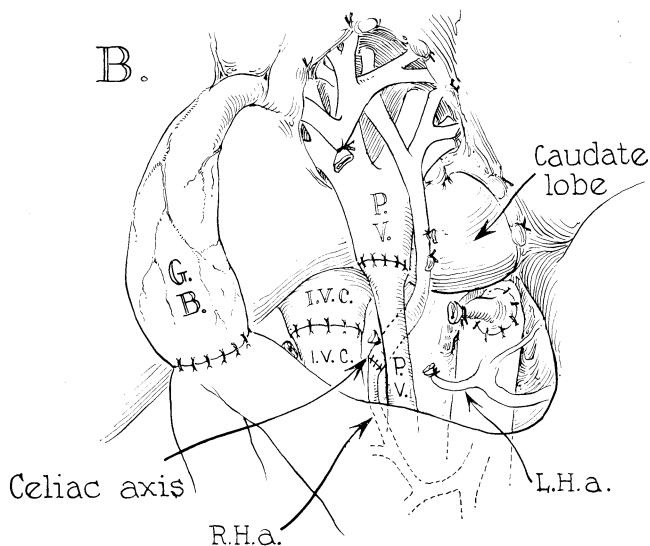
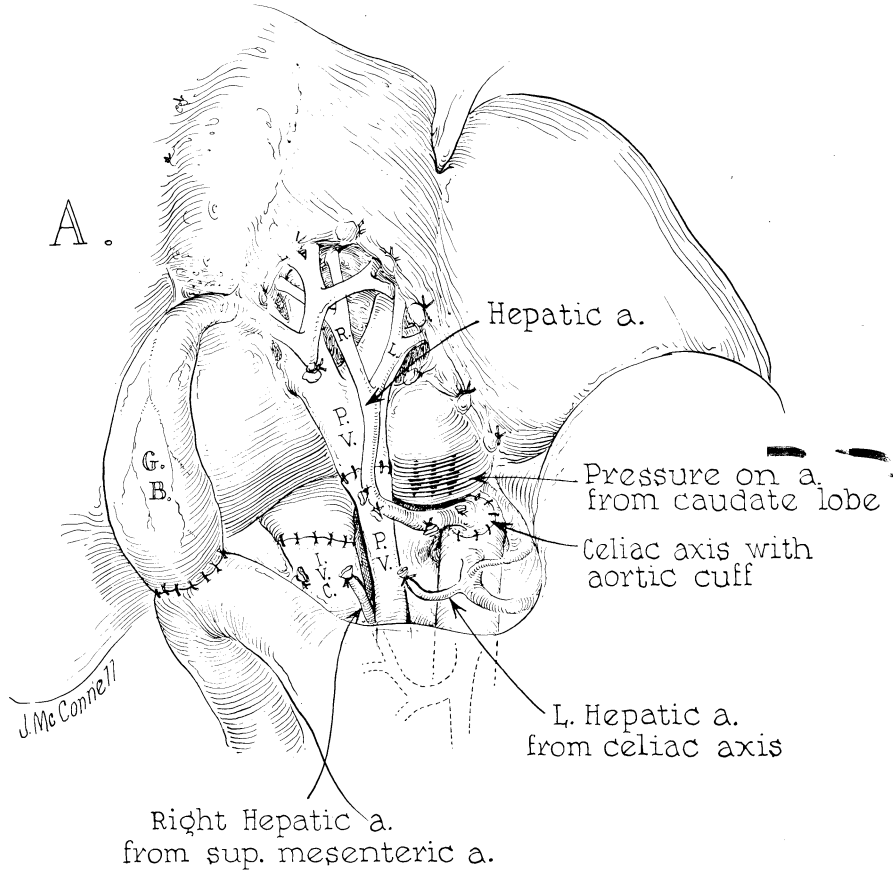


Figure 57. Anomalous hepatic arterial supply encountered in two pediatric recipients of orthotopic homografts. In each the larger right hepatic artery originated from the superior mesenteric artery; the small left branch came from the celiac axis. A, Attempted arterial reconstruction. The homograft celiac axis was anastomosed directly to the recipient aorta. Note the compression of the homograft arterial supply by the oversized homograft. This was not detected in patient OT 20 and resulted in death 15 hours later. B, In the second patient (OT 21) it was recognized that the above reconstruction would not be satisfactory; the homograft celiac axis was then anastomosed to the anomalous vessel originating from the superior mesenteric artery. However, the child died 12 hours later of portal vein thrombosis to which the size disparity of the portal vessels probably contributed; the recipient portal vein was hypoplastic. (By permission of Grune & Stratton, Inc., 1969.)

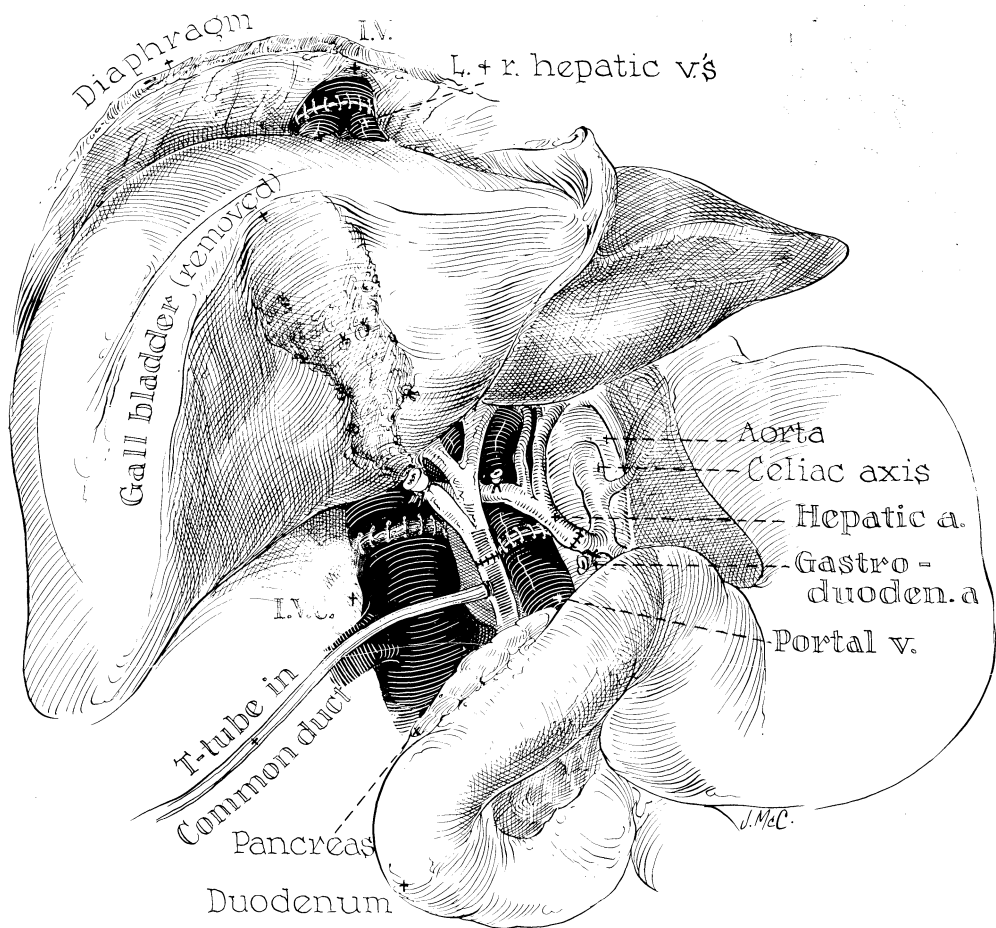


Figure 58. Method of biliary tract reconstruction used for patients OT 2 to 5. A choledochocholedochostomy was performed with an inner layer of 6-0 chromic catgut, reinforced with an outer row of 6-0 silk. A T-tube was placed through the recipient portion of the reconstructed duct. The gallbladder was removed.

However, there are important disadvantages of choledochocholedochostomy. One is the necessity of leaving a tube prosthesis and a drain in a patient who must be treated with immunosuppressive agents, and in whom the duct reconstruction must be placed in close proximity to the vascular anastomoses of the hepatic artery and portal vein. Also, there may not always be an adequate blood supply to the distal portion of the homograft common duct which normally receives its principal arterialization from retroduodenal sources,¹⁴ and which now depends on retrograde flow from arteries in the central hilum. One of our early patients died from slough of the distal common duct and consequent bile peritonitis (OT 5).

In all cases subsequent to OT 5, biliary drainage has electively been with cholecystoduodenostomy after ligation of the distal common duct of the homograft. A two-layer anastomosis was used with an inner mucosal row of continuous 4-0 chromic catgut and an external layer of interrupted 5-0 silk

through the serosa and submucosa (Fig. 52). In a few patients, a Kocher maneuver was needed to obtain adequate duodenal mobility.

The technique of biliary tract drainage had to be modified in two patients. The variation in one (OT 24) was described before (Fig. 55). In the other patient (OT 17) the change was necessitated by a concomitant gastric resection. A hepatoma which had been diagnosed three months previously had invaded the pylorus and antrum. Two thirds of the stomach was removed; the gallbladder was anastomosed to the second portion of the duodenum and gastric drainage provided by a posterior gastrojejunostomy (Fig. 59). Bilateral truncal vagotomy was added.

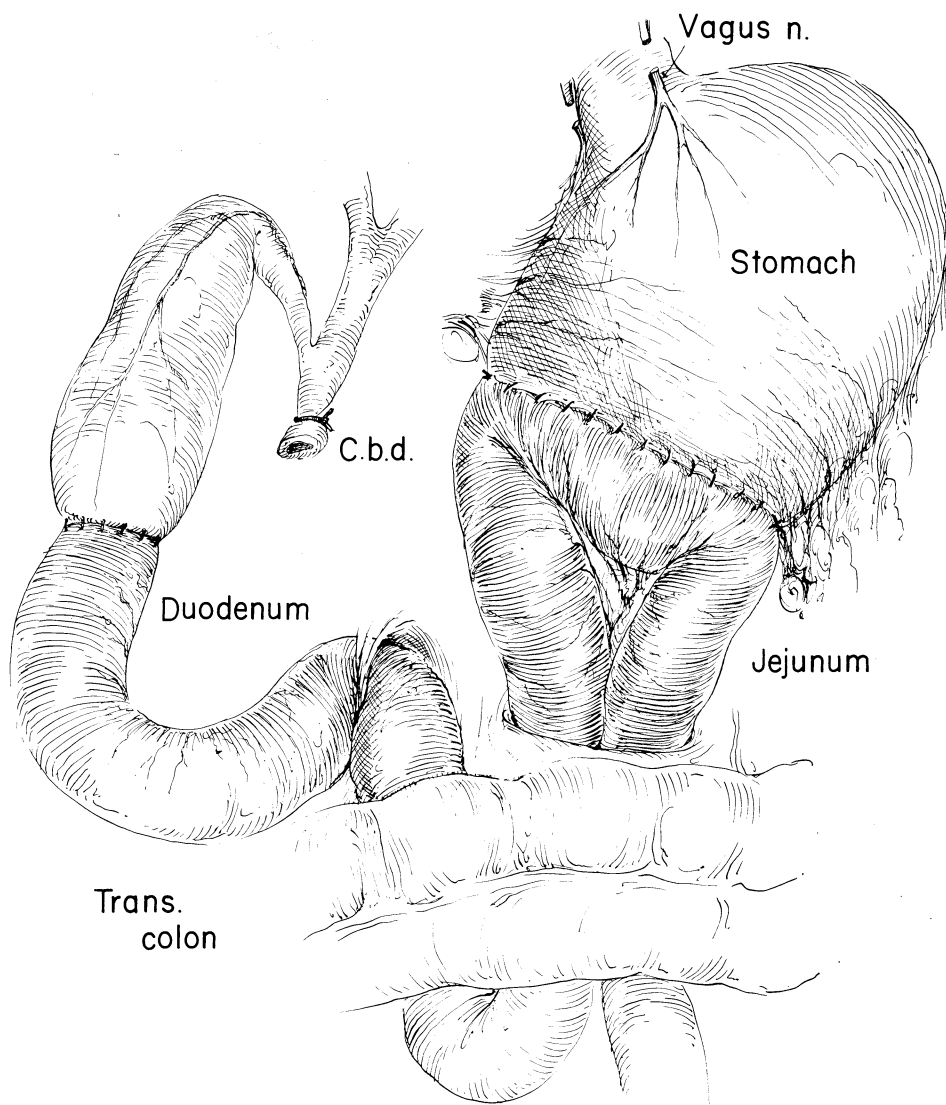


Figure 59. Method of reconstruction used in patient OT 17. The modification which included gastrectomy was necessary because of invasion of the stomach by the hepatoma. Before closing the abdominal incision, the gastrojejunostomy was drawn down and sutured circumferentially to the transverse mesocolon, eliminating the potential defect.

In one of our more recent recipients (OT 25), a primary cholecystoduodenostomy failed because of the accidental production of complete biliary obstruction. Re-exploration was carried out three days later and a conversion made to a choledochcholedochostomy. The case will be presented in detail in Chapter Nine.

Operative Staging

In four of our first patients (OT 2 to 5) the recipient procedure was done in two stages. At a preliminary operation the major structures above and below the liver were skeletonized. The incision was then closed and patient taken to the recovery room with provisions for return to the operating room at a moment's notice. In each instance, a desperately ill potential donor was in the hospital. Fonkalsrud⁸ has used the same kind of double operation.

At the time this approach was used there were no effective means of organ preservation. The staging was, of course, designed to shorten the interval between donor death and graft revascularization in the recipient. Within a few minutes after donor death the recipient incision could be reopened and the diseased liver removed.

The need for such urgency no longer exists, not only because of improvements in conservation techniques (Chapter Five), but also because of the emerging new criteria to define donor death (Chapter Two). This is fortunate since the two closely spaced recipient operations were poorly tolerated. Moreover, it was impossible to predict the time of complete circulatory arrest in the donor with any degree of certainty. In one of our cases, the interval between the first and second operation was 22 hours. In the others, it was from two to 14 days.

Liver Fixation

In animals the supporting ligaments of the liver are not reattached. A seal quickly forms between the homograft and the diaphragm. Torsion of the liver has never been observed in our experience after orthotopic transplantation in either pigs or dogs. The omission of this step in patients may have serious consequences, as will be discussed in Chapter Fifteen, probably because of the assumption of an upright position by humans. Consequently, in all of our later cases, the liver homograft was firmly reattached to the diaphragm.

The falciform ligament is the easiest structure to work with in both the homograft and the patient; the cut edges are firmly sewed together with continuous 3-0 chromic catgut. Less complete reunion of the left triangular ligament can also usually be accomplished in the same way (Fig. 60). A continuous suture cannot be used for the coronary or right triangular ligaments, but tags of these structures can usually be tacked with figure of eight sutures to portions of the retroperitoneal raw area.

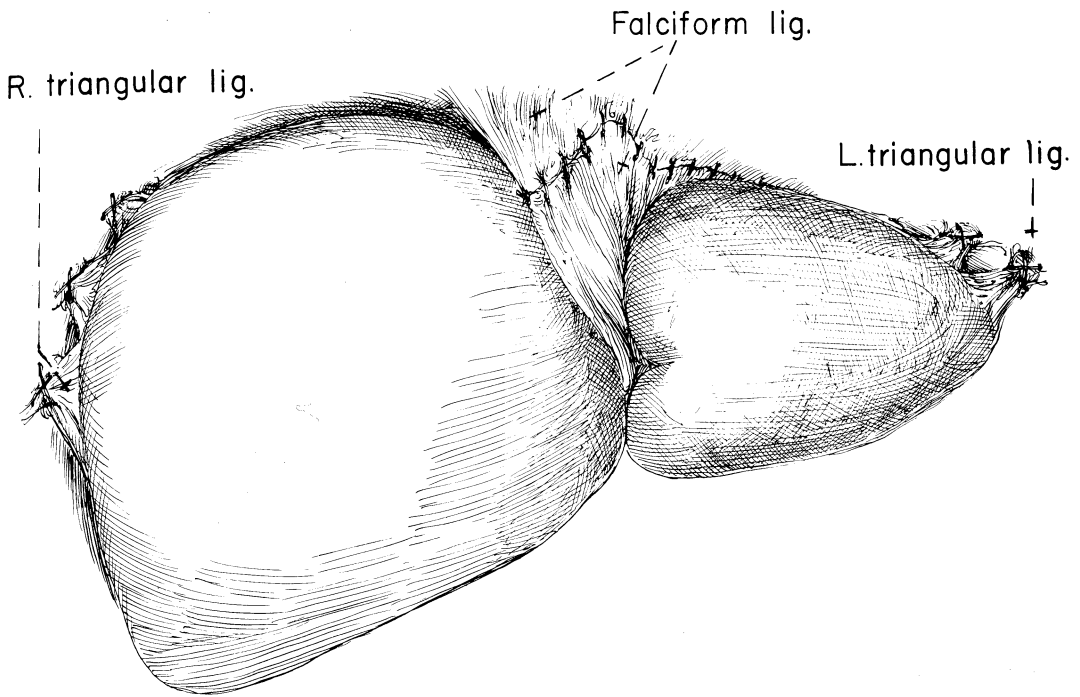


Figure 60. Resuture of the suspensory ligaments of the transplanted liver.

Splenectomy

Eighteen of the 25 patients treated with orthotopic liver transplantation have also had splenectomy. This procedure has been used in our institutions for recipients of renal homografts for a number of years, although it has not been proved that it is a worthwhile adjuvant immunosuppressive measure. The rationale for splenectomy, which has been discussed at length elsewhere,^{17, 28} is based primarily upon the demonstration in experimental animals that the response to intravenous antigens is attenuated after splenectomy. A second possible benefit could be that a restraining influence upon the peripheral white blood count might be removed by this procedure, thereby permitting better dose control of azathioprine.

In many recipients of liver homografts, particularly children with biliary atresia, there are less esoteric justifications for splenectomy. The organs are often extraordinarily large. In the children with biliary atresia in our series the spleens weighed from 93 to 415 gm. It was thought desirable to remove them for purely mechanical advantages. Moreover, secondary hypersplenism was thought to exist before transplantation in these children as well as in some of the adults with hepatoma superimposed upon cirrhosis. Preoperative leukopenia and thrombocytopenia were common in such cases.

In all instances in which it was performed, the splenectomy was carried out as the last step in the operation. By so doing, the portal hypertension had already been partially relieved by decompression through the newly placed

homograft. Difficulties with venous bleeders in the splenic bed were thereby minimized.

Splenectomy was omitted in seven patients (OT 1, 3, 4, 5, 14, 22, and 24) because the operation had already been too traumatic or because the presence of a bleeding diathesis made the undertaking too hazardous.

Closure

Drains were not used except in the early cases in which biliary reconstruction was with choledochocholedochostomy. The wounds were closed with interrupted fine silk technique. Stay sutures were not used.

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Chapter Nine

INTRA- AND POSTOPERATIVE COMPLICATIONS AND CARE

Much of the remainder of this book will be preoccupied with the therapy that is necessary to prevent rejection of homografts without at the same time causing death by overcomplete crippling of the host immunologic defenses. The risks thereby introduced are probably greater (Chapters Fourteen to Sixteen) than those which must be borne by recipients of other organs such as the kidney, heart, or lung.

There are, in addition, a number of other hazards with hepatic transplantation, many of which derive from the complicated surgical requirements for successful replacement of the whole liver as well as from the metabolic derangements that can follow even after a technically perfect operation. In this chapter, the incidence, treatment, and prevention of these essentially nonimmunologic complications will be described.

THE INTRAOPERATIVE PERIOD

Earlier the complexities of anesthetic management during and after the anhepatic phase were described. The rapid and potentially life-threatening metabolic changes during this interval could be accurately corrected only with the aid of frequent measurements of several biochemical indices. This aspect of the intraoperative care (Chapter Seven) will not be mentioned further except to draw attention to its importance in preventing sudden death or permanent brain injury on the operating table; instead, the following section will be concerned with a variety of technical problems.

Special Problems

Hemorrhage. It is not surprising that bleeding has often been difficult to control during and after orthotopic transplantation. Some of the causative factors such as portal hypertension may be mechanical, secondary to the pathology

afflicting the liver which is removed (Chapter Eight). Others are due to the decreases in the effectiveness of clot formation (Chapter Ten), which are roughly proportional to the magnitude of ischemic injury to the liver (Chapters Six and Ten). The result may be diffuse oozing from all raw surfaces. When a bleeding diathesis develops, the diagnosis usually becomes clinically evident within 30 to 60 minutes after revascularization, in some cases after an initial period of completely adequate hemostasis.

Unless the new liver has been irreparably damaged, the hemorrhage will often cease spontaneously without any specific therapy. It is important to quickly survey the entire operative field for major bleeding sites, particularly at the anastomoses. If localized hemorrhage cannot be found and controlled by ligature or suture, the best policy is usually to place the homograft in the liver fossa and avoid all manipulation for a half hour or longer. As the portal hypertension is relieved through the new liver and the organ resumes elaboration of clotting factors, the wound may become dry.

If this does not happen, the fact that a coagulation disorder may be present should not obscure the possibility that there might be a defect in the vascular system of the homograft in some inaccessible location. In several cases we have found an untied tributary in the posterior wall of the retrohepatic vena cava, and in another there was a leak in the posterior part of the suprahepatic vena caval anastomosis.

Continuation of hemorrhage from multiple locations after these maneuvers have been completed is an ominous sign. Exhaustive efforts must then be made at mechanical hemostasis of capillary bleeders with suture ligatures and cautery. As a last resort, thrombogenic agents can be given (Chapter Ten) despite the risk that these may contribute to later thrombotic complications.^{21, 22} This kind of case may gradually take on a nightmare quality when it is realized that bleeding cannot be controlled sufficiently to permit closure of the incision with any hope of survival. Two such patients (OT 5 and OT 6) required 12 and 18 hours of continuous effort, respectively, before their operations could be successfully concluded. One of our recipients (OT 1) bled to death on the operating table. The same complication has been reported from other centers.^{8, 10, 11}

Total Arterial Thrombosis. There have been two examples of main hepatic artery thrombosis within a few minutes after homograft revascularization. One was in our series (OT 18) and the other was reported to us by Dr. Allan Birtch of the Peter Bent Brigham Hospital in Boston.⁵ The events in the two cases had certain similarities.

The circumstances of donor death were unusually advantageous for the procurement of minimally damaged organs since total body hypothermia was being used in an attempt to treat the ultimately lethal brain injury. In both cases the recipient was brought to the operating room and had mobilization of the diseased liver while the donor still had an effective circulation and was being maintained on a respirator. When ventilator support was stopped, the donors died within a few minutes. Their livers were further cooled by infusion (Chapter Five), quickly removed, and immediately transplanted. Both homografts were thought to have sustained almost no ischemic damage. They

promptly excreted large volumes of bile. The wounds were noted by the surgeons in both institutions to be remarkably dry. However, within an hour the hepatic arteries had clotted at the sites of anastomoses. The livers, which were now perfused only with portal flow, became cyanotic.

Reanastomosis was performed. In the Brigham case, excision and reconstruction resulted in a second thrombosis. The right kidney of the 16 year old recipient was then removed and a renal to hepatic artery anastomosis performed. In our patient, who was 12 months old, the anastomosis was locally excised and reconstructed. Unfortunately, heparin therapy was not instituted in either case, although the Boston patient was given dextran. The vessels of both homografts reclotted during the early postoperative period with eventual disastrous consequences.

Complete thrombosis of the homograft arterial supply leads to the death of canine and porcine recipients within a few hours. The effects in the two patients just described were much more indolent and they were slow enough in evolution so that corrective surgery might have been possible had the diagnosis been made promptly. In the Boston case the rethrombosis was proved by aortography on postoperative day 21, several days after partial necrosis of the left liver lobe had already occurred and had become the focus for a gram negative septicemia. It was suspected that the dearterialization had occurred at a much earlier time, probably within 36 hours after transplantation. Except for early high increases in the transaminases, the hepatic function tests were disturbed very little at any time during postoperative life. Furthermore, the dearterialized liver visualized well with radioisotope scanography, as might have been predicted from the experimental studies of Groth.¹³ Eventually the left upper abdomen was re-explored and the partially necrotic liver was drained. The patient died six and a half weeks after transplantation with widespread sepsis. At autopsy there were scattered small mature infarcts in the periphery of the right lobe in addition to the drained septic infarct on the left. The great bulk of hepatic parenchyma was viable and structurally intact.

The downhill course of our patient (OT 18) was more rapid but illustrated some of the same points. After the transplantation she was returned to the intensive care unit in excellent condition at one o'clock in the afternoon. During the day she had hypertension, with systolic blood pressures of 150 to 200 mm Hg, and was wide awake. At 6:15 P.M. she was weighed on a bedside scale, and immediately afterward was noted to be somnolent and to have a systolic pressure of 80 mm Hg (Fig. 61).

Reclotting of the hepatic artery was suspected, but the serum transaminases then and at six-to 12-hour intervals for the next 24 hours were only slightly elevated. However, at 10:00 P.M. the following evening both the SGOT and SGPT suddenly rose to more than 10,000 units (Fig. 61). A liver scan showed almost no isotope concentration in the area of the liver. It was concluded that the opportunity to restore the arterial supply had been lost by procrastination, that massive necrosis had already occurred, and that the only hope of survival would be the transplantation of a second graft. A donor could not be found and the child died two and a half days later. Blood cultures just before death contained *Escherichia coli*.

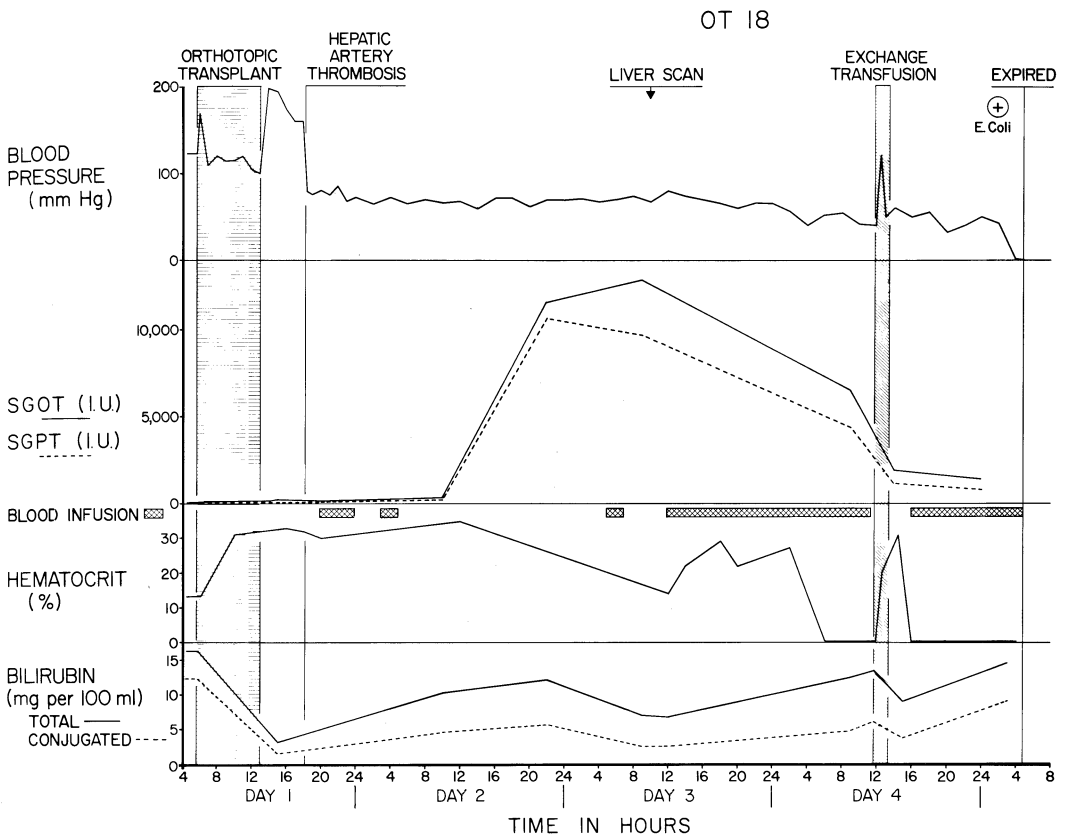


Figure 61. The course of a one year old child (OT 18) with extrahepatic biliary atresia who was treated with orthotopic liver transplantation. The hepatic artery of the homograft apparently underwent thrombosis within a few hours after operation, but this fact was not reflected in transaminase rises until almost a day later. The eventual drop of the hematocrit to almost zero was due to massive hemolysis. (By permission of Grune & Stratton, Inc., 1968.)

The infant's original disease was biliary atresia. Before operation her serum bilirubin was 16.2 mg per cent. This fell to 3.2 mg per cent and then rose progressively until death (Fig. 61). The significance of the deepening jaundice was obscured by hemolysis which began on the second postoperative day and which eventually was so overwhelming that the hematocrits fell to zero during the last 24 hours of life (Fig. 61); on peripheral smear it was impossible to find a single intact red cell except just after an isovolumetric exchange transfusion. At autopsy there was massive but incomplete necrosis of the liver (Chapter Twenty), which had a thrombosed artery and an intact portal vein. There was also widespread blood pigment deposition throughout all tissues.

Study of these two cases does not provide clear guidelines for the early detection of hepatic artery occlusion, although in our patient the sudden changes in peripheral vascular dynamics and in sensorium should have made the diagnosis possible had not a judgment error been committed. It is clear from this experience that tests such as the serum transaminases or liver scans can remain normal or at least nondiagnostic until the situation has become irreversible. It is likely that aortography will prove to be the only really decisive

way of consistently establishing the diagnosis while there is still time to attempt repair, providing this procedure is done at the first suspicion of a vascular accident. In turn, surgical restoration of arterial flow is mandatory if there is to be hope for long survival. Although hepatic arterial ligation is surprisingly well tolerated in both cirrhotic^{1, 14, 16} and noncirrhotic^{1, 7, 12} patients, this cannot be expected to be equally true early after orthotopic hepatic transplantation.

It is ironic and probably more than coincidental that these two vascular accidents occurred in patients who received superb liver grafts and who probably lost the "auto-anticoagulation" effect which follows hepatic injury. The same thing had been previously noted in laboratory experiments.⁶ With increased experience and with improved immunosuppression it was observed that there was a rise in the incidence of arterial thrombosis after orthotopic transplantation of the canine liver. Under optimal clinical circumstances of organ procurement and preservation it may, therefore, be appropriate to consider prophylactic anticoagulation after human liver transplantation. Certainly, if intravascular clotting is demonstrated during operation, as was the case in the patients just described, heparin therapy should be started at once.

Lobar Arterial Thrombosis. A homograft with two hepatic arteries underwent partial gangrene within 48 hours postoperatively, when the anastomosis of the smaller right branch thrombosed. The case (OT 9) is more fully described in Chapters Eight, Fifteen and Seventeen. Survival was for four and a half months.

Nonthrombotic Arterial Occlusion. In Chapter Eight, two cases were cited in which vascular anomalies in the recipients necessitated the use of nonstandard implantation techniques. In one of the patients (OT 20), the hepatic arterial supply was apparently pinched off, although it did not clot (Fig. 57A, Chapter Eight); death followed 15 hours later as the result of acute hepatic insufficiency, including an uncontrollable bleeding diathesis. The other pediatric recipient (OT 24) who had complex congenital malformations of the upper abdominal viscera (Fig. 55, Chapter Eight) probably had poor blood flow through a kinked hepatic artery (Fig. 56, Chapter Eight). She died of hepatic insufficiency after 11 days; at autopsy the homograft was necrotic.

Portal Vein Thrombosis. In the child (OT 21) who died 12 hours after operation from this complication, anatomic anomalies of both the recipient portal vein and hepatic arteries probably played an important role (Fig. 57, Chapter 8).

Complete Biliary Obstruction. The only patient in our series who received an orthotopic liver transplantation for Laennec's cirrhosis (OT 22) died as the result of a technical error. The cystic and common ducts of the homograft were fused, being separated by a septum. The common duct ligature occluded both lumens and prevented the passage of bile into the gallbladder (Fig. 62).

Postoperatively, jaundice inexorably developed; other indices of hepatic function were initially adequate (Fig. 63). Although the correct diagnosis was entertained, re-exploration was not carried out until 10 days later when a second cadaveric donor became available. The patient was desperately sick by this time and died on the operating table.

An opportunity was lost in this case to determine if the hepatorenal syndrome could be reversed by the provision of a new liver. During the days pre-

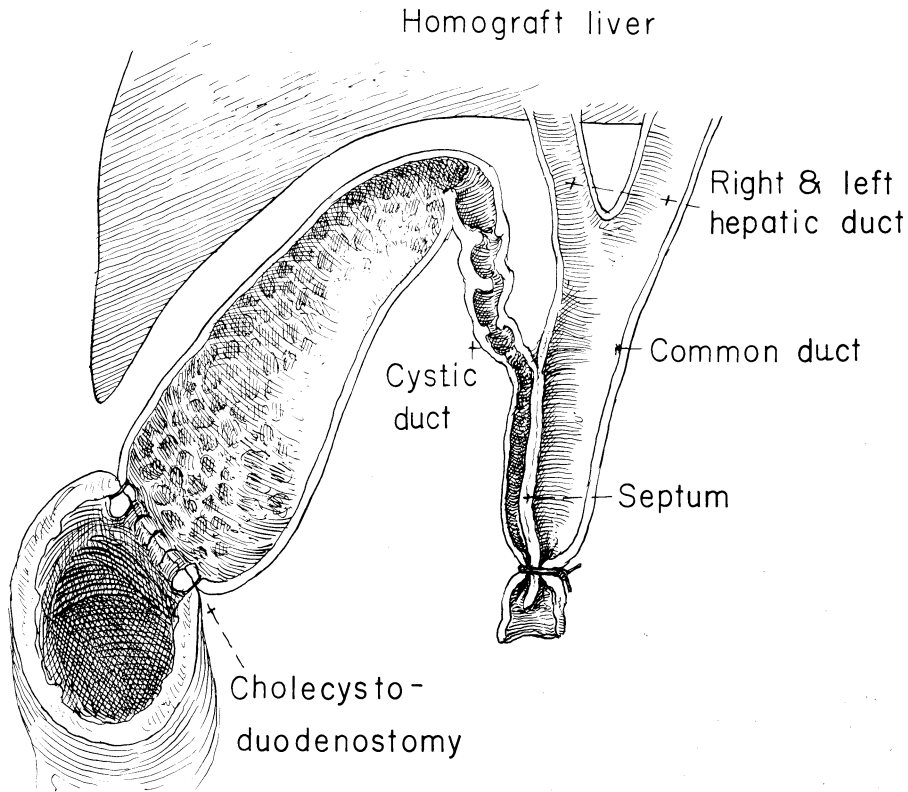


Figure 62. The anatomic basis for a technical error which cost the life of patient OT 22. Distal ligation of the double-barreled extrahepatic duct system resulted in total biliary obstruction.

ceding operation the patient had become oliguric and azotemic. After the transplantation renal function did not improve (Fig. 63).

A second patient (OT 25) was also lost because of a similar unrecognized anomaly. In this case, the homograft cystic duct passed behind the common duct and descended for almost two inches in the relationship shown in Figure 64. The obstructing ligature was placed just at its entry into the common duct.

The resulting pattern of the early postoperative course was almost identical to that of the earlier recipient in that the biochemical changes were highly suspicious of obstruction. Re-exploration was carried out after three and a half days. The gallbladder was removed, the duodenotomy was closed, and biliary drainage was re-established with a choledochcholedochostomy (Fig. 64B). The anastomosis was performed with interrupted 6-0 and 7-0 silk in bites that just stayed outside the mucosa. The cystic duct portion of the double lumened homograft structure was incorporated into the posterior wall of the suture line (Fig. 64). Neither a T-tube nor any other kind of internal splint was employed.

During the next few days there was marked clearing of the hyperbilirubine-mia. Jaundice then deepened again, apparently as the consequence of rejection. The immunologic process was controlled, but two weeks after the secondary procedure a bile fistula and peritonitis developed. The patient died 39 days after transplantation.

OT 22

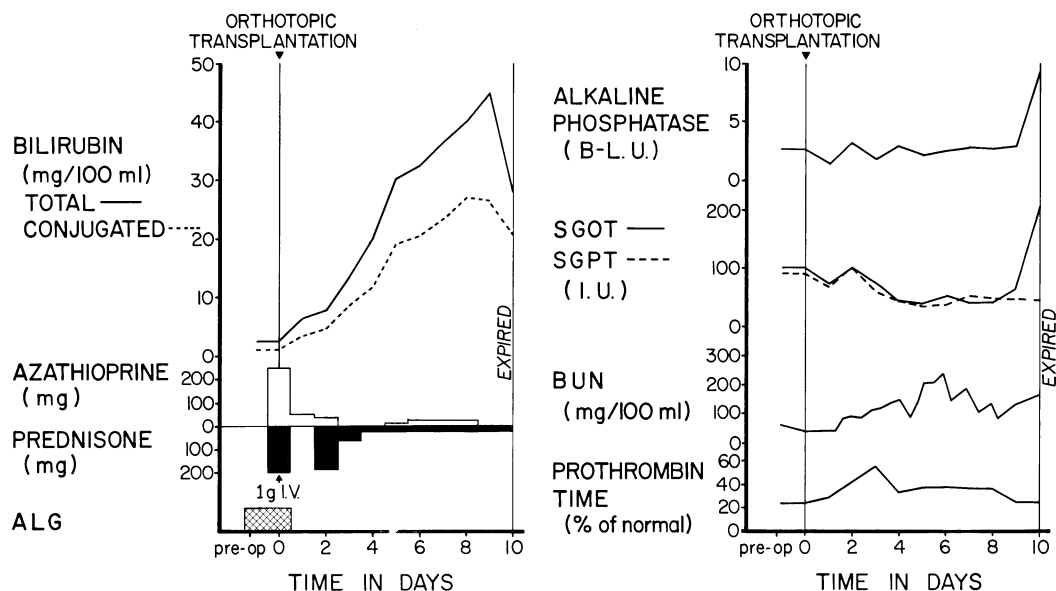


Figure 63. The course of a patient (OT 22) whose biliary duct system was completely obstructed by the accident shown in Figure 62. The alkaline phosphatase values are in Bessey-Lowry units (normal 1 to 3).

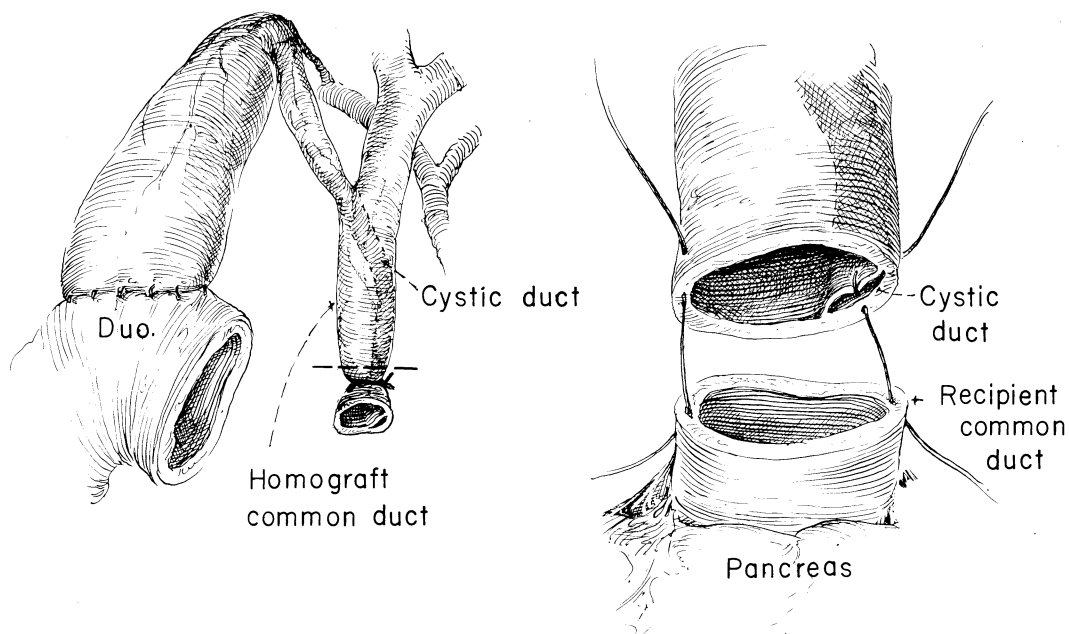


Figure 64. A. An anomaly encountered in patient OT 25. The cystic duct passed posterior to the common duct and descended for almost 2 inches as one compartment of a double-barreled lumen. The distal ligature caused total biliary obstruction. B. Three and a half days later the gallbladder was removed and biliary continuity restored with a choledochocholedochostomy.

Air Embolism. In several recipients tears were made in the inferior vena cava or its tributaries during mobilization of the diseased liver. During repair of the defects the anesthesiologist was asked to maintain continuous positive pressure ventilation. In spite of this precaution, air bubbles were observed to enter the venous system and pass into the thorax.

Only one patient (OT 23) had aftereffects which might have been attributable to this complication. Immediately after the operation he had diffuse weakness of both legs, with the predominant involvement being of the muscles supplied by the peroneal nerves. Ankle braces were required for several months to permit ambulation. It was speculated that the air might have passed to the systemic arterial circulation via a small patent foramen ovale. However, at autopsy 143 days later, the atrial septum was intact.

Adrenal Infarction. In removing the recipient liver and the enclosed segment of vena cava, the right adrenal vein must be sacrificed (Chapter Eight). The adrenal gland underwent venous infarction in two of our patients. The necrotic organ was discovered at autopsy 10 days after operation in patient OT 7. In the other case (OT 17), the congested gland ruptured 24 hours after transplantation, causing a massive intra-abdominal hemorrhage. An emergency right adrenalectomy was performed.

Right Diaphragmatic Paralysis. Four of our patients, all children (OT 8, 9, 10, and 16), were found after transplantation to have paradoxical movement of the right hemidiaphragm. The finding, which had not been present at preoperative fluoroscopy, was thought to have resulted from crushing of the right phrenic nerve by the vascular clamp which was applied to the suprahepatic inferior vena cava (Fig. 65). The diaphragmatic paralysis (Fig. 66) persisted for ten, seven, two, and six weeks, respectively. In one patient (OT 8), failure of the right upper pulmonary lobe to expand prompted its removal during the fourth postoperative week. In most cases the complication can be avoided by developing a longer host vena caval cuff and by taking care not to include any diaphragm in the bite of the clamp.

Intestinal Injury. During removal of the liver of several patients with biliary atresia, bowel trapped at the site of previous hilar dissection was perforated. Rents in both small intestine and colon were immediately closed in two layers. They healed without complication.

THE POSTOPERATIVE PERIOD

Acute Problems

The first several hours after return from the operating room must be considered as an extension of the anesthesia time and, accordingly, this interval is considered in detail in Chapter Seven. The patient is ordinarily kept on a mechanical ventilator until he resists this kind of support. The endotracheal tube is not removed until the adequacy of spontaneous respiration has been established by serial blood gas studies which can be obtained from the

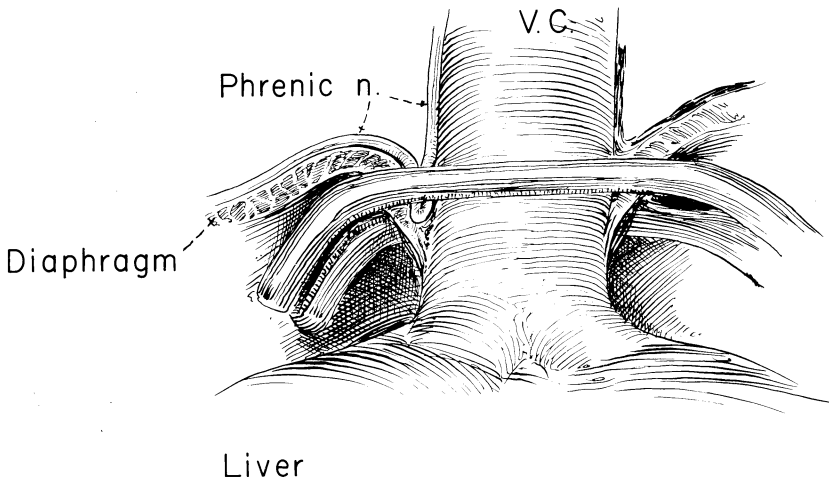


Figure 65. Probable mechanism of operative injury of the right phrenic nerve in four pediatric patients. Note the inclusion of the nerve in the bite of the vascular clamp, which has been placed across the suprahepatic vena cava and which has also included a piece of diaphragm.

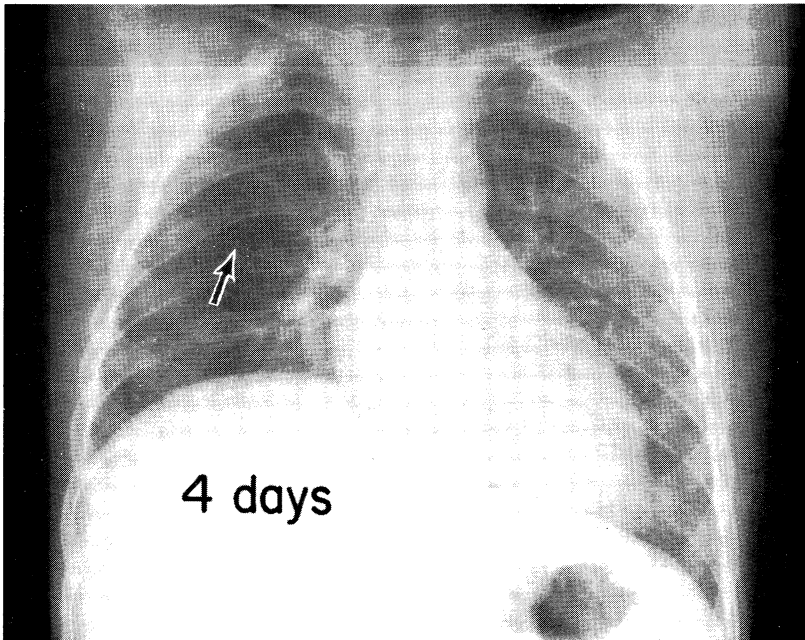


Figure 66. Paralysis of the right diaphragm four days after orthotopic liver transplantation in a 19 month child (OT 8). The arrow indicates the transverse fissure, which is displaced superiorly.

indwelling radial artery catheter. In the meanwhile the fluid replacement described later is stabilized.

Continued Respiratory Care. There are many reasons why tracheobronchial care has overriding significance in recipients of orthotopic liver homografts. First, the operation is a long and difficult one which usually involves considerable trauma to both diaphragms. Second, it has been speculated¹⁹ that the placement of a foreign organ in the subphrenic spaces might predispose to fluid accumulation or other sympathetic reactions in the overlying thorax; pleural effusions or lower lobe atelectasis have been seen in several of our patients. Finally, the patient with serious liver disease can be expected to go to the operating room with a variety of easily measurable cardiorespiratory handicaps, which have been summarized by Bashour, McConnell, and Miller.³ These can include a loss of vital capacity, an increased physiologic dead space, an inefficient kind of hyperventilation, and large volume venoarterial shunting with consequent peripheral oxygen desaturation. Fortunately at least some of these abnormalities are rapidly corrected by successful liver replacement. For example, the V-A shunts have been shown to close, at least functionally, within hours or days after transplantation in children with biliary atresia.¹⁸

The recipient should be fully awake by the time of extubation and capable of voluntary coughing. The slightest unwillingness to do so is an indication for vigorous nasotracheal suctioning. This can be done in the semi-Fowler position. Except for this concession, the patients are kept in a supine position for the first 24 to 48 hours. Humidified oxygen is given by face mask or through a nasal tube.

There may be special problems in very young children who cannot follow commands, and in whom effective nasotracheal suction may be difficult. In such cases catheters have been inserted into the trachea by direct laryngoscopy every three or four hours. To minimize upper airway trauma and irritation, continuous gastric intubation has been avoided. Instead, a tube is passed into the stomach every two hours and then immediately removed after the completion of aspiration. After one or two days postural drainage exercises are begun.

Several acute respiratory problems were encountered in our series. The four right phrenic nerve injuries were mentioned earlier in this chapter. One other patient (OT 11) developed a left pneumothorax which was treated with catheter drainage; the source of the air leak was never determined. Several patients required the needle aspiration of serous pleural fluid. Bronchoscopy was performed in two patients (OT 14 and 17), because of persistent segmental atelectasis in the lower lobes.

Two patients had very troublesome laryngotracheal complications. One was an infant (OT 11) who was not known to have had previous feeding problems. After transplantation, aspiration occurred with every meal until it was found that this could be prevented by keeping her in a chalasia chair. The difficulty spontaneously resolved in two weeks. The other patient (OT 14), a 16 year old girl, developed a circumferential granuloma in the subglottis which was thought to have resulted from prolonged endotracheal intubation during and after operation. When she developed a high degree of airway obstruction, the

area was given 1800 R local irradiation over a one-week period which resulted in amelioration of the symptoms.

Pulmonary sepsis eventually developed in almost all our first liver recipients (OT 1 to 7) and either caused or contributed to the fatal outcome. There were apparently a number of factors which played a cumulative role in the genesis of these complications (Chapters Thirteen and Sixteen), of which overimmunosuppression and use of badly damaged homografts were probably the most important. Patients treated subsequently (OT 8 onward) were less susceptible to pulmonary infection.

Intravenous Therapy and Alimentation. The first seven recipients of orthotopic livers (OT 1 to 7) either never could take a full diet or were able to do so for only brief periods. The consequent difficulties with fluid therapy, particularly when secondary renal failure developed, were recounted in Chapter Six. In contrast, most of the later patients who survived operation without serious technical complications did not require parenteral fluids for more than a few days.

In the later cases the guidelines for intravenous therapy were relatively simple. They called for the constant delivery of appropriate doses of glucose, the maintenance of a normal acid-base balance, and the avoidance of fluid overload. The first two objectives were easily and accurately met with the aid of frequent measurements of serum electrolytes and plasma glucose. Usually standard solutions of 5 per cent glucose in 0.1 or 0.2 normal saline were administered; sodium bicarbonate and potassium were added according to the calculated need. The patients were also kept relatively dry, despite which transient edema, ascites, and hypoproteinemia were frequently seen (Chapter Six). The fluid intake for the infants was 40 to 60 ml/kg per day; for adults it was usually less than 3 liters per day. In a few cases in which hypoproteinemia became severe or in which there was evidence of low blood volume, intravenous human albumin was given.

Gastrointestinal Ulceration or Bleeding. Three (OT 3, 5, and 6) of the first patients (OT 2 to 7) who survived operation developed significant gastrointestinal hemorrhage during the few remaining days of life and were found at autopsy to have erosive esophagitis with invasion by *Candida albicans* (Chapter Sixteen). These complications were largely prevented during the early convalescence of the subsequent recipients; the only GI hemorrhage during the first postoperative month was after heparin therapy had been started in Patient OT 17. The difference in these results was probably due to the better quality homografts used in the later series. However, as will be mentioned in Chapter Seventeen, the patients with protracted survival were not exempt from gastrointestinal hemorrhage many months after operation.

Pulmonary Embolization. At autopsy 22, seven and a half, and six and a half days after transplantation, multiple emboli were found in the pulmonary arteries of the first three patients (OT 2 to 4) who lived through the operation.^{20, 21} It is probable that the use of external bypasses and the administration of clot-promoting agents in these cases were at least partially responsible (Chapter Ten). After these practices were discontinued, there were no additional examples in the next 21 recipients.

Pancreatitis. There were two examples of clinically significant pancreatitis. Both were apparently caused by operative trauma to the gland. In one patient (OT 17) the pancreatic tail may have been injured during splenectomy. There were no symptoms for the first postoperative month. Then she developed episodic cyanosis which was erroneously thought to be due to pulmonary embolization; in actuality, the etiology was a *Pseudomonas* pneumonitis. On the basis of the incorrect diagnosis, systemic heparinization was instituted. Gastrointestinal and intra-abdominal bleeding followed. At re-exploration, fat necrosis surrounded the entire pancreas. The source of the intraperitoneal hemorrhage was near the tail. The tail was resected and contained histopathologic evidence of pancreatitis. Cultures from the specimen and from the left subphrenic space revealed *Candida albicans*.

Acute inflammation of the head of the pancreas and extensive fat necrosis of the surrounding tissues was found at autopsy in Patient OT 24, 11 days after transplantation. This was the child with multiple congenital anomalies whose duodenum had undergone necrosis at the time of the original operation (Fig. 55, Chapter Eight).

Pancreatic tissue was studied in all the other recipients who died from a few days to more than 13 months after orthotopic liver transplantation. There were histologic findings of mild acute, subacute, or chronic pancreatitis in some of the patients, but the disease had not apparently contributed to the fatal outcome in any of these patients.

Arterial Hypertension. An extremely perplexing observation was made in three children (OT 13, 16, and 18). After their return to the intensive care unit, they had hypertension of such severity that therapy with reserpine was started. One of these patients died after four days. The other two continued to require treatment for several months. The urines from these three recipients and from another child who did not develop high blood pressure (OT 19) were examined by Dr. Charles Chidsey⁹ of our institution and by Dr. Lawrence Krakoff of Columbia University.

The excretion of aldosterone was not increased, nor were there elevations of the plasma renin concentration. However, abnormally high levels of norepinephrine and/or tyramine were found in two of the three patients with hypertension (Table 10). The serial changes were of particular interest in the child (OT 16) who received two livers. After the first operation the initially normal values began to rise with the onset of a rejection that ultimately necessitated retransplantation. In the first weeks after placement of the second homograft, the measurements of tyramine returned to normal.

The findings provided a plausible explanation for the hypertension. It is probable that tyramine degradation by the liver was depressed in at least some of the recipients. Tyramine is an aromatic amine which is normally formed in the gastrointestinal tract by the decarboxylation of tyrosine and which is then detoxified by hepatic monoamine oxidase. The sympathomimetic properties of tyramine are thought to be by its induction of norepinephrine release from adrenergic nerve endings.

Table 10. Twenty-four Hour Urinary Catecholamine and Tyramine Determinations (μg) in Four Pediatric Recipients of Orthotopic Liver Homografts and in Seven Normal Children

PATIENT	POST- OPERATIVE DAY	EPINEPH- RINE	NOREPI- NEPHRINE	TYRAMINE	BLOOD PRESSURE (mm Hg)	ANTIHYPER- TENSIVE DRUGS	BILIRUBIN (mg%)
OT 13	177			885.0	140/85	—	0.2
	300			795.0	140/90	—	13.3
OT 16A	2	0	18.7	8.7	120/70	—	3.4
	3	0.5	5.7	10.9	160/112	—	2.9
	10	0	27.5	46.2	190/120 → 148/90	Reserpine	1.4
	16	0.7	10.4	143.5	130/76	Reserpine	4.0
	24	0	33.0	968.0	142/88	Reserpine	7.3
	25	0	33.6	892.0	150/90	Reserpine	8.0
OT 16B (2nd liver)	1			227.8	110/80	—	0.2
	2			120.5	120/80	—	1.2
	3			13.2	120/90	—	1.3
	4			13.3	135/100	—	1.9
	5			28.6	140/112	—	0.1
	6			19.0	136/90	—	0.1
	7			12.5	125/80	—	1.2
OT 18	1	25	3.5	10.6	195/110 → 70/P	Reserpine	3.5
OT 19	2	10.1	36.2	47.2	118/80	—	1.5
	4	1.0	11.4	61.1	120/64	—	2.2
Control	1	0.0	7.0	42.4			
	2	0.0	4.1	—			
	3	1.1	4.1	—			
	4	0.1	2.8	—			
	5			136.1			
	6			72.1			
	7			186.0			

Continuing Care

None of the patients who were given badly damaged organs or who had uncorrected technical complications were ever returned to a reasonable state of health. During their remaining days they passed from crisis to deepening crisis until the time of death, which was usually caused by a multiplicity of factors. In contrast, the recipients of homografts which functioned reasonably well at the beginning were usually able to begin eating and to resume a number of other normal activities within a few days.

With the latter kind of patient it was not possible to predict the magnitude of the rejection which was anticipated to follow. Consequently, all were treated with the expectation that the process of homograft repudiation would be severe and that a period of secondary hepatic dysfunction would have to be endured at some time during recovery. Prophylactic measures were always taken against the complications of hepatic failure.

For one thing, fluid balance was carefully monitored. It was noted in Chapter Six that water retention was common in the first days after operation, but that this tended to be transient unless severe injury to the homograft had occurred. Later, daily weights, intake-output records, and physical examinations were used as a guide to determine if fluid restriction should be re-instituted. This was not usually necessary except in the patients who underwent explosive acute rejection (see Chapter Fourteen).

Close attention was also paid to renal excretion, partly because of the kidney malfunction that can be caused by liver failure.^{2, 15} In addition, many of the antibiotics, which must be administered to these patients to prevent invasion of their new livers by gram negative bacteria, have pronounced nephrotoxicity. It was necessary to change the doses and frequency of administration of these agents from time to time on the basis of relatively minor alterations in renal function or urinary sediment.

Vitamin K was usually administered daily for at least the first postoperative month. Other vitamins were also given. All narcotic or sedative medications were avoided for reasons discussed in Chapter Seven.

All liver transplant recipients must be treated with large doses of ulcerogenic steroid agents. Consequently, it is mandatory that they be placed on a strict peptic ulcer regimen. The importance of such a precaution is well known from experience in renal homotransplantation.¹⁷ The need is probably even greater following hepatic replacement, especially if there is a protracted or severe rejection. Observations will be cited in Chapters Eleven and Twelve showing that both porcine and canine recipients of orthotopic liver homografts have a very high incidence of peptic ulceration with fatal hemorrhage or perforation. The patients are started on a bland diet with multiple feedings. Between meals they are given antacids or milk and cream every hour.

There is another advantage from around-the-clock feedings. The adult patients in our series who have had prolonged survival after orthotopic liver transplantation have had initial marked weight loss. The most severe example was Patient OT 15 whose indication for treatment was hepatoma. In the six months preceding the liver replacement his weight had fallen from 165 to 140 pounds. After operation, he continued to lose weight, reaching a nadir of 128 pounds despite an intake of 4000 to 8000 calories per day. It required five months to restore his original pre-illness weight.

In general, the patients were permitted to regulate their own physical activity. During periods of deteriorated liver function, which was due to the violent variety of rejection crisis (Chapter Fourteen), they usually preferred to remain in bed and sleep much of the time. When rejection developed more indolently, they were less affected.

One justification for sanctioning as much exertion as could be tolerated was the hope that pulmonary complications such as atelectasis and postoperative pneumonitis could be thereby reduced. In both the short- and long-term follow-up of these patients it is important to perform frequent chest examinations and to have chest x-rays at regular intervals. Only in this way can potentially lethal pneumonitis be detected in time to institute effective treatment.

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Chapter Ten

CHANGES IN COAGULATION

by Carl G. Groth, M.D.

Most of the hemostatic factors are hepatic in origin. In addition, the liver is involved in the clearing of several substances active in coagulation and fibrinolysis. Consequently, it is not surprising that derangements of coagulation have been responsible for bleeding diatheses and possibly also for intravascular clotting during and after clinical hepatic transplantation.^{1, 5-7, 10, 11, 22-24, 26, 27}

The potential seriousness of these problems was not fully appreciated from the early canine studies, probably because the appropriate measurements were not obtained. In one of his first articles on orthotopic transplantation Moore¹⁷ reported the absence of significant coagulation abnormalities. Our own early publications²⁵ referred only to the development of fibrinolysis, which was suggested by the secondary dissolution of clots in the operative field or in a test tube. The complication was particularly lethal in experiments in which there apparently had been severe ischemic injury to the liver. Inadequate homograft preservation as the cause for a bleeding diathesis was later more specifically indicted both in the first human recipients of hepatic homografts²⁶ and in dogs studied under similar experimental conditions at about the same time.¹⁶ The characteristic abnormalities in both species were increased fibrinolysis, decreased plasma fibrinogen, and prolongation of thrombin times. Several of the patients who survived developed peripheral venous thrombosis and pulmonary embolization at a later time. It was suggested that the initial clotting deficiency might have been succeeded in these cases by a state of hypercoagulability.^{14, 26}

Subsequently several investigations of the effect of liver transplantation on blood coagulation were carried out in dogs and pigs.^{2, 3, 13, 14, 20, 28} This experimental work will first be reviewed; then the changes in hemostasis in patients undergoing hepatic transplantation will be described. The methods used at our institutions for the assays of coagulation and fibrinolysis have been described before^{13, 14, 20} and the details of methodology will not be repeated here.

ANIMAL STUDIES

Dogs

Well Preserved Orthotopic Homografts. Most of these experiments^{13, 14, 20} involved the immediate transplantation of a homograft that was protected from ischemic injury by rapid cooling at the time of its removal. However, good livers were also obtained after preservation for as long as 24 hours⁴ by the techniques described in Chapter Five. Under both circumstances all measures of recipient hepatic function were adequate from the beginning, although rarely completely normal. Obvious bleeding diatheses did not develop. Nevertheless, changes in clotting could always be detected by the appropriate examinations (Table 11).

One of the most consistent alterations was increased fibrinolysis.^{14, 20} The euglobulin lysis time (ELT) started to decrease during the anhepatic phase and became markedly shortened immediately after graft revascularization. Subsequently the ELT started to recover and some 24 hours after transplantation it had rebounded to values well above the control levels. The concomitant depression in fibrinogen levels and the prolongation of thrombin time were of moderate degree and often transient. Besides shortening of the ELT and hypofibrinogenemia, direct evidence of fibrinolysis was obtained with the fibrin plate method as well as by the demonstration in the plasma of falls in plasminogen levels and increases in fibrinogen split products (Table 11). Analogous findings have also been reported in simulated autotransplantation experiments in which the liver was temporarily isolated from the circulation and perfused with cold electrolyte solution.¹³

A second characteristic change in the canine recipients was thrombocytopenia. This aspect of the problem was most completely studied by Hutchison et al¹³ and later by Pechet²⁰ and Boehmig³ and their associates. A drop in the circulating platelets regularly occurred immediately after the revascularization of auto- or homotransplants. Normal counts were not restored until several days later. Other factors including immunosuppression, which can accentuate thrombocyte depression, will be discussed in Chapter Thirteen.

More recent studies have stressed the complexity of the alterations in coagulation caused by hepatic transplantation^{3, 13, 20} even when using satisfactorily preserved homografts. In our own laboratories, decreases could invariably be detected of the clotting factors synthesized by the liver (I, II, V, VII, IX, and X) as well as of Factor VIII, which is also produced¹⁸ in other than hepatic tissue (Fig. 67). The changes commenced during the anhepatic phase, became most pronounced one to four hours after revascularization, and lasted for variable intervals. Factors V, VIII, and IX usually recovered in 24 to 72 hours, whereas II and X usually required three or more days.

It is of interest that the factor depressions considerably outlasted the burst of fibrinolytic activity measured by the ELT which was usually of only a few hours' duration. The evolution of the changes in the factors more closely con-

Table 11. Changes in Coagulation and Fibrinolysis in 17 Canine Recipients of Orthotopic Hepatic Homografts*

TIME	FIBRINOGEN (mg/%)	CLOTTING FACTORS (%)								PLATELETS (10 ³ per cu mm)	PLASMINOGEN (units)	ELT (min.)	THROMBIN TIME (sec.)	FIBRIN PLATES (lysed area cu mm)		FIBRINOGEN SPLIT PRODUCTS	CLOTTING TIME (min.)	
		II	V	VII	VIII	IX	X	UNHEATED	HEATED					GLASS	SILICONE			
Before removal of host liver	Mean	422	86	90	97	89	87	80	248	4.6	171	12.8	103	40	-	to +	6	14
	S.D.	165	14.6	11.4	9.35	27	10	12.3	96.8	0.72	85.4	1.62	38	7.5				
Homograft revasc.	Mean	333	70	77	86	61	68	57	205	3.0	60	15.5	252	82	-	to ++	7	6 to >6 hrs.
	S.D.	114.6	15.7	16.7	8.4	24.5	20	10.1	54.5	0.92	83.5	3.64	102	60.7				
1 hour after revasc.	Mean	320	61	58	82	50	54	48	184	2.5	105	13.9	103	24	-	to +	8	14 to >6 hrs.
	S.D.	92.4	14.8	21	11.6	16.6	17.7	11.9	58.4	0.8	62	1.91	60.6	9.8				
2 hours after revasc.	Mean	321	60	52	73	50	57	44	201	2.9	27 to >5 hrs.	13.9	82	23	-	to ++	7	
	S.D.	98.1	16.1	23.4	6.3	20.9	28.6	11.1	50.6	0.78		1.43	43.2	13.7				
4 hours after revasc.	Mean	341	57	54	70	52	61	48	212	3.5	100 to >24 hrs.	15.2	0	4	-	to +		
	S.D.	81.5	10.8	24.3	9.4	21.3	19.3	9.2	38	0.96		2.4	7	7				

*Fifteen dogs received immediately transplanted livers. In two cases the livers had been effectively preserved in vitro for 24 hours. All animals had good early postoperative liver function and survived for at least three days. The data were obtained from Pechet et al.²⁰

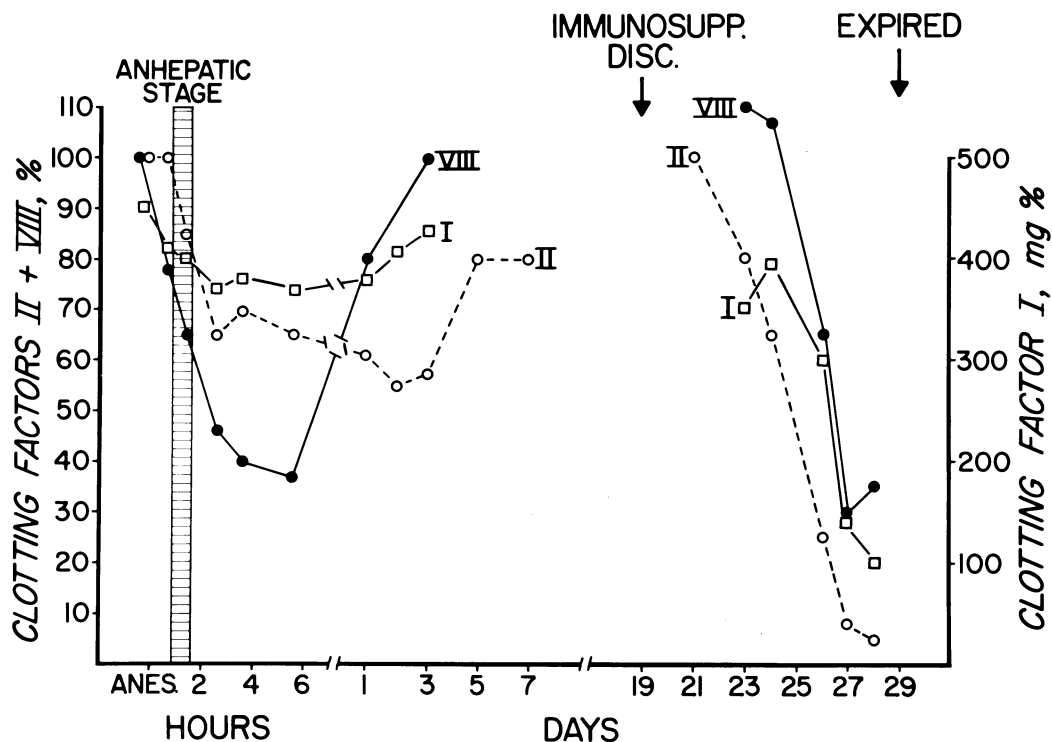


Figure 67. Measures of coagulation Factors I (fibrinogen), II (prothrombin), and VIII (antihemophilic globulin) in a canine recipient of a promptly transplanted orthotopic hepatic homograft. EACA and protamine were administered intraoperatively. No significant bleeding diathesis occurred. The coagulation factors recovered within a few days after transplantation and remained essentially normal until immunosuppression was discontinued and homograft rejection occurred. (By permission of J. Lab. Clin. Med. 73:91, 1969.)

formed to those of plasminogen. In one of five recipient dogs studied for the presence of circulating heparin, very small amounts were detectable (22 $\mu\text{g}/\text{ml}$); in vitro the antithrombin activity could be diminished by heating the plasma or by the addition of protamine. The abnormalities in coagulation and fibrinolysis were not prevented by the administration of epsilon-amino caproic acid (EACA), protamine, or heparin. Two dogs given intravenous Trasylol, a proteinase inhibitor, had somewhat less marked changes.

In the foregoing 17 experiments,²⁰ as in the previous canine studies of von Kaulla et al,¹⁴ there was little or no objective evidence from the different assays of delayed hypercoagulability. The only suggestive finding in Pechet's analysis²⁰ was a marked prolongation of the ELT. Nevertheless, two of the dogs died with a thrombus in the superior vena cava a few days after transplantation; both had received EACA and protamine during operation. Three more succumbed within one week with hepatic artery thrombosis.

Poorly Preserved Orthotopic Homografts. Four animals which received homografts damaged by inadequate 24 hour preservation all bled profusely during operation and died within 24 hours. The concomitant changes in coagulation and fibrinolysis assays were profound (Fig. 68) and there was little or no recovery (Table 12). The platelet counts were the least affected.

Table 12. Changes in Coagulation and Fibrinolysis in Five Dogs Which Received Orthotopic Hepatic Homografts That Had Been Severely Damaged by Ischemia *

TIME	FIBRINOGEN (mg%)	CLOTTING FACTORS (%)						PRO- THROMBIN TIME (sec.)	PLATELETS (10 ³ per cumm)	PLAS- MINOGEN (units)	ELT (min.)	THROMBIN TIME (sec.)	FIBRINOGEN SPLIT PRODUCTS
		II	V	VII	VIII	IX	X						
Before removal of host liver	Mean	320	75	82	100	67	81	81	10.2	177	121	14.8	+ to ++
	S.D.	40.81	13.3	12.9	0	5	20	14.4	0.44	57.4	21.3	1.66	
Homograft revasc.	Mean	269	55	57	73	55	58	63	10.7	157	29	15.0	+ to ++
	S.D.	35.65	13.2	11.6	10.1	13.5	15.4	21	0.56	57.5	2.8	7.05	
1 hour after revasc.	Mean	241	42	26	58	14	18	36	13.9	98	30	21.6	+
	S.D.	59.20	14.8	17.4	15.8	11.1	10.1	14.1	2.38	17.4	16.3	3.76	
2 hours after revasc.	Mean	216	36	10	59	13	9	29	18.3	146	10 to	31.1	- to ++
	S.D.	93.72	11	7.7	19.6	10	8.1	18	4.40	61.6	>3 hrs.	8.21	
4 hours after revasc.	Mean	234	33	2	54	7	4	29	21.2	168	125 to	52.4	- to +
	S.D.	54.65	17.5	4	22.7	3.1	3.1	14.7	1.38	48.6	>3 hrs.	32.47	

*Four livers had been subjected to inadequate 24 hours preservation; the other was injured by a prolonged surgical procedure. All animals had a marked intraoperative bleeding diathesis and died within 24 hours. The data were obtained from Pechet et al.²⁰

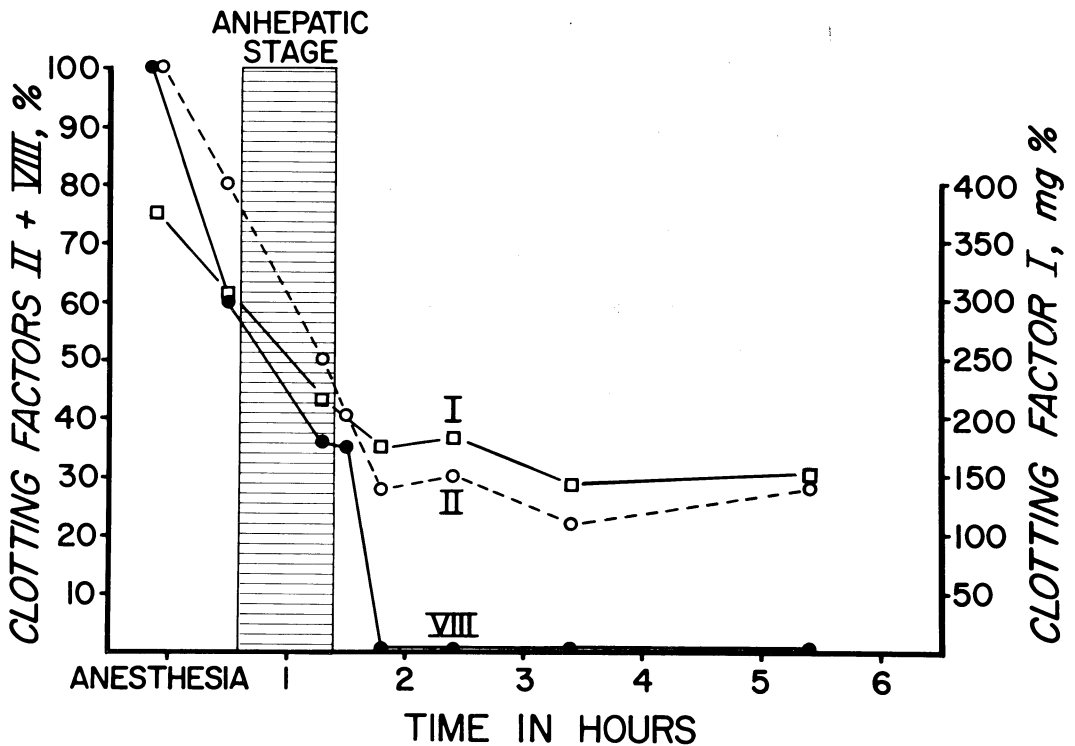


Figure 68. Changes in coagulation Factors I, II, and VIII in a dog receiving a liver homograft which was badly damaged during 24 hours of preservation. The recipient animal developed a marked bleeding diathesis and died of uncontrollable hemorrhage. Note absence of recovery of the coagulation factors. (By permission of J. Lab. Clin. Med. 73:91, 1969.)

Auxiliary Homografts. Somewhat different coagulation changes were seen by Stremple, Hussey, and Ellison after transplantation of a heterotopically placed extra liver.²⁸ When their recipients developed an intraoperative bleeding diathesis, the levels of fibrinogen, Factor V, and platelets became depressed; there was an increase in the prothrombin time. No pathological fibrinolysis could then be demonstrated. However, increased quantities of circulating endogenous plasma heparin were found concurrent with a prolonged thrombin time. In other animals in which there was no problem with bleeding, only depression in the fibrinogen and platelets was recorded. Subsequent removal of the host liver had no influence on any of the parameters studied.

Late Changes. In a number of the first dogs surviving for a prolonged period after liver transplantation, clotting studies after recovery from the acute effects of operation were observed to range from normal to a degree of depression that was in general accordance with the quality of hepatic function as judged by other measures.⁸ The same thing has been more completely documented by Pechet et al.²⁰ In his dogs both the coagulation factors and fibrinolytic activity tended to remain near normal until after the onset of delayed rejection. If the graft was then repudiated abruptly, the clotting factors fell within a few days after the onset of enzyme changes and jaundice (Fig. 67). The thrombin times

became prolonged, and there was increased fibrinolysis. Hutchison has reported that thrombocytopenia is usually not associated with either acute or chronic rejection.¹³

Pigs

Analogous studies have been carried out after orthotopic liver transplantation in pigs.² The animals received heparin during operation. The results were almost identical to those in dogs except that thrombocytopenia was much less common. An example of the latter finding is illustrated in Figure 83 (Chapter Eleven).

Interpretation of Findings

In the first quantitative studies of the bleeding diathesis during and after transplantation¹⁴ considerable emphasis was placed on the role of an explosive intraoperative fibrinolysis. It was suggested that the problem might have been contributed to by the release of plasminogen activators during the extensive surgical procedures, by a reduced clearance of these substances from the circulation during and even after the anhepatic phase, or by failure of the liver to produce fibrinolytic inhibitors. It is known from other kinds of investigations that each of these conditions can be a factor in the promotion of clot lysis.^{9, 19}

In addition, however, von Kaulla et al¹⁴ speculated that intravascular coagulation coupled with temporary cessation of the synthesis of proteins essential for coagulation must be an important component of a much more complicated total process. This point of view has been strongly supported by Hutchison¹³ Pechet,²⁰ Blecher,² and Boehmig³ and their associates. The findings that all measured clotting factors declined far more rapidly than would have been expected from their biologic half-lives and that these alterations were accompanied by thrombocytopenia and fibrinolysis were characteristic of a consumption coagulopathy.^{12, 21} The failure of heparin to prevent these changes^{2, 20} may have been due to the acidosis regularly seen during transplantation (Chapter Seven); such pH shifts suppress the anticoagulant effect of heparin.¹²

In spite of the strong evidence of intravascular coagulation, a systematic search of canine homografts as well as the recipient tissues has failed to reveal microthrombi.¹³ The only morphologic evidence of consumption was the sequestration of platelets in the perisinusoidal spaces. Presumably the absence of thrombi was ascribable to the effectiveness with which the fibrinolysins were capable of sweeping away the products of coagulation. It is probable, as Blecher² and Pechet²⁰ have suggested, that the anticoagulant effect of the fibrinogen split products¹⁵ was one element in the prolongation of the thrombin times mentioned earlier. This explanation was at variance with that proposed by Stremple,²⁸ who concluded that the antithrombin activity he observed in canine recipients of auxiliary homografts was caused by endogenous heparin.

CLINICAL OBSERVATIONS

Coagulation During and Shortly After Orthotopic Transplantation

It was noted in the introduction to this chapter that the essential features of the bleeding diathesis were actually first defined in patients and only subsequently subjected to complete analysis in laboratory animals. The studies that were carried out in the earliest human cases (OT 1 to 5) revealed very sudden but generally transient increases in plasma fibrinolytic activity, longer lasting acute hypofibrinogenemia, prolongation of the thrombin and prothrombin times, and thrombocytopenia.^{13, 14, 26}

During the procedure the first four recipients were given large quantities of intravenous human fibrinogen and EACA. One (OT 1) exsanguinated on the operating table despite the fact that the EACA seemed to partially control the fibrinolysis as judged by serial thromboelastograms; fatal bleeding has also been reported from other institutions.⁵⁻⁷ Hemorrhage was controlled in the other three patients (OT 2 to 4) who lived for another 22, seven and a half and six and a half days, respectively. In the later postoperative period the euglobulin lysis times (ELT) tended to become abnormally prolonged, now suggesting low fibrinolytic activity. At autopsy all three recipients were found to have multiple pulmonary emboli. The source of the clots could not be found in one case; in the other two the origin was in the iliofemoral system at or near the femoral venotomy sites where plastic catheters had been inserted for the purpose of decompressing the vena caval system. It was concluded that the intraoperative efforts to manipulate the clotting mechanism may have led to hypercoagulability.¹⁴

In the next patient (OT 5) an attempt was made to avoid pulmonary embolization by administering 2 mg/kg heparin and by inserting the bypass into the femoral system via the saphenous vein, which was later tied off at its proximal termination. EACA and fibrinogen were not given. Hemorrhage was controlled with great difficulty. At the time of death 23 days later there was no evidence of any clotting complications.

The role of the bypass catheters in promoting either bleeding or thromboembolic complications can only be speculated upon. In dogs submitted only to external blood bypass without transplantations Boehmig has noted consumption of various coagulation factors as well as the induction of morphologic changes in platelets.³ In patients OT 2 to 4 the generation of clots at or near the location where the inferior limb of the plastic prostheses were introduced into the venous system was probably more than coincidental. The most important precipitating event may have been mechanical trauma to the vessels.

In all the later recipients the decompressing bypasses were omitted when it was learned that they were not necessary (Chapters Seven and Eight). Subsequent to this important change in technique no patient has suffered from peripheral thrombophlebitis or has developed pulmonary emboli. Moreover, it became possible to perform studies free of the potential artefacts that might be

introduced by the temporary extracorporeal venous circulation. The results will be classified on the basis of observations in a small number of cases in which a relatively complete panel of examinations could be carried out. The findings have been confirmed in many later recipients whose data will not be included.

Poorly Functioning Homografts. In two of the first patients for whom bypasses were not employed, organs were used that had sustained a grave ischemic injury as judged by high postoperative rises in the recipients' serum transaminases, poor liver function from the outset including rapidly progressive jaundice, and death in seven and 10 days, respectively, from combined hepatic and renal failure.

The first studies were obtained in the postoperative period, at which time profound coagulation abnormalities were already present. In a 13 month old child (OT 7) the liver-produced Factors II, V, IX, and X were all less than 15 per cent of normal; fibrinogen (Factor 1) was never higher than 125 mg per cent (Fig. 69). The Quick prothrombin time and the thrombin time were both extremely prolonged. In contrast Factor VIII was supernormal for the entire 10 postoperative days. There was a progressive thrombocytopenia despite daily platelet infusions. Euglobulin lysis times were always prolonged. Hemorrhage was not troublesome until gastrointestinal bleeding became continuous during the last two days of life.

The other patient, a 29 year old man (OT 6), had similar but widely fluctuating changes. However, bleeding was a far more significant problem. During operation and the seven postoperative days he received 33 liters of blood and several hundred grams of fibrinogen.

Adequately Functioning Homografts. The next seven recipients (OT 8 to 14) benefited from more discriminating donor selection and from the application of an effective technique for interim preservation of the homografts (Chapter Five). Early liver function was satisfactory. A hemorrhagic diathesis was either not obvious or else very transient.

During operation only one of the patients (OT 9) received EACA; 0.1 mg/kg, was given just after revascularization of the liver. This recipient as well as all the others developed coagulation abnormalities that varied only quantitatively from case to case. The data for the two patients with the most and the least profound changes, respectively, are summarized in Figures 70 and 71. The factors synthesized by the liver, which began to fall during the hepatectomy, decreased further during the anhepatic phase and reached a nadir shortly after the homograft was revascularized. Factors V and IX were usually the most severely affected; Factors II, VII, and X next; and fibrinogen the least. The alterations in Factor VIII, which is not exclusively produced in hepatic tissue, varied from profound depression in some cases (Fig. 70) to a moderate decrease in others (Fig. 71). The platelets were moderately depressed in five of the seven patients.

In every case there was marked intraoperative activation of the fibrinolytic system as shown by the occurrence of lysis on heated plates, decreases in plasminogen levels, and shortening of the ELT. Concomitantly, fibrinogen split products appeared in the serum.

(Text continued on page 171.)

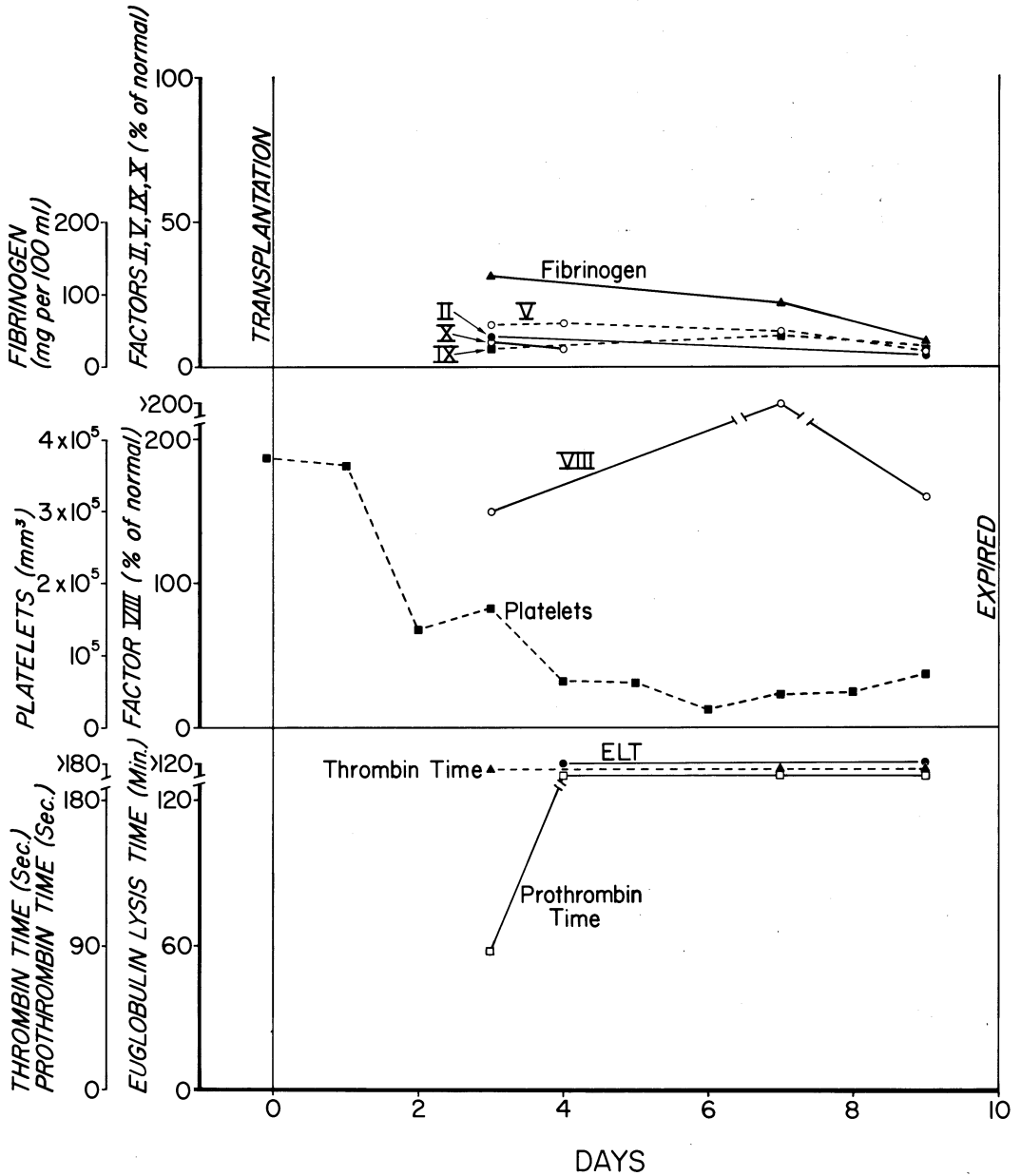


Figure 69. Measures of coagulation and fibrinolysis in a 13 month old child (OT 7) who never had satisfactory function of his orthotopic liver homograft. Note that Factor VIII remained supernormal while all the liver based coagulation factors were severely depressed. The euglobulin lysis time (ELT) was infinity. (By permission of Arch. Surg. 98:31, 1969.)

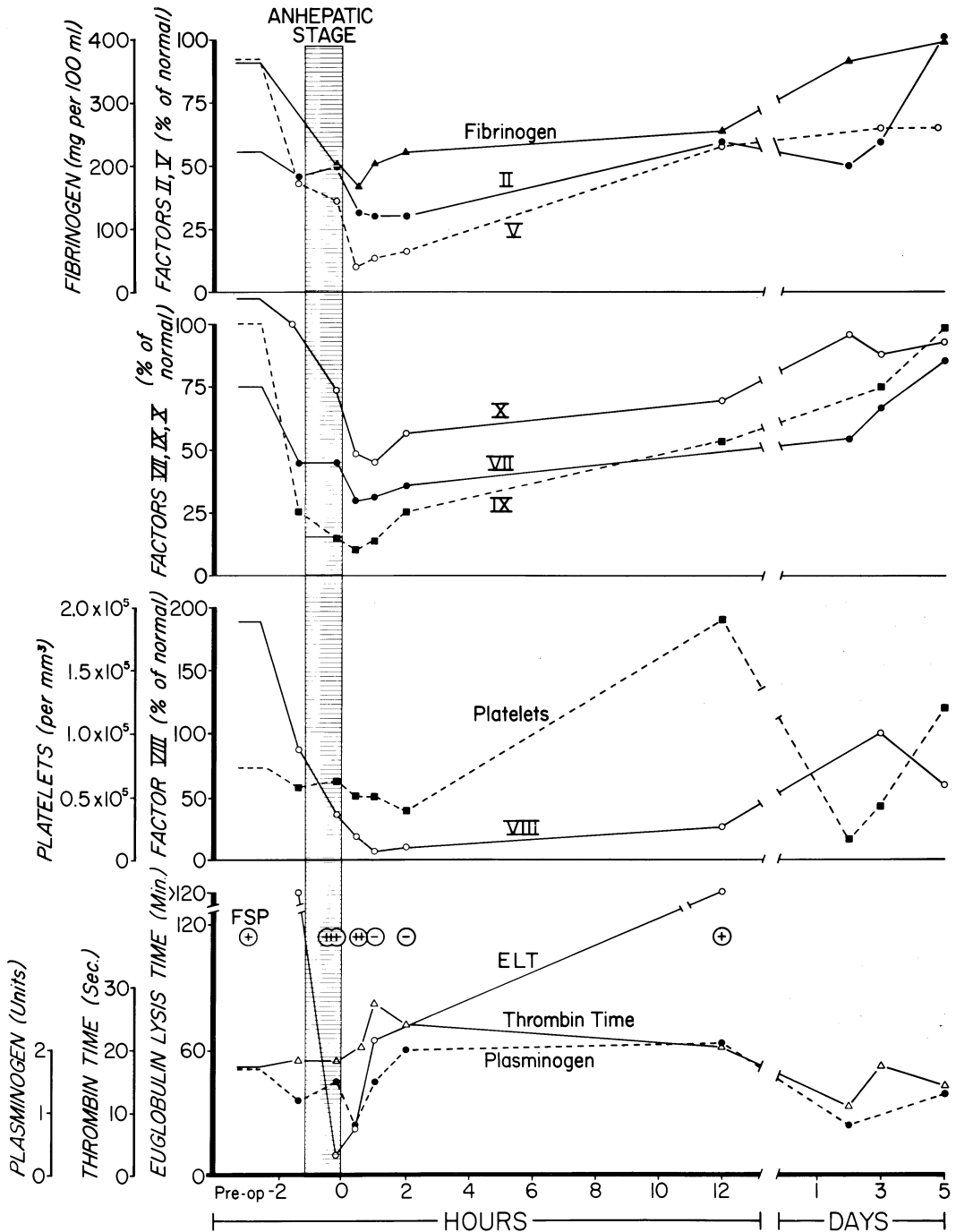


Figure 70. Intraoperative and early postoperative coagulation changes in a 16 month old child with life sustaining but impaired immediate homograft function (OT 12). All the coagulation factors studied were depressed below 50 per cent of normal. However, these gradually returned toward normal. FSP = fibrinogen split products. (By permission of Arch. Surg. 98:31, 1969.)

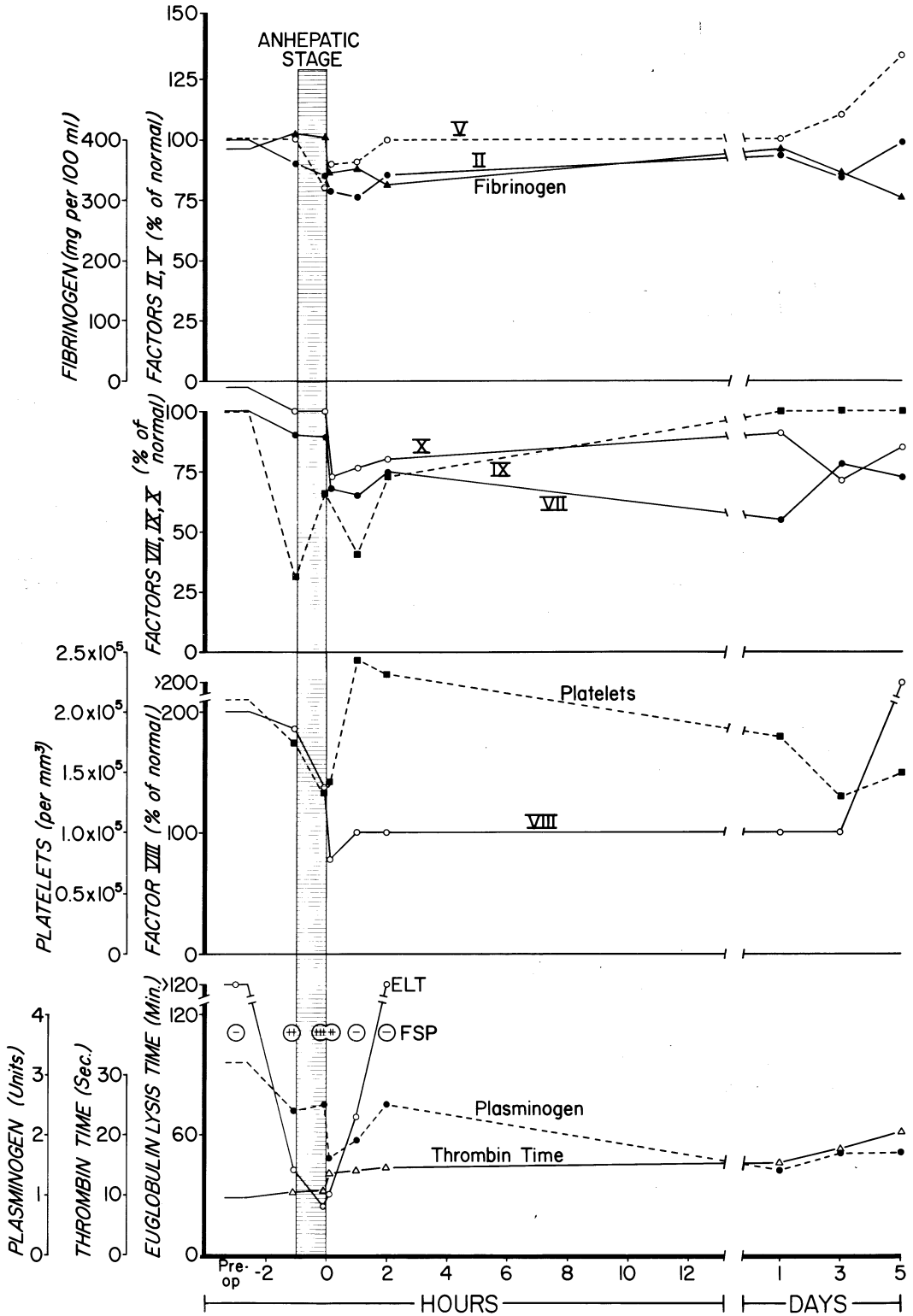


Figure 71. Changes in a 14 month old child with satisfactory immediate liver function (OT 11). All the coagulation factors studied were transiently but only minimally depressed. FSP = fibrinogen split products. (By permission of Arch. Surg. 98:31, 1969.)

Invariably there was prolongation of the thrombin time after revascularization of the homograft. In the patient with the most serious changes in hemostasis, measurable amounts of plasma heparin became detectable at this time (47 $\mu\text{g}/\text{ml}$). The small quantities may have come from the homograft, which was perfused and flushed with anticoagulant solutions during preservation. Three other patients studied had no detectable heparin in their plasma in spite of prolonged thrombin times.

Two hours after revascularization of the homograft a restitution of the clotting factors had generally begun, and during the ensuing week they usually returned to essentially normal levels. Similarly, the evidence of fibrinolysis promptly diminished or disappeared. After 24 hours the ELT had recovered, even though the plasminogen levels ordinarily remained below normal for several weeks (Figs. 70 and 71). The platelet counts were also usually quickly restored. Secondary declines a week or so later were thought to be due mainly to ALG therapy (Chapter Thirteen).

Early Homograft Devascularization. Data were collected in two patients who died within 24 hours after operation as the result of external compression of the main hepatic artery (OT 20) and thrombosis of the portal vein (OT 21); the details of these cases are given in Chapter Eight. Six to 10 hours after revascularization of the livers both recipients had marked hypofibrinogenemia (41 and 67 mg per cent). In the first patient there was a marked depression of Factors VIII (0 per cent) and IX (3 per cent). The other variables of coagulation and fibrinolysis were similar to those in patients who survived.

Later Changes

In eight patients (OT 8 to 15) coagulation studies were carried out for many weeks or months postoperatively. The clotting values were closely correlated to the quality of homograft function as measured by standard liver function tests. The patient with the longest survival (OT 8) had good liver function and essentially normal coagulation and fibrinolysis until shortly before death. Two patients who died of progressive liver failure four and a half (OT 9) and six months (OT 10) after transplantation had slowly declining levels of the clotting factors synthesized by the liver as well as decreases in plasminogen (Fig. 72). During this course, Factor VIII remained unaffected. Splenectomy had been performed in both patients at the time of transplantation.

In three of the patients who developed delayed septic hepatic infarction of the right lobe of the liver and who were subsequently proved to have thrombosed their right hepatic artery (Chapter Fifteen), clotting studies were obtained at about the time of this complication. Two of the recipients (OT 10 and 11) had essentially normal coagulation measurements. The third (OT 8) had had supernormal values of some of the clotting factors in the preceding days (Fig. 73).

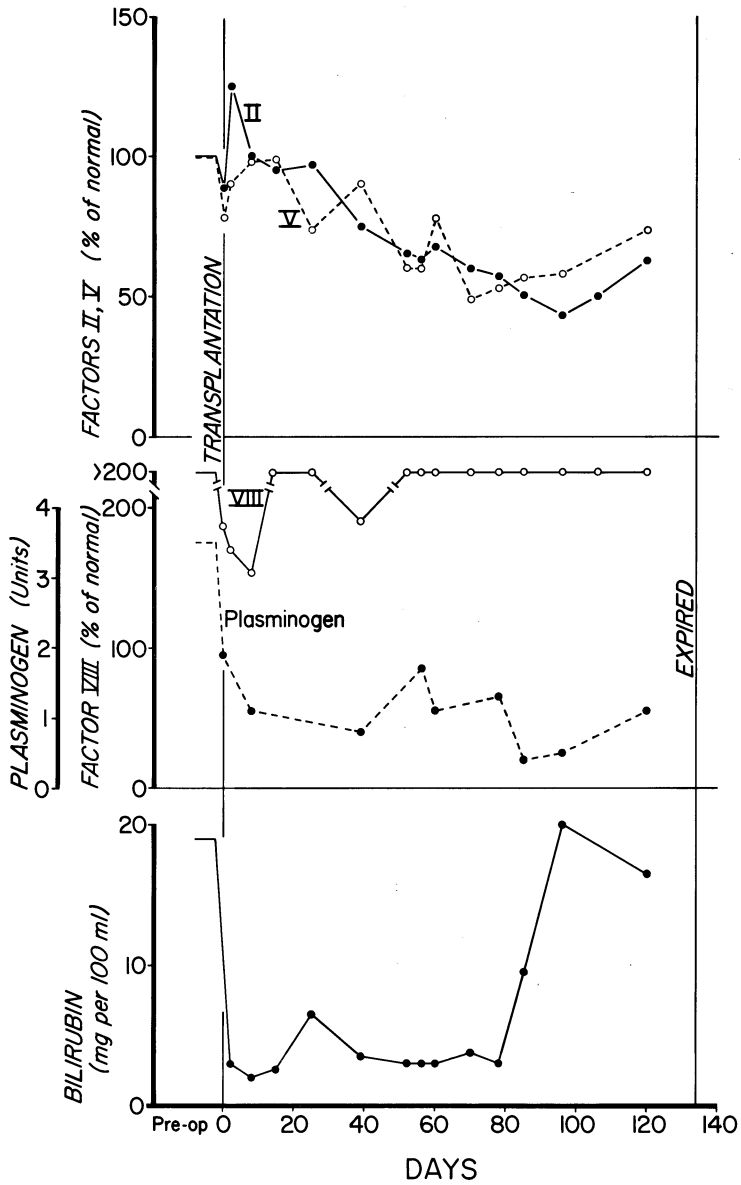


Figure 72. Postoperative changes in a 20½ month old patient (OT 9). Early homograft function was satisfactory, but progressive liver failure due to chronic rejection subsequently developed. During the late course Factor VIII remained supernormal. Plasminogen and the liver based Factors II and V ultimately declined. (By permission of Arch. Surg. 98:31, 1969.)

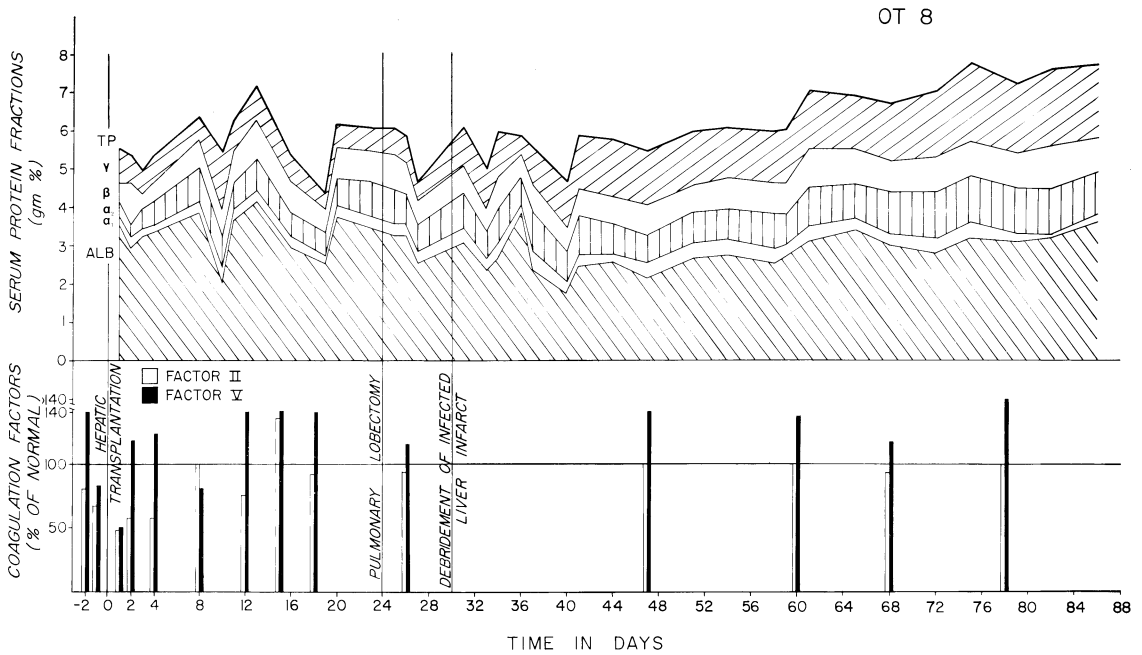


Figure 73. Serum proteins after liver homotransplantation in patient OT 8. The septic infarction of the right hepatic lobe developed about three and a half weeks postoperatively, apparently at about the same time as thrombosis of the right hepatic artery. Note the supernormal values of Factors II and V in the three weeks preceding this complication, and the maintenance of high levels even afterward. (By permission of Surgery 63:549, 1968.)

Retrospective Evaluation

It is obvious that the changes in blood coagulation in the human cases of orthotopic liver transplantation were very similar to those recorded in analogous animal experiments. The initial findings were suggestive of intravascular coagulation associated with pathologic fibrinolysis. The alterations were quickly reversible providing an adequately preserved and functional homograft was used.

A number of practical lessons have been learned about the proper management during the critical operative and early postoperative period. The most important is that the situation may be made hopeless from the beginning by the use of a poor quality organ. Under these circumstances the prevention of fatal hemorrhage may require the use of thrombogenic agents such as EACA, fibrinogen, or protamine sulfate. Even though Pechet et al were unable to report significant benefit from these substances in animal experiments,²⁰ there was evidence from clinical observations that they helped to control severe bleeding diatheses in some of our patients.^{14, 26}

With better quality homografts, there should be no need for efforts to promote clotting and, indeed, such intervention is probably strongly contraindicated since it may add to the already significant risk of major intravascular thrombosis. The iatrogenic induction of hypercoagulability may have been part-

Table 13. Homograft Vascular Thromboses in 17 Consecutive Recipients of Orthotopic Livers (OT 8 to 24)

	NUMBER CASES	LOBAR ARTERY	TOTAL ARTERY	PORTAL VEIN	TOTAL
Adults	5	0	0	0	0
Children	12	5	1	1	7

ly responsible for the three examples of thrombophlebitis and pulmonary embolization among our first four recipients. An added factor in those cases was probably the unnecessary use of external venous bypasses.

The avoidance of plastic bypasses and iatrogenic manipulation of the hemostatic mechanism has not prevented a high incidence of clotting of the homograft blood supply in our more recent experience^{22-24, 27} and in that of others.^{1, 6, 10} In our own cases vascular calamities have often occurred in the absence of striking hypercoagulability as judged from the results of a number of individual tests. However, such a panel of intermittently performed examinations may not be able to accurately track the balance of coagulation and lysis upon which normal hemostasis is dependent. This would be particularly true during the early postoperative period of very rapidly evolving change; it is during this interval that the euglobulin lysis times tend to become unusually prolonged.

In view of the past frequency of homograft vessel thrombosis, it may be warranted to consider prophylactic total body heparinization beginning at the time of homograft revascularization and continuing thereafter. Special consideration should probably be given to this possibility in pediatric patients. In such patients the very small size of the vascular structures has seemed to pose a special risk. Of 17 consecutive recent recipients of orthotopic hepatic homografts (OT 8 to 24), 12 were infants; there were seven examples of thrombotic sequelae affecting the homograft from two to 104 days postoperatively (Table 13). In contrast none of the five adults suffered this kind of complication.

There are, of course, other mechanical and flow factors which can contribute to late thrombosis. These are considered elsewhere in the book (Chapters Eight and Fifteen).

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Chapter Eleven

REJECTION IN UNMODIFIED ANIMALS

With renal homotransplantation the first clinical trials were in patients who were not altered by therapy inasmuch as techniques of immunosuppression were not then available. Important observations were made, particularly by Hume,¹⁸ about the behavior and fate of kidney homografts in uremic recipients whose immune capabilities were not further modified by iatrogenic means. That some of these transplants functioned for a surprisingly long time was at least partially explained by the fact that there is often a loss of immunologic reactivity accompanying renal failure.^{10, 53} Apparently, renal homografts inserted by Lawler,³⁰ Murray,³⁶ and Hume¹⁸ excreted urine for several months before slowly being rejected.

It is conceivable that a privilege comparable in magnitude to that accompanying uremia exists as a consequence of chronic liver failure and slows the pace of homograft repudiation. Gardner¹⁴ has provided evidence that this may be so in skin transplantation experiments carried out in normal volunteers and in patients with severe Laennec's cirrhosis. The average rejection time of skin from nonrelated donors was 11.6 days in the control group compared to 16.3 days in the victims of hepatic disease.

There will never be an occasion with liver grafts to obtain information analogous to that acquired in untreated humans during the early trials of renal homotransplantation. Instead, reliance must be placed on extrapolation to man of the events of hepatic rejection in unmodified animal recipients. In this chapter these will be described as they have been seen in dogs and pigs. Companion studies of the pathologic changes in such experiments are documented in Chapter Twenty.

DOGS

Time of Survival

At the beginning the mortality was exorbitant after orthotopic transplantation of the canine liver.^{34, 35, 44, 45} The deaths were due to deficiencies in surgical

technique, inadequate organ preservation, and anesthetic mismanagement. Later it became possible to obtain consistently good results. For example, 22 of 23 dogs (96 per cent) who received livers from nonrelated mongrel donors in one of our control series⁴⁶ survived for at least two days after operation, and 19 (83 per cent) lived for at least six days. All the animals were dead by the tenth day. The mean survival was 7.1 ± 2.2 (SD) days.

However, there have been occasional instances of relatively long survival in unmodified canine recipients, probably because of the fortuitous pairing of donor-recipient combinations with good histocompatibility matches. Examples are shown in Figures 74 and 75. The first of these dogs had evidence of spontaneous reversal of hepatic rejection. His death was caused after 20½ days by disruption of the hepatic arterial anastomosis at a time when the serum bilirubin was declining (Fig. 74). In the other experiment the liver function tests did not become grossly abnormal except for an elevated alkaline phosphatase during the three weeks of postoperative life, nor was there the reduction in hepatic blood flow which is characteristic (see later) during rejection (Fig. 75). A postoperative survival of 31 days is the longest known to have been achieved with these conditions of liver transplantation between nonrelated mongrel donors and recipients.²⁹

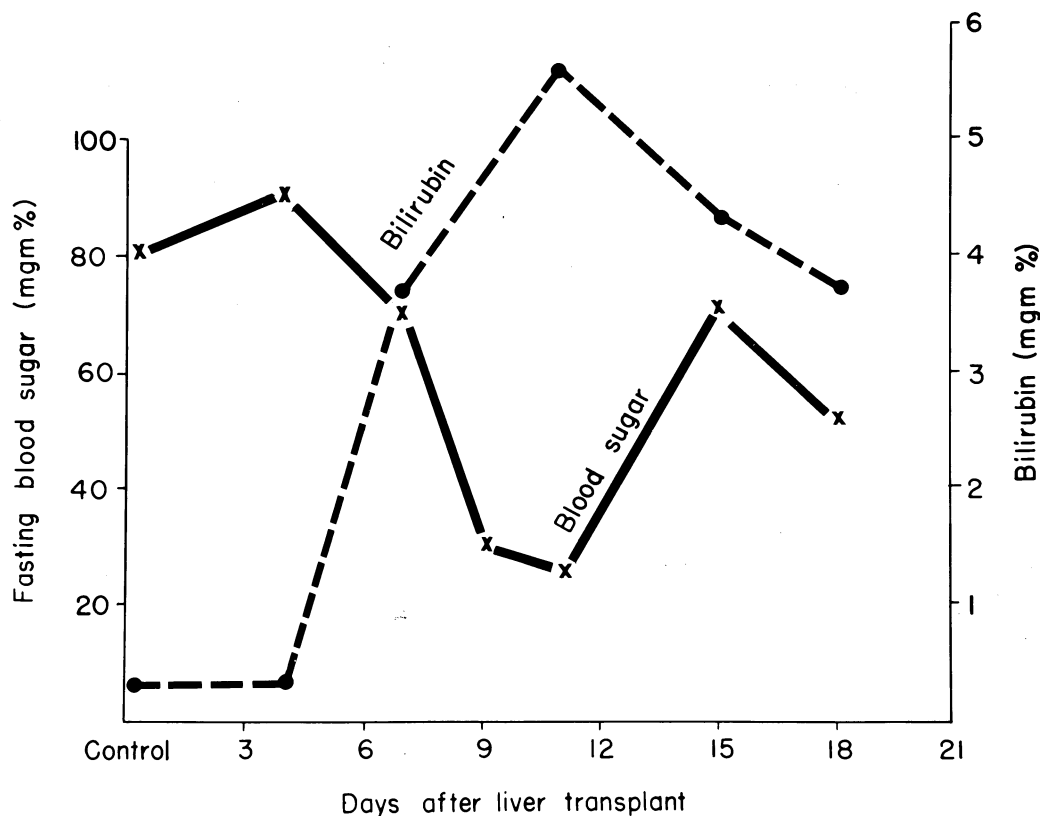


Figure 74. The course of an untreated dog observed after orthotopic liver transplantation. The experiment was carried out in 1959. The donor and recipient were nonrelated mongrels. Note the apparent spontaneous reversal of rejection. The animal died after 20½ days of rupture of the hepatic arterial anastomosis. (By permission of Surg. Gynec. Obstet. 112:135, 1961.)

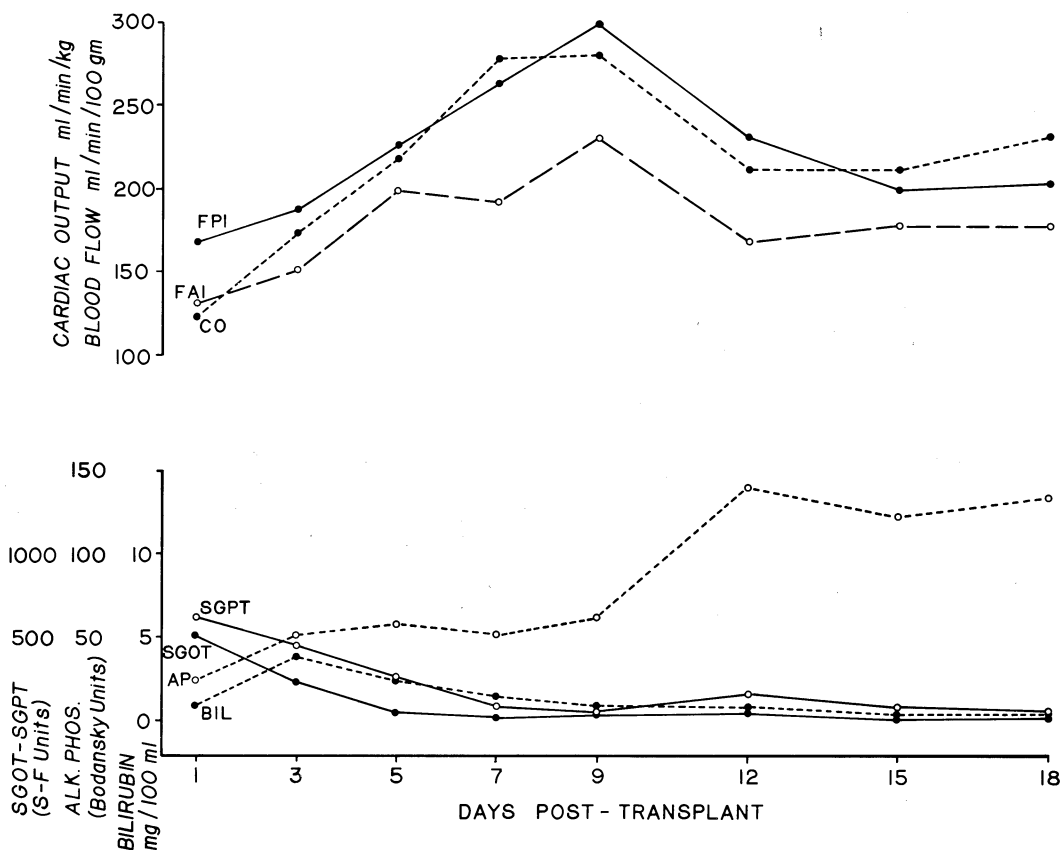


Figure 75. Absence of classic rejection after orthotopic liver transplantation in a dog; the only abnormality in liver function was an elevated alkaline phosphatase. Note that the decline in hepatic blood flow usually observed at the time of rejection (see Fig. 79) did not occur. The animal died after 21 days of wasting. Immunosuppressive therapy was never given. Under these conditions, survival of more than two weeks can be expected in only about 5 per cent of experiments.

Pathologic processes other than rejection often contribute to the death of the animals. The most frequent is pneumonitis, but ulceration of the gastrointestinal tract, intussusception, peritonitis, and atelectasis are also common.

Usual Postoperative Course

The examples just cited of long survival or spontaneous reversal of rejection are distinctly uncommon after orthotopic transplantation in untreated dogs. In the usual experiment a straightforward and almost invariable sequence of events follows a well executed operation. Immediate excellent hepatic function can be expected (Fig. 76). The animals recover promptly from either inhalation or barbiturate anesthesia; parenteral glucose therapy is usually not required. They often can resume an oral diet by the following morning, and they ordinarily appear to be quite normal. There may or may not

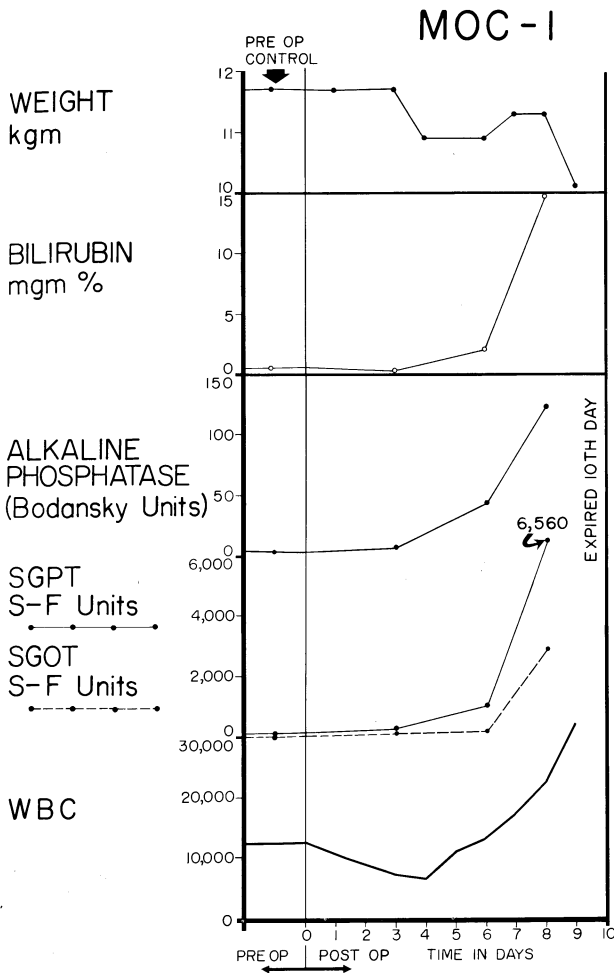


Figure 76. The biochemical changes usually seen after orthotopic liver transplantation to the untreated recipient. A brief period of good function follows operation, but deterioration of the biochemical pattern is unrelenting once it has begun. (By permission of Year Book Medical Publishers, 1966.)

be immediate transient rises in the SGOT and SGPT caused by the ischemic injury incurred during operation.

Within four or five days, however, there is manifold evidence of rejection (Fig. 76). The dogs stop eating. The SGOT and SGPT increase, usually to high levels, in rough temporal relation to parallel changes in alkaline phosphatase. Jaundice develops rapidly at the same time. The ultimate degree of hyperbilirubinemia often exceeds that which can be produced by ligation of the common duct in normal animals. With the latter procedure the serum bilirubin does not usually increase above 6 or 8 mg per cent, evidently because of moderately efficient clearance of bilirubin glucuronide by the canine kidney.⁷ In contrast, the dog with a rejecting liver may have serum bilirubins of 20 or 30 mg per cent.

The character of the jaundice in animals undergoing rejection is of interest. A variable but substantial fraction of the bilirubin is in the glucuronide form (Fig. 77). With the development of icterus, conjugated bilirubin appears in the urine, usually before the disappearance of urine urobilinogen. Finally, the stools became clay-colored. Thus, the syndrome of rejection has features of

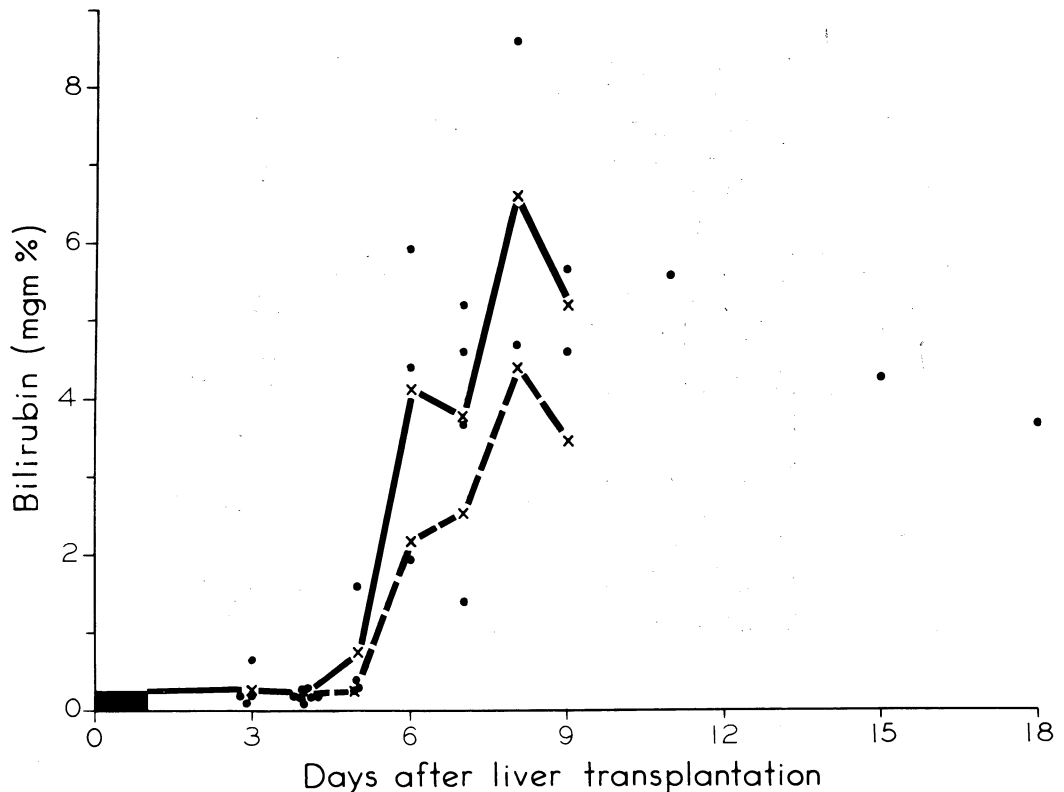


Figure 77. Changes in serum bilirubin in a series of untreated canine recipients of orthotopic liver homografts. Note that jaundice does not usually appear until the fourth to sixth days and that the conjugated or direct fraction (dashed line) contributes a major portion to the total bilirubin values. (By permission of Surg. Gynec. Obstet. 112:135, 1961.)

parenchymal cell injury and necrosis plus an element suggestive of biliary obstruction. The histologic finding of intracanalicular bile stasis (Chapter Twenty) helps to explain the component of obstruction.

The ability of the transplanted liver to promptly carry out complex functions of synthesis has been well established. In our first experiments⁴⁵ it was noted that the total plasma proteins were often well maintained, in some cases long after overt rejection had commenced (Fig. 78). A similar durability of carbohydrate metabolism in the face of unmodified rejection was also evident. Fasting plasma glucose levels were usually maintained for several days after clinical rejection was diagnosed; hypoglycemia was a terminal event.

Subsequently Kukral²⁷⁻²⁹ showed that there was actually heightened protein metabolism. He studied the synthesis rates of protein in untreated canine recipients by determining the incorporation of ³⁵S-methionine into plasma proteins. The specific activity of discrete protein fractions was separated by paper block electrophoresis and other standard radioisotope techniques. The synthesis (³⁵S uptake) was increased 40 to 200 per cent, with considerable variability in the different protein moieties. At the same time the biologic half-lives of the isotopes were shortened (increased turnover). The changes were similar in auto- and homografts, except that the latter had a disproportionate intensification of

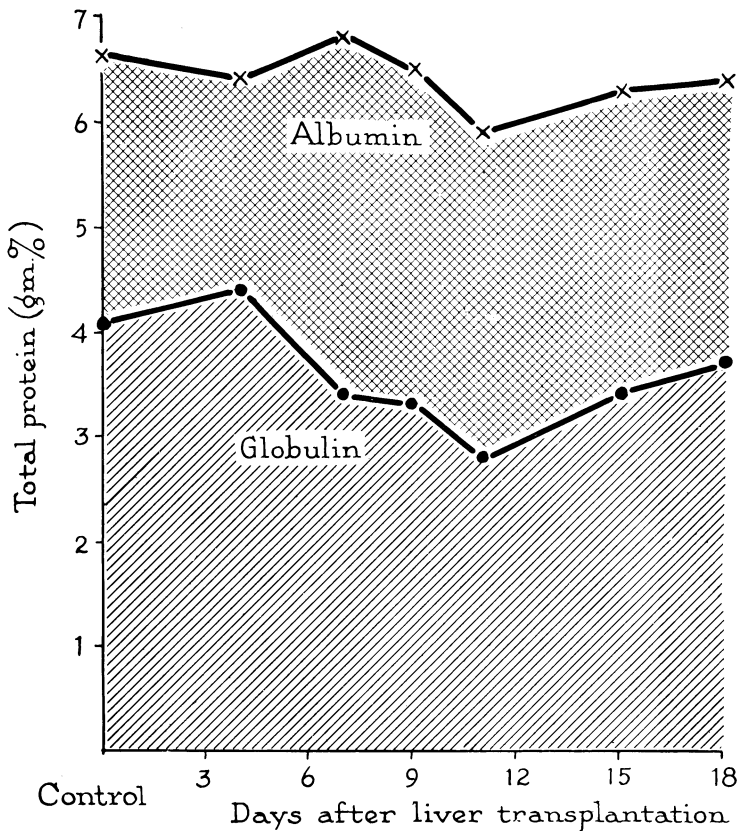


Figure 78. The serum proteins in the same dog whose course is shown in Figure 74. Serious hypoproteinemia did not develop even after the onset of moderately severe jaundice. (By permission of Surg. Gynec. Obstet. 112:135, 1961.)

gamma globulin activity. Kashiwagi's studies of haptoglobin and group specific component in human recipients of orthotopic hepatic homografts (see Chapter Eighteen) have suggested that accelerated protein turnover is also characteristic after clinical liver transplantation.²³ The failure of commensurate increases in protein synthesis because of ischemic injury to the transplant probably contributed importantly to the "third space" syndrome described in Chapter Six.

Hepatic Blood Flow

It has long been thought on the basis of observations with vital microscopy that a sudden decrease occurs in the blood flow of skin homografts at the time of their rejection.^{40, 47} This has also been shown to be a feature of rejecting renal homografts in dogs.^{20, 25, 26, 38, 41, 50, 52} Similar observations have been made by Groth¹⁶ in unmodified canine recipients of orthotopic liver homografts by use of a xenon isotope (¹³³Xe) washout technique.¹⁷

Eight animals were studied. In seven which died of rejection after six to 10 days, there was a decrease in both hepatic arterial and portal blood flows concomitant with a deterioration in hepatic function at the same time as the car-

diac output was relatively stable (Fig. 79). The other animal, in which there was never evidence of rejection until death 22 days later, did not have a diminution in hepatic blood flow (Fig. 75). It will be shown later (Chapter Twelve) that these hemodynamic changes of rejection can be prevented and/or reversed by adequate immunosuppression.

Groth's findings confirmed the suspicion that there was an important component of ischemia in the rejection of liver homografts. Earlier it had been speculated that blood flow might be choked off at a sinusoidal level^{32, 45} or, alternatively, in larger vessels within the intrahepatic portal tracts.³³ The best evidence now¹⁶ is that the immunologically mediated ischemia is due mainly to damage of the veins and sinusoidal bed of the homograft (Chapter Twenty) and that it cannot be favorably influenced by the intra-arterial administration of vasodilating agents.³³

Variations in Portal Revascularization

Early in our experience⁴⁴, three methods of revascularization were compared. After normal reconstruction of the portal system (Fig. 80C), eight of 11 dogs lived for at least four days. When a small portacaval shunt was left in place (Fig. 80B), only six of 15 animals survived this long. An important contributory cause of the mortality in many of the latter dogs was a distinctive syndrome consisting of intractible vomiting and retching which began immediately after operation and continued until death. This was thought to be due to acute liver failure. Recently Fonkalsrud¹³ has also reported poor results with a modification of this method; less than a third of his azathioprine treated recipients lived for as long as one week.

Efforts to augment portal venous flow by means of a reverse Eck fistula in 26 animals (Fig. 80A) were even less successful.⁴⁴ Only four survived for as long as four days. Often, the newly transplanted liver could not transmit the increased flow. Immediate hepatic congestion and outflow block or delayed ascites were the consequences.

The desirability of restoring a normal blood supply to orthotopic liver homografts is evident from this kind of experiment. Such considerations are even more important in auxiliary liver transplantation, as will be discussed in Chapter Twenty-one.

The Orthotopic Liver in a Composite Graft

The liver has been transplanted as part of a relatively enormous multiorgan graft^{42, 43} which also included the spleen, pancreas, omentum, stomach, small bowel, and colon (Fig. 81). The cooled viscera were used to replace the same structures which were removed en bloc from the recipient.

Only five of 38 animals survived the first 24 postoperative hours; most of the rest developed irreversible shock secondary to congestion of the intestinal portion of the homograft complex. The exceptional dogs lived for five and a

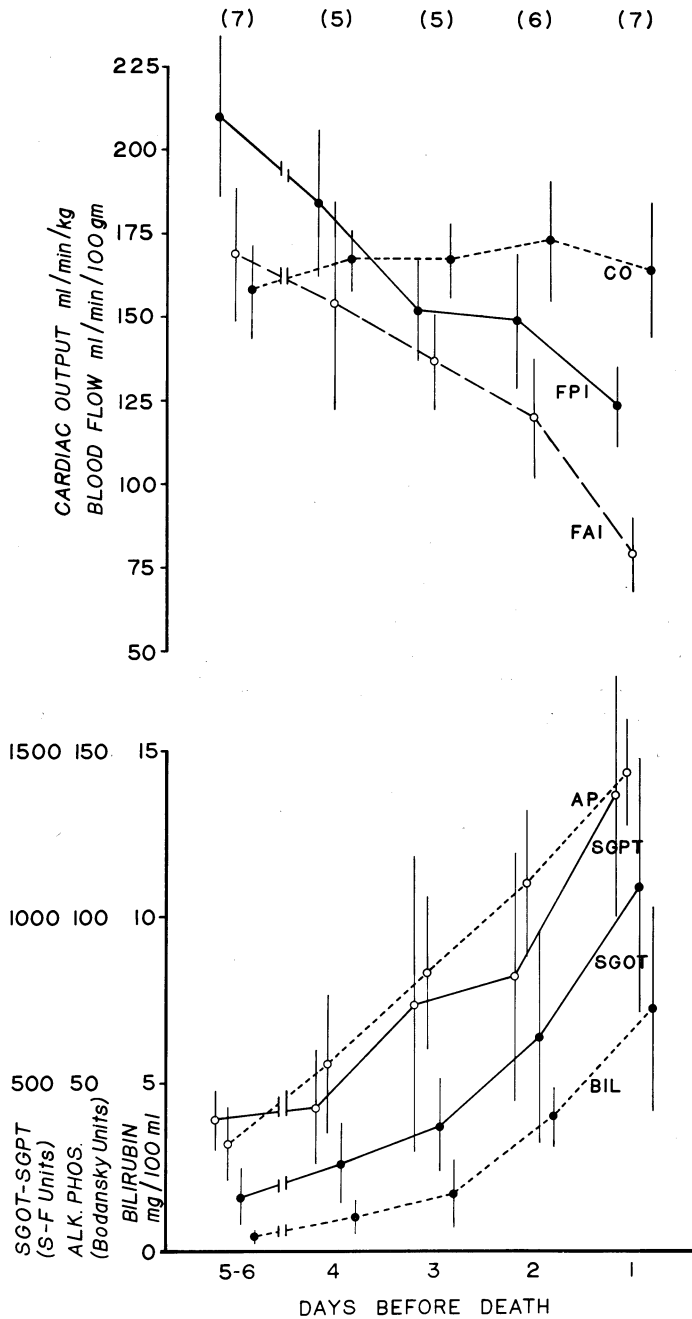


Figure 79. Liver blood flow values obtained on portal (FPI) and hepatic arterial (FAI) injection, cardiac output (CO) and liver function in seven unmodified recipients that died of rejection. The mean values \pm SE are shown, as well as the number of observations (in parentheses) for each day. Note that both the hepatic arterial and portal blood flows, as determined by the xenon isotope (^{133}Xe) washout technique, decreased concomitant with deterioration of hepatic function. (By permission of Surgery 63:658, 1968.)

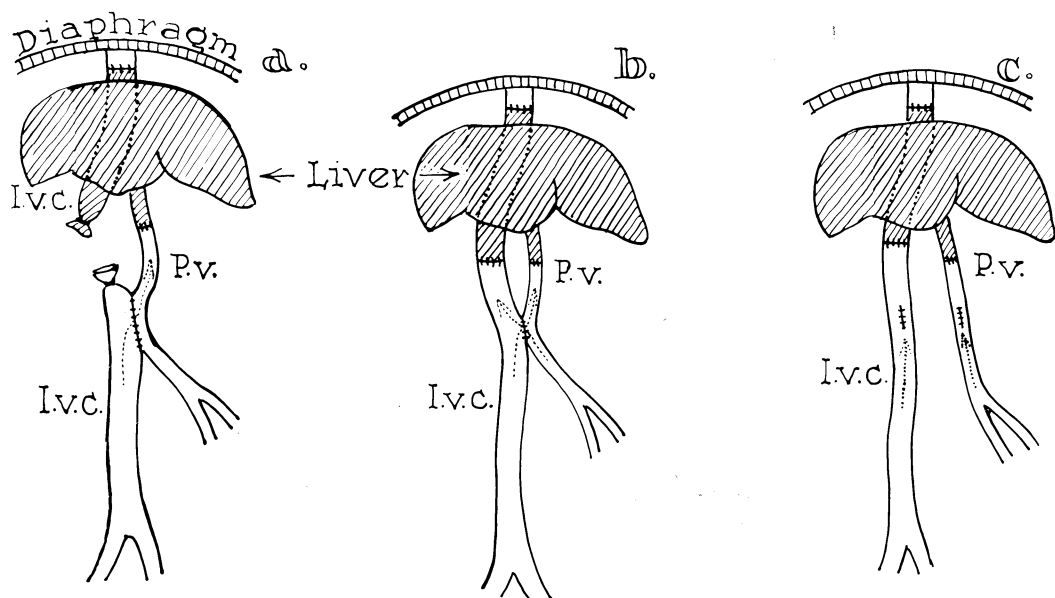


Figure 80. Methods of venous reconstruction evaluated in canine recipients of orthotopic homografts. *a*, Reverse Eck fistula *b*, Anatomic reconstruction with a small portacaval shunt left in place. *c*, Complete anatomic reconstruction with closure of the preliminary portacaval shunt. Both *a* and *b* carried a prohibitively high early mortality and were, therefore, abandoned in subsequent canine experiments. (By permission of Surg. Gynec. Obstet. 111:733, 1960.)

half to nine days, were able to eat, and maintained essentially normal glucose homeostasis. Two of them never became jaundiced. The abnormalities found in the liver after death were less advanced than those usually seen at comparable times when this organ is transplanted alone.

Potentially there could be an occasional clinical use for a composite transplant of this kind. We have seen two relatively young men with thrombosis of the celiac axis and mesenteric arteries who developed gangrene of all the same organs contained in the homograft, including the liver. In dogs the technical requirements of the procedure were no more difficult than with a standard orthotopic hepatic transplantation since only three vascular anastomoses with large caliber vessels were involved. The limiting factor was the intestinal congestion and hemorrhage which promptly killed more than 80 per cent of the recipients during and just after operation, and for which there has not yet been a satisfactory explanation.

PIGS

The foregoing background in dogs was later supplemented by analogous studies in untreated pigs. It is quite likely that the choice of the pig as a second species was influenced by the extensive investigations by Eiseman of the extirpated and perfused porcine liver.^{11, 51} Other factors were the similarity of the pig liver and its vessels and biliary tract to that of man² and the resistance of

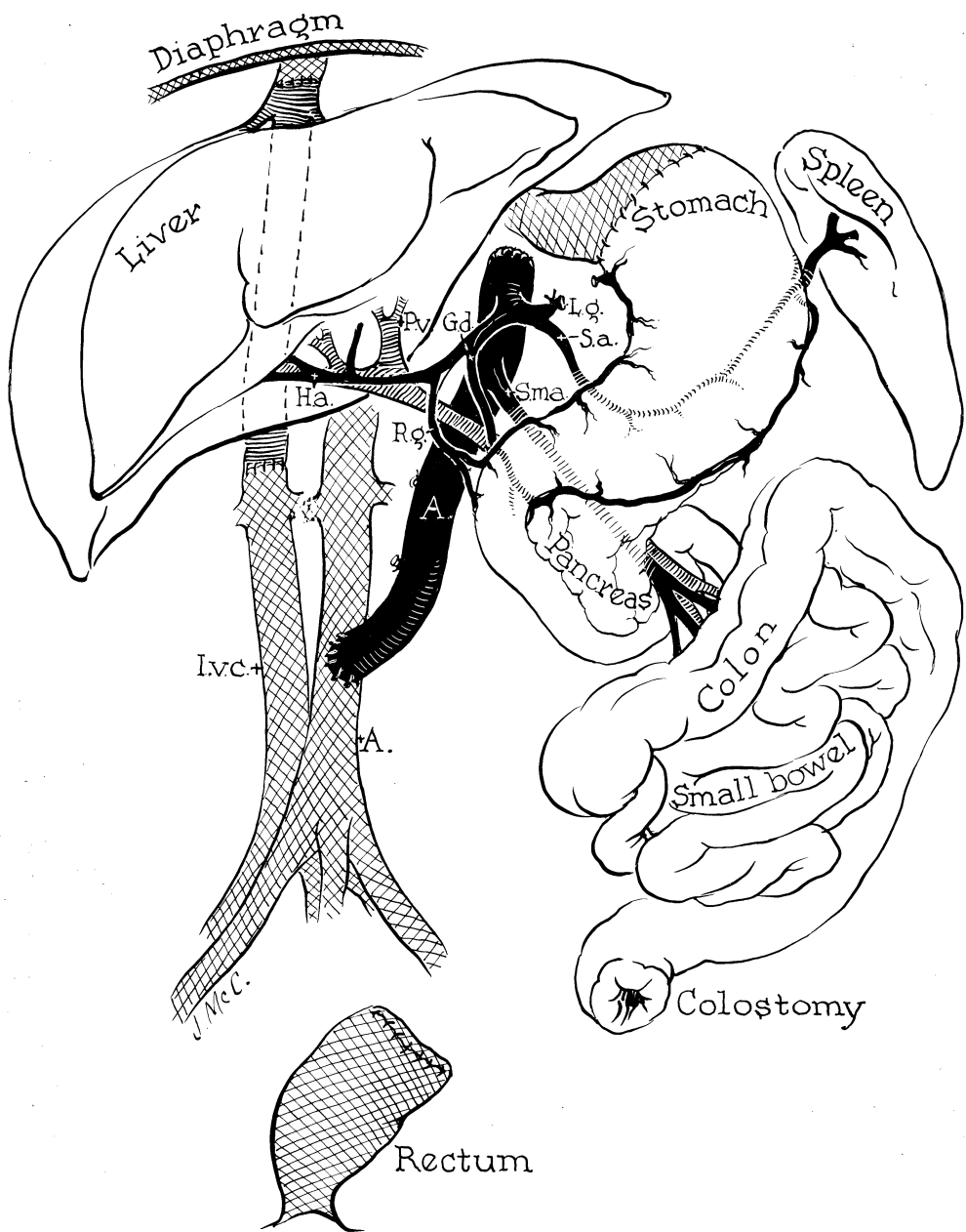


Figure 81. Schematic view of the canine multiorgan graft. The liver, spleen, pancreas, omentum, stomach, small bowel, and colon of the donor (tissues not shaded in the diagram) were transplanted en bloc following removal of the corresponding structures from the recipient. No immunosuppressive therapy was given. The longest survival was nine days. (By permission of Amer. J. Surg. 103:219, 1962.)

the porcine organ to development of "outflow block,"¹² which is a major problem in dogs (Chapter Five).

The surgical techniques were adapted from those developed in the dog.^{5, 6, 8, 15, 37, 39, 48} The only noteworthy point is that a single bypass from the splenic to the jugular vein is all that is necessary for venous decompression during the anhepatic phase, at least in our hands (Fig. 82). Systemic heparin is

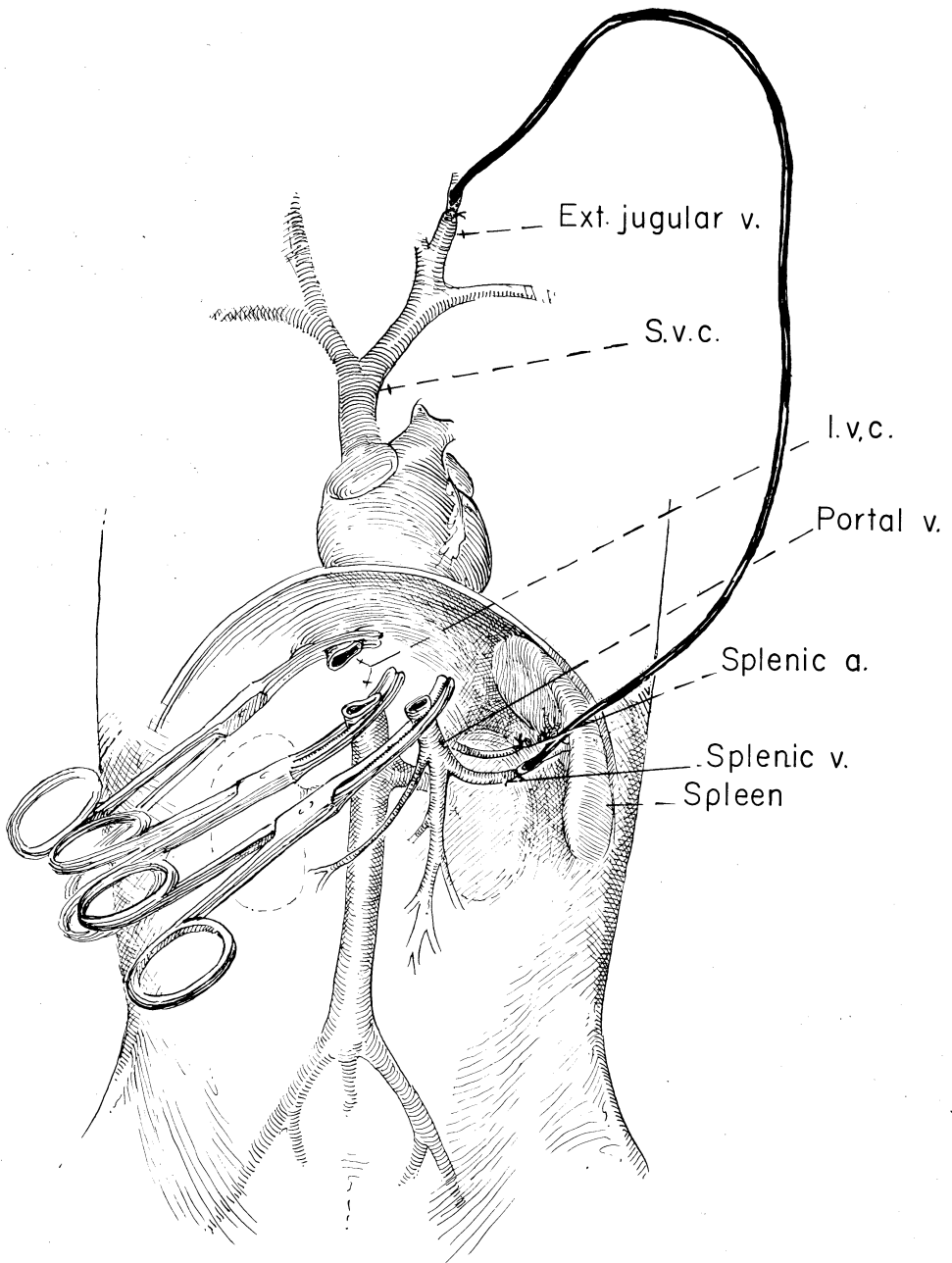


Figure 82. The bypass system used for orthotopic transplantation in pigs. Only portal decompression is necessary. The vessels entering and leaving the hepatic homograft are reconstructed in an essentially normal manner. We have provided biliary drainage with cholecystoduodenostomy.

given before removal of the recipient liver and is not subsequently neutralized. Survival for at least several days has been obtained in more than three-fourths of the porcine experiments in our laboratory (Table 14).

Professor Henri Garnier and Dr. J. P. Clot of Paris^{8, 15} were the first to investigate orthotopic porcine liver transplantation. Using unrelated large white pigs, they confirmed that the general events after operation were usually comparable to those in dogs. The biochemical changes during rejection were similar, although the rises in the transaminases were less pronounced than after canine hepatic transplantation. They also described acute peptic ulceration which led to the death of their longest survivor.

The unique contribution of Garnier's work was his observation of the mild and indolent nature of the rejection. Because of technical and anesthetic difficulties, he lost most of his animals during operation or within the first 48 postoperative hours. There were, however, seven pigs which lived as long as two days, and four of these seven survived for 25 days or longer (maximum 35).

This interesting finding was soon confirmed by Peacock, Terblanche, and Riddell in Bristol,^{37, 39, 48, 49} who began their studies without knowledge of Garnier's prior work. Of 15 of their pigs which passed the two-day postoperative mark, three survived for more than 25 days. The last animal in the series was sacrificed at two years because of failing health. Hunt¹⁹ reported serial biochemical as well as histopathologic studies in these animals which indicated that the rejection was feeble than in untreated dogs. A few of the pigs never had postoperative deterioration of liver function, and some of the others apparently passed through a rejection within four to 10 days that underwent spontaneous reversal. As in Garnier's earlier experiment, several valuable recipients ultimately died because of hemorrhage from peptic ulceration. The most common site of ulceration was at the esophagogastric junction, where this species has a long tongue of squamous epithelium extending into the body of the stomach.⁹

Jaffe, Symes, and Terblanche²¹ sought an explanation for the surprising survival of porcine liver recipients with a series of immunologic studies, including lymphocyte transfer tests, mixed lymphocyte cultures, and skin grafting. The results in the first two examinations were difficult to interpret, but the skin grafts were invariably rejected within 11 days. The authors suggested that liver tissue might be relatively nonimmunogenic, a hypothesis supported by an earlier report of Kaliss.²²

Further proof of the blandness of hepatic homograft rejection in pigs was contributed by Calne and his associates at Cambridge, England,^{5, 6} and from our own laboratories (Table 14 and Figs. 83 and 84). One of Calne's animals survived for seven months, eventually dying of intestinal obstruction; there was no evidence of active rejection and only minimal liver damage. In the second of Calne's two reports, 20 of 36 liver recipients lived for at least three postoperative days and 12 of these 20 had survival exceeding 25 days. The problem of delayed gastrointestinal hemorrhage was avoided by the performance of prophylactic gastroenterostomy and bilateral vagotomy.

The Cambridge workers also added to the evidence that a lack of recipient immunologic reactivity was not the essential reason for these results, since

Table 14. The Results in Our Denver Laboratories with Orthotopic Liver Transplantation in Untreated Pigs and in Pigs Given ALG*

EXPERI- MENT NUMBER	SURVIVAL (Days)	POST- OPERATIVE HEMATOCRIT CHANGE	LAST BILIRUBIN (mg per 100 ml)	GROSS CAUSE OF DEATH
<i>Untreated recipients</i>				
1	37	46 → 19	7.0	Rejection; esophagogastric ulcer; GI hemorrhage
2	1	—	—	Atelectasis
3	Alive	40 →	0.0	Alive 15 months
4	Alive	36 →	0.2	Alive 15 months
5	2	—	—	Congestive atelectasis
6	8	38 → 34	1.8	Rejection; esophagogastric ulcer; GI hemorrhage
7	20	40 → 33	6.6	Rejection; L. bronchopneumonia
8	8	42 → 46	6.1	Rejection; agonal intussusception
9	6	42 → 38	1.5	Not specified
<i>Recipients treated with horse antipig-lymphocyte globulin (ALG)</i>				
1	7	35 → 21	<1.0	? Rejection; ? GI hemorrhage
2	15	40 → 11	6.1	Rejection; duodenal ulcer
3	23	36 → 11	6.6	Rejection; esophagogastric ulcer; GI hemorrhage
4	1	—	—	Congestive atelectasis
5	7	46 → 34	10.2	Rejection; pneumonitis; cholangitis
6	28	50 → 32	6.4	Rejection
7	Alive	40 →	0.5	Alive 15 months
8	1	—	—	Congestive atelectasis
9	13	35 → 33	1.1	Rejection

*Outbred animals were used and donors and recipients were selected of different color and appearance. The experiments were performed by Dr. George V. Smith, who is presently located in Hartford, Connecticut.

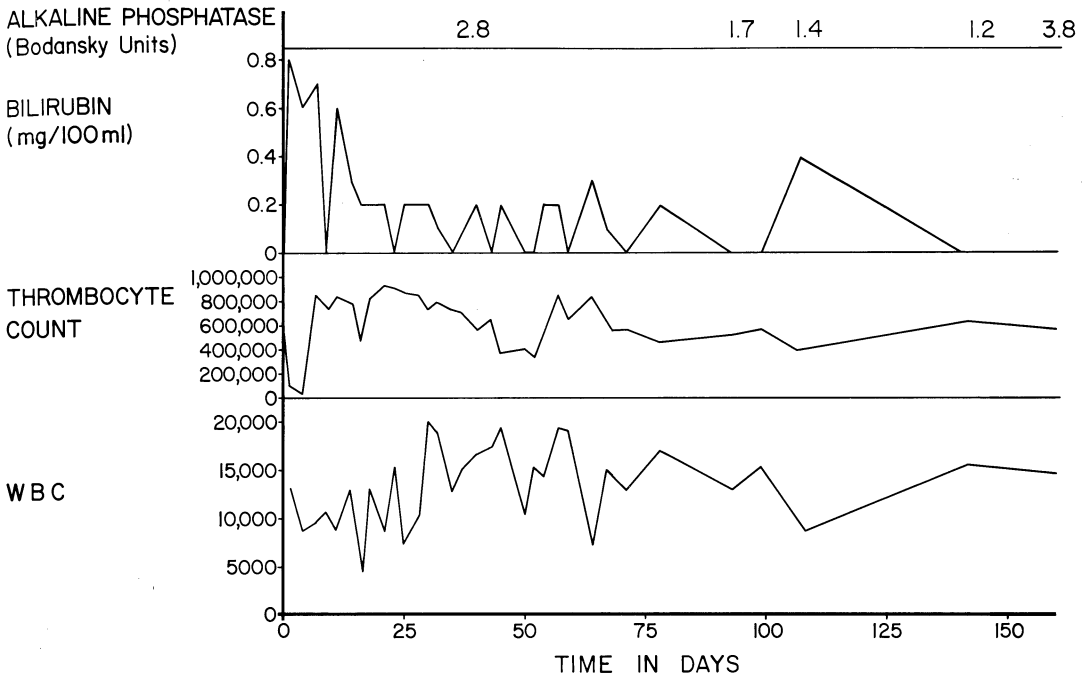


Figure 83. The first six months in the postoperative course of an untreated pig after orthotopic transplantation of the liver. Rejection was not diagnosed at any time. Note the abrupt thrombocytopenia which persisted for the first few days after transplantation. The same phenomenon has been noted by Hutchison after auto- and homotransplantation of the liver in dogs. The animal is still alive 15 months after operation. The experiment was performed by Dr. George V. Smith.

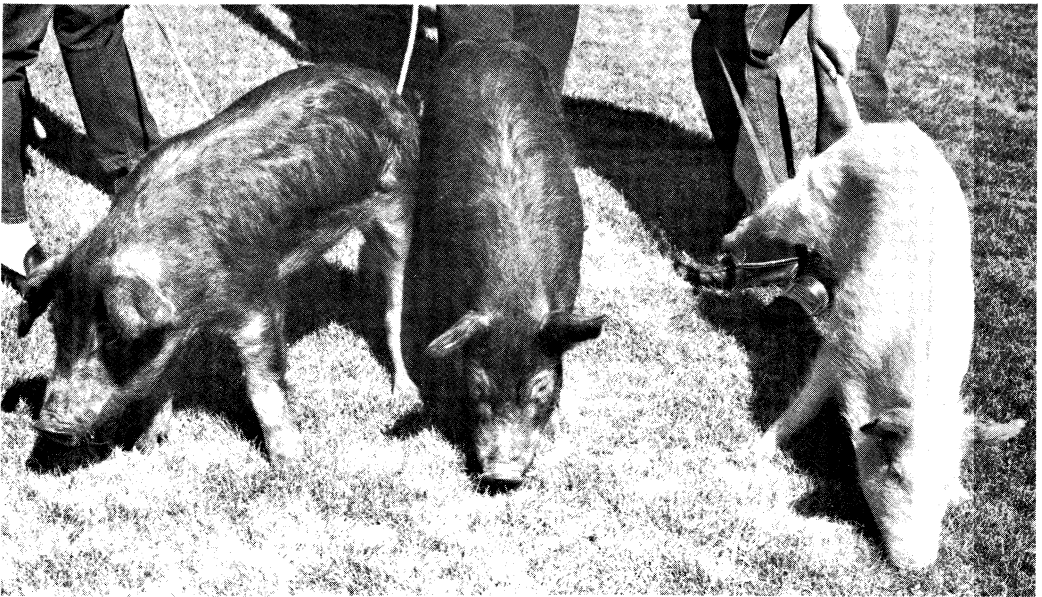


Figure 84. Three growing pigs which were subjected to orthotopic liver transplantation five months earlier. The two on the left received no treatment with immunosuppressive agents. The one on the right had 20 injections of horse antipig-lymphocyte globulin (ALG). All three animals are still alive, now 15 to 15½ months after operation. The experiments were performed by Dr. George V. Smith.

both skin¹ and kidney grafts⁵ were rejected in the expected way. In a control investigation with renal homotransplantation and bilateral recipient nephrectomy, there were 13 technically satisfactory experiments. One animal lived for 33 days but all others died of uremia within three weeks.⁵

Interestingly enough, this aggressive rejection of porcine renal homografts was substantially mitigated and/or delayed if a kidney and a liver from a common donor were simultaneously transferred to the same recipient;⁴ now the same privilege of mild rejection seen with transplantation of the liver alone extended as well to the renal tissue. The specificity of this finding is open to some question since it has also been shown, as mentioned earlier in this chapter, that other combinations of organs can lead to a less vigorous host reaction than that evoked by the individually transplanted constituent parts. The units tested have included multiple gastrointestinal viscera¹³ and the kidney plus the spleen.^{24, 31}

The observations from hepatic transplantation in pigs have given substance to earlier suspicions from dog experiments¹⁶ that, in terms of the host response it evokes, the liver may be one of the "easiest" organs to transplant. Although conceivably valid, this view could easily be overstated and, as will be emphasized in the succeeding chapters, could lead to erroneous and self-defeating conclusions about the requirements for therapy after hepatic transplantation in man.

At a research level another danger stems from the notion that the pig model is somehow qualitatively unique as compared to that in the dog or human. The differences are almost certainly only quantitative, as will be discussed in the next chapter. In the series of pig liver transplantations in our laboratories in which the recipients were either unmodified or treated with a few injections of ALG (Table 14), the majority of animals developed the overt clinical signs and the characteristic pathologic findings of fatal acute rejection (Chapter Twenty). Even when this did not occur, homograft biopsies taken later often showed evidence of healed earlier rejection (Chapter Twenty). The abnormalities were entirely analogous to, although less marked than, those in many dogs which had been given azathioprine or ALG for several postoperative weeks or months and then had had this therapy stopped with long subsequent survival.

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Chapter Twelve

EFFORTS TO MITIGATE OR PREVENT REJECTION

The immunosuppressive treatment given to human recipients of liver homografts is considered in Chapters Thirteen to Seventeen. Combination drug therapy was used for these patients in a regimen similar to that widely employed for renal homotransplantation in man. However, the most precise information about the immunosuppressive agents has come from controlled animal experimentation in which the individual drugs were used alone.¹⁶² Essentially all the meaningful research of this kind in the field of liver transplantation has been carried out in dogs. X

TOTAL BODY IRRADIATION

The only reported attempts to prolong hepatic homograft survival by host total body irradiation were completely unsuccessful.¹⁶² In these experiments 1400 R were given to dogs. Eighteen to 40 hours later, before the development of leukopenia, total hepatectomy and orthotopic homotransplantation were carried out. The recipients all died within 36 hours from diffuse hemorrhagic gastroenteritis and/or intrapulmonary hemorrhage. The combination of the operative trauma and irradiation appeared to preclude even temporary success. The intestinal injury was a particularly discouraging finding. It was probable that irradiation injury to the bowel was aggravated by transient elevations of portal pressure during the transplantation.

AZATHIOPRINE

The first efforts to prevent rejection of liver homografts with azathioprine were in 1963, at least two years after the value of this agent in protecting renal homografts had been proved.¹⁹ The initial results were extremely poor.^{126, 166} Only one of the first 25 liver recipients in our laboratory lived for as long as one

month, and in the Boston report on 10 dogs the observations were similarly discouraging. Nevertheless, there was unequivocal evidence that rejection had been at least partially controlled. The deterioration of postoperative hepatic function occurred later or, in some cases, was avoided altogether.^{166, 173} The majority of the deaths were due to pneumonitis. Other causes of failure were perforated gastric ulcer, peritonitis, liver abscess formation, and intussusception.

In retrospect, the lack of success seemed ascribable to several factors which probably played a cumulative role. First, the perfect technical performance necessary for consistently good results was achieved only with a great deal of subsequent practice. In addition, too much azathioprine was probably being given, accounting for the almost invariable development of sepsis; the susceptibility to pneumonitis under these circumstances may have been accentuated by subdiaphragmatic irritation caused by the large mass of subjacent foreign tissue. Finally, it was learned that azathioprine was hepatotoxic in dogs.^{61, 172, 180}

Hepatotoxicity

The last finding had special significance since the target tissue of rejection was also being injured by the drug used to prevent this process. This was easily demonstrated in experiments on normal animals which were given a 40-day course of azathioprine, using doses which would have been considered reasonable for antirejection therapy.¹⁷² Transient increases in the serum transaminases and the alkaline phosphatase were invariably manifest within a few days after the institution of treatment (Fig. 85). The features of the hepatic injury were suggestive of a direct toxic rather than a hypersensitivity mechanism inasmuch as the deleterious effect was dose-related, was seen promptly, and tended to diminish with continued treatment. Although none of the animals became jaundiced, the enzyme alterations were reflected in anatomic changes, as will be described in Chapter Twenty. The animals often became anemic and lost weight during therapy (Fig. 85).

Relevance of Canine Hepatotoxicity to Man. This question has never been adequately settled. A high incidence of human liver injury has been documented with the use of 6-mercaptopurine for cancer chemotherapy;^{38, 108} 6-mercaptopurine is a chemical from which azathioprine is derived. Similarly, many patients administered azathioprine for long periods after renal homotransplantation have developed either acute or chronic liver disease.^{59, 67, 137, 160, 169} However, the etiologic role of the drug has been difficult to prove since the best diagnosis in many such cases was infectious hepatitis,¹³⁷ a complication also commonly seen in uremic patients under treatment with chronic hemodialysis without subsequent transplantation.^{37, 45, 84, 146} Furthermore, it is possible that other agents used in the complicated clinical regimen of therapy could have been contributory, particularly the prednisone which was almost invariably administered in high doses. Steroids can cause hepatic parenchymal injury in rats,^{9, 71, 135} rabbits,⁶ dogs,⁶ and man.^{177, 184}

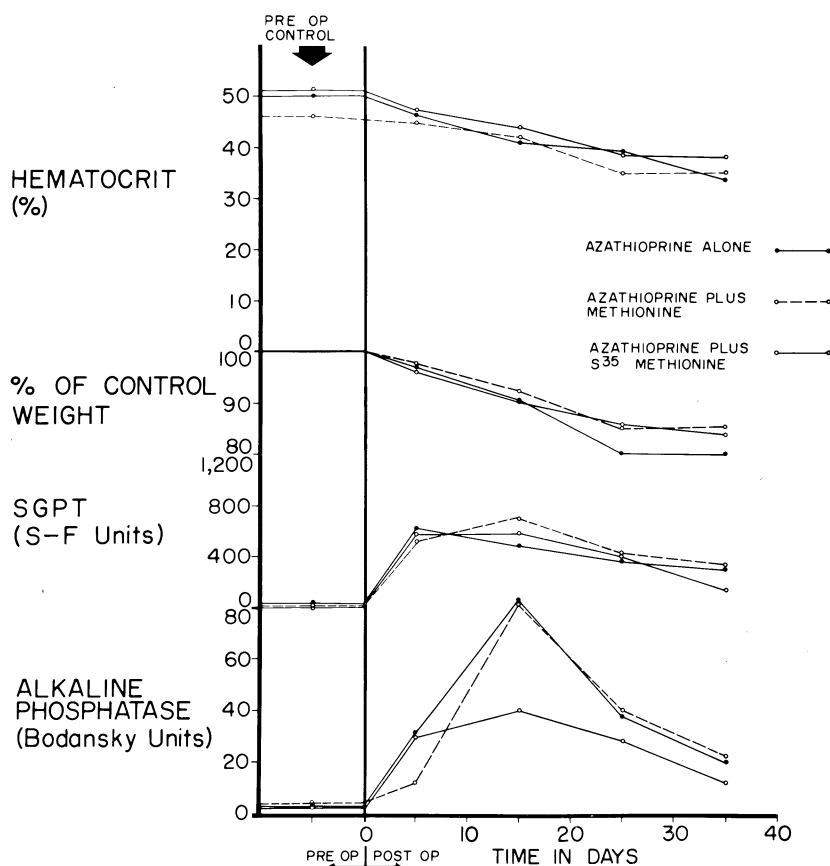


Figure 85. The toxicity of azathioprine when used alone or in combination with ^{35}S methionine or methionine. Six dogs were in each of the three test groups. There were easily detectable abnormalities of liver function but jaundice did not develop. The azathioprine doses were 1 to 4 mg/kg per day. Note that the animals tended to lose weight and become anemic. (By permission of Surgery 58:131, 1965.)

An effort was made by Hill, Porter, and Massion⁶³ to establish whether azathioprine had a consistent hepatotoxicity in man. They systematically reviewed the autopsies of 34 recipients of renal homografts who had been treated with this agent for a few days to many months before their deaths. A pattern of hepatic injury could not be found which could be attributed to the prior administration of azathioprine.

Nevertheless, there have been a number of clinical cases, as was discussed in a recent monograph,¹⁶⁹ in which it was difficult to dismiss lightly the possibility of azathioprine liver toxicity. An example is shown in Figure 86. The patient received two renal homografts. Five months after the first operation he developed jaundice which slowly receded after his azathioprine dose had been reduced from 150 to 50 mg/day. Ultimately, the kidney was rejected. A second homograft was then transplanted after two doses of 200 and 175 mg azathioprine had been given, one the evening before and the other on the day of operation. Following surgery the SGOT and SGPT rose to more than 4000 International Units (normal less than 40) and he became jaundiced. He died 25 days later and was found at autopsy to have massive liver necrosis.

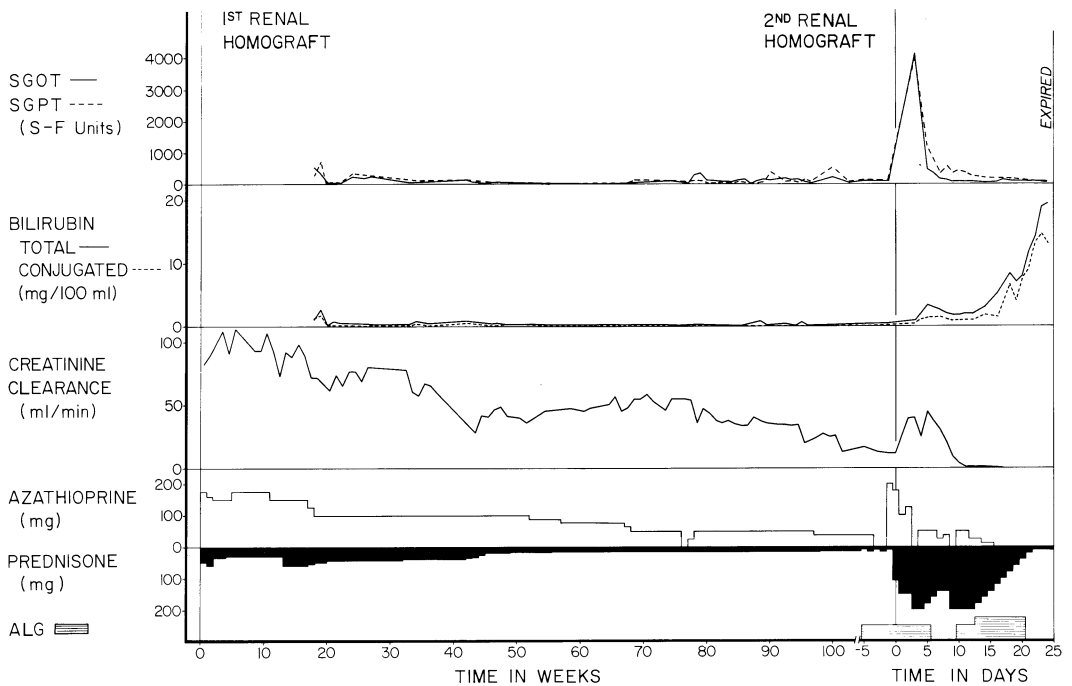


Figure 86. The course of a kidney transplant recipient who appeared to have suffered liver damage from azathioprine toxicity. About five months after his first renal homotransplantation, he developed low grade jaundice and increases in his transaminases. The azathioprine dose was cut immediately and further reductions made at subsequent intervals. The homograft eventually failed and was removed after about two years. When retransplantation was performed, four large doses of azathioprine were given just before and after operation. Immediately the transaminases rose to more than 4000 units. The patient became intensely jaundiced and died of hepatic and renal failure.

Vague though the conclusions may be about the presumed multiple etiologies of liver disease in patients receiving renal homografts while under immunosuppressive therapy, it is crucial for obvious practical reasons to have an idea of the frequency of this kind of complication before embarking on trials of liver transplantation in which the differential diagnosis and proper management could become hopelessly confused by the coexistence of rejection and liver injury from other causes. Penn's studies¹³⁷ have demonstrated the number of instances in which such a perplexing distinction might have to be made.

Penn examined the pre- and postoperative courses of 146 kidney recipients. At some time 88 (60 per cent) of the patients developed abnormalities in the serum bilirubin, alkaline phosphatase, SGOT, SGPT, prothrombin time, serum protein concentration, or bromsulphalein (BSP) clearance. The hepatic dysfunction appeared in the majority of cases within the first six postoperative months (Fig. 87). In most instances the changes were transient and mild but in 20 they were marked and persistent; four of the patients died of hepatic failure five to 18 months following operation. The derangements in the liver function were variable from case to case and were accompanied by clinical complaints in only a small minority. For example, it was extremely common to have elevations in the serum transaminases (Fig. 88) at a time of apparent good health

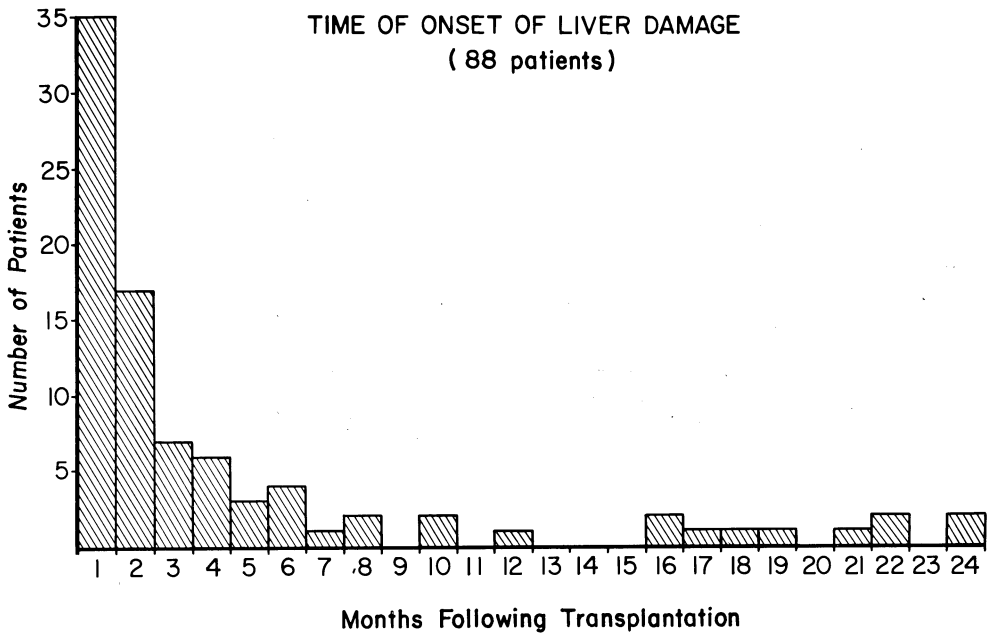


Figure 87. Time of onset in human renal transplant recipients of abnormalities of one or more liver function tests. One hundred forty-six patients were studied. Of these, 88 (60 per cent) had some evidence of hepatic damage, usually during the first six postoperative months. This was usually mild, but in 20 patients (14 per cent) it was severe. (By permission of Current Topics in Surgical Research 1:November, 1969.)

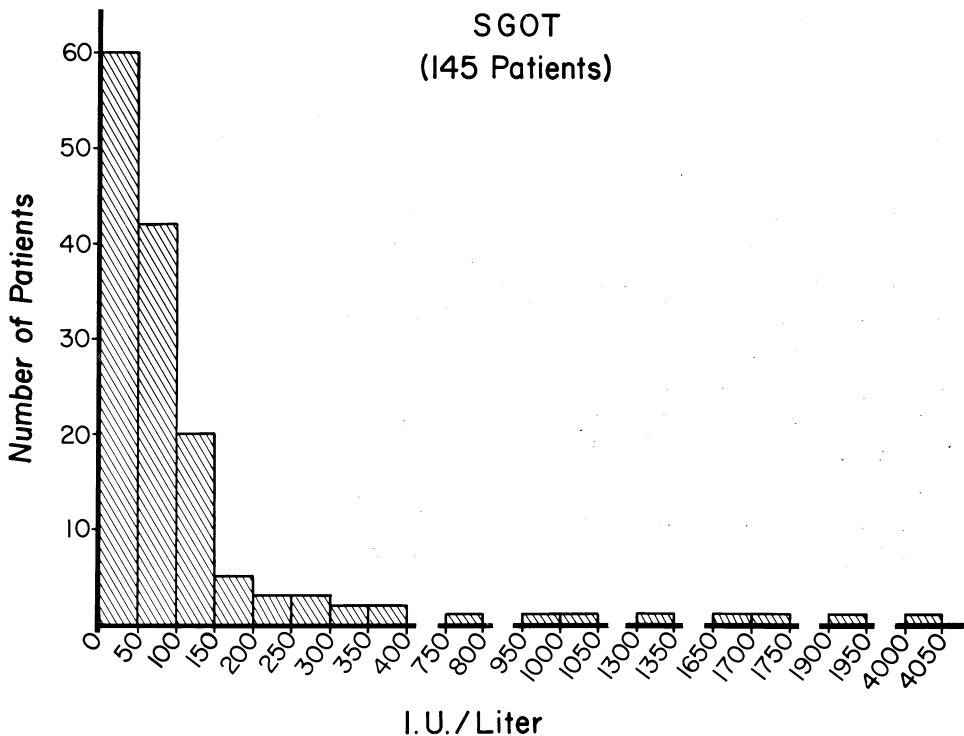


Figure 88. Serum transaminase determinations in all but one of the patients included in Figure 87. The SGOT was elevated in 59 per cent of these patients and the SGPT in 51 per cent. The normal values are less than 40 International Units (IU). The levels shown here and in Figure 89 are the most abnormal observed in any given patient. (By permission of Current Topics in Surgical Research 1:November, 1969.)

and in the absence of jaundice or other alarming findings. Even the appearance of hyperbilirubinemia was not necessarily a bad prognostic sign since 52 patients (36 per cent) had icterus (Fig. 89) of 1.0 mg per cent or greater; however, in most cases this was short-lived. The increased bilirubin concentration was usually an approximately equal combination of the conjugated and unconjugated forms and was almost invariably accompanied by increases in alkaline phosphatase. The prothrombin times, serum protein concentrations, and BSP excretion were most markedly affected in the patients who developed symptoms of serious liver disease.

Eighty of the recipients studied by Penn were treated before June of 1966 with azathioprine and prednisone. Fifty-two (65 per cent) of the 80 had evidence of liver damage; all four of the fatalities caused by hepatic failure were in this group. The next 66 patients were also administered heterologous ALG and were given reduced quantities of the other two agents (see Chapter Thirteen). Thirty-six (54 per cent) of the 66 recipients treated in the latter period also had some findings of hepatic damage, but generally to a less severe degree and never with a fatal outcome. Consequently it has been our conclusion that the magnitude of the problem has been distinctly less since the addition of ALG to the immunosuppressive regimen.

Prolonged Survival after Canine Liver Transplantation

Despite the handicap imposed by its undoubted hepatotoxic effect in dogs, azathioprine has permitted extended survival after orthotopic transplantation

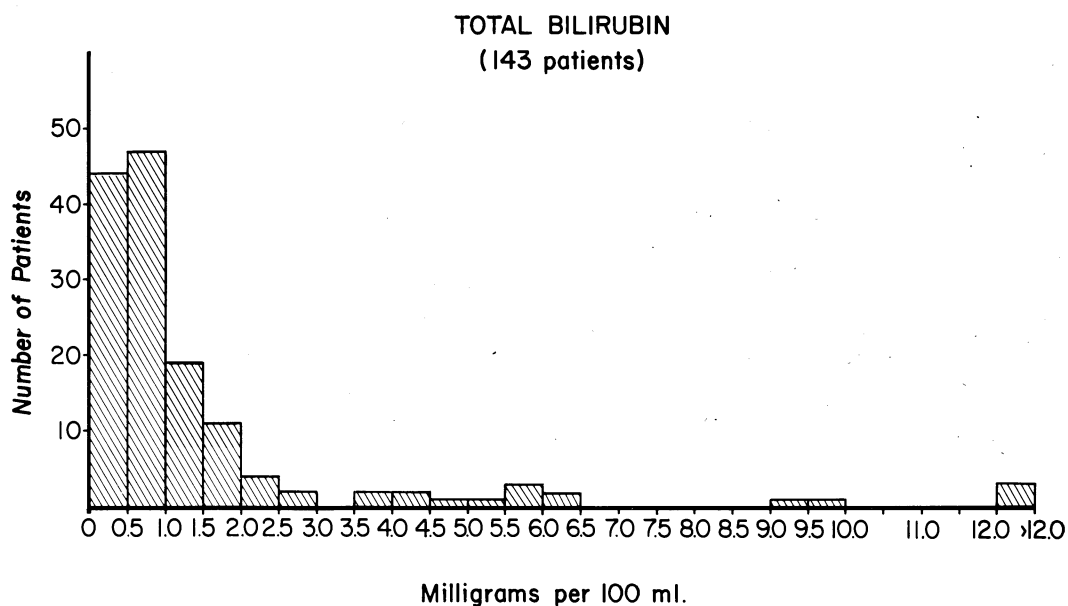


Figure 89. The maximum total serum bilirubin concentrations in all but three of the patients shown in Figure 87. At some time the bilirubin was elevated above 1.0 mg per cent in 52 cases (36 per cent). (By permission of *Current Topics in Surgical Research* 1 : November, 1969.)

of canine livers between nonrelated donors and recipients. The first of many successful experiments was performed on March 23, 1964,^{168, 169, 172} about a year after the initially discouraging results mentioned earlier. That animal and 43 and 23 others from the same era lived for at least 25 and 50 days, respectively, after operation. Nineteen of the dogs survived for three months or longer, five lived for more than a year, and two are still in perfect health four and a half and five and a quarter years later (see Frontispiece).

Dr. Philippe Mikaeloff of Lyon, who assisted in the compilation of this experimental series, was able to reproduce many of the findings promptly after his return to France that autumn;¹¹²⁻¹¹⁶ in addition, he demonstrated the feasibility of extended survival (three months) after transplantation of livers preserved for several hours after donor death (see Chapter Five). Fonkalsrud^{43, 44} and Stuart¹⁸⁰ have also extended survival of canine recipients of orthotopic liver homografts with the aid of azathioprine therapy.

The ability of azathioprine to prevent homograft repudiation was unpredictable from dog to dog, a variability which was presumably due to differences in the quality of histocompatibility (Chapter Three) fortuitously achieved by random donor-recipient pairing in the outbred canine population. The onset of rejection was delayed little if at all in slightly less than one third of our experiments.¹⁷² Destruction of the hepatic grafts in these recipients proceeded relentlessly until the time of death (Fig. 90).

At the other end of the spectrum were about 23 per cent of the recipients in which overt early rejection was not diagnosed on the basis of serial liver function tests (Fig. 91) or else occurred to a very mild degree. From this minority group came all five of the animals that eventually survived for a year or longer out of the total series of 84 technically satisfactory experiments.

Finally, almost half the animals followed a course between these two extremes. Rejection developed by the diagnostic criteria outlined in the preceding chapter on untreated recipients but, in contrast, did not cause early death (Fig. 92). In some instances there was marked hyperbilirubinemia. In others, the jaundice was not so extreme, but there were comparable elevations in the serum transaminases and alkaline phosphatases. These abnormalities eventually tended to spontaneously revert toward normal with the passage of time even though no attempt was made to intensify immunosuppressive therapy (Fig. 92). The time before reversal became obvious was as long as six weeks, during which time many of the dogs appeared to be in terminal condition with clay-colored stools, dark urine, profound anorexia and rapid weight loss. As liver chemistries subsequently improved, there was often a return of appetite.

Later, hepatic failure tended to recur with an ultimately fatal outcome, both in the animals which continued to receive azathioprine and in those in which it was stopped (Fig. 92). Nevertheless, there was survival of 50 days or more in 16 of these 41 experiments, the maximum being 204 days. Eventually death was usually due to pneumonitis or to complications of chronic liver insufficiency such as gastrointestinal ulceration and hemorrhage.

The waxing and waning of liver function abnormalities in the foregoing studies during intervals of unchanging therapy were of considerable intrinsic interest. The findings suggested that the intensity of the rejection process was

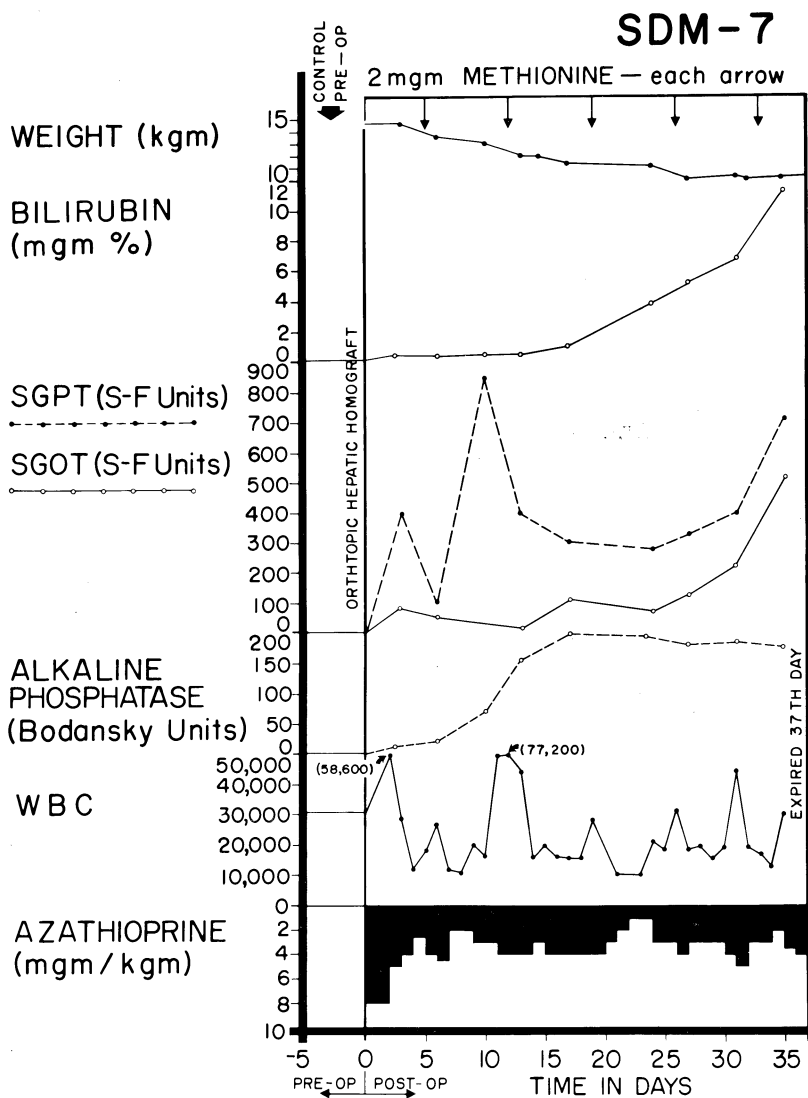


Figure 90. An example of inexorable rejection despite therapy with azathioprine. The sequence of events was almost identical to that expected in the unmodified dog (Chapter Eleven), but developed at a somewhat slower pace. The reason for the methionine administration is explained in the text. (By permission of Surgery 58:131, 1965.)

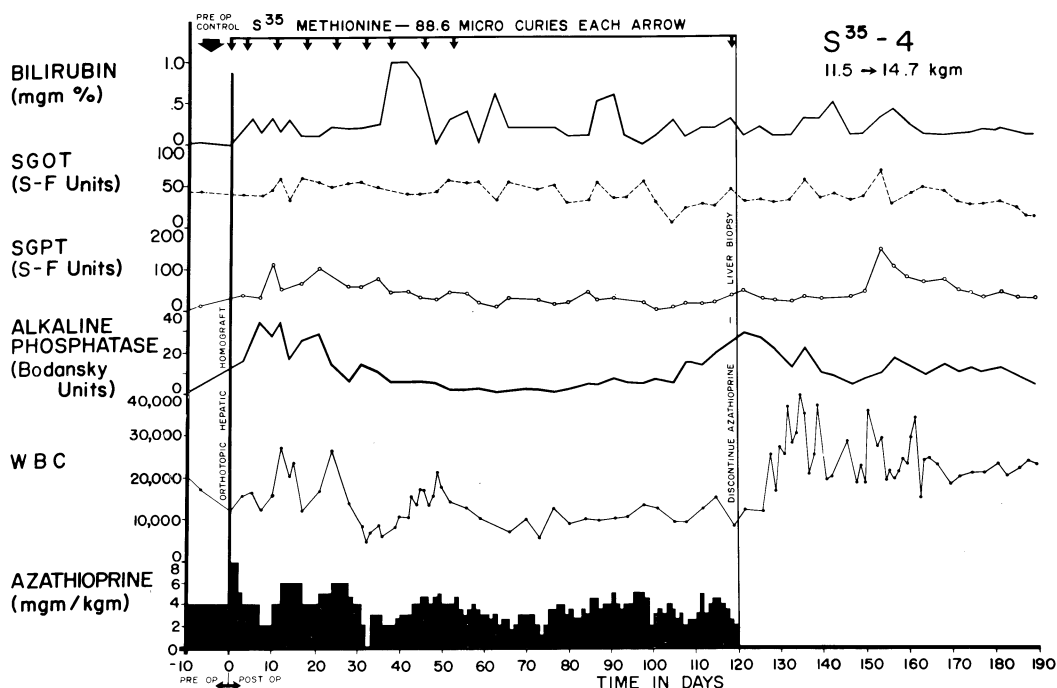


Figure 91. Course of a dog after orthotopic homotransplantation of the liver. Overt clinical rejection has never been observed. Liver biopsies after three, six and a half and 10 months were normal. The operation was performed on March 23, 1964. The animal is in good health more than five years later (see frontispiece). Note that immunosuppression was discontinued after four post-operative months without subsequent deterioration of liver function. Resumption of treatment has never been required. (By permission of Surgery 58:131, 1965.)

remitting independently of alterations in treatment in many cases. It was highly likely that the same explanation accounted for the surprising secondary recovery of the occasional dogs (Chapter Eleven) which received no immunosuppressive treatment at all. The demonstration of spontaneous reversal of rejection in these two kinds of experiments introduced a note of caution about attributing this effect to any preceding variation of therapy not only after liver transplantation but also in experiments involving other organs.

As also described in Chapter Eleven, the same thing has been seen commonly after orthotopic liver transplantation in unmodified porcine recipients. In retrospect, it can now be suggested that the use of azathioprine as the single therapeutic agent in dogs provided an experimental situation not dissimilar to that which exists naturally in the untreated pig model. The variability of host response to the foreign tissue, the spontaneous reversibility of rejection, and the eventual frequent development of a more or less complete state of graft acceptance (to be discussed later) were common observations in both varieties of laboratory studies.

Effect of Methionine or Its Radioisotope

Certain lipotropic factors including methionine have been demonstrated to protect against or facilitate recovery from various liver poisons,^{13, 34, 142} Because

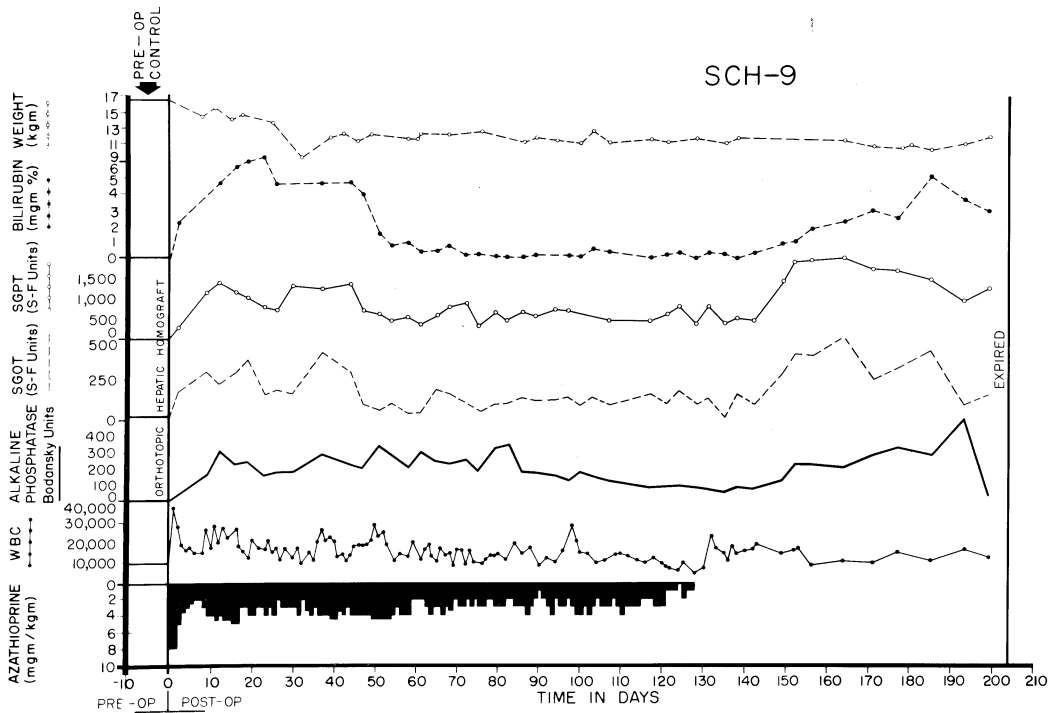


Figure 92. Course of a dog showing nearly complete reversal of a rejection episode following orthotopic liver transplantation. Note the severe jaundice which ultimately completely disappeared. The animal received ^{35}S methionine, L-methionine and choline in addition to azathioprine; no alterations in immunosuppressive therapy were made at the time of rejection. The dog died 203 days post-transplantation. Azathioprine therapy was stopped 127 days after operation. Slow deterioration of hepatic function developed. The immediate cause of death was perforation and bleeding of a peptic ulcer at the esophagogastric junction. (By permission of Surgery 58:131, 1965.)

of the therapeutic dilemma posed by the necessity of using a hepatotoxic immunosuppressive agent it seemed reasonable to see if there was a protective effect from the concomitant administration of methionine, an amino acid which has been reported to prevent the toxicity of bromobenzene,⁹⁰ pyridine,^{12, 26} chloroform,³⁵ Mapharsen,⁴⁷ and butter-yellow⁵⁶ and to accelerate recovery from chronic carbon tetrachloride poisoning.¹⁵⁵

In the nontransplanted control dogs described earlier in this chapter the pattern of hepatic injury caused by azathioprine therapy was compared to that observed when methionine or its radioisotope was also administered intravenously. The degree and duration of the abnormal changes of liver chemical values were not influenced (Fig. 85). Moreover, administration of methionine or its radioisotope did not potentiate homograft survival after homotransplantation to the otherwise untreated host. In spite of these negative findings, slightly better results were obtained after transplantation to the immunosuppressed recipient when methionine or its ^{35}S isotope was given in addition to azathioprine.¹⁷² However, the improvement in survival was of negligible statistical significance ($P=0.17$).

The difficulty of evaluating such adjuvant agents was greatly increased by the extreme variability of host response to the foreign tissue, which, as men-

tioned earlier, ranged from complete absence of rejection to uncontrollable repudiation of the liver homograft. The inability to demonstrate a statistically significant advantage with the use of methionine or its isotope may have been due to the system of testing in which the variables of uncontrolled donor-recipient selection were of far greater importance in the individual experiment than the therapeutic parameter being examined. In any event, methionine has not been used clinically.

Graft Versus Host Reaction

A hepatic homograft retains its metabolic specificity after transplantation. The interesting question of the effect upon its new host of the "foreign protein" synthesized by the organ is considered in Chapter Eighteen. It can be stated here that there is no clear evidence that a hostile liver homograft can mount a clinically significant immunologic attack upon its recipient.

However, increased red blood cell destruction has been recorded after this kind of operation¹⁷² as well as after homotransplantation of the spleen.^{41, 101} With either organ it is possible that erythroclastic activity by the transplanted reticuloendothelial system is responsible; such grafts often contain prominent hemosiderin deposits (see Chapter Twenty). A typical example of the phenomenon is shown in Figure 93. The red cell half-life was reduced to less than 50 per cent of its normal value during the first postoperative month but subsequently returned to normal. Conceivably the increased red cell destruction was at least partially due to mechanical factors secondary to ischemic injury of the transplanted organ rather than to any immunologically specific antierythrocyte activity of the reticuloendothelial portion of the graft. Excessive hemolysis has not been seen in any of our human recipients of liver homografts except one who developed this complication after complete hepatic artery thrombosis (Chapter Nine).

Although red cell destruction has not been prominent after clinical liver transplantation, thrombocytopenia has been extremely common, apparently as the result of platelet trapping in the homograft. This important subject will be discussed in Chapter Thirteen.

The Discontinuance of Therapy

The ability of 6-mercaptopurine to modify the rejection of canine renal homografts was first studied almost a decade ago. Shortly afterward an observation of great fundamental importance was made by workers in two laboratories. Zukoski¹⁹⁶ and Pierce and Varco¹⁴¹ noted that it was eventually possible to stop treatment with this drug in some of their recipients with prolonged subsequent function of the renal homografts.

Comparable findings were soon reported with the use of azathioprine for immunosuppression.^{104, 129, 160} In some experiments¹⁶⁰ it was demonstrated that discontinuance of therapy was sometimes feasible after as short an interval as

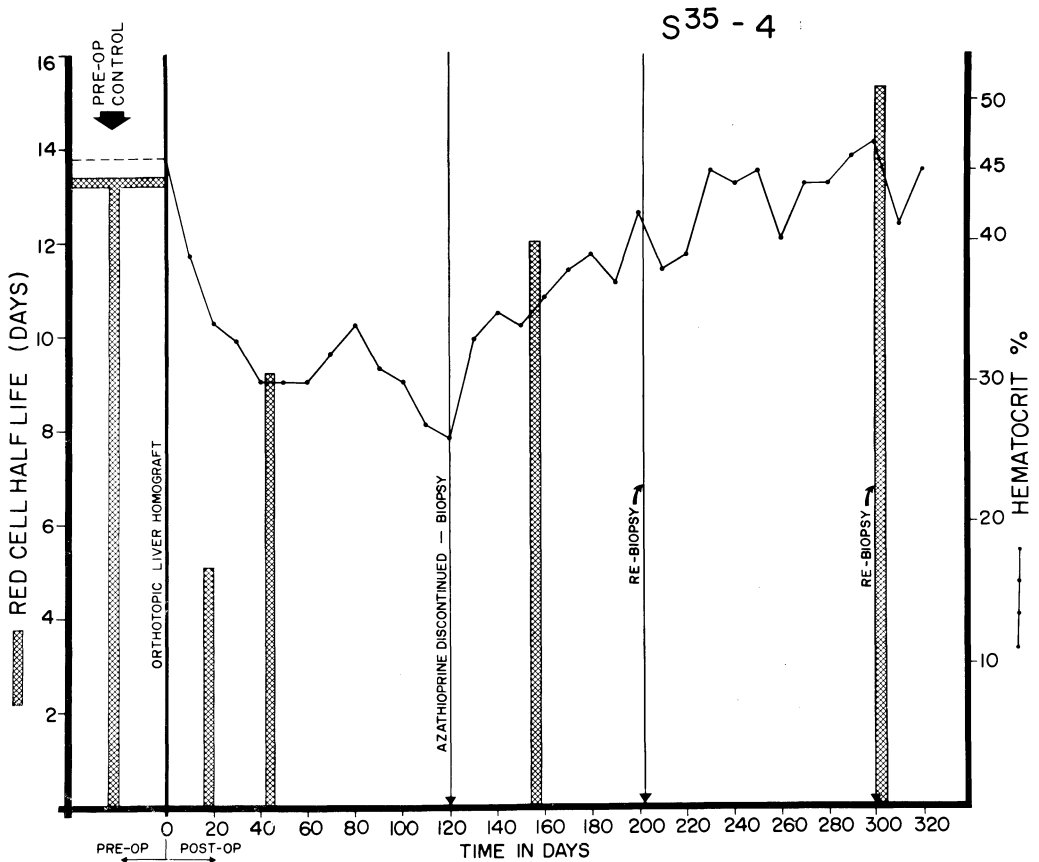


Figure 93. Red cell survival and hematocrit values in a canine recipient of an orthotopic liver homograft. Note the sharp reduction in red cell half-life in the first postoperative month, with a gradual return toward normal. Red cell survival was not altered by the withdrawal of azathioprine at the end of four months, but the depressed hematocrit rose sharply after the discontinuance of immunosuppression. The animal is still alive more than five years post-transplantation. (By permission of Surgery 58:131, 1965.)

four months; about a quarter of the animals had prompt acute homograft rejection, another half had slow repudiation of their transplanted kidneys, and the rest had continuing stable homograft function for months or years. One of our canine kidney recipients from that study is still alive with normal urine excretion six years later.

The same phenomenon has been demonstrated in dogs after orthotopic liver transplantation, probably with an even greater frequency than in the kidney experiments. Later, more will be said about the mechanism by which it becomes possible to attenuate or stop therapy, how this is apparently dependent on a progressive reduction in the immunologic interreaction of a homograft and its host, and how the extent to which this change occurs determines the need for immunosuppression and, in turn, the long-term prognosis of the recipient. For the moment, however, attention will be directed to some laboratory investigations which bear on the question of survival in dogs subsequent to cessation of all treatment.

In the series of 84 hepatic recipients cited earlier in this chapter, there

were 14 still alive at the end of four months. Treatment of 10 of these dogs was discontinued at that time or slightly before. The subsequent events in all 10 experiments are summarized in Table 15.

There were no examples of abrupt late rejection and, in fact, the measured liver chemistries slowly deteriorated in only two of the animals (Fig. 92). In the other eight the hepatic function stayed at about the same level of quality as before. If this had been subnormal (Fig. 94), it remained so and the dogs were prone in succeeding weeks or months to fatal complications such as gastrointestinal ulceration and pneumonitis (Table 15), which are common in chronic liver disease. In contrast, four animals with good four-month function survived for more than a year after the cessation of treatment (Table 15) and two are still alive almost half a canine lifetime later (see Frontispiece).

When these findings were reported, the reason for the apparently favored situation of late hepatic as opposed to renal homografts was speculated upon.¹⁷² It was suggested that the disproportionately large antigenic mass could have been a factor or, alternatively, that the liver with its enormous regenerative capacity was simply capable of sustained function in the face of continuing but minimal chronic rejection. The latter hypothesis received minimal support from histopathologic studies of the late homografts. Other possibilities might be that liver tissue is inherently less antigenic than the kidney or that the new environment created by the metabolites synthesized by the hepatic homografts is in some way made less hostile to the source of these products. Similar

Table 15. The Fate of 10 Dogs Which Lived for Four Postoperative Months and Had Azathioprine Therapy Stopped

EXPERIMENT NUMBER	DAY TREAT- MENT STOPPED	CHEMISTRIES WHEN THERAPY STOPPED			SUBSE- QUENT SURVIVAL (days)	LAST CHEMISTRIES			CAUSES OF DEATH
		Bili- rubin	Alkaline Phos- phatase* SGOT†			Bili- rubin	Alkaline Phos- phatase* SGOT†		
S ³⁵ 4	120	0.3	25	47	1820	0.1	1	20	Alive, April, 1969
S ³⁵ 27	109	0.1	43	24	1640	0.1	2	35	Alive, April, 1969
S ³⁵ 26	99	0.5	52	62	31	0.4	55	49	Pneumonitis
S ³⁵ 18	120	0.1	156	100	217	0.1	34	28	Perforated duodenal ulcer
SS 4	117	1.3	106	350	120	4.0	114	755	Rejection; GI hemorrhage; pneumonitis
HHM 2	120	0.1	41	115	443	1.0	454	310	Portal vein thrombosis; ? rejection
HHM 12	119	0.1	47	22	1041	0.5	96	590	Pneumonitis; ascites; atrophic liver
I 10	116	3.3	433	380	48	4.6	166	62	Bleeding duodenal ulcer
ICBM 13	130	5.1	293	240	27	4.8	485	280	Chronic liver failure
Schime 9	127	0.1	71	100	77	2.9	134	160	Rejection; esophagogastric ulcer with hemorrhage and perfora- tion

*Bodansky units (normal values 3 to 6).

†Sigma-Frankel units (normal values 10 to 30).

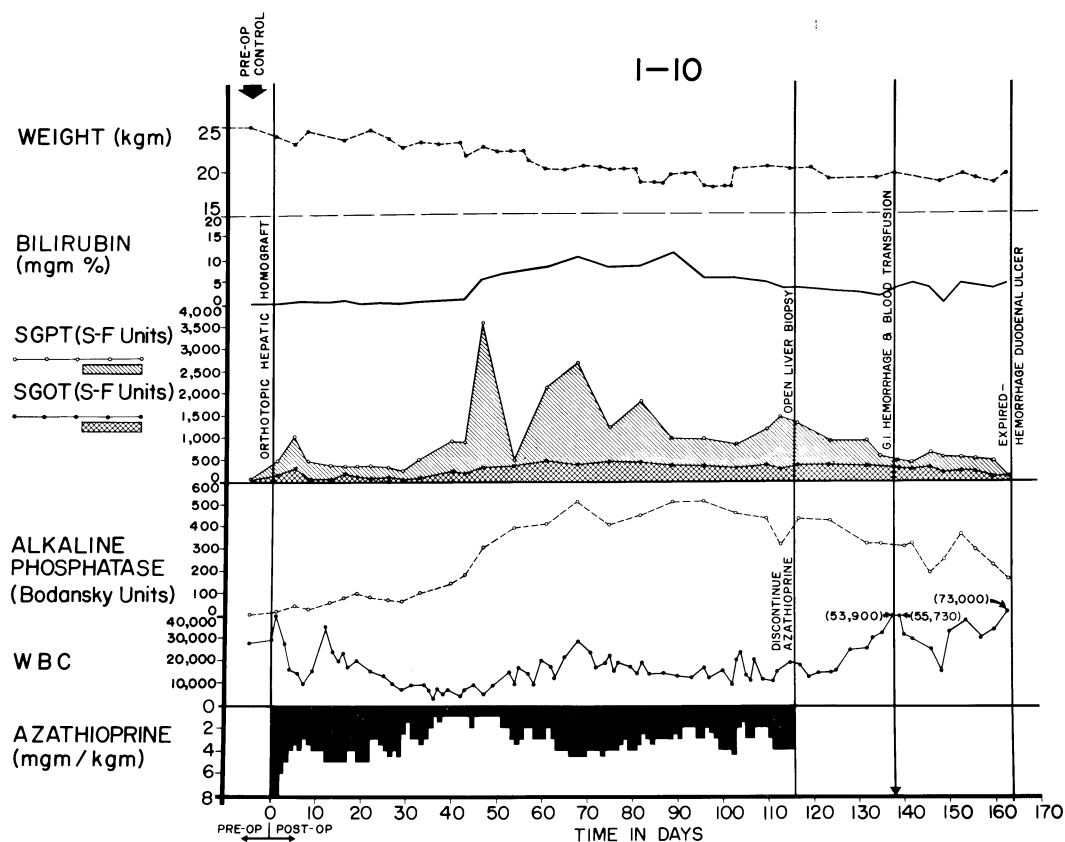


Figure 94. The course of an animal treated with azathioprine for the first 116 post-transplantation days. Under therapy a severe rejection developed which spontaneously reversed. After the azathioprine was discontinued the liver function tests actually improved. However, the dog died 48 days later of a massive hemorrhage from a large duodenal ulcer. (By permission of Surgery 58:131, 1965.)

thoughts have been expressed by Jaffe et al⁷⁴ and Calne²⁰ in attempts to interpret their observations in pigs (Chapter Eleven).

Gastrointestinal Ulceration

In the first publications on the subject of orthotopic liver transplantation to unmodified dogs, a high incidence of acute gastroduodenal ulcerations was noted.^{127, 164} The immediate cause of death in many of the animals was hemorrhage from these lesions or, frequently, perforation. Such complications seemed related to the development of rejection and were considered to represent an ulcer diathesis generated by poor hepatic function. However, Garnier,¹⁶ Peacock and Terblanche,¹³⁶ and Calne²⁰ lost a number of their untreated porcine liver recipients from gastrointestinal erosion and perforation or bleeding at a time when rejection was thought to be minimal or altogether absent.

Similar problems were encountered in dogs receiving azathioprine in sufficient quantities to mitigate rejection and to obtain chronic survival. In our early series¹⁷² there were 12 examples of hemorrhage or perforation in 69

animals which died from one week to five months after transplantation. Biliary tract reconstruction had been with cholecystoduodenostomy.

Even after this interval, there was continued risk, as noted by Faris³⁹ in a subsequent report of the residual animals from the same study. Gastric or duodenal hemorrhages or perforations were the commonest causes of late death. The most likely victims were dogs with chronically abnormal hepatic homograft function.

Subsequently Stuart¹⁸⁰ reported an even higher incidence of gastric or duodenal ulceration in dogs which had generally poor hepatic function despite treatment with azathioprine alone or with azathioprine in combination with actinomycin C and/or local homograft irradiation. Of nine dogs which lived for 25 to 88 days postoperatively, six had gastrointestinal ulceration as the direct or an important contributory cause of death. The type of biliary tract reconstruction was not a critical factor in promoting the ulcer diathesis. The finding that vagotomy and pyloroplasty was an inadequate prophylactic measure, whereas gastric resection was highly effective, prompted Stuart to speculate that the etiology was the inability of the poorly functioning liver to inactivate humoral gastric stimulants such as histamine. In contrast, Calne found vagotomy and pyloroplasty to be an almost uniformly protective procedure in pigs.²⁰

Taken together the foregoing information has raised the question of whether human recipients should automatically receive a gastric acid-control procedure at the time of orthotopic liver transplantation. The more recent clinical experience suggests that this will not be necessary if good early and sustained hepatic function can be obtained from the transplanted liver (Chapters Fourteen, Fifteen, and Seventeen).

HETEROLOGOUS ANTILYMPHOCYTE GLOBULIN

Other than azathioprine, the only agents which have been proved to prolong life when used alone after orthotopic liver transplantation in dogs have been heterologous antilymphocyte serum (ALS) and its globulin derivative (ALG). Historically, the first experiments with ALS were performed by Metchnikoff, who clearly appreciated the potential therapeutic value of such serum products. In 1899 he wrote somewhat philosophically of the struggle between the various cells of an organism and added:¹¹¹ "The time is not remote when medical art will actively intervene to maintain the integrity of the whole organism, the harmony of which is broken by the preponderance of certain cells, mononuclear cells in the atrophies, several other elements in the malignant diseases. Therefore, I undertook the study of the effect produced by the resorption of macrophages. To attain this end I initially injected guinea pigs subcutaneously with an emulsion of rat spleen or lymph nodes ground up in saline solution. Forty-seven days after this injection, guinea pig serum agglutinated and dissolved rat leukocytes. Mononuclear cells were the most sensitive and were converted into transparent vesicles. Later the granulocytes underwent the same changes and finally the mast cells." Metchnikoff pointed out that nonim-

munized serum did not have these properties. In addition, he observed that the guinea pig antirat serum which he used did not agglutinate lymphocytes from other species.

In the next 60 years there were a number of reports on the properties of ALS. These contributions as well as the subsequent literature on ALS were recently summarized by Medawar¹⁰⁵ of London who, with his colleagues at the Mill Hill laboratory, has played a leading role in the development of this field. In the following remarks no attempt will be made to present such a general survey; instead, attention will be directed to the way in which information from research laboratories concerning ALS and its by-products was reviewed and expanded with the ultimate objective of administering these substances to patients.

Preliminary Steps

The first to demonstrate the ability of ALS therapy to mitigate homograft rejection were Waksman, Arbouys, and Arnason in 1961.¹⁸⁷ They obtained prolongation of skin homograft survival. The effect was a weak but significant one. The subsequent investigations of Woodruff and Anderson^{192, 193} catalyzed widespread interest in the possibility of using such antisera for clinical therapy by demonstrating a striking protection of homografts in rats treated with ALS alone or in combination with thoracic duct drainage. Within two years antisera of comparable or greater potency were developed by many other investigators.^{49, 65, 76, 98, 99, 123, 130} In all these early studies the experimental model consisted of skin grafting in mice, rats, or guinea pigs which were treated, usually by intraperitoneal injection, with antisera raised in rabbits. The use of genetically controlled donor and recipient strains for transplantation permitted precise delineation of many of the features of the tested sera which have direct clinical applicability.

Nevertheless, a number of intermediate steps were required before heterologous antilymphocyte products could be considered for clinical trial. It was necessary to demonstrate a beneficial effect of ALS after whole organ transplantation in outbred large animals, to determine the therapeutic schedules which were the most effective with the least toxicity, and to evolve practical techniques of administration which would be acceptable for use in man. This kind of information was sought in dogs by use of heterologous ALS raised in horses,^{1, 7, 69, 72, 138, 140, 165, 171} sheep,^{120, 140} or rabbits.^{2, 95, 121}

Raising the Antiserum

One of the first practical problems to be solved was to raise an antiserum of sufficient potency so that it could be given in small volumes. The experiments with rodents cited previously usually involved the intraperitoneal administration of 0.25 to 1.0 ml of raw ALS, volumes which would be 1 to 2 liters if extrapolated on a weight basis to adult humans. In most of these earlier reports

the activity of the antisera was not quantitated except for its ability to prevent skin homograft rejection. When stated, the antiwhite cell titers as measured by leukoagglutination tests never exceeded 1:64. Consequently an attempt was made to improve this situation, using the horse as the heterologous serum source. This animal was chosen primarily because its large blood volume would permit enough ALS and ALG to be prepared for adequate trials in large animals and, ultimately, in man.

The dose of lymphoid antigen used for immunization proved to be a critical factor in raising a high potency serum.^{69, 72, 165} Early in our experience lymphocytes from lymph nodes or thymuses were given subcutaneously in doses of 0.18 to 1.4 billion cells (Fig. 95). Within a few weeks the leukoagglutinin titers rose from control values of 0 to 1:4 to a maximum of 1:256. These did not increase further despite similar repeated booster doses over periods of as long as six months.

Subsequently the dose was greatly increased by using splenic lymphocytes. This source of antigen was selected because of its bulk availability from human cadaveric sources. When 4 to 200 billion spleen cells were used the antiwhite cell titers rose within a few weeks (Fig. 95) to as high as 1:16,000 in parallel

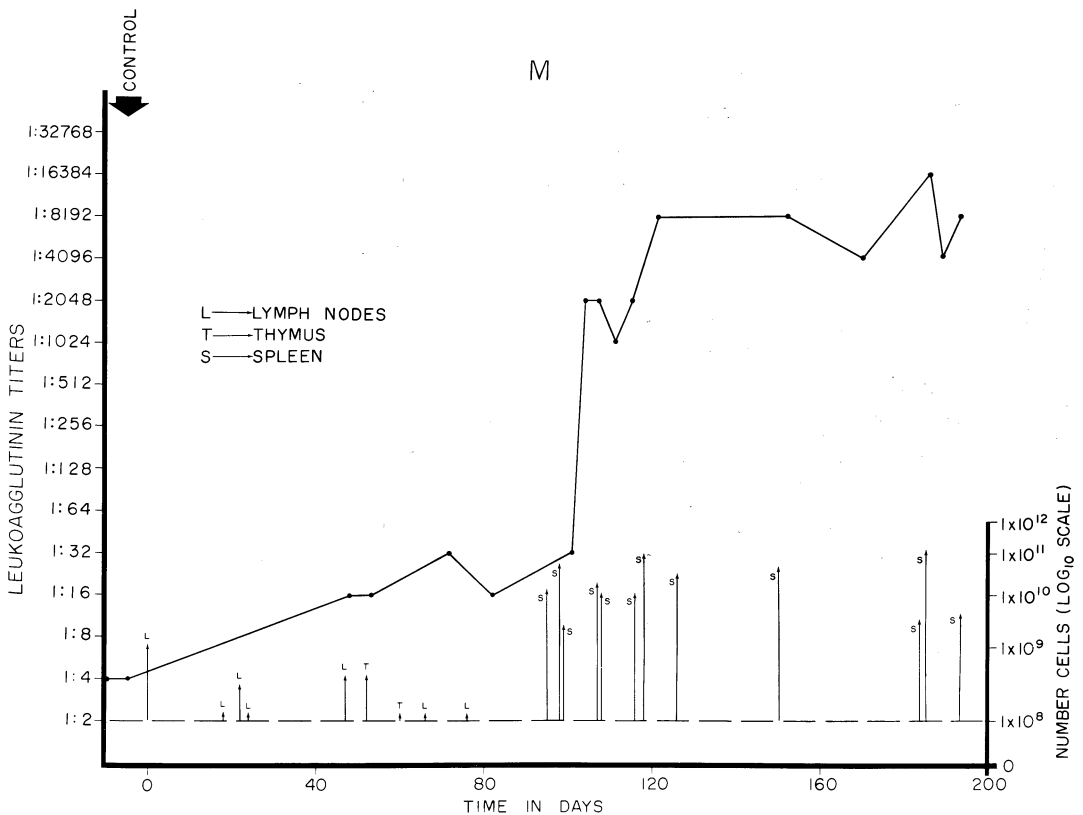


Figure 95. Effect of immunizing dose upon the leukoagglutinin titer of a horse inoculated with cadaveric human lymphoid tissue. Note that the rise in titer was very modest in the first three months, during which time small doses of cells were used. When the quantity of antigen was increased by the use of spleen cells, abrupt elevations in titer were observed within a few days. If large antigen doses are given from the beginning, a high titer antiserum can be obtained in about 60 days. (By permission of Surg. Gynec. Obstet. 124:1, 1967.)

with comparable increases in lymphocytotoxicity titers. There was some variability of the ALS or the globulin (ALG) extracted from it inasmuch as the white cells from individuals of the species against which immunization was conducted were not all agglutinated or lysed to the same dilution.^{72, 144} Putnam¹⁴⁴ explained this by differences in the antigens of the original immunizing cells as these related to the antigenic profile of the cells ultimately used for testing. He suggested that a serum to be used in the outbred canine or human populations would ideally be raised with the lymphoid tissue of the individual to be eventually treated, or else that many donors should be used to assure adequate representation of the variable immunizing antigens.

As yet there has been no convincing evidence that immunization with a particular kind of lymphocyte results in the production of an antiserum with inherently superior immunosuppressive qualities. The only investigators to systematically examine this question were Ono et al.¹³² In parallel experiments in our laboratories they raised ALS in rabbits with rat splenic, thymic, and lymph node lymphocytes. The three resulting sera, which had equal antiwhite cell titers, had exactly the same ability to mitigate the rejection of heterotopically placed hearts which were transplanted across an AgB (Wistar-Furth to Fischer) barrier. The only special drawback with the use of splenic antigens was that the ALS had a significantly higher antiplatelet titer. As will be discussed in Chapter Thirteen, this disadvantage has special implications in liver transplantation.

The work of Jooste et al.⁸⁵ has emphasized that much still needs to be learned about the optimum techniques for immunizing the heterologous serum donor. The exact technique used may have a very important effect on the immunosuppressive qualities of the ultimate product and, equally important, may determine its toxicity. In Jooste's report it was noted that the use of an adjuvant caused a marked increase in undesirable side effects.

Standardization of ALS

It was soon shown in dogs that the high titer ALS mitigated rejection in volume doses which were only a fraction of those required for the same effect with the previous low titer products.^{69, 165, 171} Nevertheless, there was initial widespread belief¹⁹¹ that the antiwhite cell titers would not be an expression of the immunosuppressive efficacy of ALS, a point of controversy that has not yet been completely resolved.^{8, 28, 77, 79} However, there now seems to be agreement that essentially all good quality antisera possess high titers, particularly when these are measured by lymphocytotoxicity tests. The converse is not necessarily true. In several laboratories occasional batches of high titer ALS have been assayed which were ineffective for the prevention of skin homograft rejection.⁸⁵

Since ALS is in no sense a standardized product like azathioprine and prednisone, this introduces special complications in the interpretation of its effects. Methods are still badly needed for the accurate prediction of the immunosuppressive properties of ALS and ALG which are prepared for use in humans. A potentially fruitful approach may be one based on the interspecies cross-reactivity of ALS which was demonstrated by Iwasaki⁷² and Putnam.¹⁴⁴ Putnam showed

that antihuman ALG lysed or agglutinated the white cells of African green monkeys almost as efficiently as those of man. Balner, Eysvoegel, and Cleton¹¹ have tested the ability of antihuman sera to prevent or to delay the rejection of chimpanzee homografts. Such an approach may ultimately shed more light on the issue of the titer-to-immunosuppression relationship, and on the other important questions of whether the source of the human lymphoid tissue or the timing and other details of immunization have an important influence on the eventual product. Until such standardization methods are evolved, it is unlikely that ALG for use in humans will be released by commercial drug companies.

Absorption of ALS

The raw serum from the clotted blood of immunized horses is usually extremely toxic because of the presence of extraneous antibodies directed against the red cells, thrombocytes, and plasma proteins of the species to be treated. For example, injections of unabsorbed or inadequately absorbed ALS or ALG can cause immediate and precipitate falls in the hematocrit of dogs (Fig. 96) or thrombocytopenia in monkeys¹⁰ or man.¹⁷⁵ The complications can be avoided or at least minimized by the absorption methods described by Iwasaki⁷² as later modified and improved by Kashiwagi.⁸⁷

Briefly, these techniques consist of absorbing the horse serum first with

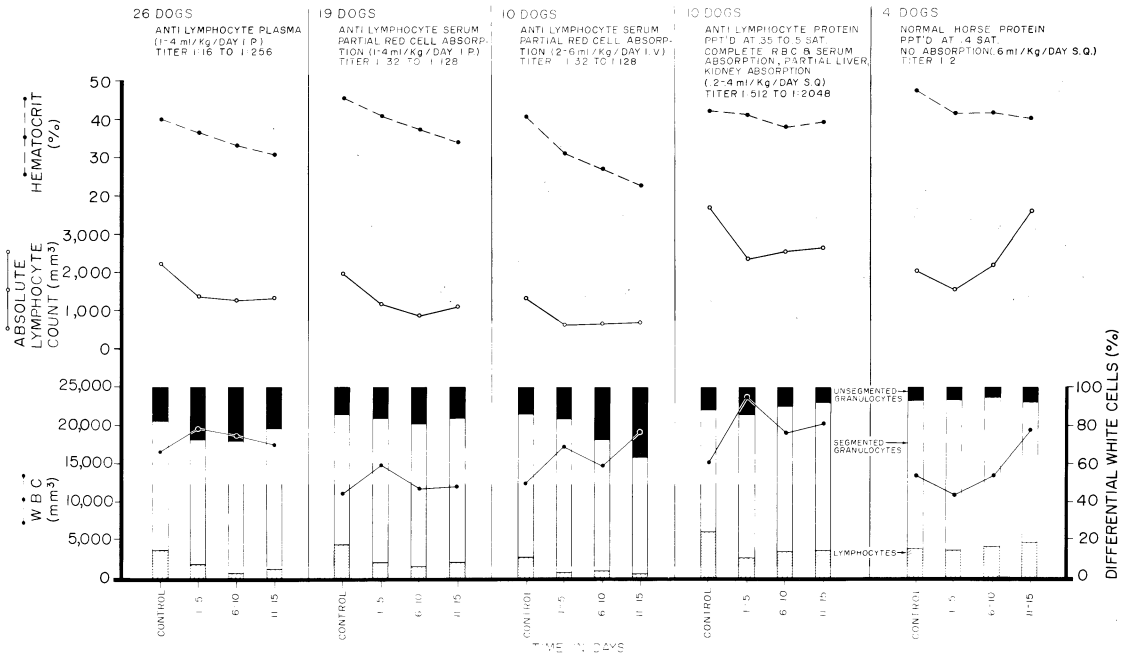


Figure 96. The effect of horse plasma or serum and crude horse globulin upon the hematocrit, lymphocyte count, total white cell count, and white cell differential during 15 days of daily administration. In all but the control experiments on the right, the agents were prepared from horses immunized against dog lymphoid tissue. Note that acute anemia was largely prevented only when complete absorption had been carried out with canine red cell pack. (By permission of Surg. Gynec. Obstet. 124:1, 1967.)

fresh human plasma, then platelets and, finally, red cells. Before the last step, the ALS is decomplexed by heating at 56° C for 30 minutes in order to avoid the hemolysis which otherwise occurs. Singly or together, these procedures have little or no effect on the antiwhite cell titers.

In contrast, absorption of the ALS by nucleated cells of the donor species causes a profound loss of titer. All workers from Pappenheimer¹³⁴ to the present time who have performed the necessary experiments agree that the antiserum is largely inactivated if it is exposed to the lymphoid tissue of the original donor species. In addition, there is a major loss of titer if the ALS is absorbed with kidney, liver, lung, or skeletal muscle,^{72, 99} an observation explained by the demonstration in other ways of ALS antibody binding to these various tissues.^{50, 149}

These findings indicated that there was a considerable antigen sharing between all nucleated cells of the species being treated. They also suggested that if antigens, which would react only with tissue-specific ALS antibodies, were uniquely represented in the lymphocytes, these unshared determinants were few in number. Levey and Medawar⁹⁹ strengthened the latter conclusion in a crucial experiment in which sera were raised in rabbits by immunization with mouse epidermal cells or even L-cells or fibroblasts from tissue culture. Mice treated with the resulting antisera developed lymphopenia and had definite prolongation of skin homograft survival.

The lack of strict immunologic specificity of the antisera has not proved to be a fatal flaw, probably because the lymphoid system is a highly vulnerable target to which ALS antibodies selectively "home" despite the fact that the liver, kidney, and other organs possess potentially competitive binding sites. Using unabsorbed rabbit antisera, Levey and Medawar⁹⁸ were able to show that fluorescein-tagged antibody was heavily concentrated in the lymphoid elements of treated mice. Furthermore, the toxicity studies to be reviewed later did not reveal a direct injury to other organ systems⁷² except under exceptional circumstances.⁵⁴ Even the renal abnormalities which will be described subsequently were thought to be due to the immunologically nonspecific lesions of "serum sickness nephritis" rather than to a direct nephrotoxic action of the ALS. Consequently, we have not used absorption with any nucleated cell suspensions in the preparation of our antihuman sera.

Purification

Although the application of absorption techniques was important, it was also necessary to obtain purified derivatives for human use. Simultaneously, several investigators^{69, 72, 75, 148, 194} found the desired antibody to be chiefly in the gamma G globulin by means of chromatographic (Fig. 97) or other techniques. However, there is good evidence, as will be re-emphasized later and in Chapter Thirteen, that the antileukocyte antibodies of horse ALS are distributed rather widely in the equine immunoglobulins and that excessive refinement of the serum may therefore lead to a serious loss of potency.

The first steps to extract the active elements of ALS were taken by Waks-

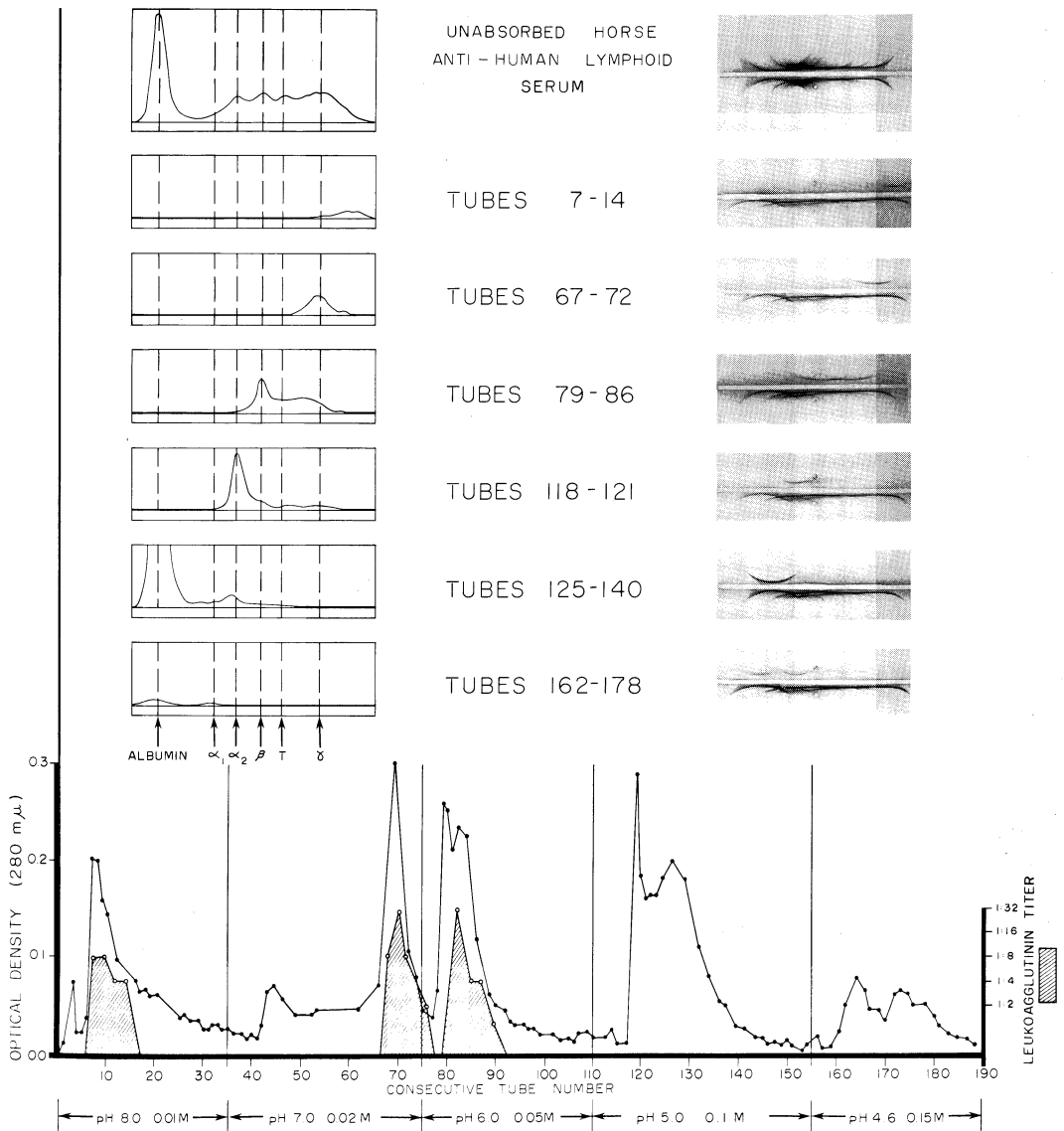


Figure 97. Studies of the leukoagglutinin-containing fraction in horse antihuman-lymphocyte serum (ALS) with the use of column chromatography, electrophoresis, and immunoelectrophoresis. The various eluates from the diethylaminoethanol (DEAE) cellulose column were analyzed spectrophotometrically for protein content (expressed as optical density), and the presence or absence of leukoagglutinins was determined for each collection tube. The shaded areas indicate leukoagglutinin activity. The electrophoresis and immunoelectrophoresis permitted relatively complete classification of the active immunoglobulins. (By permission of Surg. Gynec. Obstet. 124:1, 1967.)

man and his associates, who reported in 1961 that crude globulin obtained by ammonium sulfate precipitation retained the same properties as the original antiserum.¹⁸⁷ This finding was confirmed by later workers.^{69, 72, 122} With the proper selection of ammonium sulfate concentration⁷² most of the antibody content could be recovered in the precipitate. The product prepared in bulk quantities by this method and used for a large number of patients at our institutions from June, 1966, onward (Chapter Thirteen) is shown in Figure 98. It contained principally gamma G globulin, as well as significant quantities of T-equine globulin and traces of alpha globulin.

More recently a highly refined ALS consisting entirely of gamma G globulin (Fig. 99) has been used in a clinical trial at our institutions. The refined globulin (called ALGG) was obtained from immune horse serum by preliminary ammonium sulfate precipitation followed by batch extraction with a DEAE-cellulose technique. ALGG, prepared in this way for use in dogs, had a definite, though limited, ability to slow the repudiation of canine renal homografts,^{87, 88} a result which was not surprising since the gamma G fraction of rabbit ALS had already been proved by actual testing to retain significant antirejection properties.^{10, 125}

Unfortunately there was evidence in the patients^{88, 161} that ALGG

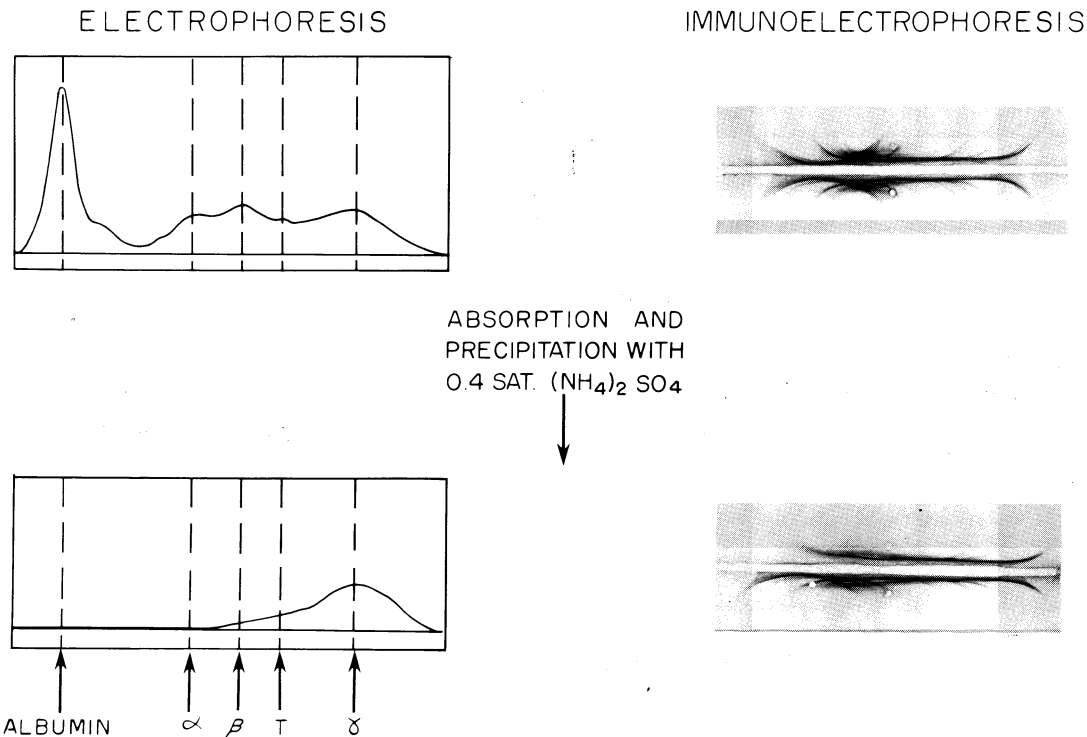


Figure 98. Electrophoresis and immunoelectrophoresis of absorbed horse antihuman-lymphocyte serum (ALS) and the globulin obtained from it (ALG) by two precipitations with 0.4 saturated ammonium sulfate, two dialyses, and lyophilization. The final product, which was used clinically, consists chiefly of gamma G and T-equine globulin. (By permission of Surg. Gynec. Obstet. 124:1, 1967.)

ELECTROPHORESIS

IMMUNOELECTROPHORESIS

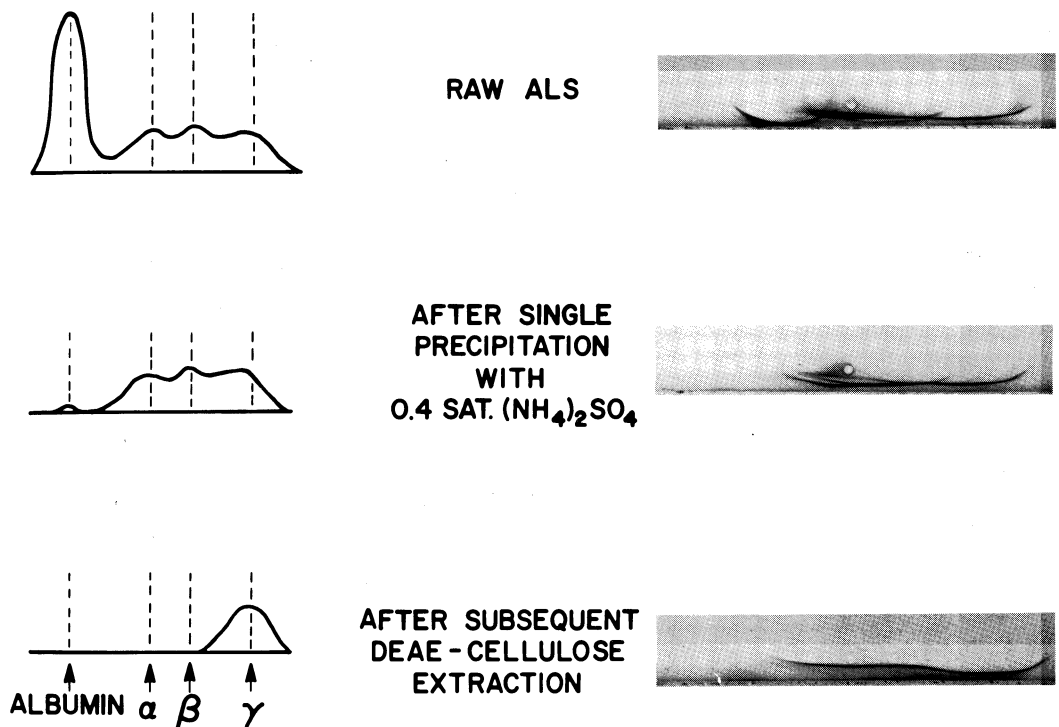


Figure 99. Electrophoretic and immunoelectrophoretic patterns of unabsorbed antihuman ALS, the raw globulin obtained with a single ammonium sulfate precipitation of absorbed ALS, and the final product (pure gamma G globulin) after subsequent DEAE-cellulose batch extraction. (By permission of Surgery 65:477, 1969.)

provided immunosuppression which was inferior to that previously observed when using the less pure ammonium sulfate-precipitated ALG (see Chapter Thirteen). The possibility that this kind of refinement may diminish the potency of horse ALG was also raised by Clunie et al²⁵ on the basis of observations in dogs that were subjected to renal transplantation.

The explanation of these findings is probably that the active fraction of horse ALG is not confined to a narrow band of the gamma G globulin, as it apparently is in rabbit serum. Iwasaki⁷² thought he could also identify antiwhite cell antibodies in the T-equine globulin component of horse serum (Fig. 97), a contention which was supported by the investigations of Pichlmayr¹³⁹ and which was not weakened by the studies of James and Medawar.⁷⁵ More recently in our laboratories Kashiwagi^{89, 161} has demonstrated conclusively that very important antileukocyte factors in horse ALS reside in the "fast" gamma (IgA) and possibly in other globulin constituents of the T-equine fraction. He also confirmed that the desired element of rabbit ALS is almost exclusively in the gamma G globulin.

The Problem of Dosage

The lack of an accepted standard assay for ALS and ALG has made it difficult to interrelate much of the research carried out in different centers. In our own laboratories the quantities of ALS or ALG administered were varied according to the weight of the treated subject and the antiwhite cell titer of the material administered. The system was an arbitrary one and based on the conceivably false (as discussed earlier) assumption that the effectiveness was directly related to the leukoagglutinating titer. Thus, 1 ml of reconstituted globulin with a titer of 1:8000 was said to contain 8000 "units." With a titer of 1:16,000 the same volume contained 16,000 units according to this nomenclature. Individual doses in our laboratory experiments were usually 500 to 1000 units/kg, a range which was eventually also used in patients.

Influence on the Rejection of Canine Kidney Homografts

Within a six month period in late 1966 and early 1967 there were several reports that the functional survival of canine renal homografts was prolonged by the intravenous, intraperitoneal, or subcutaneous administration of horse,^{1, 7, 69, 138, 165, 171} rabbit,^{2, 64, 95, 121} or sheep¹²⁰ antidog-lymphocyte serum (ALS) or globulin (ALG). These findings have since been confirmed in a tidal wave of similar publications too numerous to warrant annotation here.

The immunosuppressive effect was not necessarily dependent upon the use of doses large enough to produce lymphopenic responses such as those shown in Figure 96. Survival was maximal if ALS or ALG was started several days before operation,^{69, 171} a conclusion previously reached by Monaco with skin homotransplantation in mice¹²² and subsequently confirmed in canine kidney experiments by Clunie.²⁵ Even at best, however, the results after kidney transplantation, with or without lymphopenia, were inconsistent in our studies and those of all other investigators working with dogs. There were some extraordinarily long-term survivors, but most animals eventually rejected their homografts. The spectrum of response to therapy was not dissimilar to that observed in the evaluation of azathioprine as described earlier in this chapter. With both agents the variability of results was presumably due to differences in the quality of chance histocompatibility matching in randomly paired mongrel dogs.

Orthotopic Liver Transplantation

The same variability of effect was evident in experiments with orthotopic liver transplantation. In the first trials nine dogs were treated with unrefined ALS which was given intraperitoneally.^{18, 69, 165, 167, 170, 171} Nine more animals received subcutaneous ammonium sulfate-precipitated ALG. Treatment was started one to 26 days before operation. One of the dogs received no further

treatment and the other 17 were given additional injections for as long as 60 days postoperatively. In no case was therapy continued after two months and in most instances it was discontinued before this time.

The results in this series of experiments are shown in Table 16. Of the 18 recipients 10, 9, 7, and 6 survived for 15, 20, 30, and 50 days, respectively; four of the animals were still living at four months. Once rejection had begun, its features (including spontaneous reversal in several instances) were undistinguishable from those described earlier for the period during or after treatment with azathioprine. The magnitude of histologically evident rejection in these livers was less extreme than in renal homografts provided with comparable therapeutic protection.¹⁷¹

Table 16. The Fate of 18 Dogs Which Received Orthotopic Liver Homografts in 1965 and Early 1966

EXPERIMENT NUMBER	DAY POST-OPERATIVE TREATMENT STOPPED	CHEMISTRIES WHEN TREATMENT STOPPED			LAST CHEMISTRIES BEFORE DEATH			SURVIVAL IN DAYS	CAUSES OF DEATH
		Bili-rubin	Alk. Phos. [†]	SGOT [‡]	Bilirubin	Alk. Phos. [†]	SGOT [‡]		
VKC	None given				0.2	14.9	18	177	Volvulus
HL 3	Did not stop				6.0	535	375	33	Rejection; pneumonitis
HL 4	Did not stop				11.4	172	200	11	Rejection; pneumonitis
HL 6	Did not stop				0.6	31.6	38	7	Hemorrhagic enteritis
HL 7	Did not stop				11.7	118	410	7	P.V. Occl; rejection
HL 8	Did not stop				9.7	173	560	21	Rejection
HL 20	Did not stop				3.2	4.6	110	6	Rejection
HL 22	21	0.3	59	60	0.2	60	42	390	Cholangitis; liver abscesses; ? rejection
HL 23	Did not stop				9.1	128	700	16	Rejection; liver sepsis
SUN 1	Did not stop				0	3.1	28	7	Rejection; hemorrhagic enteritis
SUN 2	57	3.5	210	114	8.2	236	236	122	Hepatic artery thrombosis
SUN 3	Did not stop				0	52.5	81	60	Died after liver biopsy
SUN 4	55	0.2	77.8	33	4.2	221	65	128	Rejection
SOLARIS 5	Did not stop				5.3	352	275	72	Hepatic artery thrombosis
SOLARIS 6	Did not stop				5.7	108	5700	7	Rejection; pneumonitis
SOLARIS 7	Did not stop				6.8	151	370	21	Rejection; pneumonitis
SOLARIS 8	Did not stop				6.1	168	1960	11	Rejection
SOLARIS 9	Did not stop				3.5	112	570	8	Rejection; pneumonia

*: The animals were treated with ALS or ALG beginning one to 26 days before operation.

[†] Bodansky units (normal values 3 to 6).

[‡] Sigma-Frankel units (normal values 10 to 30).

Some interesting observations were made in the dogs which lived for the longest time. One animal received only six injections of antilymphocyte serum over a 26-day preoperative period. A definite lymphopenic effect was not produced (Fig. 100). After liver transplantation no further therapy was given. The dog always had satisfactory hepatic function and ultimately died of intestinal obstruction six months postoperatively. The second animal, which had pretreatment for one month, received ALS postoperatively for only 21 days. After therapy was stopped there was a striking lymphocytosis (Fig. 101), despite which acute rejection did not occur. The dog eventually died of liver sepsis 13 months after transplantation.

In many of the foregoing experiments with livers, as well as in others involving transplantation of the dog kidney,^{2, 69, 121, 171} it was proved that the subcutaneous injection of small volumes of potent ALS or ALG produced a readily detectable immunosuppressive effect. From a practical point of view this was an important advance since it pointed the way to one acceptable method for the administration of ALG to humans. Ultimately, however, it was decided to use the intramuscular route clinically since, unlike the situation in dogs, injections of irritating substances are poorly tolerated in the fatty subcutaneous tissues of man.

Other proof of the therapeutic value of ALS in canine liver transplantation was quickly forthcoming from two groups. The first was by an intra-European team headed by Rudolph Pichlmayr of Munich and by Philippe Mikaeloff of Lyon. The ALS was raised in Germany in horses and shipped to the French laboratory where the orthotopic transplantations were performed. There were 10 dogs in the series,^{117, 139} all treated with intravenous ALS; twice daily injections were started four days before operation and continued indefinitely after-

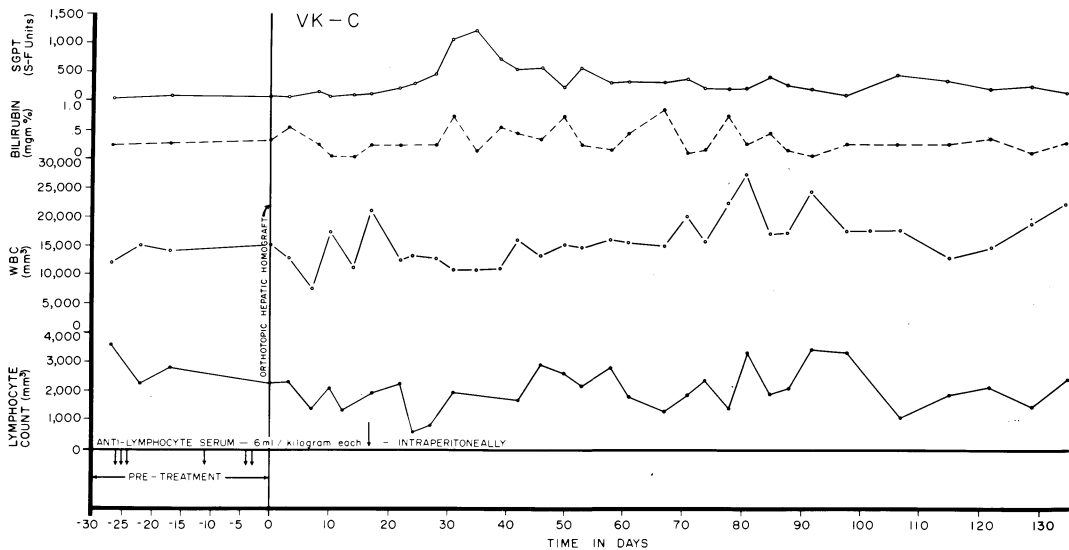


Figure 100. A dog which received an orthotopic liver homograft after six intraperitoneal injections of antilymphocyte serum (ALS). No postoperative therapy was ever given. Note that the lymphocyte count was little changed. The dog lived for six months, finally dying of an unrecognized intestinal volvulus. (By permission of Surg. Gynec. Obstet. 124:301, 1967.)

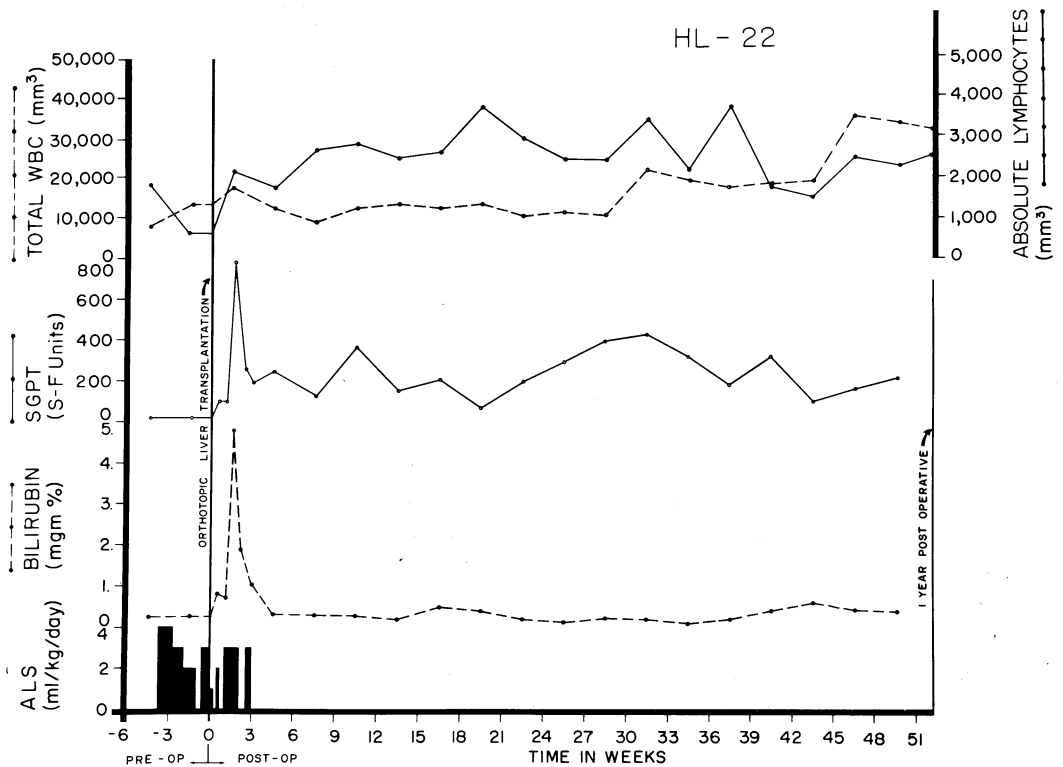


Figure 101. The first postoperative year of a dog which was treated with intraperitoneal ALS before and for 20 days after orthotopic liver transplantation. Note the pronounced lymphocytosis late in the postoperative period. The animal finally developed ascites and died of chronic liver failure 390 days after operation. (By permission of Butterworth & Co., Ltd., London, 1967.)

ward. Five of the animals lived for at least 20 days, the longest survival being four and a half months.

More recently Birtch,¹⁴ of the Peter Bent Brigham laboratories in Boston, has reported even better results from giving equine ALS or ALG by the subcutaneous route. Birtch's studies focused particular attention upon the effect of dosage on his results. Below a given threshold there was no demonstrable prolongation of recipient life.

There have been no reports of immunosuppressive therapy of any kind after orthotopic homotransplantation of the pig liver. The only attempts known to have been made were summarized in Table 14, Chapter Eleven. Horse antipig-lymphocyte serum was used as the sole means of therapy. The results in a small series were not so good as in untreated animals.

The Reversal of Rejection

In some of the early investigations with skin homotransplantation in inbred rodents it was shown that ALS given even several days after application of the homograft resulted in definite prolongation of transplant survival.^{77, 98} This was not surprising since it was already known that ALS could prevent or blunt the

expression of pre-existing immunization states,^{70, 186, 187} including that of a second set reaction.^{98, 99, 122, 124}

A logical extension of this kind of information should be that ALS would be useful in treating an established rejection. The hypothesis was tested by Smith¹⁵⁸ in mongrel dogs that were subjected to kidney transplantation and not treated with ALG until there was evidence of deterioration of renal function. Death from homograft failure was significantly delayed by the treatment and in several experiments the rejection was at least partially reversed (Fig. 102). His report also contained an account of one orthotopic liver transplantation. The recipient dog developed hepatic rejection after a week, was treated with three subcutaneous injections of ALG, and lived for an additional 100 days.

ALG Synergism

Before ALG was used in patients it was highly desirable to know about the immunologic interaction between the antilymphocyte substances and the more conventional immunosuppressive agents. At the time that a clinical trial was first being considered this was a particularly important question since an early

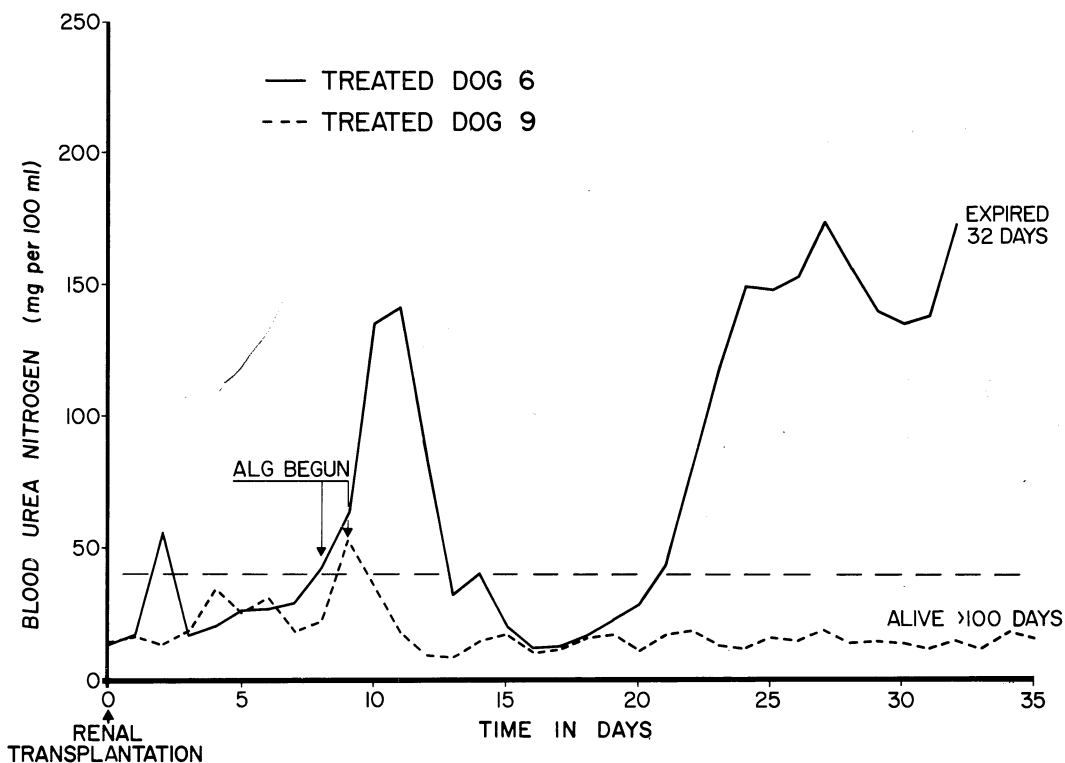


Figure 102. Blood urea nitrogen levels in two dogs which received delayed treatment with anti-lymphocyte gamma G globulin (ALG). Therapy was initiated (arrows) when the BUN secondarily rose above 40 mg per cent eight and nine days, respectively, after renal transplantation; it was continued daily thereafter. Dog 6 died of uremia 32 days postoperatively, 24 days after the onset of rejection. Dog 9 lived with normal renal function for many subsequent months. (By permission of Surgery 66:1969, in press.)

hypothesis of Levey and Medawar⁹⁸ held that the immunosuppressive properties of ALS were due to binding of the heterologous antibodies to recipient lymphocytes, thereby "blindfolding" and functionally emasculating them. Such a possibility implied that the action of the antisera was dependent upon a stable lymphocyte population and that the superimposition of agents which caused a rapid turnover of stem cells might cancel the effect.

Experiments were therefore undertaken in which ALG plus azathioprine were used for immunosuppression.^{69, 171} It was found in the canine kidney transplant model that the limited but definite prolongation of survival which could be achieved with suboptimal doses of either agent was slightly increased when the two were employed together. Although this improvement was not statistically significant, the really important observation was that survival was not made worse. Moreover, the extent of histologic damage was less in homografts retrieved from dogs that had been given the combination therapy.¹⁷¹ Later, Weil and Simmons¹⁸⁹ and Alexander et al³ reported a much more striking synergism of ALS with azathioprine under conditions of testing that were similar except that large drug doses were used.

The two agents, ALG and azathioprine, were also used together by Groth⁵² in his canine experiments on blood flow in orthotopic liver homografts. In Chapter Eleven the results of such studies in unmodified recipients were described; characteristically, there were sharp declines in hepatic blood flow with the advent of rejection. Such changes were completely avoided in four of five treated recipients (Fig. 103). Three of the five dogs lived for more than

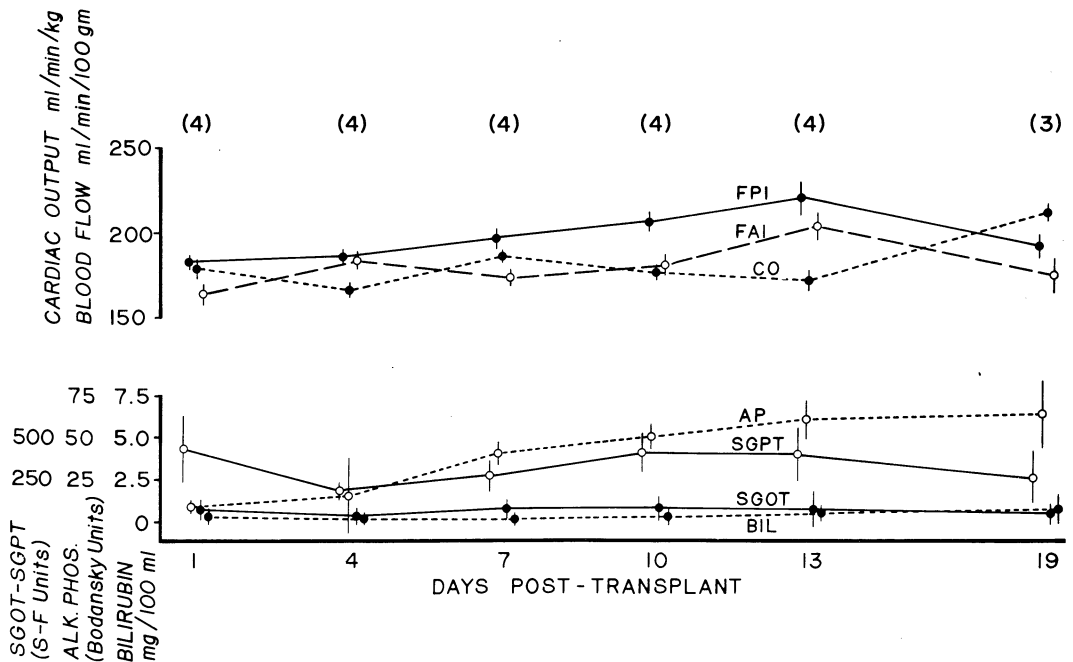


Figure 103. Liver blood flow, cardiac output, and liver function in four canine liver recipients which received immunosuppression with ALG and azathioprine and had little or no evidence of rejection. Mean values \pm SE, as well as the number of observations (in parentheses), are given. (By permission of Surgery 63:658, 1968.)

four months after operation. One of the residual animals is still alive after two and a half years; of the other two one died after 125 days of intestinal obstruction and the other after 185 days of duodenal ulceration with hemorrhage.

A complementary effect of the antilymphocyte substances with other immunosuppressive measures has also been demonstrated.^{42, 65, 66, 78, 157} The studies which were most relevant to the therapeutic regimen finally used clinically (Chapter Thirteen) were performed by Levey and Medawar.⁹⁹ They showed a profoundly synergistic effect of adrenal corticosteroids with ALS, an observation not dissimilar to that of Woodruff and Anderson,^{192, 193} who combined serum therapy with thoracic duct drainage in rats. Levey and Medawar⁹⁹ have also demonstrated that total body irradiation and immune serum treatment can be used together with benefit, providing the roentgenotherapy is given before exposure to the antigen. When irradiation was used late, well established skin homografts in serum-treated mice were rejected within a few days, long before the expected times established in control experiments with the administration of ALS alone.

ALS and Thymectomy

It has been shown in otherwise unaltered adult mice, rats, and hamsters that thymectomy causes a slowly developing loss in immunologic reactivity.^{110, 119, 156, 183} Miller^{27, 118} demonstrated with skin transplantation experiments in mice that this process could be accelerated if sublethal total body irradiation was also given. Later, Monaco,¹²⁴ Jeejeebhoy,⁷⁶ and Davis²⁹ reported that thymus excision in mice potentiated and prolonged the action of ALS.

Efforts to reproduce the latter findings in dogs treated with ALS or ALG have not been successful either in our laboratories^{69, 171} or in those of Atai and Kelly.⁷ Furthermore, no worthwhile consequence of thymectomy could be demonstrated in a series of patients subjected to this procedure before renal homotransplantation and subsequently followed for two and a quarter to four years.¹⁶³ This clinical investigation included several cases in both the thymectomy and control groups in which ALS was part of the therapeutic regimen described in Chapter Thirteen.

The failure to demonstrate an effect of thymectomy in the foregoing studies does not prove that the thymus has no immunologic function in adult dogs or humans. At the least, however, it does indicate that other factors are so much more important in determining survival and homograft function that the loss of the thymus resulted in no detectable therapeutic benefit under the stipulated experimental conditions. Conceivably, future improvements in management might permit unmasking of a presently unrecognizable subtle influence of thymectomy in these species, but at present there seems to be no justification for continued use of the procedure in clinical organ transplantation.

Mechanism of ALS Immunosuppression

In spite of numerous publications on the subject, the action of ALS and ALG are very incompletely understood. A discussion of this question is beyond

the scope of this chapter and the competence of the author. The interested reader is referred to Medawar's recent summary¹⁰⁵ which analyzes the case for or against several hypotheses, including those of simple lymphocyte depletion, coating of the lymphocyte recognition sites with the heterologous globulin (blindfolding theory), and stimulatory redirection of lymphocytes into immunologically nonspecific pathways (sterile activation). Medawar's survey of the known facts makes it clear that more than one and possibly all of these theories could have validity and that the ALS effect does not necessarily have a monolithic explanation.

From a practical point of view it is important to realize this, since the induction of overt peripheral lymphocyte or central lymphoid depletion by ALS or ALG is definitely not a requisite for the modification or prevention of homograft rejection.^{1, 69, 98, 99, 165, 171} The magnitude of the immunosuppression, even in the absence of detectable lymphopenia, is often of a much higher order than that resulting from profound lymphocyte depletion produced with mechanical techniques such as extracorporeal blood irradiation⁸⁰ and thoracic duct drainage.^{36, 192} Martin and Miller,¹⁰² Lance,⁹³ Taub and Lance,^{181, 182} Denman et al,³⁰⁻³² Leuchars et al,⁹⁷ Tyler et al,¹⁸⁵ and Medawar¹⁰⁶ have all suggested a common explanation for the immunosuppression caused by ALS in the absence of detectable lymphopenia. They have proposed a selective killing of the thymus-derived lymphocytes that subserve delayed hypersensitivity and homograft rejection, either by recognizing antigen and reacting against it, or else by activating a different kind of lymphoid cell derived from bone marrow.^{23, 24} Under these circumstances the loss of the thymus-derived lymphocytes might not be reflected in the blood because of the overproduction of other lymphoid cells which then spill out into the circulation.

The hypotheses listed above are based on essentially incontrovertible evidence that the lymphocyte is altered in one way or other or else destroyed by ALS. An alternative suggestion has been made by Guttman and his associates,⁵³⁻⁵⁵ who proposed that the antisera acted by coating the cells of the graft rather than by altering the host. They reported that donor pretreatment resulted in prolongation of skin homograft survival in unmodified mouse recipients. These observations could not be confirmed by Cerilli²² in dogs.

Toxicity of ALS and ALG in Animals

There is no question that administration of antilymphocyte substances causes a general weakening of immunologic reactivity. Nevertheless, the many investigations in rodents and dogs cited earlier have made it clear that homograft survival is often attainable without the penalty of total immunologic crippling and consequent overwhelming sepsis. Most of the canine experiments were carried out in a standard kennel environment. The incidence of infectious complications was noted to actually be less than with other effective immunosuppressive agents.

This aspect of toxicity is almost certainly dose related. Monaco et al¹²² showed that an invariably fatal wasting disease could be induced in mice heavily treated with rabbit ALS. At autopsy the animals had virtually complete

lymphoid depletion. In contrast, such lymphoid involution has not been observed in several other studies in which ALS or ALG provided significant protection of the homografts.^{72, 99, 171} The striking feature in our dogs⁷² and Lance's mice⁹³ was not atrophy of the lymphoid tissue, but the loss of small lymphocytes and their replacement by large and medium-sized pyroninophilic blast cells.

Apart from the risks of overimmunosuppression, there are other specific dangers with ALS and ALG administration. These were not appreciated until recently since many of the early investigators working with ALS in mice and rats reported freedom of their animals from obvious side effects. In such studies the periods of administration were limited to a few days or weeks. Furthermore, a systematic search was not made in various organs for evidence of the kind of damage that is classically associated with foreign protein therapy. The hope was expressed by several authors that the immunosuppressive qualities of ALS would blunt or prevent a reaction to its own alien protein. This may be partially true.

Gray et al,⁵⁰ Levey and Medawar,⁹⁸ and Lance⁹³ found that the appearance of antirabbit-protein antibodies was inhibited in mice during a course of therapy with rabbit ALS as compared to that which occurred in response to normal rabbit serum. The same thing was documented by Huntley⁶⁹ and Iwasaki⁷² in dogs receiving horse ALS. However, in all these experiments there was eventually some evidence of a host antibody reaction against the heterologous serum (Fig. 104).

Lance's study⁹³ from the Mill Hill laboratories was a particularly important one in terms of clinical applicability. He showed that intermittent "pulse" therapy with intervening rest periods resulted in a much more rapid sensitization to the injected immune serum than chronic steady administration and that the former regimen of treatment, in turn, led to a loss of the immunosuppressive effect; Ono's experiments demonstrated essentially the same thing.¹³³ This was not unexpected in view of Lance and Dresser's⁹⁴ observation that animals presensitized with rabbit gamma G globulin obtained from ALS eliminated ALG much more rapidly than the corresponding globulin prepared from normal rabbit serum.

Because of the immunogenicity of ALS and ALG (even of pure gamma G globulin), it was also not surprising that a number of dogs studied during chronic administration of ALS or ALG developed classic complications of foreign protein therapy. The most dramatic were anaphylactic reactions, which were usually mild and nonfatal^{1, 69, 72, 140, 165} and which often did not necessitate the discontinuance of therapy.

Even more alarming was the development in many of these test animals of histopathologic evidence of serum sickness nephritis after treatment with relatively large doses of ALS or ALG for longer than two weeks.^{69, 72, 172} In some of these animals in which the canine precipitating antibodies against horse protein were studied, the titers had risen little or not at all. The incidence of the complication was increased if intravenous therapy was given, and uremia was induced only with the latter route of administration. Similar lesions have been described since then by almost all investigators who administered ALS or ALG chronically to animals as the sole form of treatment. The reports of Lance⁹³ and

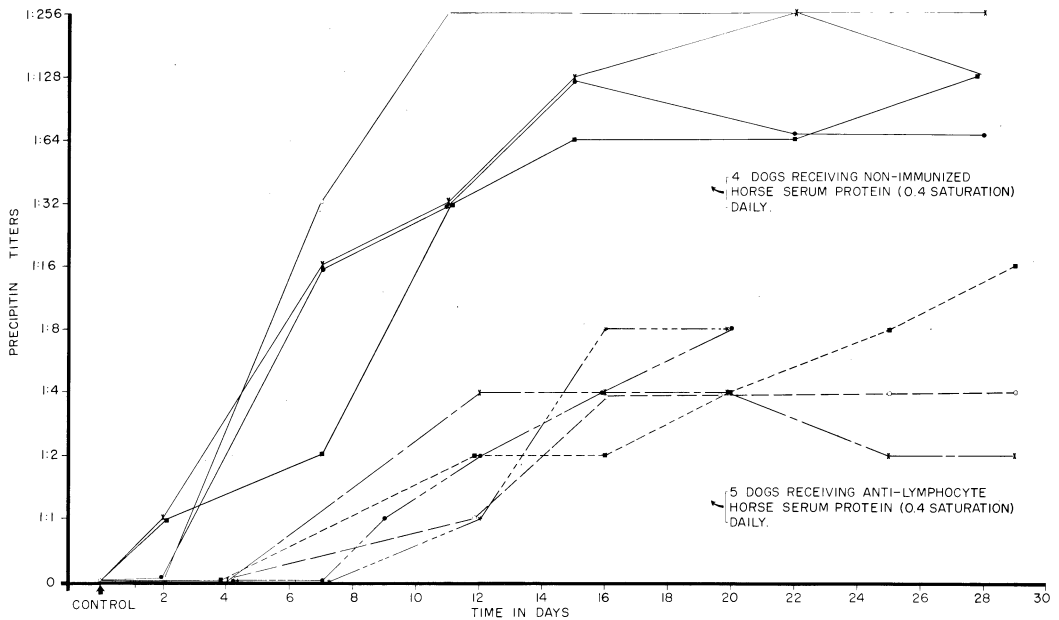


Figure 104. The response of canine precipitin titers to horse protein during daily injection of globulin prepared from the serum of nonimmunized and immunized horses. Note the striking difference in the two groups of dogs. (By permission of Surg. Gynec. Obstet. 124:1, 1967.)

Clunie²⁵ were particularly significant because their mice and dogs, respectively, had been given pure gamma G globulin. Lance showed that the incidence of the complication was reduced with continuous as opposed to “pulse” therapy. In one of Clunie’s renal homografts there was an apparent disappearance of the renal lesions 250 days after ALG treatment had been stopped.

Serum sickness nephritis is not based upon a specific reaction of the heterologous antibody with host renal tissue. Instead, the injected foreign protein causes a host antibody response, with the result that soluble antigen-antibody complexes are formed peripherally, coincident with a depression of complement. The complexes are mechanically trapped in the microcirculation of the glomeruli where they provoke a secondary inflammatory reaction.³³ The consequences are not readily reversible since heterologous globulin, host gamma globulin, and complement can be identified in these kidneys long afterward.^{40, 103} The only evidence of late clearing of the foreign protein in a transplantation experiment was cited earlier.²⁵ In Chapter Thirteen it will be shown that if ALG is used in combination with azathioprine and prednisone, the incidence of serum sickness nephritis in patients is essentially zero.

The potential reactivity of ALS or ALG with a variety of nucleated cells was mentioned under the section on absorption. This was apparently not a source of morbidity in our studies since direct binding of ALG with other than lymphoid tissues has almost never been demonstrated; specific lesions in the heart, liver, and other organs could not be found. Moreover, there was no evidence⁷² of direct binding of ALG to renal tissue (Masugi-type nephritis). The latter kind of nephrotoxicity could be produced by Guttman,⁵⁴ but he infused the ALG directly into the renal artery.

PREDNISONE

During the several years after the reports of Billingham, Krohn, and Medawar,¹⁴ and of Morgan¹²⁸ in 1951, there were several descriptions^{92, 107, 159} of the ability of adrenocortical steroids to significantly delay the rejection in rodents of first set skin grafts; the same effect has been seen in chickens.²¹ Furthermore, it was demonstrated by Peter Krohn as early as 1954⁹¹ that cortisone acetate could abolish a pre-existing state of sensitivity induced by full thickness skin homografts that were rejected in about nine days in rabbits. Thirty-seven to 60 days after the primary exposure regrafting was carried out with skin from the same donor. An accelerated rejection was avoided if subcutaneous steroid therapy (10 mg/day) had been instituted several days in advance of the reoperation and, in fact, the second transplants often survived longer than had been the case with the untreated first ones.

The crucial role of prednisone for the control and reversal of the rejection process has been unequivocally established in clinical renal homotransplantation^{48, 58, 68, 174} and there is no reason to doubt its utility in protecting hepatic homografts as well. It is ironic that the effect of steroid therapy has never been adequately tested in animals after liver transplantation. One reason is the remarkable propensity of dogs receiving prednisone to develop widespread gastrointestinal ulcerations and fatal hemorrhage.^{100, 197}

Examples have been reported of the reversal of rejection after prednisone treatment was started in canine recipients of orthotopic liver homografts.¹⁶⁶ The interpretation of these observations was later made less clear by the demonstration that reversal of hepatic rejection often occurs without any change in therapy.¹⁷² Nevertheless, the liberal use of steroid therapy is an extremely important facet of the treatment of human recipients of liver homografts (Chapters Thirteen to Fifteen).

OTHER IMMUNOSUPPRESSIVE DRUGS

Stuart¹⁸⁰ and Moore¹²⁶ and their associates were unable to demonstrate any beneficial effect of actinomycin C, local homograft irradiation, or azaserine when administered alone or in combination with azathioprine.

GRAFT ALTERATION

Many of the problems of organ transplantation could be minimized if it were possible to mitigate graft rejection by modifying the transplanted tissue rather than the host immunologic response. Efforts to achieve this objective have been unsuccessful with occasional possible exceptions,^{16, 62, 82} of which the most intriguing was described by Jolley, Hinshaw, and Peterson.⁸² They reported that rabbit skin grafts which were first immersed in homologous ribonucleic acid (RNA) and then transplanted to recipient animals which were given intravenous RNA had a survival four times longer than controls. The role

of the preliminary soaking was not analyzable in these experiments, but the authors also reported that human skin homografts subjected only to RNA soaking had unusually protracted viability when placed upon patients with burns.⁸¹ Similar findings have been reported by Lemperle in mice.⁹⁶

In our laboratories attempts to "pretreat" whole organ homografts have been made in dogs by perfusing kidneys for about 30 minutes with RNA prepared by phenol extraction from the spleens of the prospective recipients ("autologous" RNA) or other dogs ("homologous" RNA). After transplantation to unmodified recipients, about one fourth of these life-sustaining organs had prolonged homograft viability.⁵¹ Maximum survival of recipients which were subjected to simultaneous removal of their own kidneys was 123 days. The mean survival in a group of 40 recipients was more than 20 days as opposed to approximately 10 days in 30 control animals. Furthermore, there were seven homografts of the 40 which had no histologic evidence of rejection, whereas all the control homografts had the typical findings of unmodified rejection. The protection afforded by recipient specific RNA was not significantly different from that obtained with homologous RNA.

The foregoing effect was not increased by the addition of a supposed RNase inhibitor, DEAE-dextran, but it was abolished by the addition of commercial RNase. The treatment of renal autografts with homologous RNA did not result in their rejection. The latter finding and the fact that the results after homotransplantation were equivalent with either homologous or autologous RNA suggest that the homograft protection was not due to RNA-induced changes in the genetic characteristics of the homograft cells.

Seven orthotopic liver transplantations were also performed after preliminary RNA perfusion of the homografts. The transplanted organs supported life for 46, 14, 13, 11, seven, seven, and seven days.⁵¹ Specific control experiments were not performed. However, it was stressed in Chapter Eleven that survival in the unmodified canine recipient rarely exceeds 10 days and that the longest survival ever obtained after liver transplantation under these conditions was 31 days.

It is not yet certain that the foregoing findings represented more than an experimental artefact, since a logical explanation for the surprising results was not available and because the degree of homograft protection was so relatively limited that its statistical significance in terms of survival was marginal. It will be of interest in laboratory experiments to first confirm these observations and then to determine whether such graft conditioning can be advantageously combined with effective host immunosuppression.

CHANGING HOST-GRAFT RELATIONSHIPS

Since each of the systemic immunosuppressive agents mentioned earlier in this chapter can cause general immunologic crippling, it has been customary to categorize as nonspecific all the treatment protocols (Chapter Thirteen) in which they have been employed. The implied criticism of using a bludgeon where a therapeutic scalpel would be preferable is not without justification.

Nevertheless, there has been for some time an impressive body of information indicating that whole organ homotransplantation with such therapy can eventually lead to selective abrogation of the host rejection response, that the success with which this can be done is related to histocompatibility factors, and that the degree to which it is achieved is the most important determinant of prognosis in any given case. Appreciation that the immunologic relation of the graft to the host is a fluid rather than a fixed one adds an important dimension to the consideration of any kind of immunosuppression.

Rejection and Its Remission

There are two clinically identifiable phases in the chain of events under discussion. The first consists of an attack by the host's immune defenses upon the new organ, usually within a few days or weeks after its transplantation. The vigor of the process is highly variable, as judged by the magnitude of the changes caused in the morphology and function of the homograft.

Whether severe or mild, the intensity of the acute rejection ultimately tends to abate in the second phase in many cases, particularly if short-term increases in immunosuppression are instituted. However, the forcefulness of the rejection may diminish without making such changes in therapy, or occasionally in animals that have not received any treatment at all (Chapter Eleven).

Human Renal Recipients. The remission of rejection was not convincingly demonstrated in animals until it had been observed following clinical renal homotransplantation. In retrospect, it is probable that the two earliest successfully treated human recipients of renal transplants passed through mild and spontaneously reversible rejection crises; both received kidneys from fraternal twins, one in Boston¹⁰⁹ and the other in Paris,⁶⁰ after being submitted to total body irradiation. Hamburger ascribed the changes to a spontaneously reversible immunologic crisis. Without changing this view, he later speculated⁵⁷ that the long survival both in his case and in that of Merrill may have been partially due to a high grade although incomplete pre-existing tolerance such as that seen in cattle siblings that have had a shared placental circulation during gestation.^{5, 178}

Strong indications that rejection was a highly controllable and regularly reversible phenomenon, and that it was often followed by a state of relative "host-graft nonreactivity," came in a later report from our institution.¹⁷⁴ In that series of renal homotransplantations, there were 10 patients treated in late 1962 and early 1963. Seven, not including any recipients of fraternal twin homografts, had clear-cut rejection commencing four to 34 days after operation. In each instance the process was reversed by the addition of massive doses of prednisone to the pre-existing therapy with azathioprine. Within a surprisingly short time it became possible to drastically reduce the steroids that initially had been necessary to rescue the grafts. In several instances the patients were soon returned to treatment only with azathioprine, the agent which at the beginning had not been capable of preventing an acute rejection crisis. Three of these seven patients are still alive more than six years later and are now among the longest surviving recipients of non-twin renal homografts in the

world. After the remarkable effectiveness of steroid therapy in this situation had been established from our own experience, but before our findings were published, it was learned that the same kind of observation had been made by Goodwin and his associates⁴⁸ in a young woman who ultimately died of sepsis 144 days after receipt of a maternal homograft.

There is no point in commenting further about the fully accepted fact that kidney rejection can undergo remission beyond noting that such an occurrence is uncommon in dogs and probably also in humans if immunosuppression is not increased. It is also worth mentioning that the central role of steroids in promoting this event in clinical practice was probably predictable on the basis of Krohn's report⁹¹ of 1954 (see description earlier in text), although the implications of his findings were not fully appreciated until many years later.

Animal Liver Recipients. Although recovery of a rejecting kidney graft cannot usually be expected unless treatment is intensified, numerous examples were cited in this chapter as well as in Chapter Eleven of spontaneous reversal of hepatic rejection both in unmodified dogs and pigs (especially the latter) and in dogs provided with unvarying azathioprine or ALS therapy. With resolution of the process, abnormalities both of the liver function tests and hepatic blood flow tended to return toward, although often not completely to, normal.

The histopathologic changes at various stages after orthotopic liver transplantation are considered in Chapter Twenty. Here it need only be noted that all the canine hepatic homografts examined after four to six days for the next two postoperative weeks were invaded with mononuclear cells, whether or not the biochemical and clinical findings of rejection had developed. After this time the infiltrate decreased in density or even disappeared while the evidence of repair became predominant. Thus, the morphologic changes also supported the idea that there was an initial forceful host attack which subsequently tended to exhaust itself, or at least to become less effective.

The foregoing comments should not be construed as suggesting that the desired change in the host-graft relationship is dependent upon an overt rejection crisis. Earlier in this chapter it was remarked that most of the canine liver recipients in which a more or less completely "tolerant" state developed, as defined by the ability to discontinue all therapy, were those in which diagnosable acute rejection either had been very minor or had not occurred at all. In these experiments the first wave of immunologic reaction had apparently been insufficient to cause significant deterioration of homograft function. There have been well-documented examples of this kind of "subclinical rejection" in patients (Chapter Fourteen).

The Mechanism of Graft Acceptance

Although it has been well established that a homograft may come to be more or less tolerated in its new host, the explanation for the privileged status is not accepted with any more unanimity today than it was five years ago. One of the reasons probably is that more than one immunologic pathway may be involved.

Specific Immunologic Tolerance. It is almost certain that the continuous pres-

ence of a transplanted organ in a host being treated with immunosuppressive therapy often leads to a selective loss of responsiveness to the antigens of the homograft (tolerance). The evidence that chemotherapy can be used for the induction of narrow range tolerance is unequivocal. The literature on this subject will not be reviewed here since it has been well summarized by Schwartz,¹⁵⁰⁻¹⁵² who was the first to call attention to this possibility.¹⁵⁴ Suffice it to say that azathioprine, 6-mercaptopurine, amethopterin, cyclophosphamide, and even total body irradiation can be used to promote specific tolerance, providing the antigen in question is administered in an appropriate dose and in close temporal approximation to the immunosuppressive treatment.

One of the theories advanced by Schwartz^{150, 151} to explain the specific effect of chemotherapy under these circumstances is depicted in Figure 105. The illustration suggests that a clone of lymphocytes which presumably have an active metabolism as the result of stimulation by antigen should be differentially susceptible to antimetabolites. The same reasoning in connection with the convalescence after human renal homotransplantation had also been ex-

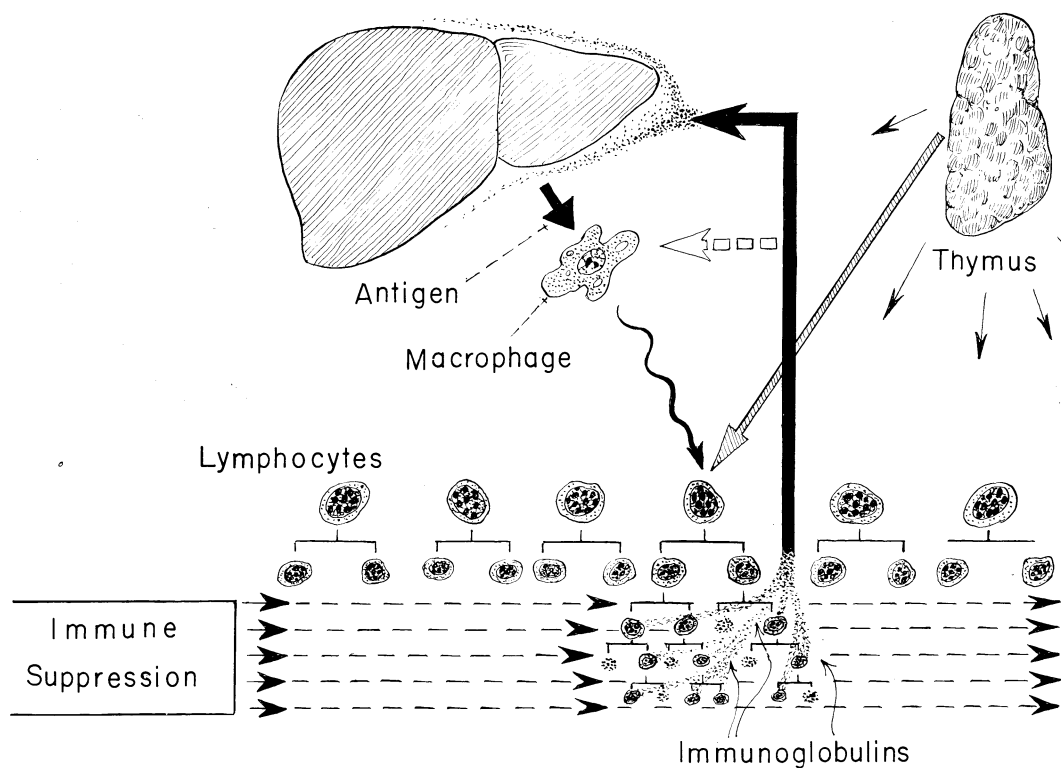


Figure 105. Hypothetical mechanisms by which nonspecific immunosuppression may lead to selective abrogation of the host immune response. Special susceptibility to these agents of a fraction of the lymphoid population could lead to exhaustion of a clone and hence, tolerance. Since maintenance of such cell lines even in adult life is apparently thymic dependent in experimental animals, thymectomy would be expected to aid the process; this appears to be true in rodents, but such an effect of thymus removal has not been detected in dogs or humans. (see discussion under ALS section). A possible protective role is also shown of immunoglobulins elaborated by the replicating cells. Conceivably the antibodies could act either at the site of the antigen (enhancement) or by affecting the macrophage processing of the antigen. See text for discussion.

pressed in the following clinical terms:¹⁶⁰ "Temporally, the first evidence of adaptation is often coincident with reversal of the rejection crisis. It has been pointed out that both events can occur in most cases without the necessity for even temporary suppression of the total white blood count below normal levels. Here, the peripheral white cells as well as the humoral antibodies with which the graft is in constant contact appear to have ultimately lost at least part of their capacity to injure the foreign tissue. . . . In these cases, the ultimate leukocyte population seemed to be inactive, at least in a relative sense, against the renal antigen. It is tempting to believe that immunosuppressive therapy caused a progressive attrition of those cells which were immunologically sensitized against the homograft antigen and that the replacement cells had an absent or reduced memory of the alien tissue." A similar hypothesis could be proposed as a plausible explanation for some of the specificity of action of ALG.

The concept of "clone stripping" in this scheme is consistent with the cyclic phenomena which occur characteristically after whole organ transplantation both in treated animals and man. With the existence of very close biologic compatibility between donor and recipient, it could also explain the apparent acceptance of weakly antigenic homografts as has apparently occurred in unmodified pigs (Chapter Eleven). Under either set of circumstances the sequence might be analogous to that demonstrated by Brent and Gowland¹⁷ in which tolerance in mice was preceded by a transient period of sensitization (Fig. 106).

Nevertheless, there has been a widespread reluctance to believe that specific immunologic tolerance has been produced with the immunosuppressive regimens described in this and the next chapter after either experimental or clinical whole organ transplantation. The article most often quoted as contravening this possibility is that of Murray et al¹²⁹ despite the fact, as the authors took pains to make clear, that the evidence in that report was inconclusive and involved only two canine experiments of a potentially crucial nature. These two dogs had been given renal homografts nine and 18 months previously and had received long-term therapy with one of the purine analogs. Throughout the postoperative course, renal function appeared from the published charts to have been unstable. Moreover, it was deteriorating at the time the other kidneys from the original donors were transplanted; in both instances, the blood urea nitrogen (BUN) had become significantly elevated by the time of the retransplantations. The second organs were rejected after 23 and three days, respectively. It could be persuasively argued that there was not good justification to believe that the first kidneys in these animals were well tolerated, in which case there would be little reason to anticipate that the second organs would be kindly accepted.

To date, few investigations have been carried out in human recipients of renal homografts surviving for prolonged periods to establish the presence or absence of tolerance to their donor tissue. One of the reasons has been the potential risk which could attend some of the testing which might be done, such as skin transplantation. However, it is worth mentioning that Dr. Fritz Bach of Madison, Wisconsin, performed mixed lymphocyte culture examinations from the peripheral blood of a number of our recipients and their donors,

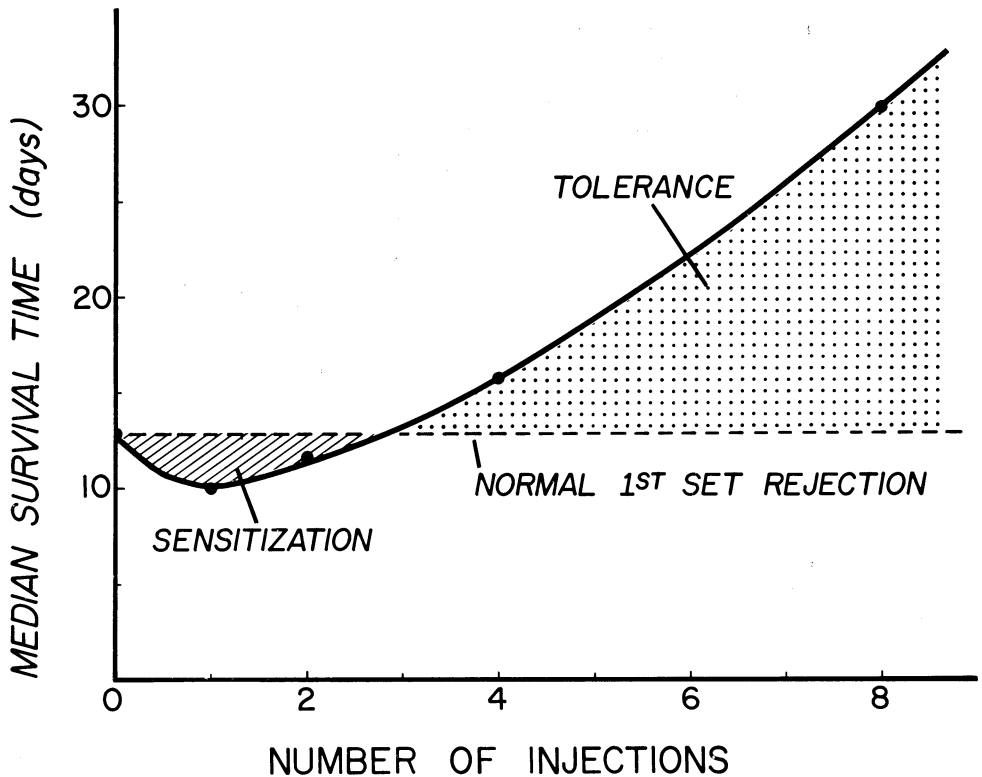


Figure 106. The experiment of Brent and Gowland¹⁷ showing the induction first of sensitization and then of tolerance in mice treated with donor-specific spleen cells. The survival times of subsequently transplanted skin homografts are plotted against the number of preconditioning injections. Note that a small number of injections sensitized the animals, but that tolerance developed if the treatment was more protracted. The analogy between these findings and those of a reversible acute rejection crisis is evident. (Modified from an illustration in *Nature* [London] 196:1298, 1962.)

two to four years after transplantation. In some cases in which clear donor-recipient histocompatibilities were detectable by serologic typing he found that the recipient lymphocytes no longer underwent blast transformation when exposed to killed donor white cells, although they reacted vigorously to third party lymphocytes.⁴ The findings were interpreted as indicating specific acquired immunologic tolerance.

Enhancement. It was shown by Kaliss in tumor systems that homografts may be protected by the presence of certain kinds of antigraft antibodies.⁸⁶ It is conceivable by a feedback mechanism that the same thing occurs under the conditions of whole organ transplantation. The process could be envisioned as shown in Figure 105, whereby immunoglobulins synthesized by the activated clone return to the target tissue and coat or protect it in some other way.

Certainly it has been possible to demonstrate antigraft antibodies in the serum of patients carrying chronically functioning and apparently well tolerated kidneys. Using an antiglobulin consumption test, Iwasaki⁷³ and also Rapaport¹⁴⁵ showed that the serum of most of the tested human recipients contained 7S immunoglobulins which could be selectively absorbed by the nucleated cells of the original donor. Moreover, there have been numerous

reports of more or less extensive immunoglobulin deposition, as detected by immunofluorescence techniques, in long functioning human kidney transplants.^{83, 143, 147, 190} By and large, however, the latter finding does not connote a favorable prognostic sign but rather the converse.

The mechanism of enhancement may explain the kind of observation originally made by Woodruff and Woodruff¹⁹⁵ and termed by them "adaptation." In their experiments with guinea pigs, bits of homograft tissue were implanted in the anterior chamber of the eye and later transferred to a subcutaneous location. There, rejection occurred very slowly or not at all. In contrast, subcutaneously placed thyroid from the same donor was repudiated promptly. Some of Murray's experiments¹²⁹ with canine renal transplantation were designed along similar lines and yielded comparable results. He showed that a secondarily placed kidney could undergo rejection while the contralateral organ which had been transplanted earlier from the same donor could continue to function. The differential ability of the first kidney to resist destruction was not due to a change in its genetic character since it could also function when replaced back in the original donor. The same general observations had been made in 1954 by Weber, Cannon, and Longmire¹⁸⁸ using skin grafts in chickens.

It is not necessary to believe that enhancement must exert its influence in an isolated way, as the recent studies of Stuart et al¹⁷⁹ have made clear. They showed that the survival of renal homografts in rats could be more consistently obtained with the combination of tolerance induction plus the administration of enhancing antibodies than when either approach was used alone.

Failure of Antigen Processing. There is the added possibility that a defect in antigen processing¹³¹ by the reticuloendothelial system could be responsible for graft acceptance, a concept for which there is not yet any firm evidence. However, it is known that antisera can under certain circumstances markedly and specifically inhibit for long periods the responsiveness to the antigen recognition.¹⁵³ The way in which an analogous sequence of events could be hypothetically injected into the picture after organ transplantation is shown in Figure 105.

↓ Nossal!! DC were not discovered, unfortunately until 1973 (6 years later), but the concept APC was the same.

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Chapter Thirteen

IMMUNOSUPPRESSION IN MAN

In the two preceding chapters attention was focused upon the kind of laboratory research which could be applied in one way or other to the problem of liver replacement in man. The investigations proved that a protracted and healthful life was possible in dogs after this kind of operation with the use of either azathioprine or ALG as the sole immunosuppressive therapy, or in pigs with no treatment at all. Nevertheless, the consistency with which really long-term survival was obtained was poor. There were at least two obvious reasons. First, complete control of rejection was usually not achieved. Second, it was difficult to prevent or to treat infectious disease complications or to provide other niceties of special care in a standard kennel or piggery environment.

Whatever the explanation, it is probable that the animal data would have indefinitely discouraged a clinical trial had it not been for the example of renal homotransplantation. With the latter procedure, the surprising frequency with which long survival was eventually achieved in man could hardly have been predicted from the prior laboratory results which, if anything, were less favorable than those after experimental liver transplantation. In addition, the human experience with the kidney did more than serve as an incentive. It also provided the conditions in which complex and effective immunosuppressive regimens could be evolved in man and applied, in turn, to the transplantation of other organs.

In this chapter the two treatment protocols will be described which were used for human recipients of both orthotopic and auxiliary liver homografts after extensive personal experience had already been acquired in clinical renal transplantation. The first program consisted of double drug therapy with azathioprine and prednisone to which less important or even questionable ancillary measures were sometimes added; there were no survivals of more than 34 days. The later liver recipients were given triple agent therapy with azathioprine, prednisone, and heterologous antilymphocyte globulin (ALG).

DOUBLE DRUG THERAPY

Background in Renal Transplantation

In the field of kidney transplantation the most important development which made immunosuppression practical was the discovery of the way in

which azathioprine and prednisone could be advantageously used together. As summarized by Mannick and Egdaahl,²⁸ there were essentially no preceding laboratory data to indicate that the benefit with this now universally accepted combination of agents would be as great as proved to be the case. Indeed, the first publication on experiments in animals²⁹ was a belated confirmation of the far more convincing observations already made in humans.⁶³

Furthermore, it is difficult even in retrospect to ascribe priority for standardization of azathioprine-steroid therapy to any single authority or transplantation group. What is clear is that by late 1962 the two drugs were being used together in one way or other and with varying degrees of conviction about their synergism for the prevention or reversal of renal homograft rejection in at least one British⁷⁵ and three American^{17, 37, 63} centers. Since then, comparable regimens have been adopted throughout the world.

The Timing of Therapy. Treatment was provided in two ways. In both azathioprine was started shortly before operation and continued indefinitely thereafter, generally in the maximum doses which were thought to be possible without causing leukopenia. Prednisone was either used prophylactically beginning on the day before or the day of operation or, alternatively, withheld until the onset of a clinically evident rejection. The techniques, virtues, and drawbacks of both approaches were analyzed several years ago⁴⁵ from our own experience. In retrospect, the most important advantage of withholding steroids until graft repudiation had clearly started was that the features of rejection and host-graft adaptation as well as the influence of drug therapy on these processes could be delineated with some precision. These concepts were discussed in Chapter Twelve. The greatest disadvantage was that the rejections which developed under treatment with azathioprine alone were sometimes very severe and difficult to reverse with delayed steroid therapy. It was found that their incidence and seriousness were reduced if prednisone was given from the beginning.⁴⁵ Consequently, it has been our policy since December, 1963, to immediately treat virtually all recipients of organ homografts with both drugs, as will be described in detail further on in the discussion of the cases of human liver transplantation.

Kountz and Cohn²⁷ recently reported that azathioprine and prednisone delivered directly into the arterial supply of renal homografts had an increased protective effect on the organs compared to that obtained with oral or intravenous administration. At the time of their publication 17 of 18 patients treated with this modification had survived from one to 13 months after transplantation of kidneys. The method has not been tested for liver transplantation.

Ancillary Measures. Other weakly immunosuppressive drugs have been added to the basic treatment with azathioprine and prednisone either in a continuing program or, more commonly, for the treatment of an acute rejection. The most widely used have been azaserine and actinomycin C (see previous chapter). The value of these agents has not been well established. We have never used azaserine. Early in our experience actinomycin C was given freely, but in recent years we have been less and less inclined to use it because of its tendency to cause sudden bone marrow depression.

The second line immunosuppressive measure that has been most generally

accepted because of its safety has been local homograft irradiation. The first description of this method was by Dempster,⁶ but Hume et al^{16, 24} and Martin and his associates³¹ have been its leading proponents. Hume's recommendation was that 150 R (at depth) be given to renal homografts on post-transplantation days one, three, five, and seven. All the foregoing authors agreed that, if homograft irradiation had an ameliorating effect on rejection, it was a feeble and transient one. We have often used local irradiation after renal homotransplantation. Although Stuart et al⁶⁷ could demonstrate no benefit after orthotopic liver transplantation in dogs, we have irradiated the hepatic homografts of several of our patients, primarily in an effort to reduce the swelling (Chapter Fourteen).

Thoracic duct drainage before and after renal transplantation was introduced clinically by Franksson¹¹ as an adjuvant immunosuppressive technique. He, and later Murray et al,³⁸ had the impression that the control of rejection was made easier. We have not constructed lymph fistulas in our patients.

Results with Double Drug Therapy for Renal Transplantation. With the combined use of azathioprine and prednisone the previous air of hopelessness about the prospects of successful clinical renal homotransplantation was dispelled almost overnight, and with good reason. It became apparent that many patients dying of terminal renal disease could be restored to relatively good health and that the benefit was apt to be long lasting, especially if a kidney was transplanted from a blood relative. The latter conclusion can still be well supported by a notation about the ultimate fate of the 64 patients treated by us who provided the material for an earlier book on renal transplantation.⁴⁵ Their operations were performed between November, 1962, and March, 1964, using kidneys obtained from volunteer living donors. At the time the book was finalized in June, 1964, 37 of the recipients were surviving. Now, five and a sixth to more than six and a half years post-transplantation, 30 of these 37 patients (47 per cent of the original 64) are still alive (Fig. 107). Furthermore, all but three of the residual group still have adequate function of their original homografts; the exceptional patients received second kidneys two and a half, five and a quarter and five and a half years after the first transplantation.

Because of its pertinence to liver transplantation, one striking observation in this early series of cases deserves special emphasis, namely the disparity in results with intrafamilial transplantation as opposed to transplantation between unrelated individuals. In the consanguineous group, which comprised 46 of the 64 cases alluded to above, the early death rate was lower. Moreover, the patients who survived through the dangerous first few months had a very good chance of living for at least half a decade (Fig. 107). Twenty-eight (61 per cent) of the 46 recipients of familial kidneys in this series survive to date; only two patients in this surviving group have had a late retransplantation.

In contrast, the acute mortality was higher in the other 18 cases in which transplantation was from nonrelative donors. One half the recipients were dead at the end of three months, and six remained at the end of one year. In addition, a steady loss rate continued into all subsequent postoperative years (Fig. 107) until, at the present time, there are only two left of the original 18 patients, one by virtue of a second kidney. In 1966 at the New York Meeting of the Trans-

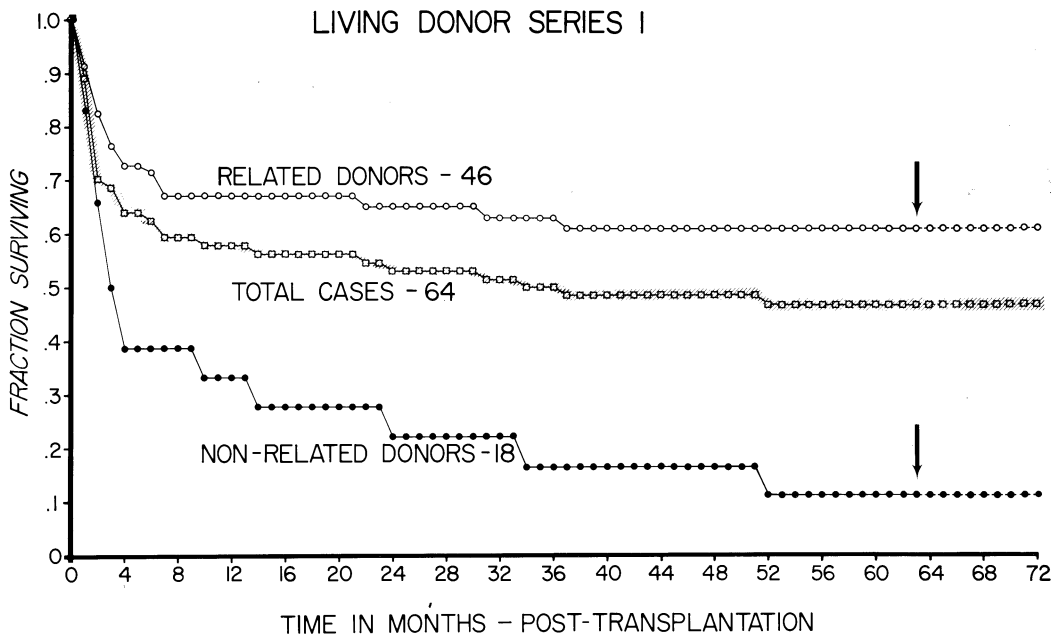


Figure 107. The life survival curve of 64 consecutive patients provided with renal homografts obtained from living volunteer donors between November, 1962, and March, 1964. Follow-ups of five and a sixth to more than six and a half years are now available; the minimum periods of observation are indicated by vertical arrows for the two subgroups. All but three of the patients were treated with one of the double regimens of azathioprine and prednisone described in the text; one of the exceptions also had 400 R total body irradiation and the other two received azathioprine only. Note the striking difference in prognosis with consanguineous as opposed to nonrelated donors.

plantation Society⁵¹ attention was redirected to the implications of the findings in this series. The statement was made that "... it seems clear that the long term prognosis of recipients of randomly selected unrelated homografts is extremely guarded. . . . The observations emphasize that development of better immunosuppressive regimens and/or perfection of tissue typing techniques will be necessary before even moderately satisfactory long term results can be expected within the unrelated donor-recipient human population."

The poor prognosis of recipients of kidneys from nonrelated donors in that era can be illustrated in another way. In March, 1968, inquiries were made to all the American and European transplantation centers which had been active from 1962 to the spring of 1964. Only nine patients were found at that time in whom there had been continuous function of nonrelated renal homografts for as long as four years. One of these patients was given an "expendible" kidney by Dr. Willard Goodwin of UCLA in June, 1963; another six recipients of cadaveric homografts had been cared for by Dr. David Hume of Richmond, Dr. Guy Alexandre of Louvain, Dr. Richard Lillehei of Minneapolis, Dr. Satoru Nakamoto of Cleveland (two cases), and Dr. Charles Zukoski of Nashville. The two others were in our original series.^{45, 46} It is worth noting that four of these nine recipients (Goodwin's, Hume's, Lillehei's and one of ours) have died since the time of the survey.

In the years following the early trials just alluded to, further experience

with double drug therapy at several centers, including our own,⁴⁶ led to a reduction in the early mortality after homotransplantation outside of family groups. This information will not be reviewed here except to say that it did nothing to allay the fear that the average nonrelated renal homograft, be it from a cadaveric or living donor, would have a functional lifetime inferior to that to be expected from a familial kidney. The point was particularly well made by Hume's group³⁶ who, using their own version of the double drug regimen, noted that the renal homograft loss rate during the second post-transplantation year was 10 times greater with cadaveric kidneys than with consanguineous organs.

Few words need to be wasted explaining the relevance of these observations to hepatic transplantation, an undertaking in which all homografts must come from nonrelated cadaveric sources. Suffice it to say that strenuous efforts were made in all cases after 1965 to upgrade the projected outlook of liver recipients by using improved methods of immunosuppression (see second section of this chapter) and by trying to select donors on the basis of prospective histocompatibility typing (Chapter Three).

Histocompatibility Matching and the Double Drug Regimen. At the time the series of renal transplantation just described was compiled, there were no practical means of assessing in advance the biologic suitability of donor-recipient combinations. The only immunologic screening that was systematically applied preoperatively was designed to avoid red blood cell incompatibilities (Chapters Two and Fourteen). Consequently, the transplantation itself served as a test system in which the recipients of histoincompatible kidneys were presumably weeded out either by death or by loss of their homografts at varying times after the transplantations. Realizing this to be the situation, a six-month moratorium on new cases was declared at the University of Colorado from March to October, 1964. During this time a program was planned, in collaboration with Dr. Paul Terasaki of Los Angeles, which for the first time would attempt donor selection by prospective histocompatibility matching with serologic methods.

The impressive evidence that the techniques of tissue typing used by Terasaki and a number of other workers in this field actually measured histocompatibility antigens is considered separately in Chapter Three. Here it will only be mentioned that efforts to improve the quality of matching in 25 new intrafamilial transplantations from October, 1964, to April, 1966, were not successful to a statistically significant degree. The reason was that a commitment was made only to find the best among whatever otherwise suitable donors were available; transplantation was not refused if a good match could not be found. Since most family groups were small, the degree of compatibility actually obtained was not essentially different from that which could have been achieved by random selection.^{68, 69} Consequently, it was not surprising to find that the results after consanguineous transplantation were not materially improved (Fig. 108). The life survival curve had a somewhat different shape from that in the original series (Fig. 108), but the number of patients who were alive after one to three years was not changed at all.

In contrast, the choice was much more discriminating for 17 patients who were treated with nonrelated homografts in the 13 months beginning in October, 1964.^{68, 69} During that time the since abandoned policy was being followed

of accepting organ donation from a pool of penal donors which contained as many as 80 volunteers for any given recipient. The matches obtained were almost always imperfect, but as a group they were significantly better than would have been expected with random selection in the nonrelated population.

There is now a follow-up in the latter 17 cases of three and a half to more than four and a half years (Fig. 108). The kidney and patient survival at one year were nine of 17 (53 per cent). Today, five of the recipients (30 per cent) are still alive, having had continuous function of their homografts for 42 to 50 months. Thus, there had been an improvement in the results in comparison to that in the original nonrelated series, but not to a satisfactory level. Moreover, the characteristic late failures after nonrelated homotransplantation had not been eliminated. The deaths which occurred in the second (one case), third (one case) and fourth (two cases) postoperative years were due, respectively, to hepatitis (one example), generalized sepsis (two examples), and quadriplegia secondary to a compression fracture of an osteoporotic cervical vertebra (one example). A contributory factor in all the late deaths was thought to be the need for chronic therapy with moderately large doses of prednisone (see later).

In reporting these cases in January, 1967,³⁰ Marchioro remarked that the

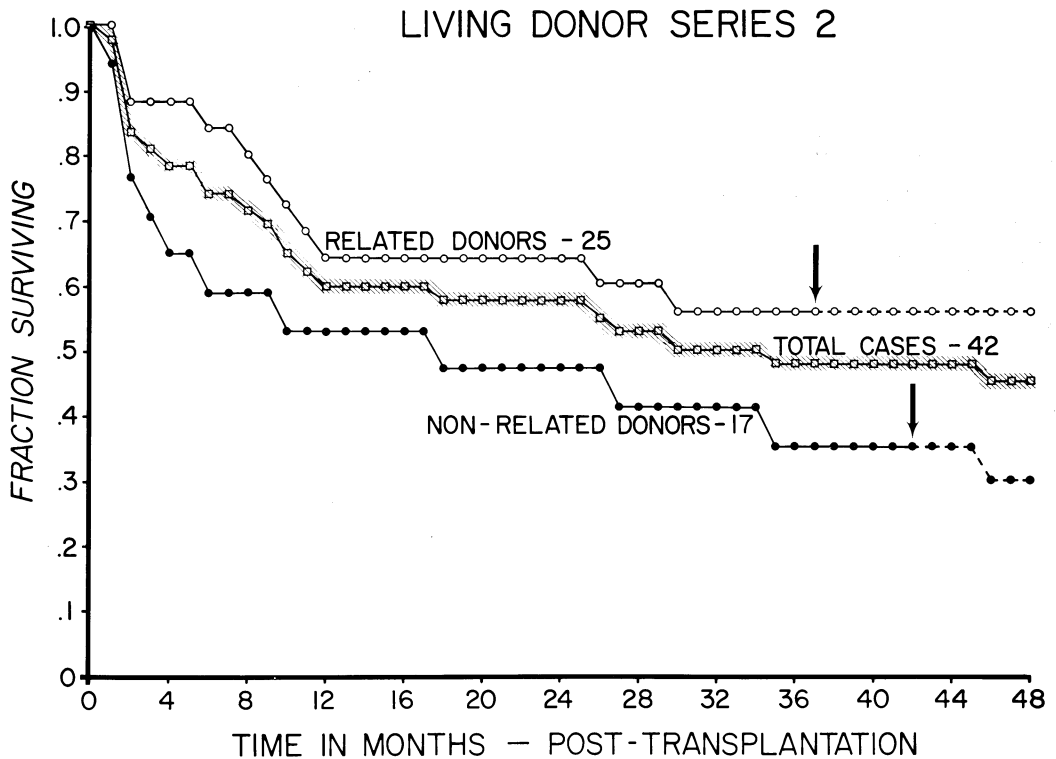


Figure 108. The life survival curve obtained when donor selection was attempted on the basis of histocompatibility matching. Since the series was compiled between October, 1964, and April, 1966, follow-ups of three to four and two thirds years are now available. The minimum periods of observation in the two subgroups are indicated by vertical arrows. Note that the acute mortality was reduced after intrafamilial transplantation, but that the ultimate prognosis was no different than in the first series (Fig. 107). However, the early and late outlook of recipients of nonrelated homografts was somewhat improved.

results did "...not imply either that the tissue typing methods then being developed were not a direct or indirect measure of histocompatibility factors, or that the use of these techniques would play an insignificant role in transplantation practices of the future. ... Instead the observations suggested that antigen matching techniques could not receive a fair trial with the treatment programs then widely being used unless transplantation was performed only between almost perfectly matched pairs. Alternatively, they required for exploitation a setting of far more adequate immunosuppression. The former approach would limit consideration to only a very small number of patients who needed this kind of therapy. The latter development would allow extension rather than restriction of the indications for acceptance into a transplantation program."

Deficiencies of the Double Drug Regimen. When azathioprine and prednisone were first used together for patients treated by renal transplantation, there were a number of deaths from drug toxicity during the early postoperative period. Many of these fatalities were due to bone marrow depression that had been caused by overdoses of azathioprine. A common story was that good initial kidney function was obtained from the homograft, that a severe rejection crisis then supervened causing a secondary return of uremia, and that leukopenia and lethal sepsis followed shortly afterward. Apparently at least part of the explanation was that the renal pathway of detoxification of this drug had been lost to a variable degree as the consequence of rejection. It was learned that the doses had to be considerably reduced under these circumstances or after the placement of cadaveric homografts which functioned poorly from the beginning.⁴⁵ As experience increased the complication of acute azathioprine overdosage was virtually eliminated.

Avoidance of the hazards of steroid therapy was less simple. In many cases continued function of the transplanted kidneys proved to be dependent upon the chronic administration of unacceptably large quantities of prednisone. The complications that often followed ranged from exceedingly troublesome to lethal. They included cosmetic deformity, bone demineralization often with spontaneous fractures, muscle wasting, arrest of growth in infants, fatty infiltration of the liver, pancreatitis, and gastrointestinal ulceration and hemorrhage. Most serious, however, was the resulting susceptibility to microorganisms of all types.

If the consequent infections were due to common pathogenic bacteria, they could be treated with properly chosen antibiotics. Very often, however, they were caused by fungi, protozoa, or viruses for which specific therapy was not available. The way in which this sequence of events manifested itself in a series of autopsies of renal transplant recipients at our institutions has been summarized by Hill.¹⁴

The therapeutic dilemma often posed by the foregoing situation, with either intrafamilial or cadaveric transplantation, but far more commonly after the latter, is evident. On the one hand, life was threatened by reduced or failing function of the homograft and, on the other, by the measures taken to prevent its further deterioration or complete loss. Largely as the result of Hume's efforts,¹⁶ one of the great lessons learned in the field of renal transplantation has been that it is often desirable to stop immunosuppression, to remove the renal

homograft, to return the patient to maintenance on an artificial kidney, and to consider retransplantation at a later date. Unfortunately, this well ordered and leisurely approach to treatment is not possible with a rejecting orthotopic liver homograft. Nevertheless, hepatic retransplantation has been shown to be feasible, as will be mentioned in Chapters Fourteen and Seventeen.

Double Drug Therapy After Liver Transplantation

An azathioprine-steroid regimen was used in all the early recipients of both orthotopic (OT 1 to 5) and auxiliary (AT 1 to 3) homografts. Whether adequate control of rejection might have been achieved in some of these cases will never be known for certain since all the recipients died in 34 days or less.^{13, 55-57, 59, 62} Invariably there were a host of complications of which only a portion were related to drug therapy or to the rejection process. However, as will be documented in Chapter Sixteen, overwhelming bacterial, fungal, and viral infections were characteristic in this early experience, indicating that gross overdosage had occurred.

The Therapeutic Schedule. In all these liver recipients, azathioprine was started on the day of operation and prednisone was also begun then or within a short time afterward. Azathioprine was given in quantities that were somewhat smaller than those used for recipients of renal homografts in that era of our program (Table 17). The average dose per day during the post-transplantation

Table 17. Immunosuppression Used in the Liver Recipients Who Survived Operation and Received Double Drug Therapy, Compared to That Administered to 18 Consecutive Recipients of Nonrelated Renal Homografts Who Were Treated in the Same Era*

No.	SURVIVAL (Days)	AVERAGE AZATHIO- PRINE	AVERAGE PREDNISONE	ACTINO- MYCIN C	HOMOGRRAFT IRRADIA- TION	WHITE BLOOD COUNT		PLATELET COUNT		EARLY LIVER	SPLEN- ECTOMY
		(mg/kg/day)	(mg/kg/day)	(Total μ gm)	(Total R)	(cu mm)	Lowest Day	(cu mm)	Lowest Day	FUNC- TION	
OT 2	22	3.51	1.45	2,200	None	1,800	16	29,000	15	Fair	Yes
OT 3	7.5	4.10	1.53	600	None	4,050	7	15,000	7	Poor	No
OT 4	6.5	3.65	0.21	None	None	12,500	2	28,000	4	Poor	No
OT 5	23	4.49	3.50	800	None	1,400	22	40,000	13	Poor	No
AT 1	22	1.34	1.78	1,200	600	2,000	19	Studies Inadequate		Good	No
AT 2	34	1.23	1.75	1,600	450	2,000	27	20,000	33	Excellent	No
Renal recips.	†	4.72	1.98	Frequently Used	Frequently Used	+		+			

*The liver patients had a much higher incidence of leukopenia and thrombocytopenia despite the fact that they were given smaller quantities of azathioprine.

†The renal homografts were obtained from living volunteers in 1963 and 1964. The average daily prednisone and azathioprine doses are given for the first three post-transplantation weeks or for how many days of that time the patients lived: 15 of the 18 recipients survived through the period of analysis and the other three died after 8, 12, and 15 days respectively. Splenectomy was performed in 17 of the 18 cases.

‡Five of the 18 renal recipients developed leukopenia (<3000) during the first three postoperative weeks but only one had severe thrombocytopenia ($<50,000$).

period and the ranges employed are summarized in Table 17 for each of the four orthotopic and the two auxiliary liver recipients who survived operation and the early postoperative period between May, 1963, and July, 1965.

Similar information is given for the use of prednisone (Table 17). The steroids were started at 0.4 to 1.5 mg/kg per day either on the day of operation or shortly thereafter. In all cases the doses were subsequently increased when liver function became worse and the cause for this was assumed to be rejection. In actuality, the ultimate explanation for the deterioration in the orthotopic cases was in large part serious ischemic injury to the transplanted organs (Chapter Six). During the post-transplantation period the lymphocyte counts of the recipients were generally lower than prior to operation (Fig. 109), probably because of the prednisone administration. Steroids are well known to cause lympholysis, lymphoid depletion, and lymphopenia^{7, 74} in experimental animals. Similarly, it has been shown in human recipients of renal homografts that the numbers of peripheral lymphocytes during the post-transplantation period are inversely related to the quantities of prednisone being given.⁶¹

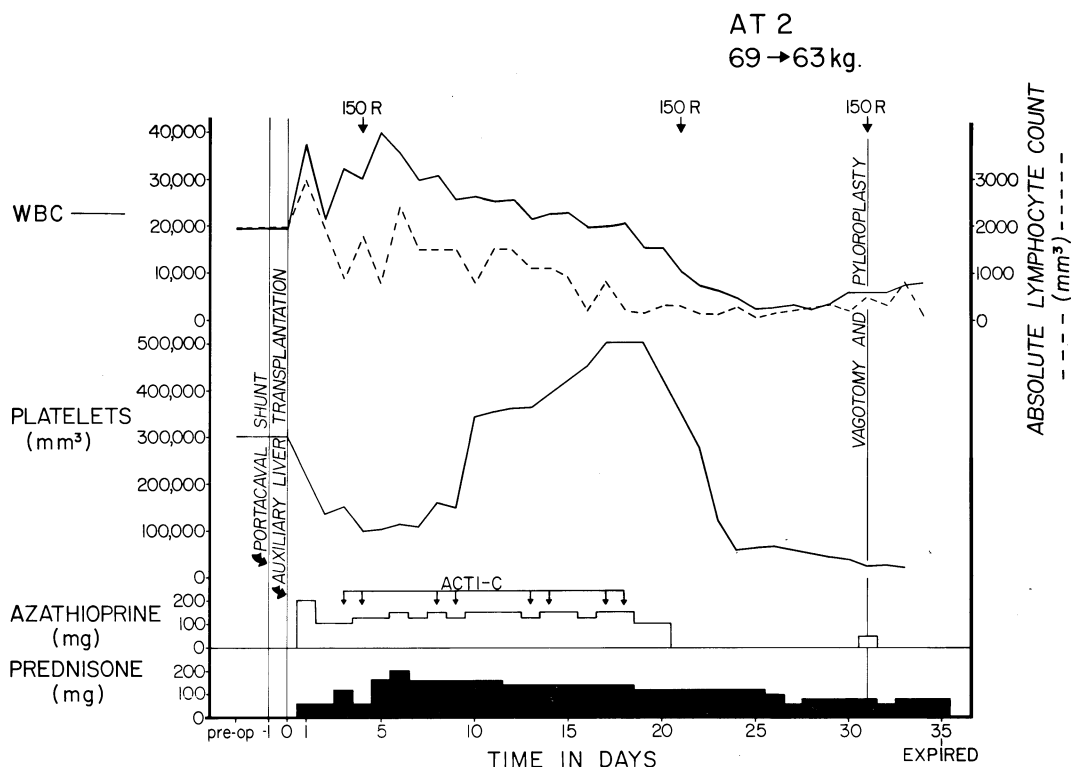


Figure 109. The double drug regimen used for one of the recipients (AT 2) of a well functioning auxiliary homograft (Fig. 252, Chapter Twenty-two). Splenectomy was not performed. Bone marrow depression and leukopenia were produced, and the patient eventually died of widespread infection from mixed microorganisms (Chapter Sixteen). The lymphocyte count postoperatively was lower than before transplantation, a change which was probably brought about by the prednisone being administered.⁶¹ Note the remittent thrombocytopenia. The first platelet falls occurred promptly after operation during a period of relative leukocytosis. The later thrombocyte declines were coincident with leukopenia. Each vertical arrow equals 200 μ g of intravenous actinomycin C. The doses of local homograft irradiation (R) were calculated at the center of the organ.

Two of the ancillary measures described in the preceding section were taken, but without demonstrable effect. These were the intravenous administration of actinomycin C and local homograft irradiation (Table 17). Thrombocytopenia (Fig. 109 and 110) developed in almost all the patients (Table 17). At the time it was suspected that the actinomycin C might have been responsible. Since then it has been established that several other factors predispose to platelet depression after liver transplantation, including the procedure itself. This important subject will be returned to later in the chapter in the discussion of triple drug therapy.

Results with Double Drug Therapy. There was a uniform inability of the patients to tolerate this kind of immunosuppressive treatment. Four of the six recipients had at least one episode of leukopenia during the brief postoperative interval of follow-up. The infectious complications which occurred at these times, or commonly even before the onset of peripheral white blood cell declines, were unmanageable (Chapter Sixteen).

In most cases the downhill course of the recipients was so rapid that the

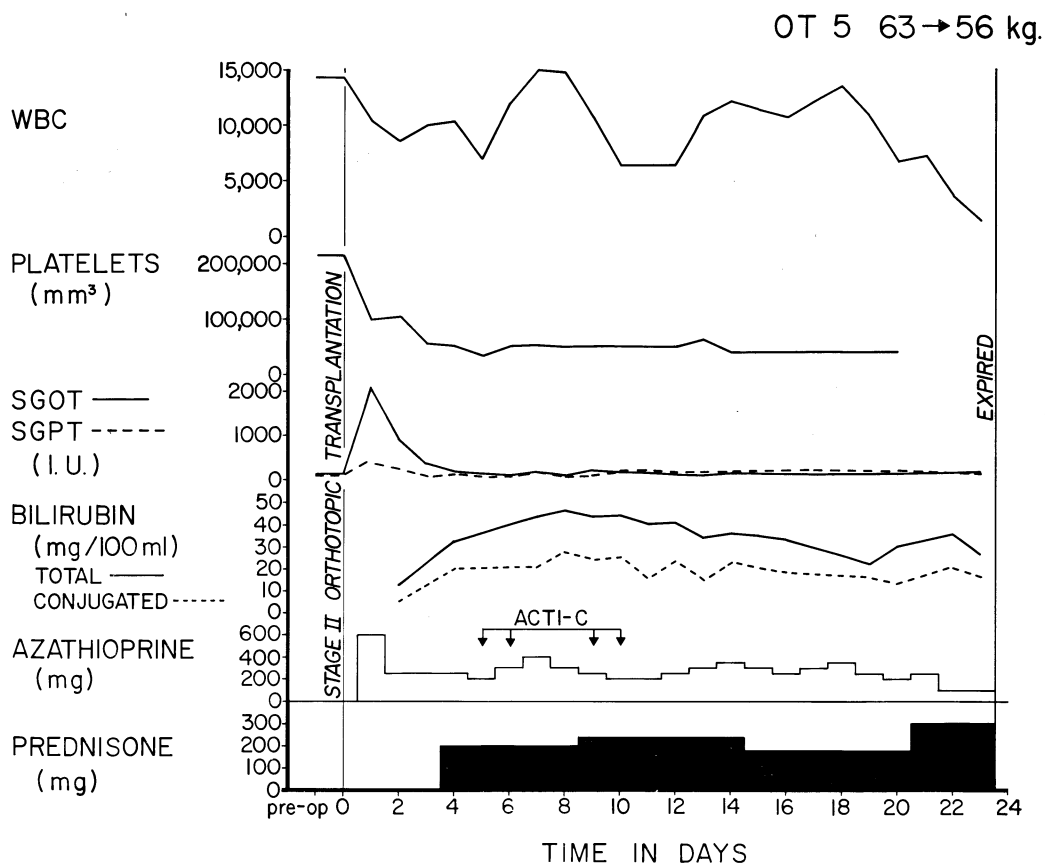


Figure 110. The effect of orthotopic liver transplantation and immunosuppression upon the peripheral white blood cell and platelet counts (OT 5). Splenectomy was not performed. Thrombocytopenia appeared almost immediately after operation at a time when there was no clear evidence of bone marrow depression. The other factors which can contribute to platelet depression in such cases are discussed in the text. Each vertical arrow equals 200 μ g of intravenous actinomycin C.

full extent of the bone marrow depression could not be completely evaluated during life. However, in both the recipients of auxiliary transplants in whom there was survival of 23 and 34 days (AT 1 and 2) the peripheral white cell counts fell to very low levels during the last five and 12 days of life, respectively.

The latter patients will be discussed separately in Chapter Twenty-two, but two observations are pertinent here. First, both received well functioning auxiliary liver homografts which then underwent severe rejection despite treatment which proved to be so toxic as to be incompatible with life. Moreover, there was never any evidence of reversal of the rejection process. Second, the doses of azathioprine which caused the lethal bone marrow depression were conservatively designed (Fig. 109) and were much smaller than those which could usually be given to kidney recipients without such uniformly fatal consequences (Table 17).

From these observations rose the strong suspicion³³ that the margin between effective and lethal immunosuppression, which at best was often slender in renal recipients being treated with double drug therapy, did not usually exist at all after clinical liver transplantation. Experience in subsequent cases to be described later has only fortified the view that azathioprine must be given to human liver recipients in incongruously small doses.

The inability of the liver recipients to support azathioprine treatment has never been satisfactorily explained. Furthermore, it was unexpected since such a striking intolerance to this drug has not been seen after canine hepatic transplantation. It is conceivable that the pre-existing liver disease in the patients was responsible by having depleted in advance the hemo- and lymphopoietic reserves. Cirrhosis, hepatitis, and a number of other liver ailments are often attended by leukopenia and thrombocytopenia. Hypersplenism may be a contributory factor in such cases, but bone marrow depression has also been well documented.^{25, 42}

Another factor which is probably variably present in every human case is injury to the transplanted liver of such an extent that the potential for azathioprine elimination is compromised to at least some degree. It was mentioned earlier that azathioprine has an important, although not exclusive, renal pathway of removal; azathioprine is excreted in the urine in unchanged form or after having been broken down to 6-mercaptopurine, thiouric acid, or other derivatives.⁸ The liver is probably an even more crucial instrument of detoxification. The studies of Elion and Hitchings and their associates^{3, 8, 9} indicated that the breakdown of azathioprine to 6-mercaptopurine should not be hepatic dependent, since a number of widely distributed organic or inorganic sulfhydryl compounds can initiate this reaction. However, it is highly likely that further steps in degradation are at least partially liver based. For example, the change from 6-mercaptopurine to 6-thiouric acid is thought to be subserved by xanthine oxidase, an enzyme which in man (but not in the dog) is principally concentrated in the microsomal systems of hepatic parenchymal cells.¹ Conceivably, species variations in the distribution of this or other enzymes could explain the differences in dose responsiveness in dogs and humans.

The practical implication of the foregoing remarks might be that any major

liver injury in humans would be analogous to that described earlier with the loss of renal function, namely unreliable dose control. This chain of events has been observed most clearly in recipients of renal homografts who have developed "hepatitis" in the post-transplantation period (see Chapter Twelve). In our experience, reductions in the quantities of azathioprine are invariably necessary in this circumstance in order to prevent the complication of bone marrow depression.

The role of steroid therapy in the deaths of the six patients was more difficult to analyze, although the unfavorable influence on the infections is undoubted. In addition, three of the patients developed gastrointestinal bleeding in the postoperative period. This complication may have partly derived from the poor liver function which was present in several of the patients, but prednisone can be responsible for the same result. The esophagogastric or intestinal ulcerations in the recipients were found at autopsy to be invaded with fungi (Chapter Sixteen).

TRIPLE DRUG THERAPY

Justification for further efforts at clinical liver transplantation came largely from the laboratory research with the globulin derivative (ALG) of heterologous antilymphocyte serum (ALS) that was described in the preceding chapter, and from the encouraging results obtained with ALG treatment after human renal transplantation. The therapeutic regimens tested clinically with the kidney model were then adapted to the problems of liver transplantation.

Background in Renal Transplantation

The Therapeutic Schedule in the First Kidney Trials. The regimen of ALG which was used in man was guided by several general conclusions that emerged from the experimentations with large animals in our laboratories and elsewhere (Chapter Twelve). These can be summarized as follows: (1) ALG had potent but imperfect immunosuppressive qualities when used alone; (2) with continued administration of the heterologous serum derivatives, there was a highly significant risk from a variety of foreign protein reactions; and (3) ALG could be used effectively and probably with increased safety in combination with other drugs. Each of these factors contributed to the initial decision to employ heterologous ALG as an adjuvant agent added to therapy with azathioprine and prednisone and to limit its use to the first four postoperative months. It was hoped that the predictability and safety with which homograft rejection could be prevented would thereby be improved and that the hazards of immunologic reactions to the serum products would be accordingly reduced with the efficient level of immunosuppression to which all three agents would contribute.

The way in which this policy decision was translated into the treatment program is shown in Figure 111. Daily intramuscular injections of ammonium sulfate-precipitated ALG (Fig. 98, Chapter Twelve) were started several days in

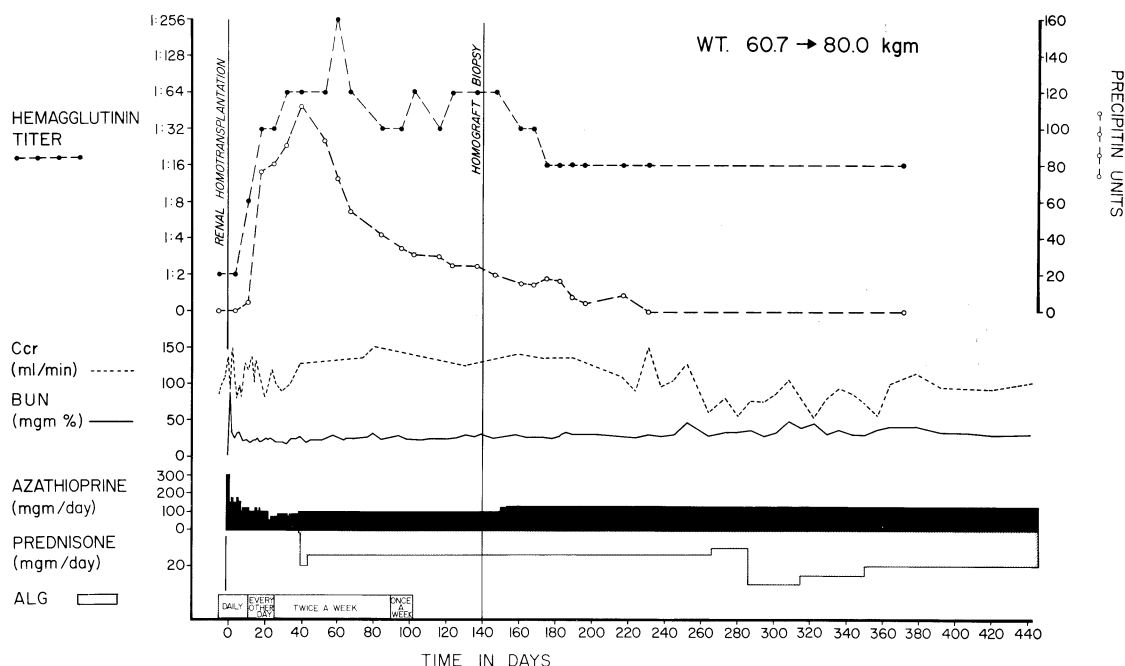


Figure 111. The course of a patient who received antilymphocyte globulin (ALG) before and for the first four months after renal homotransplantation. The donor was an older brother. The Terasaki match was grade A (see Chapter Three). There was no early rejection. Prednisone therapy was started 40 days postoperatively because of the high rises in the serologic titers which warned against a possible anaphylactic reaction. Note the insidious onset of late rejection after cessation of globulin therapy. This was treated by a moderate increase in the maintenance doses of steroids. This delayed complication occurred in only two of the first 20 recipients of intrafamilial homografts who were treated with ALG. It has been seen with about the same infrequency in subsequent cases. (By permission of Surg. Gynec. Obstet. 126:1023, 1968.)

advance of operation, continued for the first 10 to 14 days afterward, and then progressively reduced to every other day, twice a week, and once a week in the ensuing three and a half months. Azathioprine was begun on the day of operation and continued indefinitely. Prednisone therapy was either instituted immediately or alternatively withheld until the onset of rejection or until there was serologic evidence of antibody formation against the injected foreign protein (Fig. 111).

During the preoperative interval when only intramuscular ALG was being given, a number of observations were made. In these first cases the doses of the immune globulin for adults were usually 4 ml. The injectate had leucoagglutinin titers of 1:4000 to 1:16,000 and a protein content of 4.6 to 9.3 gm/100 ml. A significant lymphopenia was not produced (Fig. 112). Usually the fraction of lymphocytes in the peripheral smear was reduced, but a leukocytosis also occurred and the total peripheral lymphocyte count was relatively unchanged. Nevertheless, a striking immunosuppressive effect was detectable during the pretransplantation period.

Many of the recipients had positive skin tests to tuberculin, histoplasmin, or other allergens. These became negative when retested 48 to 72 hours after

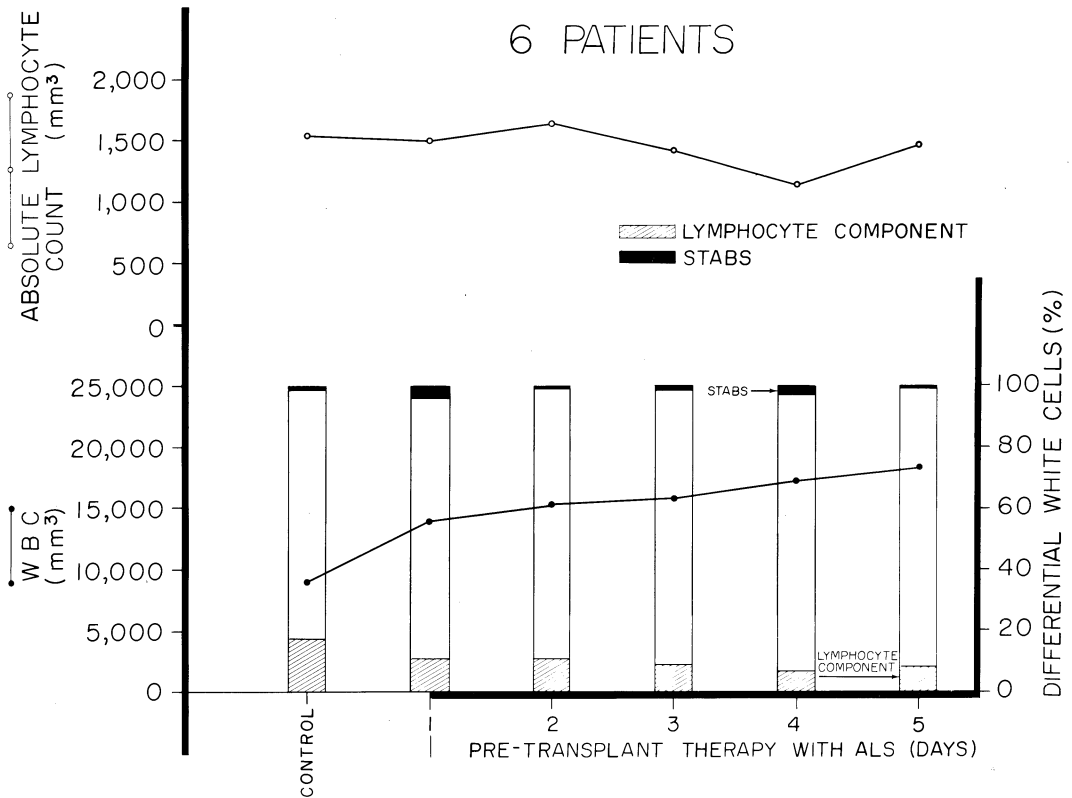


Figure 112. The effect of horse ALG in six patients treated daily for five days before renal homotransplantation. No other therapy was being given. A leukocytosis was induced. The lymphocyte fractions declined, but with the increased total white count the number of peripheral lymphocytes per cu mm was not significantly changed. Compare these results with those obtained when twice as much ALG was given in later cases (Fig. 117). "Stabs" refer to nonsegmented neutrophils. (By permission of Surg. Gynec. Obstet. 124:1, 1967.)

the institution of globulin therapy, indicating that the ALG prevented expression of a previously established delayed hypersensitivity.^{4, 65}

Similar clinical observations have been reported by others.^{5, 35, 40, 71} It should be added that in Monaco's studies³⁵ he also demonstrated prolonged survival of skin homografts in human volunteers who were treated only with ALG. During the brief periods of therapy with ALG alone changes were not detected in hematocrits or platelet counts.

The Effect on Results After Intrafamilial Renal Transplantation. The foregoing therapeutic program was used in 58 consecutive patients who received intrafamilial homografts from donors of variable histocompatibility matches from one and a half to three years ago. The outcome in part or all of these cases has been reported on several occasions^{48, 51, 52, 58, 65} but will be summarized here. The one-year survival after consanguineous renal homotransplantation was in excess of 90 per cent, the ultimate kidney function was superior to that in comparable past cases, the quantities of azathioprine and prednisone necessary to achieve these results were decreased, and lethal septic complications were virtually eliminated. Other encouraging clinical trials with ALG have been reported,

with the use of similar therapeutic protocols.^{26, 44, 66, 70, 71} When the ALG was discontinued after these intrafamilial renal transplantations, delayed rejection was uncommonly seen and, if it did occur, it was easily controlled by minor upward adjustments of prednisone dosage (Fig. 111). Most of the deaths which did occur were the consequence of technical surgical mishaps. Late retransplantation or return to chronic dialysis has become necessary in only one of the surviving patients in this series. As will be discussed later, the results after transplantation between nonrelated individuals have been far less satisfactory.

Toxicity of ALG in the First Trials of Triple Drug Therapy. A number of side effects were seen in the above experience^{20, 65} as well as in patients given comparable treatment at other institutions. There was usually pain, tenderness, erythema, and swelling at the injection sites. Fever commonly developed. Annoying systemic side effects included hives, generalized rashes, arthralgia, and periorbital edema.

The most serious toxic manifestation of ALG therapy was the development of anaphylactic reactions in 17 per cent of the patients. These were seen at the earliest three weeks after the beginning of triple drug treatment and were absolutely characteristic. Within a few minutes following an ALG injection profound anxiety developed, followed by nausea and sometimes by an urge to defecate. Many of the patients complained of low back pain and dyspnea. The usual physical findings were hypotension and cyanosis of variable severity. The blood pressure falls were usually minor, but in some the recorded values were 40 to 60 mm Hg. At this time cyanosis with distention of the neck veins was observed. A temperature spike usually followed, occasionally accompanied by chills.

In most patients no specific treatment was instituted. To some, 50 mg intravenous prednisolone and nasal oxygen were given. A few patients also received 100 mg diphenhydramine hydrochloride (Benadryl) intravenously. A liter of lactated Ringer's solution was given. Epinephrine was not required. All patients recovered in five to 90 minutes. In no instance were there any demonstrable aftereffects. Usually ALG therapy was stopped with the first evidence of an anaphylactic reaction, but several patients were given a number of additional injections.²⁰ Ultimately this practice was abandoned because the incidence of similar further difficulties was too high.

These patients and all those subsequently treated with ALG were followed with a battery of immunologic examinations, including serial skin tests to horse protein and the measurement of precipitins and heterohemagglutinins in the serum. Precipitating antibodies regularly developed against the horse globulins. Hemagglutinating antibodies against sheep red blood cells presumably represented a response to the Forssman antigen which is present in horse plasma protein.

All three immunologic monitors were of value in predicting the probability of an anaphylactic reaction, but the discrimination was imperfect (Fig. 113). Most of the anaphylactic reactions were in patients who had high grade sensitization as judged on the basis of skin testing or serologic examination, but there

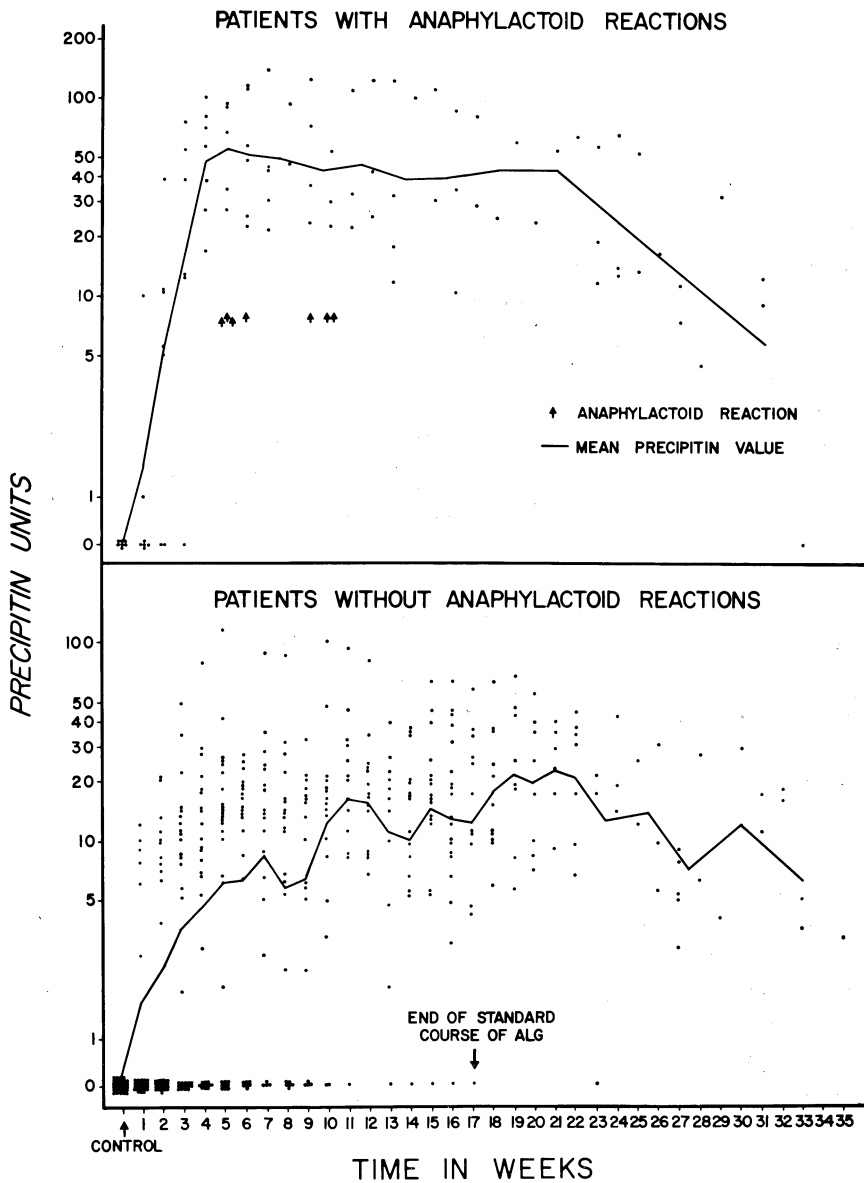


Figure 113. The development of precipitins in 40 patients receiving horse ALG. The titers of precipitating antibodies were determined by the electroimmunodiffusion technique of Merrill, Hartley, and Claman.³³ The same general information provided by this special method can be less quantitatively obtained with a simple twofold dilution measurement. Most of the anaphylactic reactions were in the patients who developed high titers, but the correlation was imperfect. The results were similar using hemagglutinin titers against sheep red blood cells as an indicator of sensitization to the horse protein. (By permission of Ann. Intern. Med. 68:275, 1968.)

were some in patients who had low antibody titers or reassuring intradermal tests.

In Kashiwagi's toxicity study²⁰ an effort was made with special techniques to determine the relative immunogenicity of the constituents of the crude globulin then being used clinically. He found that most of the precipitating antibodies which developed in these patients were directed against the horse beta and alpha globulins which the ALG contained. In only one instance could precipitins be found against the gamma G globulin. Tsirimbas et al⁷² of Munich reported similar findings after giving intravenous ALG to six patients with leukemia. These observations supported the hope that a safer ALG could be made by more complete refinement of the gamma G globulin in which the desired antibodies were thought to chiefly reside (see Chapter Twelve). As will be mentioned later, such an effort was eventually made with extremely unsatisfactory consequences.

A number of other side effects of ALG were looked for. A few of the patients had thrombocytopenia ($<50,000$) at some time in the postoperative course when leukopenia was not present. Bleeding did not occur. There were no examples of neurologic complications, myositis, abdominal pain, or pericardial and pleural effusions.

In Chapter Twelve the potential dangers of Masugi (direct nephrotoxicity) and serum sickness nephritis were discussed. Fortunately this kind of complication has not been a serious problem in the clinical cases. The first eight patients treated with ALG had biopsies of their renal homografts after completion of the course of globulin therapy. Equine protein could not be found in the renal tissues of any patient.⁶⁵ About 40 other kidneys have since been similarly studied in our series; in only one was horse globulin detectable within the renal tissue with standard immunofluorescence techniques. Recently Traeger⁷⁰ and Konomi²⁶ and their associates also reported the absence of horse protein in biopsy specimens of human kidney homografts after prolonged ALG therapy.

An ALGG Trial. Because of Kashiwagi's findings that the serologic response to the injected ALG was largely directed against its alpha and beta constituents, a technique was developed for the extraction of pure gamma G globulin, hereafter called ALGG, in bulk quantities.^{21, 22} The resulting product (Fig. 99, Chapter Twelve) was given to the next 13 recipients of intrafamilial renal homografts. The horse ALGG batches used had leukoagglutinin titers of 1:2000 to 1:8000 and protein concentrations of 1.9 to 3.5 gm per cent. The lymphocytotoxicity titers, which had usually been about 1:4000 in the original ammonium sulfate-precipitated ALG, were now also lowered. The intramuscular doses were 4 to 8 ml in adults, depending on the leukoagglutinin titers.

Measurable precipitating antibodies against the ALGG developed in only one of the 13 patients.⁴⁸ The hemagglutinin response against sheep red blood cells was about the same as in the original series. Although minor toxic manifestations were seen with about the same frequency as with the previously used ALG, there were no anaphylactic reactions. Unfortunately it soon became apparent that the immunosuppressive effect of the product had largely been lost.^{22, 48}

All 13 of the patients had a satisfactory post-transplantation diuresis. However, seven developed severe rejection crises from one half to 14 days later,

requiring prednisone dose increases to as much as 400 mg per day. The rejection was so severe that five of the recipients had secondary elevations of the BUN to more than 150 mg per cent, and in four, return to the artificial kidney became necessary for one to five weeks before adequate urine excretion resumed. The incidence of these complications was about 10 times as great as in the patients treated with ammonium sulfate-precipitated ALG. Three of the 13 recipients died before the end of the first postoperative year. The clinical difficulties in these cases¹⁸ were entirely similar to those during the time when the azathioprine-prednisone double drug combination was being used without adjuvant globulin.

The Penalty of Overrefinement. As the consequence of this experience it was necessary for us to return for the time being to the clinical use of the crude globulin obtained with ammonium sulfate precipitation. A possible explanation of the apparent loss of potency of the ALGG was discussed in Chapter Twelve. First, it was pointed out that the antilymphocyte antibodies in horse ALS are more widely distributed than in the immune sera of certain other animals. Significant activity is not limited to the IgG; it can also be found in the T-equine fraction, just as with the preparation of tetanus or other antitoxins.¹⁸

The implication of discarding the latter portion of the raw globulin, as was done in the preparation of the ALGG, can be appreciated by the analysis performed by Kashiwagi et al^{23, 48} of the serum of horses that had been immunized with splenic lymphocytes for six to nine weeks (Fig. 114). The three horses were considered to be early serum donors since essentially no leukoagglutinins or lymphocytotoxins were in the IgM, as has been described by Fateh-Moghadam at a later time.¹⁰ It was estimated by Kashiwagi that more than three-fourths of

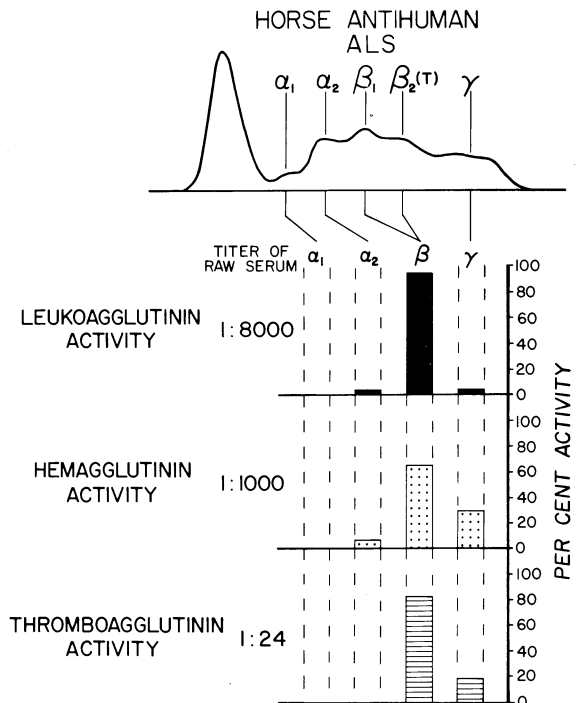


Figure 114. The location of leukoagglutinins, hemagglutinins, and thromboagglutinins in a horse that had been immunized with human splenic lymphocytes for a little more than two months. Note that the T-equine globulin contained the preponderance of all three kinds of antibodies. The cytotoxins (not shown) were mainly in the IgG.²³

the leukoagglutinins in the sera of his horses were in the T-equine fraction; the lymphocytotoxins were predominantly in the IgG. Similar findings have been reported by Carraz et al of Lyon.⁵ Moreover, Kashiwagi showed in companion canine experiments involving differential separation that the discarded T-equine globulin had a lymphopenic and immunosuppressive effect at least as great as that caused by the highly refined and cytotoxin-rich gamma G globulin from which it had been separated.²³

The more recent studies of Kashiwagi et al^{23, 48} alluded to previously have not seriously weakened the previously held proposition that most of the active portion of equine ALS is gamma G (7S) globulin. Rather, such investigations have only emphasized how difficult it may be in the immunized horse to retrieve all the desired globulin in unadulterated form. Because of its sluggish electrophoretic mobility, the part referred to as "slow" gamma G globulin remains sufficiently isolated to permit its easy removal. In contrast, the antibody-rich "fast" gamma G globulin, with its slightly greater electrophoretic mobility, remains buried in the heterogenous T-equine globulin which, in turn, also contains variable quantities of irrelevant beta and alpha globulins. Separation of the "fast" gamma from the other components of the T-equine globulin is the practical problem that has not yet been satisfactorily solved for mass production. Better techniques are needed for this purpose.

Alternatively, it may eventually prove more expedient to use other animals than the horse as an ALS source. For example, both the rabbit (Fig. 115) and the goat have less overlap of the different immunoglobulins, thereby greatly simplifying the methodology for removal of pure gamma G globulin. In addition, the

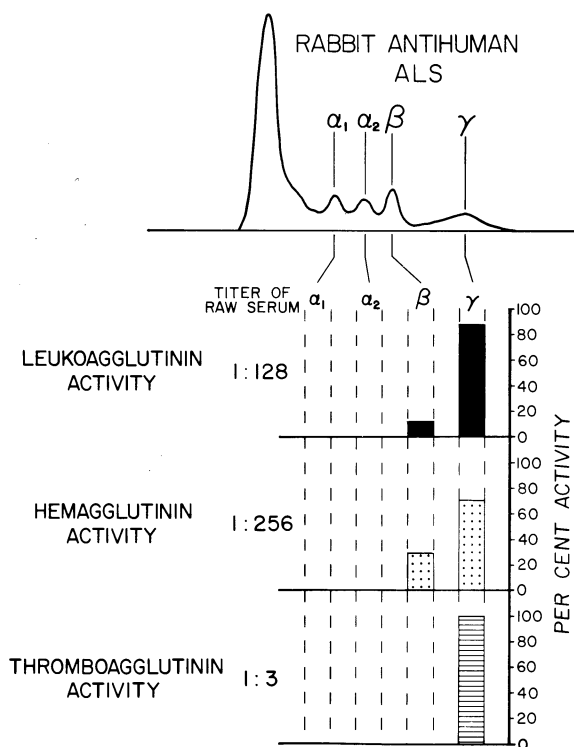


Figure 115. The location of leukoagglutinins, hemagglutinins, and thromboagglutinins in the serum of a rabbit that had been immunized with human splenic lymphocytes for two months. The antibody concentration is almost entirely in the gamma globulin. Moreover, a heterogenous area comparable to the T-equine fraction of the horse (Fig. 114) is not present. The lymphocytotoxic antibodies (not shown) were also in the IgG.

problem of refinement should be made easier by the fact that there is probably less tendency for the location of the active antibodies to change during a course of immunization.

Cadaveric Renal Transplantation. In discussing double drug therapy in the first part of this chapter, a distinction was made between the long-term prognosis in recipients of consanguineous as opposed to nonrelated homografts. Before the advent of ALG use, there was an unacceptably high mortality in both kinds of cases during the first few postoperative months after renal transplantation. However, the recipients of homografts donated by family members who survived this critical period in good condition had a good chance of living for years with stable homograft function. The same was not true when kidneys from nonrelatives were used. Not only was the early death rate greater but, even in those who survived, delayed homograft failure proved to be the rule, not the exception.

The addition of a four-month course of ALG to the immunosuppressive regimen reduced the early mortality in our hands after both consanguineous and unrelated renal transplantation. It did not, however, prevent the delayed homograft deterioration so often previously seen in the latter kind of case during the era of double drug therapy. Our own experience with cadaveric renal transplantation, while not extensive, has provided incisive information on this point.⁴⁸ It was found that rejection of the cadaveric kidney could be easily controlled during the period of triple drug therapy. However, after the discontinuance of ALG, progressive deterioration of renal function was soon detectable in virtually all recipients of histoincompatible cadaveric kidneys, and even in some cases in which good compatibility was thought by Terasaki to have existed. A typical example is shown in Figure 116. In this case, there was a donor-recipient mismatch in the HLA group 5 as well as in another group termed Te 3 which has not yet been universally accepted as an HLA antigen. After an initial acute tubular necrosis the transplanted kidney functioned stably for several months. Then, within a few weeks after cessation of globulin therapy, the creatinine clearance slowly began to decline and the blood urea nitrogen to rise. The organ has supported life for almost two years but it is highly unlikely that it will do so for much longer.

The foregoing has not been an isolated observation. We have followed 13 cadaveric kidney recipients treated with the triple drug regimen after their operations from one to almost three years ago. In one patient there was no opportunity to evaluate the immunosuppression since the homograft was lost on the operating table by hyperacute rejection (see Chapter Fourteen). Of the remaining 12, 10 (83.3 per cent) lived for at least one year, with continuous function of the homograft; the other two recipients had good renal function before they died of pulmonary embolization three months postoperatively.

Although the one-year patient and kidney survival was not discouraging, the term "successful" could be applied to the series only with qualifications. In eight of the 10 cases in which one-year survival was obtained, ALG was stopped after four months or less. Deterioration of function followed after variable intervals in five patients, and in two of these the homografts eventually failed altogether and had to be removed during the second postoperative year. It is

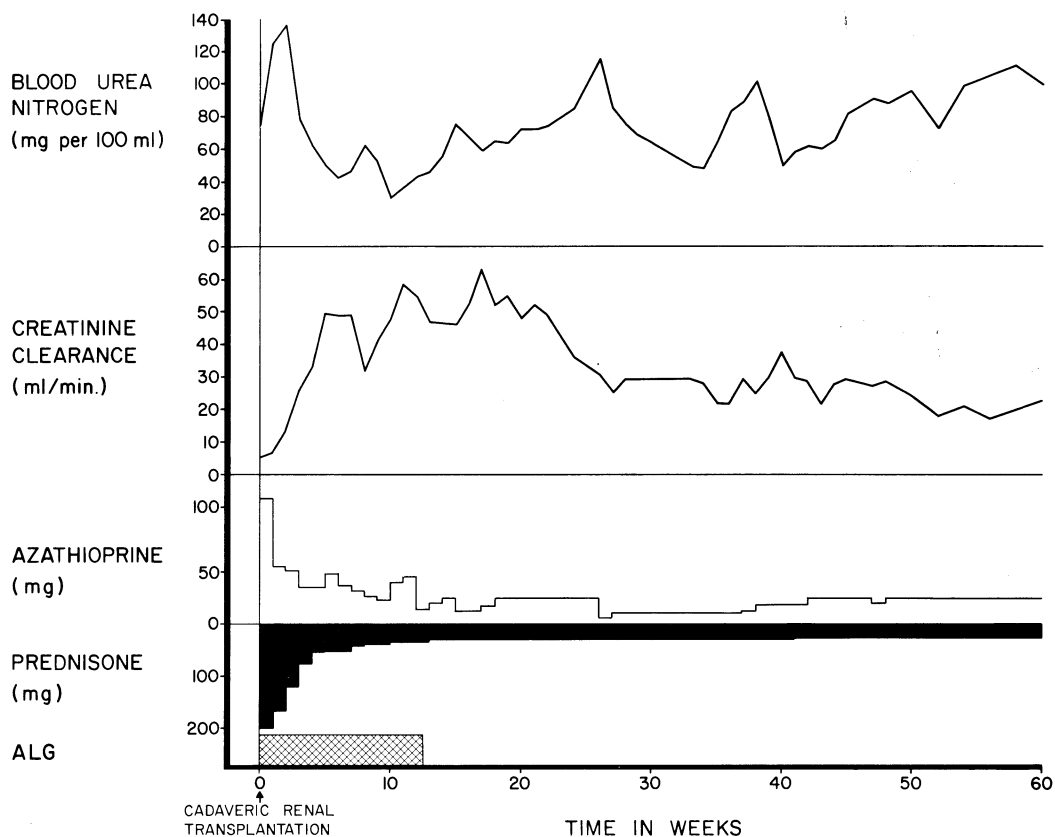


Figure 116. The course of a patient who received a kidney from a histoincompatible (HLA 5; Te 3) cadaveric donor. He was given triple drug therapy. After an initial period of poor urine excretion due to acute tubular necrosis, there was excellent renal function. However, within a few weeks after completion of the four-month ALG course, the homograft began to deteriorate slowly. The patient is now 22 months post-transplantation. His creatinine clearance is 20 ml/min and his BUN is 70 mg per cent.

worth mentioning that the same adverse chain of events was not seen in either of the two patients in whom ALG was not stopped after four months but continued for another four to six months.

It will become apparent in the succeeding chapters that the kind of "escape" from immunosuppressive control just described, after stopping ALG, was commonly seen in recipients of orthotopic liver homografts. Thus, the way in which ALG was initially used was eventually judged to be unsatisfactory and was systematically altered in later cases.

Alternative ALG Regimens. The previous observations^{48, 52} as well as similar ones reported by Traeger et al⁷⁰ of Lyon, France, have underscored the potential inadvisability, especially in cadaveric cases, of arbitrarily stopping ALG therapy after any preordained interval. The decision to continue is not difficult in the kind of patient who continues to tolerate the globulin injections without complaint and who does not develop the signs of dangerous foreign protein sensitization mentioned earlier. In the majority of patients, however, horse ALG cannot be given indefinitely and one of several alternate possibilities must be

selected after variable periods. The first is to stop globulin therapy and place reliance on the efficacy of azathioprine and prednisone; the frequently undesirable consequences of this approach were described in the preceding section. Second, an attempt at desensitization to the horse protein can be made, as will be described later. Finally, it may be possible to switch to ALG prepared from the sera of a different heterologous species. Rabbits, goats, and cows have been immunized in our laboratory against human lymphoid tissue in order to permit a broader choice of second line immune globulins. In our experience it has been safe to change from one kind of ALG to another.^{48, 64}

The foregoing modifications of ALG administration have in common the objective of extending the permissible duration of globulin treatment. A different approach that is currently under investigation is to use larger doses of ALG with the other two agents of the triple drug regimen in an attempt to induce an early and long lasting tolerance of the kind often achieved with ALG alone in rodents^{32, 34} or, less frequently, in dogs.⁵⁸ The mechanism by which such an effect might be achieved with any good immunosuppressive agent is considered separately in Chapter Twelve. The following remarks will be concerned only with a description of a clinical trial begun in June 1968 and continued until the present.

In these patients an effort was made with a "blitzkrieg" approach to therapy to achieve lymphoid depletion before and immediately after arrival of the antigen. High-titer ammonium sulfate-precipitated ALG was started in elective cases three to five days before operation, using twice the previous doses. The individual injections in adults were usually 8 ml of horse globulin which had a protein concentration of 4 to 6 gm per cent and a leucoagglutinin titer of 1:4000 to 1:16,000. In children the quantities given were individualized according to size. After two to four days, about 3 mg/kg of prednisone per day was started. The first dose of azathioprine was given on the day before transplantation. For cadaveric cases, the regimen had to be modified by starting all three drugs on the day of operation. Except for the increased quantities, the subsequent ALG schedule was about the same as in the earlier trials.

The double doses of ALG were generally well tolerated, inasmuch as the complaints of pain at the injection sites were not increased, nor was the incidence of other clinical side reactions greater than in the earlier cases. However, the resulting hematologic response was substantially different from that previously observed.

Whereas the 4 ml doses had not caused significant acute lymphopenia, the 8 ml injections did so without fail. Both the lymphocyte fractions and the total lymphocyte counts were abruptly decreased within one day after the first intramuscular injection (Fig. 117); leukocytosis was almost always observed. In the usual patient the lymphopenia was further accentuated when steroids were added. Concomitant falls in the hematocrit did not occur.

In these patients lymphopenia was usually maintained for several weeks as an isolated finding. Then a secondary depression of the platelet count commonly developed. From this point onward it was necessary either to give the ALG in smaller quantities, to reduce the frequency of administration (Fig. 117), or to make both adjustments. Several of the patients developed nosebleeds or

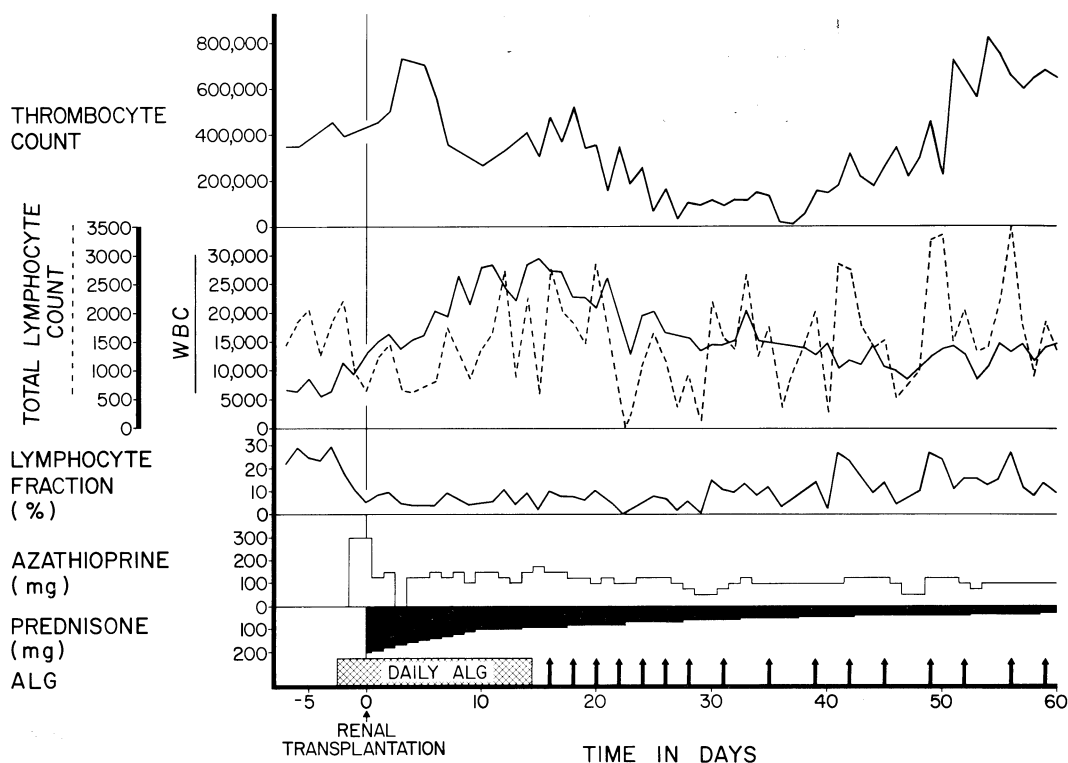


Figure 117. Hematologic changes observed in a recipient of a renal homograft who was treated with triple drug therapy including double doses of horse ALG. Splenectomy and bilateral nephrectomy were performed on the same day as the transplantation. Lymphopenia was immediately produced by the first injection of ALG and was moderately well maintained until the globulin injections were reduced in frequency. Note the development within three weeks of thrombocytopenia. The latter complication has been common in patients given increased quantities of the heterologous ALG (see text for discussion).

hematuria. The complication of thrombocytopenia proved to be only a nuisance, since it could be quickly corrected by fresh platelet transfusions. It is suspected, however, that if such a blood bank service had not been available, the consequences could have been serious.

When the frequency of ALG injections was reduced, sustained lymphopenia could often no longer be maintained, especially in those patients in whom the development of thrombocytopenia made it necessary to give the globulin on an irregular basis (Fig. 117). The lymphocytes returned to the peripheral smear in numbers that sometimes exceeded those present before operation. Thereafter, sporadic lymphopenia seemed to be produced shortly after individual injections, but frequently there were concomitant and parallel falls in the platelet counts.

The ALG used for these patients had been raised with splenic lymphocytes and absorbed with human platelets. After absorption it contained either undetectable or very low titers of antiplatelet antibodies, as measured by a standard thromboagglutinin method. It is probable that part of the delayed thrombocytopenic effect was due to equine antibodies raised against antigenic deter-

minants which are shared by both platelets and lymphocytes. This explanation was suggested by Pichlmayr,⁴⁰ who noted a reduction in the leukoagglutinin titers of his horse ALG after absorption with platelets from the species against which immunization was conducted. Kashiwagi¹⁸ was unable to confirm these findings but he provided support for the concept of cross reactivity by showing that leukoagglutinins and thromboagglutinins in raw horse antidog ALS were reduced in parallel by absorption with washed canine lymphocytes (Fig. 118). Whatever the explanation for the thrombocytopenia, its appearance has imposed a very practical limitation on the doses of ALG that can be given to humans. Ono's studies from our laboratory have suggested that the magnitude of the problem should be reduced by special pains to remove contaminating platelets from the immunizing cell suspensions and by more complete subsequent absorption with thrombocytes.³⁹

The usual relationship of the other immunosuppressive measures to ALG therapy in this series of kidney transplantations is shown in Figure 117. The steroid doses in adults were ordinarily reduced steadily at the rate of 10 mg/day, so that within two weeks the daily quantities were 40 to 60 mg/day. In the event of rejection during this interval, attenuation of the steroid doses was stopped or slowed. Later reductions were determined on an individualized basis. Thus, someone with a smooth postoperative course would be apt to have reached a maintenance prednisone level of 15 to 30 mg per day within three weeks. On the other hand, difficulties with rejection prolonged the time before

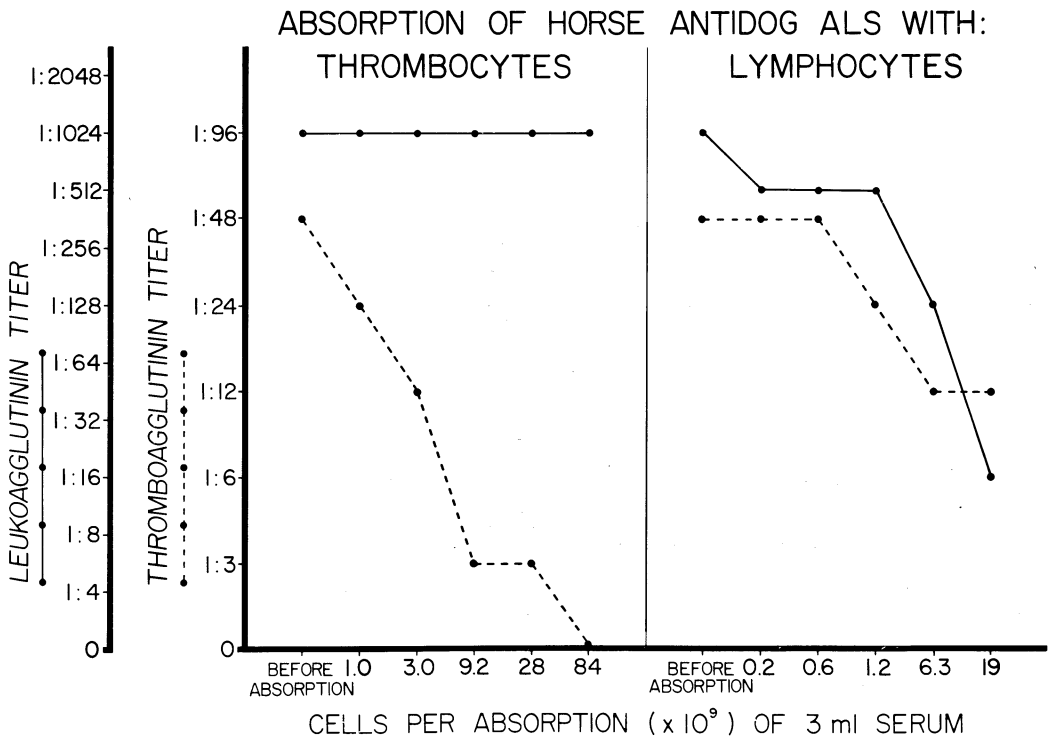


Figure 118. Absorption studies of raw antidog ALS. Left. Absorption with platelets sharply reduced the thromboagglutinin titer without affecting the leukoagglutinins. Right. In contrast, absorption with lymphocytes caused declines in both kinds of antibodies.

this level was reached. Azathioprine was administered in small enough quantities to avoid bone marrow depression.

There have been more than 40 renal transplantations in the high dose ALG series, including six cadaveric cases. All but two of the recipients are still alive, with function of their originally transplanted kidneys from three months to more than one year after operation. The two deaths were caused by pulmonary emboli 52 and 51 days after transplantation. It is, of course, too early to speculate about the induction of homograft tolerance in these patients and, conceivably, such a determination may never be possible with certainty under the conditions of such a trial. All that can be said at present is that the therapeutic regimen employed was exceptionally effective and relatively safe.

Triple Drug Therapy in Liver Transplantation

As was made clear earlier in this chapter, there was little evidence from the early experience with human liver transplantation that long survival could ever consistently be achieved with the double drug therapy then being used. Despite the administration of the azathioprine in quantities that almost always resulted in complex and fatal infections or other lethal side effects, rejection was apparently not well controlled. The most discouraging notations were in those patients in whom the presence of satisfactory initial hepatic function allowed the clinical diagnosis of homograft repudiation to be made with certainty and in whom the failure to control this process was equally well demonstrated (Chapter Twenty-two). The addition of heterologous ALG to therapy with azathioprine and prednisone changed this picture to the extent that a number of patients became available for the kinds of studies and more chronic observations that form the basis for several of the next chapters.

The way in which azathioprine, prednisone, and heterologous ALG were used together was not precisely the same in any two cases since the doses and timing of the agents were constantly adjusted. The variability of treatment can best be appreciated by comparing the graphic records of the many individual patients whose courses are illustrated in the balance of the book. Nevertheless, the general guidelines of therapy were well defined.

The First Triple Drug Schedule. Twelve consecutive orthotopic liver recipients (OT 6 to 17) were treated with the basic triple drug regimen used in the first ALG kidney series.^{47, 49, 50, 64} Azathioprine, prednisone, and horse ALG were all started on the day of operation. The doses of the same ammonium sulfate-precipitated horse globulin described earlier in this chapter were 4 ml in the adults. The children, whose ages averaged about 18 months,* received 1 or 2 ml per injection. Lymphopenia was not consistently produced (Fig. 119). In the patients who had extended survival, ALG was eventually stopped in all but one (OT 15) either because the end of a four-month course had been reached or because local or systemic toxic reactions had occurred before this time.

The administered quantities of prednisone differed from patient to patient

*For exact ages and weights of all patients see Chapter Twenty-four.

OT 13

11 → 13.5 kg.

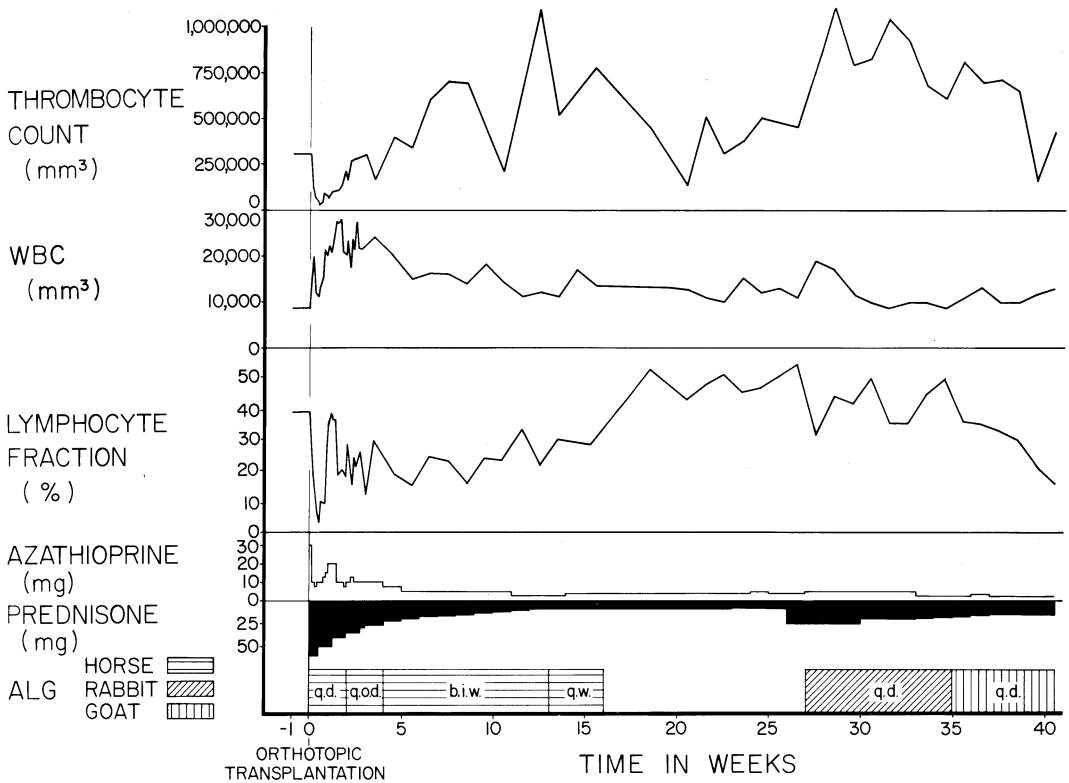


Figure 119. Peripheral white cell and platelet changes in an orthotopic liver recipient (OT 13) who received heterologous ALG prepared from sera from three different species. The indication for transplantation was extrahepatic biliary atresia; splenectomy was also performed. Horse globulin was begun in 1 ml doses on the day of operation and continued for four months, with a gradual reduction in the frequency of the injections. When jaundice recurred about two months after the horse ALG had been stopped, the prednisone dosage was increased and a course of immune rabbit globulin (4 ml daily) was instituted. Eight weeks later a reaction to the second globulin prompted the transition to goat ALG, also in 4 ml doses. The relative lymphopenia present during therapy with horse ALG was succeeded by lymphocytosis when this agent was discontinued. The lymphocyte increases were not affected by the rabbit ALG but appeared to decline when goat ALG was started. Note the acute thrombocytopenia immediately after operation and compare to the same finding (Fig. 83, Chapter 11) in a pig that received no treatment at all.

and are summarized for the first three postoperative weeks in Table 18. These were somewhat higher than had been used in the earlier experience with hepatic transplantation using double drug therapy. The most important change from the policy followed with the previous liver patients was a drastic reduction in azathioprine. (Compare Table 18 with Table 17). With this adjustment, the dread complication of leukopenia was avoided. The peripheral white blood counts of none of the 12 recipients dropped to below 3000 cu mm in the first three postoperative weeks.

In Table 18 the therapeutic schedules for the first 21 postoperative days in

Table 18. Triple Drug Immunosuppression Used During the First Three Postoperative Weeks for All Liver Recipients Treated Since the Autumn of 1966, Excluding Only Three Patients (OT 18, 20, 21) Who Died Just After Operation of Technical Complications (Chapters Eight and Nine)*

NO.	SURVIVAL (Days)	AVERAGE AZATHIO- PRINE (mg/kg/day)	AVERAGE PREDNISONE (mg/kg/day)	ACTINO- MYCIN C (Total μ gm)	HOMOGRAFT IRRADIA- TION (Total R)	WHITE BLOOD COUNT (cu mm) Lowest Day		PLATELET COUNT (cu mm) Lowest Day		EARLY LIVER FUNC- TION	SPLEN- ECTOMY
Standard ALG doses											
OT 6	7	1.06	1.7	None	None	4,900	1	15,000	2	Poor	Yes
OT 7	10	2.1	4.2	None	None	13,000	2	37,000	6	Poor	Yes
OT 8	400	1.2	1.6	None	None†	7,500	20	78,000	6	Excellent	Yes
OT 9	133	2.0	2.4	None†	None†	10,000	1	7,700	13	Good	Yes
OT 10	186	1.7	1.2	None	None†	22,000	5	12,000	15	Excellent	Yes
OT 11	61	1.1	2.6	120†	450	15,700	6	32,000	15	Fair	Yes
OT 12	105	1.3	5.7	120	300	15,800	6	19,000	19	Fair	Yes
OT 13	Alive	1.2	4.3	None	None	12,900	2	25,000	4	Good	Yes
OT 14	Alive	0.7	3.0	None	None†	4,900	5	42,000	5	Good	No
OT 15	339	1.0	2.4	None	None	7,600	21	158,000	2	Fair	Yes
OT 16A B	Regraft	0.7	4.9	None	None†	7,300	3	14,000	4	Good	Yes
	Alive	0.2	3.5	None	None	3,200	22	49,000	5	Excellent	
OT 17	35	1.3	2.8	None	None	9,600	5	28,000	12	Fair	Yes
Mean		1.2	3.1								
High dose ALG											
OT 19	Alive	1.0	4.0	None	None	7,700	7	14,500	14	Excellent	Yes
OT 22	10	0.6	3.0	None	None	7,800	5	24,000	3	Poor	No
OT 23	143	2.3	1.9	None	None	11,900	8	224,000	5	Good	Yes
OT 24	11	0.5	3.9	None	None	5,000	11	25,000	7	Poor	No
Cadaver kidney recips.	+ +	1.6	2.5	Rarely Used	Commonly Used	§		§			

*The immunosuppression is contrasted with that employed in a parallel series of 12 consecutive cadaveric renal transplantations compiled from 1966 to 1968. The survival notes are to April 1, 1969.

†Received this kind of therapy at some time after three weeks.

‡Two of the patients died after three months of pulmonary embolization. The other 10 lived for at least one year with original homograft function. Concomitant splenectomy was performed in only three of the cases. Results are means.

§None of the kidney recipients developed leukopenia (<3000) during the first three postoperative weeks. During the same time five of the 12 exhibited thrombocytopenia ($<50,000$).

the liver recipients are also compared to those used in 12 consecutive patients who had cadaveric kidney transplantation while under the same kind of triple drug therapy and during the same general period. The renal recipients were able to take considerably larger average quantities of azathioprine without a single incident of leukopenia.

During the period of analysis, and afterward for as long as any of our liver recipients have lived, efforts to increase the azathioprine doses have proved to be dangerous. Even among the longest survivors, the largest quantities consistently tolerated have ranged from as little as 0.15 mg (OT 14) to a maximum (OT 15) of about 1 mg/kg per day. In most of the latter patients, ALG was eventually stopped. In the ensuing months, efforts to increase the quantities of azathioprine usually resulted in leukopenia, necessitating its temporary discontinuance. The fact that the permissible azathioprine doses were about the same whether or not ALG was also being given suggested that the globulin had not contributed to the azathioprine intolerance.

The complication of postoperative thrombocytopenia was alluded to earlier in describing our initial unsuccessful experience with human liver transplantation. Severe platelet depression was even more troublesome and persistent when heterologous globulin was added for the later hepatic recipients. In 11 of the 12 liver cases listed in Table 18 thrombocytopenia of less than 50,000 cu mm appeared within the first three weeks, invariably in the absence of other evidence of bone marrow depression. The ALG clearly contributed to the severity of the problem by the mechanism described in the preceding section on kidney transplantation with triple drug therapy. However, additional factors were also undoubtedly significant in the hepatic cases since thrombocytopenia can develop after liver transplantation to untreated animals (Fig. 83, Chapter 11) or in human liver recipients not treated with ALG (Table 17).

Hutchison et al¹⁹ have formally evaluated the problem in dogs after orthotopic hepatic transplantation. Both in untreated animals and in those administered ALG (Fig. 120), he found significant platelet depressions which often began immediately after revascularization of the hepatic homografts and continued for several days thereafter. There appeared to be pronounced platelet clearing by the new livers, a conclusion supported by the histologic demonstration of thrombocyte sequestration in the spaces of Disse. Collateral data showing a shortened platelet half-life and abrupt reductions of fibrinogen prompted the authors to speculate on the possibility of intravascular coagulation within the transplanted organ. The studies of Boehmig and his associates² have strengthened this suspicion and, in addition, have confirmed the fact that the declines in platelets and other clotting factors may actually begin during host hepatectomy. (See more complete discussion in Chapter Ten.)

It is of interest that similar but somewhat less severe platelet changes were also detected by Hutchison in animals subjected to simulated autotransplantation.¹⁹ Thus, the risk of acute thrombocytopenia after liver transplantation is probably due at least in part to a nonimmunologic etiology such as ischemic injury to the transplant rather than to any specific interaction between the liver and the platelets of the host. The practical problem is apparent. Because thrombocytopenia can be regularly caused by the procedure of liver transplanta-

ORTHOTOPIC LIVER TRANSPLANTS WITH ALG

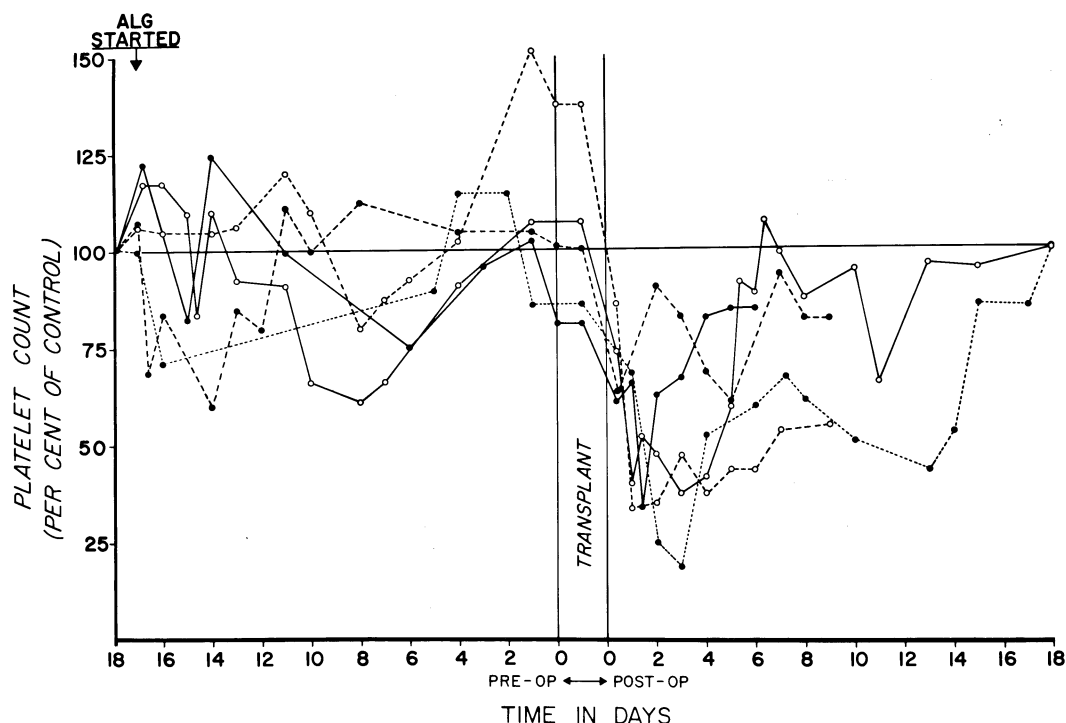


Figure 120. The effect of orthotopic liver transplantation on the platelet counts of five dogs. The animals were treated with doses of horse ALG that did not cause thrombocytopenia. With the transplantation there were sharp declines in the peripheral thrombocytes and these persisted for several days afterward. The same kind of platelet depression was noted in the absence of all therapy or even after simulated autotransplantation. (By permission of Arch. Surg. 97:27, 1968.)

tion as well as by the ALG or, presumably, even by the azathioprine given in the immunosuppressive regimen, platelet counts must be considered a standard and frequently obtained laboratory determination in this kind of case, and the results used for the planning of the drug therapy.

Variations in ALG Use. Earlier, an attempt at "blitzkrieg" therapy was described in a series of renal transplant recipients. ALG was given in double the doses used in the first triple drug therapy trials. The justification for the change was the high incidence in nonrelated cases of delayed rejection after ALG was stopped. As will be made clear in succeeding chapters, the same thing had also been very common after hepatic transplantation. It was hoped to avoid this complication after transplantation of both organs by intensifying the initial immunosuppression and thereby inducing a longer lasting state of tolerance. The trials in kidney transplantation have already been discussed.

Efforts to apply this principle after liver transplantation were not very successful when 8 ml of ALG per dose were administered to adults and 2 to 4 ml were given to children (Table 18). At these levels, relative lymphopenia was promptly produced (Fig. 121). However, downward revisions of the quantities and frequency of the injections were soon necessitated by the appearance of

OT 19 14.3 → 14.8 kg.

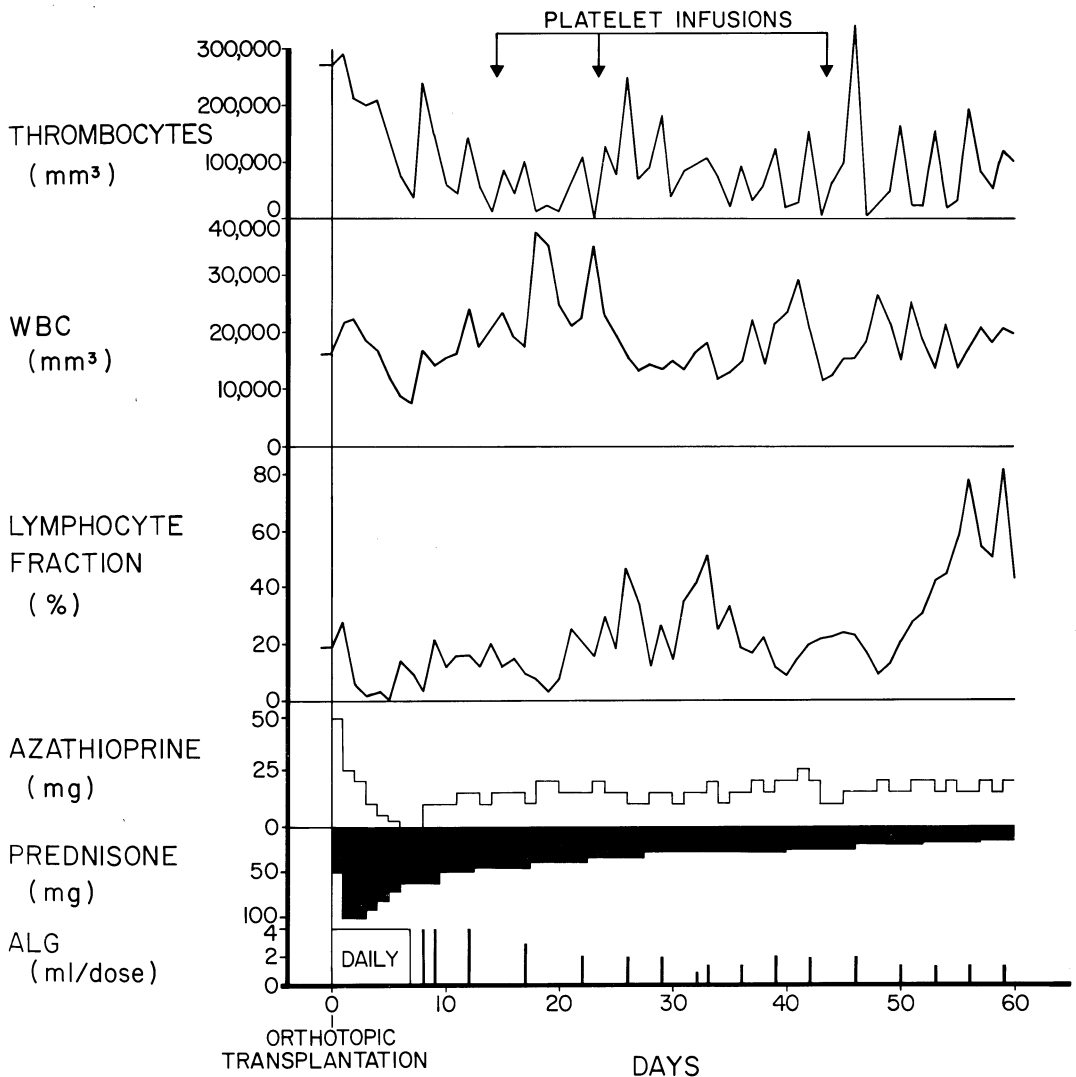


Figure 121. Hematologic changes in an orthotopic liver recipient (OT 19) who was given triple drug therapy. The donor-recipient histocompatibility was poor, since there were mismatches in the HLA antigens 2, 3 and 7. High dose ALG treatment was attempted (see text). Initially, striking lymphopenia was obtained; however, thrombocytopenia was concomitantly induced with almost every injection after the first week and the frequency and quantity of the ALG administration had to be reduced. Thereafter, lymphocytosis gradually became evident. At the end of four months the ALG was not stopped, as in many earlier cases. The child whose original diagnosis was biliary atresia is now almost one year post-transplantation and has completely normal liver function.

thrombocytopenia which was sometimes severe enough to require platelet transfusions (Fig. 121), but which was never accompanied by evidence of bone marrow depression. The lymphocytes then returned to the peripheral blood in large numbers. In the long run the irregularity of globulin treatment often resulted in smaller total volumes being given than when more conservative doses were used from the outset.

Thus, it became apparent that the best hope of improving the ALG regimen was to extend its use beyond the four months to which its administration was originally restricted. This was not possible indefinitely because of the eventual development of toxic reactions. The longest courses of continuous horse ALG treatment have been eight months (OT 15 and 19). One of these patients ultimately died of metastases from the hepatoma for which liver replacement was undertaken. The other still has perfect hepatic function (see Chapter Seventeen and Figure 128, Chapter Fourteen).

Desensitization to the horse protein was attempted in two patients (OT 14 and 16) who had equine ALG discontinued because of severe local reactions ten and a half weeks and 18 days after operation, respectively. In both patients progressive jaundice appeared soon afterward. The desensitization procedure involved the repeated injection of initially minute but progressively larger quantities of the ALG, first by the subcutaneous and then the intramuscular routes. The schedules and techniques were those of classic tetanus antitoxin desensitization.⁴³ Both patients developed high fevers and became so ill that the effort was abandoned.

In a number of other recipients who had toxic reactions to the horse globulin, the better alternative was taken of switching to ALG prepared from the serum of immunized rabbits or goats. In the first two of these patients (OT 13 and 16) rejection had already developed after stopping treatment with the horse globulin. The consequent deterioration of hepatic function was not reversed with the institution of the new ALG although its progression was halted (Fig. 119). The other patients were immediately transferred from horse to goat or rabbit ALG (see Chapter Seventeen).

The doses and titers of the immune globulin prepared from rabbit and goat serum are given in Table 19. It was of interest that neither lymphopenia nor

Table 19. The Leukoagglutinin and Lymphocytotoxin Titers of the Rabbit and Goat Antilymphocyte Globulins Administered to Several Orthotopic Liver Recipients After Sensitization to Horse ALG Had Occurred*

SPECIES	LEUKO- AGGLUTININ TITER	LYMPHO- CYTOTOXIN TITER	DOSE (ml)	PROTEIN CONTENT (gm per 100 ml)
Rabbit	1:2000—1:4000	1:1000—1:2000	1—4	2.3—5.2
Goat	1:2000—1:8000	1:1000—1:2000	1.5—4	4.2—8.3

*The doses were adjusted according to the leukoagglutinin titer and the patient's weight.

thrombocytopenia was caused by the secondarily employed ALG. There was, in fact, an apparent lymphocytosis with the rabbit globulin (Fig. 119), suggesting that there had been suboptimal dosage.

Ancillary Measures. Intravenous actinomycin C and local homograft irradiation were occasionally used during the first three postoperative weeks, or in some instances after this time. The x-ray therapy was ordinarily prompted by swelling of the liver. In the usual patient two or three doses of 150 R each (at depth) were given. There was no demonstrable effect.

Splenectomy was performed in about two thirds of the liver patients. This adjuvant measure has been used in our institution since 1962^{45, 60} as part of the treatment protocol for kidney transplant recipients. It has been omitted in our renal patients only when nephrectomies were not performed simultaneously with transplantation or when splenic extirpation was judged to be technically dangerous. The latter criterion was the only contraindication to splenectomy in the liver recipients. The rationale for the procedure^{45, 60} was based on the known primary participation of this organ in the immune response to intravenous antigens. That the value of splenectomy in mitigating rejection is unproved can be appreciated by noting the published articles on the subject of splenic extirpation alternately appearing to oppose^{15, 73} or to support^{12, 41} its use. In liver transplantation, however, there are other special reasons why splenectomy is advisable if it can be conveniently and safely done. These were discussed in Chapter Eight.

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Chapter Fourteen

EARLY LIVER REJECTION IN PATIENTS WITHOUT HEPATIC GANGRENE

In many of our patients it was impossible to define the features of rejection with any degree of certainty. This was particularly common at the beginning of the experience, but even later there were several instances in which it could not be determined to what extent immunologic repudiation of the homograft contributed to an early unfavorable outcome. The reason was that the abnormalities in postoperative hepatic metabolism were predominantly reflections either of tissue injury inflicted during donor death and organ transfer or else of grave technical complications. If there was an added element of dysfunction due to homograft rejection, this could not be readily distinguished during the short survival periods. There were no recipients who had really poor initial graft function for nonimmunologic reasons who recovered and became available for long-term observation. Such patients have been considered in other portions of the book (Chapters Six, Eight and Nine).

In this chapter only cases will be reviewed in which the presence of reasonably satisfactory initial homograft function made it possible to ascribe subsequent deterioration to an immunologic etiology. The patterns of rejection were not dissimilar to those after orthotopic liver transplantation to modified dogs (Chapter Twelve) or untreated pigs (Chapter Eleven). The only special problem which had not been anticipated on the basis of previous animal experimentation was regional hepatic necrosis. This complication will be considered separately in Chapter Fifteen. However, it will be suggested both now and in Chapter Fifteen that the incomplete control of rejection was an important etiologic factor in the development of the partial homograft gangrene.

HYPERACUTE REJECTION

Red Cell Group Incompatibility

There have not yet been any fully documented examples of the "rejections on the operating table" which have not infrequently led to the immediate loss

of renal homografts. With kidneys, this kind of disaster was first (although not invariably) seen^{16, 25} when donors and recipients had different ABO red cell groups in violation of the rules shown in Table 1, Chapter Two. An effective blood flow was not restored when the vascular anastomoses were opened. Later the small vessels of the excised kidneys were found to be plugged with erythrocytes, platelets, white cells, and fibrin. A rational explanation was available since the blood group substances which allow red cells to be typed are also found in other tissues including the kidneys.^{9, 27} Thus, if the kidney of an A or B donor were placed in a patient of O blood type, the naturally occurring anti-A and anti-B isoagglutinins, respectively, in the serum of the recipient might be expected to bind with the renal red cell antigens. Serologic studies by Wilson and Kirkpatrick³³ provided evidence that this actually occurred. Subsequent authors have reached similar conclusions about the role of red cell isoagglutinins in precipitating accelerated rejections.^{14, 34}

Hepatocytes also contain erythrocyte antigens,^{9, 27} for which reason it may well be equally dangerous to perform transplantation of this organ in the face of a red cell group incompatibility. It is unlikely that future liver transplantations will be carried out under these circumstances in view of the information now available. However, it is of interest that before the adverse consequences of erythrocyte mismatches were fully appreciated, two such attempts were made, one by Moore¹² and the other by Absolon.¹ The ABO incompatibilities were B to A and A to O, respectively. The organs functioned for 13 and 11 days and at autopsy did not have any obvious distinguishing pathologic features suggestive of a hyperacute rejection.

Red Cell Group Compatibility

Hyperacute rejection of renal homografts has also been seen in the absence of red cell group incompatibility. The first case was described briefly by Terasaki²⁸ and many others were added later;^{11, 24, 29, 31, 32} they occurred usually in patients who had preformed lymphocytotoxic antibodies. The sequence was typical. After the opening of the renal vessels, the kidney surface was not well perfused and became cyanotic. The consequence was complete or nearly complete cortical necrosis. Circumstantial evidence that the destruction was caused by preformed antibodies directly binding to the grafts has been presented by several authors.^{11, 29, 31, 32} In other cases, the possibility has been raised that an inherently less violent antigen-antibody reaction in combination with depressed reticuloendothelial function of the recipient precipitated a generalized Schwartzman-like reaction²⁴ that secondarily resulted in the plugging of the renal vessels with fibrin filtered by the kidney microvasculature from the perfusing blood. The analogy between the consequences of such states of host presensitization and those of heterograft reactions will be discussed in Chapter Nineteen.

Sooner or later it is almost inevitable that hepatic homografts will be placed, by accident or with foreknowledge, into recipients with preformed antibodies induced by prior blood transfusion, pregnancy, previous homotrans-

plantation, or in other ways. Presumably an accelerated rejection of the liver would follow if these immunoglobulins had an avidity for the tissues of the transplanted organ. There is also the possibility, if an antigen-antibody reaction in the liver were capable of triggering a Schwartzman reaction, that the normal host kidneys could be damaged as innocent bystanders by virtue of their propensity for clearing the products of generalized fibrinogen degradation.

There is no proof that hyperacute rejection of a hepatic homograft has occurred in any case to date. However, Hume and Williams¹⁰ have told us of an experience that may represent an example. An infant with extrahepatic biliary atresia was given an orthotopic homograft at the Medical College of Virginia under almost ideal circumstances of organ procurement. The new liver promptly excreted bile. About 10 hours after completion of the operation, the abdomen was re-explored because of evidence of hemorrhage. By this time the transplant had become mottled; the left lobe was now selectively indurated. A biopsy revealed widespread necrosis. In some of the portal veins there was margination of polymorphonuclear leukocytes similar to that found by the same authors in immediately rejecting kidneys.³¹ When the child died seven days later, the liver was almost completely necrotic even though its major vessels were patent. Immunofluorescence studies disclosed no significant immunoglobulin deposition in the graft. However, eluates of the autopsy specimen caused lysis of the cultured renal cells of the original donor; the same effect was also produced by the serum collected preoperatively from the recipient. At no time during life could lymphocytotoxic antibodies be detected in the serum by the Terasaki method.

ACUTE REJECTION (FIRST TWO MONTHS)

The variety of rejection which has been compared to the cell-mediated delayed type hypersensitivity response has been more or less clearly identifiable in all the patients who received orthotopic hepatic homografts at our institutions and who survived for more than a month;^{17-23, 26} the same has been true in other centers.^{2, 3, 6, 13} In our own patients the criteria upon which the diagnosis was made were highly variable and subject to the review of a large number of facts on a day-to-day basis. These included the results of multiple liver function and hematologic tests, measures of abdominal girth and body weight, and serial liver scans, to mention only a few. Judgments about the presence or absence of rejection or the need to adjust therapy were never made on the basis of limited observations. Instead, an attempt was made to develop a panoramic view of the whole course. This was best done with the use of large wall charts which were brought completely up to date each evening before rounds.

As experience was accumulated, it became evident that there were several distinctive clinical syndromes of early rejection which could be expected. These have been used as the basis for the following classification of eight orthotopic homotransplantations performed prior to October 26, 1968, in seven patients. In each instance the postoperative course began satisfactorily enough

to permit the meaningful analysis of subsequent events. All the recipients were treated with the triple drug regimen of azathioprine, prednisone, and ALG.

Subclinical Rejection

Only two of the patients (OT 13 and 23) failed to manifest an unequivocal rejection during the first two postoperative months. The recipients, who were two and 16 years old at the time of their transplantations, had no overt symptoms and no depression of appetite. Nevertheless, there were sound reasons to suspect that they passed through very mild rejection episodes, beginning in the fourth (OT 13) and second (OT 23) postoperative weeks.

Physical Signs. The most objective evidence was swelling of the homografts. The enlarged livers were firm, had well defined inferior edges, and were nontender. The degree of hepatomegaly could conveniently be quantitated with serial scans (Figs. 122 and 123) using ^{99m}Tc -technetium.⁷ This isotope, which is picked up by the reticuloendothelial portion of the graft, had a special advantage after liver transplantation. Since its physical half-life is only six hours, the background activity of the isotope was gone by the time of each succeeding examination even if this was performed on the same day. Moreover, the calculated irradiation dose to the liver was only about one rad, no more than that delivered during performance of an intravenous pyelogram. The homografts of patients with early subclinical rejection had an essentially normal intensity of isotope concentration (Figs. 122 and 123).

Although jaundice did not develop, the stools of the recipients intermittently became light colored and the urine dark. These changes were fleeting, coming and going within a few hours or in the course of a day. Temperatures remained relatively normal. The patients were discharged from the hospital after 14 and five weeks, respectively.

Laboratory Studies. Major secondary rises in the serum bilirubin were not observed. However, there were variations in the serum transaminases and the alkaline phosphatase (Figs. 124 and 125). Other measures of liver function were generally not altered. These included the total serum proteins and the albumin fraction (Figs. 124 and 125) as well as the liver-based clotting factors I (fibrinogen), II (prothrombin), V (accelerator globulin), VII (proconvertin), IX (Christmas), and X (Stuart). The gamma globulin tended to remain at normal levels.

Treatment. Major adjustments were not made in the doses of azathioprine and ALG. The quantities of prednisone, which were already being reduced, were slowly dropped even more despite the fact that an anicteric rejection was thought to be evolving. The rationale of this approach will be returned to later in discussing the treatment of more severe problems of graft repudiation. Other aspects of general care have been described in Chapter Nine.

Reversibility. The various changes described above gradually returned toward, although not necessarily completely to, the pre-rejection state. Eventually the liver size decreased (Figs. 122 and 123). The abnormalities in hepatic function, which were mainly detectable in the enzyme determinations, slowly improved (Figs. 124 and 125).

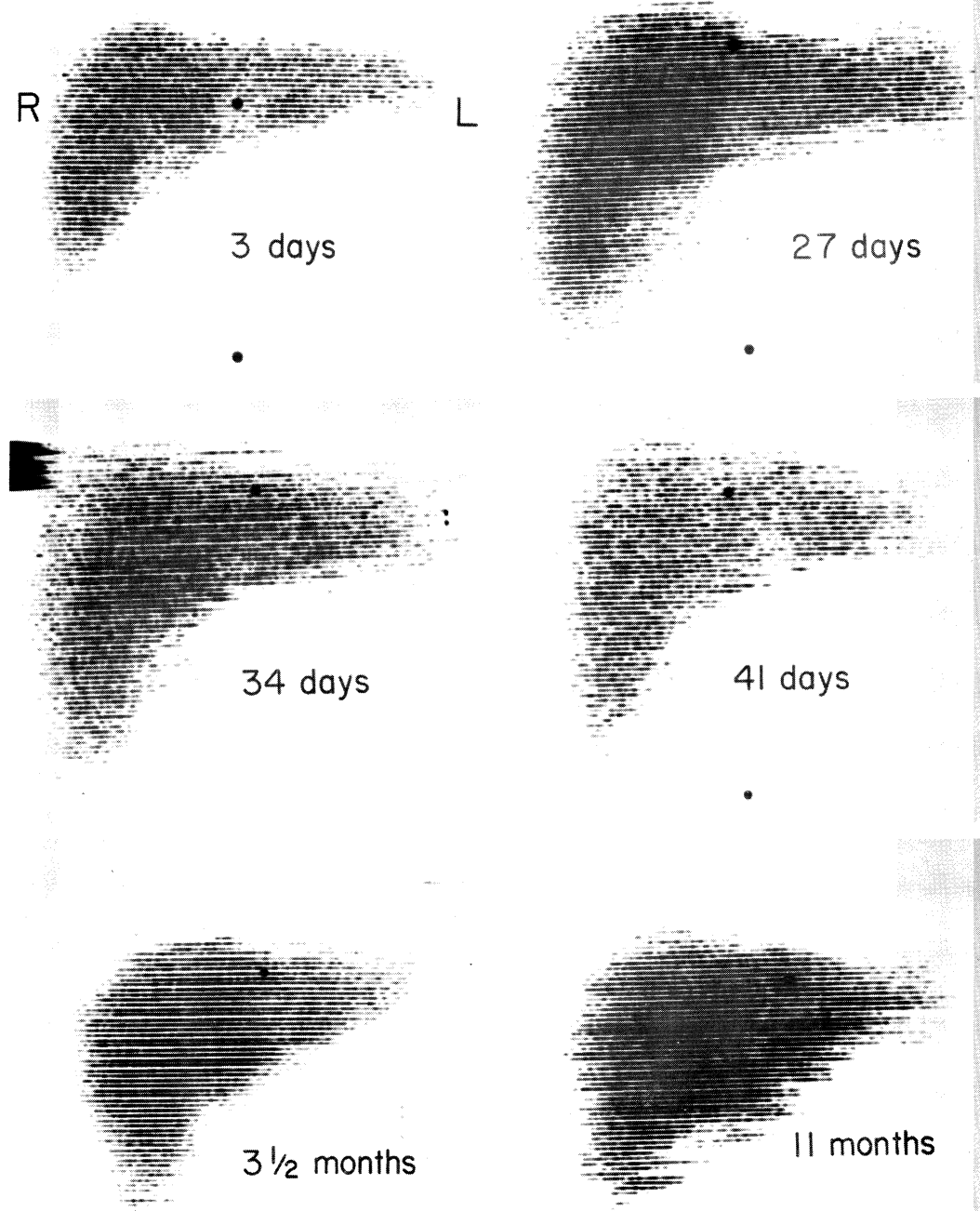


Figure 122. Anteroposterior liver scans performed with ^{99m}Tc -technetium in orthotopic recipient OT 13. The times after operation are shown. Note the marked hepatomegaly during the third to fifth postoperative weeks at the time of an anicteric rejection; isotope concentrating ability was not perceptibly altered. Compare with Figure 124 and note that swelling of the organ occurred coincident with rises in the serum enzymes. Much later in the course jaundice developed. At that time the liver scan did not show hepatomegaly, as exemplified by the 11 month examination.

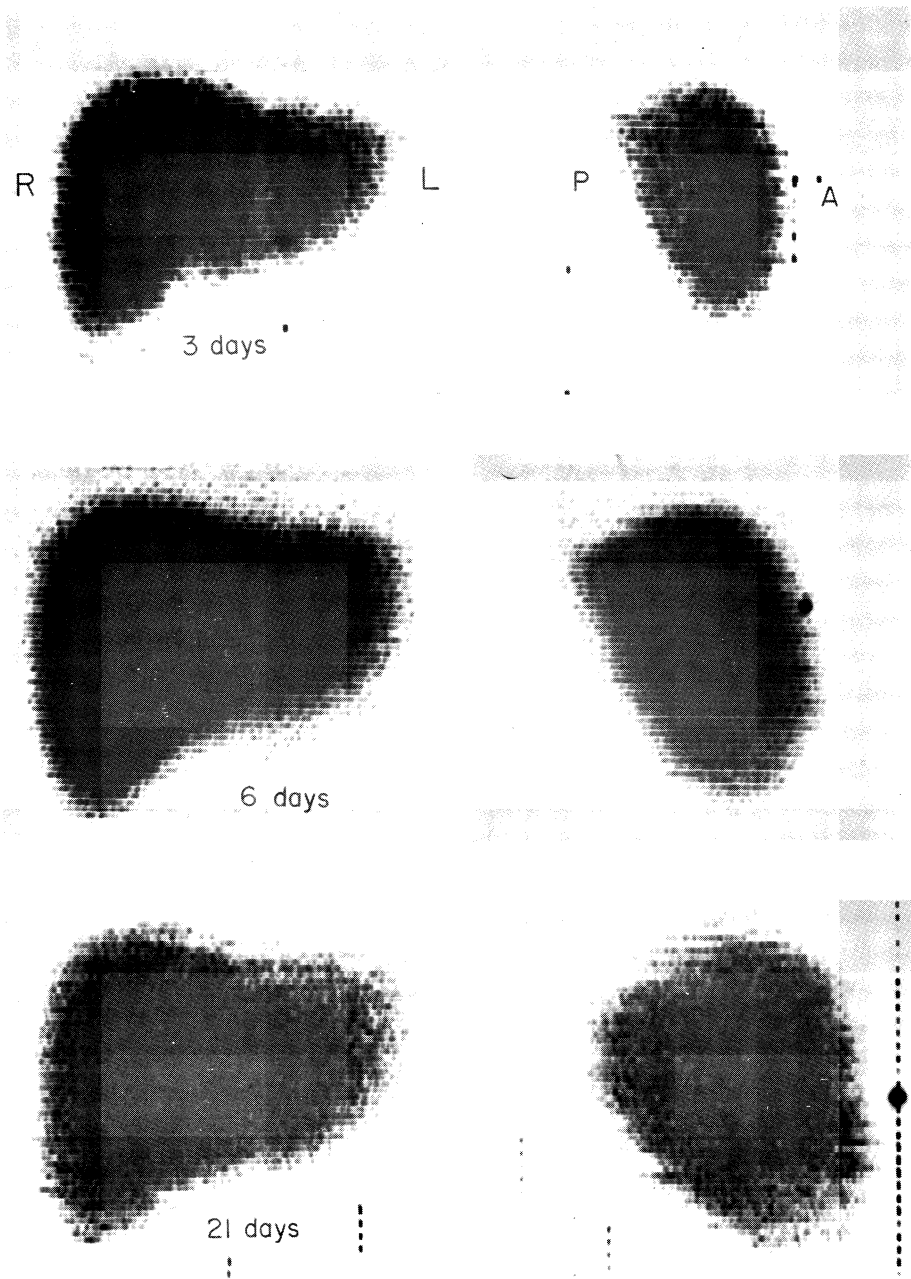


Figure 123. See facing page for legend.

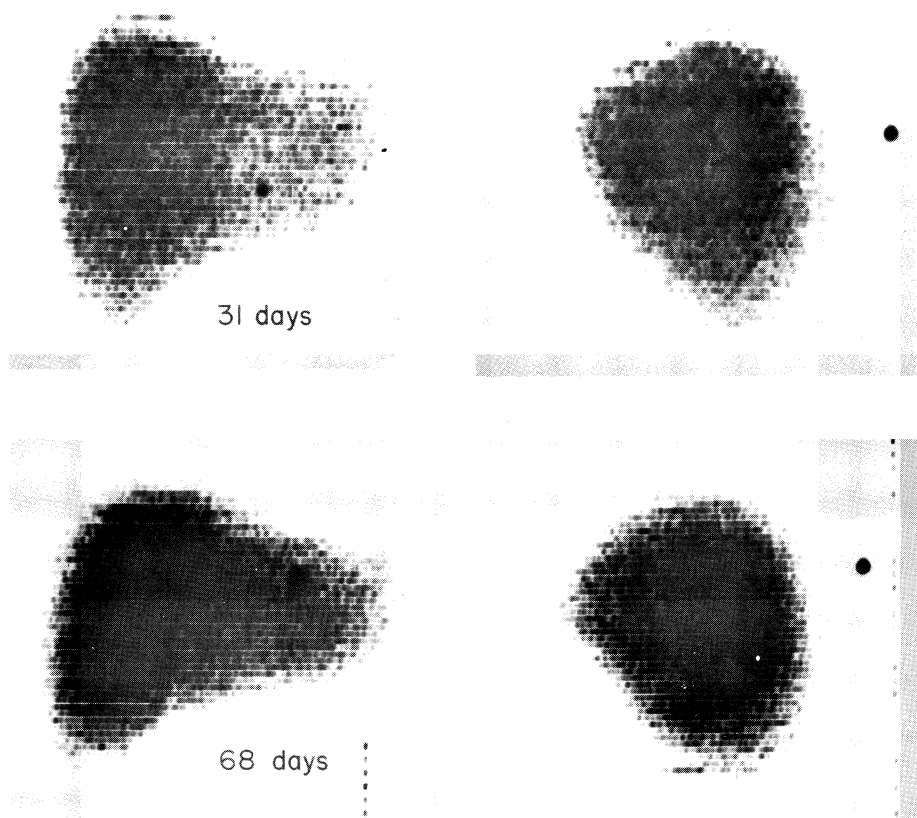


Figure 123. Anteroposterior and lateral liver scans in patient OT 23, using ^{99m}Tc -technetium. The patient, whose diagnosis was hepatoma, was 15 years old. He received the liver of a six year old cadaveric donor. The evidence for rejection is shown graphically in Figure 125, beginning about one week after operation. Note the remarkable liver swelling which occurred at this time and which lasted for more than two subsequent weeks. By 68 days the anteroposterior view appeared to have returned to about the same dimensions as had been present shortly after operation. However, the lateral view showed that the liver mass was increased; liver function was then completely normal. Note that the pickup of the isotope remained homogeneous throughout the period of observation, except possibly at 31 days. More scans of this patient taken at the time of invasion of the homograft by recurrent tumor are shown in Chapter Seventeen.

Long-term Prognosis. Avoidance of a florid rejection crisis during the early course did not assure stable long-term hepatic function. This aspect of the problem is going to be considered in Chapter Seventeen. Here it will be noted only that both the patients who passed through an anicteric early rejection became jaundiced several months later, although the delayed deterioration in one case was due to invasion of the homograft by recurrent carcinoma.

Rejection Crisis

There were three recipients (OT 15, 17, and 19) who passed through rejections which evolved so suddenly that they have been termed "crises." The

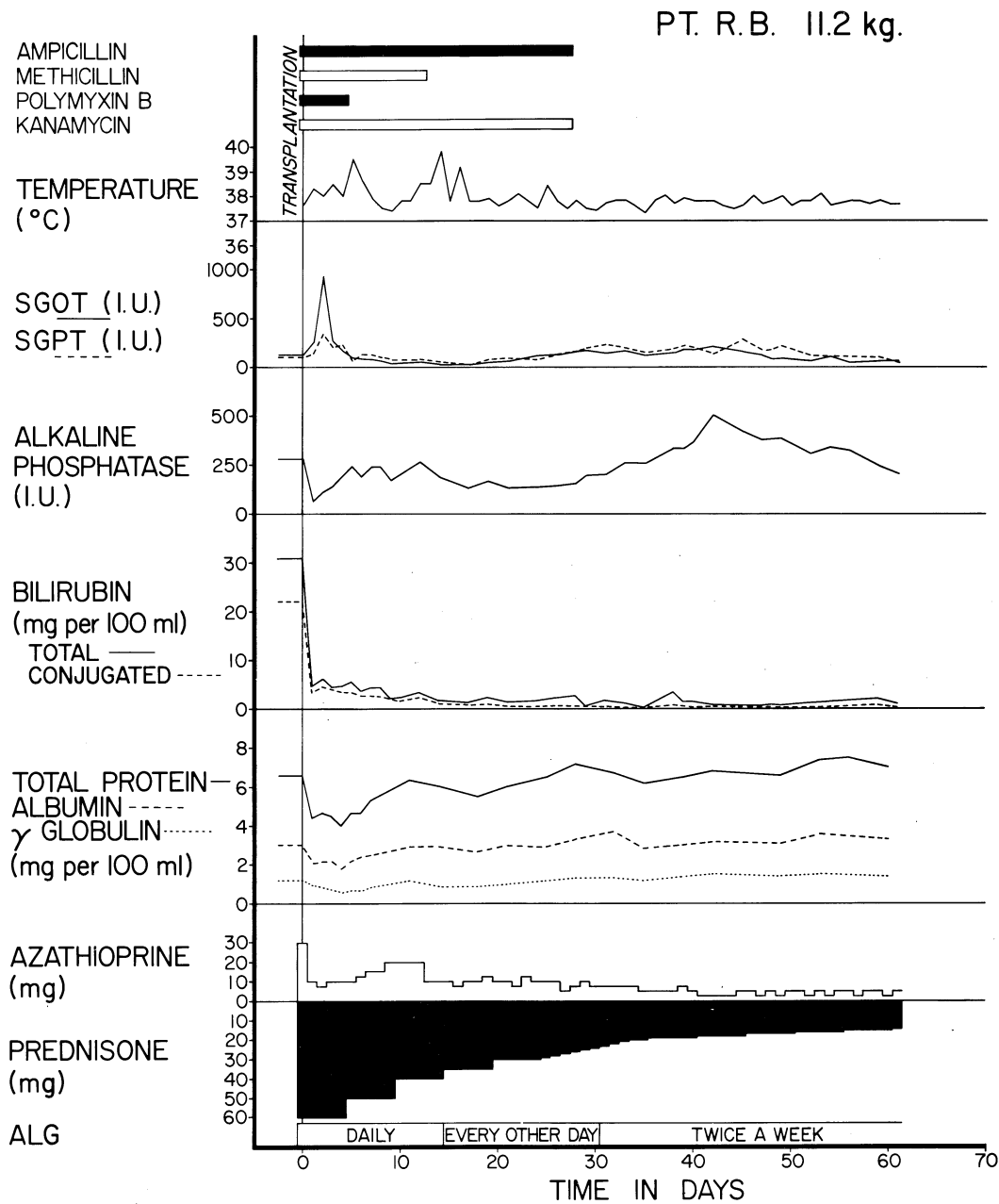


Figure 124. Example of subclinical rejection (OT 13). The indication for operation was extra-hepatic biliary atresia. The bilirubin fell almost immediately from more than 30 mg per cent to less than 5 mg per cent. There was no recurrence of the jaundice in the interval shown. However, there were rises in the alkaline phosphatase, SGOT, and SGPT from the third to the seventh postoperative weeks. Antibiotic therapy was stopped after four weeks. The temperatures shown are the maximums for each day. Several months later a delayed rejection developed: the late course of the child is graphically depicted in Figure 176, Chapter Seventeen. (By permission of Ann. Surg. 168: 392, 1968.)

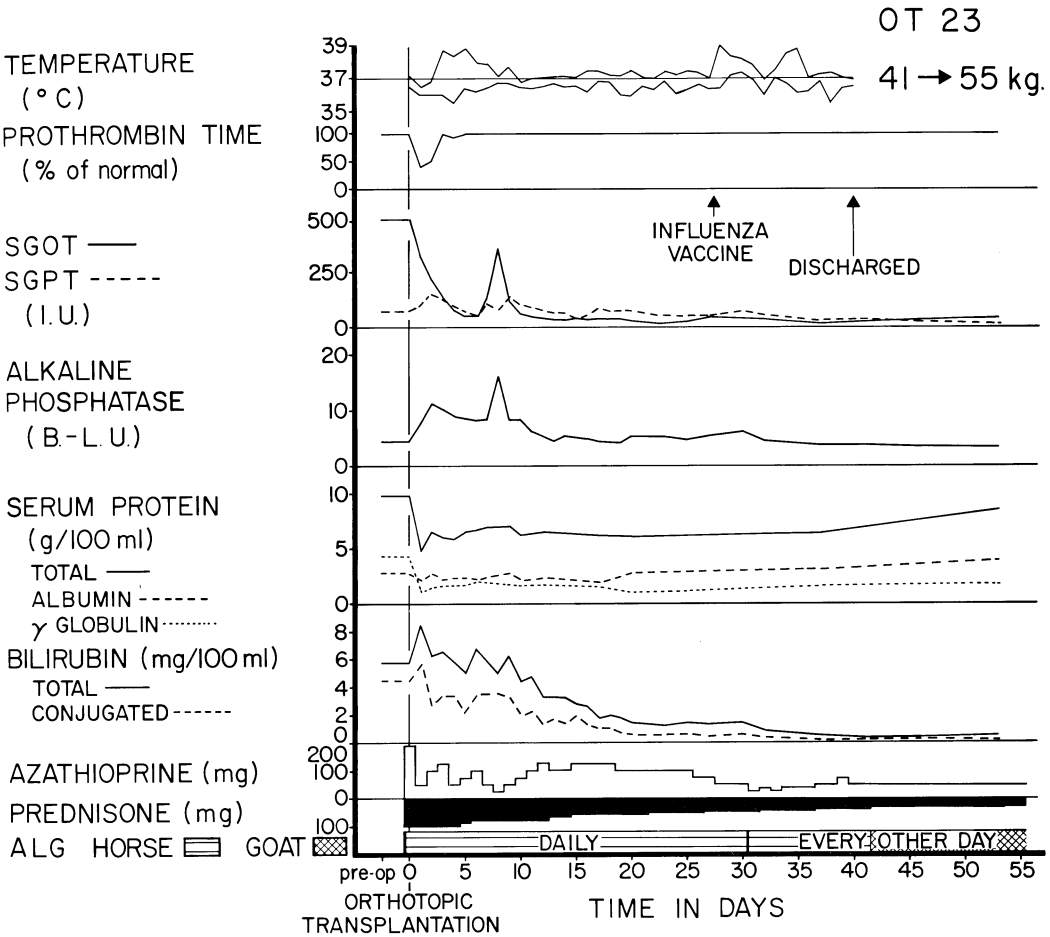


Figure 125. The first two post-transplantation months of a 15 year old boy (OT 23) with hepatoma who received an orthotopic liver from a six year old donor. An overt rejection was not diagnosed during this time. Nevertheless, there was evidence of a subclinical rejection beginning on day seven. See text for discussion. The febrile episodes at the end of the fourth week were probably related to the administration of influenza vaccine.

onset came six, six, and 29 days after operation. The process developed with extreme abruptness, reached a peak within a day or two, and then receded with surprising rapidity. Two of the three patients (OT 15 and 17) became acutely ill.

The recipients were aged 42, 23, and four years. All three, especially the two older ones, complained of pain in the center of the back which occurred intermittently, sometimes extended to the right flank or right upper quadrant, and was relieved by mild analgesics. Extreme fatiguability and anorexia were prominent complaints but there was no loss of mental acuity.

This was the most explosive kind of rejection observed in our experience and in two of the patients it could be described as violent. However, even in this very acute form, rejection did not precipitate anything resembling an anhepatic state; there were, for example, no recognized bouts of hypoglycemia. This was fortunate since there are no effective techniques available for liver

support during a protracted functionless interval analogous to those which can tide over temporarily anuric recipients of renal homografts.

Physical Signs. At first glance, two of these three patients looked like victims of fulminating viral hepatitis. There was an increase in the size and consistency of the transplanted organs. Paradoxically, however, this was not nearly so prominent a finding as with the milder rejection described earlier. Moreover, the liver scans did not permit as precise delineation of the dimensional anatomy since there was a loss of the ability to concentrate isotope in two of the three patients. Other important observations were made from the scans which will be returned to later.

With the onset of the crisis, the stools immediately became white and the urine dark colored. The rapidity with which visible jaundice developed and deepened was surprising, but the visual impression was in conformity with concomitant chemical determinations. Liver percussion caused a very poorly localized abdominal pain.

Two of the patients had high fevers. It is well known that hyperpyrexia is a characteristic sign of renal transplant rejection in patients being treated with azathioprine alone, but that the fever can be prevented if heavy doses of prednisone are given concomitantly.¹⁶ Steroids also blunt the febrile response often seen after the administration of heterologous ALG (Chapter Thirteen). Prednisone had been given from the beginning in large quantities to all three of the recipients who underwent acutely evolving rejection crises; consequently, temperature elevations which developed during these episodes of apparent immunologic repudiation were construed as infectious in etiology. The point of view will be developed later that at least some of the fevers may have been caused by bacterial invasion of the besieged and vulnerable homografts.

The two patients with the most severe crises had moderate fluid retention. Coincident with a weight gain of 3 to 6 kg, they could be seen to have slight periorbital and dependent edema; at the same time, x-rays of the abdomen were interpreted as showing fluid between the intestinal loops. Associated with these findings was a fall in urinary sodium concentration to below 20 mEq/liter.

Laboratory Studies. The most striking feature in this group of patients was the suddenness with which hepatic malfunction developed. The details varied, but in all the serum bilirubin concentration rose from essentially normal to relatively high levels (6, 14, and 18 mg per cent) within a 24- to 48-hour period (Figs. 126, 127 and 128). About half the bilirubin was in the conjugated form. Associated with, or slightly preceding, the icterus were increases in the alkaline phosphatase.

The changes in the serum transaminases were of special interest in view of the findings in the liver scans to be described later. Both the SGOT and SGPT rose, although not to alarming levels in patients OT 15 and OT 19 (Figs. 127 and 128). However, there was good reason to suspect massive hepatic necrosis in the third recipient (OT 17); her SGOT and SGPT (Fig. 126) were recorded at peaks of 2600 and 3200 International Units (normal 9 to 45 and 5 to 35, respectively). A liver scan was obtained at this time.

There was a reduced uptake of ^{99m}Tc-technetium in her swollen transplant

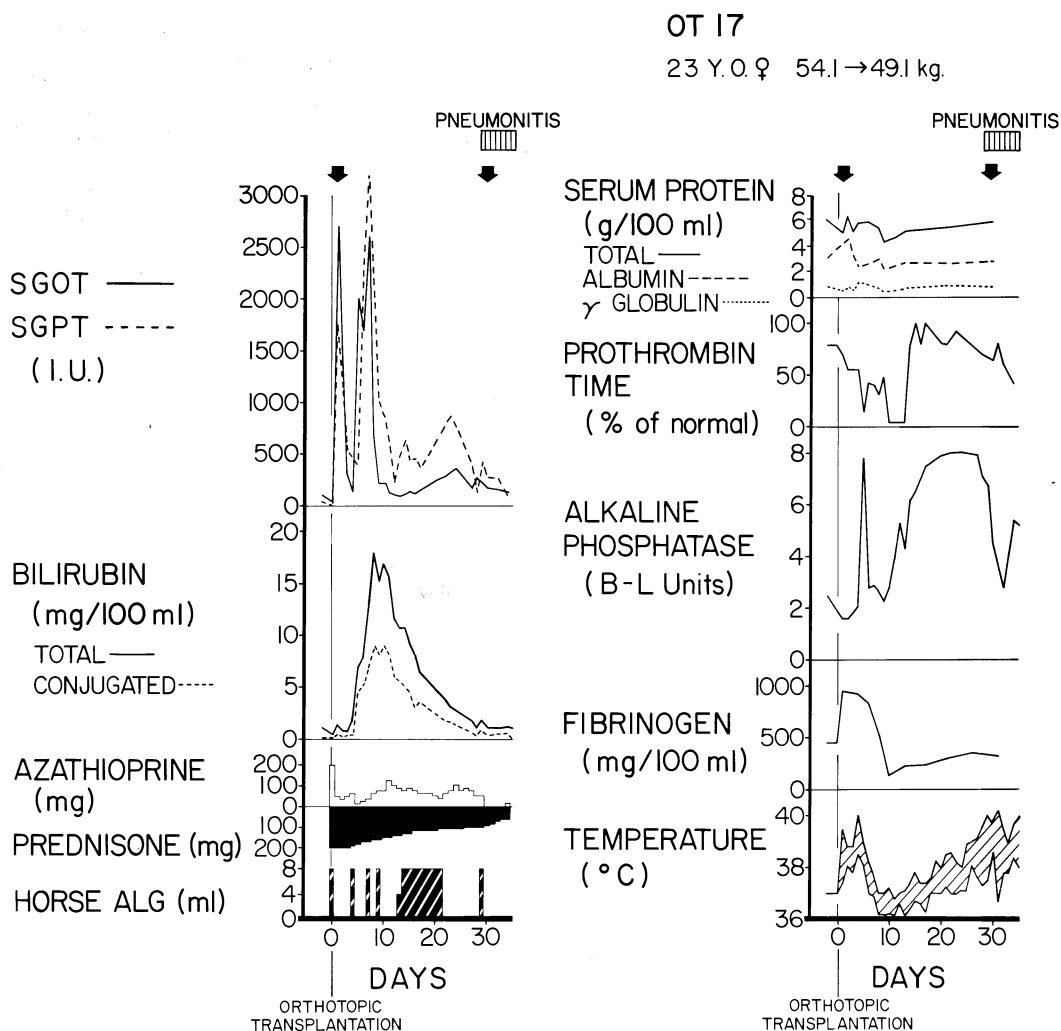


Figure 126. The course of a patient (OT 17) who underwent a violent rejection crisis after orthotopic liver transplantation. The first abdominal re-exploration (arrow) was for the control of bleeding. Even before the onset of jaundice, her course was markedly febrile. Note the drastic depression of prothrombin time on postoperative days 10 through 13. Recovery from the rejection crisis was prompt, but the patient died of *Pseudomonas pneumonia* after 35 days. The radiographic changes in the lung were first thought to be the consequence of pulmonary emboli; consequently intravenous heparin therapy was started. This resulted in intra-abdominal hemorrhage, necessitating the second laparotomy (arrow). The alkaline phosphatase values are in Bessey-Lowry units (normal 1 to 3). Normals for the SGOT and SGPT are less than 50 units.

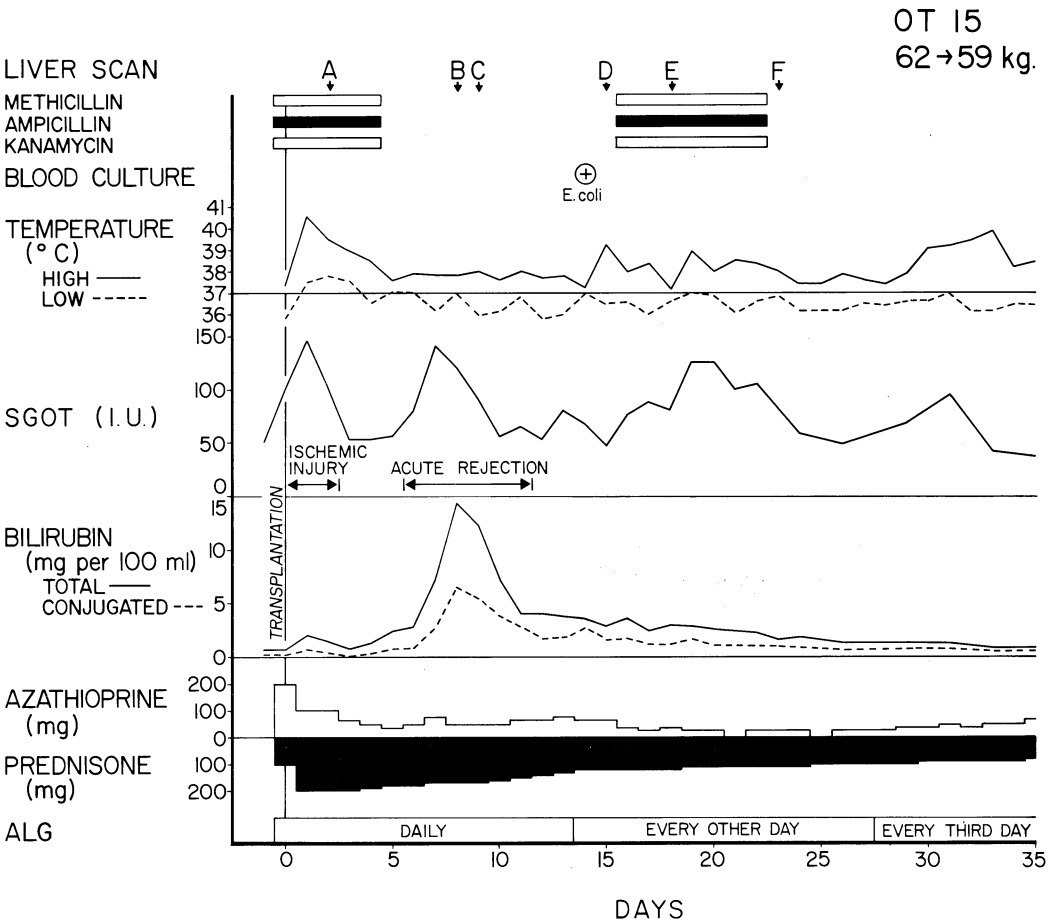


Figure 127. The postoperative events in patient OT 15. An explosive rejection began on the sixth day, reached a peak within two days and receded promptly. Gram negative bacteremia was diagnosed from a blood specimen obtained on post-transplantation day 14. The liver scans indicated by the letters A to F are shown in Figure 130. Note that the immunosuppressive therapy was actually lightened with the development and evolution of the rejection episode.

which it was feared had undergone irreparable injury (Fig. 129, left); the distribution of the isotope was relatively homogeneous. The magnitude of the impairment of the hepatic reticuloendothelial function was demonstrated by the large fraction of the technetium which was located in extrahepatic sites (Fig. 129, top, left). Within a few days the situation improved and the homograft was able to carry out this function far more completely (Fig. 129, top, right). By three weeks the organ swelling had markedly subsided (Fig. 129, bottom, right).

Even more alarming changes were seen in the liver scans in one of the patients (OT 15) whose serum transaminases had not risen to such extreme levels (Fig. 127). Coincident with the onset of the severe rejection crisis there was palpable hepatomegaly. However, the homograft actually appeared smaller on the scan, presumably because of its generally reduced uptake (Fig. 130B). Moreover, there seemed to be a regional loss of isotope concentration affecting mainly the left hepatic lobe (Fig. 130B); on lateral view a wedge of the dome of

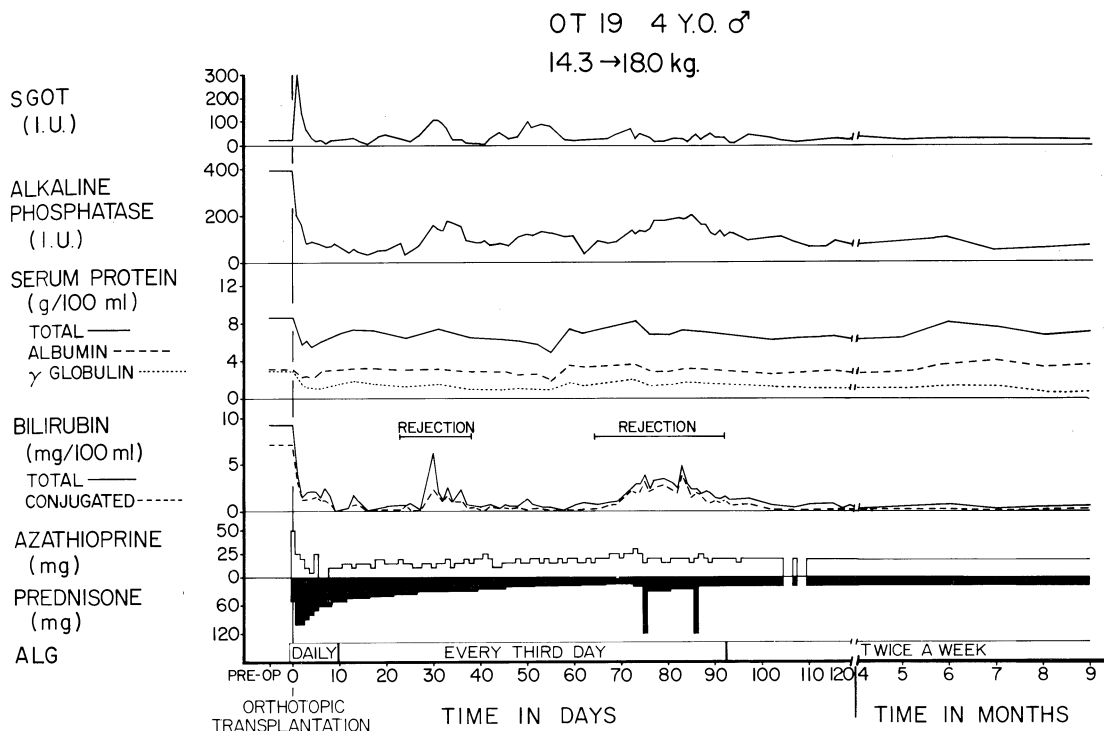


Figure 128. A four year old child (OT 19) with intrahepatic biliary atresia who was treated with orthotopic liver transplantation. A very transient rejection occurred after one month. This underwent almost immediate and complete remission. A late rejection which began on postoperative day 72 was also easily controlled. Note the change in time scale after four months. The patient still has perfect liver function after 10 months. He is still receiving ALG. The normal enzyme values in international units at this age are: alkaline phosphatase, 57 to 151; SGOT 3 to 27; and SGPT, 2 to 30.]

the liver was not visualized (Fig. 131, left). One day later a more normal scan was obtained (Fig. 130C).

During the next several days most of the liver functions improved. On the morning of the fifteenth postoperative day the patient's temperature rose sharply coincident with the appearance of *E. coli* in a peripheral blood culture. At the same time, a liver scan (Fig. 130D) showed only a fraction of the transplant. Again, nearly the full dimensions of the organ had been restored by the time of the succeeding examination (Figs. 130E and 131, right) a short time later.

Probably serial liver scanning in such cases provided a crude means of obtaining the sort of information that has been sought in dogs with more direct techniques. Groth⁸ showed striking reductions in hepatic blood flow at the time of canine liver rejection (Chapters Eleven and Twelve). It is not difficult to conclude that the same kinds of flow changes, with remissions and exacerbations, were present in the clinical cases to account for the bizarre and volatile abnormalities of the liver shadows as viewed by scanography.

The concept that drastic reductions in hepatic blood flow were responsible for the "disappearing liver" are consonant with the other findings at about the

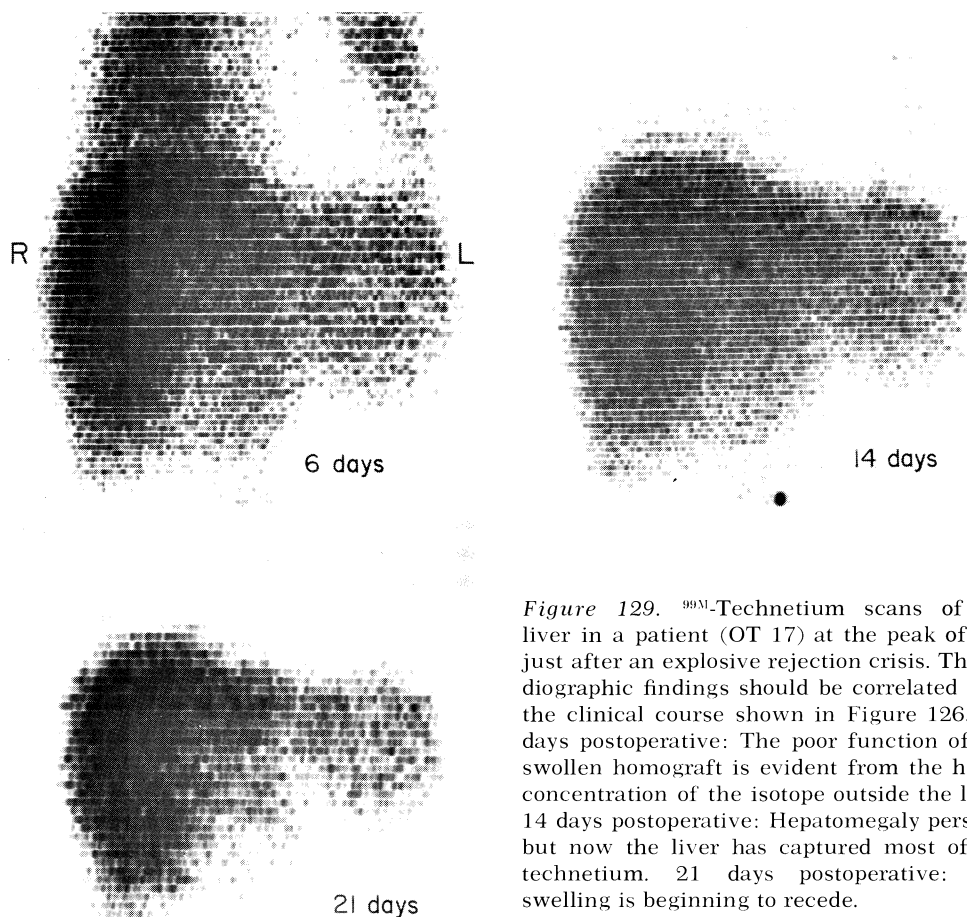


Figure 129. ^{99m}Tc -Techetium scans of the liver in a patient (OT 17) at the peak of and just after an explosive rejection crisis. The radiographic findings should be correlated with the clinical course shown in Figure 126. Six days postoperative: The poor function of the swollen homograft is evident from the heavy concentration of the isotope outside the liver. 14 days postoperative: Hepatomegaly persists, but now the liver has captured most of the technetium. 21 days postoperative: The swelling is beginning to recede.

same time, including the biochemical measures in the serum suggesting hepatocyte necrosis. The invasion of the ischemic homograft by microorganisms from the adjacent intestinal tract under these circumstances would not be surprising. This was apparently the pathogenesis of the gram negative septicemia (Fig. 127).

Other determinations of hepatic function were also often affected by immunologic repudiation of the homograft, but not with such consistency as those already described. In the most mild example of abrupt rejection (OT 19) there were no changes at all in the concentration of the total or fractional serum proteins (Fig. 128) or in the prothrombin times. The picture was not really distinguishable from that of a very transient intra- or extrahepatic biliary obstruction. It was in this patient that serial liver scans did not reveal any striking change in the homograft size.

In contrast, the young woman (OT 17) with the most severe rejection crisis manifested deterioration of every liver function that was measured. Her total serum protein and albumin concentrations began to fall within a few days (Fig.

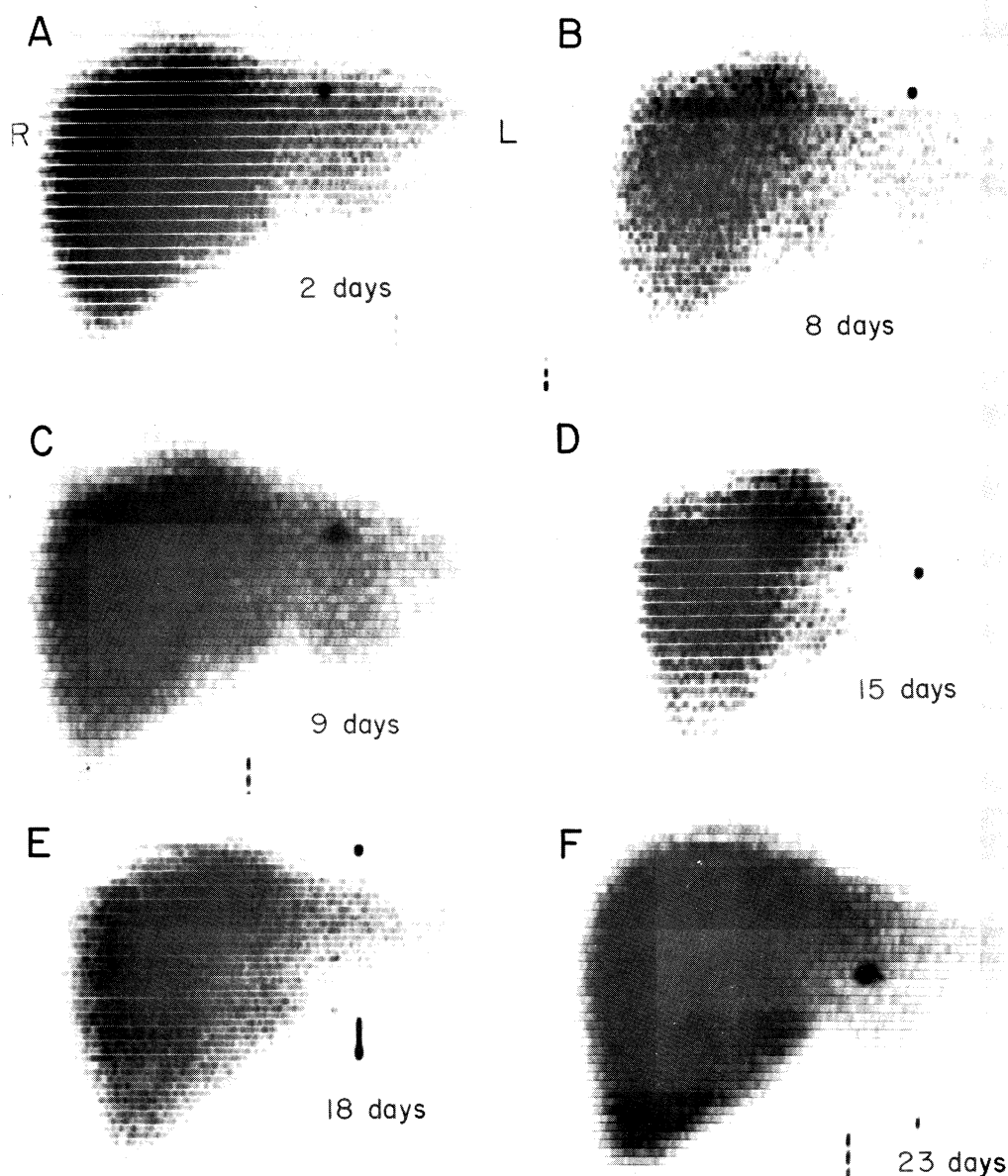


Figure 130. Anteroposterior liver scans attained with ^{99m}Tc -technetium in patient OT 15. Note the temporary disappearance of portions of the homograft on the eighth and fifteenth postoperative days; the first of these occasions was at the beginning of an explosive rejection crisis. At the time of the 15-day scan, the patient had developed gram negative septicemia, presumably from a hepatic focus. The remittent changes in the liver scans were thought to be due to variations in hepatic blood flow during the rejection crisis. The patient recovered completely from these episodes and was discharged from the hospital 65 days postoperatively. Correlate the scans with the clinical events shown in Figure 127.

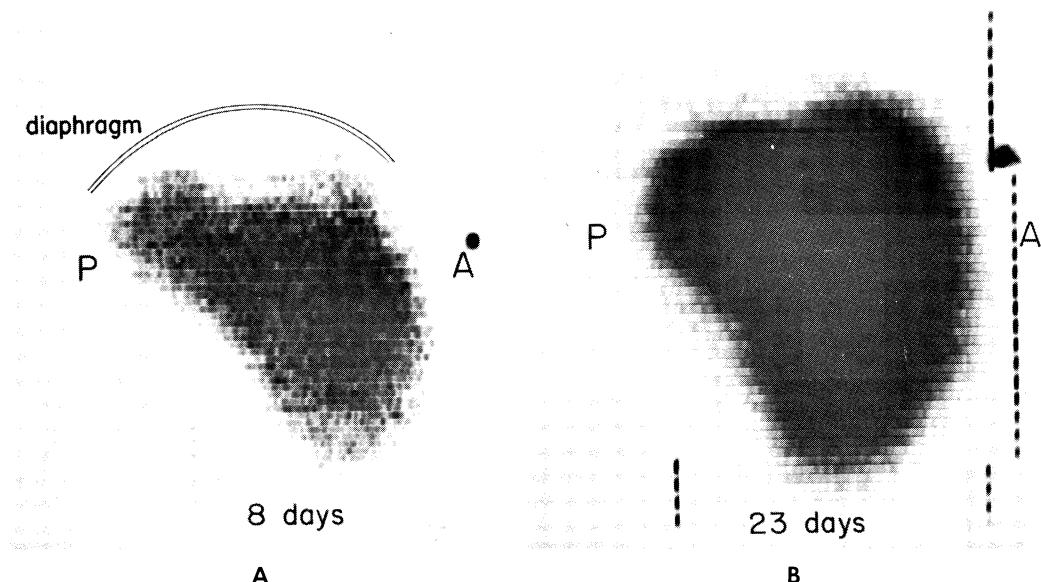


Figure 131. Lateral scans obtained at the same times as the studies shown in Figures 130B and E at eight and 23 days postoperatively in patient OT 15. A, Note the wedge of absent isotope pick-up at the dome of the liver. B, This area is now well seen 13 days later, although a small defect persists.

126). There were life-threatening changes in the clotting factors. For example, the prothrombin times were prolonged to 45 to 75 seconds (control 13 seconds); expressed in percentages, these values were essentially zero for almost a week (Fig. 126). At the same time the plasma fibrinogen (Factor I) fell to a low of 146 mg per cent.

The other patient (OT 15) was between these extremes. Although there were temporary declines in the serum protein concentrations, the predominant findings were those of obstructive jaundice. The prothrombin times were not affected.

All three patients had a leukocytosis at the time of the rejection episodes with maximum total white blood cell counts of 18,000 (OT 15), 39,000 (OT 17), and 19,000/cu mm (OT 19). The preponderance of the increased numbers of cells was of the unsegmented polymorphonuclear variety. Thrombocytopenia was common before, during, and after rejection but did not seem to be made worse by this process; the multiple factors contributing to platelet depression were discussed in Chapter Thirteen. Acute anemia of the kind that might have been caused by accelerated hemolysis (see Chapter Twelve) was not observed. During the immunologic crises, none of the patients developed hemorrhage or other evidence of the gastrointestinal ulcerations that have often been seen at comparable times in animal experiments.

Treatment. The general care of the patients was along the guidelines described in Chapter Nine, with emphasis on the provision of a strict antacid diet, vitamin supplementation (especially K), and bronchopulmonary care. Fluid intake was restricted if edema became detectable or in the event of excessive weight gain; with amelioration of the crises, there was a brisk diuresis.

Because of the evidence that the transplanted organs were in danger of being invaded by intestinal bacteria, or that this had already happened in one case (OT 15), intensive antibiotic treatment was maintained either continuously or intermittently (Fig. 127) throughout the rejection episodes and for some time afterward. This important subject is covered in Chapters Fifteen and Sixteen.

In the two patients in whom the rejections were the most severe, significant changes were not made in the schedules of azathioprine, ALG, and prednisone that were in effect at the time the process began and, in fact, the steroid doses which were already being attenuated were further cut (Figs. 126 and 127). The third patient (OT 19) was kept on the pre-existing daily quantities of prednisone (Fig. 128).

This approach during episodes of hepatic rejection was different from that which is standard practice in our institution after renal transplantation. When renal homografts undergo deterioration, steroid doses are invariably increased and maintained at whatever levels are necessary to restore good kidney function. With the liver, the assumption was made that return of hepatic function would occur without such heroic measures. Whether this was an appropriate policy is a matter that has still not been completely settled. However, there were specific reasons for the decision to follow a conservative approach with immunosuppression.

The expectation that the reversal of hepatic rejection would be less dependent on the intensification of treatment than is the case with the kidneys was based on the results of the animal investigations described in Chapters Eleven and Twelve. It will be recalled that hepatic rejection in dogs and pigs often underwent spontaneous resolution without any change in therapy or in some animals in the absence of any therapy at all. The explanation for these events has been of great interest to investigators of transplantation immunology and was speculated upon earlier (Chapter Twelve).

The prospect of achieving the same result in humans was especially appealing because of the overwhelming infectious complications which developed in all the unsuccessfully treated liver recipients in our initial experience (see Chapters Thirteen and Sixteen). The high dose immunosuppressive therapy in these first cases had seemed to be incompatible with survival. The experimental observations alluded to previously had raised the possibility that such stringent treatment might not actually have been necessary to achieve continued graft viability.

Reversibility. The point of view just mentioned received support from the prompt improvement of these three patients. Liver function began to return rapidly toward normal (Figs. 126–128). The recipient (OT 17) whose transplanted organ had suffered the most violent assault made an amazingly quick recovery and she began to leave the hospital for most of each day by the end of the third week. Unfortunately she developed several infectious complications including a monilial granuloma in the left subphrenic space which hemorrhaged and necessitated laparotomy for control. She died 35 days postoperatively of pneumonitis caused by *Pseudomonas*. The other two recipients (OT 15 and OT 19) had a very benign subsequent course and were discharged in excellent condition five and four weeks after their transplantation. By this time,

measurements of their hepatic function were at least as normal as in the patients whose homografts had passed through the much milder anicteric rejections. With the improvement in liver function, the abdominal and back pain ceased. Liver size became stable both by palpation and by scanography.

It was established from these observations that complete reversal of hepatic homograft rejection could occur without increasing the level of the pre-existing immunosuppression. However, it might have been safer to have been more aggressive, particularly with the use of prednisone. In the group of patients to be described in the next chapter it is almost certain that efforts to keep the steroid doses as small as possible contributed to eventual devitalization of large areas of the homografts. Even in the recipients of the group now under discussion, one of the patients (OT 17) nearly underwent total destruction of the transplant and another (OT 15) apparently came perilously close to developing large areas of focal necrosis.

Long-term Prognosis. An early rejection crisis, even a very severe one, did not have adverse implications for long-term survival. The two patients who returned to their homes enjoyed long periods of good health. Their later courses will be described in Chapter Seventeen. One of these recipients had a very minor late rejection in the third postoperative month. The other survived for almost a year until he died of metastases from his original hepatoma.

Indolent Rejection

A third variety of rejection, having certain features of both the kinds already described, proved to be the most difficult of all to treat. The process now to be discussed affected three homografts in two patients. In one of the recipients the first transplant eventually had to be removed and replaced with another graft. The immunologic attack on the second organ was similar to, but less severe than, that which had destroyed the first.

The two recipients were 16 (OT 14) and two (OT 16) years old. The rejections began on the sixth, 13th, and 32nd post-transplantation days, and developed in a disarmingly slow way that required from four to seven weeks to reach a peak. On no occasion did the patients seem ill, at least at the outset. They became icteric but had good appetites and were physically active. Eventually the older child complained of a dull but very annoying pain in the back which persisted in spite of treatment with analgesics.

Physical Signs. With the onset of jaundice, the stools became progressively whiter and the urine darker in color. At the same time there was gradually developing palpable hepatomegaly (Figs. 132 and 133). Eventually the liver

Figure 132. Liver scans with ^{99m}Tc-technetium of a patient (OT 14) who developed an indolent rejection that could never be completely reversed. 4 days: The isotope is not very well concentrated. However, there was no evidence of rejection. 9 days: With the onset of an indolent rejection there was no striking change in liver size. 16 days: The rejection persisted. Definite swelling of the homograft is now obvious, especially on the lateral view. 10 weeks: The dimensions of the transplant had nearly returned to those first observed. Subsequently the patient developed a very chronic late rejection and, eventually, striking shrinkage of the transplanted organ became evident (Fig. 182, Chapter Seventeen).

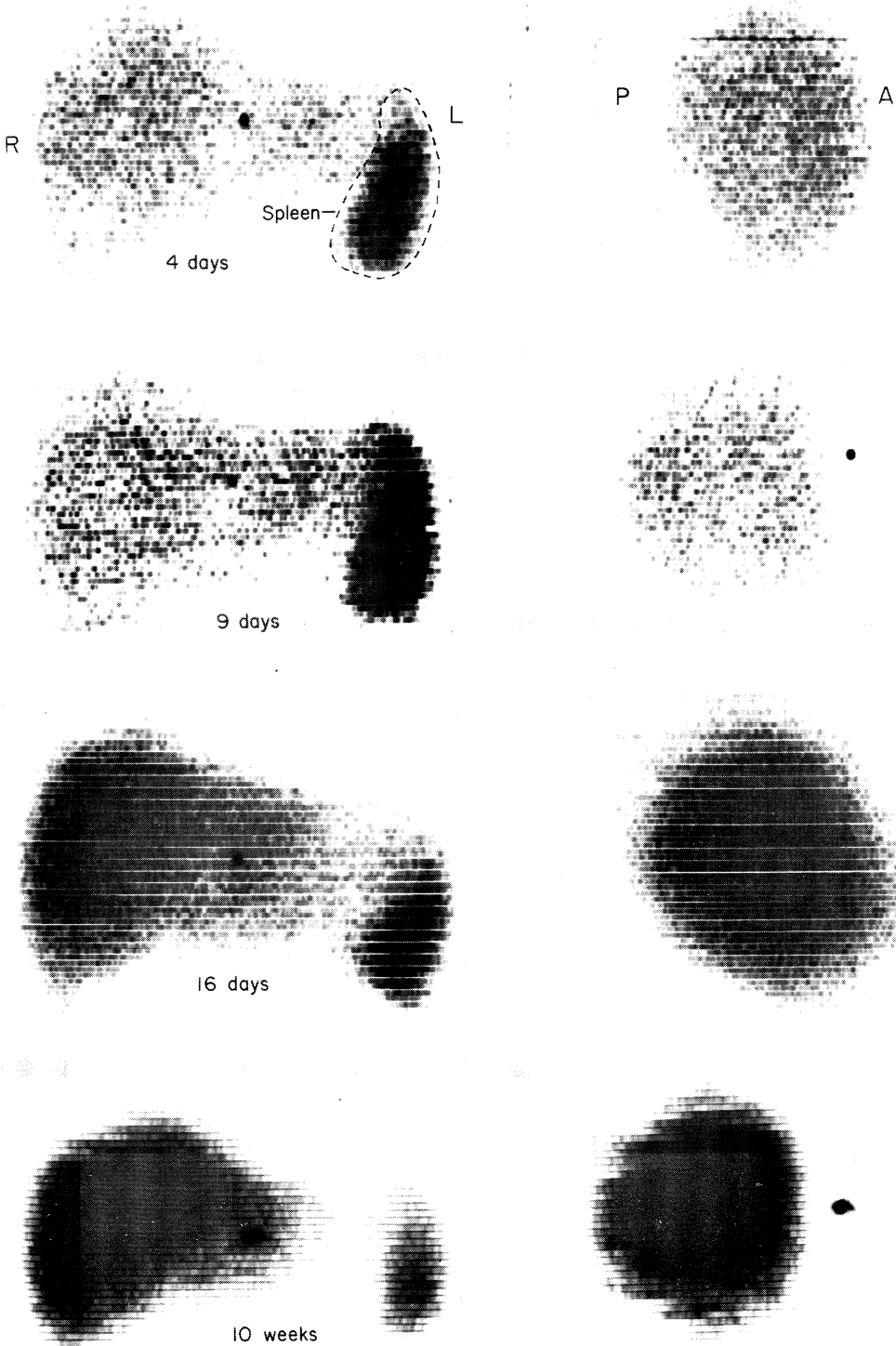


Figure 132. See opposite page for legend.

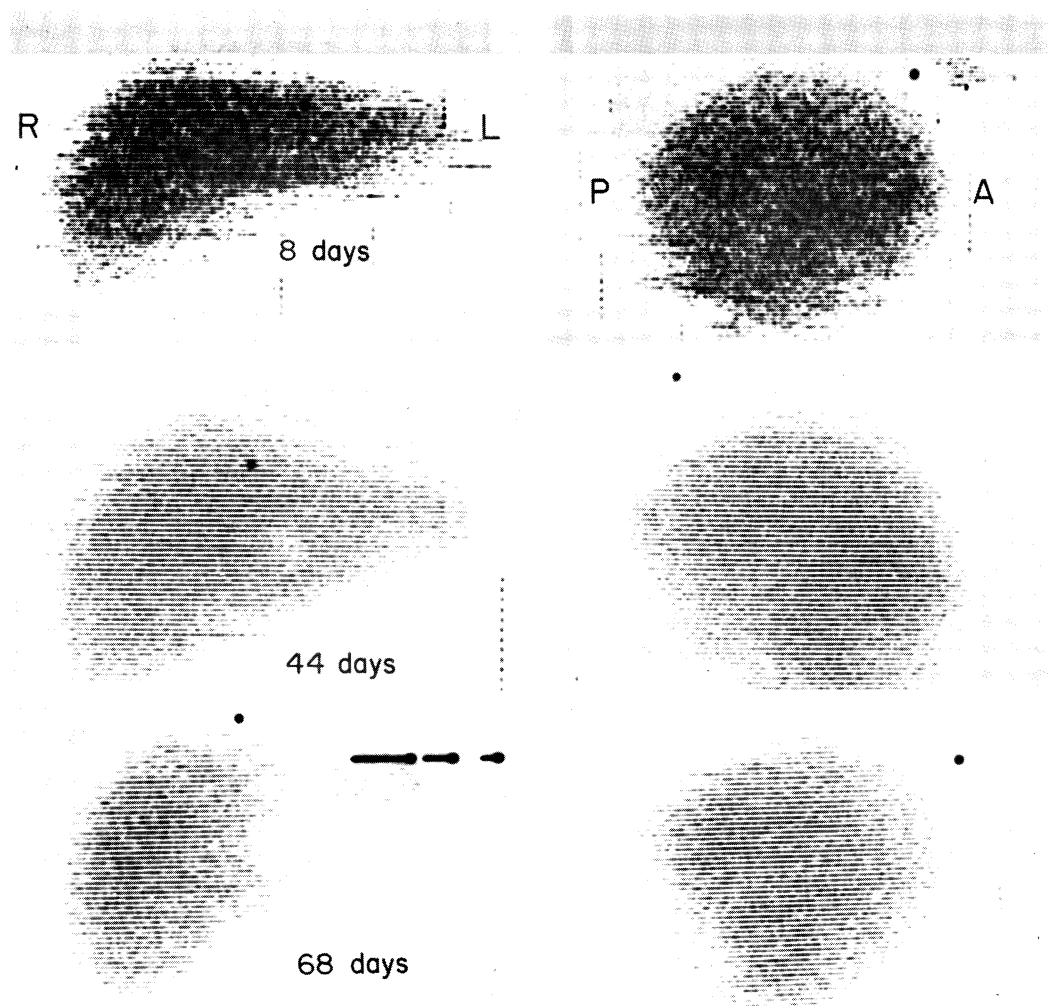


Figure 133. Liver scans performed with ^{99m}Tc -technetium after the first orthotopic liver transplantation in patient OT 16. 8 days: In this patient the first scan was obtained on the day after operation (Fig. 7, Chapter Five). By eight days organ swelling had already started, but the isotope concentration was essentially normal. 44 days: The marked hepatomegaly and the reduced concentration of isotope are evident. 68 days: The liver appears smaller than at 44 days. In actuality it was probably even larger since, when it was removed later the same day, it weighed 880 gm. Note the large patches of radiolucency in the left lobe. At the time of homograft excision these areas as well as the right portion of the liver contained multiple small infarcts (Fig. 138). For several weeks before the retransplantation the child had almost continuous high fever.

enlargement became at least as pronounced as in the patients with subclinical or explosive rejections. The swelling could be only incompletely documented on the liver scans in one case (OT 16A) since eventually there was a loss of isotope concentration. Shown in Figure 133 is the changing appearance of the first homograft in this patient. The last examination at 68 days was obtained shortly before the organ was excised and replaced. The size of the radiographic shadow looked very much smaller than the surgical specimen proved to be at the time of its removal. There were other abnormalities on the scans which will be discussed later.

This child, who received two homografts, had extensive venous collaterals on the surface of the abdomen and lower chest prior to both transplantations. After each procedure the abnormal vascular pattern disappeared transiently only to return with the onset and persistence of rejection. The infant also had intermittent mild generalized edema. Neither he nor the other patient (OT 14) ever developed detectable ascites.

Hyperpyrexia was not a prominent early feature of the indolent rejections. The temperature of the older patient (OT 14) remained normal as the icterus deepened. The infant (OT 16) had intermittent low grade fevers for several weeks after the first homotransplantation. However, he then became toxic and almost continuously febrile just before the removal of the organ. There was prompt defervescence with receipt of the second homograft, followed later by a return of a low grade fever.

Laboratory Studies. When the syndrome of indolent rejection was first observed, it was assumed that the process would be relatively self-limited. There were two reasons for this: first, the patients did not immediately become ill; second, the degree of deterioration in liver function occurred gradually. Initially the abnormalities were mainly those which are commonly found with incomplete biliary tract obstruction. The serum bilirubin, which rose slowly and irregularly over a period of many days, had an exceptionally high glucuronide fraction which was almost invariably 75 per cent of the total (Figs. 134 and 135). Paralleling the insidiously deepening icterus were rises in the alkaline phosphatase (Figs. 134 and 135).

There was no reason to suspect massive, or even moderate, hepatic necrosis from the transaminase determinations. The SGOT and SGPT were transiently elevated immediately after operation, presumably due to ischemic injury. They did not rise again to extreme levels as the jaundice progressed. The greatest abnormalities were in case OT 14 (Fig. 134) in which there was a dissociation between the SGOT's which underwent very minimal changes and the SGPT's which rose to a maximum of 700 International Units (normal 5 to 35). The child who received two homografts did not have early increases in either kind of transaminase to much more than 200 International Units.

An additional paradox was noted in that the complex functions of synthesis were exceptionally well preserved for long periods. For example, the patient who required eventual retransplantation had improving total serum protein concentrations for many weeks as his condition deteriorated (Fig. 135). He maintained a prothrombin time of greater than 80 per cent until the day the extensively damaged transplant was removed. The only severe hypoproteine-

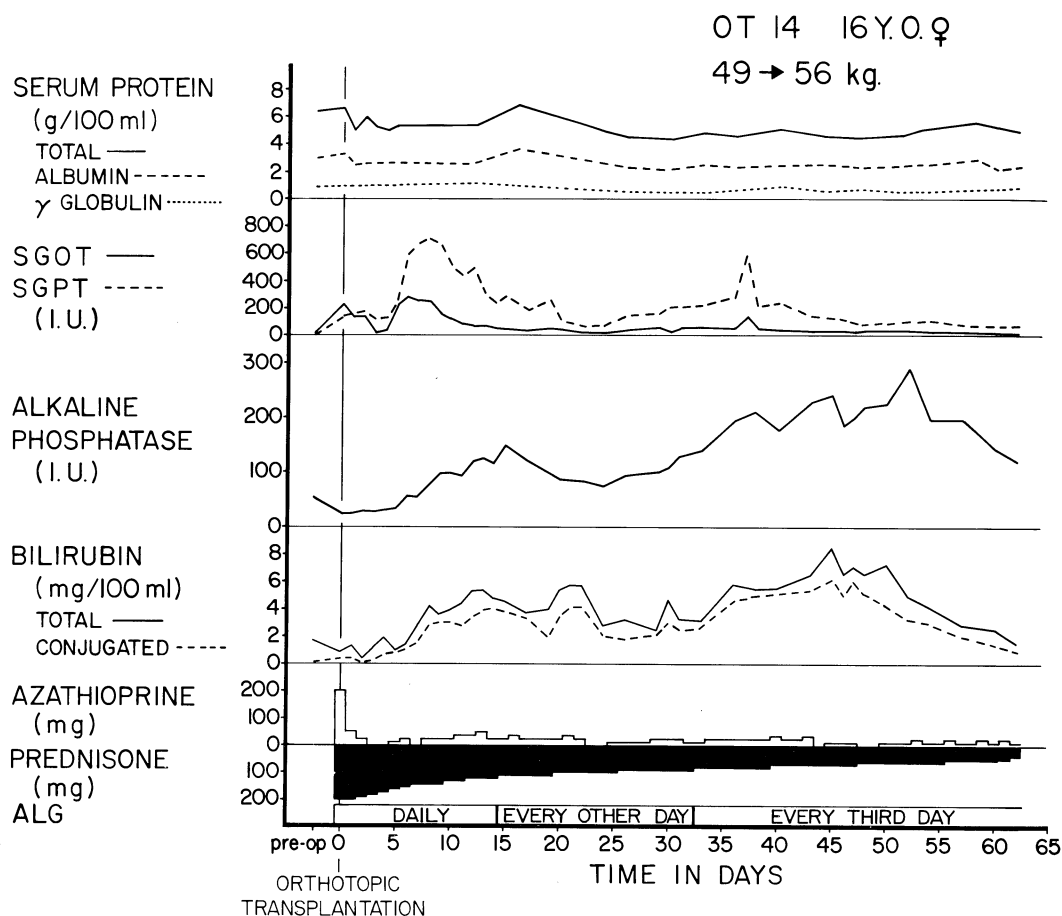


Figure 134. The early course of a 16 year old girl (OT 14) who received orthotopic transplantation for the treatment of a hepatoma. She developed an indolent rejection which lasted for almost two months before remission was obtained. Note the hypoproteinemia that appeared several weeks after rejection had started.

mia within the first six weeks was in the 16 year old girl. Her serum protein concentration fell below 5 gm per cent one month after transplantation, three weeks after the onset of the rejection. There were no striking early changes in gamma globulin although this fraction increased to 2 gm per cent in one patient after about two months (Fig. 134).

The hematologic changes were generally similar to those in the patients described earlier who had explosive rejections. The child who received two livers had a leukocytosis on both occasions. However, the teen-age girl had virtually no white cell response. Thrombocytopenia was seen in both cases but not in particular relationship to the rejections. Anemia did not develop.

Treatment. Antibiotic therapy was maintained for a longer time than usual because of the fear that focal hepatic necrosis might occur with the protracted rejections. As will be described later, focal infarcts did in fact develop in the homograft that was eventually removed.

The other aspects of general care were no different than those described

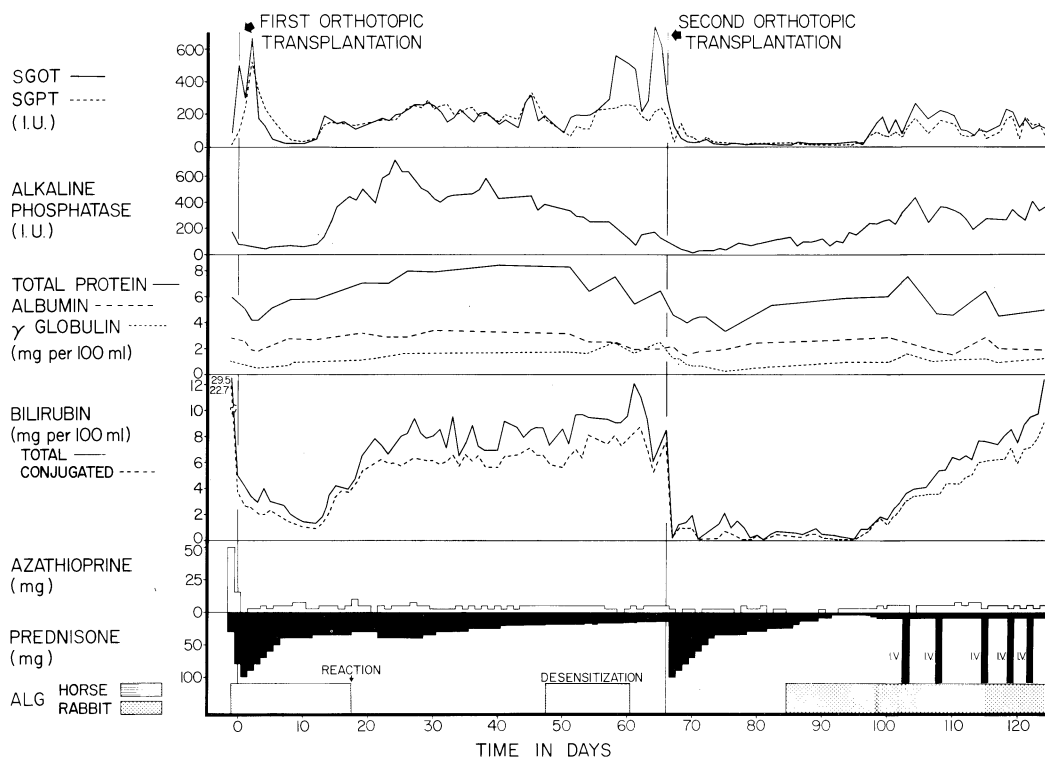


Figure 135. A slowly developing rejection in patient OT 16 which led to the destruction of the homograft. The patient was a two year old child with extrahepatic biliary atresia. It was necessary to stop horse ALG after 18 days because of local reactions at the injection sites. The progressive jaundice then developed. Other measures of liver function were well maintained for many weeks. However, the child then became febrile and extremely ill. Desensitization was attempted with the hope of resuming horse ALG treatment, but it was eventually abandoned. Note the hypergammaglobulinemia toward the end of the second postoperative month.

earlier. However, the details were extremely complicated in case OT 16 after the second transplantation. Most of the problems resulted from the transfer of a liver from a seven year old donor to the two year old recipient. After operation the consequent abdominal overcrowding made it difficult for the recipient to breathe and almost impossible for him to eat for several postoperative weeks. The situation was also later aggravated by the swelling of the homograft that occurred with onset of a rejection which fortunately was delayed for a month.

Eventually the child was able to resume a full diet. Thereafter anorexia was never a significant problem. On the contrary it was later necessary to prescribe a diet in order to prevent serious obesity. The same restrictions of caloric intake were imposed on the older recipient as well.

The older of the two patients received triple drug immunosuppressive therapy for more than two months. ALG was finally stopped after 73 days because of a suspected anaphylactic reaction. The daily doses of all three agents are shown graphically in Figure 134. It should be noted that the quantities of azathioprine that could be given to her without causing leukopenia were very small, averaging less than 0.5 mg/kg/day. On the other hand, steroid doses were kept at levels which have been shown from past experience in renal

transplant patients to be dangerous (see Chapter Thirteen). Even after two months she was still receiving daily doses of 50 mg/day.

A highly significant deviation from the aforementioned regimen was required in the infant who received two livers. It was necessary to discontinue the horse ALG after 18 days because of severe reactions at the injection sites. It was almost immediately after this that the indolent rejection began its inexorable course. An attempt at desensitization (Chapter Thirteen) was made but ultimately abandoned.

When retransplantation was undertaken, ALG raised in another species was not yet available. Almost three weeks after the second operation a course of rabbit ALG was started and continued for the next six and a half weeks. A final switch was then made to goat ALG for three and a half months. Eventually therapy with immune globulin had to be discontinued because of the suffering that attended every injection.

It was necessary to maintain very high doses of steroids after the first transplantation, probably because of the inability to continue ALG. Following the second operation, the quantities of prednisone were cut more quickly. As soon as a maintenance schedule of 5 to 10 mg/day had been established, intermittent single boosters of 100 mg were given intravenously every five to 20 days. In this patient as in OT 14, it was never possible to consistently exceed an azathioprine dose of more than 0.5 mg/kg/day without causing leukopenia.

Reversibility. In the final analysis, the fate of these patients became dependent upon reversal of an immunologic disease in which the inability to affect bile drainage seemed eventually to contribute to a slow later deterioration of all other facets of hepatic function. It was well known from histopathologic studies of homografts in animal experiments that the cause of the obstructive jaundice was apt to be found within the lobules of the liver (Chapter Twenty). Nevertheless, efforts were made in the clinical cases to prove the patency of the extrahepatic ducts. Operative cholangiography was finally resorted to (Chapter Seventeen) in one of the patients (OT 14). In both recipients, nonoperative diagnostic tests were obtained.

It was possible to visualize the cholecystoduodenostomies with gastroduodenal x-rays after a barium meal. The dye passed through the anastomosis and more or less completely outlined the gallbladder (Figs. 136A and B). It did not regurgitate into the common duct, but from time to time air could be detected in the major or minor radicles (Figs. 136B and C). The only conclusion that was possible was that the distal segment of the reconstructed biliary tract was open. Intravenous cholangiography was also attempted. Good visualization was not obtained because of poor dye concentration in these jaundiced patients.

The use of ^{131}I -rose bengal liver scanning⁵ yielded equally indecisive results. This isotope is processed by hepatocytes and, except for a normally minor extrahepatic "leak," is excreted through the minor and major biliary duct radicles into the small bowel. It has been reported^{14, 15, 30} that high grade intrahepatic retention of the isotope is a diagnostic sign of biliary obstruction. The scans obtained with this method revealed prolonged trapping of the isotope in the homografts (Fig. 137), but it could not be determined whether the site of the block was intra- or extrahepatic.

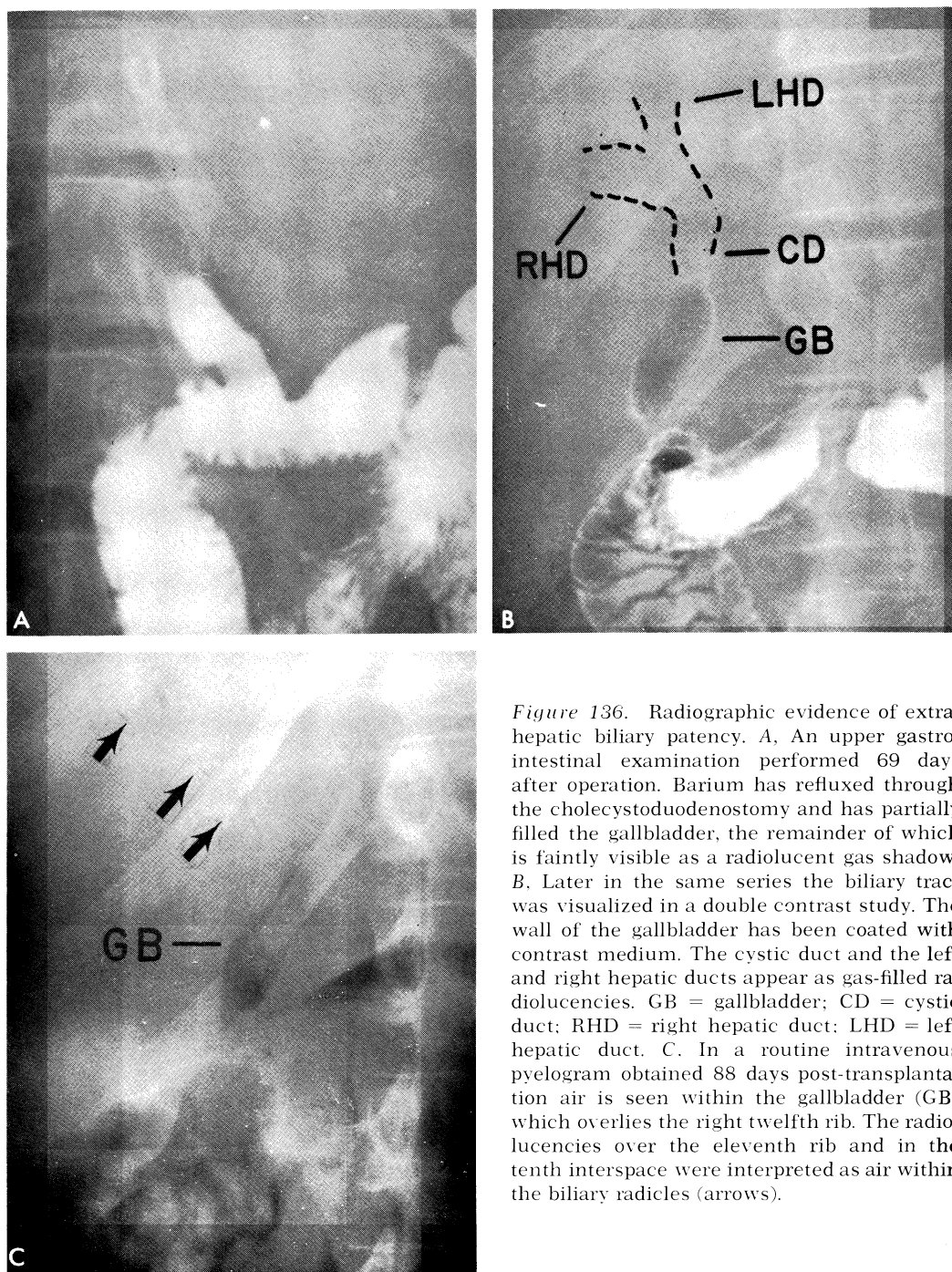


Figure 136. Radiographic evidence of extra-hepatic biliary patency. A, An upper gastrointestinal examination performed 69 days after operation. Barium has refluxed through the cholecystoduodenostomy and has partially filled the gallbladder, the remainder of which is faintly visible as a radiolucent gas shadow. B, Later in the same series the biliary tract was visualized in a double contrast study. The wall of the gallbladder has been coated with contrast medium. The cystic duct and the left and right hepatic ducts appear as gas-filled radiolucencies. GB = gallbladder; CD = cystic duct; RHD = right hepatic duct; LHD = left hepatic duct. C, In a routine intravenous pyelogram obtained 88 days post-transplantation air is seen within the gallbladder (GB) which overlies the right twelfth rib. The radiolucencies over the eleventh rib and in the tenth interspace were interpreted as air within the biliary radicles (arrows).

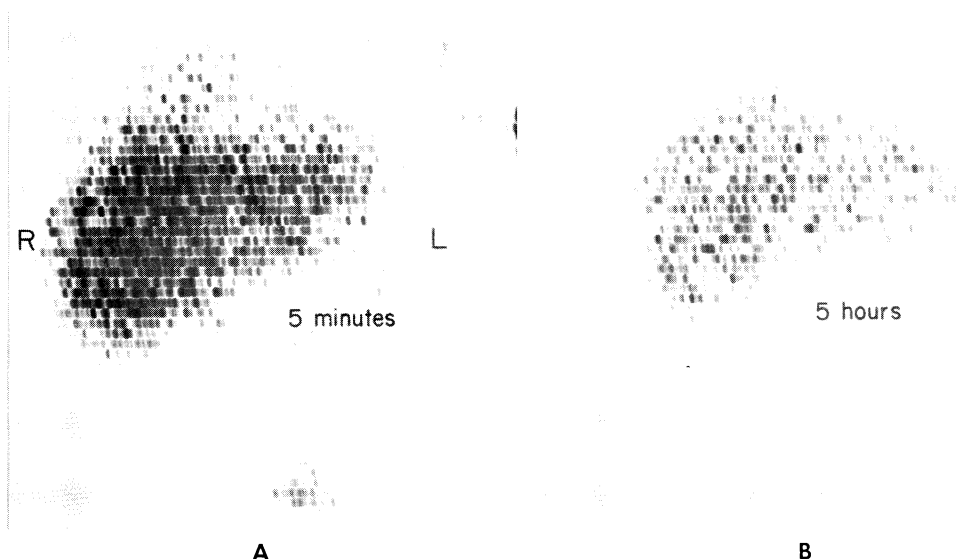


Figure 137. A ^{131}I -rose bengal scan in a patient whose liver homograft underwent an indolent but inexorable rejection, eventually necessitating retransplantation (OT 16). A, Five minutes following intravenous injection of the isotope. The examination was performed 38 days after orthotopic transplantation. The liver concentrates the rose bengal well. B, Five hours after the rose bengal injection. Much of the isotope is still entrapped in the homograft and very little is detectable in the intestine. The delayed scan was performed in an effort to prove the patency of the extrahepatic biliary tract.

It was eventually concluded that a very persistent and relatively nonreversible form of rejection was responsible for the jaundice. Remission was achieved in only one instance (OT 14) and then only incompletely. In this case, the icterus persisted somewhat variably for almost two months before it significantly lightened (Fig. 134). During all this time the SGPT and alkaline phosphatase remained slightly elevated even though the SGOT was usually essentially normal. The SGPT and alkaline phosphatase levels began to fall slowly several weeks before the alleviation of the hyperbilirubinemia.

As the weeks went by, the patient gradually developed muscle weakness which was attributed to the continuing high dose steroid therapy. Otherwise, she did not seem to be particularly ill. When the indolent rejection very slowly reversed, the hepatomegaly partially receded. She was discharged from the hospital on the 68th postoperative day.

Neither of the transplants in the other patient had a delayed improvement in function. Once jaundice had started it progressively increased until a plateau was reached. Increases in the transaminases also became fixed at a slightly elevated day-to-day level (Fig. 135). The alkaline phosphatases began to fall after the first postoperative month.

The most important difference between the two recipients was that the child who ultimately required retransplantation became violently ill with hyperpyrexia, tachycardia, and profound dyspnea. At frequent intervals during the two weeks preceding reoperation he had many episodes of transient cyanosis and appeared to be moribund. During the same period hepatic function began to deteriorate further. After two months there were striking increases in

the serum transaminases for the first time. Moreover, hypoproteinemia then began to develop (Fig. 135).

The extreme swelling of the homograft in this case was mentioned earlier. Now, another finding appeared which suggested that the prognosis had become hopeless; on the liver scan a large portion of the left hepatic lobe failed to visualize (Fig. 133). This was interpreted as a beginning devitalization of this area of the liver which was progressing despite the most intense immunosuppression that seemed compatible with recipient survival. The radiographic findings were very similar to those described earlier during the explosive kind of rejection. However, it was concluded that reversal of the process and restoration of an adequate blood flow to the endangered region could not be expected. Septicemia secondary to the impending hepatic gangrene was anticipated to be the next complication. Before this occurred, another organ became available and retransplantation was carried out.

The rejection of the second transplant followed very much the same pattern as that of the first, although it was less severe. The jaundice which was temporarily relieved became stable between 15 and 20 mg per cent and persisted for many months without the slightest indication of receding (Chapter Seventeen). Other measures of liver function were adequate although not normal.

Retransplantation. By the time it was decided to replace the homograft in case OT 16, the child was gravely ill, although not primarily from liver failure. The reason for the toxic syndrome became immediately evident when the old incision was reopened and the abdominal cavity entered. The liver was enormously enlarged. Its rounded inferior surface protruded through the wound. The entire presenting surface of the organ was mottled (Fig. 138A). There were many cyanotic areas interspersed with islands of pink tissue. The discoloration affected both lobes but was most severe on the left side in about the same location as the poorly visualized region identified with a scan the day previously. It was suspected that there were many foci of fresh infarction. At that time the transplant had been in residence in the new host for a full 68 days.

After its removal, the homograft weighed 880 gm compared to its estimated weight of 250 gm at the time of the original procedure. The impression of multiple infarctions was confirmed when the organ was transected (Fig. 138). Some of the necrotic areas appeared to have been devitalized very recently, whereas others seemed to be older. Both varieties of infarcts tended to be near the surface. The main hepatic artery, the portal vein, and the major divisions of both vessels were patent and contained no clots whatsoever. Far distally within the liver there were very tiny but easily visible clots in some of the terminal portal ramifications. The extrahepatic duct system was patent from the cholecystoduodenostomy as far into the hilum as the ducts could be traced. The histopathologic findings are reviewed in Chapter Twenty.

Technically, extirpation of the rejecting organ was not very difficult. Adhesions had formed between its capsule and the diaphragm but these could be stripped away with blunt dissection. Similarly the vessels in the hilum were easily reisolated. The new organ was placed with the same techniques as those described in Chapter Eight with one possibly significant deviation. When the



Figure 138. A rejecting homograft in patient OT 16. The badly damaged and swollen organ was removed 68 days after its transplantation. A. The protruding homograft as it was seen when the transverse abdominal incision was reopened. Note the enormous swelling, as well as the heterogeneous appearance of its surface. B. After extirpation and slicing, gross subcortical infarcts (lighter colors) were easily seen.

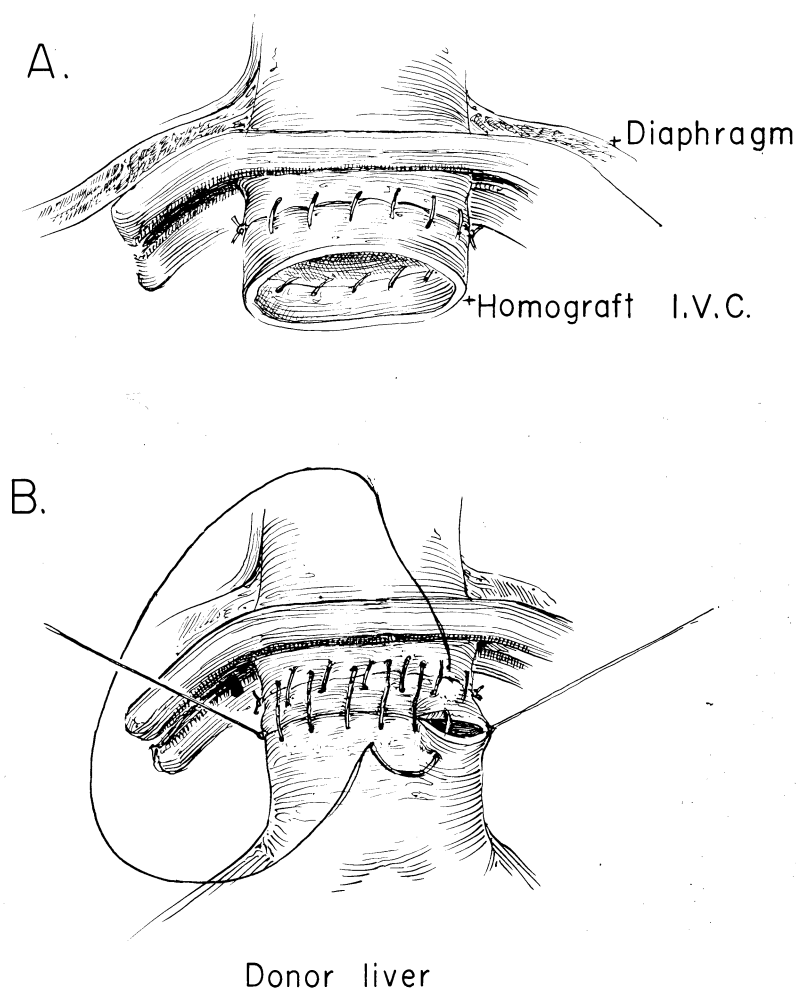


Figure 139. Technique of anastomosis of the suprahepatic inferior vena cava at the time of retransplantation in patient OT 16. A, After cross clamping of the recipient inferior vena cava, the vessel was transected below the original suture line, thereby leaving a cuff of the original homograft. B, The suprahepatic vena cava of the new homograft was anastomosed to the recipient vessel, incorporating the cuff of the retained vena cava in the suture line. The technique used was designed to prevent the sacrifice of vena caval length.

upper vena cava was cross clamped just below the diaphragm, it was obvious that the cuff was excessively short. Consequently the original suture line was left with the patient. When the new anastomosis was performed, the old suture line was incorporated in the freshly formed one (Fig. 139). In this way, the length of the host vena cava was not unnecessarily sacrificed.

Prognosis with Indolent Rejection. It will already have become evident that the ultimate course of these two patients was difficult. The rejection was temporarily controlled and reversed in the teen-age girl. However, within a few weeks after discontinuation of ALG therapy, the process recurred in an even more severe form (Chapter Seventeen). Nevertheless, the homograft supported life for more than a year.

The same kind of chronic course was seen in the infant after the second transplantation. He lived in a jaundiced state for many months. His late fate will be returned to in Chapter Seventeen.

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Chapter Fifteen

ACUTE REJECTION AND HEPATIC GANGRENE

In the preceding chapter two examples of rejection were cited that apparently nearly resulted in devitalization of discrete fractions of orthotopic hepatic homografts. It was suggested that the etiology of the threatened necrosis was an immunologically mediated reduction in the blood flow to the transplanted livers and that the most affected portions of the organs became differentially susceptible to invasion by bacteria from the gastrointestinal tract. The dire sequelae that would have been expected if frank gangrene had followed were circumvented either because the process initiating the ischemia spontaneously reversed (OT 15) or because the stricken homograft was removed and replaced (OT 16).

An extension of these events will now be described as they were observed in five consecutive earlier cases (OT 8 to 12) in which orthotopic hepatic homografts actually did undergo regional necrosis. The five recipients were the first humans to survive for long periods after this kind of operation,^{16-21, 23} and the reasons for the complication were anything but clear at that time. Later it was concluded, partly because of the greater insight provided by the further experience documented in Chapter Fourteen, that a primary element in the pathogenesis had been incompletely controlled rejection. In addition, evidence was uncovered that contributory mechanical factors determined, at least in part, the localization of the gangrene.^{15, 20}

It was not anticipated from the animal experimentation described in Chapters Eleven and Twelve that liver infarction and secondary infection would be such a major deterrent to success in the clinical trials. Nevertheless, it was appreciated in advance that homograft sepsis might be a special problem for several reasons. First, the graft duct system must be exposed to the endogenous flora of that portion of the gastrointestinal tract to which it is anastomosed. Second, a liver which receives its portal inflow from the nonhepatic splanchnic bed may be directly inoculated with bacteria to the extent, whether great¹⁴ or small,¹² that microorganisms are present in the intestinal venous effluent. Finally, the reticuloendothelial element of the liver homograft, which is also

under immunologic attack, is thought to be normally an important and effective bacterial filter.^{2, 4, 10} The degree to which this function might be lost, either because of hepatic rejection or because of the immunosuppression given to prevent this process, could be expected to influence the efficiency of host defense against both local and systemic infection.

THE SYNDROME OF SEPTIC INFARCTION

The consequences of septic hepatic infarction were characteristic^{7, 8, 19, 20} and resulted in three clinical or laboratory findings that together were absolutely diagnostic. The components of the triad were gram negative septicemia, evidence from transaminase determinations of massive liver necrosis, and the development on serial liver scans of large areas of persistently absent isotope concentration in the homograft.

Symptoms and Signs

The complication of regional hepatic gangrene occurred in children who were 13 to 20½ months old at the time of transplantation. The young age distribution may have had no more profound significance than the fact that only children were treated with orthotopic liver transplantation during this era of our experience. The diagnosis was made 24 (OT 8), two (OT 9), 25 (OT 10), 52 (OT 11), and 104 (OT 12) days after operation.

All but one of the patients had previously been in good condition, although variable fevers had been observed in each recipient for several days or even weeks before the onset of obvious liver necrosis and septicemia. Thereafter, their temperatures rose to very high levels (Figs. 140-143) and they were prostrated. Hypotension was always observed, either in a transient or more persistent and serious form.

There were few other diagnostic clues from observation or examination. The children all became extremely irritable and had labored respirations. One (OT 12) stopped breathing altogether and would have died if temporary ventilator support had not been instituted; a sample of her peripheral blood drawn at the time of the apnea was later found to have a glucose content of 0 mg per cent. This child and another developed repeated retching and vomiting. All five recipients appeared to be moribund within a few hours.

Bacteriologic Findings

Invariably bacteria could be found in the peripheral blood. Persistently positive blood cultures were first documented in four of the five patients coincident with or just after the moderately sudden onset of severe hyperpyrexia (Figs. 140-142). In the other patient (OT 12), who also had a pre-existing mixed

(Text continued on page 314.)

OT 8
9.6 → 9.7 kg.

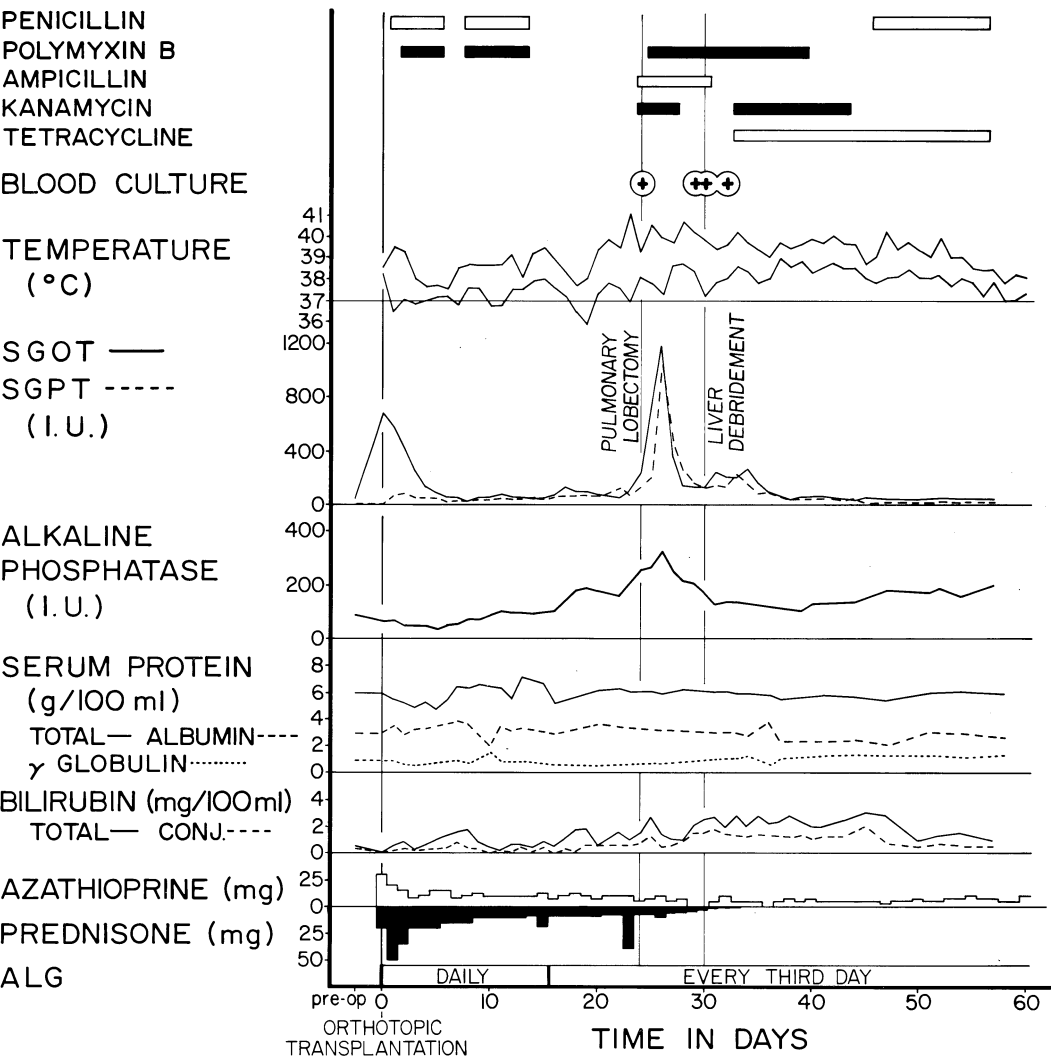


Figure 140. The first 60 post-transplantation days in patient OT 8. During the third postoperative week there was evidence of an “anicteric” rejection, but the significance of the function changes was not appreciated at the time. The lung resection was carried out because the right upper lobe was collapsed (see Chapter Nine) and it was suspected that this was the source of the unexplained fever. In retrospect, the pulmonary lobectomy was probably not indicated. One day later the definitive evidence of the septic hepatic infarction had appeared. All the positive blood cultures were of *Aerobacter-Klebsiella*. This patient was the first to survive for a prolonged period after human liver transplantation. The indication for operation was hepatoma. The late course will be described in Chapter Seventeen.

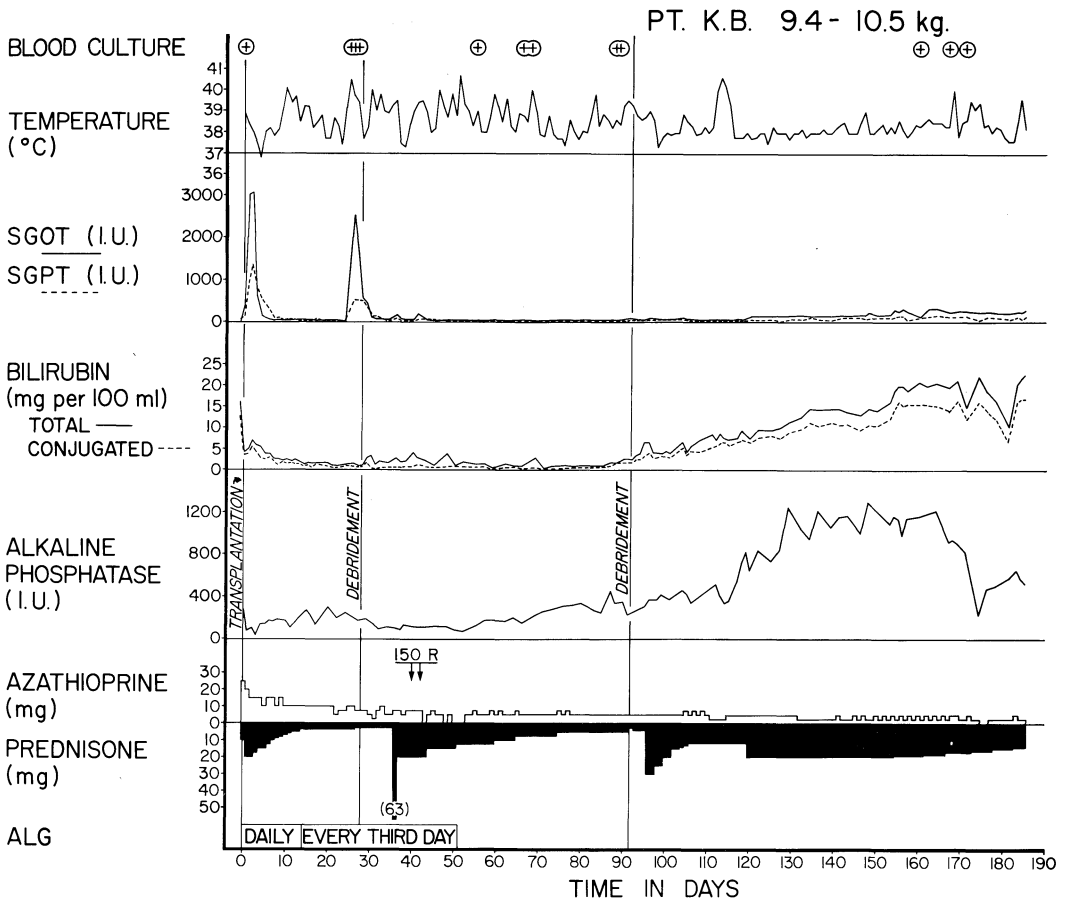


Figure 141. The course of patient OT 10 after orthotopic liver transplantation for extrahepatic biliary atresia. Homograft function prior to the onset of a septic hepatic infarction on the twenty-fifth day was excellent; the only consistent abnormality was an elevated alkaline phosphatase. Even after the infarctions destroyed large portions of the central and right lobar liver tissues, function remained good until the end of the third postoperative month. Thereafter, liver failure was progressive. Note the late parallel increases in alkaline phosphatase and bilirubin. The immediate cause of death was intraperitoneal rupture of an undrained residual abscess of the left lobe. Survival was 186 days. Liver irradiation was with 150 R depth dose at each arrow. The temperatures are the daily maximum. At autopsy the right hepatic artery was the site of a completely occlusive old thrombosis. (By permission of *Ann. Surg.* 168:392, 1968.)

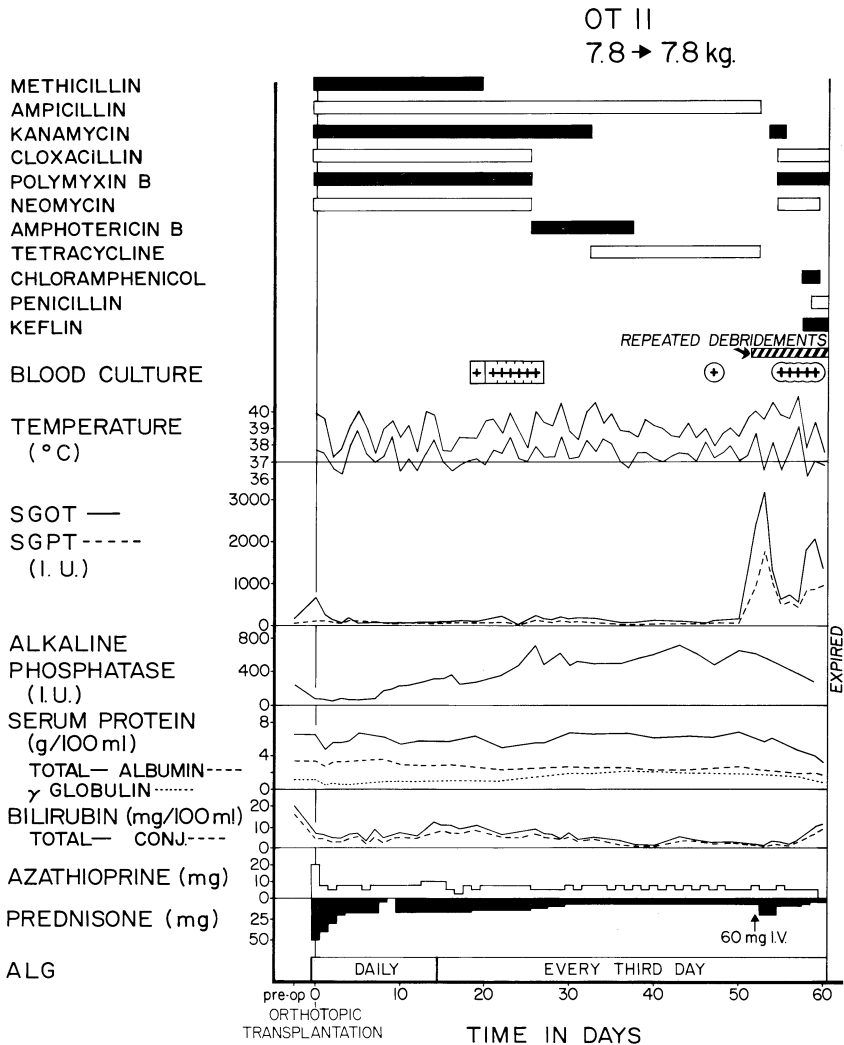


Figure 142. A child (OT 11) who developed a rapidly fatal septic hepatic infarction which was superimposed upon an indolent rejection of almost two months' duration that was undergoing very slow remission. In the second and third postoperative weeks numerous blood cultures of an unidentified yeast were obtained (+'s in squares). The source of the microorganisms was never determined, but the fungemia cleared with amphotericin B therapy. With the onset of the regional hepatic gangrene, gram negative organisms were found in the peripheral blood (+'s in circles). At autopsy the right liver lobe was necrotic and the right hepatic artery was thrombosed.

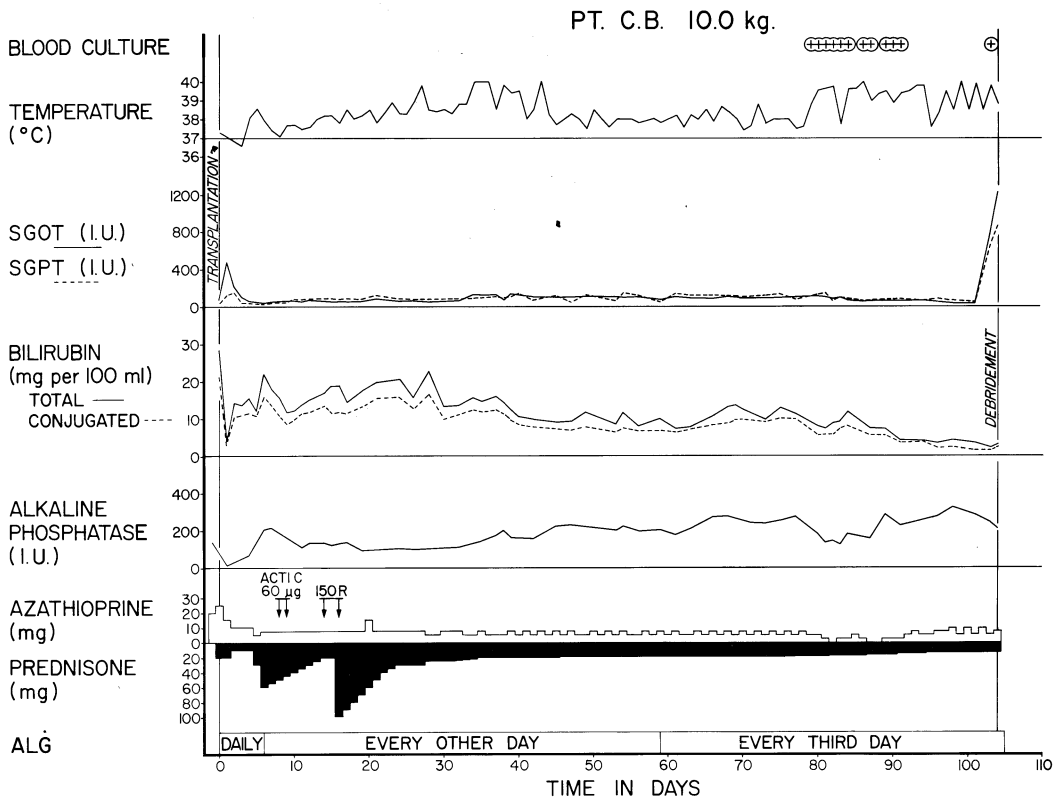


Figure 143. Course in patient OT 12 after orthotopic liver transplantation for extrahepatic biliary atresia. A vigorous and protracted rejection began postoperatively within a few days and was not reversed for 10 weeks. Function was improving when persistent gram negative septicemia presaged liver sepsis. Complete right lobar infarction finally occurred, causing death within a few hours. At autopsy the right hepatic artery was thrombosed. Acti C=intravenous actinomycin C in micrograms, 150 R=homograft irradiation. (By permission of Ann. Surg. 168:392, 1968.)

bacterial, protozoal, and viral pneumonitis (Chapter Sixteen), gram negative bacteremia presaged the impending regional hepatic gangrene for more than two weeks (Fig. 143).

The microorganisms collected from the blood stream in these five patients at the time of septic hepatic infarction are shown in Table 20. They were all the type normally found in the intestinal tract. In one case (OT 12), two species were identified. Antibiotic agents were given according to the sensitivities determined from the cultures.^{7, 19, 20} In the three patients who survived at least temporarily, such therapy was necessary on a continuing basis and undoubtedly contributed to the high incidence of extrahepatic infections from opportunistic organisms, as discussed in Chapter Sixteen.

RELATION TO REJECTION

The occurrence of regional liver gangrene in these five consecutive patients made it appear for a time that the operation of orthotopic liver transplantation was fundamentally unsound and that further attempts at its application for the treatment of human disease should not be made. The most compelling justification for not accepting this point of view was that the feasibility of the undertaking had already been established in animals. It would have required an extraordinary pessimism to believe that the same objective could not be achieved in man.

Nevertheless, the dismay with which the septic hepatic infarctions were viewed was heightened by three exceptionally disquieting features. First, partial homograft gangrene was seen without fail in each of the five first patients who lived for more than a few weeks after transplantation. Second, it was also observed in at least one other institution by an independent team that employed a significantly different orthotopic procedure.⁶ Finally, any explanation for the deadly complication had to be considered highly speculative at that time. Although it was suggested from the beginning^{19, 20} that incomplete control of

Table 20. The Bacteria Cultured from the Blood Stream at the Time of Septic Hepatic Infarctions

NO.	TIME OF DIAGNOSIS (HR)	ORGANISM
OT 8	24	Aerobacter-Klebsiella
OT 9	2	Bacteroides fragilis
OT 10	25	Aerobacter-Klebsiella
OT 11	52	Escherichia coli
OT 12	104	Aerobacter-Klebsiella Escherichia coli

rejection must be one responsible factor, evidence in support of this contention from several indices of liver function was initially thought to be weak.

In retrospect, such an equivocating position need no longer be taken. As the later experience recounted in Chapter Fourteen accumulated and the distinctive patterns of rejection were delineated under less confusing circumstances, it became increasingly clear that all the homografts that underwent regional necrosis had contemporaneously been under immunologic attack. Furthermore, failure to recognize this fact at the time the patients were being cared for led to the error of minimizing immunosuppression in several of these cases at precisely the moment when this therapy should have been intensified (Figs. 140 and 141). The underlying rejections were highly variable in their antecedent manifestations.

Subclinical Rejection

Three of the recipients (OT 8, 9, and 10) probably belonged in the category of bland rejection described in Chapter Fourteen. However, only two will be analyzed in this section. In the third (OT 9), two arteries of the homograft were individually anastomosed to the host right and left hepatic arteries (Fig. 52, Chapter Eight). The smaller of the vessels which supplied part of the right lobe apparently clotted about two days later. It will have to be assumed that the accident was due to a technical error in the performance of the tiny anastomosis, although this evaluation may be incorrect since right hepatic arterial clotting regularly occurred in subsequent cases despite more favorable anatomic situations.

The two patients to be considered had extraordinarily smooth early postoperative courses. One of the children (OT 8), who was not previously jaundiced, did not become icteric after transplantation (Fig. 140). The elevated serum bilirubin concentration in the other (OT 10) promptly fell to near normal (Fig. 141). Serum proteins and all clotting factors were well maintained.

Nevertheless, both recipients had a number of physical and biochemical findings which would now be regarded as significant but which then did not cause alarm. Their new livers became sufficiently enlarged and firm so that the alterations were quite evident by palpation; from time to time the children seemed to be suffering abdominal pain. Persistent or remittent fever developed. There were small but recurrent elevations in the serum transaminases and a slow but steady rise in the alkaline phosphatases (Figs. 140 and 141).

The changes made so little impression that adjustments in the immunosuppressive regimen were not even seriously considered. The failure to react was doubly tragic since the doses of steroids given in the days preceding the actual infarctions would now be considered to be dangerously low. This can be illustrated by comparing the average daily quantities of prednisone given these two patients for the first 24 postoperative days (1.27 mg/kg) with those administered during the same time (2.9 mg/kg) to the two more adequately treated

recipients with the same kind of subclinical rejection whose early convalescence periods were described in Chapter Fourteen.

Another reason why septic hepatic necrosis was not immediately identified as a consequence of rejection in patients OT 8 and 10 was our failure even after its onset to observe acute deterioration in most kinds of liver function. As already mentioned, there always followed very dramatic and sudden increases in the SGOT and SGPT (Figs. 140 and 141). However, this was a relatively dissociated finding. Jaundice did not promptly develop, although there were slight rises in the serum bilirubin concentrations in both patients (Figs. 140 and 141). Moreover, the patients maintained their serum proteins and prothrombin times at about the same levels as had been present before (Fig. 140).

The retention of generally good function in spite of the evidence of major hepatic necrosis was explained by the localized nature of the process as seen on the liver scans (Figs. 144 and 145). Isotope uptake was retained in most parts of the homografts although the concentration was somewhat reduced. In contrast, the afflicted areas, which were predominantly in the right lobe, had essentially no specific activity at all. The devitalized region remained relatively constant in one case (OT 8) (Fig. 144), but it extended very rapidly in the other (OT 10) to several times its original size (Fig. 145).

Since immunosuppression was not increased even at this late time, continued survival of the patients is probably attributable to the inherently self-limited nature of the rejection. Significant changes in treatment were made only after the regional necrosis had already gone to completion. With the subsequent advent of low grade jaundice in the ensuing days or weeks, prednisone was restarted or given in larger daily quantities (Fig. 141). A slow subsequent deepening of the icterus was not thereby prevented, a problem which will be discussed more extensively in Chapter Seventeen.

Persistent Rejection

The evidence that an underlying rejection was present in the other two cases (OT 11 and 12) was not vague. The pre-existing disease in both instances was extrahepatic biliary atresia. After operation there was a prompt but transient alleviation of the jaundice. Within a few days, the serum bilirubin concentration began to rise again (Figs. 142 and 143). Heavy steroid therapy was instituted and continued for weeks or months.

Eventually improvement was obtained, but the reversal was very slow and incomplete in both recipients. The secondary increases in bilirubin began to recede in four to six weeks (Figs. 142 and 143) but they never returned to normal. During the remainder of life the alkaline phosphatases were always

Figure 144. Radioisotope liver scans in OT 8 obtained with ^{99m}Tc -technetium. A. Seventeen days after transplantation. The isotope uptake is diminished but there are no defects. B. Large nonopacifying areas are demonstrable (arrows) 29 days after transplantation in the posterior part of the right lobe. The patient had septicemia. C. Three days later. The necrotic tissue had been debrided. D. The defects are no longer seen 252 days after operation. Note that the liver has progressively increased in size. (By permission of Ann. Surg. 168:392, 1968.)

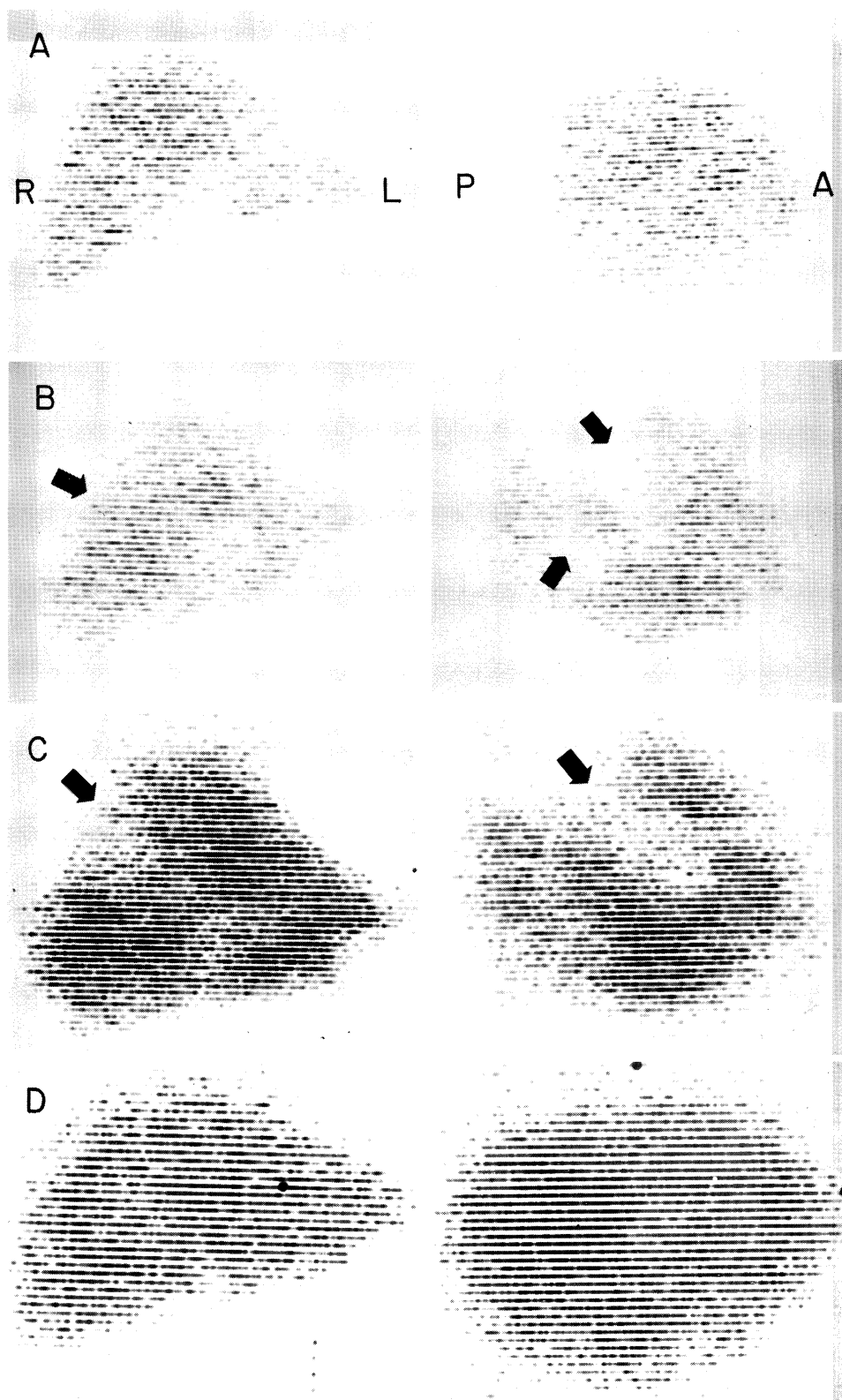


Figure 144. See opposite page for legend.

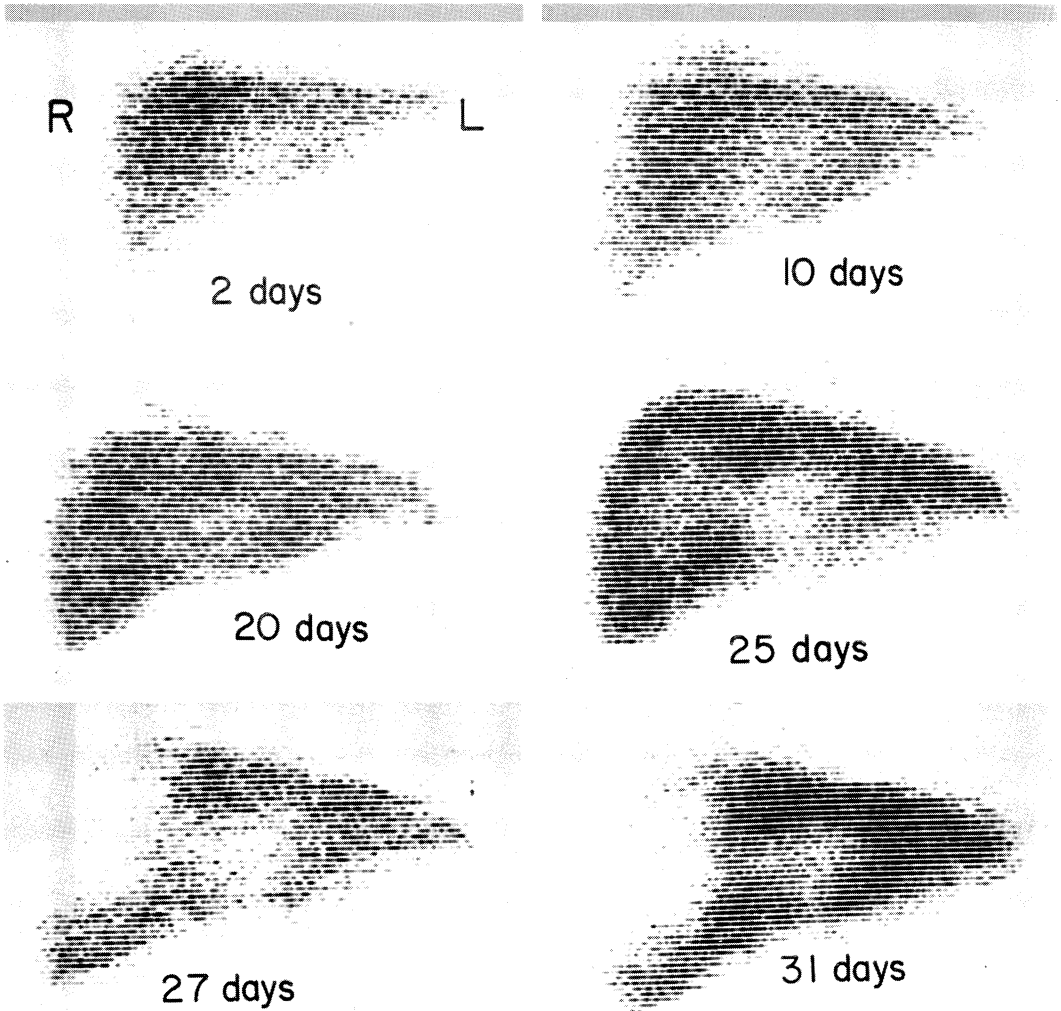


Figure 145. Postoperative technetium scans of the liver in patient OT 10. 2 days: The small homograft is normal. 10 days: An increase in size is evident although the general configuration of the organ is still normal. 20 days: No further change is noted. 25 days: The examination was conducted as an emergency when the child developed gram negative septicemia and very high increases in the transaminases (Fig. 141). Areas of decreased isotope uptake are obvious in the right lobe and the central part of the liver. 27 days: A striking extension of the process can be seen less than 48 hours later. A debridement procedure was carried out the same evening. 31 days: Four days after debridement the radiographic appearance was improved.

elevated and there were minor fluctuations of the transaminases. Intermittent fevers were recorded. The homografts became and remained large (Figs. 146 and 147) and firm.

When regional liver gangrene finally developed, 52 (OT 11) and 104 (OT 12) days after transplantation, its features including the explosive rises in the transaminases (Figs. 142 and 143) were not qualitatively different from when it presented against the background of the milder kind of rejection. However, its evolution was considerably more fulminant inasmuch as the defects on the liver scans expanded with great rapidity. Death followed within a few hours or days. The magnitude of the problem can be appreciated by reviewing the scans in Figure 146. Initially, necrosis involved only the inferior portion of the right lobe; this was debrided under local anesthesia. By the next day the process had begun posteriorly. At the time of death eight days later, virtually the entire right lobe was dead. The unsuccessful attempts at surgical therapy will be described later.

EFFORTS AT SURGICAL TREATMENT

Initial Procedures

Removal or drainage of the infarcted area was attempted shortly after the diagnosis was made. A formal hepatic resection was carried out in one patient (OT 9) eight days after transplantation. Through the original abdominal incision, about half of the anatomic right lobe was removed. Two weeks later a large subphrenic abscess required drainage. This was the patient in whom two arteries supplying the homograft had been individually anastomosed to the recipient right and left hepatic arteries. The excised portion of liver was that which had been supplied by the smaller right artery.

The right lobe of the liver was explored in the other four recipients through incisions designed to avoid entry into the chest or abdomen. These were placed in the tenth intercostal space with subcostal extensions of variable length (Fig. 148). A thin fluid was found in the right subphrenic space and patchy necrotic areas were evident on the surface of the homograft. The dead liver tissue was traced into the interior of the right lobe where ramifying tracks were invariably found. Frank pus was never present. The dead tissue had a yellow cheesy appearance. There was no evident fibrous reaction around the necrotic tracks. The cavities created by the debridement were packed.

Additional Procedures

Two of the five patients (OT 11 and 12) died eight days and 12 hours, respectively, after the initial debridements. The outlook was made hopeless in both cases by rapid extension of the infarctions, which were initially relatively small. As already mentioned, efforts to keep up with the process by repeated debridements in the child who survived for several days (OT 11) resulted in the eventual piecemeal removal of almost all the right lobe.

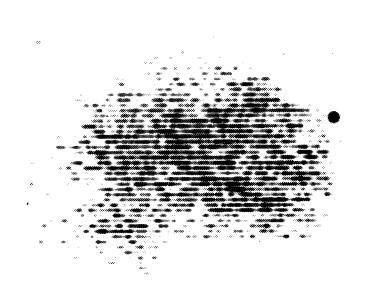
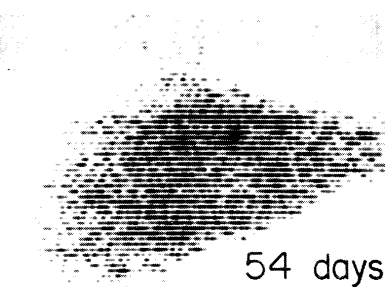
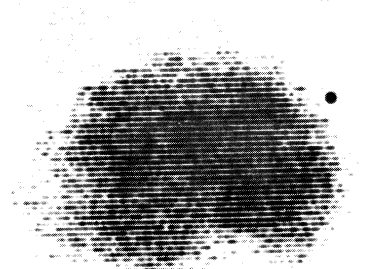
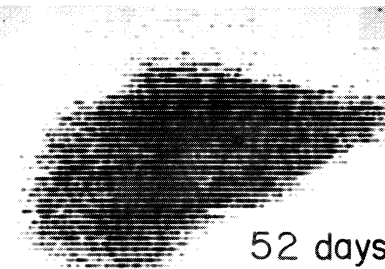
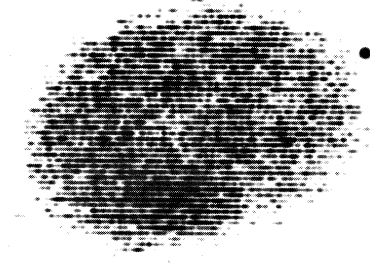
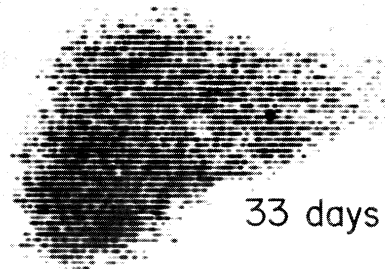
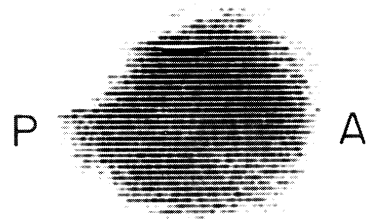


Figure 146. See facing page for legend.

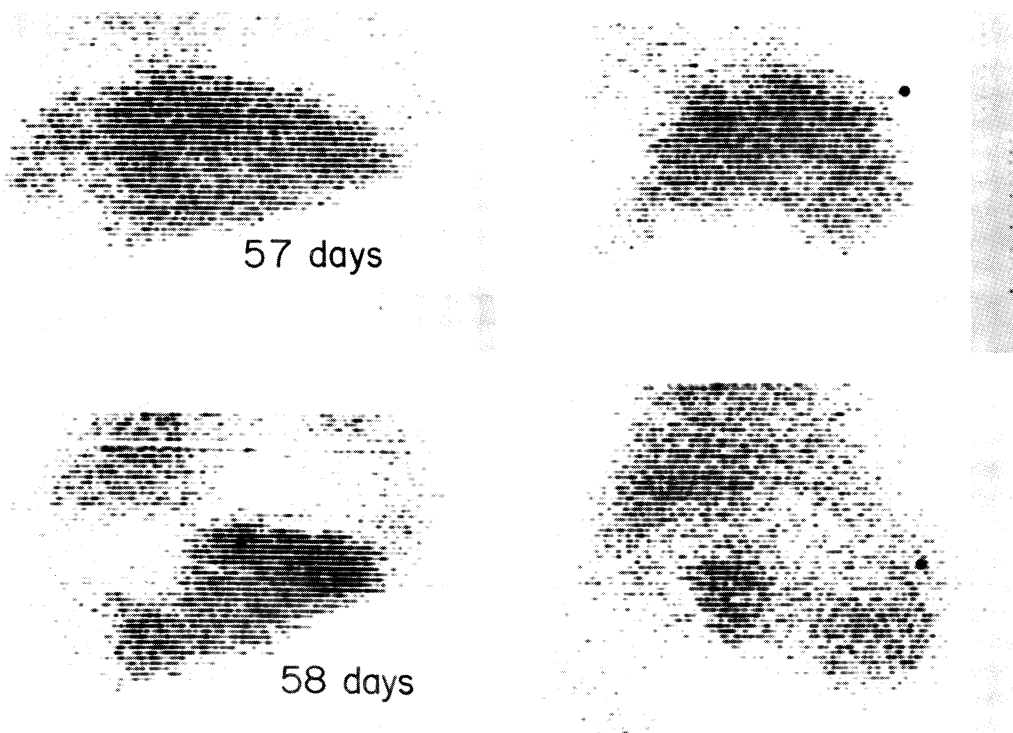


Figure 146. Serial PA and lateral liver scans (technetium) in a child (OT 11) who received an orthotopic liver transplant. 2 days: Normal organ. 33 days: The homograft is greatly increased in size. 52 days: A defect is visible in the lateral projection. It was localized to the inferior portion of the right lobe. Gram negative septicemia had developed (Fig. 142). 54 days: The defect persists. Later the same day the necrotic portion of the right lobe was debrided through an extraperitoneal incision. 57 days: The right lobe is becoming more extensively involved. 58 days: Further spread can be seen. Note that the ability of the homograft to concentrate the isotope well has been lost. A disproportionate fraction of the technetium is now found in extrahepatic areas, particularly in the thorax. The child died three days later.

Two other recipients (OT 9 and 10), including the one treated with partial hepatectomy, were temporarily rescued but died of chronic liver failure four and a half and six months after transplantation. In both, sepsis in the right subphrenic space and the contiguous liver tissue persisted until the time of death. The infected and draining serpiginous intrahepatic tracts were irrigated twice a day. Fragments of dead tissue were repeatedly removed from the exposed hepatic parenchyma. These were usually plugs, adjacent to portal vessels, which could be pulled out of the remaining viable surface. Failure to keep the wound meticulously clean often seemed to dispose to septicemia (Fig. 141), although not always with the same bacteria then present in the depths of the wound. At first there was evidence of liver regeneration, but eventually the organs began to shrink (see Chapter Seventeen). Both homografts had residual sepsis at the time of autopsy. The patient who survived for more than six months was found to have ruptured an unrecognized satellite abscess of the left lobe into the free peritoneal cavity.

Only one patient (OT 8) recovered relatively completely from the complication of septic liver infarction. After extensive debridement, the defect was

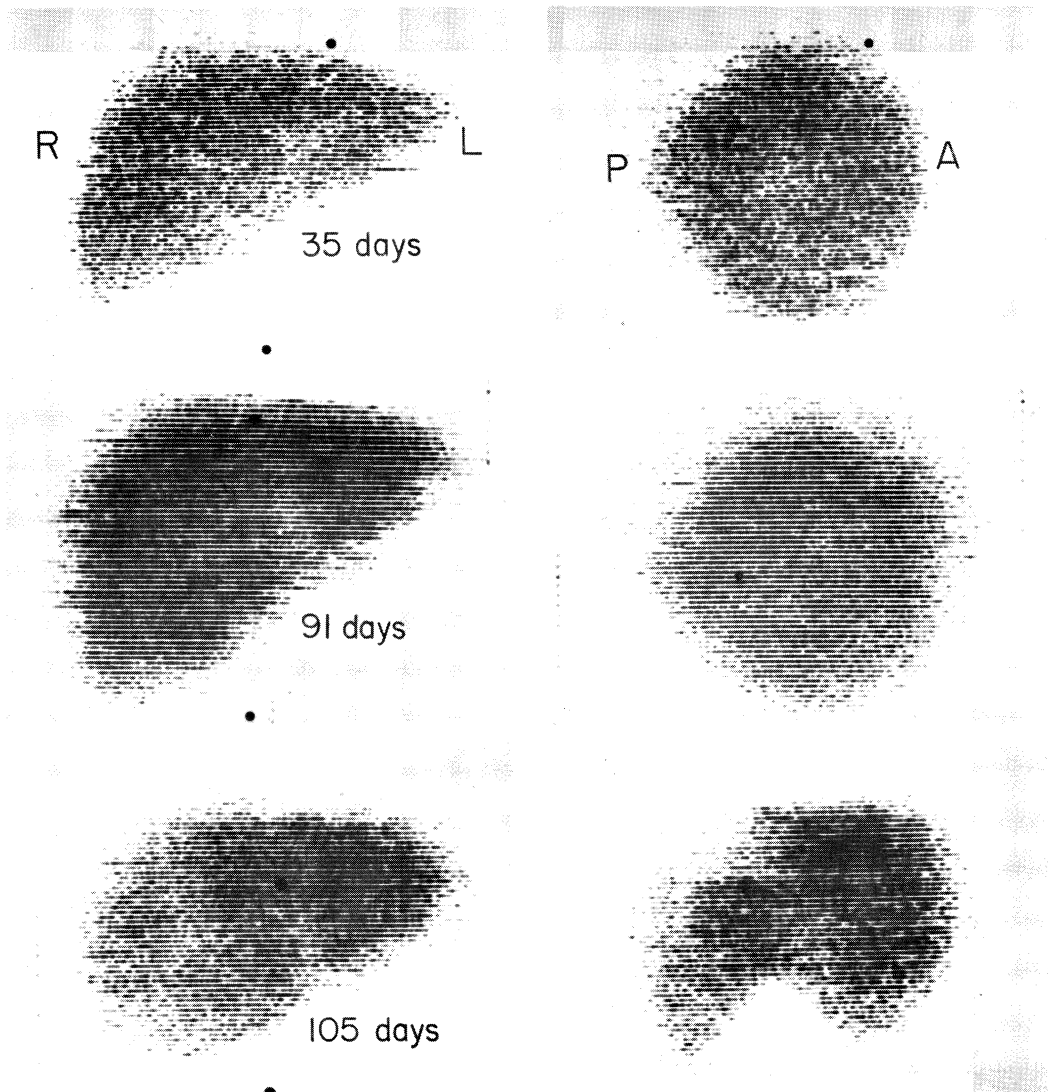


Figure 147. ^{99m}Tc -Technetium liver scans in patient OT 12. 35 days: The homograft is already considerably enlarged by comparison with previous scans. 91 days: The hepatomegaly has become extreme. The absence of a filling defect with this examination was unexpected since gram negative septicemia had developed (Fig. 143). 105 days: The infarcted region of the right lobe of the liver is evident, especially on the lateral view. An emergency debridement procedure was attempted but the patient died a few hours later.

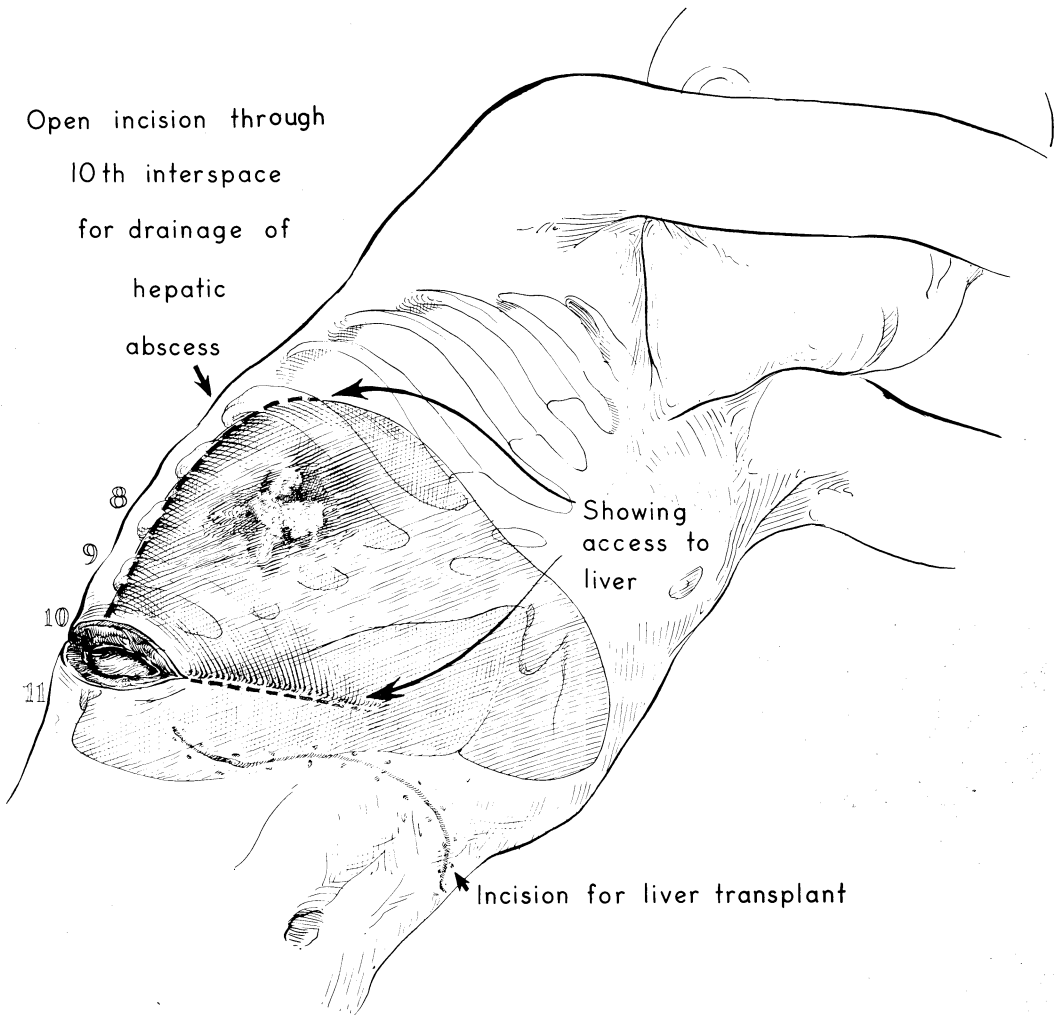


Figure 148. Operative approach to septic liver infarctions through the tenth intercostal space. Neither the pleural nor peritoneal cavities were entered. (By permission of *Ann. Surg.* 168:392, 1968.)

slowly filled in, apparently by hepatic regeneration. The process required eight months to become almost complete (Fig. 144). Until the time of her death from carcinomatosis more than a year later, a sinus tract to the right subphrenic space required daily irrigation.

MECHANICAL CONSIDERATIONS OF ETIOLOGY

The Homograft Arterial System

Emphasis has so far been placed on the role of rejection in the genesis of regional hepatic infarction. The studies of Groth⁹ in dogs left little doubt about the importance of this factor (see Chapters Eleven and Twelve). His results

indicated that the vascular supply to the homograft was endangered by an immunologic assault. However, it was not necessary to believe that the blood flow to all parts of the liver was equally reduced during rejection. Presumably the most deprived portions would be the ones to undergo ischemic necrosis. The first to suggest the latter hypothesis was Moore¹¹ more than five years ago, on the basis of canine experiments.

It follows that a number of nonimmunologic conditions could also contribute to regional devascularization. This possibility was eventually examined with special care in the human recipients of orthotopic livers because of two findings that were present in each case. First, it was always predominantly the right lobe which was affected by the gangrene. Second, selective thrombosis of the right hepatic artery was proved at autopsy in the four children who either died promptly after the development of septic infarction or who lingered on in poor condition and then succumbed several months later (Chapter Twenty).

Later, evidence was found from studies in fresh cadavers²⁰ that an anatomical distortion of the course of the right hepatic artery could accidentally be produced during transplantation in man (or at least small children), partly because of the erect position assumed by higher primates. In humans the right hepatic artery is longer than the left branch (Fig. 149) and usually traverses behind the other central hilar structures, where it is held by surrounding tissues.

When the restraining ligaments of the liver were cut and when the vascular structures entering and leaving the liver were skeletonized in fresh cadavers, the released right lobe rotated to a somewhat more posterior and inferior location than normal. If the head of the x-ray table was now elevated to 60 degrees, an incompletely occlusive kink of the proximal right hepatic artery could often, although by no means invariably, be demonstrated (Fig. 149). Presumably such a dangerous situation could be further aggravated in the post-transplantation period either by the diminutions in blood flow alluded to earlier or by the swelling of the organ at the time of rejection. The constellation of circumstances would be especially hazardous in infant livers with their fine caliber thin-walled arterial branches.

OTHER FACTORS

Insufficient attention was paid to the problem of sepsis in the transferred organs in all the early studies of homologous liver transplantation to modified or unmodified animals (Chapters Eleven and Twelve). Usually heavy antibiotic treatment was given postoperatively. In spite of the latter precaution, there were a number of examples of purulent cholangitis or liver abscess formation in every large canine series.^{5, 22, 24} Later, Alican and Hardy¹ showed that such complications could not all be ascribed either to rejection injury of the homografts or to the agents which were used to prevent this process, and which coincidentally rendered the recipients more susceptible to infection. In the studies of Alican and Hardy dog livers were autotransplanted, thereby automatically eliminating both the immunologic barrier and the need for immunosuppression. Abscesses developed in several of the autografts.

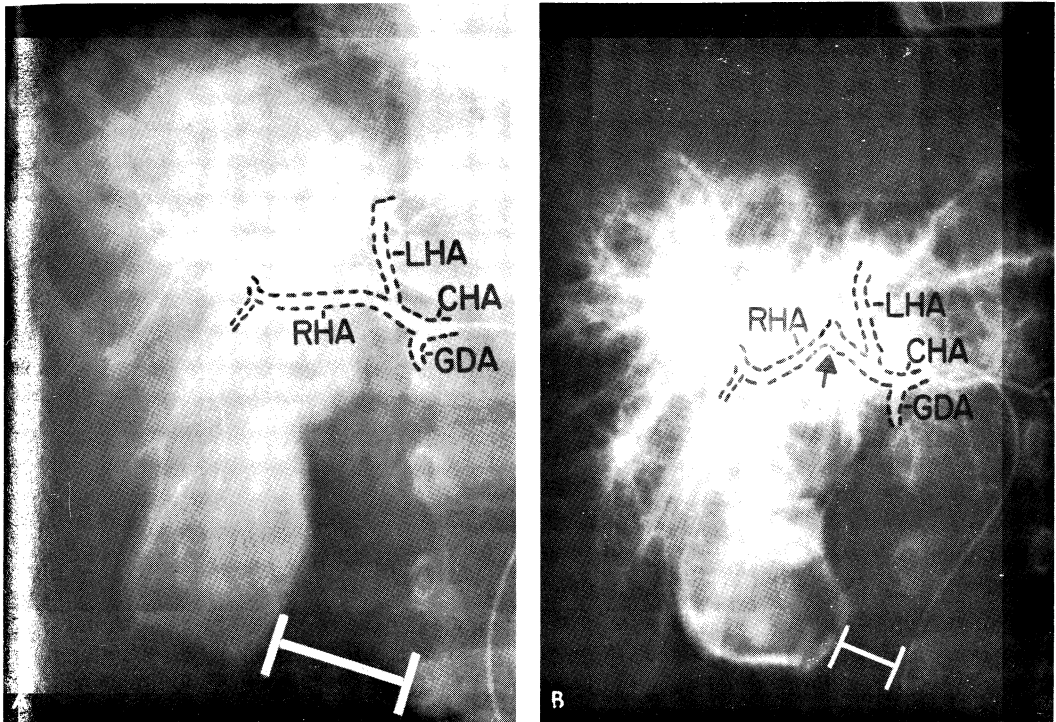


Figure 149. Angiographic studies performed in a five year old child immediately after her death from head injuries. Dye was injected into the common hepatic artery (CHA) proximal to the gastroduodenal artery (GDA). A, Initial injection. Note the smooth course of the right hepatic artery (RHA). B, The restraining ligaments of the liver have been incised, a cholecystoduodenostomy performed, and the head of the x-ray table elevated to 60 degrees. The right lobe of the liver has rotated down and medially. The course of the left hepatic artery is undisturbed. However, the right hepatic artery (RHA) is now severely kinked where it passed beneath the common duct. See text for discussion. (By permission of *Ann. Surg.* 168:392, 1968.)

After the septic hepatic infarctions were observed in the clinical cases, a number of experiments were performed in our own laboratory³ in order to define more accurately how the liver came to be invaded by microorganisms after a variety of insults. Dogs and pigs were used and treatment with antibiotics was omitted. It was shown that the normally low incidence of positive cultures from the liver tissue of both species was increased even after sham operations in which the abdomen was simply opened for several hours and the liver not disturbed. When a hepatic ischemic injury was added by performance of simulated autotransplantation, all livers became contaminated, primarily with the same microorganisms shown to be concomitantly present in the upper intestine. Bacterial counts were somewhat lower if the common bile duct was left intact than when the duct was ligated and bile drainage restored with a cholecystoduodenostomy. The bacteriologic changes were even more pronounced in liver homografts transplanted either to unmodified or immunosuppressed canine or porcine recipients.

The conclusion was that the numbers of bacteria that could be harvested from the liver reflected more or less accurately the magnitude of hepatic injury from mechanical causes as well as from rejection. Presumably any necrotic

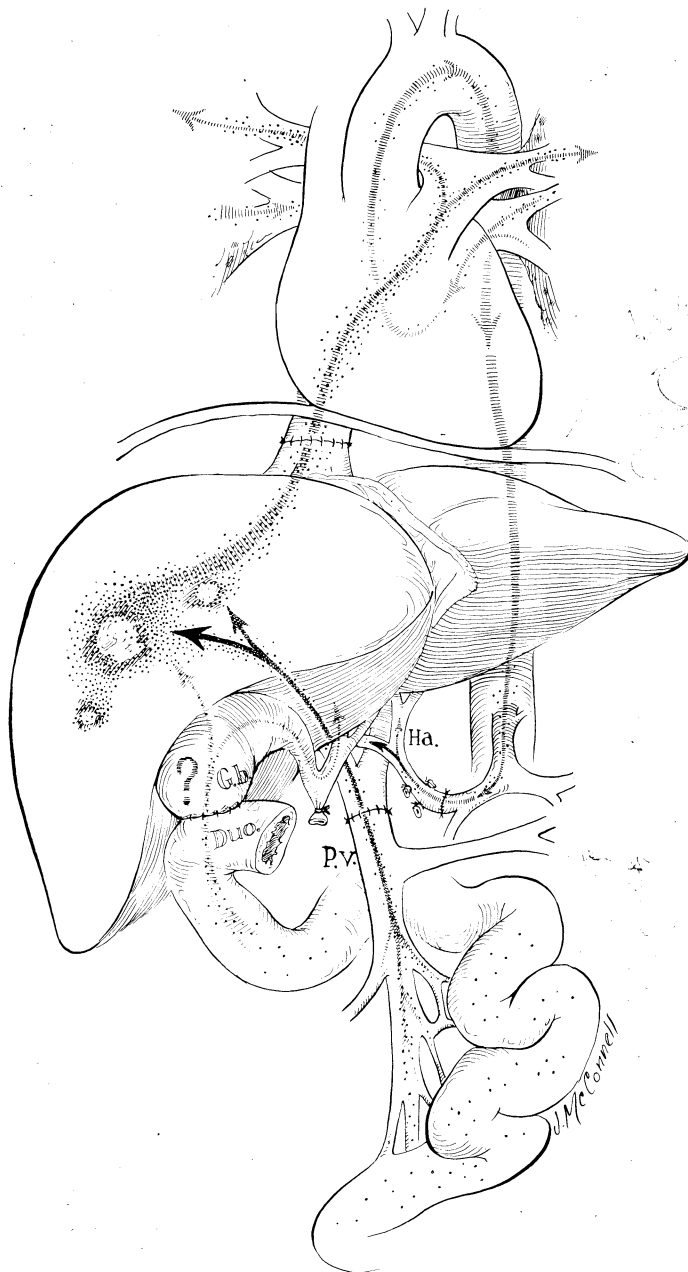


Figure 150. An explanation of the predisposition of the liver to bacterial sepsis. Presumably the invading microorganisms enter via the portal vein or through the reconstructed biliary tract. (By permission of *Ann. Surg.* 168:392, 1968.)

area, whatever its cause, could become a septic focus (Fig. 150), particularly if the host were being given immunosuppressive therapy and could not respond normally to the invading microorganisms.

PREVENTION OF SEPTIC HEPATIC INFARCTIONS

It is almost ironical to state that one of the most important ways to prevent this peculiar form of liver infection is to provide very heavy immunosuppression, especially during the early postoperative period. Adherence to the converse policy of minimum immunosuppression at the time when the five patients discussed in this chapter were treated was a key factor in at least some, and probably all, of the consecutive tragedies of that era. Subsequent recipients were given much higher doses of prednisone, beginning during operation. This was the only real adjustment that could be made since there was little maneuverability in the use of azathioprine and ALG.

The second step that should be taken is to prevent distortion of the right hepatic artery. This can be done by the simple expedient of fixing the falciform and other ligaments of the homograft to the companion structures in the recipient, as described in Chapter Eight.

Finally, a systematically designed program of antibiotic therapy should be used. In the early postoperative period a narrow spectrum antistaphylococcal drug such as methicillin may be advisable for several days. In addition, ampicillin, cephalothin, kanamycin, chloramphenicol, or polymyxin should be given prophylactically by the intravenous route for one to four weeks. The latter drugs require change every few days from one to the other in order to avoid the toxicity peculiar to each agent and to prevent the overgrowth of resistant strains; although they are potentially dangerous antibiotics, their effectiveness against gram negative bacteria makes them indispensable in liver transplantation. The importance of beginning therapy in the operating room can be appreciated by the fact that a highly significant and easily detectable ischemic injury must be imposed on all liver grafts, rendering them immediately vulnerable to bacterial invasion. How fast this can occur was illustrated in one of our patients (OT 10) in whom *E. coli* was cultured from the homograft gallbladder during transplantation. Within four hours the same microorganism was found transiently in the blood stream (Fig. 141).

The foregoing guidelines have been followed in all patients treated since February, 1968, with liver transplantation. There have been no further examples of regional hepatic gangrene. Furthermore, it has been possible to discontinue safely the prophylactic antibiotic treatment within two to six weeks. The avoidance of chronic antimicrobial therapy in the later cases has been accompanied by a reduction in the incidence of serious extrahepatic infections (Chapter Sixteen).

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Chapter Sixteen

INFECTIOUS COMPLICATIONS, EXCLUDING PARTIAL HEPATIC GANGRENE

Increased susceptibility to infection is a problem common to the transplantation of all organs as the result of the measures taken to prevent repudiation of the grafts. The role of the immunosuppressive drugs in lowering host reactivity to inimical environmental antigens is undoubted even when they are given at medium or low dose ranges which do not cause bone marrow depression.

The mechanism by which immunologic responsiveness is variably lost is not known. However, the consequences are well recognized. Infections often develop which are comparable in many respects to those seen in children who have a congenital lack of cellular immunity with or without impaired immunoglobulin synthesis.^{7, 11, 15} Functional impairment of the small and medium lymphocyte is probably one common link between the naturally occurring and iatrogenic disorders, as Gowans' recent expanded views of lymphocyte function would indicate.⁵

The infection of necrotic regions of hepatic homografts was discussed separately in Chapter Fifteen. A variety of other septic complications were also seen in the liver recipients. Many of these were similar to those previously reported after transplantation of the kidney.^{3, 6, 8-10, 13, 18-20, 21, 23, 24} Common features after both kinds of operations included the predisposition to pneumonitis; the tendency for localized sepsis to quickly become multifocal; and the frequent etiologic role of gram negative bacteria, fungi, and other less common microorganisms of normally low pathogenicity. *Sepsis is similar to kidney*

In the description of the infectious complications after liver transplantation^{4, 25, 26} the patients will be divided into those treated early in our experience, at an intermediate stage, and in a final series. Major changes in surgical technique and/or immunosuppressive therapy (Fig. 151) were made during these times which slowly transformed treatment from a hopeless to a feasible undertaking. One of the most important lessons that was learned was that the quality of the transplanted liver played a prime role in determining the frequency and

E = EARLY CASES (OT 2 - 7)

I = INTERMEDIATE CASES (OT 8 - 12)

L = LATER CASES (OT 13 - 17)

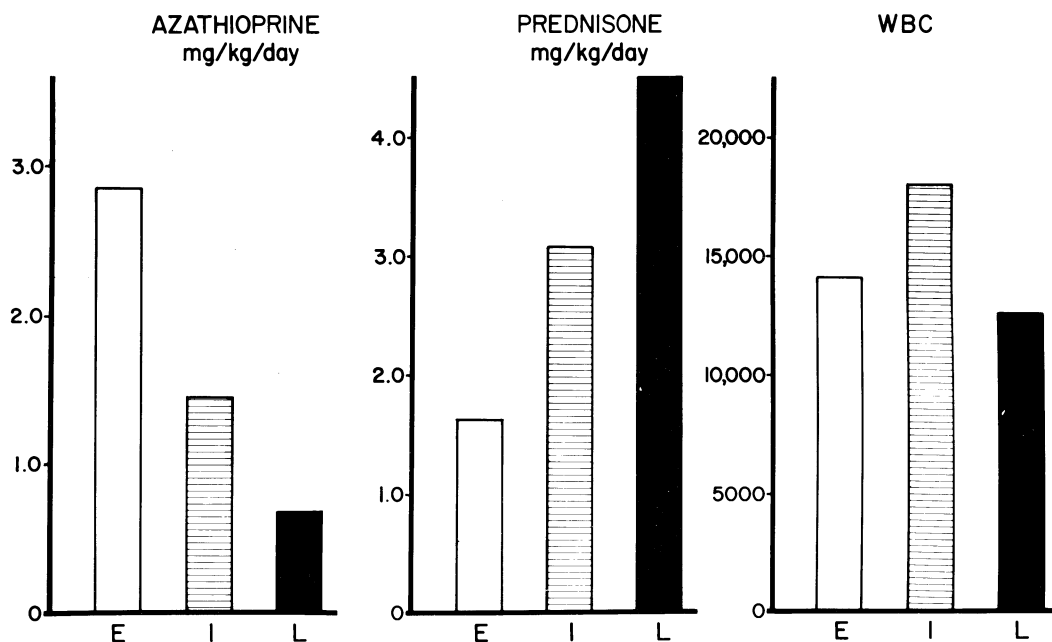


Figure 151. Pooled data from the first six postoperative days showing the changing policy of immunosuppression which evolved in cases of orthotopic liver transplantation. All the early patients died in 23 days or less. Each of the recipients in the intermediate period developed a septic hepatic infarction. The later patients survived the operation and immediate postoperative period and were spared the complication of partial liver gangrene. Note that the therapeutic adjustments consisted of progressive reductions in the daily quantities of azathioprine and increases in the amounts of prednisone. More complete information about the treatment of the individual recipients is given in Chapter Thirteen.

severity of sepsis elsewhere in the body. Patients who received promptly and adequately functioning organs tended to recover very quickly from the acute insult of operation. Those whose transplants were badly damaged remained bedridden and almost invariably fell prey to explosive and lethal infections.

During the entire period of the study, the approach to diagnosis and antibiotic therapy was similar. On admission of the recipient, cultures were taken from the skin, trachea, urine, and feces. During operation, additional specimens were cultured from the donor and recipient duodenum and the donor gallbladder bile. After transplantation, bacterial cultures were taken daily or at least twice a week from the wounds, nose, throat, trachea, urine, feces, and peripheral venous blood. In addition, virus isolation was attempted, as reported by Rifkind^{17, 18} and Fulginiti,⁴ in specimens obtained from the nose and throat, feces, and urine.

Methicillin or one of the other narrow spectrum antistaphylococcal drugs was started intravenously before or during operation and continued afterward. Other antibiotics were selected on the basis of the endogenous flora demon-

strated in the preoperative bacterial cultures. These were changed according to the cultures and sensitivities in subsequent specimens from the multiple sampling sites. For reasons discussed in Chapters Fourteen and Fifteen agents were often used which are usually effective against gram negative organisms. These included ampicillin, cephalothin, kanamycin, chloramphenicol and polymyxin. In the later cases, one or more of these potent drugs were always started at the time of operation, rather than later when infection was already evident.

EARLY CASES

There were 10 patients treated between March 1, 1963, and May, 1967, seven with orthotopic and three with auxiliary transplantation. One patient in each group (OT 1 and AT 3) died during operation and will not be considered further in this analysis. The first four recipients had staged procedures, hepatic mobilization being carried out one to 14 days before the actual transplantation. Biliary reconstruction in all these cases was with choledochcholedochostomy and T-tube drainage. The other two recipients of orthotopic homografts had a one stage operation, and provision for biliary drainage was with cholecystoduodenostomy. The two auxiliary homografts (AT 1 and 2) were placed in the right paravertebral gutter three and one days, respectively, after the performance of portacaval shunts (Chapter Twenty-two); biliary drainage was with a Roux-Y cholecystojejunostomy. In most of the orthotopic cases, the livers were gravely or seriously injured and functioned poorly (see Chapter Six).

General Postoperative Condition. Five of the eight patients were more than 47 years old and seven were badly debilitated before operation. After transplantation most were further enfeebled by the absence of satisfactory hepatic function. The consequences were that prolonged ventilatory support was always needed, that tracheostomies became necessary in three instances, that bladder catheters usually had to be left in place for many days, and that the resumption of an oral diet was delayed or poor, if this was ever achieved at all.

Immunosuppression. Azathioprine and prednisone were given to six of the patients and supplemented irregularly with intravenous actinomycin C and/or local homograft irradiation. The other two recipients (OT 6 and 7) were also treated with horse ALG. The errors made in drug therapy in this group of patients, particularly with respect to azathioprine, were described in Chapter Thirteen. Briefly, the latter agent was given in average daily quantities that proved to be overdoses. Five of the eight patients became leukopenic at some phase of their course, with total white blood cell counts of 4000/cu mm or less; in four, the white cell counts fell to 2000/cu mm or lower. During the postoperative period the average prednisone doses were about 2 mg/kg per day (see Chapter Thirteen for details).

Nonhepatic Infections. Septic complications were prompt and overwhelming in these physically invalided and immunologically crippled patients. The localization, etiology, timing, therapy, and outcome of the extrahepatic septic episodes are summarized in Table 21. Seven of the eight patients had a total of 29 separate infections. Of these only one, an *E. coli* urinary tract infection (OT

5), definitely existed before operation, although in another case (OT 3) sterile biliary peritonitis before transplantation probably contributed to the subsequent bacterial invasion of the peritoneum. The diagnoses were made from the first to the last day (maximum 34) of postoperative survival or at autopsy. There were eight, five, four, three, and three examples, respectively, of pneumonitis, urinary tract sepsis, peritonitis, esophagitis and bacteremia.

Multiple foci of infection with the identical organism were common. Thus, three patients had pneumonitis and septicemia with the same bacteria. Two of the four examples of peritonitis were in patients who had the same culture in the trachea or blood stream. Two of the five urinary tract infections were associated with septicemia (Table 21).

It is noteworthy that pyogenic cocci were not responsible for any of the foregoing infections. Gram negative enteric bacilli were the causative agents in 14 instances, including six of the eight pneumonias. The organisms were *E. coli*, *Aerobacter-Klebsiella* species, *Pseudomonas aeruginosa* and *Proteus* species.

There were also infections with *Candida albicans* resulting in erosive esophagitis (OT 3, 5, and 6), pneumonitis (OT 2 and 3), pyelonephritis (AT 2), fungemia (OT 6) and panenteritis (AT 2) (Table 21). The invasive moniliasis of the small bowel in AT 2 was responsible for an eventually fatal hemorrhage. Examples of fungus infections in different locations are shown in Figures 152 and 153.

In one patient (AT 2) cytomegalovirus (CMV) infestation was found at autopsy in the lungs, in the intestinal tract, and in the homograft.

Hepatic Sepsis, Excluding Gangrene. A single microabscess was detected with histologic study of the postmortem homograft of one patient (OT 7). The right lobe of another (OT 5) was found at autopsy to contain a discrete 2 cm abscess.

In the patient (AT 2) with widespread cytomegalovirus, inclusion bodies were found in the small intrahepatic bile ducts (Fig. 154).

INTERMEDIATE CASES

These five orthotopic liver recipients were treated between July 23, 1967, and November 27, 1967. Biliary tract reconstruction was with cholecystoduodenostomy (Chapter Eight). None of the patients was older than two years of age. Their homografts, which were moderately or even minimally damaged by ischemia, performed reasonably well from the beginning, although there were always some initial abnormalities in hepatic function. Eventually, recovery was jeopardized or prevented in each case by the development of the septic hepatic infarctions described in Chapter Fifteen.

General Postoperative Condition. All the children were strong after transplantation, even one (OT 12) who had been in terminal condition before operation. They were removed from mechanical respiratory support after two to 12 hours, were not exposed to the risks of prolonged bladder catheterization, and were all able to resume eating within 48 to 72 hours.

Immunosuppression. Therapy was with azathioprine, prednisone, and

Table 21. Infectious Complications Encountered in the Early Cases of Orthotopic and Auxiliary Liver Transplantation at Our Institution

PATIENT	AGE (Yr)	SURVIVAL (Days)	ONSET OF INFECTION (Days)	TYPE OF INFECTION	ETIOLOGY	THERAPY*	COURSE	COMMENT
OT 2	48	22	15	Pneumonia	<i>Pseudomonas</i> , <i>Proteus</i> , <i>C. albicans</i>	— —	Progressive, fatal	Massive pulmonary infarction
OT 3	68	7.5	1	Pneumonia	<i>Pseudomonas</i> , <i>Aerobacter-Klebsiella</i> , <i>C. albicans</i> , cytomegalovirus	Str, Chl	Persistent	Multiple pulmonary emboli
			4	Peritonitis	<i>Pseudomonas</i> , <i>E. coli</i>	Str, Chl	Persistent	Bile peritonitis 2 weeks before transplant
			?	Esophagitis	<i>C. albicans</i>	— —		Autopsy finding
OT 4	52	6.5		None		— —		Healed tuberculous granuloma
OT 5	29	23	Pretransplant 6	Urinary tract infection Peritonitis	<i>E. coli</i> <i>E. coli</i>	Na, Sulfsox, Chl, Po Meth, Chl, gamma globulin	Persistent Progressive	Necrotic biliary anastomosis
			7	Urinary tract infection	<i>C. albicans</i>	— —	Persistent	
			21	Bacteremia	<i>E. coli</i>	Po, Sulfsox	Terminal	
			22	Pneumonia, empyema	<i>E. coli</i>	Po, Sulfsox	Terminal	
			?	Esophagitis	<i>C. albicans</i>	— —		Autopsy finding
			?	Liver abscess (single, 2 cm)	?	— —		Autopsy finding
			?	Microabscesses of myocardium	?	— —		Autopsy finding
OT 6	29	7	3	Pneumonia	<i>Pseudomonas</i>	Pen, Kef	Progressive, fatal	
			4	Fungemia; esophagitis; peritonitis	<i>C. albicans</i>	— —		
			7	Bacteremia	<i>Pseudomonas</i>	Pen, Kef	Terminal	

*Abbreviations for antibiotics: Str = streptomycin, Chl = chloramphenicol, Na = naldixic acid, Sulfsox = sulfisoxazole, Po = polymyxin B, Meth = methicillin, Kef = cephalothin, Amp = ampicillin, Pen = penicillin G.

Table 21. Infectious Complications Encountered in the Early Cases of Orthotopic and Auxiliary Liver Transplantation at Our Institution (*Continued*)

PATIENT	AGE (Yr)	SURVIVAL (Days)	ONSET OF INFECTION (Days)	TYPE OF INFECTION	ETIOLOGY	THERAPY*	COURSE	COMMENT
OT 7	11/12	10	Pretransplant	Right upper lobe atelectasis and bronchiectasis	?	—	—	Autopsy finding
			?	Interstitial pneumonia	?	—	—	Autopsy finding
			?	Hepatic microabscess	?	—	—	Total slough of jejunal mucosa at autopsy
AT 1	50	22	1	Pneumonia	<i>Pseudomonas</i>	Po	Progressive	Multiple lung abscesses
			3	Urinary tract infection	<i>C. albicans</i>	—	Progressive	Multiple renal abscesses
			15	Urinary tract infection	<i>Enterococcus</i>	Po, Kef	Controlled	
			?	Peritonitis	Aerobacter-Klebsiella	Po, Kef	Persistent	Autopsy finding
			?	Epididymitis	<i>C. albicans</i>	—	—	Autopsy finding
AT 2	47	34	7	Urinary tract infection	<i>C. albicans</i>	—	Persistent	
			10	Urinary tract infection	<i>E. coli</i>	—	Persistent	
			20	Hemorrhagic enteritis	<i>C. albicans</i>	—	Persistent, progressive	Etiologic diagnosis at autopsy
			21	Pneumonia	<i>E. coli</i>	Amp, Kef, gamma globulin	Persistent, progressive	
			34	Bacteremia	<i>E. coli</i> , Aerobacter-Klebsiella	Amp, Kef, gamma globulin	Terminal	
			?	Pneumonia; enteritis; hepatitis; cholangitis	Cytomegalovirus	—	—	Autopsy finding

*Abbreviations for antibiotics: Str = streptomycin, Chl = chloramphenicol, Na = naldixic acid, Sulfsox = sulfisoxazole, Po = polymyxin B, Meth = methicillin, Kef = cephalothin, Amp = ampicillin, Pen = penicillin G.

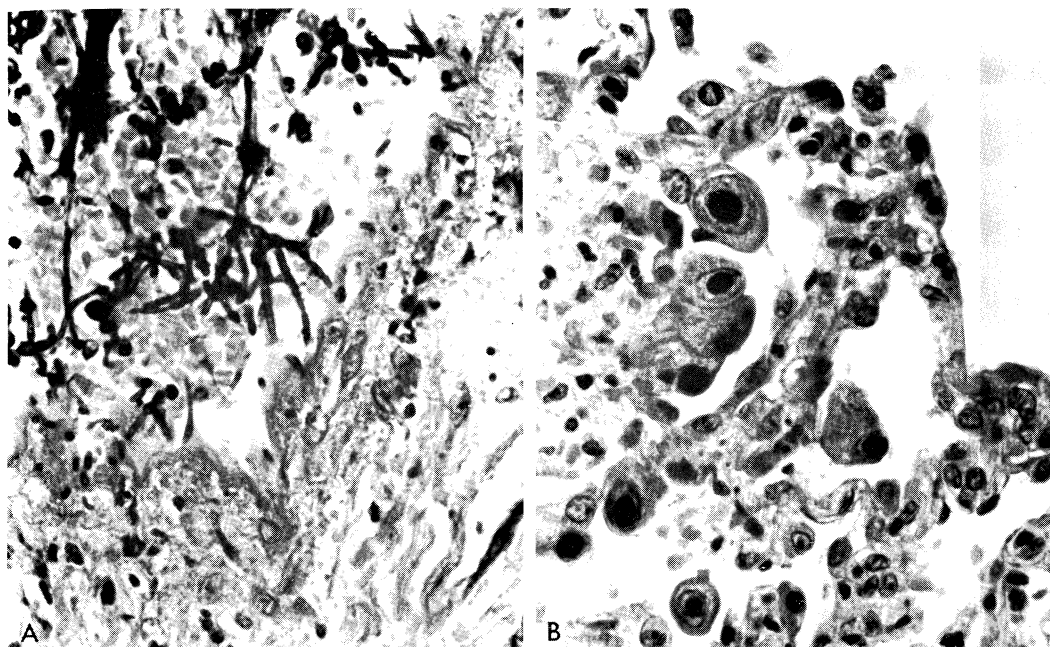


Figure 152. Opportunistic organisms identified in the lungs of patient AT 4, 24 days after auxiliary hepatic transplantation. *Aerobacter-Klebsiella* and *Candida albicans* were recovered from tracheal aspirates during the postoperative period. A, *C. albicans* infiltration of the pleura (PAS stain, $\times 400$). B, Large intranuclear inclusions of cytomegalovirus in alveolar lining cells (hematoxylin and eosin stain, $\times 400$).

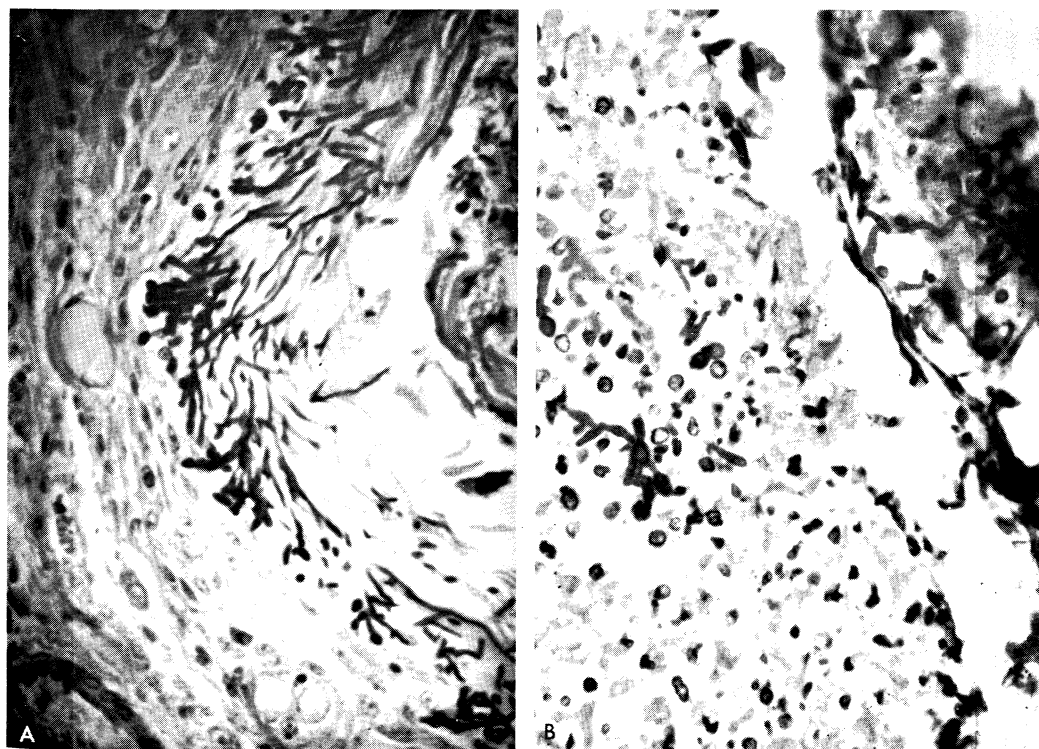


Figure 153. *Candida albicans* infections of the GI tracts of two recipients of hepatic homografts. The specimens were obtained at autopsy, seven and a half and 34 days, respectively, after transplantation. A, Ulcerative esophagitis in patient OT 3 (PAS stain, $\times 200$). B, Enteritis in patient AT 2 (PAS stain, $\times 200$).

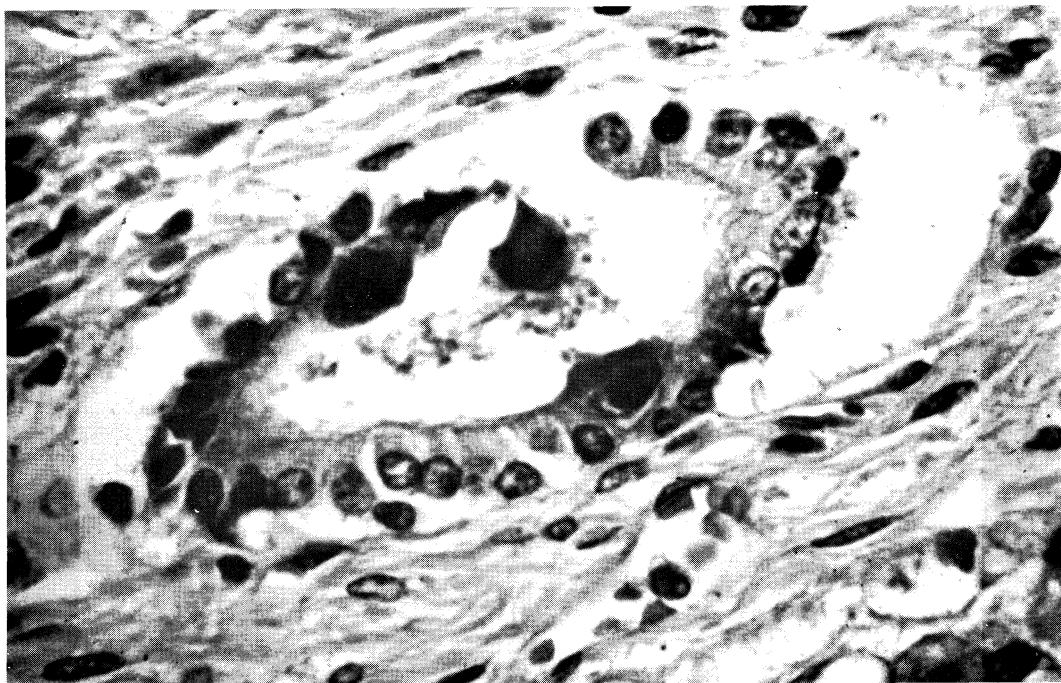


Figure 154. Cytomegalovirus in a small bile duct of the auxiliary homograft of patient AT 2, 34 days after operation. (hematoxylin and eosin stain, $\times 200$). (By permission of New Eng. J. Med. 279:619, 1968.)

heterologous ALG. The azathioprine doses per kg per day for the first six days were 1.5 mg, about one half on a body weight basis of those used in the earlier series (Fig. 151). Leukopenia was avoided. The quantities of steroids were larger (Fig. 151).

Nonhepatic Infections. Although leukopenia was avoided, there were 26 episodes of extrahepatic infection (Table 22) which were at least partly explained by the prolonged antibiotic treatment made necessary by the partial liver gangrene. Apparently the consequences of chronic antimicrobial therapy were the emergence of bacterial populations which were resistant to the agents used, as well as the overgrowth of fungi, viruses, and protozoa. This "unbalancing" effect of antibiotics on the natural flora has often been observed in non-transplant patients^{12, 14, 16, 27, 28} and has also been reflected in the etiology of infectious deaths after renal homotransplantation.⁹

There were 16 bouts of gram negative bacteremia which did not occur at the same time as the septic liver infarctions. A few of these positive blood cultures occurred before the appearance of hepatic gangrene, but most appeared sporadically afterward. When the sinus tracts into the liver were cultured in the three patients who survived debridement, they sometimes contained the same bacteria as those simultaneously found in the blood stream, but often they did not. Because of this frequent lack of association, the intermittent late gram negative bacteremias were classed as nonhepatic in origin (Table 22). Nevertheless, it is highly likely that the homograft was the responsible focus since recurrent gram negative septicemia has not been nearly so common in

Table 22. Infectious Complications in Five Patients Whose Orthotopic Liver Homografts Underwent Regional Infarction and Subsequent Gangrene*

PATIENT (Yr)	AGE (Yr)	SURVIVAL (Days)	ONSET OF INFECTION (Days)	TYPE OF INFECTION	ETIOLOGY	THERAPY*	COURSE	COMMENT
OT 8	1-7/12	400	23	Bacteremia	<i>E. coli</i>	Po, Ka	Cleared	
			24	Septic liver infarction; bacteremia	Aerobacter-Klebsiella	Po, Ka, Chl, Tet	Open tract slowly healed; redebridement at 144 days	
			66	Gastroenteritis	Salmonella, group B	Amp, Tet, Ka, Chl	Carrier state to 127 days; cleared	
			137	Bacteremia	<i>Pseudomonas</i> species	Po	Cleared	
			141	Bacteremia	<i>B. fragilis</i>	Tet	Cleared; hepatic debridement at 144 days	
			171	Fungemia	<i>C. albicans</i>	Amphotericin B	Cleared; no recurrence	
OT 9	1-9/12	133	179	Bacteremia	<i>B. fragilis</i>	Tet, Ery	Cleared	
			240	Urinary tract infection	<i>E. coli</i> , <i>Proteus</i>	Variable	Controlled	
			2	Septic liver infarction; bacteremia	<i>B. fragilis</i>	Tet, Ery; R lobar resection	Bacteremia cleared; septic infarction persisted	
			14	Hip abscess; bacteremia	<i>B. fragilis</i>	Tet; abscess drainage	Healed	
			23	Bacteremia; subphrenic abscess	<i>B. fragilis</i>	Tet; abscess drainage	Persistent	
			??3	Pneumonia	Cytomegalovirus	— —	Virus isolated on 8, 22 and 42 days and from lung at autopsy	Inclusions present in lung
			60	Fungemia	<i>C. albicans</i>	5-Fluorocytosine	Fungemia cleared	Cerebellar abscesses
			81	Bacteremia	Aerobacter-Klebsiella	Tet, Amp	Cleared	
OT 10	1-1/12	186	92-105	Bacteremia	Aerobacter-Klebsiella; <i>E. coli</i>	Tet, Amp	Cultures positive on 112 and 133 days	
			?	Interstitial pneumonia	<i>P. carinii</i>	— —	Patient asymptomatic; x-ray appearance clear	Autopsy finding
			0	Bacteremia	<i>E. coli</i> ; Aerobacter-Klebsiella	Amp, Ka, Chl, Po	Cleared	
			25	Septic liver infarction; bacteremia	Aerobacter-Klebsiella	Str, Ka, Po	Debridement on 27, 90 and 92 days; remained open	Persistent
			55	Bacteremia	Aerobacter-Klebsiella	Ka, Tet	Cleared	
			66	Bacteremia	<i>E. coli</i>	Chl, Amp	Cleared	
			87	Bacteremia	<i>E. coli</i>	Chl, Po	Cleared	
			160	Bacteremia	<i>E. coli</i>	Ka, Amp	Cleared	
			168	Bacteremia	<i>E. coli</i>	Po	Cleared	

*The complication of septic hepatic infarction is considered separately in Chapter Fifteen, but the culture data and therapy are included in this table since the consequent need for chronic antibiotic treatment probably was contributory to the genesis of the many extrahepatic infections. See text for discussion.

†Abbreviations for antibiotics: Str = streptomycin, Chl = chloramphenicol, Po = polymyxin B, Kef = cephalothin, Amp = ampicillin, Ka = kanamycin, Tet = tetracycline, Ery = erythromycin.

Table 22. Infectious Complications in Five Patients Whose Orthotopic Liver Homografts Underwent Regional Infarction and Subsequent Gangrene* (Continued)

PATIENT (Yr)	AGE	SURVIVAL (Days)	ONSET OF INFECTION (Days)	TYPE OF INFECTION	ETIOLOGY	THERAPY*	COURSE	COMMENT
OT 10 (Cont)			185	Interstitial pneumonia	<i>E. coli</i> ; Aerobacter-Klebsiella Cytomegalovirus	Po	Terminal	Cytomegalovirus isolated, but no inclusions in lung at autopsy
			185	Peritonitis	<i>E. coli</i>	Po	Terminal rupture of liver abscess	
OT 11	1-2/12	61	19	Fungemia	Unidentified yeast	Amphotericin B	Cleared	
			52	Septic liver infarction; bacteremia	<i>E. coli</i>	Po, Kef; drainage	Persistent until death; re-drained 58 days	
			58	Meningitis	<i>E. coli</i>	Po, Kef		
			59	Bacteremia	<i>E. coli</i> ; <i>E. intermedia</i>	— —		
			?	Interstitial pneumonia	?	— —	Present at autopsy	
OT 12	1-4/12	105	27	Pneumonia	Cytomegalovirus, Aerobacter-Klebsiella, <i>P. carinii</i>	Str, Kef, Tet, Ka, Po, gamma globulin	Positive cytomegalovirus; isolation on 27, 81, 84, and 105 days and from lung at autopsy	Inclusions in lung
			79	Bacteremia	Aerobacter-Klebsiella	Str, Kef, Tet, Ka, Po, gamma globulin	Positive blood cultures for 13 days	
			104	Septic liver infarction; bacteremia	Aerobacter-Klebsiella, <i>E. coli</i>	Str, Kef, Tet, Ka, Po	Rapidly fatal	
			?	Cholangitis	?		Secondary to partial biliary obstruction	

*The complication of septic hepatic infarction is considered separately in Chapter Fifteen, but the culture data and therapy are included in this table since the consequent need for chronic antibiotic treatment probably was contributory to the genesis of the many extrahepatic infections. See text for discussion.

†Abbreviations for antibiotics: Str = streptomycin, Chl = chloramphenicol, Po = polymyxin B, Kef = cephalothin, Amp = ampicillin, Ka = kanamycin, Tet = tetracycline, Ery = erythromycin

the course of subsequent patients in whom liver sepsis was largely avoided. An *E. coli* meningitis (OT 11) found at autopsy in one of the patients was probably hematogenously "seeded" during the septicemic episodes.

There were two bacterial pneumonias, these being caused by gram negative microorganisms (Table 22). In addition, cytomegalovirus (CMV) was isolated by Dr. Vincent Fulginiti from the lungs of three children (OT 9, 10, and 12) at the time of autopsy (Fig. 155, A). During life the same virus had been repeatedly isolated from the tracheal aspirates in two of these patients. In both the CMV was only part of a complex infestation which also included *Pneumocystis carinii* (Fig. 155, B) and *Aerobacter-Klebsiella* (Fig. 155, C) in one patient (OT 12), and *Pneumocystis carinii* in the other (OT 9). Premortem, the former patient had clinically evident pneumonia (Fig. 156), whereas the latter recipient did not.

Three of the five recipients in this series developed fungemia (Table 22), two with *Candida albicans* (OT 8 and 9) and one (OT 11) with an unidentified yeast. The blood cultures of all three patients cleared with antifungal therapy (amphotericin B or 5-fluorocytosine*). However, silent residual abscesses of the cerebellum were found in one (OT 9) at autopsy (Fig. 157).

These five patients lived from two to more than 13 months after operation (Chapters Fifteen and Seventeen). Their postoperative courses were dominated by the septic hepatic infarctions. The numerous extrahepatic infections contributed to the morbidity but were not directly responsible for any of the deaths.

Hepatic Sepsis, Excluding Gangrene. The direct cause of death in patient OT 10 was intraperitoneal rupture of an abscess in the left hepatic lobe 186 days after transplantation. This portion of the liver had not been involved by the septic infarction that developed at the end of the first postoperative month (Chapter Fifteen). However, the later infection may have spread by local invasion from the chronically suppurating wound.

All five of the homografts from these patients have now been examined at autopsy from 60 to 400 days after transplantation. There was one example of bacterial cholangitis (Chapter Twenty). In this transplanted liver (OT 8), the intrahepatic ducts after 400 days were dilated and filled with a chalk-like debris which consisted of polymorphonuclear leukocytes, gram negative bacteria, and an amorphous material. In some areas the bile epithelium had sloughed (Chapter Twenty). In this case, there was obstruction of the duct system by a large recurrence of tumor (Chapters Seventeen and Twenty).

LATER CASES

In the last cases of liver transplantation collected from July 23, 1967, to November 10, 1968, attempts were made to avoid the circumstances that had previously made infectious disease control either exceptionally difficult or im-

*Roche Company, Professional Service Department, 212 Oxford, New York, New York.

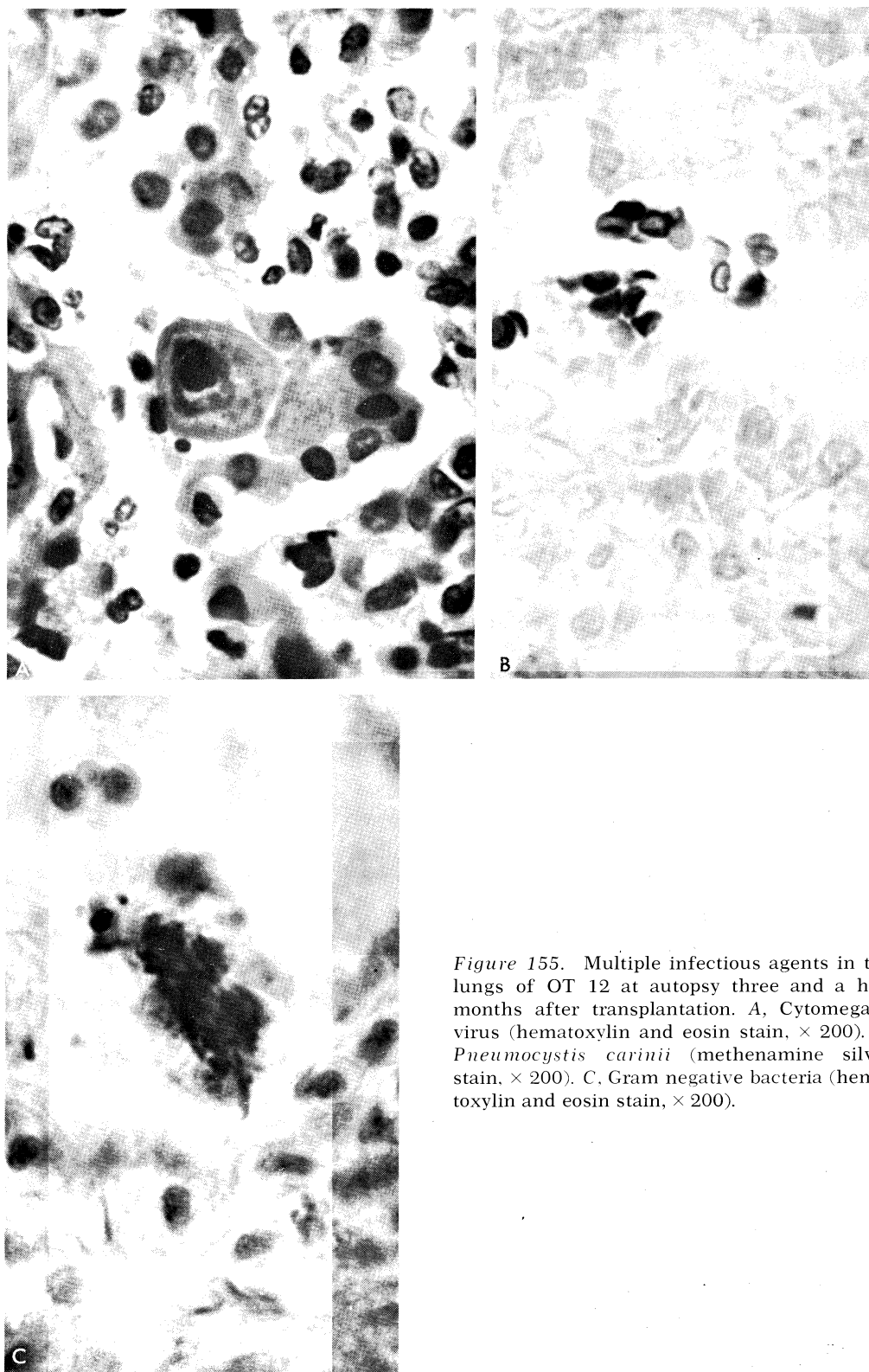


Figure 155. Multiple infectious agents in the lungs of OT 12 at autopsy three and a half months after transplantation. A, Cytomegalovirus (hematoxylin and eosin stain, $\times 200$). B, *Pneumocystis carinii* (methenamine silver stain, $\times 200$). C, Gram negative bacteria (hematoxylin and eosin stain, $\times 200$).

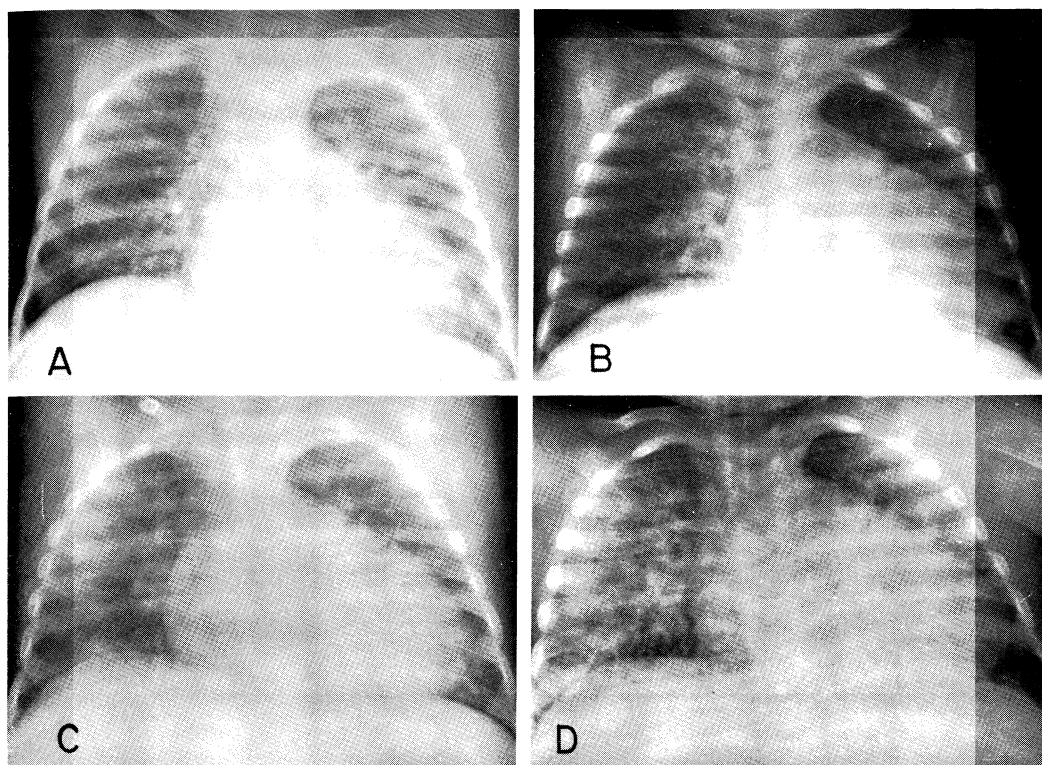


Figure 156. Chest x-rays in patient OT 12. A, 82 days after transplantation: There are prominent bronchovascular markings but no evidence of pneumonitis. B, 93 days: Bronchopneumonia on the right is evident. C, 104 days: Diffuse pneumonitis has developed. D, 104 days, 12 hours after the x-ray shown in C: The infiltrate has increased. The child died 105 days after transplantation, shortly after an attempt to debride a septic hepatic infarction. At autopsy cytomegalovirus (CMV), *Pneumocystis carinii* and *Aerobacter-Klebsiella* were found in the lungs.

possible. There were 13 patients in this final series, of whom 12 received orthotopic homografts. Three of the 13 recipients died shortly after operation of technical complications (Chapter Nine) and will not be included in this discussion. None of the remaining 10 developed partial gangrene of their transplants, although in two patients (OT 15 and 23) the homografts were invaded by recurrent hepatoma many months later (Chapter Seventeen).

General Postoperative Condition. Seven of the patients who obtained well functioning livers recovered quickly from the immediate effects of the operation and were able to eat and ambulate within a few days. The other three (OT 22 and 24 and AT 4) suffered from severe hepatic insufficiency and were bedridden; they died after 10, 11, and 24 days, respectively.

Immunosuppression. The agents were used during the first week in the same way as in the intermediate series except that the doses of azathioprine were even smaller and the average quantities of prednisone were further increased (Fig. 151). There were no bouts of leukopenia.

Nonhepatic Infections. The three recipients with inadequate homograft function all developed overwhelming infections. In two of these patients (OT 24 and AT 4), *Candida albicans* had been identified in preoperative cultures of the

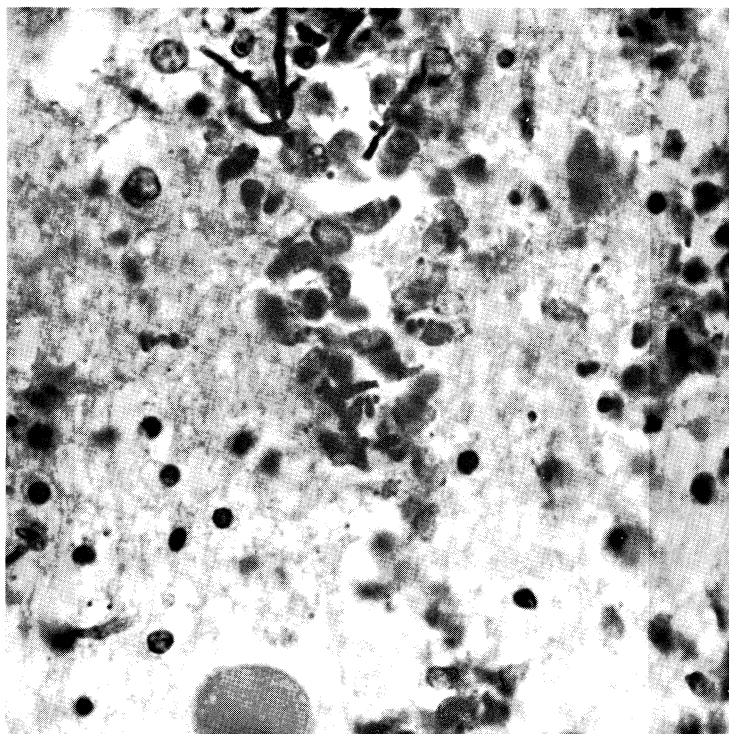


Figure 157. Cerebellar monilial abscess found at autopsy (OT 9). More than two months previously she had had *Candida albicans* cultured from the peripheral blood on several occasions. The blood stream had cleared with amphotericin B therapy. (PAS stain, $\times 400$.)

tracheal aspirate or stool. In both instances the fungus apparently became invasive in the postoperative period (Table 23). The problems were compounded by the appearance of gram negative bacteria in the lungs, urinary tract, and blood stream. A third patient who received a liver with an obstructed duct system (OT 22) died of multifocal gram negative sepsis.

In contrast, the seven patients with good early liver function had fewer and more controllable infections. These are listed in Table 23. Early death resulted only in patient OT 17, who died after 35 days of pneumonitis caused by *Pseudomonas*.

The nonlethal infections in the remaining six recipients were of great interest (Table 23). Bacteremia was documented at some time in all but patient OT 14. In four of the five patients the microorganisms grown from the blood stream either early or long after transplantation were of varieties that most likely originated from the intestinal tract. These included *E. coli*, *Aerobacter-Klebsiella*, *Enterococcus*, *Pseudomonas*, diptheroids, *Clostridium perfringens*, and *Corynebacterium bovis*.

At the time of most of the septicemias there was no evidence from transaminase determinations or liver scans that major focal necrosis was occurring within the transplanted livers. Furthermore, the patients were sometimes not clinically ill on these occasions. This was especially surprising in the two patients (OT 16 and 19) whose peripheral blood contained clostridia. In each

Table 23. Infectious Complications in the Later Cases of Liver Transplantation

PATIENT	AGE (Yr)	SURVIVAL* (Days)	ONSET OF INFECTION (Days)	TYPE OF INFECTION	ETIOLOGY	THERAPY†	COURSE	COMMENT
OT 13	2	431, alive	315 342	Bacteremia Bacteremia	Hemophilus <i>D. Pneumoniae</i>	Pen	Cleared Cleared	Origin not determined Origin not determined
OT 14	16	397, alive after 2nd transplant	177	?Interstitial pneumonia	? <i>P. carinii</i>	Pent.	Cleared	No tissue diagnosis
OT 15	44	339	14 200 241 277 282 284 314 315 316	Bacteremia Bacteremia Bacteremia Urinary tract infection Bacteremia Bacteremia Bacteremia Bacteremia Urinary tract infection	<i>E. coli</i> Aerobacter-Klebsiella <i>E. coli</i> Pseudomonas, Proteus <i>E. coli</i> Pseudomonas Pseudomonas Enterococcus Pseudomonas	Amp, Ka Amp, Ka Ka, Kef Ka, Kef Ka Ka, Po Po Amp Amp, Ka, Po	Cleared Cleared Cleared Cleared Cleared Cleared Persistent Cleared Persistent	Source probably liver Not clinically ill
OT 16A	1-11/12	Graft replaced 68 days	26	Bacteremia	<i>Staph. albus</i>	Pen	Cleared	Origin not determined
OT 16B		324, alive after 2nd transplant	10 17 43 64	Bacteremia Gastroenteritis Bacteremia Bacteremia	Diphtheroids Salmonella, group B <i>Cl. perfringens</i> <i>Corynebacterium bovis</i>	Chl Chl Pen	Cleared Cleared Cleared Cleared	Origin not determined Origin not determined Origin not determined Origin not determined
OT 17	24	35	3 18 29 30 35	Pneumonia Gastroenteritis Pneumonia Left subphrenic granuloma Bacteremia	Pseudomonas Salmonella, group B Pseudomonas <i>C. albicans</i> Pseudomonas	Amp, Ka Amp Amp Amp, Po	Cleared Cleared Progressive; fatal Persistent Fatal	
OT 19	4	270, alive	16 43	Urinary tract infection Bacteremia	Proteus <i>Cl. perfringens</i>	Pen, Tet	Cleared Cleared	Origin not determined
OT 22	33	10	4	Pneumonia Peritonitis	Pseudomonas, Aerobacter-Klebsiella Aerobacter-Klebsiella; yeast	Ka, Po	Progressive Terminal finding	

*To April 15, 1969

†Abbreviations for antibiotics; Str = streptomycin, Chl = chloramphenicol, Po = polymyxin B, Meth = methicillin, Kef = cephalothin, Amp = ampicillin, Pen = penicillin G, Ka = kanamycin, Tet = tetracycline, Ery = erythromycin, and Pent = pentamidine.

Table 23. Infectious Complications in the Later Cases of Liver Transplantation (*Continued*)

PATIENT	AGE (Yr)	SURVIVAL* (Days)	ONSET OF INFECTION (Days)	TYPE OF INFECTION	ETIOLOGY	THERAPY†	COURSE	COMMENT
OT 23	15	143	5	Bacteremia	Aerobacter-Klebsiella	Amp, Ka	Cleared	
			7	Urinary tract infection	Aerobacter-Klebsiella	Amp, Ka	Cleared	
			124	Urinary tract infection	Aerobacter-Klebsiella	Amp, Ka	Persistent	
			124	Bacteremia	Aerobacter-Klebsiella	Amp, Ka	Persistent	
OT 24	3	11	Pretransplant	Enteritis	C. albicans		Persistent	
				Urinary tract infection	C. albicans		Persistent	
				Pneumonia	C. albicans		Persistent	
				Fungemia	C. albicans	Amphotericin B	Persistent	
				Urinary tract infection	Pseudomonas	Kef	Persistent	
				Wound infection	C. albicans	Amphotericin B	Persistent	
				Bacteremia; pneumonia	Pseudomonas	Kef	Fatal	
AT 4	48	24	Pretransplant	Tracheal aspirate	C. albicans		Persistent	
				Urinary tract infection	C. albicans		Persistent	
				Urinary tract infection	E. coli	Nitrofurantoin	Cleared	
				Urinary tract infection	Aerobacter-Klebsiella	Nitrofurantoin	Persistent	
				Wound infection	E. coli		Persistent	
				Pneumonia	Aerobacter-Klebsiella	Str, Chl	Persistent	
				Bacteremia	E. coli	Str, Chl	Persistent	

*To April 15, 1969

†Abbreviations for antibiotics; Str = streptomycin, Chl = chloramphenicol, Po = polymyxin B, Meth = methicillin, Kef = cephalothin, Amp = ampicillin, Pen = penicillin G, Ka = kanamycin, Tet = tetracycline, Ery = erythromycin, and Pent = pentamidine.

instance the infections were quickly controlled by the institution of antibiotic therapy (Table 23).

In addition to the microorganisms that probably came from the intestinal tract, bacteremias were also seen (OT 13 and 16A) with *Diplococcus pneumoniae*, *Hemophilus* and *Staph. albus*; the same organism could not be isolated from the upper airway, the trachea, or any of the other sampling sites.

In fact, the source of the blood stream bacteria in the six patients could be found only in two (OT 15 and 23) who, coincident with one of several bouts of septicemia late in their lives, had the same microorganisms in the urine.

Hepatic Sepsis. Infection within the homografts was probably caused in two of the long surviving patients by metastases (Chapter Seventeen). It was in these two cases (OT 15 and 23) that the same bacteria were simultaneously cultured from the urine and blood stream. The biliary ducts of one graft (OT 15) were obstructed by tumor with secondary cholangitis (Chapter Twenty); the other transplant was nearly replaced with metastases.

There was one example of histologically diagnosed cholangitis in the absence of duct obstruction (Chapters Seventeen and Twenty). The homograft was removed and replaced after 380 days (OT 14).

THE INFECTIOUS PROBLEM IN RETROSPECT

Early in this chapter, emphasis was placed on the general role of immunosuppression in causing infection in recipients of different whole organ homografts. It has become increasingly obvious that the consequent risks may be disproportionately great after liver transplantation. This fact was appreciated from the beginning of our experience, but the reasons were not well defined. Initially it was thought that drug overdosage (Chapter Thirteen), ischemic injury to the homografts (Chapter Six), and the need for chronic antibiotic therapy in patients with partial liver gangrene (Chapter Fifteen) were the primary factors that were responsible for the extraordinarily high incidence of extrahepatic septic complications. Observations of more recent cases of liver transplantation, in which these adverse conditions were avoided, have added a more sinister dimension.

In the later patients there was strong justification for belief that the transplanted liver was itself the portal of entry by which microorganisms of all kinds gained direct access to the blood stream. The variety of bacteria that were cultured from peripheral veins, early or many months after operation, was strikingly similar to that found by Brettschneider et al¹ in dogs and pigs subjected to liver injury or hepatic transplantation. In the patients the failure to find any other focus of infection necessitated indictment of the homograft by the process of exclusion, even when the microorganisms were gram positive cocci.

The exposed relation of the orthotopic liver to gastrointestinal flora carried to it through the portal venous and duct systems was discussed in Chapter Fifteen in describing how gangrene developed in large focal areas of hepatic necrosis. The same routes of contamination were probably responsible for the sporadic bacteremias from the intact homografts. If the bacterial "leak" oc-

curred through the ducts, this was apparently possible without the presence of significant cholangitis since histopathologic evidence for the latter diagnosis has not commonly been found (Chapter Twenty). It should be added that portal phlebitis had not been diagnosed on morphologic grounds in any of the grafts; nevertheless, visible injury to the intrahepatic portal veins with consequent increased "porosity" is a hallmark of immunologic injury of transplanted livers (Chapter Twenty).

The ease with which bacteria seemed to enter the circulation through the hepatic homografts may be partly due to the kind of alteration that cannot be seen through a microscope, namely, a subtle decline of a particular kind of graft reticuloendothelial activity. Conceivably this could lead to the loss of the normal function of bacterial filtration. Not only would this result in greater permeability of the transplant to microorganisms, but it could in a more general way undermine the total host defenses against infection of other organ systems.

In spite of the special problems of infectious disease control which are introduced by liver transplantation, long-term survival after such procedures has not thereby been precluded in dogs (Chapter Twelve), pigs (Chapter Eleven), or humans (Chapter Seventeen). Many factors which are essential for success have already been discussed in this and the preceding chapters. Of these, only the need for accurately controlled antibiotic administration will be mentioned again. Treatment must be guided by repeated cultures and sensitivities. Otherwise, the overgrowth of resistant populations will be promoted. For the same reason, chronic antibiotic therapy should be avoided if at all possible.

As was demonstrated in the later patients in the series, most of the bacterial infectious complications could be promptly cleared with the institution of carefully selected antimicrobial drugs. An aggressive attempt at accurate diagnosis may be lifesaving even with infestations by opportunistic organisms such as fungus, cytomegalovirus, or *Pneumocystis carinii*. It has been reported that control of infections caused by these agents can sometimes be obtained by intensive courses of uncommon experimental drugs or by the administration of immune globulin.^{2, 4, 18, 22}

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Chapter Seventeen

LATE RESULTS AND COMPLICATIONS

In this chapter attention will be focused primarily upon observations that were made two months or longer after orthotopic liver transplantation.^{29, 75-81} Before doing this a capsule summary will be presented of the survival statistics in our total experience from March 1, 1963, to February 11, 1969.

OVERALL RESULTS

Throughout the book details have been given about all the 25 recipients of replacement homografts. Included have been analyses of the causes of early failure as well as suggestions for the avoidance of a number of acute or delayed complications of a serious or lethal nature. In the course of this exposition the length of survival of each recipient has been mentioned on one or more occasions. Since the same information will be documented in tabular form in Chapter Twenty-four, additional discussion now about this aspect of the problem might seem superfluous. Nevertheless, there may be merit in examining the highly variable quality of the results in the different phases of our experience.

The outcome in the total series is shown in Figure 158. Twelve of the 25 patients, including the first seven, did not live through the first postoperative month. As a consequence the life survival curve had a shape that was very similar to that with our first trials five to six years ago of renal homotransplantation between nonrelated individuals (Fig. 107, Chapter Thirteen). The reasons for the steep acute loss rate have been thoroughly described in other chapters. In all seven of the earliest cases, the use of damaged homografts played an important role (Chapter Six). The death of subsequent recipients within the first four weeks was invariably related directly to a technical surgical accident of one kind or other (Chapters Eight and Nine). Even in the second month two more patients (OT 17 and 25) died of complications which had their genesis from difficulties at the original operations (Chapter Nine).

The absolute necessity of having a technically perfect transplantation was particularly well illustrated by the experience which began on July 23, 1967.

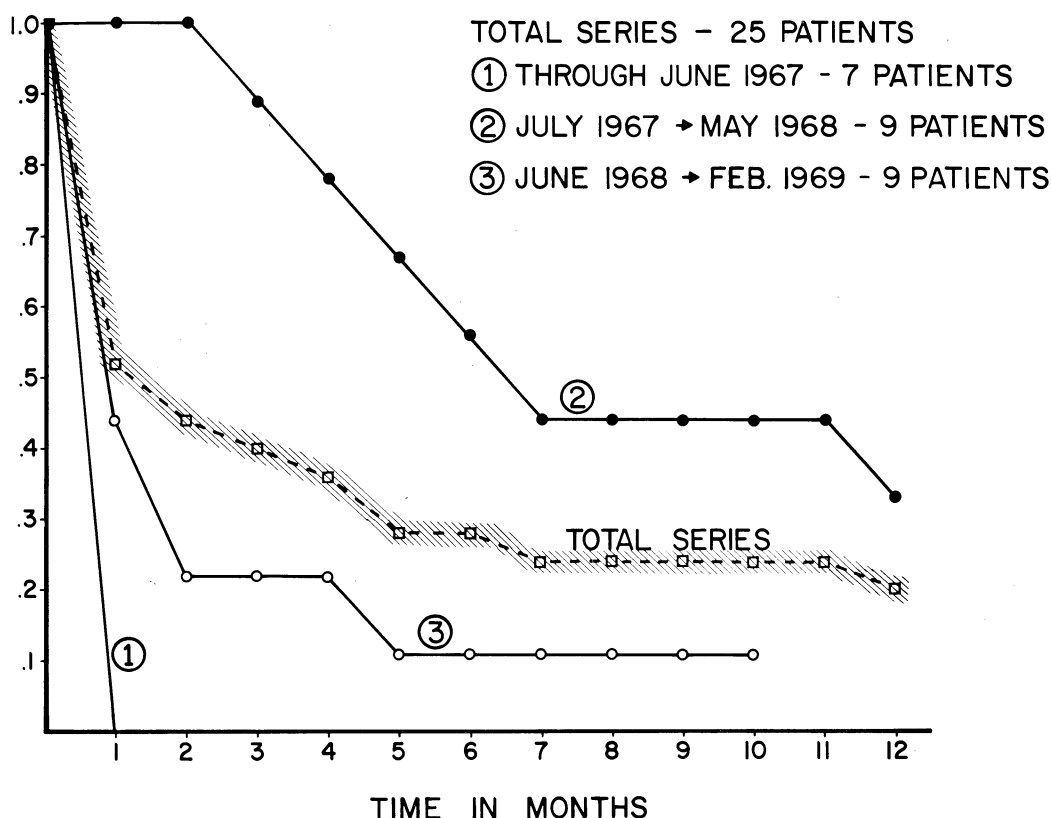


Figure 158. Life survival curve of 25 patients treated at our institutions with orthotopic liver transplantation. The shortest follow-ups for recipients who are still alive on May 15, 1969, are 10 (OT 19) and 12 (OT 16) months. Since Patient OT 19 still has perfect hepatic function, the curves may be considered essentially complete to one year. The results have also been divided according to the first, second, and third intervals of our total experience. The ordinate indicates the fraction surviving.

All patients treated after that date had the benefits of efforts at histocompatibility matching (Chapter Three), better immunosuppression (Chapter Thirteen), and an improved quality of the homografts (Chapter Six). The next nine consecutive recipients lived for at least two postoperative months. At 3, 6, 9, and 12 months there were 8, 5, 4, and 3 patients, respectively, still surviving (Fig. 158).

In contrast, the early results were much poorer in a final series of nine cases compiled from June, 1968 to February, 1969, in which an array of anomalies was encountered (Chapters Eight and Nine) of either the host or homograft vascular system or of the graft extrahepatic biliary tree. There were only two of the nine later patients living 60 days after transplantation, and by the end of the fifth postoperative month only one was still surviving (Fig. 158). An abnormal anatomic disposition of the hilar structures was an important factor in all but two of the seven lethal technical calamities which were responsible for the early failures. In Chapters Eight and Nine recommendations were made which might help in future cases to avoid similar accidents. Conceivably an additional useful practice would be to perform aortography prior to transplantation, at least in the recipients and possibly also in the donors. The

detection of unusual vascular patterns could lead to a decision against operation or, at least, the information obtained could aid in the advance planning of a variant procedure.

RECURRENCE OF MALIGNANT DISEASE

There were 11 recipients of 12 homografts who survived for at least 60 days after operation. With a single possible exception (OT 9), the subsequent events could not be said to have been influenced by earlier technical surgical complications. Nevertheless, not all these cases could be used to study in a clear way the life expectancy of patients with chronically functioning orthotopic livers. The reason was that several deaths were caused by the neoplastic process for which treatment was initially undertaken.

As mentioned in Chapter One, the most absolute indication for liver replacement was thought at first to be primary hepatic malignancy that had advanced to the stage at which a standard resection was no longer possible. This was the diagnosis in 11 of the 25 recipients of orthotopic liver homografts in our series. In each case extrahepatic metastases were not identifiable before or at the time of operation. Consequently it was thought that all the total hepatectomies were potentially curative procedures. This proved to be an overly optimistic projection.

Clinical Observations in Liver Recipients

Early Death. Seven of the 11 patients with primary cancer of the liver died from six and a half to 39 days after orthotopic hepatic homotransplantation (OT 2 to 6, 17, and 25). At autopsy an exhaustive search was made to determine if any metastases had been missed with the preoperative survey and the operative examination. No residual tumor was found in six of the seven cases. In the other patient (OT 4), there had been spread to one of the lumbar vertebrae.

Prolonged Survival. The other four recipients (OT 8, 14, 15, and 23) lived through the immediate effects of the operation and became available for longer term studies. In all four hepatic cell carcinoma (hepatoma) was the histologic diagnosis (Fig. 159A). One of the patients is still alive after 14 months, but with slowly growing metastases (OT 14). Recurrent neoplasm was responsible for the death of the other three after 143, 339, and 400 days, respectively.

The general features of the four cases are summarized in Table 24. In the three patients who died of carcinomatosis, the diagnosis of recurrent malignant disease was first made from 29 days to 13 weeks postoperatively on the basis of new abnormalities in the chest x-rays. After the first lesions became visible, these and other deposits enlarged with great rapidity (Figs. 160 to 162).

The first chronic survivor after orthotopic hepatic homotransplantation was a 19 month old child. More than three months after operation and a few days after pulmonary metastases had been diagnosed, she was found to have a mass in the right upper quadrant between the transverse colon and the liver.

(Text continued on page 355.)

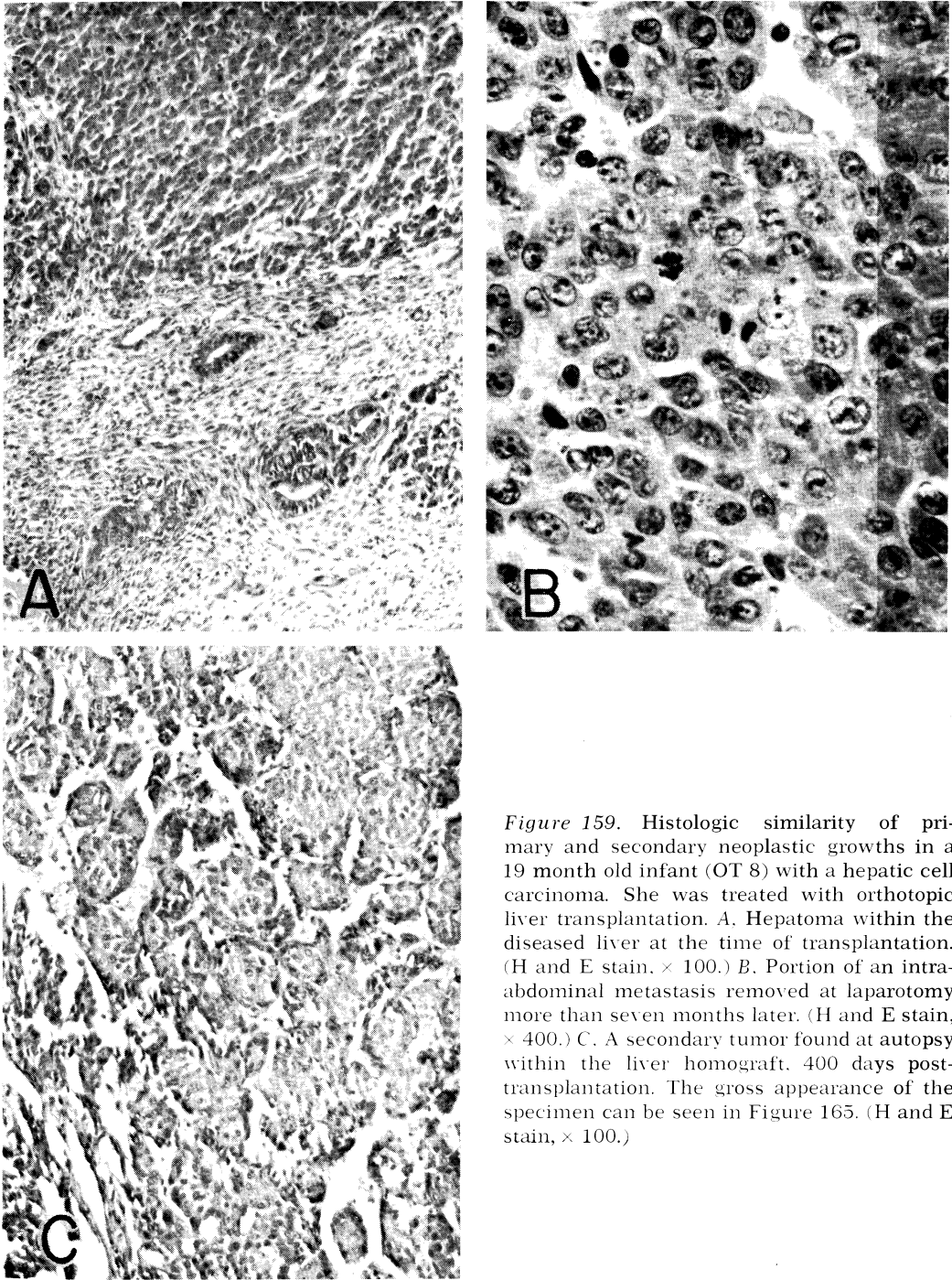


Figure 159. Histologic similarity of primary and secondary neoplastic growths in a 19 month old infant (OT 8) with a hepatic cell carcinoma. She was treated with orthotopic liver transplantation. A, Hepatoma within the diseased liver at the time of transplantation. (H and E stain, $\times 100$.) B, Portion of an intra-abdominal metastasis removed at laparotomy more than seven months later. (H and E stain, $\times 400$.) C, A secondary tumor found at autopsy within the liver homograft, 400 days post-transplantation. The gross appearance of the specimen can be seen in Figure 163. (H and E stain, $\times 100$.)

Table 24. Four Patients with Hepatoma* :

PATIENT NUMBER	METASTASES FIRST DETECTED (Days Postop)	LOCATION FIRST METASTASES	TREATMENT OF METASTASES	METASTASES TO HOMOGRAFT	ORGANS ULTIMATELY INVOLVED	CAUSE OF DEATH & TIME
OT 8	90	Lungs	Vincristine sulfate; 5 FU; surgical excision of intra-abdominal masses; local x-ray therapy to pelvis	Yes	Brain, lungs, liver, other abdominal organs	Carcinomatosis 400 days
OT 14	380	Diaphragm, liver, retroperitoneal space	—	Yes	Diaphragm, retroperitoneal space, ribs, liver, ? pancreas	Alive 14 months
OT 15	60	Lungs	—	Yes	Lungs, liver, diaphragm	Carcinomatosis 339 days
OT 23	29	Lungs	—	Yes	Brain, lungs, liver, retroperitoneal space	Carcinomatosis 143 days

*The fate of four patients who received liver replacement for the indication of hepatoma and who lived long enough after operation to permit observations about the course of the malignancy.

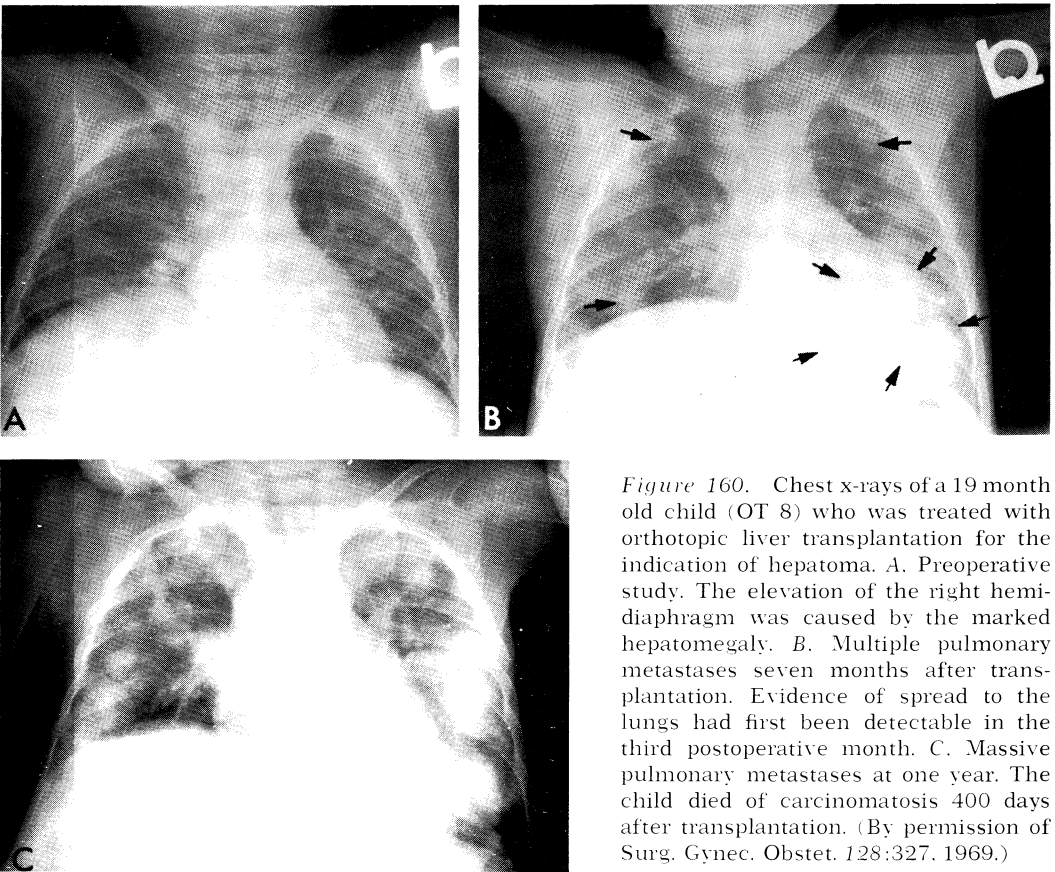


Figure 160. Chest x-rays of a 19 month old child (OT 8) who was treated with orthotopic liver transplantation for the indication of hepatoma. A. Preoperative study. The elevation of the right hemidiaphragm was caused by the marked hepatomegaly. B. Multiple pulmonary metastases seven months after transplantation. Evidence of spread to the lungs had first been detectable in the third postoperative month. C. Massive pulmonary metastases at one year. The child died of carcinomatosis 400 days after transplantation. (By permission of Surg. Gynec. Obstet. 128:327, 1969.)

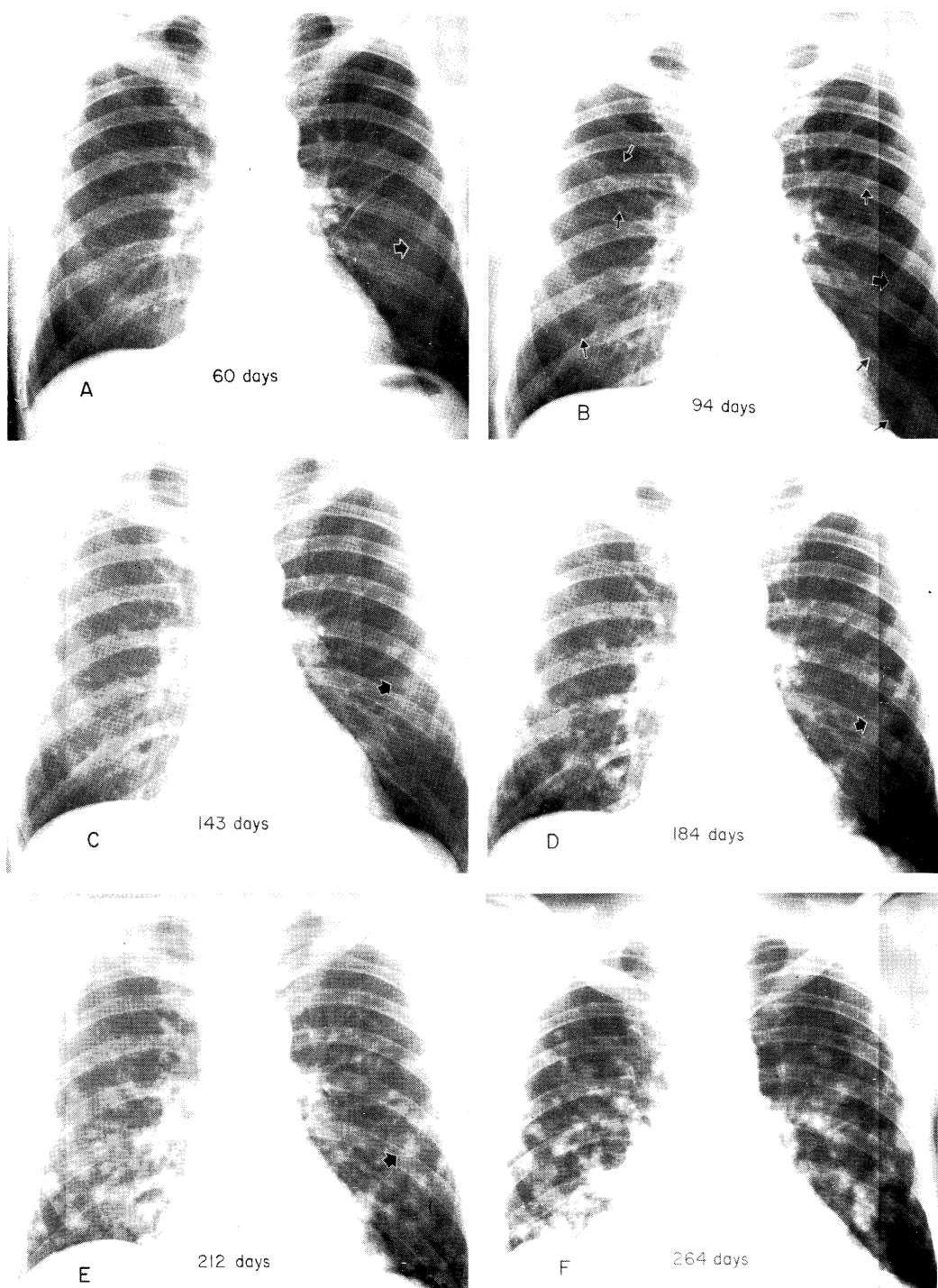


Figure 161. Appearance and evolution of pulmonary metastases in patient OT 15, who received liver replacement for hepatoma. At the time of operation there was no evidence of extrahepatic tumor growth. A. Chest film obtained 60 days after transplantation. A single neoplastic nodule was detected (arrow). In retrospect, the lesion was probably present on earlier examinations. B. Ninety-four days postoperatively. The metastasis seen earlier has enlarged (heavy arrow) and at least six more are visible. C. During the seven weeks since the previous x-ray, the nodule indicated by the arrow has more than doubled in size. D. Six months postoperative. E. Seven months postoperative. F. Nine months postoperative. The patient died 339 days post-transplantation of pulmonary insufficiency.

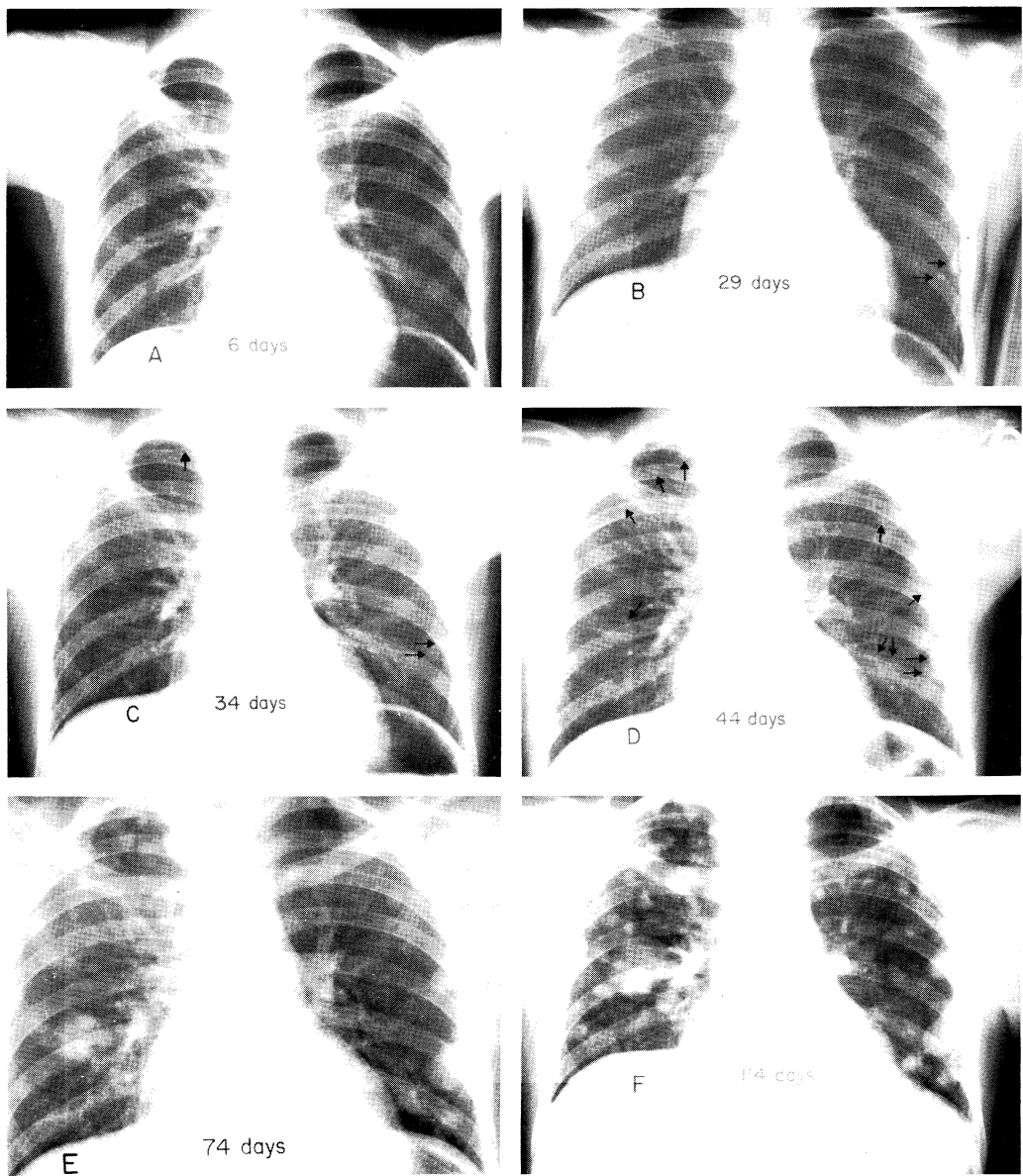


Figure 162. The extremely rapid development of pulmonary metastases in patient OT 23. *A*, The chest is clear six days after liver replacement for the indication of hepatoma. *B*, Twenty-nine days postoperative. Two metastases are visible in the left lower lung field (arrows). *C*, Five days later the tumor deposits previously seen have grown in size (horizontal arrows) and a third focus is now present in the right upper lobe (vertical arrow). *D*, Forty-four days. Only 10 days have elapsed since the last examination. Metastatic growths are scattered throughout the lungs (arrows). *E*, Seventy-four days postoperative. *F*, Four months after operation. Transient dyspnea was first noticed a few days later. The patient died of pulmonary insufficiency 143 days after transplantation.

Because of its proximity to the cholecystoduodenostomy, the recurrence was excised; it weighed 28 gm. Other intra-abdominal masses soon appeared. The largest of these was in the left lower abdomen and pelvis. It eventually caused obstruction of the sigmoid colon and both ureters (Fig. 163A and B). More than seven months after transplantation, 164 gm of the bulky pelvic tumor were removed piecemeal along with the uterus and one ovary (Fig. 159B). Small metastatic nodules were present throughout the rest of the abdomen. Temporary palliation was obtained (Fig. 163C and D).

Eventually a huge metastasis became evident in the same approximate subhepatic location as the first one that had been resected. It appeared to compress the cholecystoduodenostomy and it was probably at least partly responsible for the jaundice that developed during the last few weeks of life (Fig. 164), since, at autopsy, the intrahepatic ducts were dilated and there was evidence of cholangitis (Chapter Twenty).

Terminally, the child developed Jacksonian seizures, lapsed into coma and died 400 days after the homotransplantation. At autopsy large deposits of tumor were found within the calvarium, thorax, and abdominal cavity. It was of special interest that the liver homograft was the site of two moderately large neoplastic nodules (Figs. 159C and 165) (the probable route of the metastatic spread will be discussed later); the transplanted organ weighed 520 gm.

The distribution of the recurrences in the other three patients is given in Table 24. The most explosive metastases were in patient OT 23, beginning in the lungs (Fig. 162). However, the unique feature in this patient was the suddenness and completeness with which tumor destroyed the homograft. This was eventually reflected in the development of hepatic failure.

The benign early course of the recipient was considered in Chapter Fourteen. The only rejection that was encountered in the first two months was the mild "anicteric" variety. Then, about 13 weeks after transplantation, jaundice appeared and progressed until the time of death 143 days after operation. Ultimately, deterioration occurred of all other hepatic function tests (Fig. 166), although the immediate cause of death was pulmonary insufficiency. Late in the course there were two bouts of gram negative septicemia (Fig. 166).

The reason for the homograft functional abnormalities was at first suspected to be delayed rejection. However, liver scans revealed very rapidly enlarging areas of poor or absent isotope concentration in both hepatic lobes of the enlarged transplant (Fig. 167). Since these changes were not initially accompanied by fever, septicemia, or the high transaminase rises seen with septic hepatic infarction (Chapter Fifteen), the diagnosis was made of recurrent hepatoma. An aortogram showed the arterial blood supply to be intact.

At autopsy the liver weighed 3000 gm compared to an estimated weight of 750 gm at the time of transplantation. Its blood supply was intact and the biliary duct system was unobstructed (Fig. 168). An estimated 95 per cent of the organ consisted of tumor. The only tissue that resembled hepatic parenchyma grossly was in the central portion. The outer shell of carcinoma had the same necrotic appearance (Fig. 169, *upper*) and crumbly consistency of the widespread pulmonary metastases (Fig. 169, *lower*). The cell type of the recurrences was similar to that of the original hepatoma.

(Text continued on page 364.)

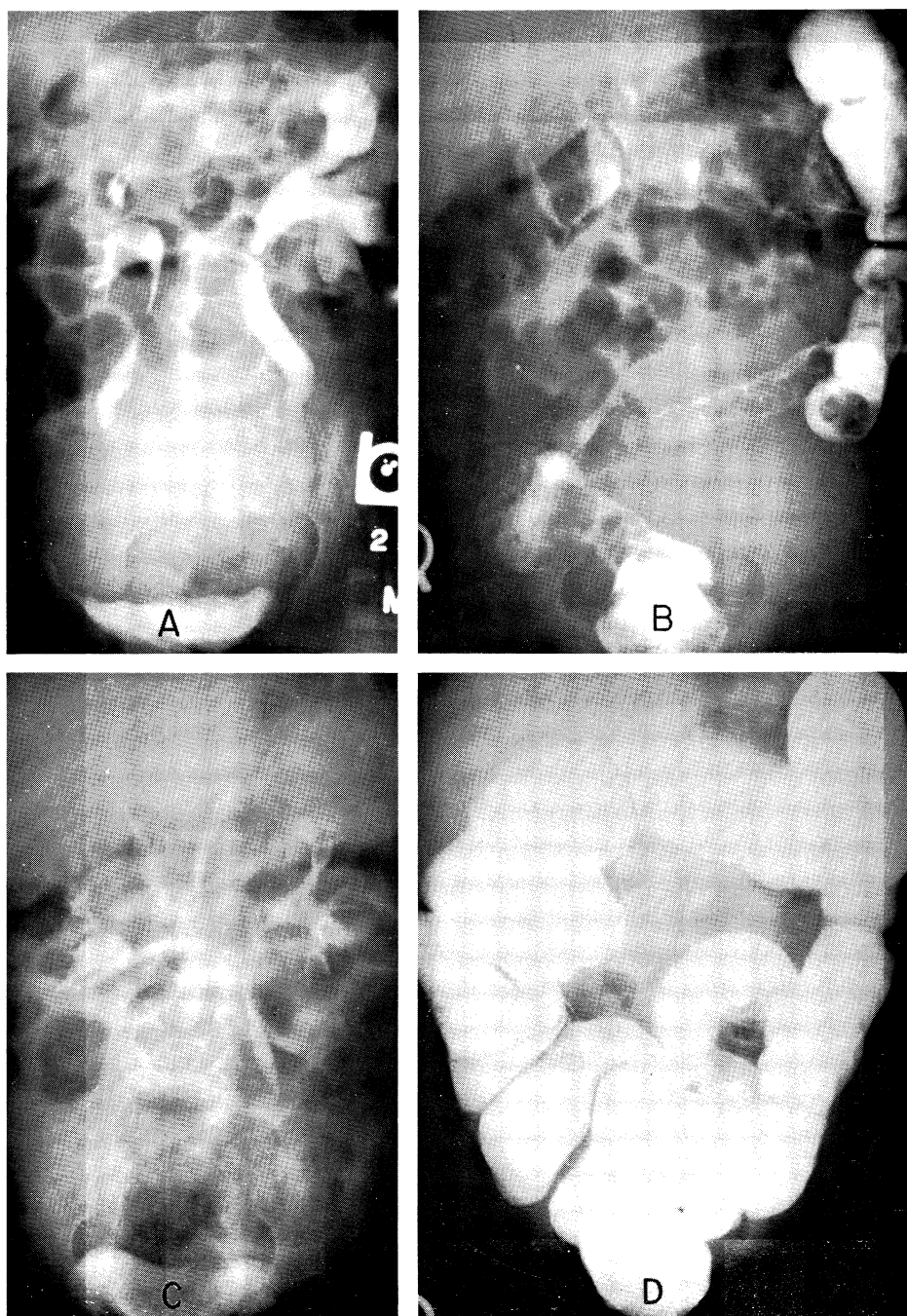


Figure 163. Palliation achieved in patient OT 8 following intra-abdominal tumor recurrences. A 28-gram metastasis in the right upper quadrant had been excised 99 days post-transplantation. Four months later exploration was again necessary in order to relieve obstruction of the sigmoid colon and both ureters. A. Intravenous pyelogram obtained 219 days after transplantation. B. Barium enema performed the same day. The rectum is deflected to the right; the sigmoid colon is displaced upward and posteriorly. C. Shortly after the above examinations, 164 gm of the tumor mass were removed from the pelvis. An IVP performed two weeks later demonstrates relief of the bilateral ureteral obstruction. D. Barium enema examination 241 days post-transplantation. The colon and rectum appear normal and without displacement.

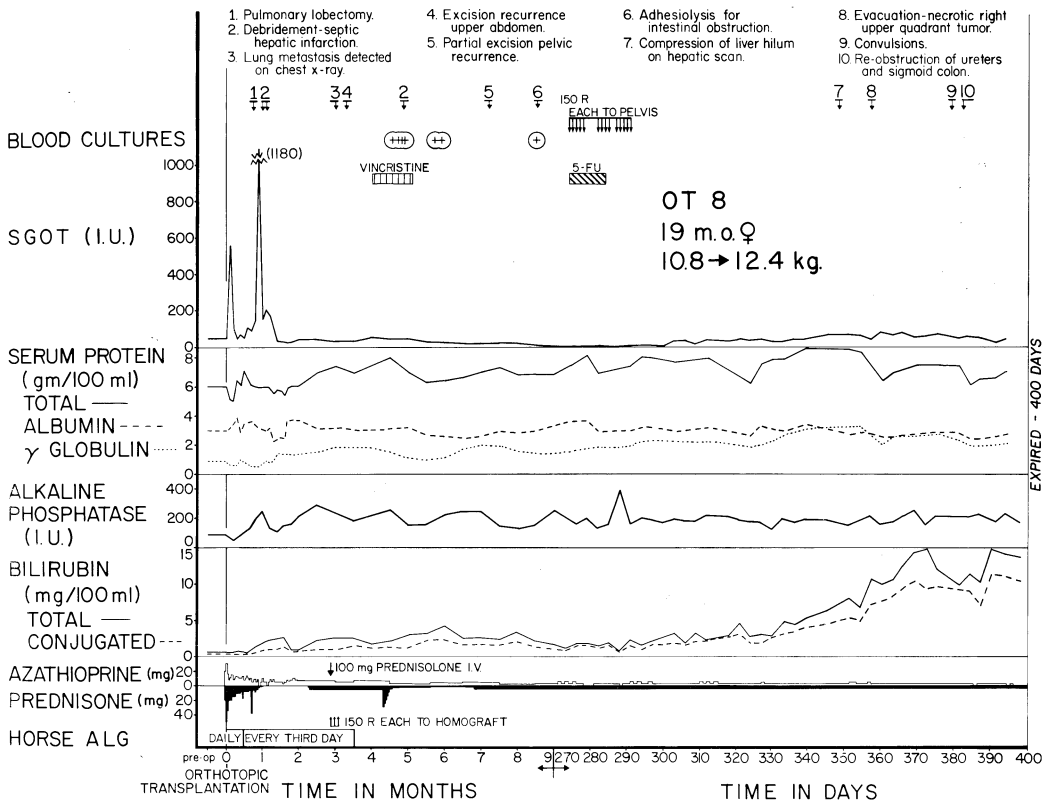


Figure 164. The total course of the first chronic survivor after orthotopic liver transplantation. The 19 month old child (OT 8) had a hepatoma which eventually caused multiple recurrences in the lungs, homograft, other abdominal viscera, and brain. The early convalescence was complicated by a septic hepatic infarction, the details of which are documented in Figure 140, Chapter 15; both the early and the later positive blood cultures with gram negative organisms probably came from the liver. The administration of vincristine and 5-fluorouracil (5-Fu) had no effect on the rate of metastatic progression, nor did the local irradiation of the pelvic tumor. The increasing hyperbilirubinemia during the last three months of life was thought to be due to compression of the biliary drainage system by a huge right upper quadrant mass. Note that an expanded time scale has been used in the last 130 days of life, during which time there was marked hypergammaglobulinemia. The normal ranges at this age for the enzyme measurements were: alkaline phosphatase (75 to 225 I. U.) and SGOT (less than 70 I. U.).



Figure 165. The hepatic homograft removed more than 13 months after orthotopic liver transplantation in patient OT 8. Note the two large metastatic nodules. (By permission of Surg. Gynec. Obstet. 128:327, 1969.)

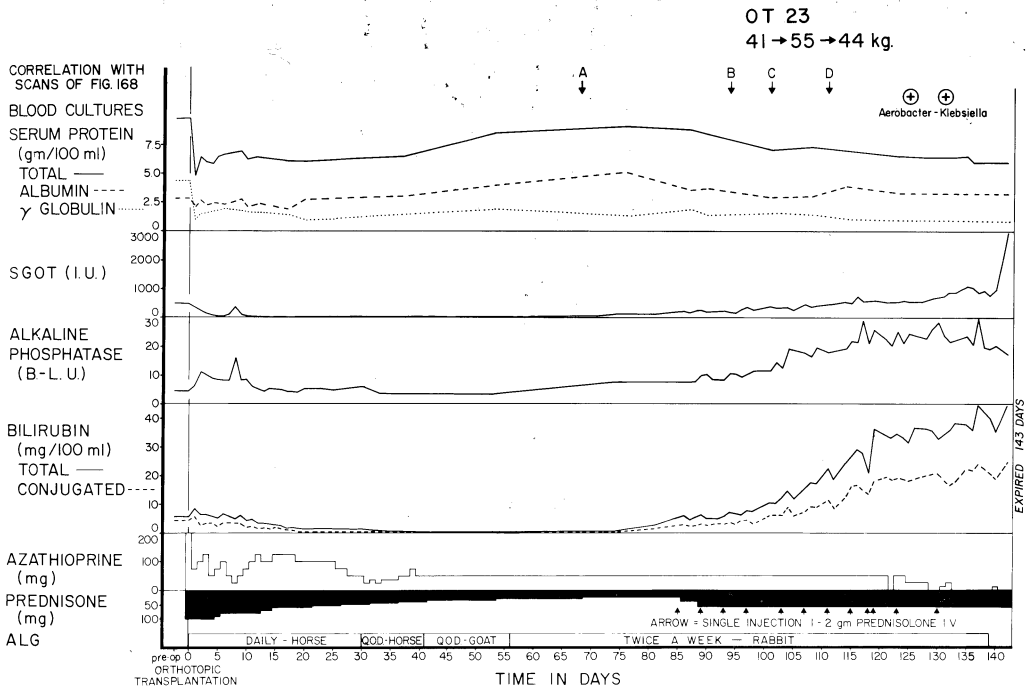


Figure 166. The manifestations of the invasion and nearly complete destruction of an orthotopic liver homograft by recurrent hepatoma (patient OT 23). The sporadic large doses of intravenous prednisolone were given because of the possibility that the deteriorating liver function was due to delayed rejection. At autopsy almost all the transplanted hepatic tissue was replaced by tumor (Fig. 169). Note that the patient had hypergammaglobulinemia before transplantation and that this finding persisted during most of the postoperative period. The normal range for Bessey-Lowry (B-L) alkaline phosphatase units is 1 to 3. The normals for the SGOT and SGPT units at this age are 60 to 100 and 5 to 35, respectively.

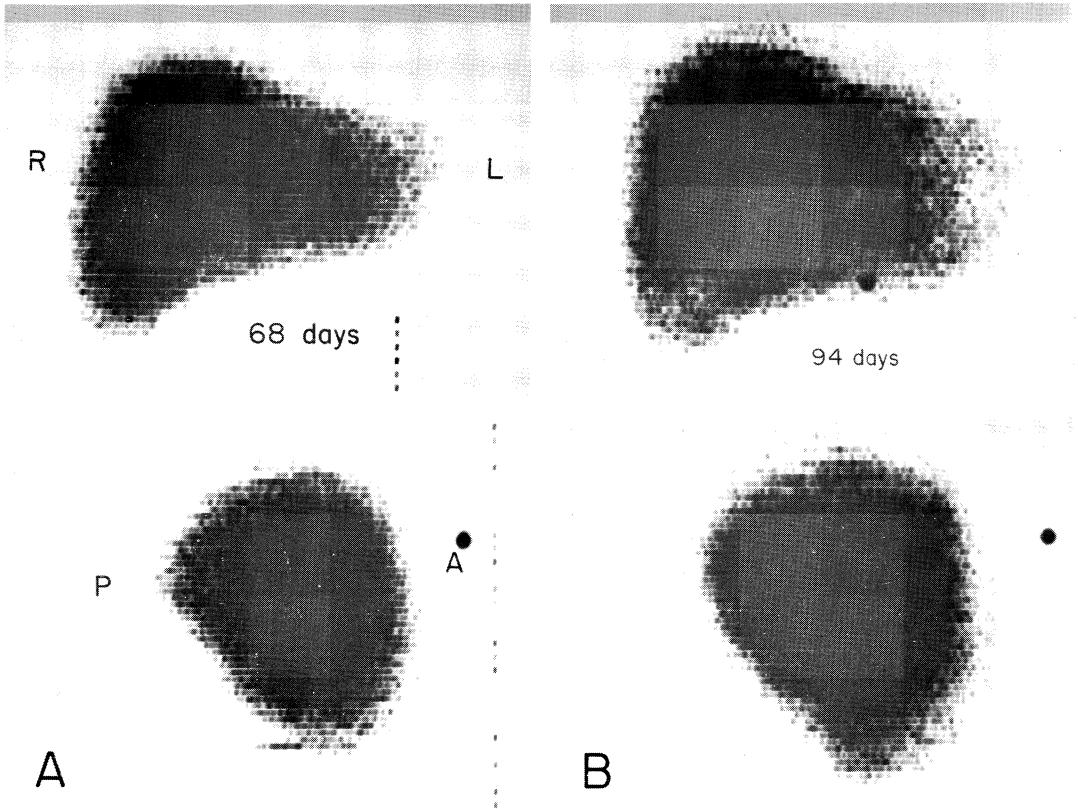


Figure 167. Destruction of the homograft in patient OT 23 by tumor recurrence. The postero-anterior and lateral liver scans were obtained with ^{99m}Tc technetium. *A.* 68 days: The scan is the same as the last one shown in Figure 123, Chapter 14. *B.* 94 days: The patient had become jaundiced. Hepatomegaly is evident.

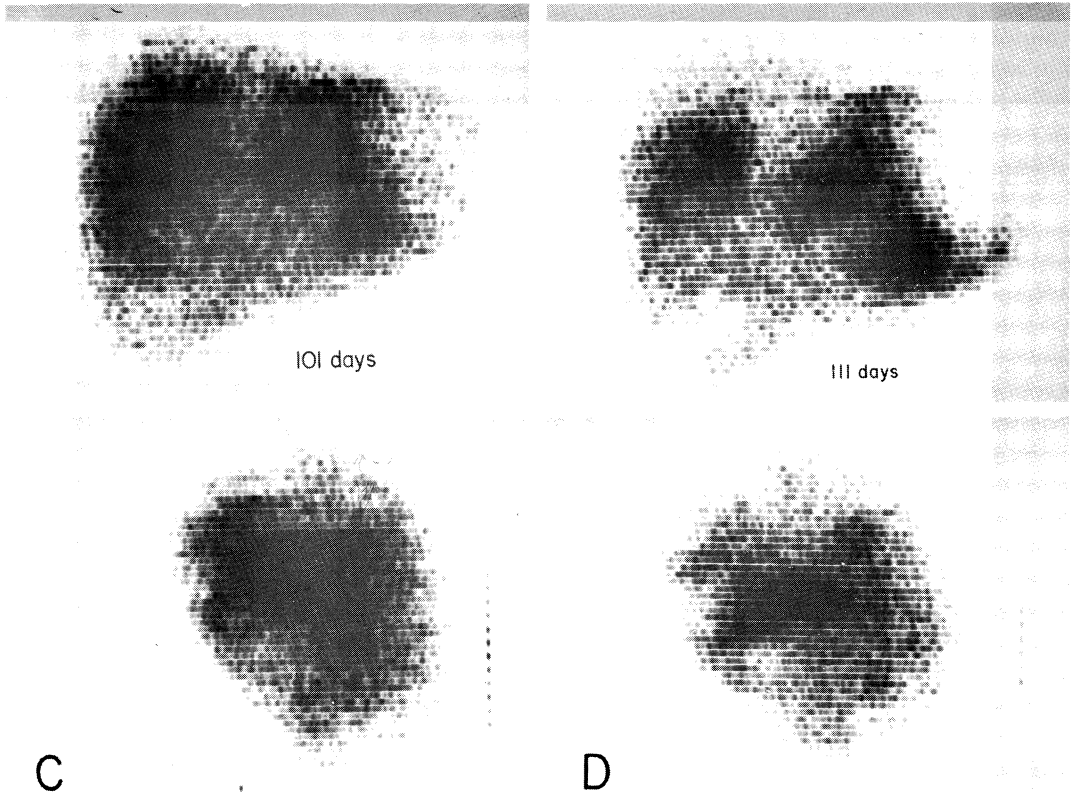


Figure 167. *Continued.* C. 101 days: Multiple areas of poor isotope concentration are now visible. D. 111 days: The process has continued its rapid progression. By the time of death one month later, the homograft was almost completely replaced with carcinoma.

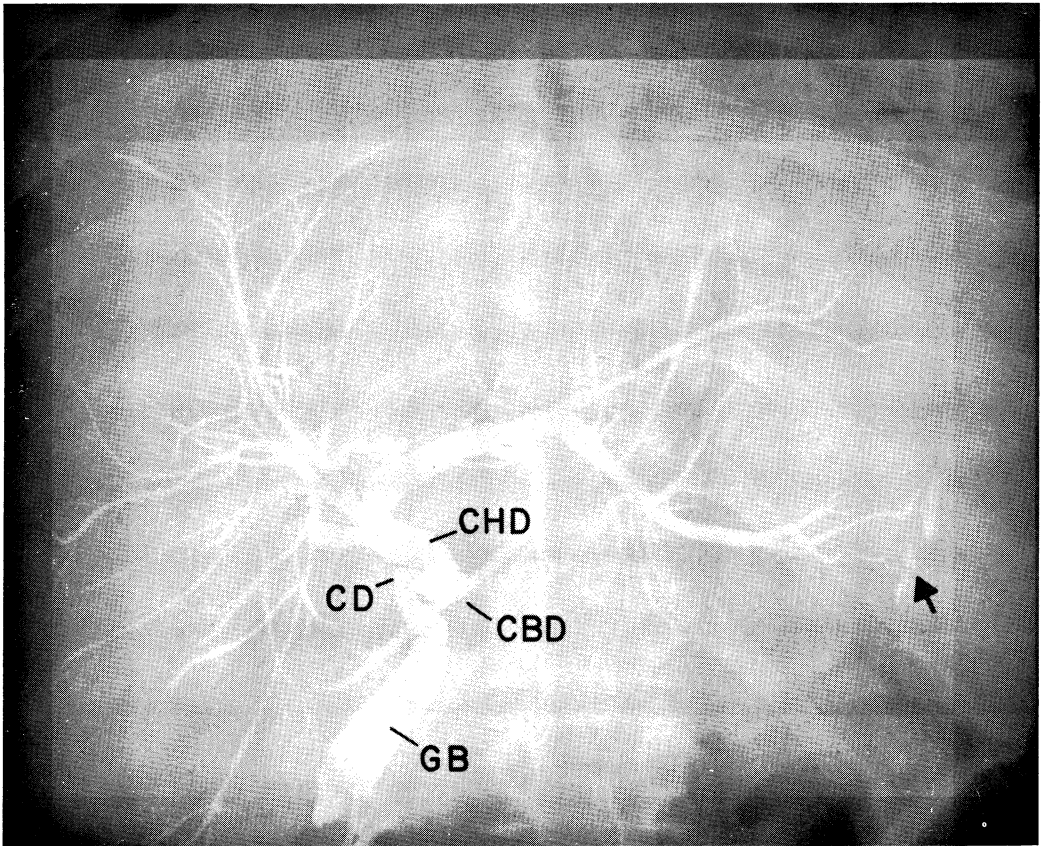


Figure 168. The postmortem cholangiogram in patient OT 23. At transplantation, 143 days earlier, biliary reconstruction was with a cholecystoduodenostomy. The autopsy study was made by tying a catheter into the gallbladder (GB). CBD=common bile duct; CD=cystic duct; CHD=common hepatic duct. The parenchymal extravasation indicated by the arrow was due to removal of a specimen from the left lobe.

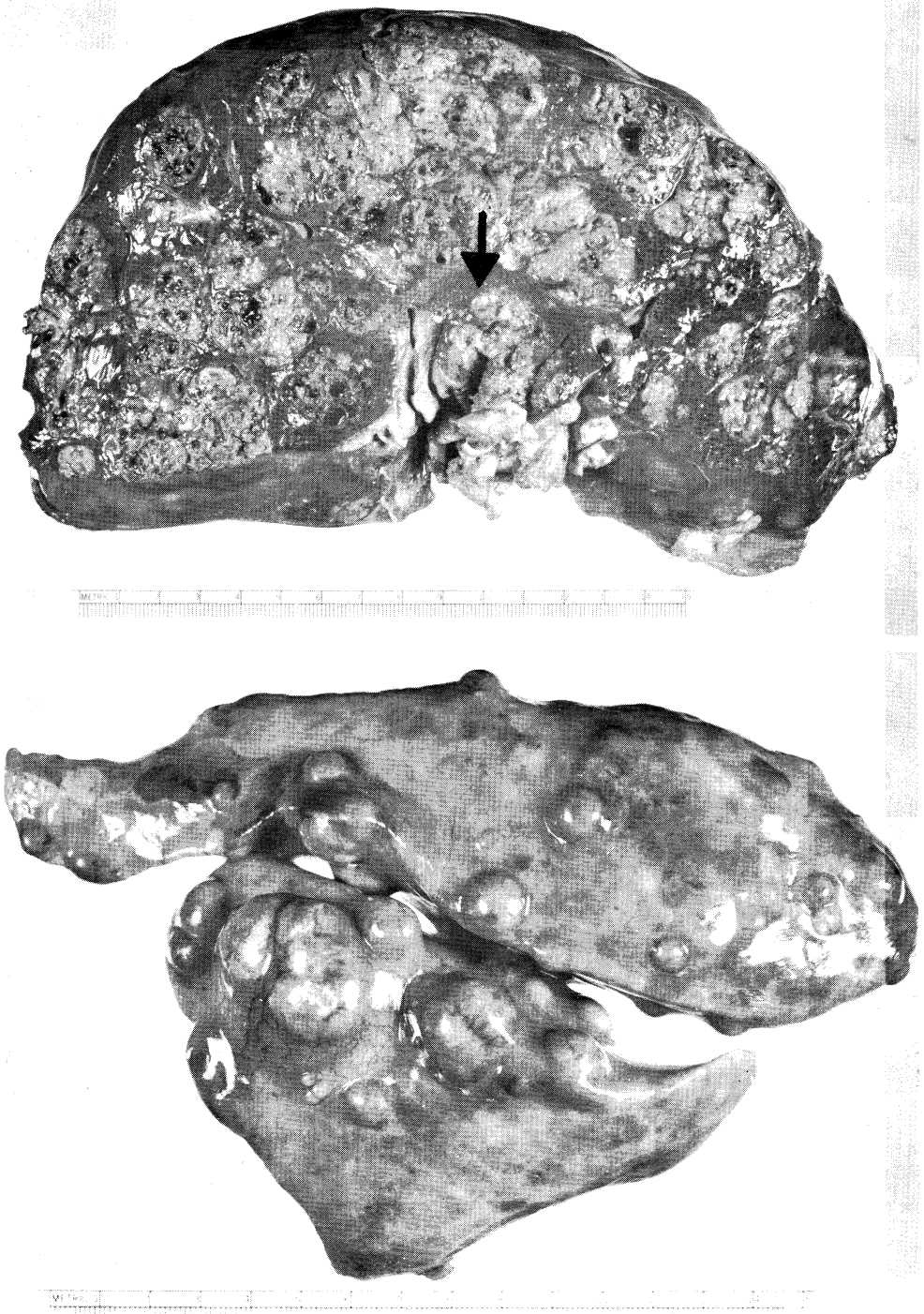


Figure 169. Metastases in patient OT 23, 143 days after orthotopic liver transplantation. A, The liver homograft. It has been replaced by tumor except for a very small residual area of hepatic parenchyma (arrow). B, Lower. Extensive pulmonary metastases.

A third patient (OT 15) lived for 339 days despite the presence of lung metastases (Fig. 161) during most of this time; death was caused by pulmonary insufficiency. During the first few weeks after operation he had passed through an explosive rejection crisis (Chapter Fourteen), but homograft function was thereafter excellent for more than two thirds of a year (Fig. 170). In the tenth postoperative month jaundice suddenly appeared, which waxed and waned for the remaining two months of life, usually in parallel with variations of the alkaline phosphatase. Moderate transaminase rises were also recorded, but there was serious deterioration of protein metabolism only terminally.

During the period of survival numerous liver scans were obtained. In retrospect, it was obvious that the homograft had begun to increase in size several weeks before the abnormalities in hepatic function became detectable. At the same time an area of decreased isotope uptake had become evident in the

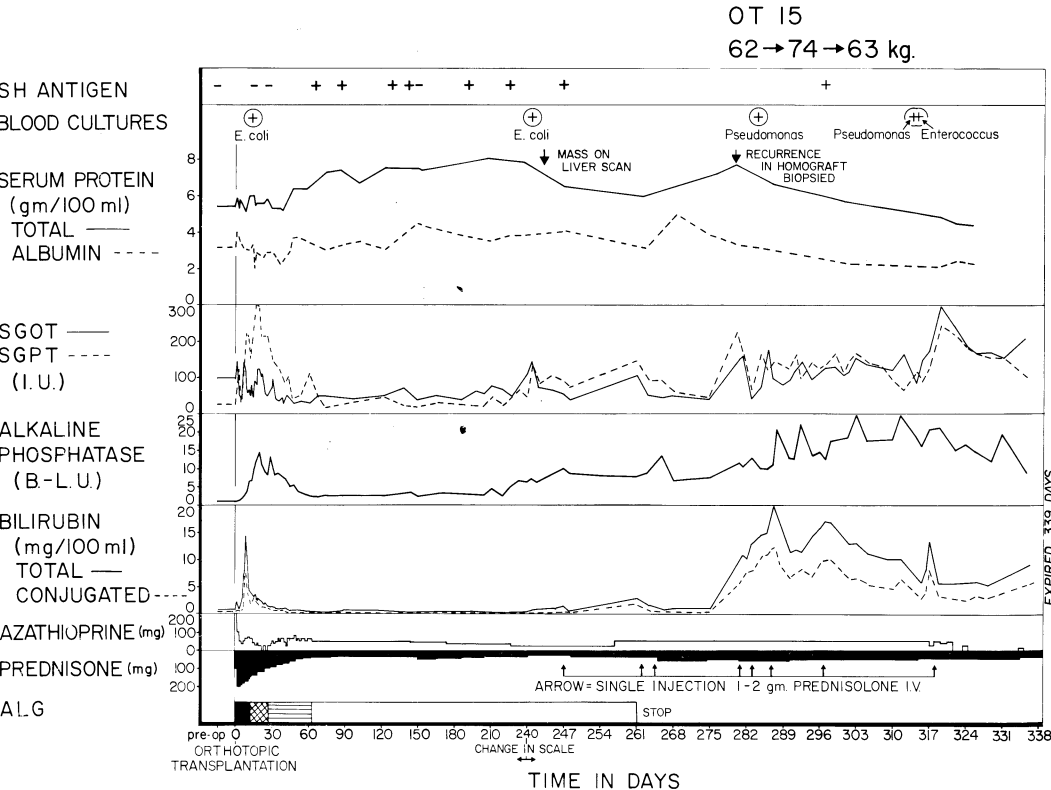


Figure 170. The course of a patient (OT 15) who died of recurrent hepatoma despite total hepatectomy and orthotopic liver transplantation. He passed through an early and vigorous rejection crisis but then had normal liver function for many months. Note that a condensed scale has been used for the first 240 postoperative days; the details of the early portion of the convalescence are shown in Figure 127, Chapter 14. The recurrent tumor was first diagnosed with the liver scan about eight months after transplantation (Fig. 171) and biopsy confirmation was obtained more than a month later. The episodic septicemia and deterioration of liver function were probably the consequence of invasion by tumor of the extrahepatic ducts (Fig. 172). The intermittent large steroid doses were given on the chance that rejection was also occurring. From left to right the different shadings in the ALG bar indicate that the injections were daily, every other day, every three days, and twice a week. The normal alkaline phosphatase range in Bessey-Lowry units is 1 to 3. For the SGOT and SGPT the normal ranges are 60 to 100 and 5 to 35 units, respectively. See text for discussion of the SH antigen.

hilum (Fig. 171). Shortly after the development of icterus, positive blood cultures of *E. coli* or *Pseudomonas* began to appear remittently (Fig. 170). It was decided to explore the suspicious region because of the possible diagnoses of liver abscess, septic hepatic infarction, or tumor metastases. At operation the liver in this area was somewhat firmer than normal but there was no evidence of necrosis. When a needle was placed into the zone of selective reticuloen-

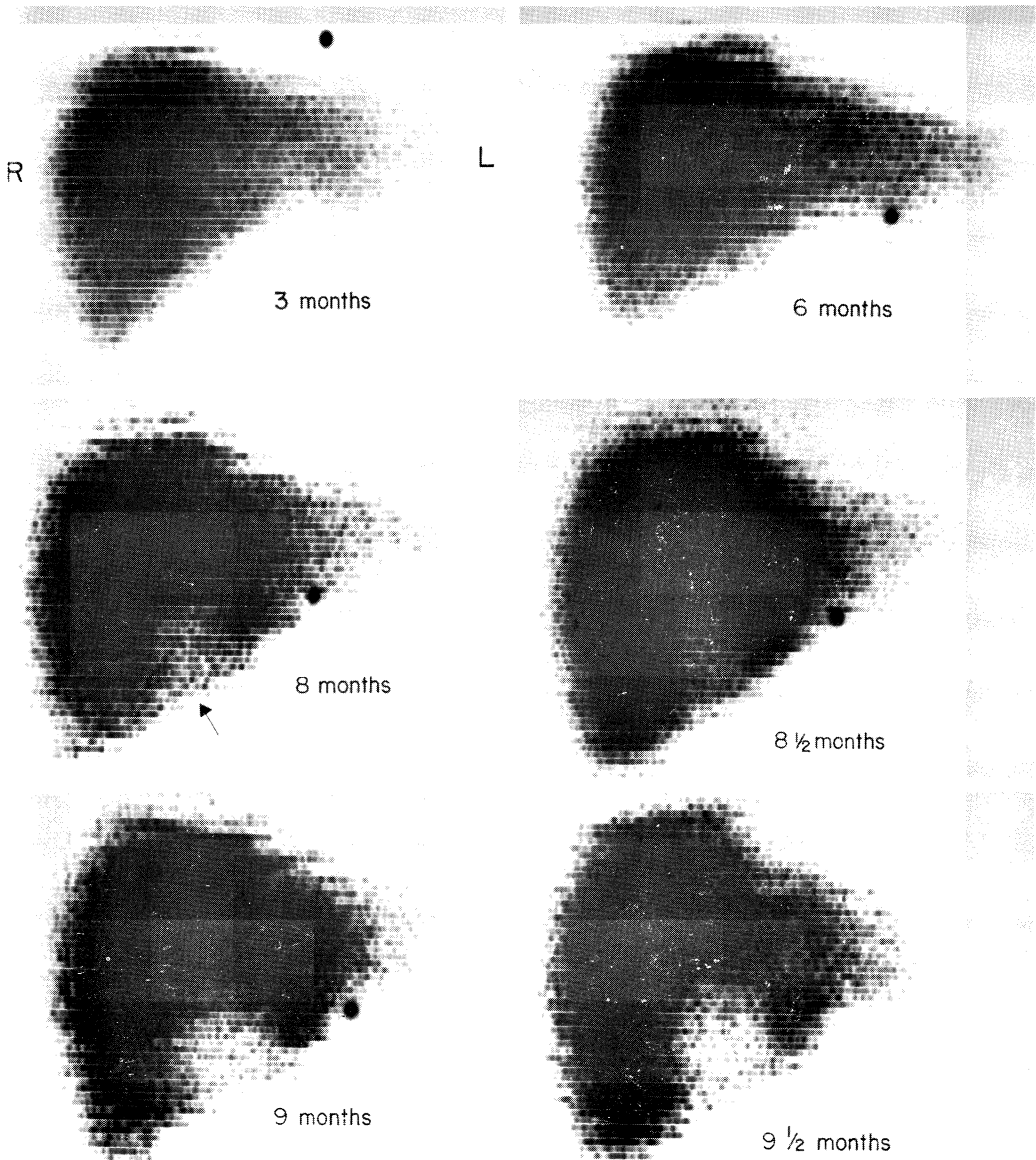


Figure 171. The progression of recurrent hepatoma in a transplanted orthotopic liver (OT 15). 3 months: The examination is essentially normal. 6 months: There has been no significant change. 8 months: A notch in the hilum is now visible. 8½ months: Two weeks later the defect has become much more evident. Moreover, the liver has increased in size. 9 months: Further progression. 9½ months: At the time of this scan, jaundice appeared. The patient eventually died 339 days after transplantation.

dothelial nonfunction, increased resistance was encountered for several centimeters. The needle then passed into softer hepatic tissue. A biopsy extracted through the needle contained carcinoma.

At the time of autopsy the diaphragmatic surface of the liver appeared grossly normal. By postmortem angiography, its blood supply was demonstrated to be unobstructed. Cholecystograms and cholangiograms were also made which showed that the gallbladder was collapsed and that the cystic duct was open. Nevertheless, there appeared to be a dye extravasation in the hilum as well as a general dilation of the entire intrahepatic collecting system (Fig. 172).

When the 1950 gm homograft was sliced in a coronal plane (Fig. 173, A), two large metastases were found. The one previously identified in the hilum by the liver scan was 10 cm in diameter. Its postero-inferior portion impinged upon the common hepatic duct. In this area there was a cavity which contained necrotic tissue and bile. Erosion of the duct system at this point was thought to

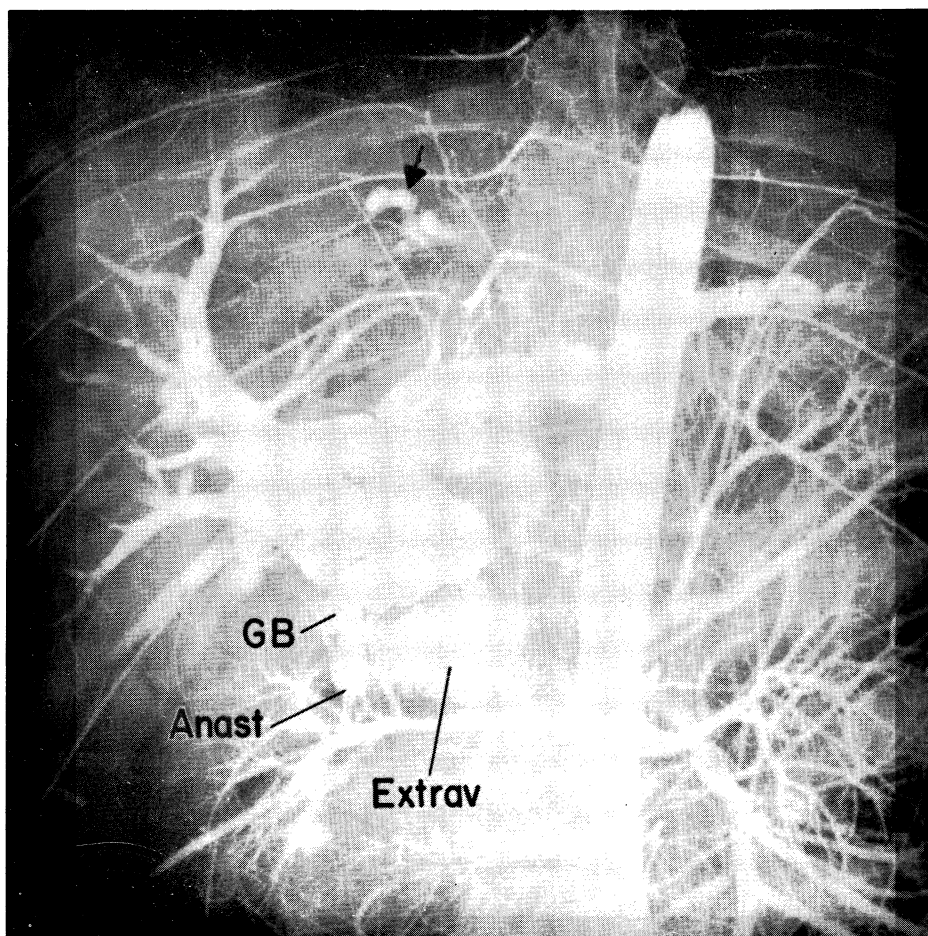


Figure 172. Postmortem cholangiogram in patient OT 15. Biliary reconstruction had been with a cholecystoduodenostomy. The dye was injected through a catheter tied into the collapsed gallbladder (GB). Note the extravasation of contrast material (extrav) and the general dilatation of the duct system. The correlation of these findings with the development of metastases is discussed in the text. The vascular shadows resulted from an aortogram. The artifact indicated by the arrow was caused by removing a parenchymal specimen.



Figure 173. Large metastases 339 days after transplantation in the liver homograft of patient OT 15. *A*, Mass in hilum seen on the liver scans during life (Fig. 171); it apparently had eroded into the distal duct system (Fig. 172). *B*, Larger metastasis in the superior portion of the right hepatic lobe. Its presence had not been detected with premortem scans. *A* shows the right lobe; *B*, the left.

have occurred, accounting for the dye extravasation seen on the cholangiogram (Fig. 172). The dilated biliary radicals within the liver contained a few stones and extensive "sludge." The duct obstruction may have been the basis for the episodic septicemia in the last two months of life; cholangitis was present within the homograft (Chapter Twenty).

An even larger recurrence which had not been suspected on the basis of the liver scans during life was found in the center of the right lobe; it was 13 cm in diameter (Fig. 173, *B*). The only other metastasis in the homograft originated in the suprahepatic vena caval suture line. It had a tail that was floating up the superior vena cava almost to the right atrium.

The lungs were nearly replaced with tumor. Metastases were also found in several plaques in or near the central tendon of the diaphragm.

The fourth patient (OT 14) was thought to be free of metastases for more than a year after operation, during which time her homograft function slowly deteriorated. After 380 days retransplantation was carried out, as will be discussed later in this chapter. While the liver was being removed, plaques of localized neoplasm were encountered in both halves of the diaphragm, in the right retroperitoneal space near the adrenal gland, and in the posterolateral parts of several of the lower ribs. In addition, several tiny nodules of tumor were found imbedded in the convex surface of the right lobe of the homograft; all these were less than 1 cm in diameter. There were no detectable metastases within the interior of the transplanted organ.

Hepatic Arterial Thrombosis. As mentioned previously, the patency of the homograft blood supply was proved by postmortem angiography in two of the four recipients with recurrent malignant disease (OT 15 and 23). In a third (OT 8), the hepatic artery had thrombosed (Fig. 174), presumably a long time before her death at 400 days. The observations in this case were germane to the ques-

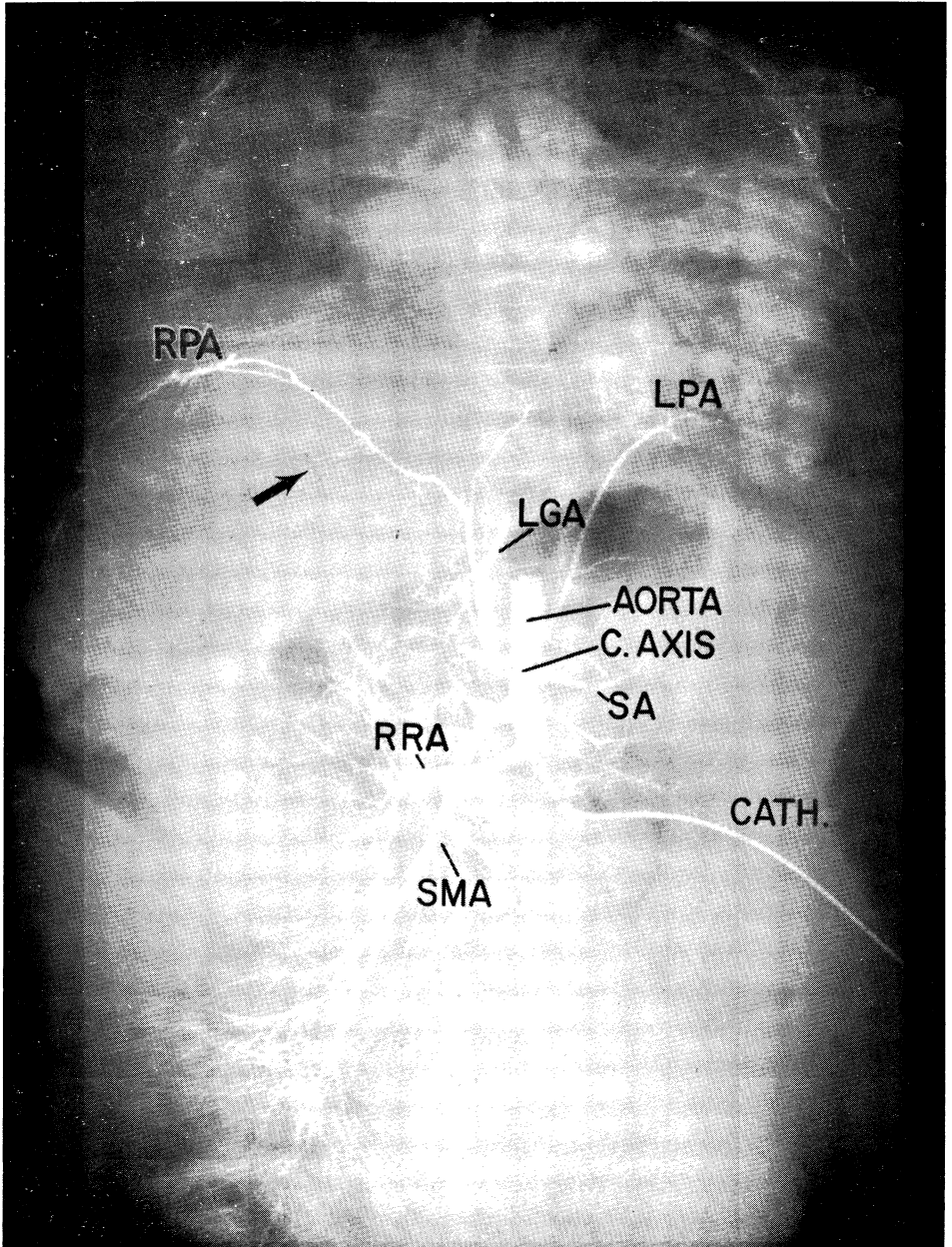


Figure 174. Postmortem aortogram obtained 400 days after orthotopic liver transplantation. Note that the hepatic artery is not present and that the only demonstrable arterial supply to the liver is from some small twigs (arrow) of the right phrenic artery (RPA). C. axis=celiac axis; LGA=left gastric artery; LPA=left phrenic artery; RRA=right renal artery; SA=splenic artery; SMA=superior mesenteric artery.

tion⁴² of "whether or not metastases metastasize." They had apparently done so (Fig. 165) and via the portal venous route.

At the time of retransplantation of patient OT 14, the reconstructed common hepatic artery was also found to be occluded by an old thrombosis. There was no tumor in the vicinity of the arterial anastomosis to suggest that metastases were responsible for the complication.

It is of interest that one of Calne's patients who lived for more than two postoperative months also had clot formation in the hepatic artery. Emboli apparently passed from this source to cause infarcts in the distal tissue, including the gallbladder. The recipient died after 72 days.¹⁸

The Possible Effect of Immunosuppression on Tumor Growth

It might be argued that the frequency and seriousness of the recurrences were approximately predictable from what is already known^{11, 33, 49, 60, 73} about the highly unfavorable natural history of primary hepatic malignancies. However, there is the additional possibility that the metastatic growth may actually have been accelerated as a consequence of the immunosuppressive therapy in those patients who were unknowingly left with residual neoplasm.^{76, 81, 82} There has been increasing acceptance of the concept that the immunologic system provides a "surveillance" function^{17, 44} by which mutant neoplastic cells are identified and either eliminated or restricted in their growth potential. The individuality of such cells, which allows their recognition as foreign, has been thought to be due to tumor specific antigens.^{28, 31, 40, 43, 44, 48, 59, 71, 72.}

Should the surveillance hypothesis be valid it would follow that neoplastic sequelae of one kind or other would constitute a threat in immunosuppressed patients after clinical transplantation procedures. Several observations that have been made in human recipients of renal homografts have tended to confirm this expectation.

Immunosuppression and the Transplantability of Tumors. A few years ago three different teams employed renal homografts that had been obtained from patients whose deaths were caused by carcinoma of the lung^{53, 88} or of the pyiform sinus.⁵⁵ In each instance the transplanted kidney was not thought to be involved by tumor at the time of the organ removal. Good homograft function was obtained with the aid of azathioprine and prednisone therapy. Four to 18 months later neoplastic growth of the same histologic type as that which had been present in the donor was found in the transplanted organs.

In all three cases the accidentally transplanted tumors had become autonomous by the time the diagnosis was made.^{53, 55, 88} Even though immunosuppression was discontinued, metastases developed in two of the recipients and led to death. The third patient recovered after drug therapy was stopped and after radical but incomplete excision was carried out of the renal homograft and the local neoplastic growth in the transplant wound.⁸⁸ It was concluded that the remaining tumor had undergone rejection coincident with recovery from partial immune paralysis.

The Spontaneous Development of New Tumors. Since neoplastic and non-neoplastic tissues are rejected by a common mechanism and are subject to similar rules of histocompatibility,^{17, 26, 30, 41, 48, 58, 59, 69, 70} protection of inadvertently transferred tumor by antirejection therapy in the renal patients was hardly surprising. A much more specific example of the oncogenic effect of immunosuppression has been the development of new malignancies in a number of renal homograft recipients whose kidneys were obtained from healthy donors. This complication was first reported from our institutions^{61, 74, 80, 82} on the basis of four of our own cases and two more contributed from other centers (Table 25). In five of the six patients the neoplasia was of cells of mesenchymal origin. More than a dozen other published^{25, 68} or unpublished instances of de novo malignancies after renal transplantation have since been brought to our attention. About half of these were carcinomas and the rest were lymphomas.

The diagnosis of an occasional neoplasm in any patient group of substantial size would not be particularly alarming. However, the incidence of malignant disease in our renal recipient pool far exceeded that which would have been expected by chance. Before May, 1967, approximately 170 patients were treated with renal homografts. In about 70 per cent of the patients survival of six months or longer was obtained. It was within this group of approximately 120 chronic survivors that the four malignancies were detected, giving an incidence of between 3 and 4 per cent in those patients who lived long enough for meaningful observations to be made.

The diagnosis of malignant neoplasia was made post mortem in one of the four patients followed by us. In that patient renal homotransplantation had been carried out more than two and a half years previously. Death occurred six days after emergency vagotomy and gastrectomy were performed to control massive upper gastrointestinal bleeding. The resected portion of the stomach contained several ulcers in the bases of which were small foci of reticulum cell sarcoma that were strikingly similar histologically to larger deposits found at autopsy in many other organs.

In the other three patients the presence of neoplasm was recognized during life. In one patient the diagnosis of reticulum cell sarcoma was made by craniotomy a few days before the very extensive tumor of the brain caused the patient's death almost six and a half months after renal transplantation. A third patient developed a mass in the basal diencephalon a few months following renal transplantation. A biopsy was taken with a stereotaxic instrument and the lesion proved to be a plasmacytoma. The doses of the immunosuppressive agents were drastically reduced and the brain stem was treated with local irradiation. Fortunately the kidney did not reject, but the tumor apparently underwent involution, since the patient has now been well for more than a year. The fourth patient developed a squamous cell carcinoma of the ear, two and a half years after renal transplantation (Table 25). Radical surgical excision resulted in an apparent cure.

In recipients of renal homografts there could be many ways in which biologic surveillance might be eroded, beginning with the loss of the immunologic reactivity that may accompany the pre-existing uremia (See Chapter Eleven). In addition, each of the main immunosuppressive agents, azathio-

Table 25. Clinical Features of de novo Malignancies in Renal Transplant Recipients*

NUMBER	PATIENT	TRANS- PLANT CENTER	AGE	SEX	DATE OF TRANS- PLANT	DONOR	DATE MALIG- NANCY DIAGNOSED	ORGANS INVOLVED	SPLENEC- TOMY	THYMEC- TOMY	IMURAN	PREDNI- SONE	ALG	TYPE OF TUMOR	OUTCOME
1	P.H.	Denver	42	M	9-30-63	Unrelated	3-1-66	Ear	Yes	No	Yes	Yes	No	Squamous cell carcinoma	Cured—surgical excision
2	I.C.	Denver	14	M	5-29-67	Mother	11-16-67	Brain	Yes	No	Yes	Yes	Yes	Reticulum cell sarcoma	Died 12-4-67
3	S.D.	Denver	23	M	6-15-65	Father	12-6-67	Thyroid, lung, liver, stomach, prostate, pituitary, skin, psoas muscle	Yes	Yes	Yes	Yes	No	Reticulum cell sarcoma	Died 12-6-67
4	E.C.	Denver	20	F	9-15-67	Father	4-11-68	Brain	Yes	No	Yes	Yes	Yes	Possible plasma-cytoma	Alive & well 4-15-69
5	W.A.	Minneapolis†	27	M	Sept. '64	Brother	June '65	Liver, brain, bone marrow	Yes	No	Yes	Yes	No	Lympho-sarcoma	Died 11-6-65
6	M.M.	Edinburgh Scotland‡	26	F	1-17-66	Mother	2-1-68	Mediastinal lymph nodes, pleura	No	No	Yes	Yes	Yes	Reticulum cell sarcoma	Died 2-16-68

*Since our original reports, about 12 more examples of malignant neoplasia have been reported to us from different centers. These have not been included in this account because the details are not fully known in many of these cases.

†C. R. Hitchcock: Personal communication. The case has been extensively reviewed since it was first reported to us by Dr. Hitchcock. On retrospective evaluation, many of the consulting pathologists believe the actual diagnosis to be undifferentiated carcinoma rather than lymphosarcoma.

‡M. F. A. Woodruff: Personal communication. The ALG was given in this case after there was radiographic evidence of an intrathoracic mass.

prine,^{19, 20} prednisone,^{1, 2, 8, 9, 89} and ALG,^{4-7, 15, 23, 24, 36} has been shown in animals either to: (1) increase the incidence of spontaneous, virus-induced, or chemically initiated tumors, (2) facilitate the ease with which malignant cells can be transplanted, or (3) accelerate metastatic growth. In addition, thymectomy^{4, 23, 32, 47, 54, 57} or splenectomy³ has a similar but less certain effect.

Another factor was suggested in our reports^{61, 74, 80} to explain the disproportionate number of mesenchymal tumors in the patients. The possibility was raised that chronic stimulation of the host reticuloendothelial system by antigens of the homograft was responsible for the nature of the malignancies. The role of antigenic stimulation in increasing the incidence of experimental lymphomas has been well established.^{22, 56, 67, 86}

The Effect on Metastases. As already mentioned, each of the immunosuppressive measures used in our patients can increase the rapidity of metastases of experimental tumors under the appropriate circumstances. Acceleration of spread has been described with the administration of prednisone,^{1, 2, 9, 89} ALG,³⁶ and azathioprine,¹⁹ or after the performance of splenectomy.³ There is no real reason to think that the same factors would not be significant clinically. The very explosive behavior of the recurrent disease in all but one of the liver transplant recipients was compatible with this concept.

In the field of kidney transplantation, an observation by Williams et al⁸⁷ may be relevant to the question of metastatic acceleration. They performed renal homotransplantation in a child six months after excision of a Wilms' tumor. Sixteen months after transplantation, at a time when cure of this kind of neoplasm would usually have been assured under normal conditions, metastases became apparent and led to death within a few weeks. The experience was reminiscent of an earlier one reported by Kuss.⁴⁶ One of his first renal recipients died of carcinomatosis 129 days after transplantation for the indication of hypernephroma of a solitary kidney.

General Conclusions

Even though all four patients developed recurrences of their hepatic carcinomas, there is no question but that life was prolonged and at least temporarily made more pleasant by the removal of the primary neoplastic focus. If only for this reason, it would seem premature at the present time to abandon the hope of using liver replacement for the treatment of hepatomas or cholangiocarcinomas. In addition, the feasibility of achieving a more lasting benefit has not necessarily been precluded. The original tumors in these cases were so large that it may have been unrealistic to hope that microscopic spread had not already occurred.

Nevertheless, if it is elected to attempt this form of therapy in future cases, it will be of great importance to be even more careful than in the past in screening prospective candidates. The experience acquired so far suggests that, if the neoplasm is not completely removed, a progressive downhill course from carcinomatosis can be expected. Under these circumstances there is a good

possibility that the immune suppression can contribute to the rapidity of growth of the secondary deposits.

Even if orthotopic liver transplantation proves to be curative for the occasional patient with hepatoma, it can be predicted that the greatest value of this procedure will be for the treatment of non-neoplastic hepatic diseases. In a historic perspective the experience with hepatomas will probably be viewed as significant principally because the efforts at therapy were responsible for demonstrating that the operation of liver replacement could be successfully performed in man.

LATE REJECTION (AFTER TWO MONTHS)

In the patients with hepatoma a complete and decisive assessment of the potential value of liver transplantation was not possible. First, the mortality of the pre-existing malignant process within the first 400 postoperative days proved to be 75 per cent despite total hepatectomy and otherwise successful liver replacement. Second, studies of the effectiveness of the therapeutic protocols designed to prevent graft repudiation became obscured in three of the four patients with malignant recurrences since the transplanted organs were invaded by tumor and either thereby destroyed (OT 23) or seriously compromised by obstruction of the biliary duct systems (OT 8 and 15). Finally, it could be speculated that the vigor of the host response to the homografts may have been initiated at a low level in these recipients since patients or animals with carcinoma may have a reduced immunologic reactivity.^{16, 38, 39, 50, 71, 72, 85} Under the latter circumstance a deceptive idea could be obtained of the requirements for control of rejection if the conclusions were extrapolated against a background of non-neoplastic hepatic disease.

Fortunately there were a number of opportunities to study chronically surviving patients without the complicating factor of serious injury to their homografts by recurrent hepatoma. There were eight recipients of this kind who lived for two months or longer. The original diagnosis was hepatic malignant disease in only one patient (OT 14); in the others it was biliary atresia. The complete courses of patients OT 11 and 12 have already been presented (Chapter Fifteen) and will therefore be omitted from the following account. In these two patients death occurred a short time after the development of gangrene of part or all of the right lobe of the liver. Survival was for 61 and 105 days, respectively. The other six patients contributed to a delineation of the problems to be discussed now.

Time of Onset and Relation to Previous Rejection

In the field of renal transplantation the term late rejection has been loosely used to describe the failure of kidney grafts, either suddenly or gradually, months or even years after operation. An analogous delayed deterioration of

hepatic homograft function has commonly been seen after liver transplantation. The histopathologic findings in a number of the livers will be described in Chapter Twenty.

In the six cases under discussion, delayed homograft repudiation was diagnosed from 63 to 175 days after transplantation. The exact timing in each instance is given in Table 26, as well as a notation about the kind of course that had been encountered earlier. Two (OT 10 and 13) of the six patients, including one with a prior septic hepatic infarction, had previously passed through what was called an "anicteric rejection" (Chapters Fourteen and Fifteen) which began during the first postoperative month. They then became significantly jaundiced for the first time in the post-transplantation period after a variable subsequent interval.

The other four recipients had suffered either an explosive but quickly reversible early rejection (OT 19) or else the indolent and persistent variety (OT 9, 14, and 16). In all but one of the latter four patients the hyperbilirubinemia which resulted from the first assault upon the homografts had more or less completely abated. An intervening jaundice-free period of 27 days to five months followed before the arrival of the second bout of icterus. The exceptional patient (OT 16) was one who received two consecutive livers. The fate of his primary homograft was described in Chapter Fourteen. The next transplant underwent a vigorous and refractory rejection beginning during the fourth

Table 26. Late Rejection in Six Transplant Patients*

PATIENT NUMBER	PREVIOUS EARLY REJECTION			ALG STOPPED (days)	LATE HOMOGRAFT DETERIORATION			OUTCOME†
	Onset (days)	Type‡	Revers- ible		Onset (days)	Development	Revers- ible	
OT 9§	17	Indolent	Yes	49	63	Gradual	No	Died hepatic failure, 133 days
OT 10§	14	Anicteric	Yes	51	84	Gradual	No	Died hepatic failure & sepsis, 186 days
OT 13	25	Anicteric	Yes	112	175	Gradual	No	Alive & jaundiced 14 months; received second course ALG
OT 14	6	Indolent	Yes	73	84	Gradual	No	Alive 15 days after re-transplantation at 380 days
OT 16A	13	Indolent	No	18	Contin. of early rejection	Gradual	No	Retransplantation, 68 days
OT 16B	32	Indolent	No	134	Contin. of early rejection	Gradual	No	Alive & jaundiced 8 months after second transplantation
OT 19	29	Crisis	Yes	Still given, 9 mos	71	Sudden	Yes	Alive 9 months, normal liver function

*Features of late rejection in six patients whose delayed deterioration of liver function was not related to invasion of their homografts by recurrent hepatoma.

†Classification defined in Chapter Fourteen.

‡As of April 15, 1969.

§Early septic hepatic infarction (see Chapter Fifteen).

postoperative week. The consequent jaundice could never be reversed, although the organ has continued to support life for many subsequent months; the processes of early and late rejection were inseparable by clinical and biochemical criteria.

Relation to Adjustments in Immunosuppression

In Chapter Thirteen it was pointed out that the extent to which immunosuppressive treatment could eventually be lightened without precipitating rejection was one of the most important determinants of long-term prognosis after human renal transplantation. It was also made clear that the frequent feasibility of attenuating antirejection therapy has been established in both animal (Chapter Twelve) and human (Chapter Fourteen) recipients of orthotopic liver homografts. Now the evidence must be considered that the poorly understood alterations in the host-graft relationship that make such therapeutic adjustments possible have proved in the clinical cases of liver transplantation to be less complete than had been hoped for.

A striking observation in several instances was that of an apparent long-term dependence upon ALG therapy. In five of the six patients globulin treatment was started on the day of or the day before transplantation. Afterward, injections were continued for 18 days to nine months, sometimes with a change from one heterologous species to another. They were finally stopped in all but one of the patients either because of a variety of toxic reactions or because the preplanned interval of treatment had ended. Within a few days to two months, a progressive deterioration of hepatic function (Fig. 175) became detectable (Table 26). It was particularly disquieting to have this happen very promptly after discontinuing ALG in one patient (OT 13) whose course had been excellent and very stable for the previous five months (Fig. 176). The most satisfactory long-term result in the entire series (Fig. 128, Chapter Fourteen) was in a recipient who is still being given horse ALG more than nine months after liver replacement for intrahepatic biliary atresia. He has normal hepatic function despite the fact that he had a very poor histocompatibility match with his donor (Table 5, Chapter Three).

The circumstances of treatment were different in the sixth patient, since ALG was not given following retransplantation until after the onset of a severe rejection (Fig. 177). Immune globulin prepared from rabbit or goat ALS was then administered for the next four and a half months. Homograft function worsened slightly during this time. After the injections were finally stopped there was no immediate dramatic change in the measured hepatic metabolism (Fig. 177).

The Presentation of Late Rejection

The hepatic function in five of the six patients (OT 9, 10, 13, 14, and 16) became progressively abnormal over a period of several weeks. The biochemical manifestations were similar to those of complete or nearly complete biliary obstruction. Bile disappeared from the stools and appeared in the urine.

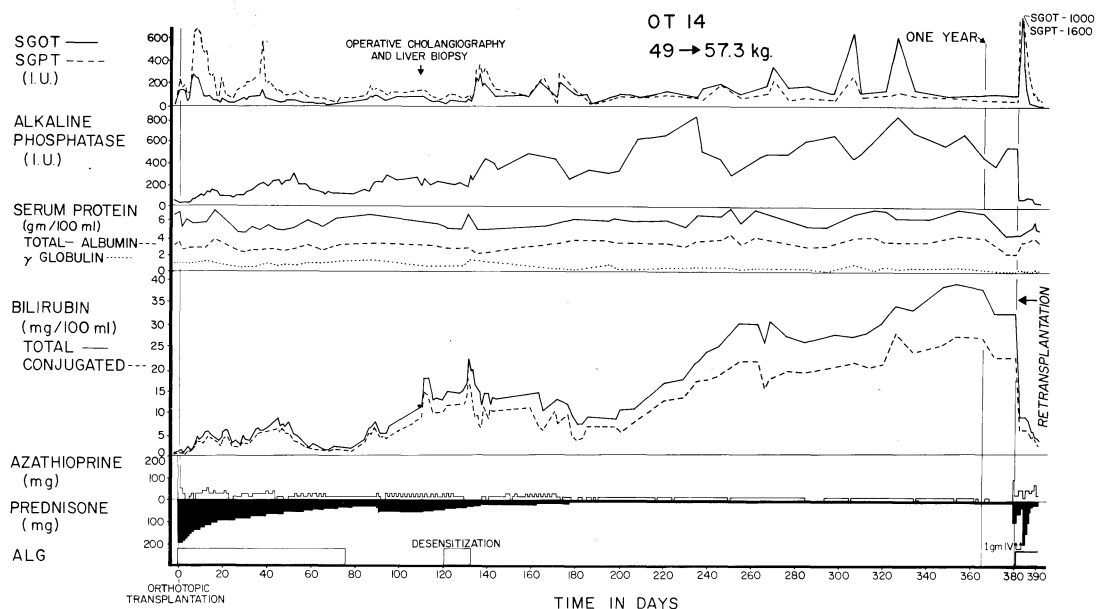


Figure 175. The course of a patient (OT 14) after two orthotopic liver transplantations. The second liver replacement was 380 days after the first. At the time of retransplantation, local recurrence of the neoplasm was demonstrated but there was very little tumor in the liver to account for its functional deterioration. However, the hepatic artery of the discarded homograft was completely occluded by an old thrombosis. Throughout the period of follow-up very small doses of azathioprine were given, usually 12.5 mg per day. Shortly after the discontinuance of the first course of ALG, a chronic rejection developed. An effort at desensitization to the horse globulin was not successful. Despite the deepening jaundice, the synthetic functions of the liver were well maintained for a long time. Note the low gamma globulins late in the course. The early convalescence of this patient after the first transplantation is depicted in more detail in Figure 134, Chapter 14. The liver function tests were performed with microchemical techniques. With these methods, the normal ranges in International Units (I.U.) of alkaline phosphatase, SGOT and SGPT, respectively, are 60 to 260, 0 to 65, and 0 to 55.

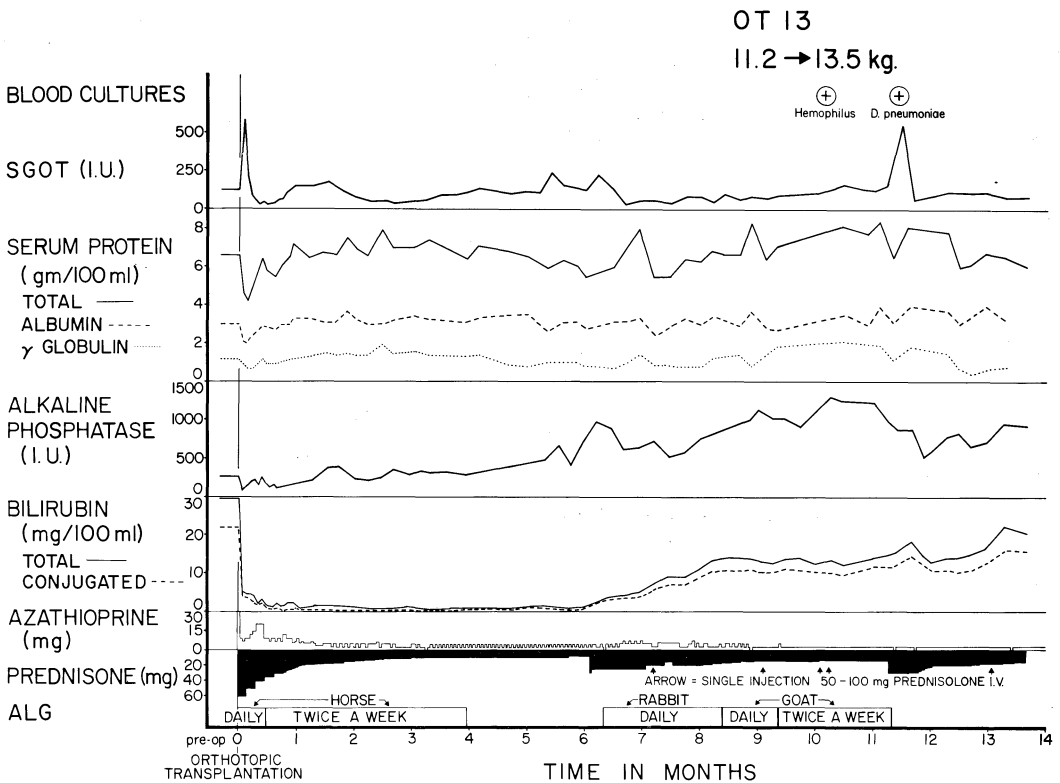


Figure 176. The first 14 months after the orthotopic liver transplantation of patient OT 13. The original diagnosis was extrahepatic biliary atresia. An overt early rejection did not occur; however, delayed repudiation of the homograft became apparent a few weeks after discontinuation of horse ALG. The manifestations of late rejection were principally those of obstructive jaundice. Biochemical evidence of severe hepatic necrosis was noted only at the time of the late septicemias. The source of the blood stream bacteria was never found. Normal ranges of serum enzymes: alkaline phosphatase, 75 to 225; SGOT, 0 to 6; SGPT, 0 to 55.

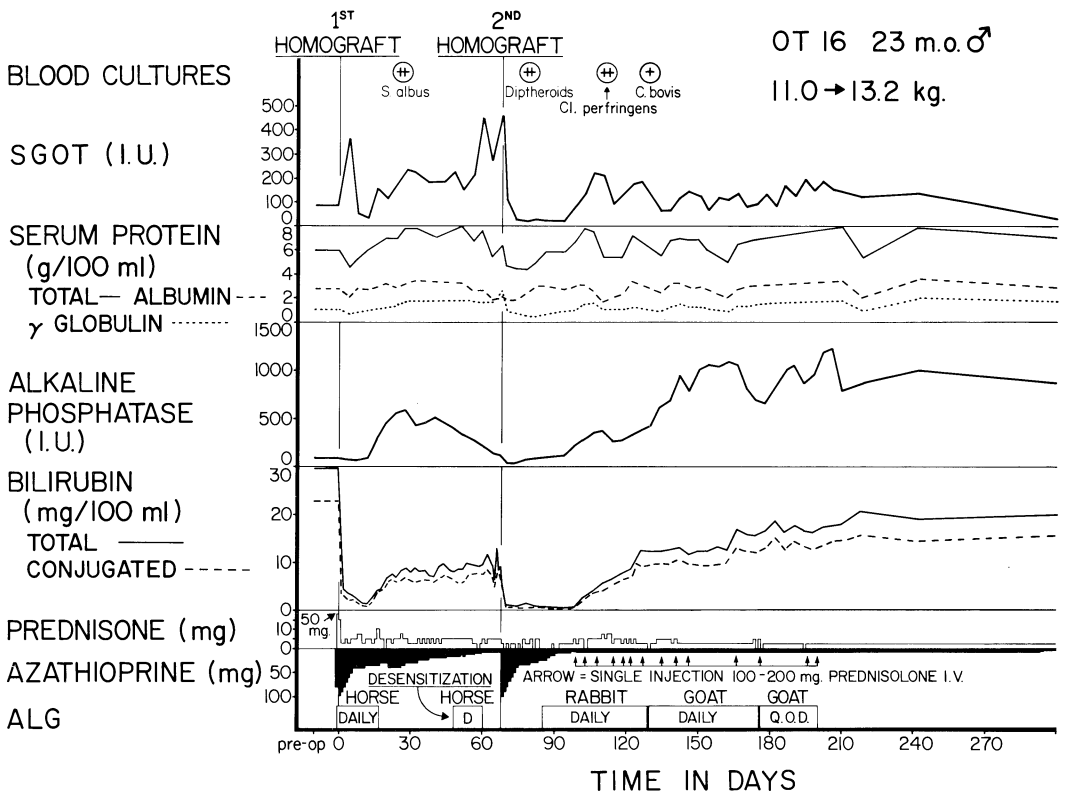


Figure 177. The course of a 23 month old child with biliary atresia who received two orthotopic liver transplantations. A severe indolent rejection was encountered on both occasions, but the function of the second homograft eventually became relatively stable. The patient is now one year post-operative. The normal ranges for the enzymes in international units are the same as in Figure 176.

The hyperbilirubinemia in all five patients consisted predominantly of the glucuronide (direct) fraction (Figs. 175 to 178; Fig. 128, Chapter Fourteen; Fig. 141, Chapter Fifteen). Increases were seen in the alkaline phosphatase either coincident with or before the deepening icterus.

None of the patients immediately became seriously ill as a consequence of the delayed rejection. They frequently had low grade fevers, but these could not always be proved to be related to the events within the transplanted organs. Bacteremia reappeared in two of the three recipients who previously had suffered septic infarctions (Fig. 178; Fig. 141, Chapter Fifteen). Positive blood cultures were obtained as the rejection developed in only one (OT 16B) of the three patients in whom the liver had not undergone partial necrosis earlier in the postoperative period; the microorganisms identified in this patient were *Clostridium perfringens* and *Corynebacterium bovis*.

There was usually little evidence of major generalized hepatic necrosis with the advent of the indolent late rejections. The SGOT and SGPT values rose to very high levels (exceeding 1000 International Units) only in patient OT 9 (Fig. 178). In the remainder (Figs. 175 to 177; Figure 141, Chapter Fifteen), the increases ranged from moderate to almost undetectable.

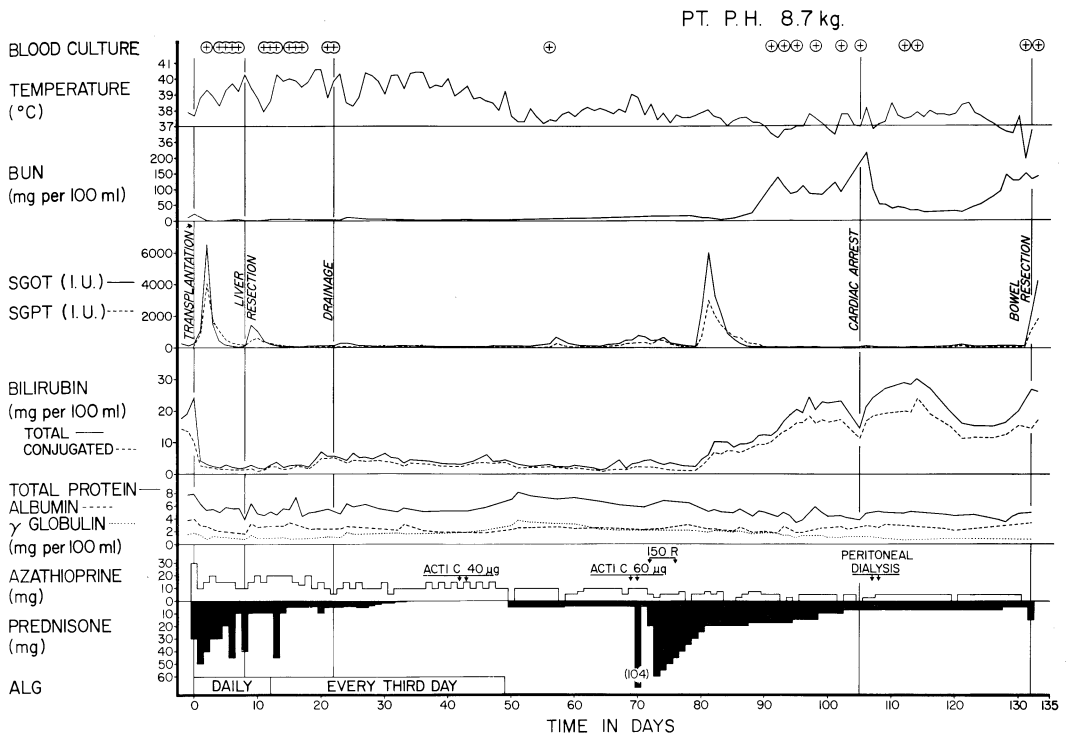


Figure 178. The course of a child (OT 9) who developed late rejection which was superimposed on an earlier septic hepatic infarction. The original diagnosis was extrahepatic biliary atresia. Jaundice did not recur until the third postoperative month. Eventually hepatic and renal failure developed, the latter leading to hyperkalemia and cardiac arrest. Bacteremia and later fungemia were intermittently demonstrated until eventual death from massive intestinal necrosis. Survival was four and a half months. Acti C=intravenous actinomycin C in micrograms; 150 R=depth dose of homograft irradiation. The temperatures are the maximums for each day. (By permission of Ann. Surg. 168:392, 1968.)

Moreover, there was little reason to suspect from the liver scans that the patients with previously established partial hepatic gangrene (OT 9 and 10) were having a significant extension of this process or that the recipients who entered the later rejection with intact homografts (OT 13, 14, and 16B) were developing fresh focal infarctions. Generally the transplants had a transient secondary increase in size, although the swelling was less than that observed during the early postoperative rejections (Chapters Fourteen and Fifteen).

The onset of delayed rejection in the sixth patient (OT 19) differed only quantitatively from that in the other patients. Its onset was slightly more abrupt, but the resulting abnormalities in hepatic function were minimal (Fig. 128, Chapter Fourteen); there was no detectable hepatomegaly.

The Course of Irreversible Late Rejection

The delayed homograft deterioration was decisively reversed only in patient OT 19 (Fig. 128, Chapter Fourteen). This was a recipient who developed the

abrupt but mild late rejection while still on treatment with horse ALG; the only change in therapy was a small upward adjustment in the prednisone. The secondary icterus in all the other patients became permanent despite increases in the steroid doses which were sometimes drastic. The subsequent courses of the latter five recipients will now be described, with subdivision into two groups according to the presence or absence of earlier septic hepatic infarctions.

Previous Septic Hepatic Infarction. The functional deficiencies of these two homografts (OT 9 and 10) tended to become gradually worse despite the intensification of steroid therapy. The bilirubins slowly climbed back to levels as high or higher than those caused by the original disease of extrahepatic biliary atresia. For a long time the serum protein concentrations were reasonably well maintained, but eventually these began to fall (Fig. 178); there were also declines in the liver-based clotting factors (Chapter Ten). Ultimately both the patients manifested multiple findings of chronic hepatic insufficiency with abdominal wall collaterals and spider angiomas, radiographic evidence of pulmonary veno-arterial shunting, peripheral edema, and ascites. Secondary renal failure in one of the children led to an electrolyte disequilibrium and a cardiac arrest. Resuscitation was successful, but she died a month later of massive intestinal gangrene (Fig. 178). The immediate cause of death in the other patient (Fig. 141, Chapter Fifteen) was rupture of an unrecognized abscess of the left hepatic lobe into the free peritoneal cavity.

In both the foregoing cases the homografts weighed 320 gm and were grossly fibrotic (Chapter Twenty). The latter finding had been anticipated from the results of liver scanning during life, since the transplanted organs had begun to shrink (Fig. 179). The final result as judged by the radiographic examination was indistinguishable from that described by Christie²¹ and Rozen-⁶⁶tal and their associates in end stage cirrhosis. In addition to the reduction in size, the ability of the transplant reticuloendothelial system to concentrate the isotope was diminished.

Previously Intact Homografts. As discussed in Chapter Fifteen, the reasons for the foregoing progression of events could not be determined with certainty at the time the patients with septic hepatic infarction were being cared for. An alternative explanation to that of rejection might have been that the daily debridements, dressings, and irrigations of the draining intrahepatic tracts were partly responsible for the shriveling and fibrosis of the homografts. It was not hard to imagine that the late functional deterioration of the livers could have been due to the continuous presence of bacterial infections in the residual hepatic tissue adjacent to the debrided areas. However, there is no longer justification to suspect an explanation other than delayed rejection, since many of the same observations were subsequently made in three patients in whom early regional gangrene of the transplanted organs was avoided (OT 13, 14, and 16).

In the latter recipients the delayed rejections had been of the indolently developing variety. In all three patients the essential features for long periods were those of obstructive jaundice with selective preservation of the functions of hepatic synthesis (Figs. 175 to 177). Once late icterus appeared in these cases, it never again diminished. A plateau in the serum bilirubin concentration was reached on which a very gradual further trend upward was based; alkaline phosphatases were also consistently elevated (Figs. 175 to 177).

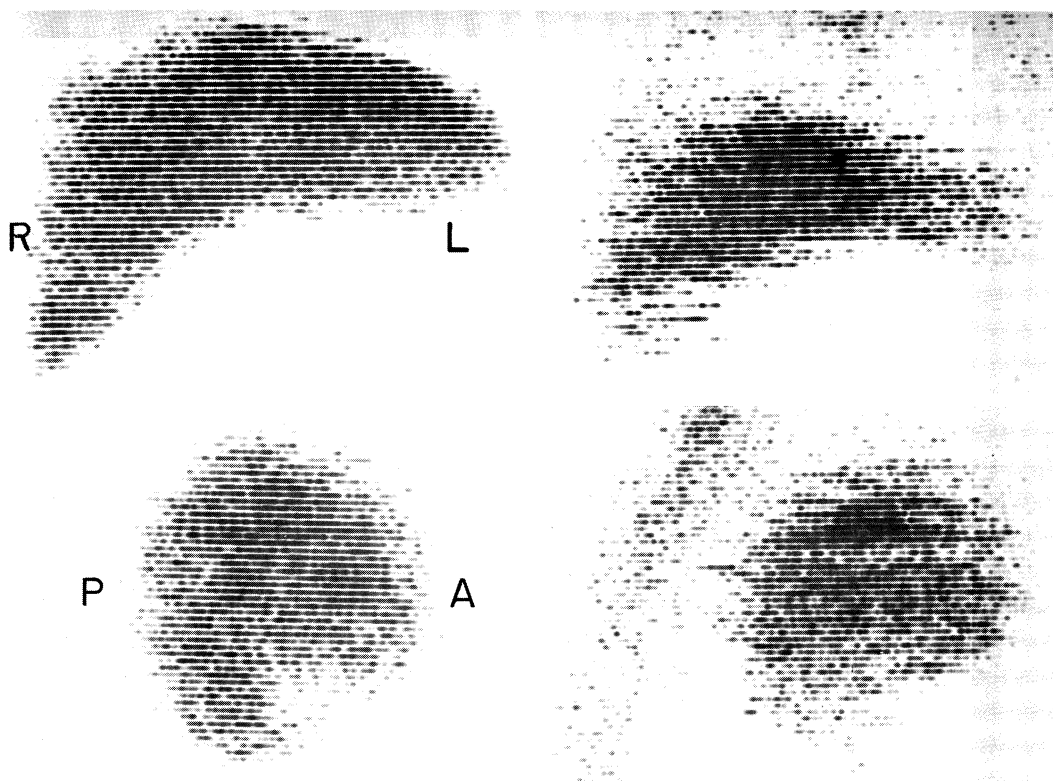


Figure 179. Liver scan in a 21 month old female made two (left) and four (right) months after orthotopic hepatic homotransplantation. Between the time of the two studies, the bilirubin had risen from 2 to 16.3 mg per cent, as a consequence of chronic homograft rejection. In the second study, note the marked decrease in liver size and isotope uptake and a commensurate increase of radioactivity in the bone marrow. The liver appears moth-eaten. The patient (OT 9) died 11 days later of massive intestinal gangrene. (By permission of Surg. Gynec. Obstet. 127:808, 1968.)

As the months went by it was surprising to find so few serious effects upon other tests of hepatic function. The day-to-day measures of serum transaminases were not alarming. For a long time total serum protein and albumin concentrations were depressed very little, if at all (Figs. 175 to 177). As long as vitamin K was administered parenterally or orally in a water soluble form, the prothrombin times remained at or near 100 per cent. Failure to give the vitamin led to marked prolongation of the prothrombin times within a few days.

At one time or other thought was given to the possibility of re-exploring the homografts in all three of the patients in order to rule out mechanical obstruction of the extrahepatic duct system. This was eventually done in patient OT 14. The duodenum was opened and the cholecystoduodenostomy was found to be widely patent. A dye study was obtained (Fig. 180) which did not reveal any dilatation of the intrahepatic or extrahepatic duct system (Fig. 181). The fine peripheral intrahepatic ducts did not fill well, giving the cholangiogram a "stripped tree" appearance. A liver biopsy was taken which revealed intrahepatic cholestasis (Chapter Twenty).

All three of the patients (OT 13, 14, and 16B) who developed a late indolent rejection in the absence of partial hepatic gangrene are still alive, one (OT 14) by virtue of a second homotransplantation more than a year after the first

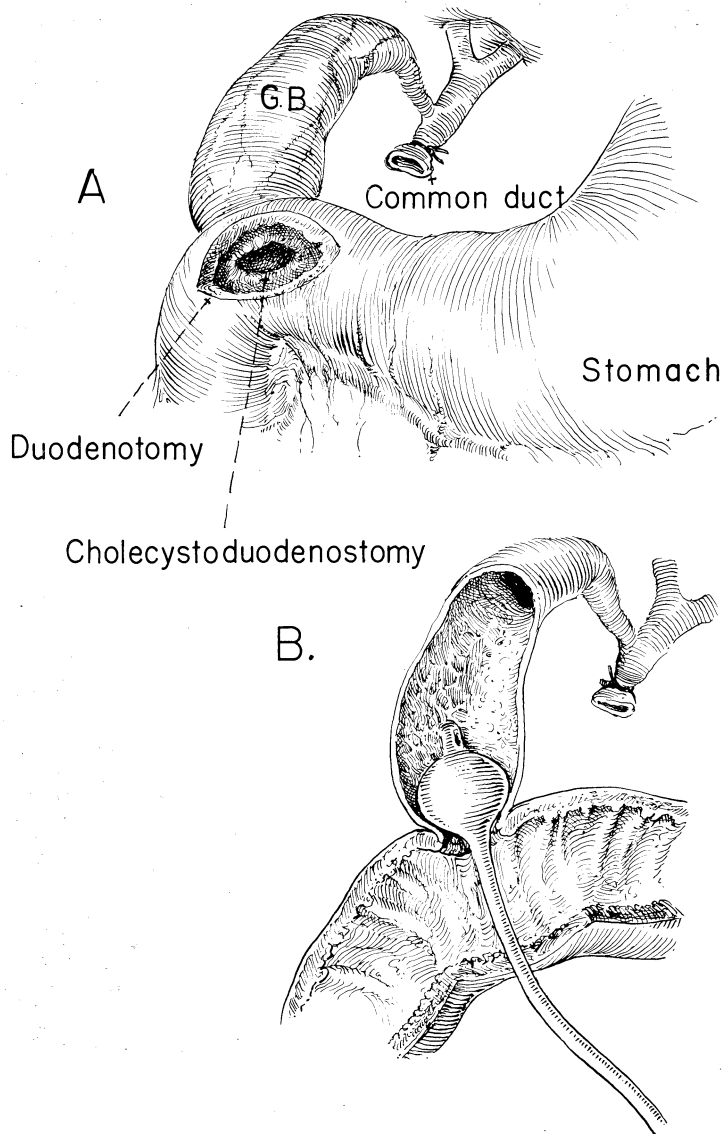


Figure 180. Operative technique used to study the duct system of the homograft in patient OT 14. Biliary reconstruction had been with a cholecystoduodenostomy. A. Counter-incision in duodenum. B, Insertion of a Foley catheter through the anastomosis and inflation of the balloon.

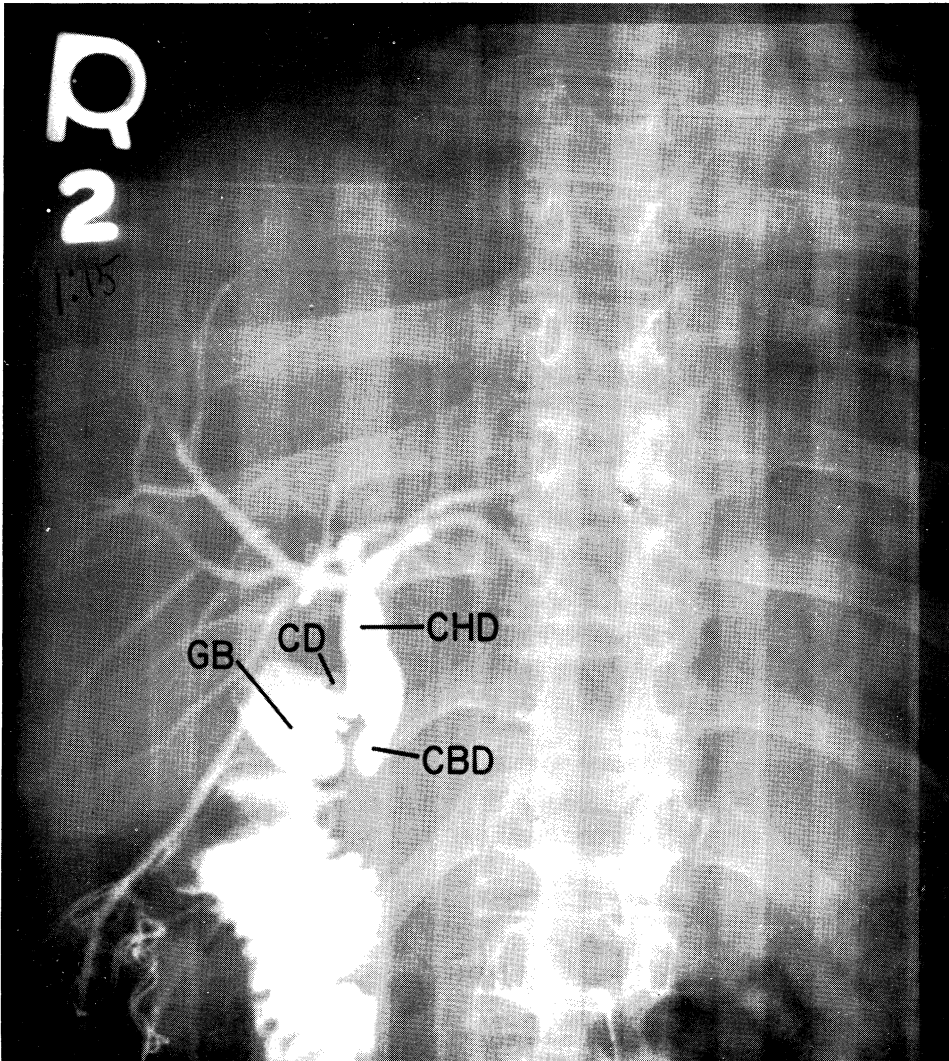


Figure 181. Operative cholangiogram of a 16 year old girl (OT 14) obtained three and a half months after orthotopic liver transplantation for the indication of hepatoma. Re-exploration was carried out because of persistent jaundice. The dye was injected after inserting a Foley catheter through the cholecystoduodenostomy into the gallbladder (Fig. 180). Note that the biliary drainage from the homograft is not obstructed but that the fine ramifications of the intrahepatic ductal system are not seen. CBD=common bile duct; CD=cystic duct; CHD=common hepatic duct; GB=gallbladder. (By permission of Surg. Gynec. Obstet. 128:327, 1969.)

procedure (see later). Although the effects of the chronic hepatic dysfunction were supported surprisingly well for protracted periods, there were eventual signs of decompensation. The transplanted organs in two of the three recipients (OT 14 and 16B) underwent a slow but steady shrinkage (Figs. 182 and 183). In all three patients the ability of the homograft reticuloendothelial system to concentrate the isotope remained permanently diminished.

The slender margin by which life was still being maintained was illustrated in one of the patients (OT 13) when pneumococcal bacteremia developed in the twelfth postoperative month (see Chapter Sixteen). With the infection came hypoglycemia which required intravenous glucose therapy for the next several days. The same patient has had several significant gastrointestinal hemorrhages from an undetermined source and has recently developed intermittent ascites.

The Question of Late Arterial Thrombosis

In the section on tumor recurrence, two examples were cited of apparently delayed complete hepatic arterial thrombosis. It is unlikely that the vascular occlusions were related to the underlying malignant disease; instead, they probably represented a complication which will also be seen in patients treated with liver transplantation for non-neoplastic hepatic disease. The most important etiologic factors in the dearterialization were probably immunologic (Chapter Twenty).

So far, this concept has not been proved in the clinical series. Since none of the recipients with pre-existing benign disease has died after seven months, the state of their homograft vascular systems has not been accurately studied. However, one of the chronically surviving patients (OT 13) has been submitted to aortography. Fourteen months postoperatively a stenosis was found at or near the arterial anastomosis (Fig. 184A). Striking abnormalities were found in the distal vascular bed. The small terminal arteries were serpiginous and there were areas of dye staining that may have been sites of arteriovenous shunting (Fig. 184B). The angiographic changes were reminiscent of those found in cirrhosis.²⁷

The Feasibility of Retransplantation

As described in Chapter Fourteen the feasibility of retransplantation was first established under the urgent circumstances imposed by uncontrolled early postoperative rejection. The courses of the patients with delayed rejection provided a clear indication that most recipients with late failing hepatic homografts could be considered under more elective conditions for the same kind of treatment since the extreme slowness with which the organ deteriorated allowed considerable time to try to find a new liver. Parenthetically it may be suggested that similar opportunities to replace deteriorating cardiac homografts will almost certainly become available. The experimental studies of

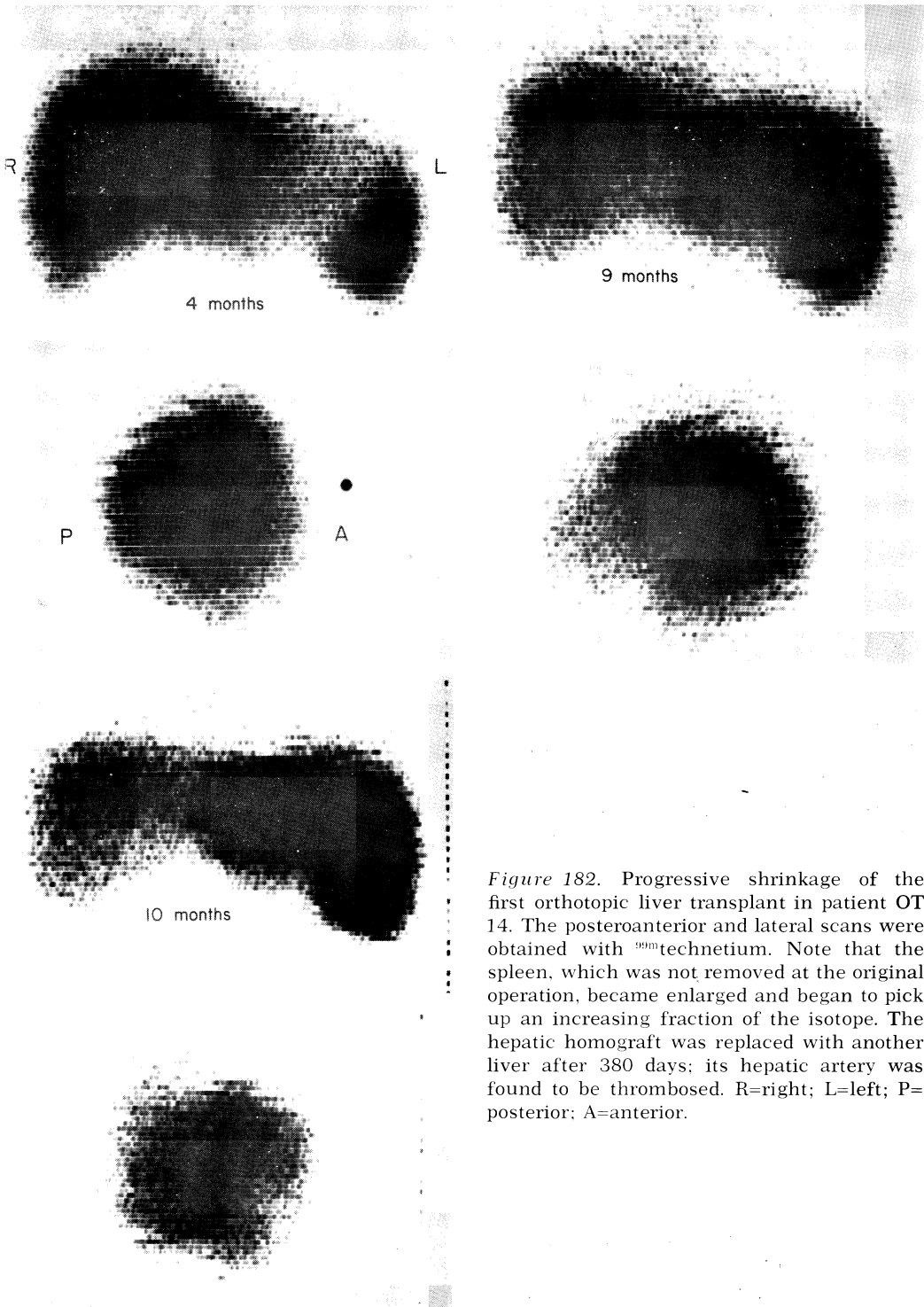


Figure 182. Progressive shrinkage of the first orthotopic liver transplant in patient OT 14. The posteroanterior and lateral scans were obtained with ^{99m}Tc technetium. Note that the spleen, which was not removed at the original operation, became enlarged and began to pick up an increasing fraction of the isotope. The hepatic homograft was replaced with another liver after 380 days; its hepatic artery was found to be thrombosed. R=right; L=left; P=posterior; A=anterior.

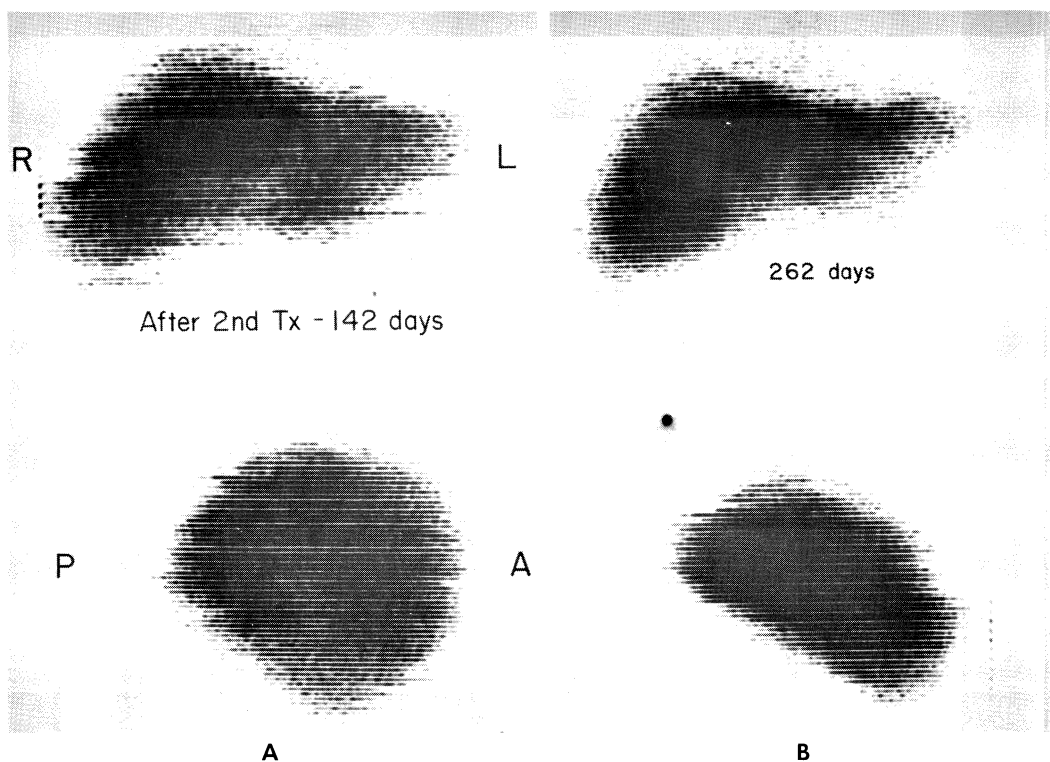


Figure 183. Homograft shrinkage seen late after a second orthotopic liver transplantation in patient OT 16. The scans were obtained with ^{99m}Tc technetium. A, 142 days after replacement of a failed first homograft with a second organ. B, 262 days after the retransplantation. Note the diminution in size in the intervening four months.

Lower and Shumway and their associates⁵² have shown that the late rejection of transplanted hearts often occurs gradually enough so that repeat operations could be considered. However, there may be serious judgment problems with either organ about when reintervention is justified. For example, the two longest survivors in our liver series remained continuously jaundiced for eight (OT 13) and 11 (OT 14) months. When the icterus first developed it was thought that a second homograft would be required within a very short time. Fortunately, suitable donors could not then be found. The anticipated rapid downhill course did not materialize. The realization that prolonged survival was often possible under these circumstances led to a conservative attitude about recommending a second organ replacement. However, one of the patients (OT 14) was eventually provided with another orthotopic liver after the initial organ had sustained life for 380 days. The histocompatibility match was a better one (Table 5, Chapter Three) than had been present with the first donor.

The technique of operation was approximately the same as for the other retransplantation described in Chapter Fourteen. The homograft which was removed did not have a cirrhotic appearance although its weight (1250 gm) and size were subnormal, as would have been predicted from the shrinkage that had been seen on the liver scans in the preceding months (Fig. 182).

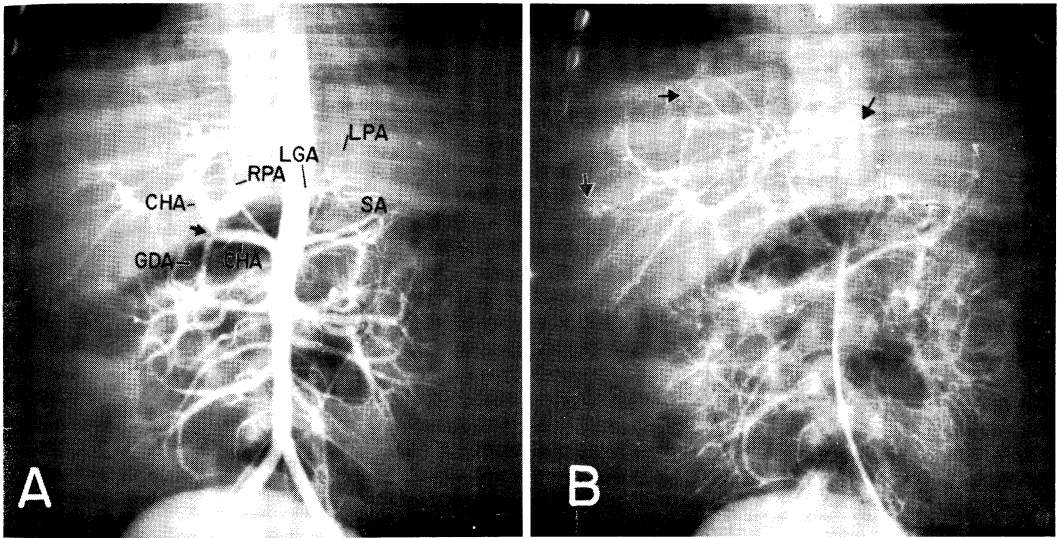


Figure 184. Aortogram obtained 425 days after operation in patient OT 13. The original arterial anastomosis had been between the host proper hepatic artery just beyond the gastroduodenal artery (GDA) and the homograft celiac axis. *A*, There is a stenosis (arrow) at or near the anastomosis. Note the large caliber of the right phrenic artery (RPA), probably as the result of its participation in a collateral blood supply. CHA=common hepatic artery; LGA=left gastric artery; LPA=left phrenic artery; SA=splenic artery. The x-ray is one second after injection. *B*, The terminal vessels are race-mose. There are dye accumulations (arrows) suggestive of intrahepatic arteriovenous shunts 1.5 seconds after injection.

The recipient hepatectomy was not particularly difficult since the adhesions attaching the liver to adjacent structures could easily be broken down. When the new homograft was inserted, it was necessary to attach its celiac axis to the celiac axis of the recipient inasmuch as the recipient common hepatic artery had thrombosed (see earlier section). A cholecystoduodenostomy was constructed for biliary drainage at the same site as had been previously used for the earlier anastomosis to the duodenum.

There was prompt and adequate function of the homograft (Fig. 175). Although the patient had been sensitized to the horse ALG a year previously, it was possible to start a new course of the equine globulin without any systemic reactions. Because of the recurrent tumor found at the time of retransplantation (see section, Recurrence of Malignant Disease), there is little or no hope for protracted continuing survival even if good liver function can be maintained.

OTHER CAUSES OF LIVER MALFUNCTION

In all the liver recipients thus far followed more or less chronically, any late bouts of hepatic malfunction have seemed ascribable to the two main etiologies to which most of this chapter has been devoted, namely metastases to the homograft or immunologic rejection. As further experience accumulates it is probable that other causative factors will be found to be important, although these may be difficult to identify.

Drug Toxicity

The liver injury that can be induced by immunosuppressive agents was reviewed at length in Chapter Twelve, with particular attention being focused upon azathioprine and prednisone. It will be recalled that deranged hepatic function tests were detected at some time in more than 50 per cent of human recipients of kidneys who were treated postoperatively with the same agents as those given to the liver patients. A differential diagnosis between drug toxicity and viral hepatitis was not generally possible.

For obvious reasons it was even less feasible to estimate the extent to which hepatotoxic effects of the drugs occurred after orthotopic liver transplantation. However, an interesting observation was made in two patients who received large quantities of steroids for long periods because of the persistence of jaundice. Eventually both recipients became chronically ill, for which reason the prednisone doses were drastically reduced. Their general condition improved remarkably. The liver function remained relatively stable for long periods despite the lightening of the immunosuppression (Figs. 175 and 177).

Hepatitis

The difficulty of diagnosing viral hepatitis is understandable since the agents responsible for this kind of disorder have not been isolated.^{37, 45} Recently there has been increasing interest in techniques to detect specific antigens associated with the virus.

The Australia and SH Antigens. Antigenic factors have been described in the blood of patients with viral hepatitis. To identify the antigens, blood from hemophiliac patients has been used as a source of antiserum. Because these donors had received multiple transfusions (as many as 10,000), they were considered by definition to have had multiple exposures to the hepatitis virus.

Blumberg et al^{12, 83} first described the Australia antigen in a high percentage of native aborigines who were thought to represent a hepatitis reservoir. Later he also found an increased incidence in certain patient populations within institutions in which there was also apparently endemic hepatitis.^{13, 51, 84} Finally, it has been noted that the hospitalized patients in whom the Australia antigen has been found with high frequency have often had diseases characterized by a reduced immunologic reactivity.¹²⁻¹⁴ The argument has been advanced that the partial immune paralysis in the latter cases was responsible for an increased susceptibility to infection by the virus.

Prince and his associates of New York^{34, 35, 62, 65} have studied a similar factor, termed the SH (serum hepatitis) antigen which is rarely found (0.1 per cent) in the normal United States population. Their most decisive observations were in recipients of blood transfusions. In the patients who subsequently developed serum hepatitis, they demonstrated the appearance of the SH antigen during the incubation period and the early clinical course.

Cross reaction studies by Prince^{63, 64} have stressed the high degree of similarity between the Australia and SH factors. However, he has also em-

phasized that there is insufficient evidence to conclude that they are identical.^{63, 64} Electron micrographic studies of the serum from patients with either the Australia¹⁰ or SH⁶² antigen have revealed virus-like particles similar in size and appearance to the picorna group.

Studies in Liver Transplant Recipients. In January, 1969, the resident physician on the liver service developed severe hepatitis. Within a 10 day period two of the chronically surviving recipients for whom he had been caring also became jaundiced after having had many previous months of normal homograft function. Because of the highly suspicious association of events, the sera of the physician and the two patients were submitted to Dr. Alfred M. Prince.

It was found that one recipient (OT 15) was SH positive and had been since the sixty-sixth postoperative day (Fig. 170); during his transplantation he had received 7000 ml of blood. The sera of the other patient (OT 23) and the house officer were negative. In actuality, the primary explanation for the deterioration of hepatic function in both the patients proved to be the invasion of their transplants by recurrent hepatoma (see earlier section).

Thirty-six sera from nine other orthotopic liver recipients were also examined by Dr. Prince. None contained the SH antigen (Table 27). These negative findings did not exclude the diagnosis of hepatitis since the SH antigen can be found in only about half the patients in whom this diagnosis is made on other grounds.^{34, 35, 62, 65}

Table 27. Immunoassays for SH Antigen*

PATIENT OT NUMBER	POSTOPERATIVE SAMPLES (days)	RESULTS
8	P†, 1, 15, 82, 117	Negative
10	48, 97, 147	Negative
11	22, 60	Negative
12	1, 17, 67	Negative
13	39, 120, 200, 221, 237, 283	Negative
14	66, 119, 186, 255, 271, 325	Negative
15	P, 14, 27, 66, 87, 129, 143, 145, 192, 226, 247, 297	Positive†
16A	32	Negative
16B	21, 35, 79, 166	Negative
17	P, 9, 14, 28	Negative
19	3, 51, 148, 176	Negative
23	P, 26, 39, 53, 103	Negative

*The results of immunoassays for SH antigen in the sera of 11 recipients of orthotopic liver homografts. The analyses were performed by Dr. Alfred M. Prince of New York. See text for discussion.

†P : Preoperative.

‡The results of the individual tests are shown in Figure 170.

Extrahepatic Obstruction

At autopsy the extrahepatic duct system of three of the homografts was found to be partially obstructed; provision for biliary drainage had been with a cholecystoduodenostomy. As described earlier in the chapter, recurrent tumor was responsible for death in two of the patients (OT 8 and 15). In the other (OT 11), a 16 month old child who died after 105 days of septic hepatic infarction (Chapter Fifteen), there may have been a kink at the junction of the cystic and common ducts. The dilated intrahepatic ducts of these three livers were filled with inspissated bile and also contained several soft stones.

CHOLANGITIS

So far there are insufficient data to reach a definitive conclusion about the incidence of cholangitis which will be seen in chronic survivors. However, Chapter Twenty will report some encouraging pathologic observations made in 13 livers which had been transplanted from three weeks to more than a year before their examination. Histologic evidence of cholangitis was found in only three of the homografts; the duct system of two of the three affected organs had become obstructed with recurrent tumor (OT 8 and 15). Biliary tract reconstruction in 12 of the 13 recipients was with cholecystoduodenostomy.

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Chapter Eighteen

SPECIAL IMMUNOCHEMICAL STUDIES

by Noboru Kashiwagi, M.D.

About five years ago studies were published analyzing the immunologic capabilities of human recipients of kidney homografts.^{13, 31} These investigations provided a quantitation of the changes in immunologic reactivity that could be produced with antirejection treatment and still be compatible with life in a relatively uncontrolled social environment. Such information is generally applicable for the transplantation of other organs.

With hepatic transplantation, additional intriguing possibilities are introduced because of the liver's essential participation in all pathways of protein metabolism. It is with the questions raised by this fact that the present chapter will deal.

THE SOURCE OF NONIMMUNOGLOBULIN PROTEINS

There is strong evidence that liver homografts retain their metabolic specificity after transfer to new hosts. This was first shown by studies of serum haptoglobin^{12, 21, 26} and the group-specific component of the alpha globulin fraction¹² in our patients before and after orthotopic liver transplantation.

The recognition of discrete protein fractions in orthotopic liver recipients was made possible by the earlier studies of Smithies^{24, 25} on haptoglobin (Hp) and of Hirschfeld^{8, 9} on group-specific component (Gc). These authors had demonstrated that three kinds of Hp and Gc were identifiable in the human population, that the type present in any individual was subject to genetic control, and that the phenotypic expression could be detected with electrophoretic techniques.

In several of our cases of orthotopic transplantation, the donors have had different Hp and/or Gc types than the recipients. Within a few hours to a few days after operation, only the donor protein fractions were present (Figs. 185 and 186); the changes were complete and permanent. The potential therapeutic

OT 12

DONOR (TYPE 2-2)

RECIPIENT - PRE-OP (2-1)

" POST-OP 24 HOURS

" " 2 DAYS

" " 3 "

" " 5 "

" " 10 "

" " 15 "

" " 31 "

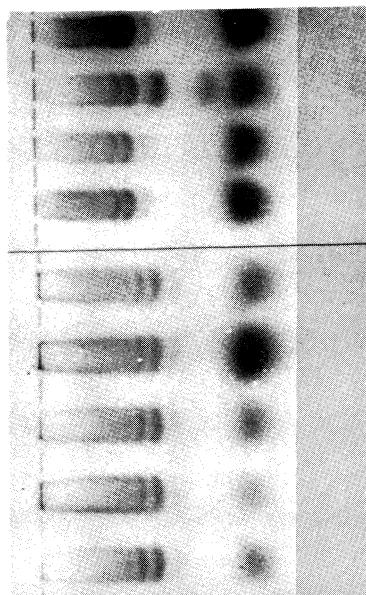


Figure 185. Effect of orthotopic liver transplantation on serum haptoglobin (Hp). The studies were in patient OT 12, who preoperatively had Smithies Hp type 2-1; her donor was Hp type 2-2. After transplantation only the donor type was detectable in the recipient sera. The determinations were with starch gel electrophoresis.

OT 8

DONOR (TYPE 1-1)

RECIPIENT - PRE-OP (2-1)

" POST-OP 10 DAYS

" " 52 "

" " 102 "

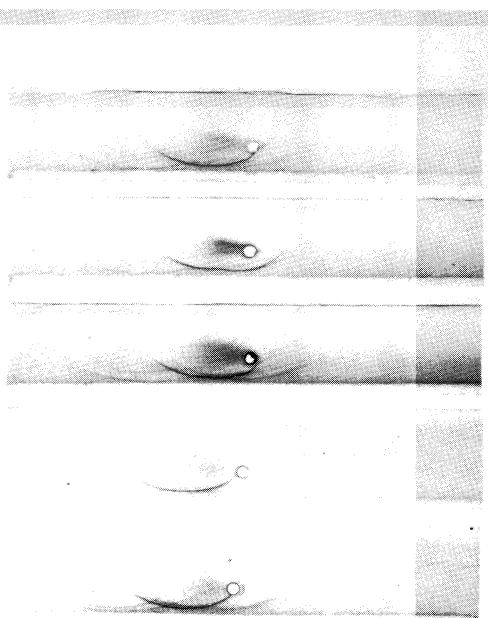


Figure 186. The effect of orthotopic liver transplantation on group specific component (Gc). Before operation the recipient (OT 8) had Gc type 2-1, whereas the donor had type 1-1. After transplantation, only the donor type was detectable. The recipient, whose original disease was hepatoma, died more than 13 months after transplantation of widespread metastases. The immunoelectrophoretic studies were performed with commercial anti-Gc antisera.

implication of these findings in terms of treating hepatic-based inborn errors of metabolism was discussed in Chapter One.

It is highly likely that similar information will be forthcoming about a number of other protein genotypes. Alper et al have traced a C'3 complement phenotype from a donor to a human liver recipient treated in Boston.¹ There was substitution of the new complement for the old type during the 45 days of postoperative survival.

IMMUNOGLOBULINS

In other parts of the book, particularly in Chapters Fourteen, Fifteen, and Seventeen, data were given on the plasma protein concentrations, including the gamma globulins. These day-to-day determinations were performed with cellulose acetate membrane electrophoresis. Now, a more detailed breakdown of the immunoglobulin fractions will be described. The different studies were carried out in six to 10 of the patients who lived for two months or longer after operation.

Serum Concentrations

There were six recipients of seven homografts (OT 10, 12, 13, 14, 16, and 19) in whom the gamma G, A, and M globulins were serially measured with a single radial immunodiffusion method.* Except for one patient with hepatoma (OT 14), the pre-existing disease was biliary atresia. The results are summarized in Figure 187.

Immediately after transplantation the serum concentrations of all three of the measured immunoglobulins fell sharply from preoperative levels, which on the average were slightly higher than normal. This occurred in parallel with similar declines of the total proteins as described more fully in Chapter Six. The changes were transient in patients who received adequately functioning livers and who recovered from the acute effects of the operation.

The Individual Fractions. After the immediate postoperative period had passed, the most consistent finding was a tendency for increased concentrations of gamma G globulin to develop. The elevations came at variable times but they never preceded the overt manifestations of rejection; rather, they were first seen from three to 62 days after this diagnosis was made (Table 28). In some patients the delayed rises of gamma G globulin were persistent, but in others they were either transient or else they waxed and waned from week to week (Fig. 187). The average gamma G concentrations weeks or months after transplantation were distinctly above normal and they were generally higher than those present before operation.

In contrast, the levels of both gamma M and gamma A globulin usually had a decline relative to the supernormal values which had been present preoper-

*Using an Immunoplate, Hyland Laboratories, Los Angeles, California.

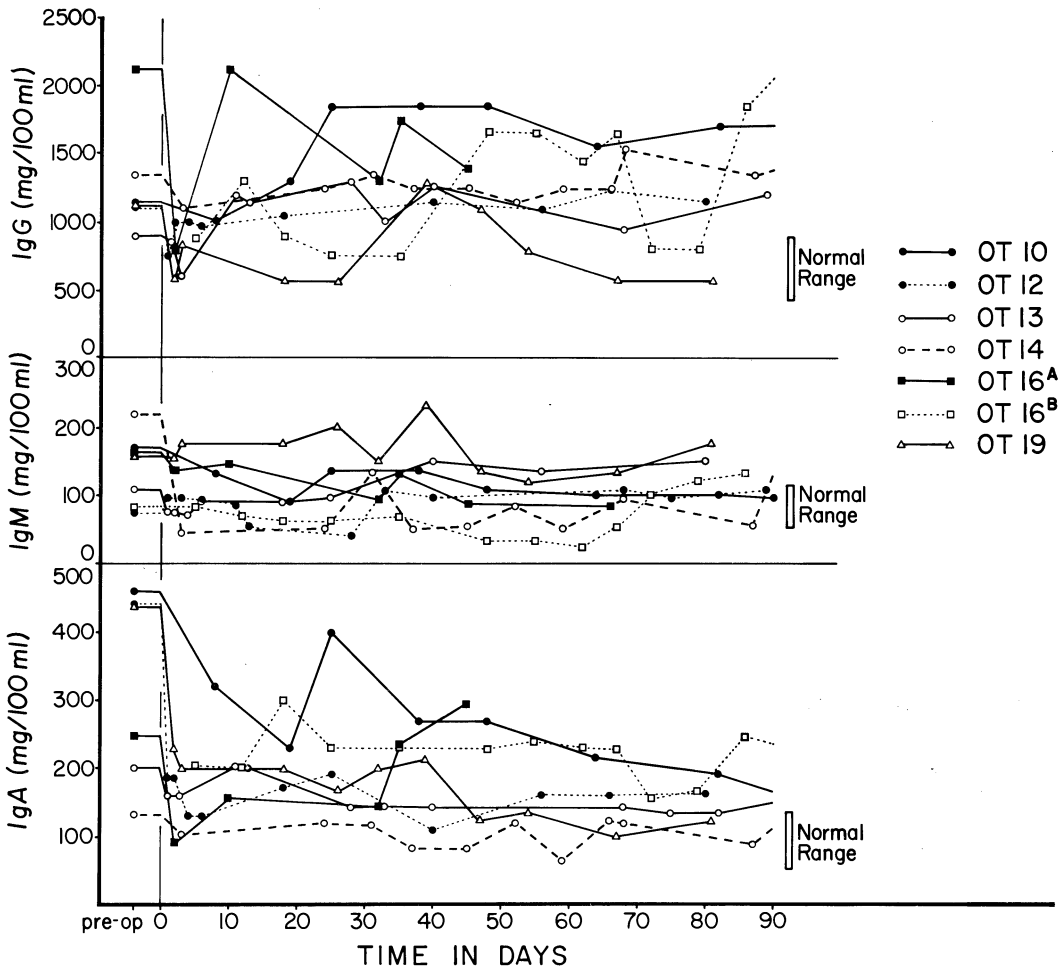


Figure 187. The changes in serum gamma G, A, and M after seven orthotopic hepatic homo-transplantations in six patients. (See text for discussion.)

actively. Even so, the average concentrations throughout the courses were still slightly above the normal for healthy persons.

Fractional Interrelationships. In a number of instances the serum concentrations of the three measured classes of immunoglobulins changed in a rough parallel to one another; thus, a rise or fall in the gamma G globulin was apt to be accompanied by a similar fluctuation of the gamma M or gamma A components (Fig. 188). However, an association of the alterations was by no means invariable.

The Source of the Immunoglobulins

In some pathologic conditions in which the liver contains abnormal numbers of plasmacytes, gamma globulin has been demonstrated by immunofluorescence techniques in the latter cells.^{4, 23} However, it has been said that there is normally little or no hepatic synthesis of immunoglobulins.^{2, 5, 14, 22}

Table 28. Elevations of Gamma G Globulin*

PATIENT NUMBER	TIME ELEVATIONS GAMMA G GLOBULIN NOTED (days)	ONSET OF REJECTION (days)
OT 10	25	14
12	40	4
13	28	25
14	68	6
16A	35	13
16B	48	32
19	39	29

*The times when definite elevations of gamma G globulin were observed in relation to the diagnosis of homograft rejection.

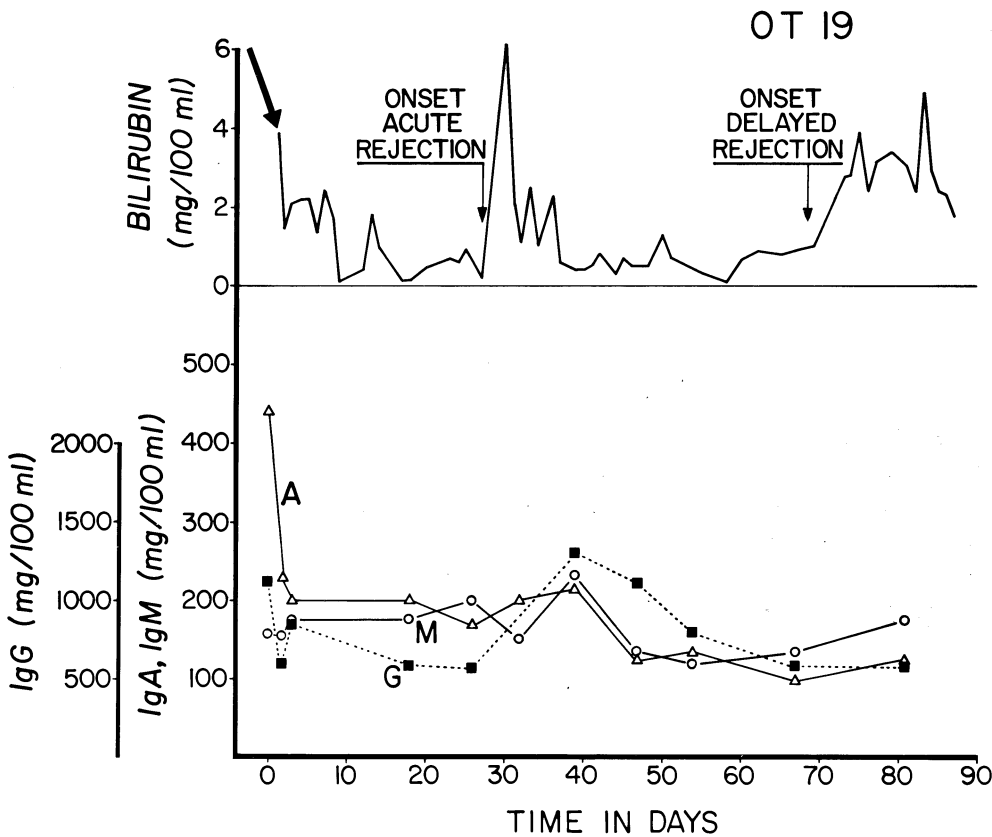


Figure 188. The changes in serum immunoglobulins in a patient (OT 19) who had two rejection episodes during the 90-day period of the study.

It has been possible to examine this question in 10 recipients of orthotopic homografts by the use of phenotype tracing analogous to that described earlier in this chapter for haptoglobin, group-specific component, and C'3 complement. It was found that the transplanted livers made a substantial contribution to the total immunoglobulin pool of the recipients. Since the data have not been previously published, the details of the analyses will be fully documented.

General Principles. At least 21 gamma G globulin phenotypes have been identified in man with the hemagglutination-inhibition test.²⁸ Seventeen of these types constitute the "Gm system"⁷ for which it is presently thought that the genetic determinants are all located in either three or four alleles of a single chromosome.^{27, 29} The Gm marker has been used to determine the success or failure after clinical bone marrow transplantation.²⁰

Detection of Phenotypes. The Gm types 1, 2, and 12 were determined in our studies by a modification of Martensson's hemagglutination-inhibition method.¹⁹ In principle the technique involved the addition of phenotype-specific antisera* to the tested samples. The presence or absence of consumption of the commercial antisera by the patients' gamma G globulin was quantitated by adding the mixture to an indicator suspension. The latter was made by adding anti-D antiserum* of the same Gm type being tested to O positive human red cells. If the coated red cells underwent agglutination, it was considered that the Gm type had not been present in the recipient serum; if agglutination did not occur, the presence of the phenotypes was considered to be established. The quantity of a Gm substance was designated with the number of the highest dilution of the patient's serum with which the hemagglutination was inhibited, the first tube being counted as 8.

There was only one modification¹⁶ from Martensson's original technique. As a preliminary step each volume of test serum was diluted by adding three volumes of phosphate-buffered saline. The diluted sera were then heated for 10 minutes at 63° C to inactivate the rheumatoid factors that were present in six of the 10 recipients before transplantation and in all 10 afterward. With a few exceptions,¹⁸ rheumatoid factors are gamma M globulins which are directed against gamma G globulin;^{6, 15} consequently they can interfere with the just described indicator system by reacting with the anti-D red cell coat and causing agglutination, which may result in a false negative test. More will be said later about other implications of the rheumatoid factors.

Conversion of Phenotypes. In five of the patients the presence or absence of the Gm types was determined without efforts at quantitation, before transplantation and on a single occasion afterward. The postoperative sera were examined from one to 25 days following the liver replacement. In every instance it was possible to find phenotypes 1, 2, and 12 whether or not these had been previously represented in the serum of the donor or the recipient (Table 29). The indiscriminate conversion of the gamma G globulin types was thought to be ascribable to the administration of blood or blood products, which were required in large volumes in these patients during the intraoperative and early

*Antisera against Gm types 1, 2, and 12 as well as the anti-D antisera were obtained from the Certified Blood Donor Service, Inc., Woodbury, New York.

Table 29. Gm Types in Five Liver Transplant Recipients*

GM TYPE	PATIENT				
	OT 10	OT 11	OT 12	OT 17	OT 23
Gm (1)					
Preop, donor	+	-	-	-	+
Preop, recipient	+	-	+	-	+
Postop, recipient	+	+	+	+	+
Days postop	25	15	6	13	1
Gm (2)					
Preop, donor	-	-	-	-	-
Preop, recipient	+	-	-	-	-
Postop, recipient	+	+	+	+	+
Days postop	25	15	6	13	1
Gm (12)					
Preop, donor	+	+	+	+	+
Preop, recipient	+	+	+	+	+
Postop, recipient	+	+	+	+	+
Days postop	25	15	6	13	1

*Qualitative studies of the Gm types in five orthotopic liver recipients. The examinations were one to 25 days after transplantation. Note that all three of the phenotypes became detectable after transplantation whether or not these had been present in either the donor or recipient; the indiscriminate conversion was probably due to multiple blood transfusions.

postoperative period. The artifact introduced by transfusions has been well documented under less complex clinical circumstances.²⁸ Moreover, the gamma G globulin apparently infused by this means was noted in some of our other more completely studied recipients to disappear completely within a few days or weeks (see patients OT 14 and 16A in Table 31).

Serial quantitative studies were carried out after six homotransplantations in five patients. In three of the six sets of observations, the donor did not have one or more of the gamma G globulin phenotypes that were in the recipient. After the transplantation the pre-existing Gm types of the recipients were not altered during follow-up periods of from six months to almost a year (Table 30).

In the other three transplantations the situation was the converse (Table 31) in that the donors each had one Gm type not naturally found in the recipient. During follow-ups of 60 to 326 days the phenotypes could now be detected in the recipient serum in highly significant quantities. In some instances the amounts of the new gamma G globulin maintained a consistent level, whereas in others there was a very gradual diminution but never a complete loss of the titer (Table 31). The persistence of the new phenotype for such long intervals was strongly indicative of its synthesis and release by the homograft.

Exactly the same examinations were performed in 11 recipients of renal homografts and their respective donors. There were several examples of postoperative rises of Gm types that had not been present before transplantation. However, these disappeared within two months. There were no permanent changes such as those described following liver transplantation.

Table 30. Transplantations Between Differing Phenotypes*

PATIENT NUMBER	DAYS	GM (1)	GM (2)	GM (12)
OT 13	Donor, Preop	< 8	< 8	
	Recip, Preop	256	64	
	1	128	32	
	14	256	32	
	40	256	64	
	68	256	32	
	96	512	64	
	124	512	64	
	194	512	64	
	250	512	64	
	284	512	64	
	325	256	64	
OT 15	Donor, Preop	256	< 8	
	Recip, Preop	512	16	
	3	512		
	10	512	32	
	24	512	32	
	38	512	32	
	71	512	32	
	101	512	32	
	129	512	64	
	157	512	64	
	185	512	64	
	213	512	64	
	241	512	64	
	276	512	32	
OT 19	Donor, Preop	256	128	< 8
	Recip, Preop	512	16	16
	1	256	16	16
	2	512	16	16
	18	256	64	16
	39	512	64	16
	69	512	64	16
	151	512	64	16
	179	512	16	8

*Three liver homotransplantations in which the recipients possessed gamma G globulin phenotypes not found in their donors.

Table 31. Transplantations Between Differing Phenotypes*

PATIENT NUMBER	DAYS	GM (1)	GM (2)	GM (12)
OT 14	Donor, Preop	512	< 8	128
	Recip, Preop	256	< 8	< 8
	3	256	64	128
	24	256	16	64
	52	256	< 8	64
	87	256	< 8	64
	129	256	< 8	64
	179	256	< 8	32
	213	128	< 8	64
	244	256	< 8	32
	272	256	< 8	16
	306	256	< 8	16
	326	256	< 8	16
OT 16A	Donor, Preop	256	< 8	128
	Recip, Preop	< 8	< 8	128
	2	256	64	128
	10	256		128
	32	256	8	128
	45	256	8	128
	60	256	< 8	128
OT 16B	Donor, Preop	512	< 8	256
	Recip, Preop	256	< 8	128
	8	32	< 8	64
	28	64	< 8	128
	49	128	8	128
	77	64	8	128
	111	64	8	128
	152	32	8	128

*Three liver homotransplantations in which the donors possessed gamma G globulin phenotypes not naturally present in the recipients. Note that the recipients were thereby endowed with the new Gm types for very long intervals and perhaps permanently.

The Rheumatoid Factors

The foregoing studies indicated that the maintenance of a specific gamma G globulin phenotype was not hepatic dependent, a conclusion in accord with that reached by Mathé et al²⁰ on the basis of their observations after bone marrow transplantation. However, the investigations in the hepatic recipients also showed that the liver could introduce and support a new Gm type not previously present in a given patient. There were no easily detectable specific adverse consequences in these cases of having a genetically heterogenous admixture of immunoglobulins. However, studies of the rheumatoid factors were

conducted to examine the possibility of an inter-reaction between the immunoglobulins of host and graft origin.

Methods of Detection. It was mentioned earlier that the rheumatoid factors are anti-gamma G globulin antibodies and that their presence may invalidate the results of Gm typing by their reacting with the coated red cells of the indicator system and causing agglutination. It follows that the same indicator system can be used as a direct test of anti-gamma globulin antibodies.

This was done by serially diluting recipient sera after first decomplementing them by heating for 30 minutes at 56° C. The diluted test sera were then added to the O positive red cells that had been sensitized by mixing with commercial anti-D serum that was known to contain the Gm (1+, 2+, 12+) types. The agglutination of the coated erythrocytes by the patient sera was indicative of anti-gamma globulin activity to a degree that could be quantitated by the highest dilution at which this effect was observed.

The Incidence of the Rheumatoid Factor. The sera of 10 recipients were examined before and serially after transplantation. Preoperatively, anti-gamma globulin antibodies were present in six of the 10 patients; after operation, positive results were obtained in all 10.

In Table 32 the results are shown from two patients whose serum contained anti-gamma globulin antibodies prior to transplantation. Afterward the titer was reduced temporarily only to return to very high levels during some stage of convalescence. A similar waxing and waning of titer was seen in all the other eight patients.

The Specificity of the Anti-gamma Globulin Antibodies. This information was sought by assessing the effect of sera of known Gm phenotypes on the rheumatoid factor present in patient OT 14's serum before and 87 days after

Table 32. Serial Studies of Antigamma Globulin Antibodies
(Rheumatoid Factor)*

OT 14		OT 16A		OT 16B	
<i>Days</i>	<i>Titer</i>	<i>Days</i>	<i>Titer</i>	<i>Days</i>	<i>Titer</i>
Preop	64	Preop	64	Preop	32
3	4	2	16	8	< 2
24	< 2	10	< 2	28	4
52	32	32	< 2	49	4
87	1000	45	4	77	4
129	64	60	32	111	16
179	4			152	512
213	4				
244	4				
272	4				
306	< 2				
326	< 2				

*Studies in two human recipients of orthotopic liver homografts. Patient OT 16 received two livers, the second 68 days after the first (see Chapter Fourteen).

transplantation. The absorbing sera were from the liver donor (Gm 1+, 2- and 12+), professional blood donor A (Gm 1-, 2-, 12+) and professional blood donor B (Gm 1+, 2+, 12-). These were individually added in an equal volume to the patient's twofold diluted test sera and the rheumatoid factor titers were redetermined by the same indicator hemagglutination system described previously.

The results are summarized in Table 33. The serum of the liver donor as well as blood donor A caused a drastic reduction in the titer of the rheumatoid factor in the pre- and post-transplantation samples from the recipient. These absorbing sera had gamma G globulin of Gm (12) type. In contrast, absorbing serum B (Gm 1+, 2+, 12-) had no such effect. It was concluded that the anti-gamma globulin (rheumatoid) antibody was directed against Gm (12) gamma G globulin both before and after transplantation.

To strengthen this conclusion the patient's serum was now compared with commercial anti-Gm (12) serum in the determination of the Gm (12) phenotype in her own serum, using the hemagglutination-inhibition test of Martensson. The results were essentially the same with use of the commercial antiserum and the donor serum (Table 34), confirming that the rheumatoid antibody in the patient had an anti-Gm (12) specificity.

The Significance of the Immunoglobulin Studies

As mentioned earlier, it is improbable that the normal liver elaborates detectable quantities of gamma G globulin. This contention has been supported by several studies not involving any kind of transplantation.^{2, 5, 14, 22} Moreover the observations of Mathé in human recipients of bone marrow homografts have strengthened this position.²⁰ In Mathé's patients the Inv phenotypes became those of the donor, whereas the types originally represented only in the recipients completely disappeared in the event of a bone marrow take. In these cases the presumably normal livers made no detectable contribution to the circulating immunoglobulins. Consequently it is necessary to believe that the gamma globulin production by the transplanted liver is an abnormal occurrence and one that may be similar to that in certain chronic hepatic disorders

Table 33. Effect of Absorption with Sera of Known Gm Phenotypes upon the Antigamma Globulin (Rheumatoid) Antibody
Titers in Patient OT 14

	TITERS AFTER ABSORPTION WITH			
	<i>None</i>	<i>Donor Serum</i> [*]	<i>Serum A</i> [†]	<i>Serum B</i> [‡]
Pretransplantation	64	Not done	4	64
87 days Postoperative	1000	32	64	500

^{*}Donor: Gm (1+, 2-, 12+).

[†]A: Gm (1-, 2-, 12+).

[‡]B: Gm (1+, 2+, 12-).

Table 34. Detection of Gm (12) Phenotype*†

DAYS	GM (12) WITH COMMERCIAL ANTISERUM	GM (12) WITH PATIENT'S OWN SERUM
Donor, Preop	128	64
Recip, Preop	<8	<8
3	128	64
24	64	64
52	64	64
87	64	64
129	64	64
179	32	64
213	64	64
244	32	32
272	16	16
306	16	32
326	16	32

*The detection of the Gm (12) phenotype in the serum of patient OT 14 by the use of anti-Gm (12) antibodies in her own serum. The results are compared with those obtained with a commercial anti-Gm (12) serum.

†Martensson's hemagglutination-inhibition test.

such as lupoid hepatitis¹⁷ and cirrhosis.^{4, 23} In these diseased livers²³ and in the hepatic homografts as well, intrahepatic lymphoid depots are presumably responsible for synthesis of the immunoglobulins. In the case of liver transplantation, the immunoglobulins released from such donor-specific lymphoid accumulations could be construed as being directed against host antigens. At the same time the immunoglobulins of host origin could be viewed as a response of the recipient against the graft and possibly also against the protein synthesized by the graft.

There has been no clinical indication that the immunoglobulins released simultaneously from the transplanted livers and the host tissues have had a mutually antagonistic effect or one that was inherently detrimental to the host. The universal presence of anti-gamma globulin (rheumatoid) antibodies in the liver recipients postoperatively could suggest that an interaction actually occurred, particularly since the rheumatoid factor in at least one case was specifically directed against a Gm phenotype found only in the donor. The force of the argument in this instance was weakened by the fact that the rheumatoid test was positive preoperatively and that the anti-gamma globulin specificity predated the transplantation.

Moreover it is clear that the presence of anti-gamma globulin activity is by no means a special feature of hepatic transplantation. In 60 of our kidney recipients studied by Bravo, Herman and Smyth,³ 56 developed a positive rheumatoid factor; two of the four exceptions were identical twins and a third was a fraternal twin. Similar observations have been reported by other authors.^{10, 11, 30} In these cases, it could be suggested that lymphoid tissue transferred in the hilum of the renal homografts was at least partly responsible for the temporary elaboration of an alien gamma globulin and that this gamma

globulin evoked the rheumatoid response. The studies of Wilson and Kirkpatrick^{13, 31} have provided evidence that functioning lymphoid tissue is usually transplanted along with the renal homografts, since the pre-existing delayed hypersensitivity reactions of the donors were conferred upon the recipients and thereafter remained for some time before eventually dying away.

With hepatic transplantation it may be that donor lymphoid depots which are accidentally carried with the livers (see Chapter Twenty) are even more durable, accounting both for the much more permanent support of new Gm phenotypes by the transplanted livers and for the presence long after operation of anti-gamma globulin activity. Porter's sex identification studies (Chapter Twenty) do not support the idea that reinforcement lymphocytes of donor genotype are differentiated from the graft Kupffer cells.

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Chapter Nineteen

ORTHOTOPIC HETEROTRANSPLANTATION

A serious limitation to the widespread use of liver transplantation is logistic in nature as emphasized in Chapters One, Two, and Four. Because artificial livers are not available with which to support a patient dying of hepatic disease, the chance for treatment with transplantation is very quickly lost if a suitable donor does not become available at precisely the right time. In practice the proper circumstances to proceed arise with distressing infrequency.

An ideal solution to the problem might someday be the use of organs from lower animals. The donors could be carefully selected from within a given species on the basis of size as well as by criteria of biologic suitability such as tissue matching. Moreover, operation could be carried out in an elective and highly planned way. Such an attempt at treatment was made at the University of Colorado in a child who was dying from end stage liver disease. Before describing this unique case, a brief review would be appropriate of the preceding clinical experience with heterotransplantation of other organs.

HETEROTRANSPLANTATION OF EXTRAHEPATIC ORGANS

The first attempts to transplant vital whole organs from animals to man were made in Europe at the beginning of the twentieth century. On about a half dozen occasions between 1906 and 1923 kidneys were removed from a pig, goat, lamb, or subhuman primate and transferred to patients who were terminally ill with uremia. These historically important cases have been reviewed and tabulated in more recent publications.^{34, 42} Interest quickly waned because of the lack of benefit from the procedures and was not aroused again until the results became known of several much more encouraging clinical trials with heterotransplantation in 1963 and 1964.

Clinical Observations

In the latter two years more than a dozen renal heterotransplantations were performed. Eventually all the patients died, but not before it was established that function of kidney heterografts could be maintained for many weeks or even months. It had become evident that the ability to exploit animals as organ sources for humans was almost, but not quite, within the grasp of surgeons using the immunosuppressive techniques then available. All but one of the recipients were treated with azathioprine and prednisone, to which were added intravenous actinomycin C and/or local homograft irradiation.

The therapeutic exception was in the first of the modern trials, made by Dr. Claude Hitchcock of the Hennepin County Hospital, Minneapolis. On February 16, 1963, he revascularized a single baboon kidney in the right femoral triangle of a 65 year old woman and established urine drainage with a skin ureterosotomy. After a brisk diuresis, the organ continued to function for four days. It then became anuric and was removed; the renal artery had thrombosed. Unfortunately the experience was not reported by publication or even by word of mouth until more than a year later,¹⁴ after the much more significant and successful clinical investigations of Dr. Keith Reemtsma of New Orleans had become well known.³⁷⁻³⁹ In Reemtsma's first case,³⁹ in which a *Macaca mulatta* (rhesus) monkey was the donor, the outcome was similar to that observed by Hitchcock.

Chimpanzee to Man Heterotransplantation. For subsequent patients Reemtsma used chimpanzees, with strikingly different results.^{34, 38} Several heterografts in the latter series functioned at least temporarily in the same way as might have been expected with homografts and one patient led a full life, much of it outside the hospital, for nine months before dying of infection and an electrolyte disorder. At autopsy^{30, 31, 38} some although not all of the transplanted chimpanzee kidneys had histologic abnormalities which were in no way different from those to be expected in homografts after similar periods of time. However, in the majority of cases there were special anatomic features which were most dramatically seen with gross examination. These included obvious swelling and scattered areas of hemorrhagic infarction.³⁴

In Reemtsma's first six chimpanzee to man heterotransplantations, there were two recipients with survival and graft function for more than two months. In the rest, rejection was very severe and led to loss of the organ or death of the patient in less than three weeks. Six more attempts by the same investigator under similar circumstances were less successful.³⁴ None of the latter group survived for as long as one month.

Other clinical attempts have been made at heterotransplantation of chimpanzee kidneys, using the same general immunosuppressive regimens. A patient of Hume died as the consequence of an uncontrollable postoperative diuresis which totalled 54 liters on the first postoperative day.¹⁶ Traeger of Lyon, France, reported three more cases.⁵⁰ In one, the graft failed immediately because of a technical accident. In another, the kidney was rejected abruptly after six days. The third patient died of sepsis after 50 days, but the transplant was

said to contain no histopathologic signs of rejection. Finally, Cortesini of Rome⁸ lost a patient from peritonitis 31 days after renal heterotransplantation. There had been no evidence of rejection at any time. The histologic appearance of the transplanted chimpanzee kidney was described as normal; host immunoglobulins could not be detected with immunofluorescence techniques.

During the same era an orthotopic cardiac heterotransplantation was performed by Dr. James Hardy of Jackson, Mississippi. The chimpanzee heart contracted promptly after revascularization but could not support the circulation of the adult human recipient and stopped beating after an hour. It was suggested in the report of this case that the organ had failed acutely more because of its small size than for any immunologic reasons.¹³

Baboon to Man Heterotransplantation. Within a few months after Reemtsma's first experience, the East African baboon (*Papio doguera*) was given a systematic trial in our institutions as an organ donor for man. Six renal heterotransplantations were performed in December, 1963, and January, 1964, using both the baboon kidneys in each case.^{42, 46} Good heterograft function was obtained immediately in all instances. Urine excretion of a variable quality^{27, 42, 46} continued for nine to 60 days. Although immunologic repudiation could be at least partially controlled or even reversed, all the organs eventually were completely or nearly completely rejected, as judged by serial studies of their function and by subsequent pathologic examination.

The six recipients died 19 to 98 days after operation, including two in whom the heterografts were ultimately removed. The results were better when there was identity of the ABO blood groups or else compatibility as shown in Table 1, Chapter Two. The degree of histologic injury to all the heterografts was considerably greater^{30, 31, 40, 46} than had been the case with many of the chimpanzee organs; damage to the vascular system was particularly striking. It was concluded that the conditions were not yet propitious for further efforts at animal to man transplantation, but that when the time came the chimpanzee would be the preferred subhuman primate donor.

Immunologic Studies

In both the chimpanzee and baboon series of renal heterotransplantations, special attention was paid to the humoral antibodies which were either present in the patient serum before arrival of the grafts or else developed subsequently. The studies in Reemtsma's cases were carried out by DeWitt^{9, 10, 34, 38} and in ours by Kirkpatrick and Wilson.^{18, 46}

Chimpanzee to Man. Blood groups O and A have been found in chimpanzees with frequencies of about 10 and 90 per cent, respectively,⁵¹ using standard blood bank detection procedures. In animals of A erythrocyte type, it is almost certain that the isoantigen is also found in the renal and other tissues, just as has been well established in humans.^{15, 47} Consequently the guidelines of red cell compatibility between humans, discussed in Chapters Two and Fourteen, apply equally in chimpanzee to man combinations. Reemtsma and DeWitt have reported that the consequences of violating these rules are indistinguishable

from those following human homotransplantation under such circumstances. In one well documented case involving the transfer of an A chimpanzee heterograft to a B group patient, the kidney was immediately destroyed. Concomitantly, there were sharp falls in the heat-stable anti-A isoagglutinins of the recipient serum, suggesting that the antibody had promptly fixed to and adversely affected the revascularized organ.¹⁰ The same kind of accident also apparently occurred in one of their later patients.³⁴

The problems of blood group mismatching are avoidable. However, other antibodies (heat-labile heterohemagglutinins) which react with chimpanzee red cells irrespective of their blood type are almost invariably present in the serum of humans. Fortunately there was no evidence from Reemtsma's observations that these heterohemagglutinins fixed to the chimpanzee grafts. After operation the titers usually remained stable for a few days and then transiently increased; there was no apparent correlation between such variations and the development of rejection crises.

In contrast lymphocytotoxic antibodies similar to those described by Terasaki after homotransplantation (Chapter Three) usually appeared at the time of overt heterograft rejection. The cytotoxins, which were never present preoperatively, became detectable subsequently in all but one of Reemtsma's recipients.³⁴ The killing ability of the serum waxed and waned with the onset and remission of rejection episodes. The antibody was characterized as a heat-stable, complement-dependent immunoglobulin located in the gamma G and gamma M fractions.³⁴

Baboon to Man. All baboons studied so far belong to red blood cell groups A, B, or AB.⁵¹ Since the red cells of these animals do not react with human anti-A and anti-B typing sera, the "blood group substances" of the donor baboons were determined by Dr. J. Moor-Jankowski with indirect methods, including saliva analysis and reciprocal agglutination testing.^{18, 46}

The penalty of using mismatched donors in respect to the red cell groups was by no means so severe as with the chimpanzees. Immediate urine excretion was obtained in three O type patients who were given the kidneys of B or AB baboons. Nevertheless, the average functional survival of the organs was only 28 days compared to 44 days in the other three patients in whom red cell compatibility had been present. Moreover, those isoagglutinins in the sera of the mismatched recipients, which would have been predicted to be absorbed on the kidney if the "blood group" antigens were represented there, tended to decrease in titer after the transplantation.

Before operation the sera of all six of the recipients also contained heterohemagglutinins for baboon erythrocytes. These antibodies were probably less innocuous than had apparently been the case after the chimpanzee to human transplantations. In each instance the titers were depressed within hours or days after revascularization of the heterografts, only to rise again, often with the advent of a rejection crisis. Electron micrographic studies of one of the baboon kidneys 49 days after its placement revealed subendothelial deposits in the small vessels and glomerular capillaries.¹⁸ It was speculated that these might have been the site of fixation of heteroagglutinins and other pre-formed heterospecific antibodies, that the antibodies did not immediately de-

stroy the graft because of a comparatively low avidity with the binding sites, but that they could have contributed to the massive injury to the vascular system seen by light microscopy in all the specimens.

Experimental Clarification

It is no exaggeration to state that the hope of transplanting animal organs to humans was universally conceded to be completely unfounded until after the trials with subhuman primate organs just described. The pessimism was not based primarily on prior work with skin transplantation, since skin transferred between some species of rodents may evoke a rejection which is not obviously different but only more vigorous than that of homografts. Instead, it was apparently founded on the disastrous results of transplanting animal kidneys to recipients of widely disparate species.

The first clear example of a hyperacute heterograft reaction was reported in 1926 in the classic and neglected paper of C. S. Williamson.⁵⁴ In the course of investigating the fate of renal auto- and homografts, he also transplanted goat kidneys into two dogs. The organs failed to be revascularized. Histologic examination of the kidneys revealed the vessels to be plugged with tightly clumped red cells, but without real clot formation. The recipients both died. Thirty years later Brull² also noted that the blood flow through cat kidneys ceased rapidly after transplantation to dogs. For the same reason Calne³ and Mowbray²⁵ found the canine environment to be almost immediately lethal for goat and rabbit kidneys, respectively. Calne's description was particularly graphic. After revascularization the goat kidney appeared initially to have a good blood supply, but within a few minutes the cortex first became pale and then deeply cyanotic. Microscopic examination of the removed kidney showed interstitial edema, disruption of cortical vessels, and numerous hemorrhages. Calne suggested that naturally occurring humoral antibodies might have been responsible.

The short and unsatisfying nature of these experiments helps to explain the paucity of further reports until after the trials of clinical heterotransplantation. Since then, many studies have been undertaken with the objectives of better understanding whole organ heterograft rejection or, alternatively, of preventing this process. The need to divide such investigations on the basis of genetic diversity has been recognized by Reemtsma^{32, 35} in his suggestion that the term heterograft be used only if the donors and recipients are closely placed on the phylogenetic scale and that the word xenograft be reserved for distant relationships. This classification will be observed in the following discussion.

Wide Genetic Disparity. The studies of Clark and Gewurz and their associates^{5, 6, 7, 12} from the University of Minnesota were the first serious efforts to dissect the events of whole organ xenograft rejection. Rabbit kidneys were placed into the circulation of dogs. Blood flow through the transplant was vigorous at first but rapidly diminished within three to 10 minutes. During this time from 25 to 50 per cent of the dog complement presented to the organ was consumed as well as a significant fraction of the heterospecific hemolysins and hemagglutinins. There was also a striking clearance of formed blood elements other than erythrocytes. Up to 70 per cent of the white blood cells and 90 per

cent of the platelets supplied to the grafts failed to emerge in the venous effluent. The remarkable sequestering properties of a variety of other xenografts have been confirmed by Marceau and his associates,²² who were unable to modify the changes with a panel of antiseritonin, antihistamine, and antiprotease agents or with azathioprine. Only intra-arterial prednisolone significantly delayed the total devascularization. In some of their pig kidneys that had been perfused with dog blood, there was "pavementing" of polymorphonuclear leukocytes on the vascular endothelium. A similar finding has been reported by Williams⁵² in renal homografts rejected immediately by patients whose blood contained lymphocytotoxic antibodies.

Clark⁶ concluded that the violent rejection was caused by naturally occurring humoral antibodies in the recipient, which reacted with the xenograft renovascular tissue. In an effort to reduce these hostile immunoglobulins, he exposed the canine blood repetitively to rabbit kidneys; the last organ had prolongation of function by several hours.^{5, 7} Survival was also slightly increased by reducing dog complement with aggregated gamma globulin⁷ or by the injection of Egyptian cobra venom.^{5, 12} Snyder has also reported that the venom increased by more than tenfold the viable period of pig kidneys transplanted to dogs.⁴¹

Perper and Najarian²⁸ have added to the circumstantial evidence that humoral antibodies may be responsible for immediate xenograft destruction. They transplanted pig kidneys into dogs and vice versa. With either combination there was a sharp fall in complement within a few minutes. However, the pig xenografts underwent rejection 10 times more rapidly than the dog kidneys. The authors explained the difference by their demonstration that heterospecific cytotoxins and hemagglutinins in the canine serum reacted with porcine renal cells; the converse was not true when canine renal cells were exposed to pig serum. The severity of rejection after transplantation in either direction could not be altered by treatment with azathioprine, prednisone, and actinomycin C.

Major genetic diversity does not always lead to the kind of hyperacute rejection just described. This was demonstrated several years ago by Eiseman and his associates, who were able to circulate human blood through isolated pig livers for as long as 30 hours.²¹ The organs could clear bile, ammonia, and other metabolites from the perfusate and could carry out synthesis of porcine protein.^{1, 21, 26} Eiseman has since connected pig livers directly to the circulation of patients dying of hepatic failure, usually for six-hour treatment periods.¹¹ He and others²⁶ have reported temporary benefit from the procedure with a minimum of side reactions.

In an extension of these observations, Calne tested orthotopic pig xenografts in seven baboons.⁴ The first three recipients bled to death in six to nine hours; the other four were given human fibrinogen to prevent this complication and lived for 19 hours to three and a half days. The animals awoke from inhalation anesthesia and were temporarily alert. In the longest survivor the hepatocytes of the xenograft were preserved at the time of autopsy, although there was mononuclear cell infiltration of the portal tracts. This baboon had been treated with steroids.

Thus far all the comments in this section have applied to whole organ transplantation. The experiments of Lance and Medawar²⁰ with skin xenografts must be mentioned because of their potential clinical significance. Using ALS as the sole immunosuppressive therapy, they were able to transplant skin from rats, guinea pigs, rabbits, and humans to adult male CBA mice with prolongation of survival in each instance. Some of the human xenografts were in good condition as long as two months after operation. By all odds these have been the most encouraging efforts at mitigation of xenograft rejection. The results also established that the repudiation of skin xenografts, at least in these species combinations, is due to an immunologic etiology and not to mechanical or other factors.

Minor Genetic Disparity. Perper and Najarian²⁹ exchanged kidneys between unmodified sheep and goats; neither species possessed easily detectable natural antibodies against the tissues of the other. The behavior of the heterografts was compared to that of goat homografts. Although the homografts functioned slightly longer, the results with all three donor-recipient combinations differed only quantitatively. Erythroagglutinins and cytotoxins appeared in the sera of each group of recipients, but rose to higher titers after the heterotransplantations. If a heterograft recipient was challenged with the other kidney from the same donor, the second set rejection appeared to be entirely mediated by humoral antibodies.

Reemtsma has carried out renal heterotransplantation between monkeys of the same genus but a different species as well as across a genus barrier.³³ The recipient animals were treated with azathioprine, prednisone, actinomycin C, and local homograft irradiation. Prolongation of survival was obtained, but only to a maximum of 38 days. The pathologic findings were similar to those of homograft rejection.

Relevance to Clinical Heterotransplantation. The general conclusions from the foregoing animal experiments were, by and large, confirmatory of those reached from the preceding clinical heterotransplantations. With close genetic similarity the events of rejection are apparently served by cellular immunity to which is later added a humoral antibody component that is far more easily and consistently detectable than after homotransplantation under the same conditions. With progressively greater donor-recipient disparities, an increasingly prominent immediate role in the destruction of the homograft is played by naturally occurring heterospecific antibodies, which probably primarily attack the vascular system of the transferred organ. The consequences under the latter circumstances have many analogies to situations in which renal homografts have undergone hyperacute rejection after placement in recipients who have had preformed isohemagglutinins that attached to the kidneys^{42, 45} or lymphocytotoxins^{19, 44, 48, 49, 52, 53} that proved to be hostile in one way or another to the transferred tissue. If the preformed antibodies have a low avidity for the heterograft, as was apparently the case in the baboon to human transplantations, such a dramatic immediate outcome may not occur.

The successful restoration of a blood supply does not, of course, preclude subsequent immunologically mediated damage to the vascular supply of the kind that is a vital factor in both the short- and long-term prognosis of homografts (see Chapters Eleven, Twelve, and Twenty). The threat of delayed

devascularization of heterografts is probably significantly greater than with the average homograft, even in the relatively favorable chimpanzee to human combination. Although some of the better tolerated chimpanzee kidneys have been free of arterial lesions after residence in human hosts for several weeks or months,^{8, 30, 31, 38, 50} many others have developed multiple areas of parenchymal infarction similar to those after baboon to human heterotransplantation.^{34, 36} As will be mentioned, the same thing was seen in the chimpanzee orthotopic liver heterograft.

CLINICAL HEPATIC HETEROTRANSPLANTATION

Liver heterotransplantation for a 28 month old child was the product of desperation. The procedure was finally decided upon because of the encouraging results already obtained after renal homotransplantation with the triple drug immunosuppressive regimen that included heterologous ALG (Chapter Thirteen). Moreover, the efficacy of ALS in mitigating skin heterograft rejection had been learned by personal communication with Monaco²⁴ and Medawar.²⁰ Finally, it was thought that a donor might be selected on the basis of histocompatibility antigens that were shared by both species. The potential value of this approach was later supported by the studies of Metzgar and Zmijewski.²³

The patient had intrahepatic biliary atresia which was diagnosed by open biopsy in the first month of life. Subsequent mental development was normal, although the child was physically stunted. His condition had progressively deteriorated from the age of 18 months onward, with recurrent gastrointestinal hemorrhages and severe ascites that required multiple paracenteses.

Preoperative Evaluation

At admission the boy was 88 cm in length. He weighed 13.6 kg, of which a large fraction was ascites and edema. There were numerous spider angiomas in the skin of the anterior thorax and abdomen, and large venous collaterals were evident in both locations. He was very pale.

Liver chemistry determinations were markedly deranged. The values included: bilirubin, 16 to 21 mg per cent; alkaline phosphatase, 7.4 Bessey-Lowry units (normal, 0.8 to 2.3); total protein, 4.5 gm per cent; albumin, 1.6 gm per cent; SGOT, 86 International Units; SGPT, 43 International Units. Clotting studies included a plasma fibrinogen of 352 mg per cent, a partial thromboplastin time of 38.6 sec (normal, 37 to 50 sec), a prothrombin time of 17.2 sec (50 per cent), a bleeding time of 8.5 minutes and a platelet count of 37,000/cu mm. Fibrinolytic activity was subnormal, with a euglobulin lysis time that was greater than five hours. The hematocrit was 18 per cent. His red blood cell group was O positive.

During the two weeks of preoperative hospitalization, no cadaveric donors became available. In this interval the child's condition became grave. He had almost continuous slow bleeding from the gastrointestinal tract. Repeated para-

centeses were required in order to permit adequate respiration. Finally it was decided that he could wait no longer for the possibility of a cadaveric liver. Arrangements were made with Colonel Clyde H. Kratochvil of the Holloman Air Force Base, New Mexico, for the shipment to Denver of a 12.2 kg chimpanzee.

The animal was a young adult male of O blood type which had been under medical scrutiny at the Federal research facility for almost two years. He was thought to have been free of infectious disease and in good health for all of that time. The chimpanzee lymphocytes were found by Dr. Paul Terasaki to react to his antihuman-lymphocyte typing sera. Incompatibilities, as defined by antigens in the donor but not the recipient lymphocytes (Chapter Three), were detected by nine of the 51 antisera in the panel then being used in Los Angeles; there was one group mismatch of what is now known as the HLA 5 antigen. The sera of both the donor and recipient caused agglutination of the red cells of the other species. Cytotoxins were not detected in the recipient serum.

The Operation

Orthotopic transplantation was performed on July 15, 1966. The chimpanzee was anesthetized with a combination of gallamine triiodide (Flaxedil) and phencyclidine hydrochloride (Sernylan) and cooled to 31° C by immersion in an ice bath. Removal of the liver was by the same technique described in Chapter Five except that the vascular supply was preserved until the last possible moment in the same way as has been employed in canine experiments.⁴³ An aberrant small left hepatic artery originated from the left gastric artery, necessitating retention of a stump of the latter vessel in continuity with the celiac axis; the main artery supplying the right three hepatic segments had the same course as is ordinarily found in man. Just as it was excised, the liver was further cooled and washed free of blood by intraportal infusion of 2 liters of chilled (4° C) lactated Ringer's solution.

In the recipient, massive vascular adhesions around the enlarged and cirrhotic liver, coupled with severe portal hypertension, made the hepatectomy exceptionally difficult. It was nearly eight hours before this stage could be completed. Otherwise there were no unusual technical features of the recipient procedure. The heterograft was inserted with the standard technique, attaching the graft celiac axis to the recipient proper hepatic artery (Fig. 52A, Chapter Eight). Biliary reconstruction was with a cholecystoduodenostomy.

When blood flow was restored to the transplanted organ after a total hypothermic ischemic interval of 102 minutes, the characteristic flush normally seen immediately after revascularization was not apparent. Instead, the liver appeared diffusely cyanotic. Later, a few tiny pink islands of tissue could be seen on the surface but these never coalesced to give a completely homogeneous bright color to the presenting surface. For a time, it was feared that an immediate rejection was occurring.

The anxiety was reinforced by the development within the next hour of a serious bleeding diathesis. Extensive coagulation studies showed about the

same clotting abnormalities as had been present preoperatively, including a platelet count of 40,000/cu mm. The only significant change from the pre-existing studies was a moderate fibrinolysis as indicated by a euglobulin lysis time of less than 40 min. Clot promoting agents were not used. Instead, almost five hours were spent in mechanical efforts at hemostasis with little apparent benefit. At the end of this time the generalized hemorrhage spontaneously ceased as suddenly as it had begun. It was then possible to perform splenectomy and to close the abdomen. The total operating time was more than 20 hours. Blood transfusions totalled 3500 ml. The patient awakened within a few minutes after returning to the recovery room and seemed to be in excellent general condition.

Immunosuppressive Therapy

Treatment was with the triple drug therapy employed for the later cases of hepatic homotransplantation (Chapter Thirteen), including azathioprine, prednisone, and horse ALG. The actual daily doses are shown in Figure 189. Depression of the white blood count was avoided (Fig. 189).

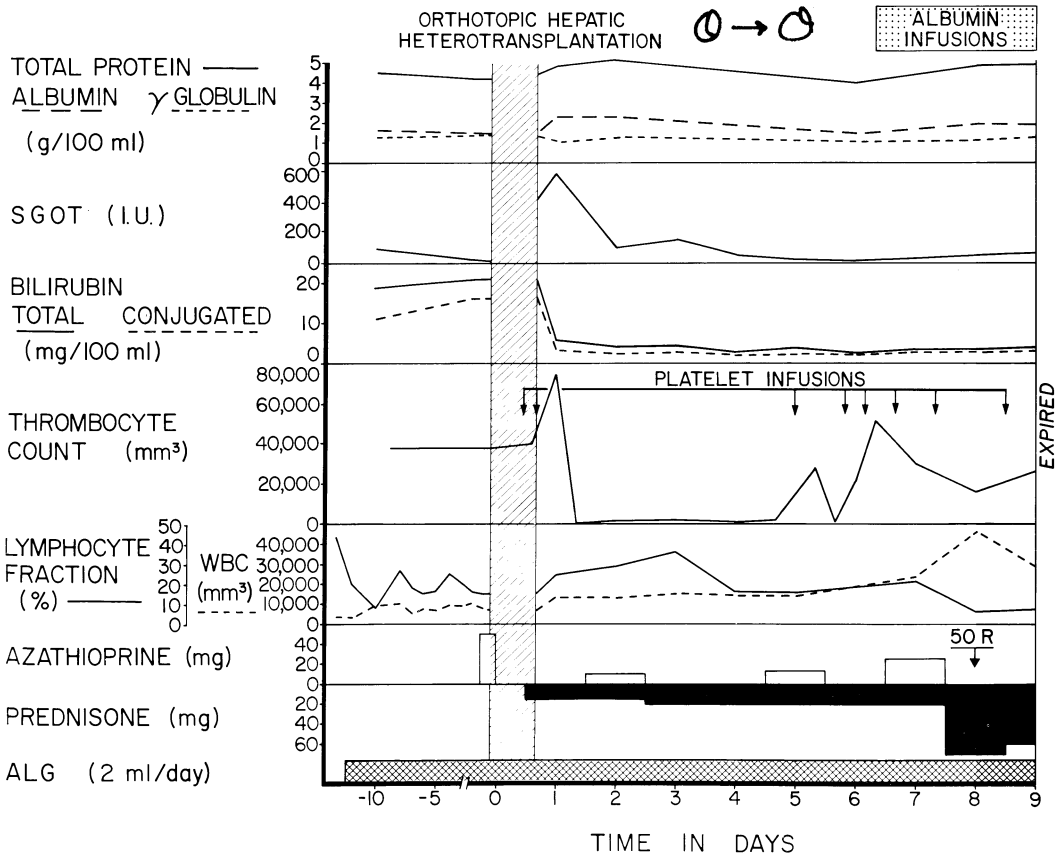


Figure 189. The course of a child with intrahepatic biliary atresia who received an orthotopic chimpanzee heterograft on July 15, 1966. ALG was started two weeks in advance of operation. The 50 R indicates local homograft irradiation.

In contrast, there were early and serious difficulties with thrombocytopenia beginning on the second postoperative day (Fig. 189). Because of the low platelet counts the patient developed epistaxis and wound bleeding which ceased promptly after platelet transfusions. At the time it was assumed that thrombocytes were being sequestered in the heterograft, as was subsequently shown by Hutchison¹⁷ to occur to a lesser extent in liver homografts or even autografts. In retrospect, an even more important role was probably played by the heterologous ALG which can also produce severe thrombocytopenia (Chapter Thirteen) when given in as large doses as were used for this patient. The heterograft was irradiated with 50 R the day before death.

Postoperative Course

In spite of the extraordinarily long and traumatic operation, the recipient had a very satisfactory early course, except for a remittent fever which first appeared after 48 hours. He began to eat on the third postoperative day. On the morning of the sixth postoperative day it was noted that marked ascites had developed during the preceding night. Because of respiratory distress, 1050 ml of the fluid was removed. It reaccumulated with such rapidity that three more needle paracenteses and finally the insertion of an indwelling catheter for continuous intra-abdominal drainage became necessary during the next 48 hours. Several cultures of the peritoneal fluid contained *Aerobacter-Klebsiella*. The patient remained mentally alert until the last few hours of life. He died at 8:30 A.M., July 24, almost nine days after insertion of the heterograft.

The fatal outcome was not well explained by any changes in the serial liver function tests (Fig. 189). On the day after operation the bilirubin had fallen from a preoperative level of 21 to less than 5 mg per cent, where it remained for the entire period of survival. The serum transaminases were transiently elevated but never to the extreme levels associated with massive necrosis secondary to dearterialization of homografts (Chapters Nine and Fifteen). Hypoglycemia was not a problem until two days before death.

The measures of protein metabolism were less satisfactory. The total serum proteins never rose above 5 gm per cent despite repeated albumin infusions that totalled 75 gm during the postoperative period (Fig. 189). Nevertheless, there was evidence of protein synthesis; for example, as late as the seventh postoperative day, the plasma fibrinogen was 350 mg per cent. The liver-based clotting Factors II (prothrombin) and VII (proconvertin) were measured at the same time at 88 and 59 per cent, respectively. The gamma globulin did not vary significantly during the postoperative period (Fig. 189).

At autopsy the heterograft weighed 340 gm, approximately the same as at the time of its transplantation. All the vascular anastomoses were patent and the major vessels contained no clots. The cholecystoduodenostomy was intact and was open. The surface was predominantly tan, but there were numerous discolored areas which appeared to be ecchymoses. After sectioning, similar hemorrhagic foci were found in the interior of the liver, as were scattered but extensive islands of obvious necrosis. The latter were most heavily concen-

trated near the surface. Histopathologic studies of the specimen are described in Chapter Twenty.

In addition, there were focal hemorrhages in the skin, brain, and GI tract, which were presumably secondary to the persistent thrombocytopenia. The lungs were affected by moderate pulmonary edema and contained several very small emboli. The lymph nodes had many germinal centers but few small lymphocytes.

Retrospective Analysis

The immunosuppression given to the patient was badly managed. First, the relation of the ALG to the profound thrombocytopenia was not appreciated at the time, and the appropriate adjustments in dosage were not made.

An even more fundamental error was administration of azathioprine and prednisone, particularly the latter, in such small doses. The quantities of both agents were less (average 0.9 and 2.2 mg/kg/day, respectively) than have frequently been used in subsequent patients who survived after orthotopic homotransplantation (Table 18, Chapter Thirteen). Rejection was uncontrolled, probably accounting for the multiple areas of parenchymal infarction by the same mechanism as that described for homografts in Chapters Fourteen and Fifteen.

In turn, the necrotic foci in the liver undoubtedly became infected and were responsible for the *Aerobacter-Klebsiella* peritonitis. Blood cultures were not systematically obtained, as has been our practice in subsequent cases of homotransplantation. If they had been, it is highly likely that a septicemia would have been demonstrated.

Even if the immunosuppressive drugs had been administered more efficiently, it is doubtful that prolonged survival could have been obtained; there have been difficulties enough in achieving this objective in subsequent cases using homografts. Consequently, no plans have been made for additional heterotransplantations unless there is a fundamental improvement in the treatment protocol which can be offered.

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Chapter Twenty

PATHOLOGY OF THE ORTHOTOPIC HOMOGRAFT AND HETEROGRAFT

by K. A. Porter, M.D., D.Sc.

Before discussing untreated and treated orthotopic hepatic homografts, consideration must be given to the structural changes that are encountered in the livers of animals that are subjected to sham operations, and in autotransplanted livers. Use of these two control groups is essential if one is to distinguish surgical artifacts from immunologically produced changes.

THE LIVER AFTER SHAM OPERATION

In this procedure all the vascular attachments to the liver are mobilized, plastic bypasses are inserted for venous decompression during the period of liver isolation, and many other details of the orthotopic operation are carried out including some of the vascular anastomoses.^{9, 17} The most detailed examinations of the histopathologic consequences of this kind of instrumentation were reported by McBride and his colleagues¹⁴ on the basis of studies in 11 dogs. Their animals, after making a good recovery and returning to a normal dietary intake, were killed at periods ranging up to 63 days. Various lesions were found. A few of the livers contained areas of infarction caused by thrombosis at the anastomotic site in the hepatic artery. In others there was evidence in the early postoperative period of outflow obstruction (see Chapter Five). This phenomenon of outflow block is particularly frequent in dogs and is usually ascribed to constriction of the hepatic veins with trapping of large amounts of blood in the liver.²¹ It is characterized by a swollen, firm, cyanotic organ. The centrilobular sinusoids become distended with blood, the adjacent hepatocytes undergo necrosis, and fat droplets accumulate in the liver cells of the middle zone of the lobules (Fig. 190).

In the longer survivors of McBride's series the hepatic architecture was normal, but dilated lymphatics were frequently seen in the walls of the central

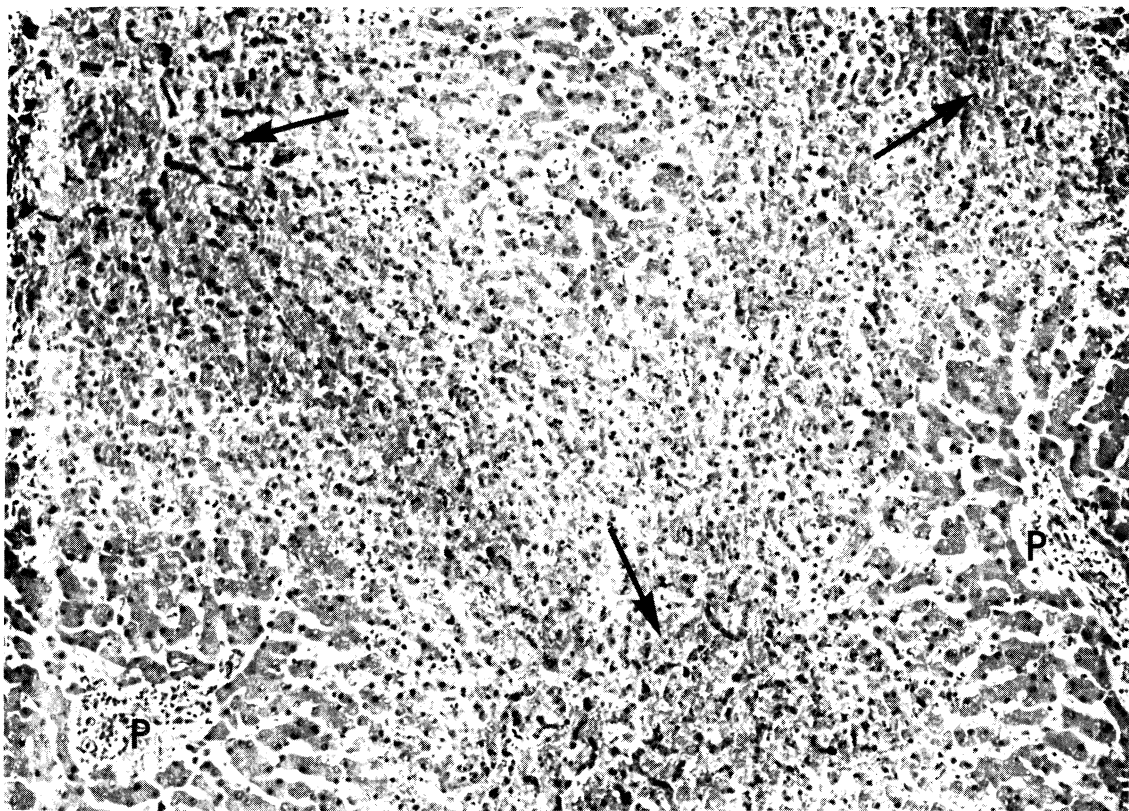


Figure 190. Outflow block in a canine hepatic homograft four days after transplantation. The centrilobular sinusoids (arrows) are distended with blood and the adjacent hepatocytes are necrotic. There is an accumulation of fat in the liver cells of the middle lobular zone. Only hepatocytes adjacent to the portal tracts (P) are normal (Hematoxylin and eosin stain, $\times 40$.) (From *Advances in Surgery*, 1966, by permission of Year Book Medical Publishers, Inc.)

and portal veins. There was edema of the portal connective tissue accompanied by fibroblastic proliferation, as well as infiltration by variable numbers of neutrophilic polymorphonuclear leukocytes and a few mononuclear cells. Some centrilobular intracanalicular bile stasis was present in a few animals. Polymorphonuclear infiltrates have also been produced in dog livers within five to 15 days after the simple performance of cholecystoenterostomy in conjunction with common duct ligation.^{3, 22}

WHOLE LIVER AUTOGRAFTS

Technically liver autotransplantation is more difficult to perform than homotransplantation because the vessels to be anastomosed are shorter. Consequently, there are relatively few reports on the structural alterations in long surviving hepatic autografts.

Within the first two weeks the abnormalities have been variable. In four of the autografts examined in the Northwestern laboratories seven to 15 days after operation there were essentially no changes.^{3, 22} On the other hand,

McBride and his associates studied seven such grafts for periods of as long as 14 days and found the same changes that had been encountered in the sham operated livers. In addition, there was sometimes ascending suppurative cholangitis. In a later study the Boston group²⁹ found slightly increased amounts of portal fibrosis in two autografts in which a biopsy was taken seven months after transplantation.

A more extensive study of canine hepatic autografts by Alican and Hardy,¹ in which biopsies were obtained at intervals of up to 17 months after transplantation, confirmed that the only abnormality was a mild degree of pericholangitis with acute inflammatory cells in the portal tracts. This change was thought to be due to the presence of the cholecystoduodenostomy. Mononuclear cell infiltration and vascular narrowing were not found.

Similar findings have been reported in porcine hepatic autografts.⁷

UNTREATED CANINE HOMOGRAFTS

Gross Features

At autopsy the rejecting or rejected liver homografts usually are larger and heavier than normal. When sectioned the parenchyma bulges over the cut capsule. The surface has a nutmeg appearance like that seen in prolonged and severe cardiac failure.^{14, 22, 24}

Light Microscopy

In the first 48 hours the central zones of the hepatic lobules become congested, a few of the hepatocytes adjacent to the central vein undergo necrosis, and the Kupffer cells enlarge. Macrophages, neutrophilic polymorphonuclear leukocytes, and occasional lymphocytes appear around the portal and central veins. These changes are nonspecific because they may also occur in the liver after sham operation and in hepatic autografts.

From the third day after transplantation, changes characteristic of rejection commence (Fig. 191). Mononuclear cells accumulate around and within the walls of the small portal and central veins, particularly the former (Figs. 192 and 193). Approximately 40 per cent of these cells possess pyroninophilic cytoplasm, and occasionally a cell is seen in mitosis. The number of infiltrating cells steadily increases, and by six days the infiltration is generally very dense.

As cells accumulate in the graft, the hepatocytes in the central and middle zones of the lobules undergo necrosis until only a thin rim of living liver cells remains around the portal tracts. This zonal liver necrosis is associated, first, with distortion of the centrally located sinusoids and, later, with collapse of the central part of the lobular reticulin framework. This leads to condensation of reticulin and some early fibrosis around the central veins in the longest survivors. The hypertrophied Kupffer cells contain bile pigment and hemosiderin

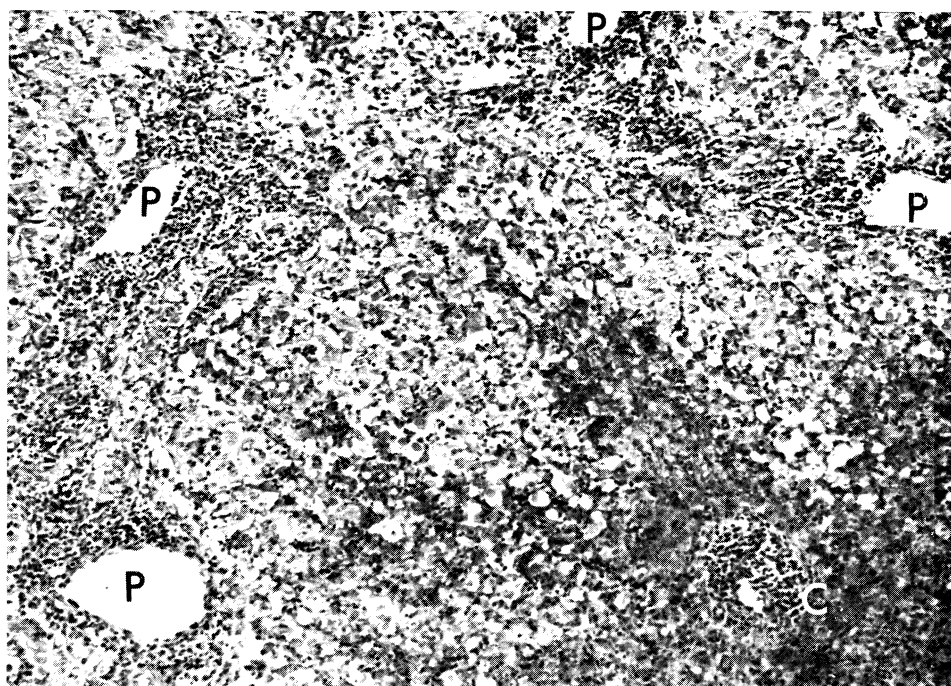


Figure 191. Untreated canine hepatic homograft at six days. Portal veins (P) and central vein (C) are surrounded by dense cellular infiltration. There is centrilobular necrosis with hemorrhage. The cytoplasm of the surviving hepatocytes in the middle and peripheral zones of the lobules contains abundant lipid. (Hematoxylin and eosin stain, $\times 30$.) (From *Advances in Surgery*, 1966, by permission of Year Book Medical Publishers, Inc.)

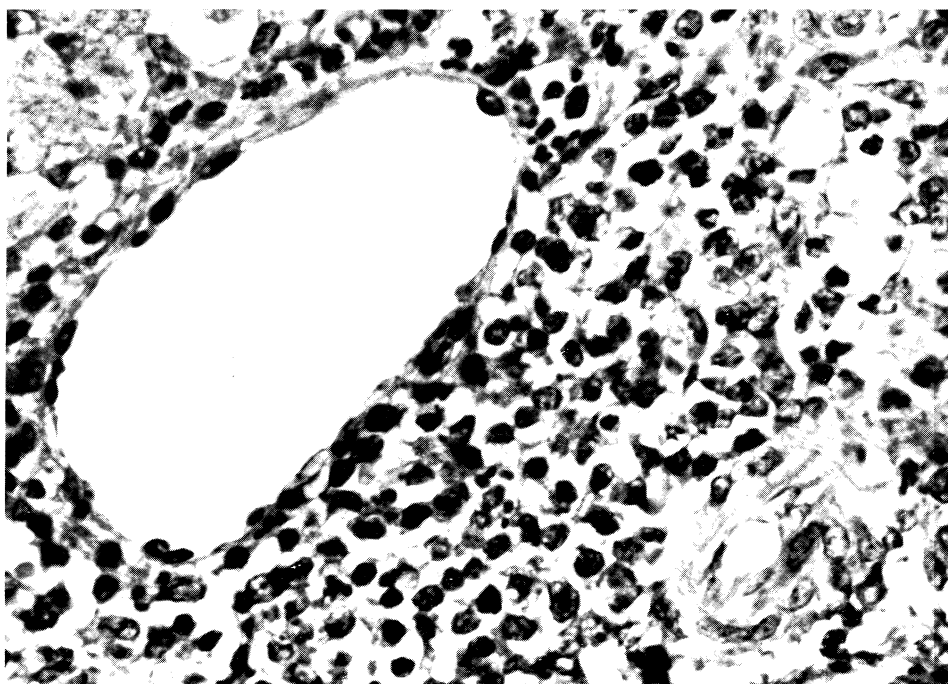


Figure 192. Portal tract of the untreated canine hepatic homograft shown in Figure 191. The portal vein is surrounded by mononuclear cells. Some of these cells can be seen in the wall of the vessel. The hepatic artery is unaffected. (Hematoxylin and eosin stain, $\times 500$.)

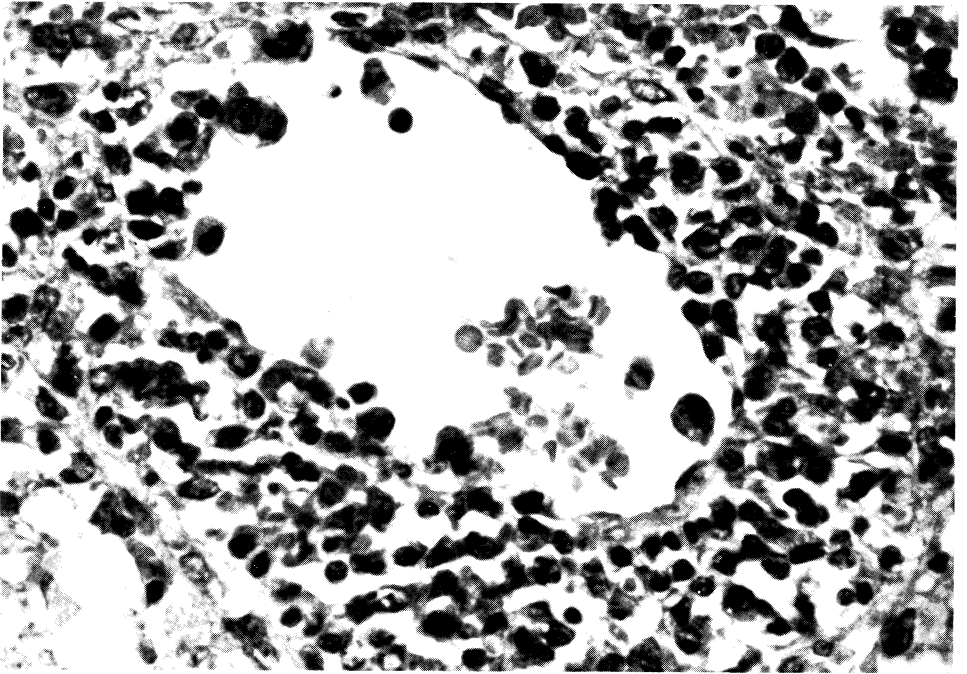


Figure 193. Central vein, surrounded by mononuclear cells, from the untreated canine hepatic homograft shown in Figures 191 and 192. Some of the cells are adhering to the endothelium and others are infiltrating the vein wall. (Hematoxylin and eosin stain, $\times 500$.) (From *Advances in Surgery*, by permission of Year Book Medical Publishers, Inc.)

and there is intrahepatic bile stasis. Fibrinoid necrosis is sometimes seen in the walls of the small hepatic arteries but this is not common, occurring in less than 25 per cent of the homografts.^{3, 13-15, 17, 22, 26}

Immunofluorescence

In the first three days no immunoglobulins are found either in the infiltrating cells or coating any of the vascular or biliary channels. From the fourth day onward the cytoplasm of about 5 per cent of the infiltrating mononuclear cells "stains" positively for IgG. At about eight days, when rejection is usually complete, IgG and complement may appear in the walls of some of the small hepatic arteries.⁸

Electron Microscopy

Forty-eight hours after transplantation any lymphocytes in the graft are usually the small variety with few organelles in their scanty cytoplasm (Fig. 194). After that time there is a progressive accumulation of large lymphoid cells with abundant cytoplasm that is full of polyribosomes but lacking in rough endoplasmic reticulum (Fig. 195). Many of these cells have large Golgi

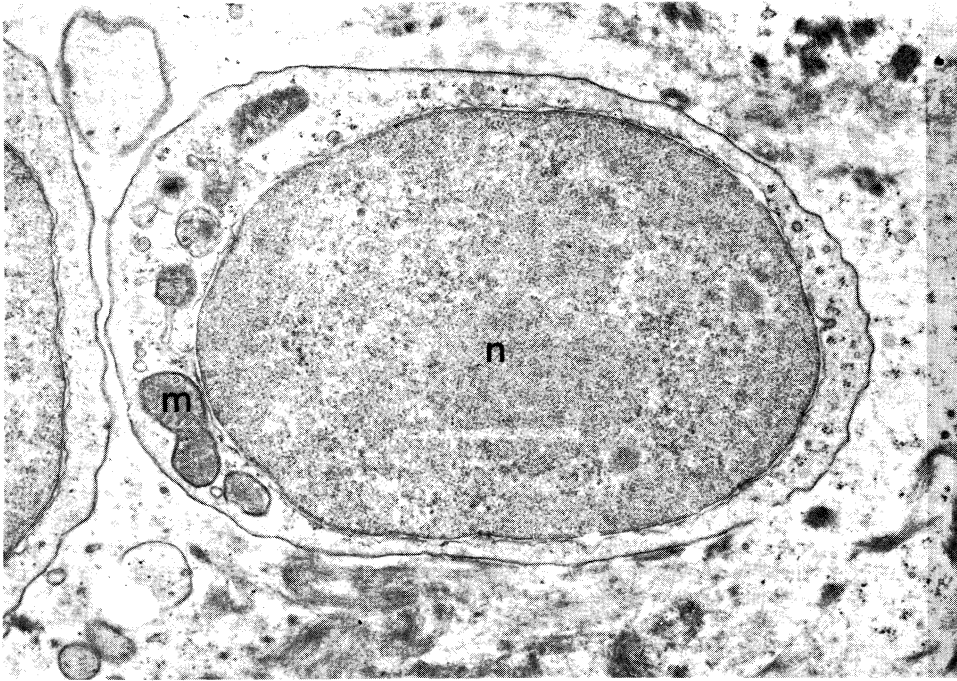


Figure 194. Canine hepatic homograft 48 hours after transplantation to an unmodified recipient. A small lymphocyte lies within the widened space of Disse. The cytoplasm is scanty and lacking in organelles. n=Nucleus; m=mitochondrion. (Lead stain, $\times 11,000$.)

bodies. The large lymphoid cells squeeze through the cell junctions of the endothelium of the portal veins and accumulate in the subendothelial space before passing through the vascular basement membrane to enter the portal connective tissue (Fig. 196).

The infiltrating lymphoid cells also accumulate in the space of Disse. Associated with this process the tenuous walls of many of the centrilobular sinusoids appear to disrupt (Fig. 197) and fibrin accumulates in the venous subendothelial spaces (Fig. 198). The hepatocytes adjacent to the damaged sinusoids are injured, as shown by swelling and clumping of the mitochondria, loss of the rough endoplasmic reticulum, and shedding of cytoplasm. A few plasma cells with abundant rough endoplasmic reticulum (Fig. 199) are always present in the cellular infiltrate, but they only appear in appreciable numbers late in the course of rejection. Macrophages, with typical ultrastructure, are common in the portal tracts (Figs. 200 and 201). When there is cholestasis the bile canaliculi lack microvilli and contain bile plugs (Fig. 202). Fibrinoid necrosis with deposition of IgG and complement is associated ultrastructurally with a homogeneous, finely granular deposit between the endothelium and the internal elastic lamina of the affected arteries.⁸

UNTREATED PORCINE HOMOGRAPTS

In contrast to the behavior of canine hepatic homografts, the rejection of liver transplants in pigs is a relatively weak and slow process even when donor
(Text continued on page 435.)

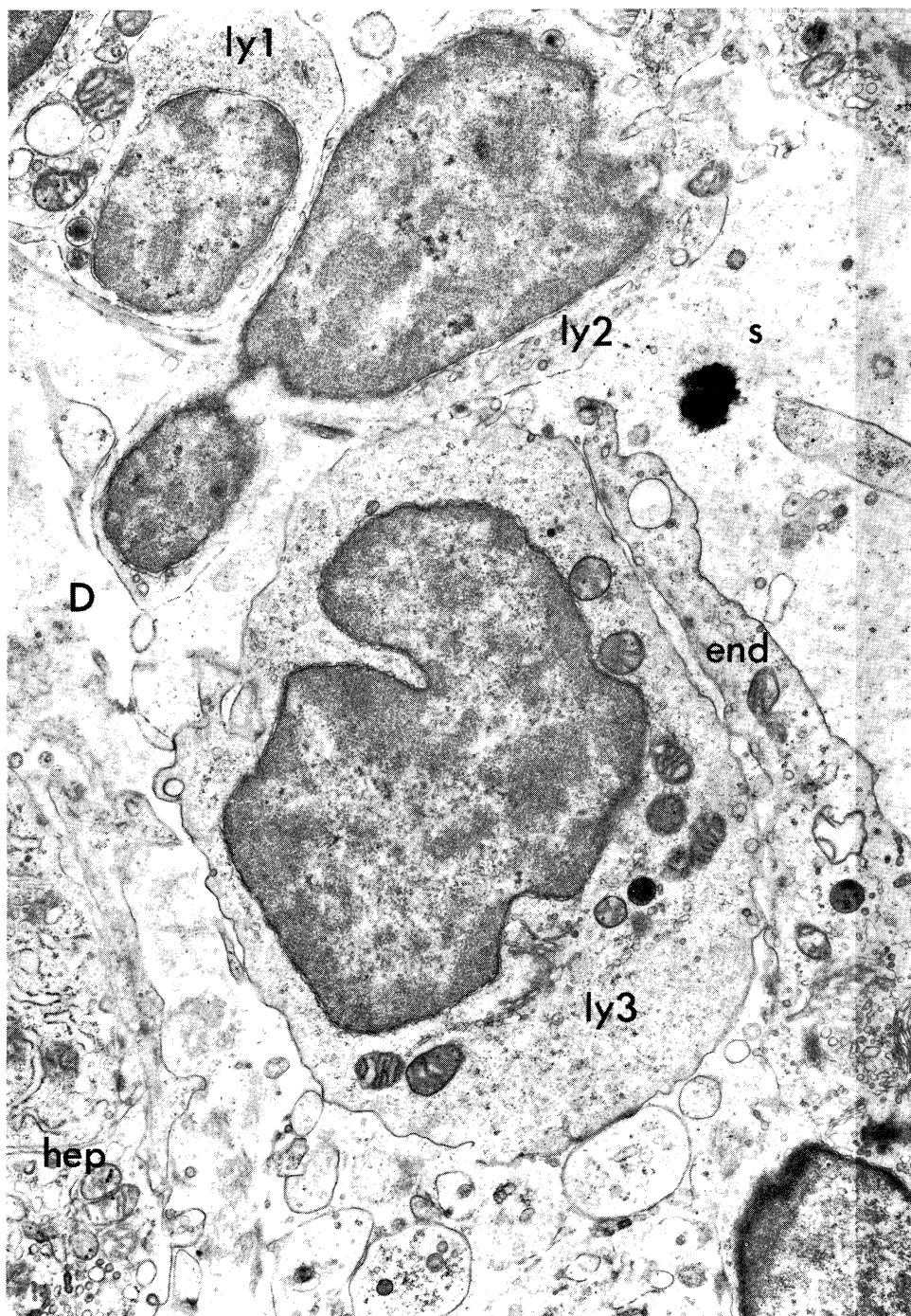


Figure 195. Canine hepatic homograft four days after transplantation. One small lymphoid cell (ly 1) lies in a sinusoid (s). A second lymphoid cell (ly 2) is in the process of migrating from the sinusoid into the space of Disse (D) while a third lymphoid cell (ly 3) is in Disse's space between a hepatocyte (hep) and the sinusoidal endothelium (end). The third cell has abundant cytoplasm that is full of ribosomes. (Lead stain, $\times 7000$.)

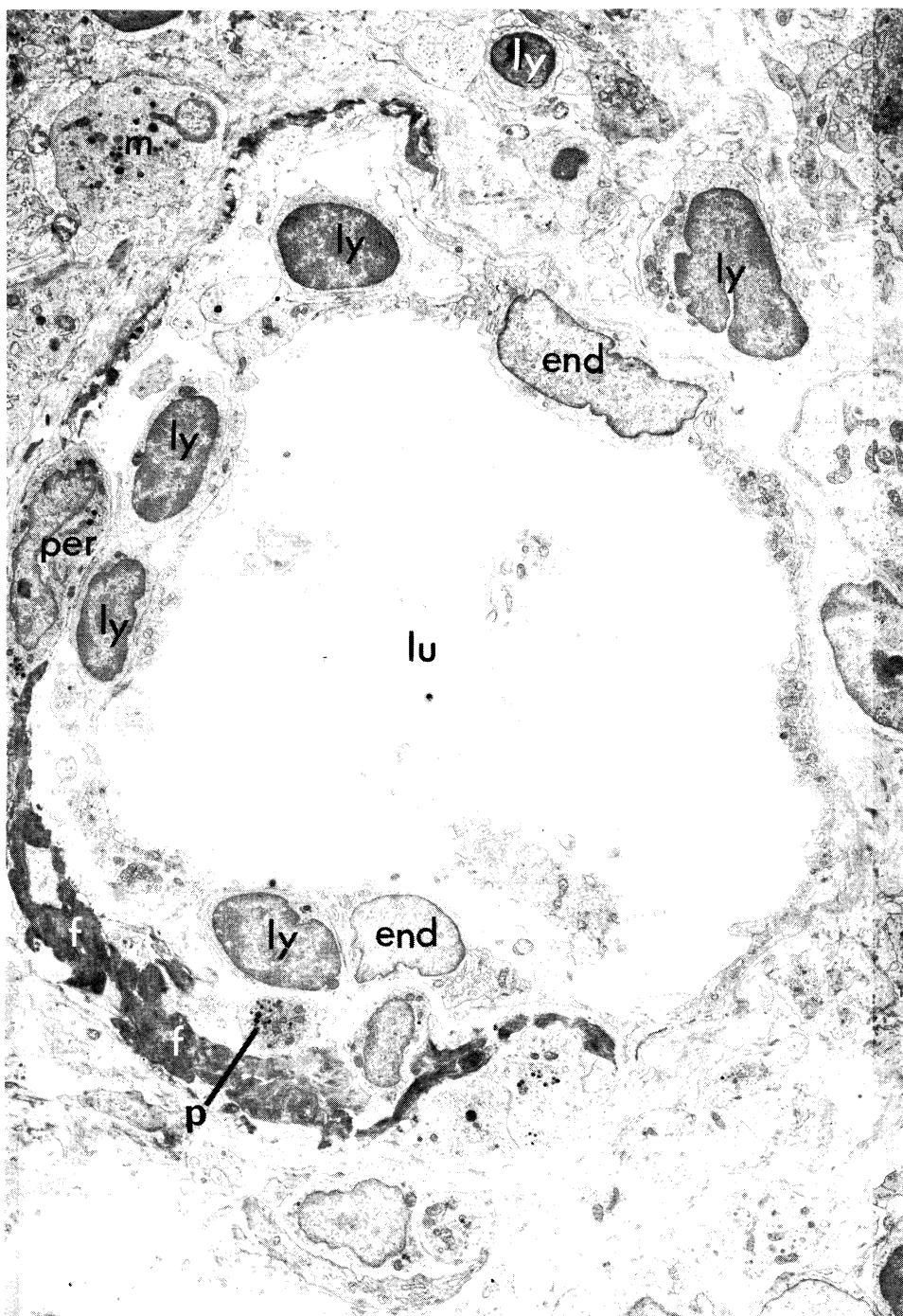


Figure 196. Biopsy made four days after transplantation of a hepatic homograft in an untreated dog. Electron micrograph showing a central hepatic vein. Lymphocytes (ly), platelets (p), and fibrin (f) lie beneath the endothelial lining (end) of the vessel. Fluid and cells have accumulated in the perivascular space. per=Pericyte; m=macrophage; lu=lumen of vein. (Lead stain, $\times 2250$.) (By permission of Surgery 63:658, 1968.)

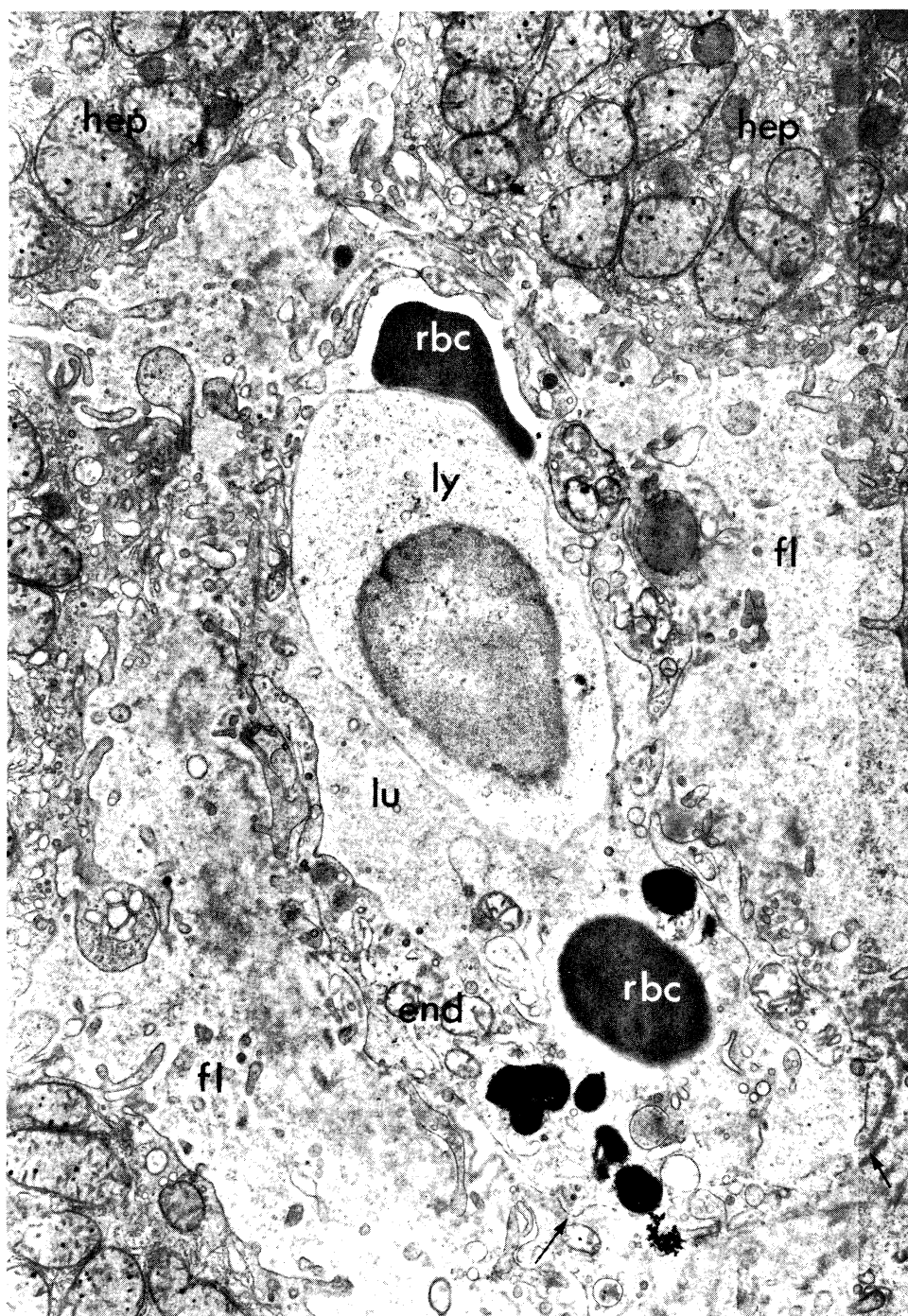


Figure 197. Biopsy of canine hepatic homograft four days after transplantation to an unmodified recipient. Electron micrograph showing a centrilobular sinusoid. The endothelial lining (end) is ruptured at the points marked with arrows. The space of Disse is wider than normal and contains fluid (fl). A lymphoid cell (ly) and two erythrocytes (rbc) are present in the lumen (lu) of the sinusoid. The adjacent hepatocytes (hep) are injured, as shown by swelling and clumping of their mitochondria and loss of rough endoplasmic reticulum. (Lead stain, $\times 6000$.) (By permission of Surgery 63:658, 1968.)

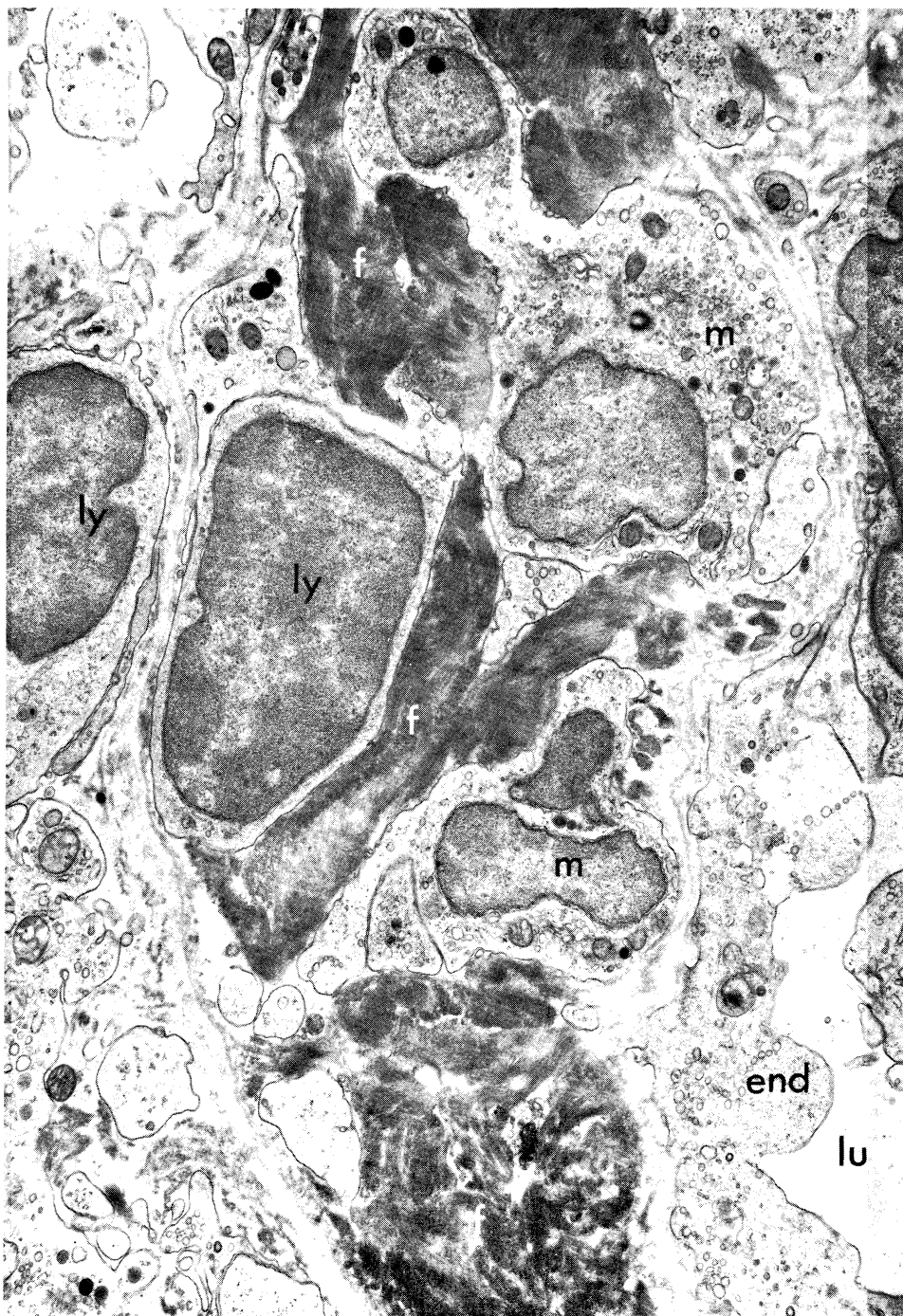


Figure 198. Biopsy of untreated canine hepatic homograft four days after transplantation. Electron micrograph showing part of wall of a vein in a small portal tract. The wall contains lymphocytes (ly), macrophages (m), and fibrin (f). lu=Lumen of vein; end=endothelial lining cells. (Lead stain, $\times 6000$.) (By permission of Surgery 63:638, 1968.)

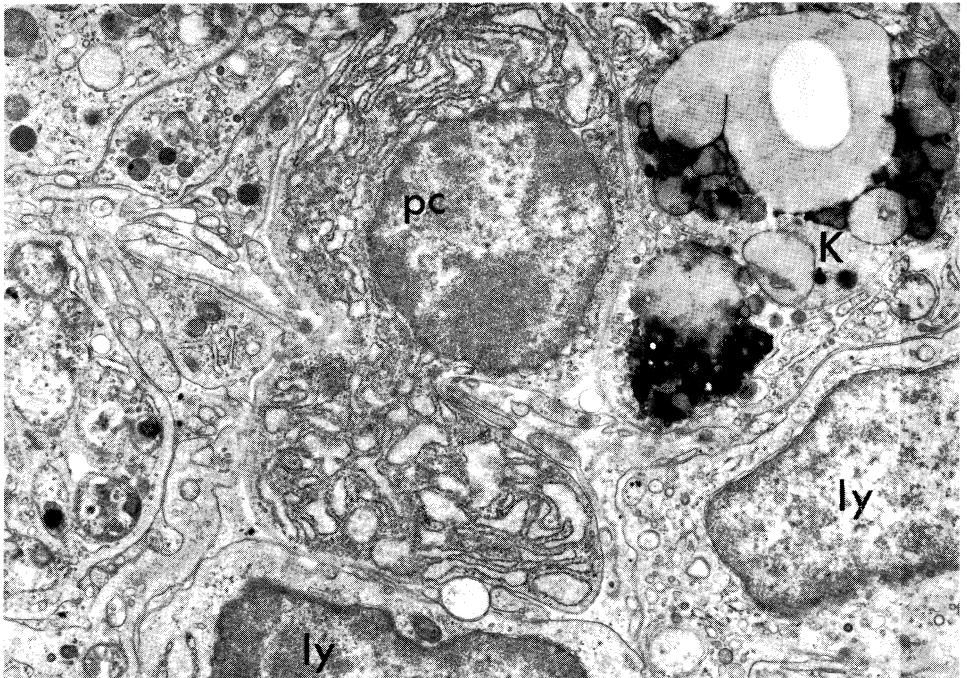


Figure 199. Untreated canine hepatic homograft 10 days after transplantation. A plasma cell (pc) with abundant rough endoplasmic reticulum is leaving a sinusoid and migrating into the space of Disse which already contains lymphocytes (ly). A Kupffer cell (K) is filled with fat droplets, lipofuscin and bile pigment. (Lead stain, $\times 6800$.)

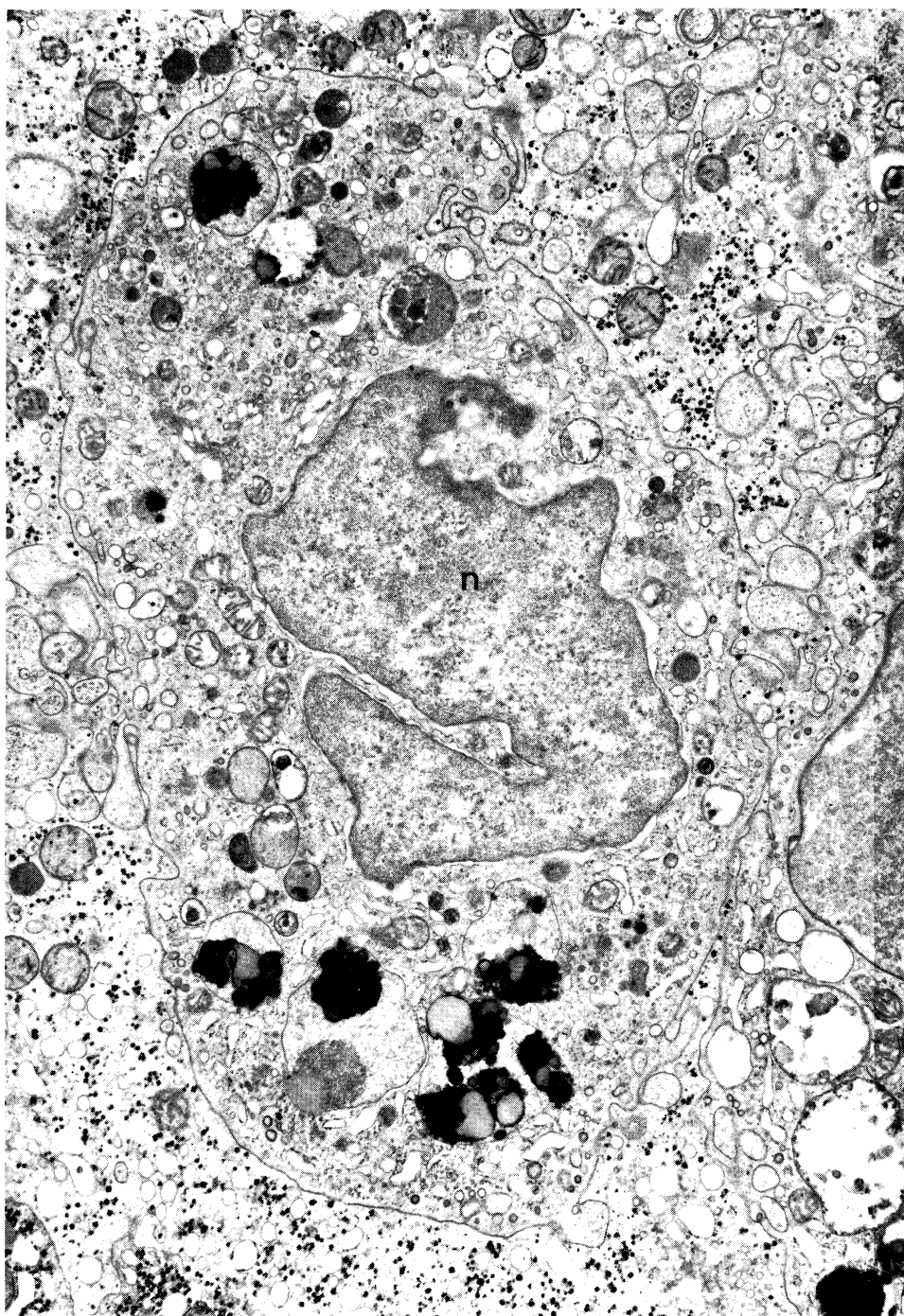


Figure 200. Untreated canine hepatic homograft 10 days after transplantation. A macrophage lies in the portal tract adjacent to the peripheral hepatocytes. n=Nucleus. (Lead stain, $\times 6000$.)

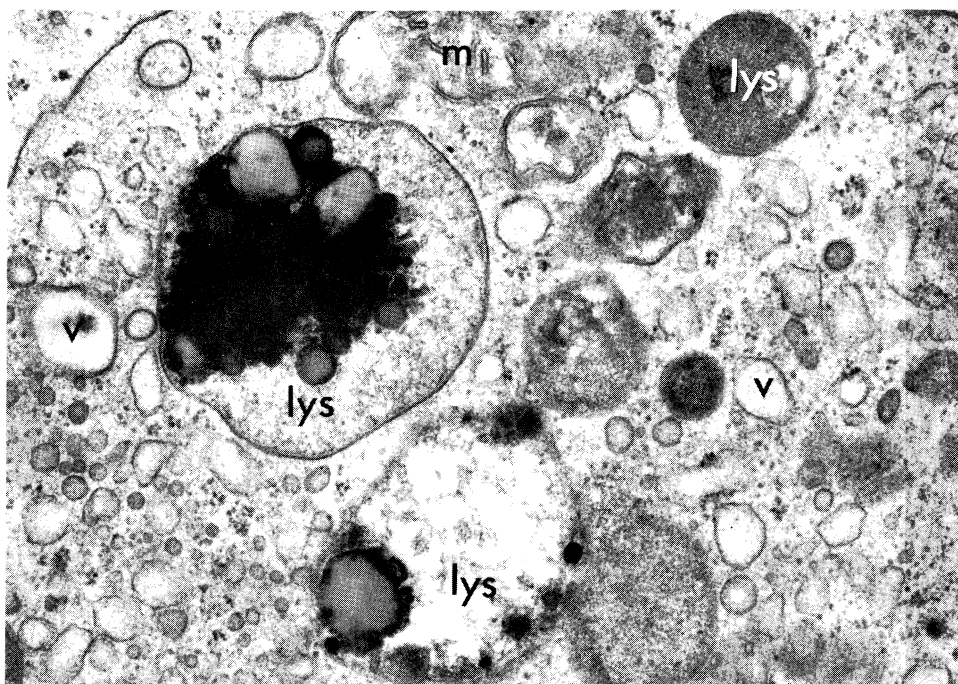


Figure 201. Higher power view of cytoplasm of upper part of macrophage shown in Figure 200. There are several lysosomes (lys), two of which contain lipid and bile pigment, and several vacuoles (v). m=Mitochondrion. (Lead stain, $\times 31,000$.)

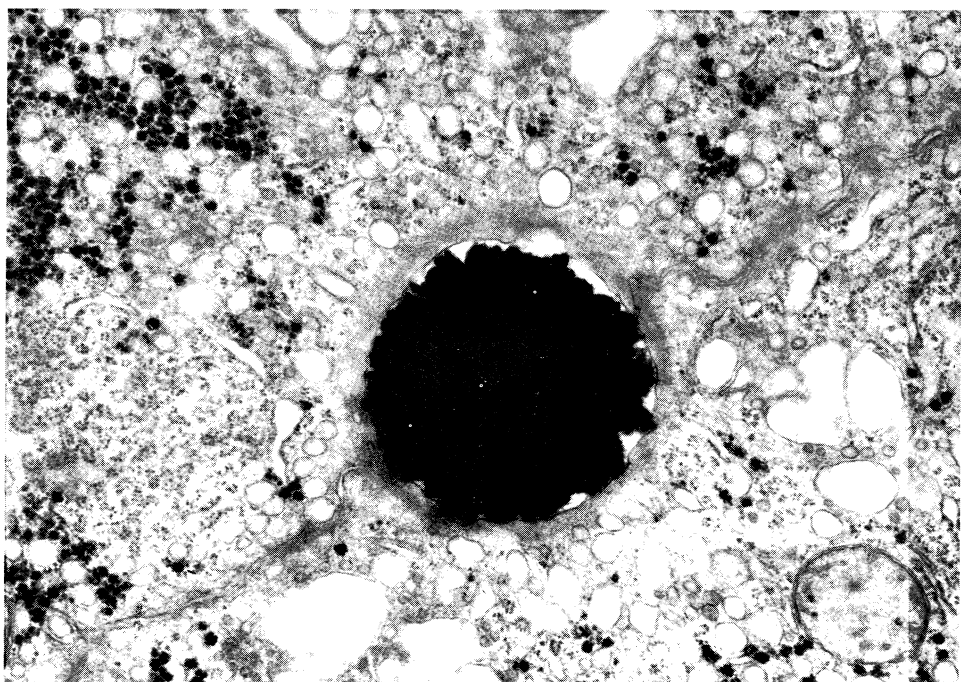


Figure 202. Untreated canine hepatic homograft seven days after transplantation. A bile canaliculus lacks microvilli and its lumen is blocked by inspissated b.le. (Lead stain, $\times 14,000$.)

and recipient are of completely different breeds. When Cordier, Garnier, and their associates⁶ studied two liver transplants that had survived for 35 and 51 days, respectively, they found only edema and a few focal necroses, but no cellular infiltration or other stigmata of rejection, even though the donor and recipient were unrelated.

A detailed description of the structural changes that occur in porcine hepatic homografts has been given by Hunt,¹⁰ who studied 16 such livers. He found that in the first few days after transplantation there was only edema of the interlobular septa, but that at about the fourth day mononuclear cells began infiltrating the portal areas and the septa. The proportion of pyroninophilic cells in the infiltrate varied from 20 to 50 per cent in different grafts and in different areas of the same liver. Ultrastructurally these cells were large lymphoid cells with abundant free ribosomes and prominent Golgi bodies. Some of these "immunocytes" were found in the space of Disse, others lay between hepatocytes, and a few even appeared to be actually within hepatocytes. Eosinophils were also present in the infiltrate. The majority of the hepatocytes lacked glycogen and had dilated rough endoplasmic reticulum with loss of membrane-bound ribosomes.

Focal necroses were present in all the livers studied by Hunt, particularly adjacent to the interlobular septa, but they were never extensive. Centrizonal necrosis was rare. Intracanalicular bile plugs were scarce before eight days, but were present in a few of the longer surviving livers. Arterial fibrinoid necrosis was not encountered. In the longest survivor a biopsy of the liver six months after transplantation revealed a commencing fibrosis with increased amounts of fibrous tissue in the interlobular septa and a few strands of collagen running into the substance of the lobules.

Similar findings have been reported by Calne and his associates⁴ in nine liver homografts examined microscopically more than four days after transplantation. They found only slight mononuclear cell infiltration in the portal tracts and interlobular septa and, in some long-term survivors, an increased amount of fibrous connective tissue in the portal tracts and interlobular septa and around the central veins. In two animals there was slight fibroelastic intimal thickening of some hepatic arteries. A variable degree of cholangitis was present in seven of the nine animals.

In the Denver series of seven porcine hepatic homotransplantations in which there was survival of six days or more (Table 14, Chapter Eleven), four animals died of rejection at eight, eight, 20, and 37 days, respectively. Microscopically, the portal tracts of all four grafts were infiltrated with mononuclear cells, 50 per cent of which possessed pyroninophilic cytoplasm. Similar cells were also present in small numbers around the central veins and adjacent to the sinusoids. A few of the lymphoid cells were in mitosis (Fig. 203). There was both focal and centrizonal necrosis of hepatocytes (Fig. 204). Intracanalicular cholestasis was not found, but there were focal bile lakes. Centrilobular reticulin collapse was not a feature of these livers.

In the 20-day and 37-day survivors there was slight reticulin increase in the portal tracts and interlobular septa. Biopsies of the homografts in the two living

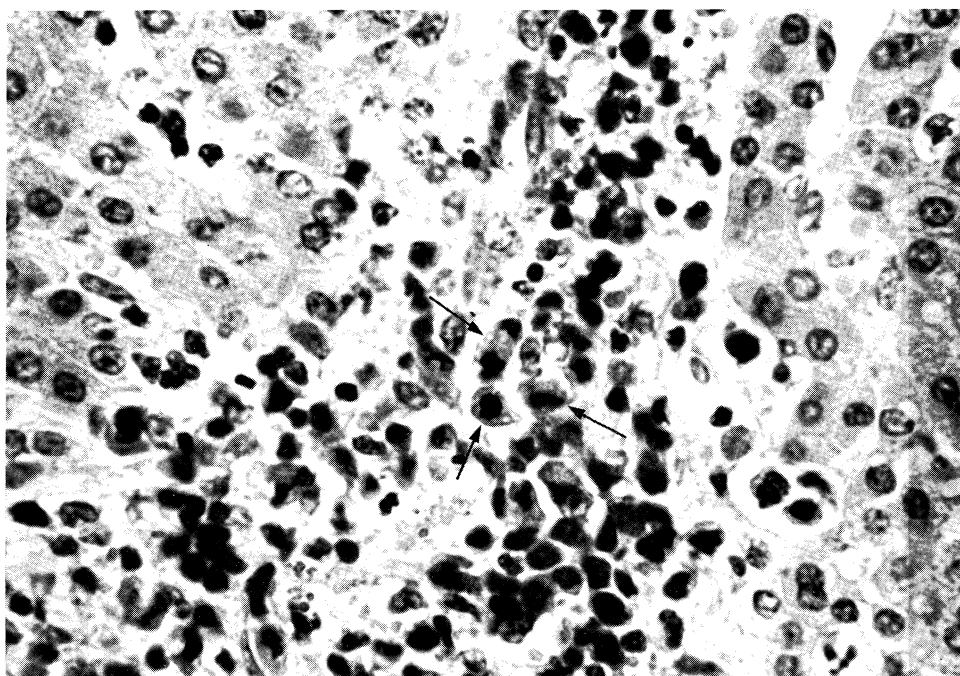


Figure 203. Untreated porcine hepatic homograft 20 days after transplantation. The animal had become jaundiced (Experiment 7, Table 14, Chapter Eleven). A portal tract is densely infiltrated with mononuclear cells that have basophilic cytoplasm: three of these cells are in mitosis (arrows). (Hematoxylin and eosin stain, $\times 600$.)

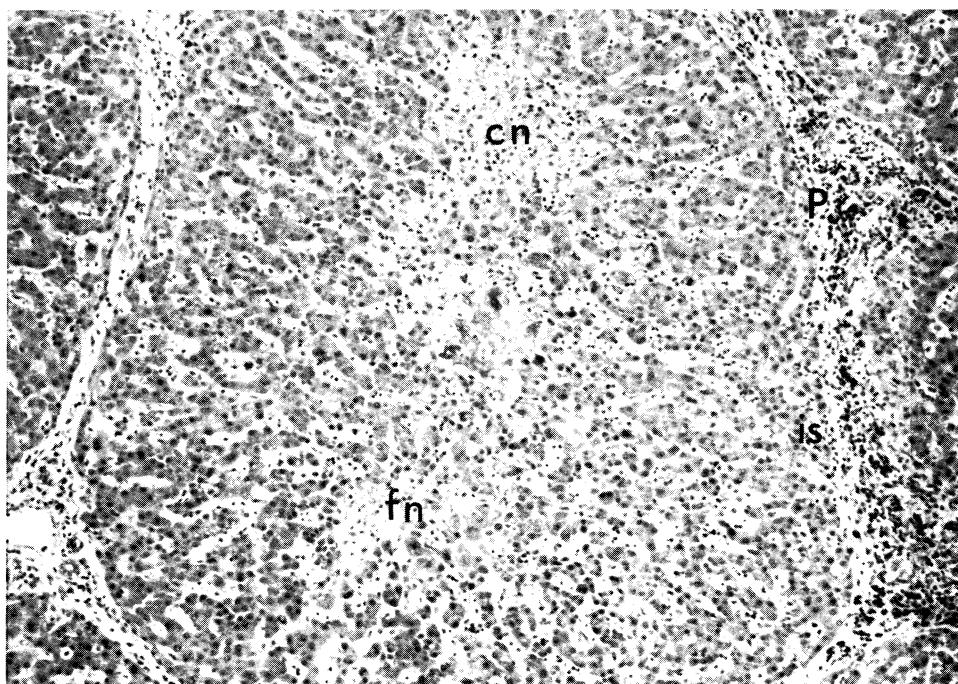


Figure 204. Untreated porcine hepatic homograft eight days after transplantation. The animal died of rejection (Experiment 8, Table 14, Chapter Eleven). A hepatic lobule contains areas of centrilobular (cn) and focal necrosis (fn) of liver cells. A moderately dense infiltrate of mononuclear cells lies in the portal tract (p) and interlobular septa (is). (Hematoxylin and eosin stain, $\times 150$.)

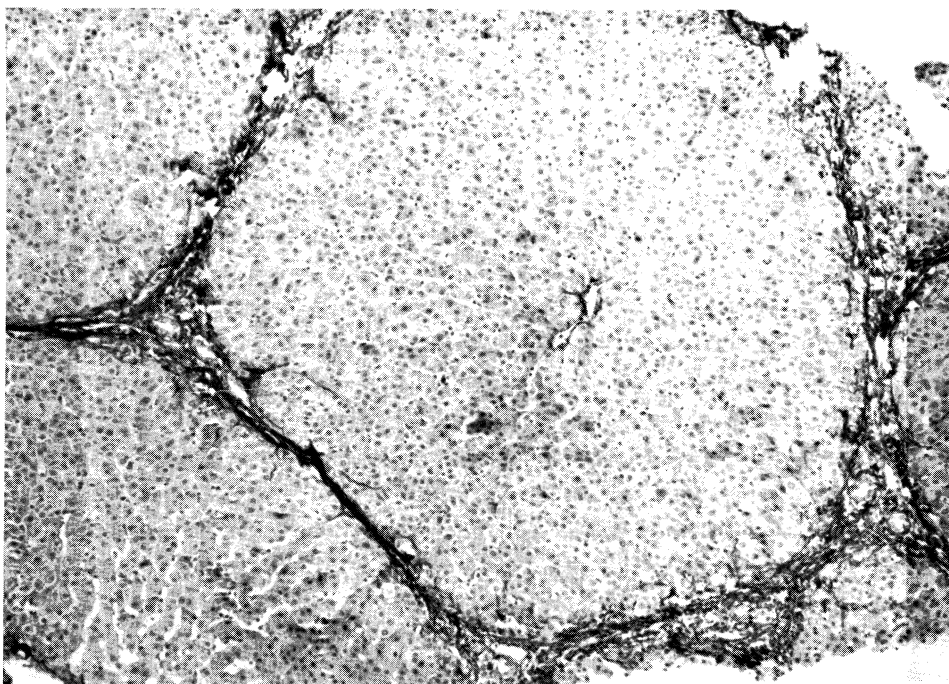


Figure 205. Biopsy of untreated porcine hepatic homograft five and a half months after transplantation (Experiment 4, Table 14, Chapter Eleven). The liver appears normal. (Van Gieson and elastic stain, $\times 150$.)

pigs five and a half months after transplantation showed normal liver tissue (Fig. 205). The latter animals are still alive 15 months postoperatively.

These findings indicate that although porcine liver homografts do undergo rejection, this is usually a much milder and more protracted process than in the untreated dog. This is particularly interesting since porcine renal or skin grafts are promptly rejected in a normal manner.^{4, 12} Moreover, in the pig the presence of a liver transplant seems to delay the rejection of kidney or skin grafts done simultaneously.⁵ The implications of these observations are discussed in Chapters Eleven and Twelve.

TREATED CANINE HOMOGRAFTS

Continuous Treatment with Azathioprine

At the beginning of the chapter the need was stressed for differentiating artifacts due to operation from the changes caused by unmodified rejection. In dogs treated with azathioprine, an additional difficulty in pathologic interpretation was introduced by the fact that this drug can damage the canine liver. The biochemical manifestations of hepatotoxicity were described in Chapter Twelve in 18 otherwise normal animals which were given therapeutic doses of azathioprine for 40 days and then sacrificed. There were histologic abnormali-

ties in 12 of the 18 livers.^{24, 26} These included the presence of centrizonal hepatocyte pallor or necrosis (seven dogs), bile thrombi in the central and midzonal canaliculi (five dogs), and intracytoplasmic fat accumulation in the hepatocytes of the midzone (two dogs). As was mentioned previously, some or all of these alterations can also be caused by rejection. However, the pharmacologic injury was relatively mild compared to that caused by immunologic repudiation, and it was never associated with mononuclear cell infiltration.

Consequently most of the structural changes to be described now in azathioprine-treated orthotopic liver recipients were ascribed to an immunologic etiology. A total of 116 dogs were studied^{24, 26} from a few days to more than four years after operation. It was found that graft rejection was almost invariably delayed. The livers examined in the first seven days after transplantation generally showed only those changes that are seen in autografts and in untreated dogs before the third day. Mononuclear cells with pyroninophilic cytoplasm were rare at this stage.

However, rejection did develop in many of the treated animals in the period seven to 15 days after transplantation, as manifested by changes that usually occur by four to seven days in the unmodified recipient. The livers became swollen and congested. The outstanding microscopic features were dense cellular infiltration (Fig. 206) and marked centrizonal and midzonal necrosis of hepatocytes (Fig. 207). Thirty to 40 per cent of the mononuclear cells possessed pyroninophilic cytoplasm and a few were in mitosis (Fig. 208). Centrilobular

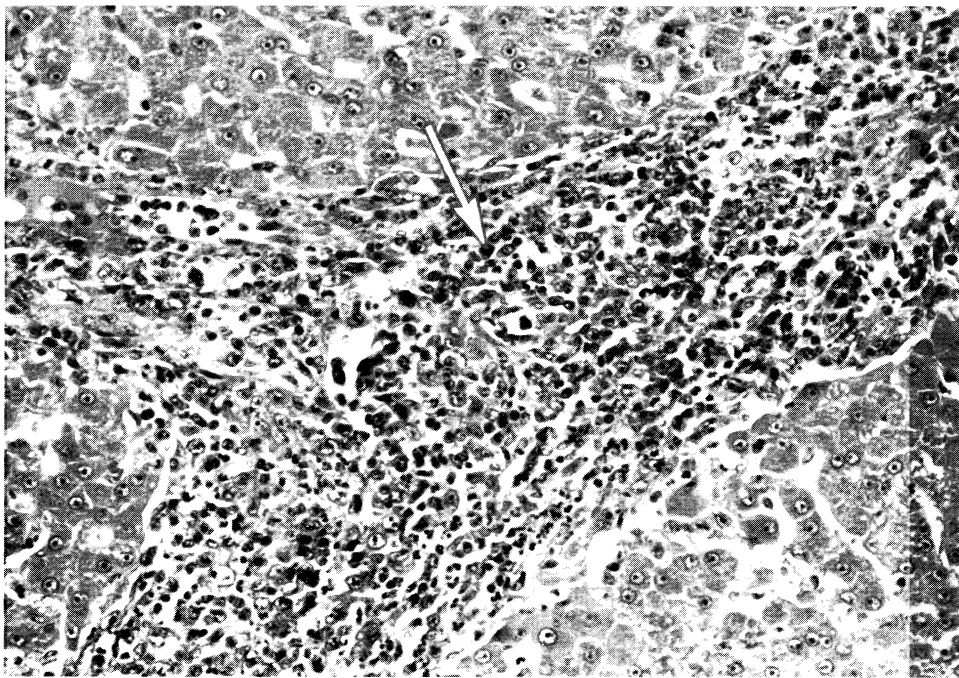


Figure 206. Treated canine hepatic homograft which was undergoing rejection seven days after transplantation. The portal tract is densely infiltrated with mononuclear cells. The cell indicated by an arrow is shown in greater detail in Figure 208. (Hematoxylin and eosin stain, $\times 250$.) (From *Advances in Surgery*, 1966, by permission of Year Book Medical Publishers, Inc.)

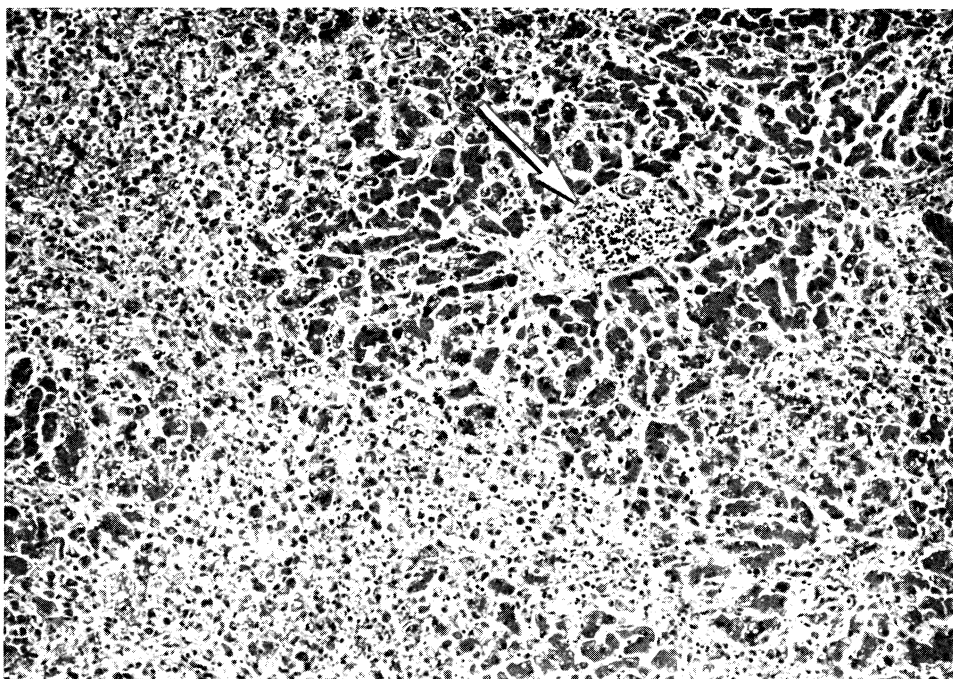


Figure 207. Canine hepatic homograft which had been rejected by 15 days despite azathioprine treatment. There is widespread destruction of hepatocytes in the central and middle zones of the lobules. Only a rim of liver cells remains around the small portal tract (arrow), which is densely infiltrated with mononuclear cells. (Hematoxylin and eosin stain, $\times 40$.) (By permission of Surgery 58:131, 1965.)

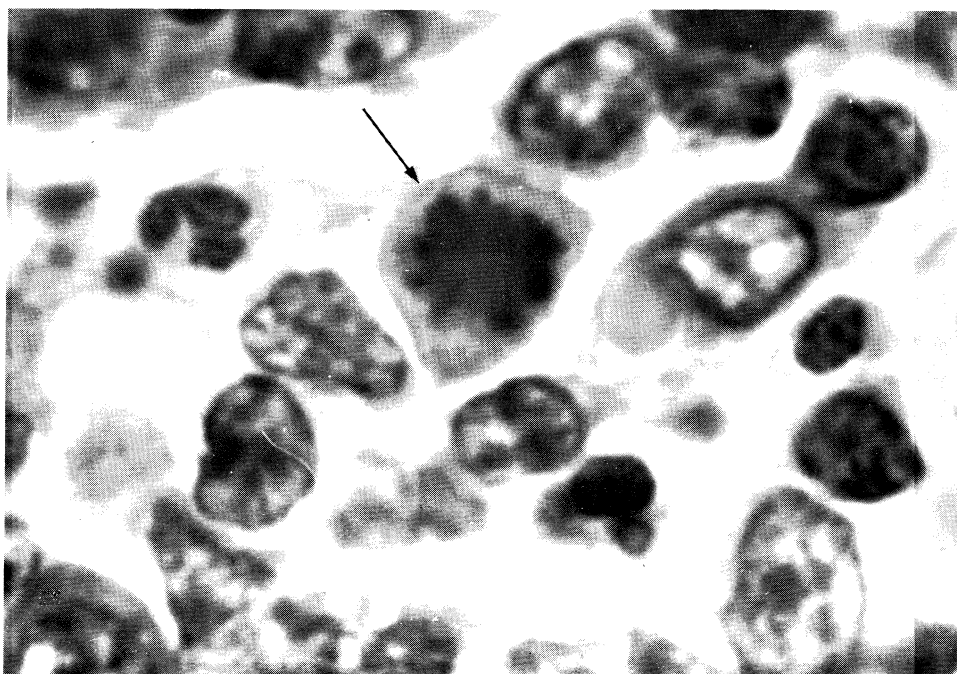


Figure 208. Details of cellular infiltrate in portal tract of hepatic homograft shown in Figure 206. Many of the cells are large lymphoid cells with several nucleoli and basophilic cytoplasm. One is in mitosis (arrow). (Hematoxylin and eosin stain, $\times 600$.)

collapse and condensation of the reticulin framework was usual (Fig. 209) and was often accompanied by bile stasis in the centrilobular bile canaliculi.

Ultrastructural examination of three such treated homografts showed more plasma cells and plasma cell precursors among the large lymphoid cells in the cellular infiltrate than in untreated grafts. The vascular and sinusoidal changes were similar to those seen in the untreated grafts. The central bile canaliculi were dilated and lacked microvilli and were often blocked by masses of bile pigment (Fig. 210). The hepatocytes in the central and middle zones of the lobules first showed mitochondrial and other changes indicating injury and then underwent necrosis.

Immunofluorescence studies demonstrated that about 15 per cent of the infiltrating mononuclear cells contained IgG. This was seen about three times as frequently as in untreated grafts. The increased incidence may have reflected the presence of greater numbers of plasma cells in the infiltrate in the treated animals. In a few grafts IgG and complement were found in the walls of the small hepatic artery radicles, in some of the sinusoids as a delicate deposit, or as a linear deposit in the portal and central veins.

From 15 days onward most of the grafts appeared to be undergoing repair following subsidence of the first wave of rejection. When animals died it was commonly due to pneumonia or other nonhepatic causes (see Chapter Twelve). The livers were either normal in size or a little shrunken and finely granular. The cut surface was often stained yellow and showed an accentuated lobular pattern. Microscopically, although some cellular infiltration was usually present around the portal and central veins, it was almost always scanty and few of

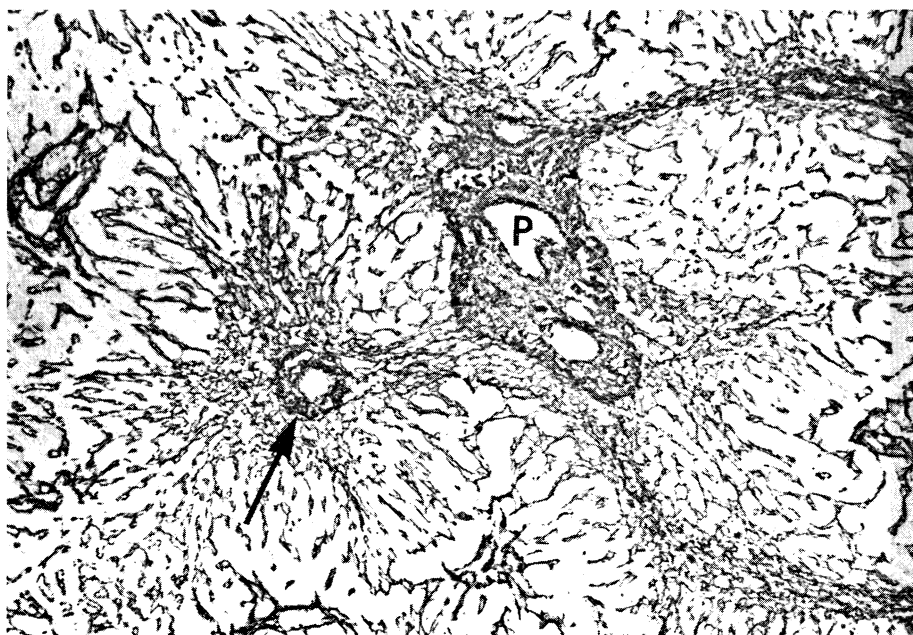


Figure 209. Treated canine hepatic homograft 15 to 20 days after transplantation. Centrilobular loss of hepatocytes has been followed by collapse and condensation of reticulin framework around central vein (arrow). P=Portal tract. (Reticulin stain, $\times 40$.) (By permission of Surger, 58:131, 1965.)

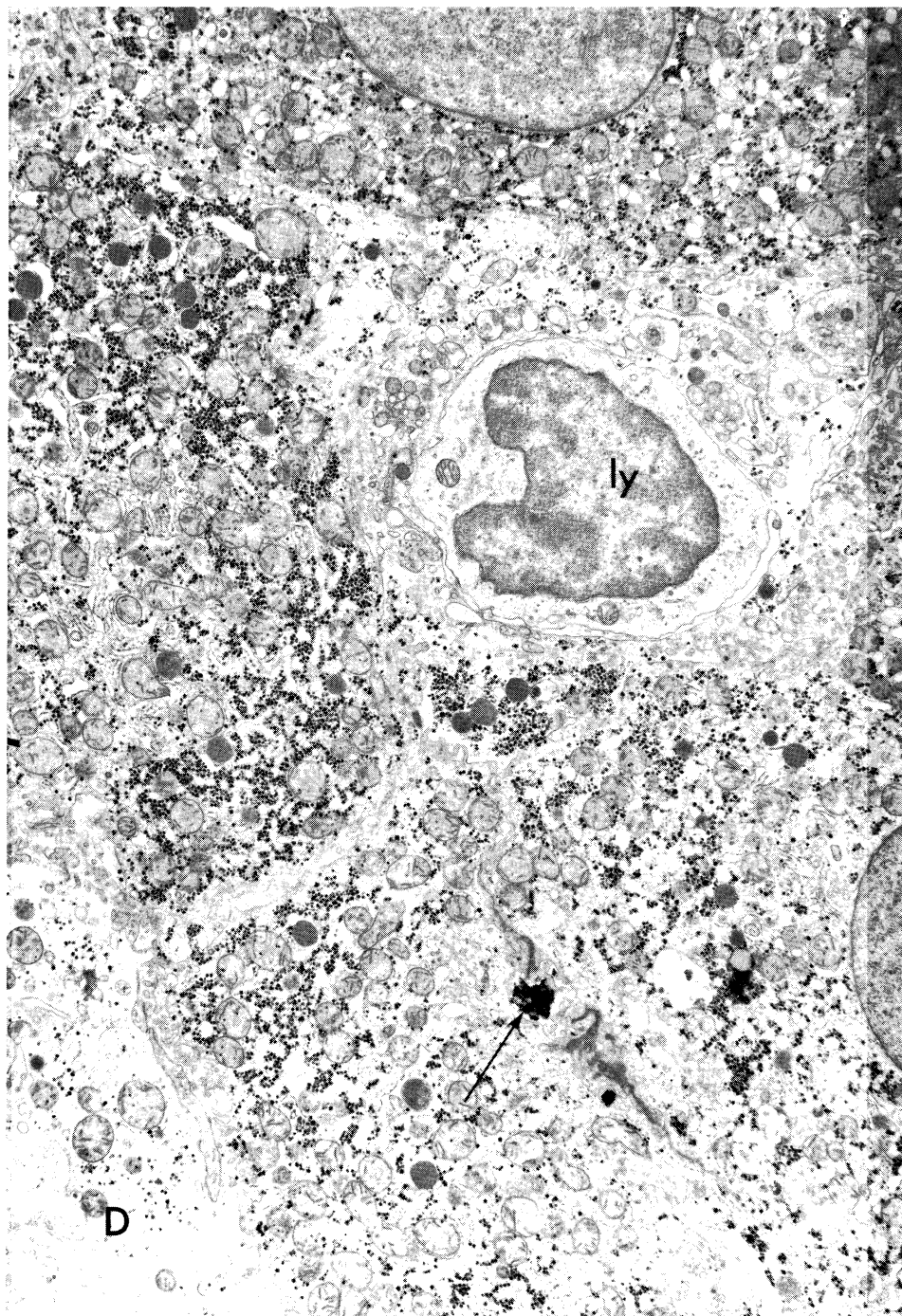


Figure 210. Treated canine hepatic homograft seven days after transplantation. Parts of four hepatocytes are shown. Between them there is an infiltrating lymphoid cell (ly). A bile canaliculus (arrow) contains inspissated bile. The space of Disse (D) is widened. (Lead stain, $\times 6500$.)

the cells were pyroninophilic. Large lymphoid cells were uncommon. The majority of cells were small lymphocytes, hemosiderin-laden macrophages, and plasma cells. The high iron content of the macrophages and of many Kupffer cells was apparently closely related to the increased red cell destruction that occurred in the first month after liver transplantation (Chapter Twelve). There was centrilobular atrophy, the hepatocytes often containing lipid droplets and excess lipofuscin. Intrahepatic cholestasis, maximal in the central zones, was present in most of these livers (Fig. 211).

The features of healing and regeneration after the first two weeks varied according to the magnitude of the initial injury. Death or atrophy of the centrilobular hepatocytes was accompanied by collapse and condensation of the central part of the lobular reticulin framework. Linking of adjacent central veins by connective tissue bands was present in about half the specimens examined after 25 days. Increased connective tissue in the portal tracts was a less consistent feature, but when present, the portal and central scars were sometimes connected, leading to hepatic fibrosis (Fig. 212). Evidence of regeneration was usually obtained between 25 and 50 days, and occasionally thereafter. Hepatocytes at the periphery of the liver lobules showed increased basophilia, occasional mitoses, and polyploidy indicative of proliferation. In a few of the livers there were many small bile ductules in the portal tracts. Regeneration nodules were present in about one third of the homografts after 50 days. These nodules were often associated with hepatic fibrosis and focal areas of hepatocyte necrosis, constituting a cirrhosis of portal type.

Similar findings have been described by other authors.^{15, 29}

Treatment with Azathioprine for Four Months, Followed by Withdrawal of Therapy

Ten dogs had azathioprine therapy discontinued about four months after liver transplantation (see Table 15, Chapter Twelve). In no case did abrupt rejection occur. However, in two instances (Schime 9 and SS 4) there was progressive cellular infiltration, centrilobular atrophy of hepatocytes, and central fibrosis. The animals died 77 and 120 days later, respectively.

Four other dogs (S³⁵ 26, S³⁵ 18, I 10, and ICBM 13) had an abnormal hepatic homograft at the time therapy was withdrawn, in that there were fibrosis and cellular infiltration. Three of the livers remained in much the same state with no appreciable histological deterioration for a further 31, 48, and 27 days. The fourth animal (S³⁵ 18) had developed cholangitis when the homograft was examined 84 days later, but after this period the biliary tract infection died down. When the animal perished after another 133 days from a perforated duodenal ulcer, the homograft showed less cellular infiltration and no more fibrosis than had been present at the time therapy was stopped.

Of the four animals that survived for more than one year after cessation of treatment, the two that were still alive in April, 1969, had normal livers at all times (Figs. 213 and 214). The homografts of the other two dogs (HHM 2 and HHM 12) showed some fibrosis and cellular infiltration at the time therapy was

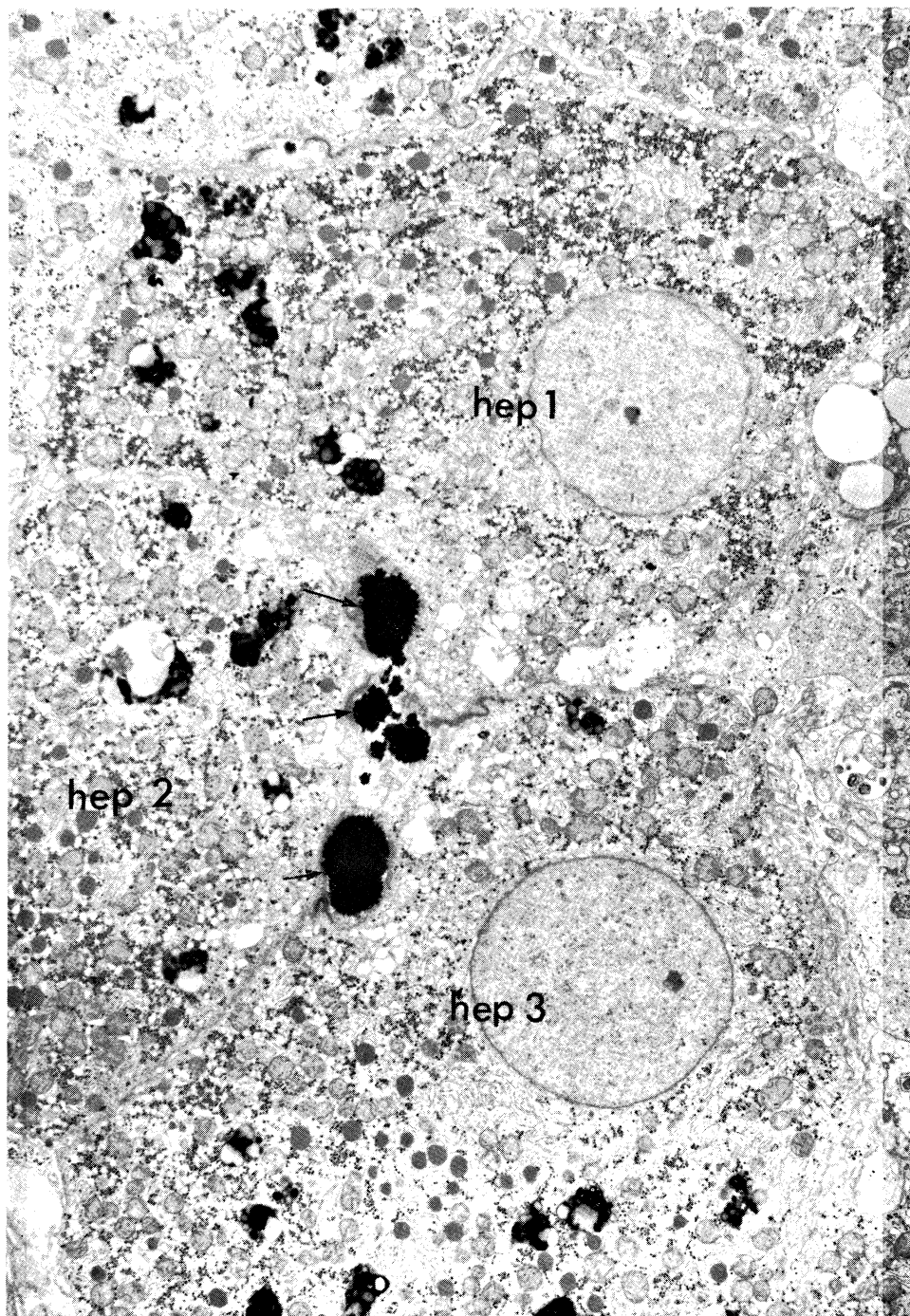


Figure 211. Treated canine hepatic homograft 21 days after transplantation. Parts of three atrophic centrilobular hepatocytes are shown (hep 1 to 3). The cytoplasm of the hepatocytes is compact and contains deposits of lipofuscin and bile pigment. The rough endoplasmic reticulum is dilated. Several bile canaliculi are distended with inspissated bile (arrows). (Lead stain, $\times 3000$.)

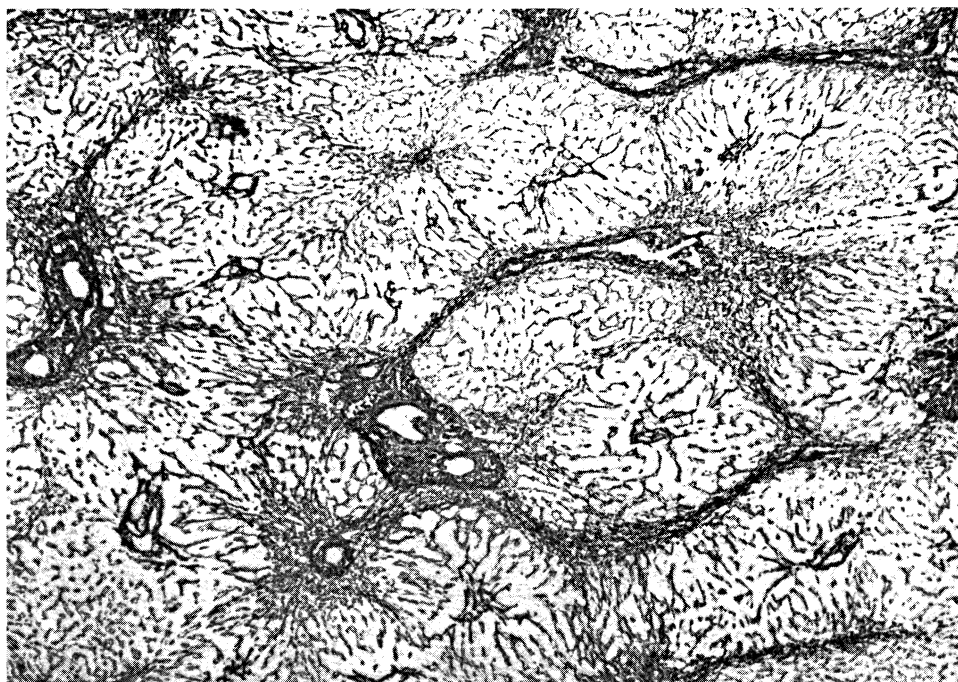


Figure 212. Hepatic homograft at 32 days from a dog treated with azathioprine. The reticulin framework of the lobules has collapsed around the central veins, and bands of reticulin now connect the central veins to each other and to portal tracts. (Reticulin stain, $\times 20$.) (By permission of Surgery 58:131, 1965.)

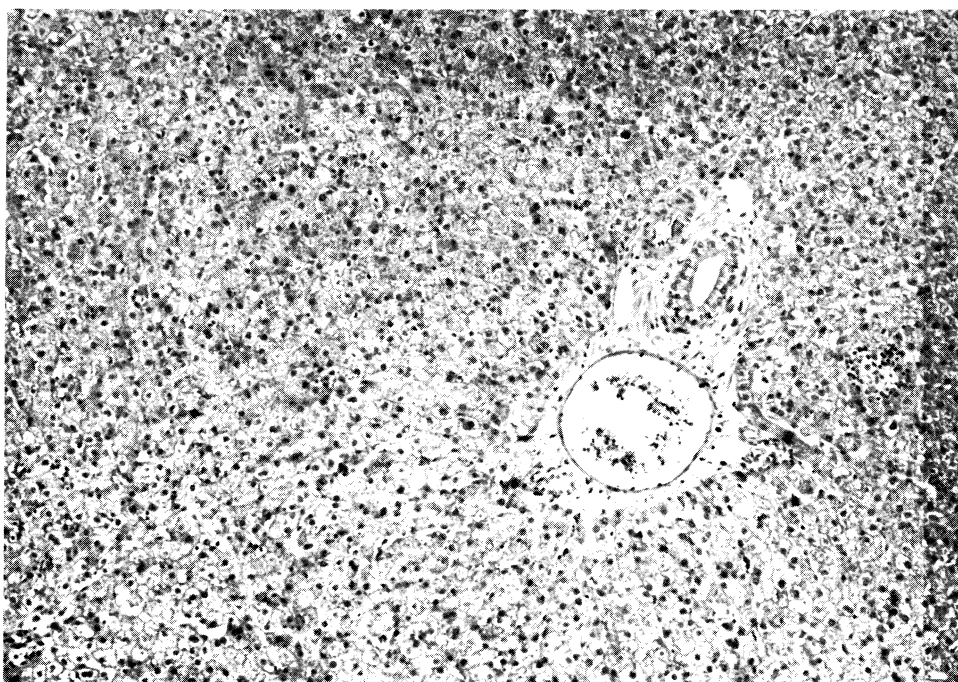


Figure 213. Biopsy from a canine hepatic homograft taken 302 days after transplantation. The dog received azathioprine for the first 120 days; the drug was then stopped. The liver appears normal. (Hematoxylin and eosin stain, $\times 40$.) (By permission of Surgery 58:131, 1965.)

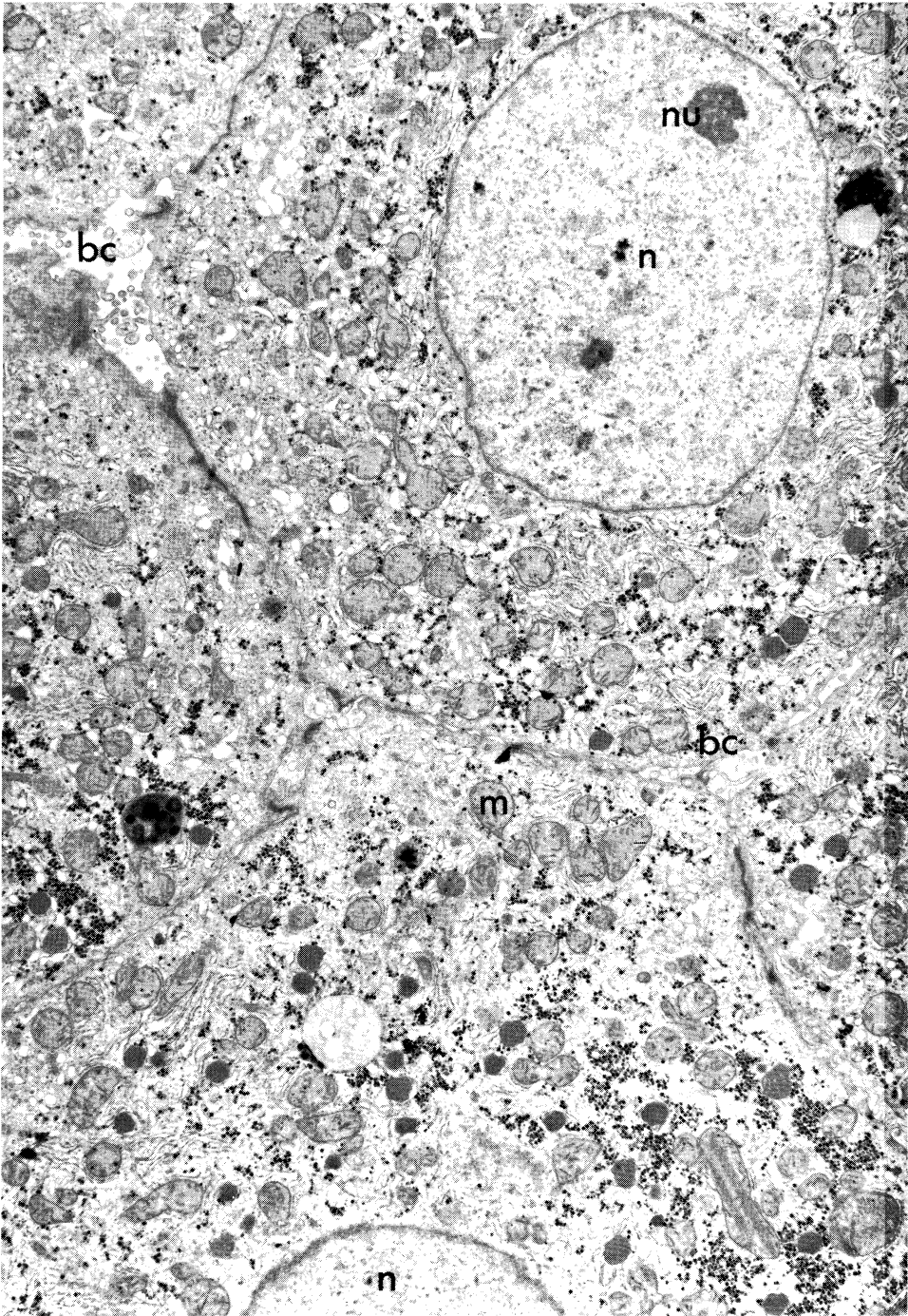


Figure 214. Electron micrograph from canine hepatic homograft shown in Figure 213. Parts of four hepatocytes are shown. The ultrastructure appears normal. n=Nucleus; nu=nucleolus; m=mitochondrion; bc=biliary canaliculus. Fine black dots are glycogen granules. (Lead stain, $\times 6400$.)

stopped. The cellular infiltration diminished over the ensuing 443 and 1041 days, respectively, but the fibrosis progressed to severe cirrhosis and eventually killed the animals.

Treatment with Heterologous Antilymphoid Globulin

The contention that the abnormalities in the foregoing azathioprine experiments were reflections of rejection rather than of pharmacologic injury was supported by subsequent trials with ALS and ALG. The changes in the homografts protected by antilymphocyte agents which were free of hepatotoxicity¹¹ were not significantly different from those in the animals treated with azathioprine.

In 18 experiments with ALS or ALG,²⁵ it was found that animals dying in the first three weeks after liver transplantation had centrizonal and, usually, midzonal necrosis of hepatocytes in the graft, infiltration of mononuclear cells around the portal tracts and central veins, and centrilobular cholestasis. The six homografts examined after three weeks showed variable degrees of centrilobular hepatocyte atrophy, central reticulin condensation, hepatic fibrosis, and proliferation of bile ductules in the portal areas. Five of the six livers contained mononuclear cells, but in two grafts the numbers were low. In all the transplants pyroninophilic cells were sparse and active rejection appeared to have ceased.

Similar findings have been reported by Mikaeloff and his colleagues.¹⁶

TREATED PORCINE HOMOGRAFTS

Of nine pigs that were treated with horse anti-pig lymphocyte globulin in Denver, seven survived for seven days or more (Table 14, Chapter Eleven). Six of these animals died of rejection at seven, seven, 13, 15, 23, and 28 days after transplantation. Pyroninophilic lymphoid cell infiltration was prominent in four of the homografts; in the other two livers lymphoid cells were less frequent, but centrizonal and focal necrosis of hepatocytes and bile lakes were common. Cholangitis was a complication in two of the pigs that died from rejection. The one long-term survivor had a histologically normal liver when a biopsy was taken five and a half months after transplantation.

The ALG used in these experiments appeared to have had no beneficial effect.

HUMAN HOMOGRAFTS

There were 27 orthotopic liver homotransplantations performed in Denver between March, 1963, and March, 1969; 25 were primary procedures and the other two were retransplantations. By April, 1969, two of the patients with first

transplants (OT 13 and 19) and the two patients with second grafts (OT 14 and 16) were still alive and no biopsy had been taken of their grafts. Tissue obtained by needle biopsy, open operation, and autopsy was available from the other 23 livers. The pathologic findings in a number of these cases have been reported before.^{19, 20, 23, 24, 27, 28}

Homografts Examined During the First 11 Days After Transplantation

Ten of the 27 Denver hepatic homografts became available at autopsy during the first 11 postoperative days and were examined histologically.

Massive Hepatic Injury Before Transplantation. The first hepatic homograft in the Denver series (OT 1) did not function and the recipient died within a few hours. This case is described in detail in Chapter Six. The liver transplant was ischemic at body temperature for 60 minutes and the total time from donor death to revascularization of the graft was 420 minutes. At autopsy the liver was swollen and pale. Microscopically there was massive necrosis of the hepatocytes, particularly in the central and middle zones of the lobules.

Severe Hepatic Injury Before Transplantation. The homograft of another patient (OT 6) functioned poorly and the patient died after seven days. The donor was a 73 year old man who had no detectable blood pressure for nine hours before death. After transplantation of the liver the recipient suffered repeated intra-peritoneal and gastrointestinal hemorrhages and he had to be surgically explored twice (see Chapter Six).

At autopsy the liver was swollen and pale; the capsule was coated with fibrin. Microscopically there was necrosis of all the hepatocytes in the central and middle zones of the liver lobules and of many of the hepatocytes in the peripheral zones. Only a thin rim of liver cells survived around the portal tracts. There was collapse and condensation of the central parts of the lobular reticulin. A few bile "thrombi" were present among the surviving hepatocytes. The portal tracts were lightly infiltrated with lymphoid cells, a few neutrophilic polymorphonuclear leukocytes and several eosinophils. None of the mononuclear cells had pyroninophilic cytoplasm. The portal and central veins, hepatic artery, and bile ducts were normal.

The long period of inadequate perfusion in the donor was responsible for much of the hepatic injury in this graft; further injury undoubtedly occurred during the stormy postoperative course. There was no evidence of rejection of the liver.

Hepatic Artery Occlusion. In three liver homografts the hepatic artery was occluded. This was due to compression in two instances and thrombosis in the third.

In patient OT 20 the hepatic arterial supply was compressed by the caudate lobe of a very large homograft (see Chapters Eight and Nine). Death followed 15 hours later as the result of acute hepatic insufficiency, which included an uncontrollable bleeding diathesis. At autopsy the liver was swollen and pale. Microscopically many of the hepatocytes were necrotic and there were

moderately dense collections of small lymphocytes around the portal vein tributaries in several of the smaller portal tracts.

Another child (OT 24) had complex congenital malformations of the upper abdominal viscera (Fig. 55, Chapter Eight) and a kinked hepatic artery (Fig. 56, Chapter Eight). She died of hepatic insufficiency after 11 days. At autopsy the swollen graft showed widespread severe necrosis of hepatocytes in the central and middle zones of the lobules. There were a few nonpyroninophilic mononuclear cells in the portal tracts.

Hepatic artery thrombosis occurred in a one year old patient (OT 18; see Chapter Nine). Within an hour of completion of the operation the hepatic artery clotted at the site of the anastomosis and the transplant became cyanotic. Re-anastomosis was performed, but the vessel again clotted during the early post-operative period. The child died on the fourth day after transplantation. At the autopsy the liver was completely necrotic except for a single layer of surviving liver cells just beneath the capsule.

In none of these three patients was there evidence that rejection played any part in the failure of the graft.

Portal Vein Thrombosis. One child (OT 21) died 12 hours after operation from this complication. Anomalies of both the recipient portal vein and hepatic arteries probably played an important role in producing this complication (Fig. 57B, Chapter Eight; Chapter Nine). At autopsy there was necrosis of the hepatocytes in the central and middle zones of the lobules. The surviving hepatocytes contained fat droplets; there was no cellular infiltration of the liver.

Bile Duct Obstruction. One patient (OT 22) died as the result of iatrogenic biliary obstruction. The cystic and common ducts of the homograft were fused, and at operation the common duct ligature occluded both lumina and prevented the passage of bile into the gallbladder (Fig. 62, Chapter Nine). The patient became jaundiced and died when exploration was attempted 10 days later.

At autopsy the liver was swollen and green. Microscopically the bile ducts in the portal tracts were dilated and filled with inspissated bile, and there were intracanalicular bile "thrombi." There was necrosis of the centrilobular hepatocytes, and a few infiltrating polymorphonuclear leukocytes and nonpyroninophilic lymphocytes were present in the portal tracts. There was no evidence that rejection was occurring in this graft.

Pulmonary Emboli Plus Poor Graft Function. Multiple pulmonary emboli seven and a half days (OT 3) and six and a half days (OT 4) after liver transplantation were contributory to the deaths of two patients. The peripheral clots may have been caused by the use of external bypasses and the administration of clot-producing agents in these patients (Chapters Nine and Ten). The transplanted livers did not provide good function (Chapter Six).

At autopsy both grafts showed some atrophy of the hepatocytes and congestion of the sinusoids in the central zones of the lobules. A moderate number of infiltrating cells were present in the connective tissue of the portal tracts. Most of these cells were small lymphocytes. Several small hepatic artery branches showed focal areas of fibrinoid necrosis affecting the intima and media; a few others showed fibroelastic thickening of the intima.²⁷ In some of the smaller portal tracts there was proliferation of bile ductules.²⁴

The arterial changes in these cases were thought to have been present before transplantation. The donor for OT 3 was aged 69 years and had died from a cerebrovascular accident; the donor for OT 4 was aged 73 years and had died from a coronary thrombosis. Both donors had suffered from hypertension. The cellular infiltrate was probably nonspecific. It lacked the large pyroninophilic mononuclear cells characteristic of rejection.

Acute Rejection. One 11 month old patient (OT 7) apparently died from acute rejection. His liver transplant came from a one year old microencephalic girl who died 48 hours after aspirating a feed. The liver was not a good one because the donor was hypotensive for the last 14 hours of her life. The normothermic ischemia time was 14 minutes and the total ischemia interval 371 minutes (Table 6, Chapter Six). Immediately after operation the serum bilirubin fell from 10.9 mg to 4.7 mg/100 ml; shortly afterward it began to rise again (Fig. 28, Chapter Six). The SGOT and SGPT rose to very high levels, ascites appeared at the end of the first week, and the patient became comatose and died 10 days after transplantation.

At autopsy the liver was swollen and coated with fibrin. Near the surface there were focal areas of necrosis. Microscopically the portal tracts were densely infiltrated with large numbers of lymphoid cells and a few eosinophils (Fig. 215). About 30 per cent of the lymphoid cells possessed pyroninophilic cytoplasm. The infiltrating cells were particularly numerous around the portal vein tributaries and were present in the walls of these vessels. The hepatic

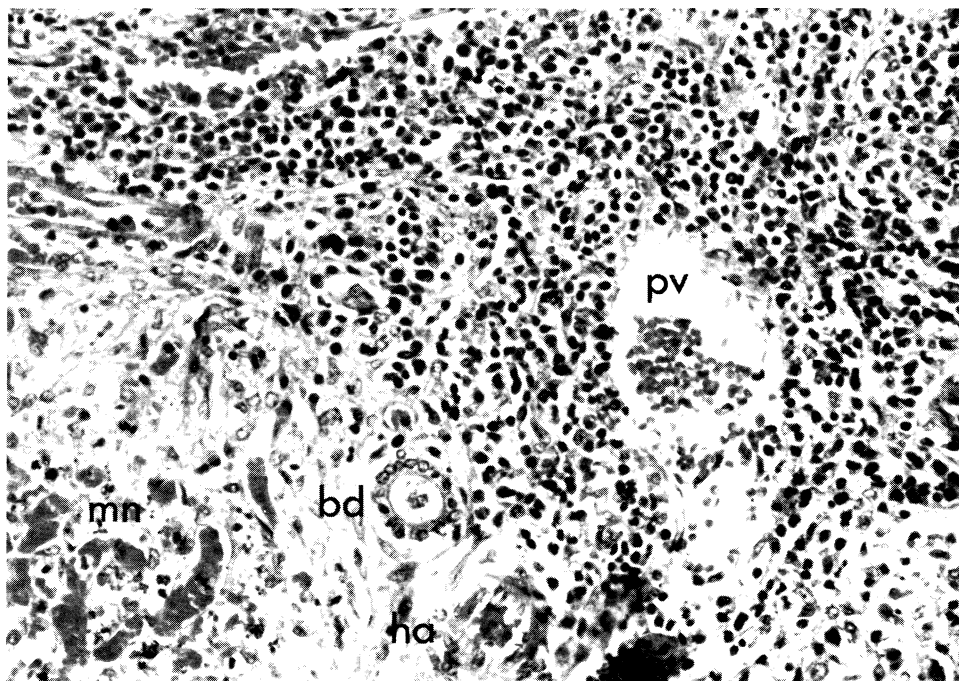


Figure 215. Human hepatic homograft (OT 7) 10 days after transplantation. The liver shows acute rejection. The portal tracts are densely infiltrated with mononuclear cells with basophilic cytoplasm. There is hemorrhagic necrosis of the midzonal hepatocytes (mn). pv=Portal vein; bd=bile duct; ha=hepatic artery. (Hematoxylin and eosin stain, $\times 400$.)

artery branches and bile ducts were normal; the lymphatics were dilated. There was complete hemorrhagic necrosis of the hepatocytes in the middle and central zones of the lobules, leaving only a thin rim of surviving, fat-containing liver cells around the portal tracts. The centrizonal and midzonal sinusoids were congested. There was a moderately dense infiltration of lymphoid cells around the central veins and within the walls of these vessels. The central parts of the lobular reticulin framework were collapsed, but there was no cholestasis and the Kupffer cells lacked hemosiderin.

Although the severity and extent of the hepatic necrosis were undoubtedly partly due to the ischemia the homograft was subjected to before transplantation, it is impossible to dismiss the massive infiltration with large pyroninophilic lymphoid cells as simply reaction to ischemic injury. Acute rejection undoubtedly contributed greatly to the early failure of this hepatic homograft. This is interesting because the donor differed from the recipient at two major histocompatibility antigens, HLA2 and HLA5, and was classified as a grade D match by Terasaki (Table 5, Chapter Three).

Cholangitis. There were no examples of this complication in the 10 specimens.

Homografts Examined Three Weeks to 400 Days After Transplantation

Tissue from 13 of the 27 Denver hepatic homografts became available for examination from three weeks to more than a year after transplantation.

Acute Rejection. On the basis of the findings in untreated and treated canine liver homografts described earlier, the assumption was made that dense infiltration of the portal tracts with large pyroninophilic lymphoid cells indicated an active rejection process. Open biopsies from three of the Denver hepatic homografts (OT 9, 10, and 8) taken eight, 27, and 30 days after transplantation all showed this change.¹⁹ In each patient there was good early liver function. However, the grafts became swollen, a fever developed, and ultimately large areas of the transplanted organs became necrotic (Chapter Fifteen). When surgical exploration was carried out for the purpose of debridement and drainage of the gangrenous areas, viable pieces of tissue were also removed for histologic examination.

The portal and central vein branches in the living portions of the homografts were surrounded and infiltrated by large numbers of mononuclear cells. Ten to 40 per cent of these cells had pyroninophilic cytoplasm and were large lymphoid cells with many free ribosomes in their cytoplasm. Some of the cells lay beneath the stripped up venous endothelium. A few were in the widened space of Disse. The sinusoidal endothelium was disrupted in many places (Figs. 216, 217, and 218). Several of the infiltrating cells contained IgG in their cytoplasm (Fig. 219). The centrilobular hepatocytes showed loss of microvilli, mitochondrial swelling, dilatation or disappearance of the rough endoplasmic reticulum, and loss of glycogen and membrane-attached ribosomes. Some centrilobular intracanalicular bile stasis was present in all three livers.

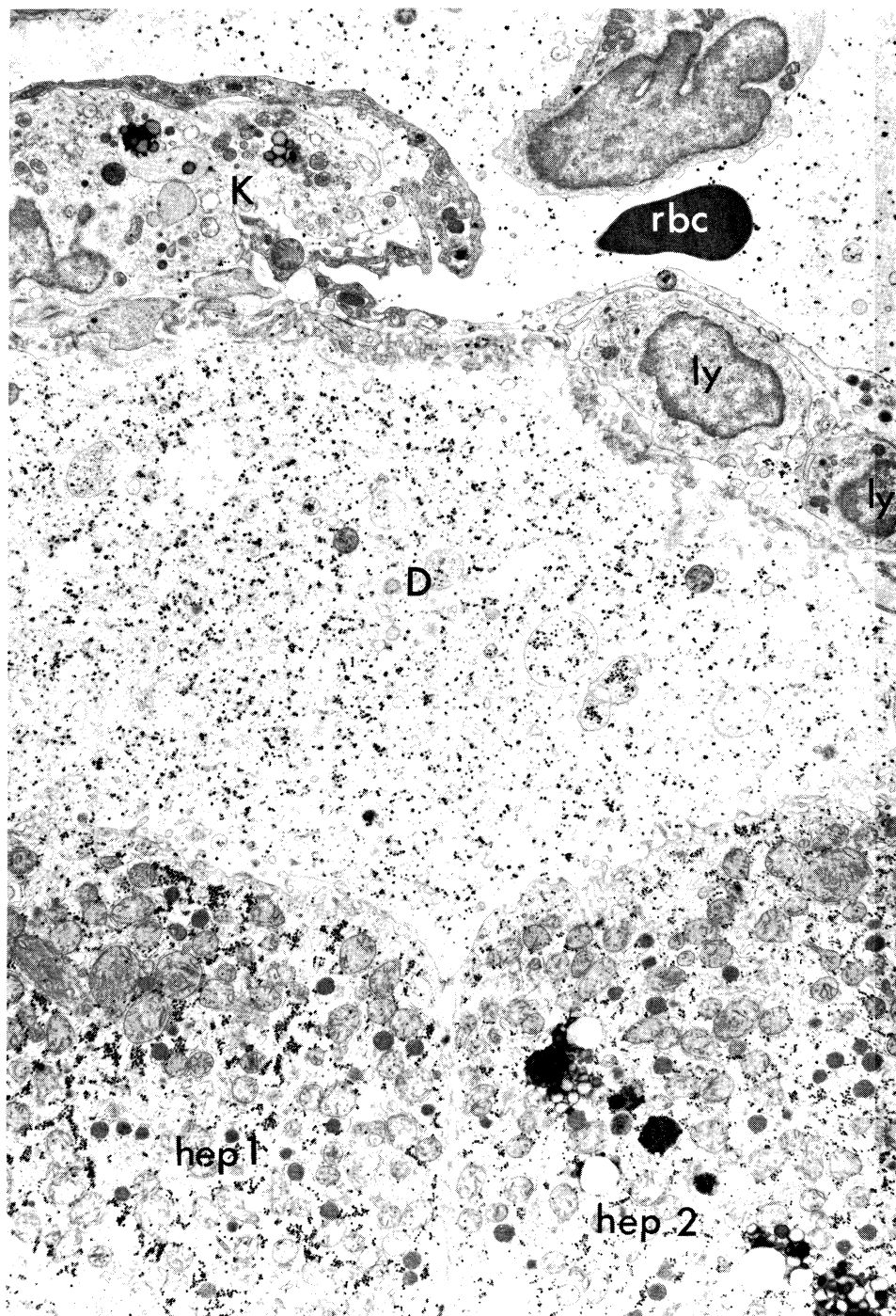


Figure 216. Human hepatic homograft (OT 10) 27 days after transplantation. The space of Disse (D) is widened by fluid and contains glycogen and cell fragments. Two lymphoid cells (ly) lie beneath the sinusoidal endothelium. A Kupffer cell (K) contains lipofuscin and bile. Rbc=erythrocyte in sinusoid; hep 1 and 2=hepatocytes. (Lead stain, $\times 6500$.)

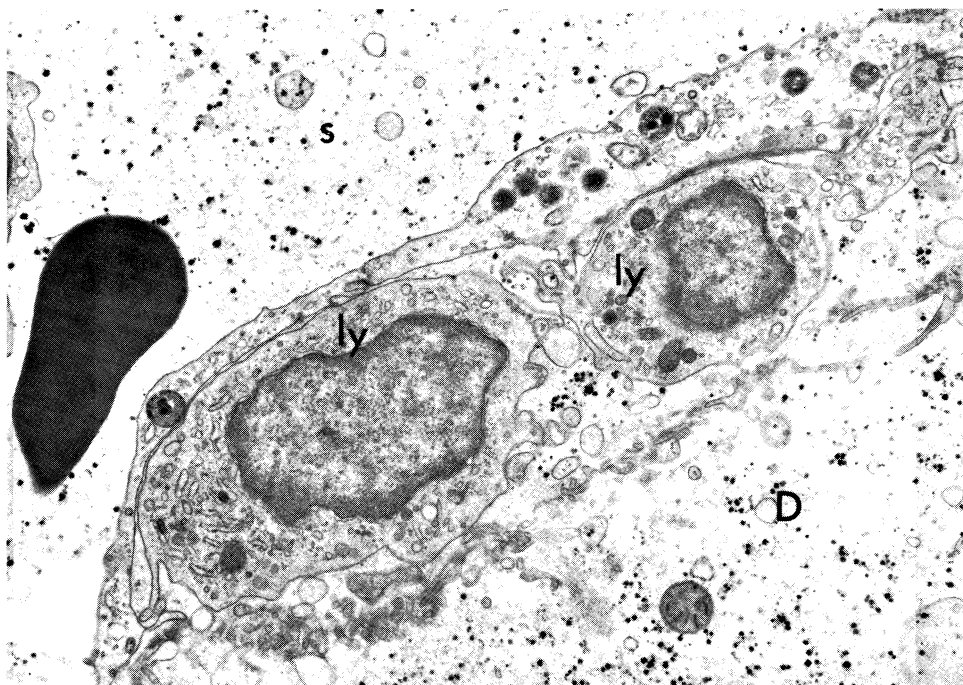


Figure 217. View of lymphoid cells lying beneath stripped-up sinusoidal endothelium in human hepatic homograft (OT 10) shown in Figure 216. ly=Lymphoid cell; s=sinusoid; D=space of Disse. (Lead stain, $\times 5600$.)

The similarity between these changes in treated human hepatic homografts and those encountered in untreated canine liver homografts was striking.

Repair Following Acute Rejection. Dogs that start to recover from rejection, either spontaneously or as a result of treatment with immunosuppressive drugs, characteristically lose the lymphoid cell infiltration and develop prominent centrilobular bile stasis, atrophy of the centrilobular hepatocytes, collapse and condensation of the centrilobular reticulin, and increased amounts of reticulin and collagen in the portal tracts.^{24, 26}

These changes were present in eight of the human hepatic homografts (OT 2, 5, 8, 11, 12, 15, 23, and 25) examined at 22, 23, 100 (and 400), 6, 105, 339, 143, and 39 days, respectively, after transplantation. Each of these patients had developed some evidence of rejection during the first two postoperative months according to the diagnostic criteria outlined in Chapter Fourteen. All the patients had some degree of hyperbilirubinemia at the time the specimens were obtained, even though the jaundice was not clinically detectable in patient OT 8 when a biopsy of the liver was taken at 100 days.

Microscopically plugging of the centrilobular and sometimes the midzonal bile canaliculi with inspissated bile was the most striking feature of these liver homografts. Ultrastructurally the microvilli of the canaliculi were reduced in number or absent and the bile plugs were in the form of aggregates of dense, needle-shaped bodies. Dilatation of the bile canaliculi was frequent. Material

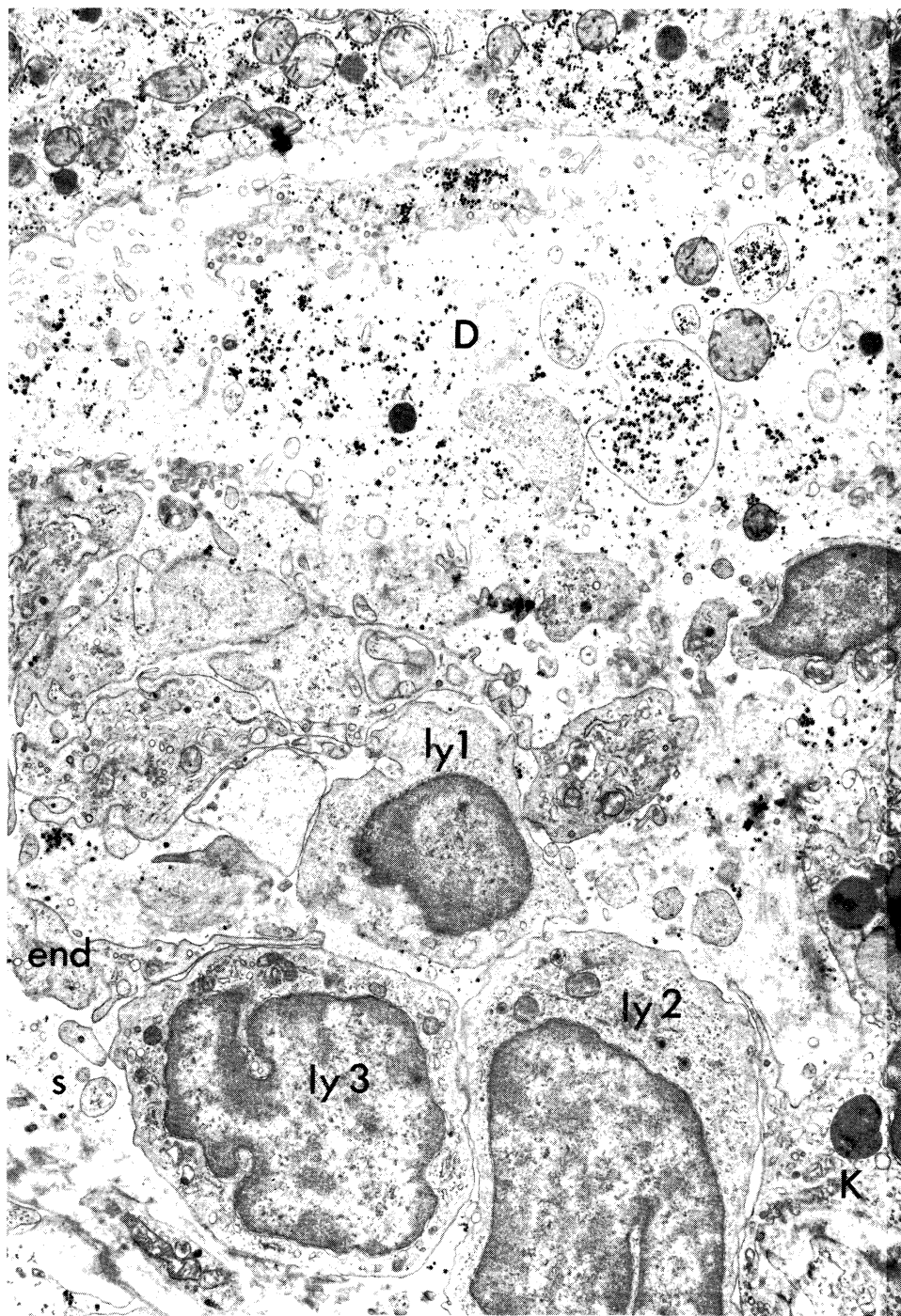


Figure 218. Human hepatic homograft (OT 8) 30 days after transplantation. Three lymphoid cells (ly 1 to 3) are in the process of leaving the sinusoid (s) to enter the enlarged space of Disse (D). end=Sinusoidal endothelium; K=Kupffer cell. (Lead stain, $\times 7500$.)

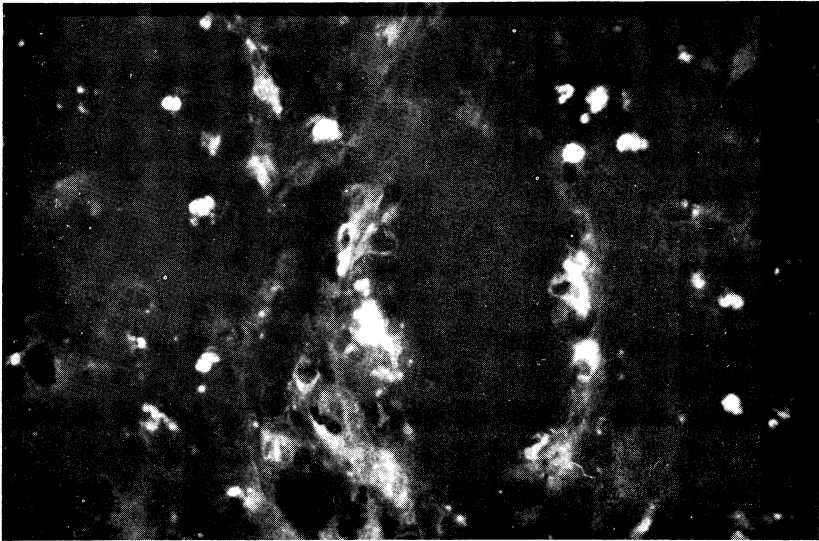


Figure 219. Human hepatic homograft 30 days after transplantation (OT 8). A frozen section has been treated with goat-antihuman IgG and then viewed by fluorescent microscopy. Several infiltrating mononuclear cells within the wall of a portal vein stain positively for IgG. ($\times 300$.)

similar to that in the bile canaliculi was often deposited in hepatocytes and sometimes in the sinusoidal lining cells (Figs. 220 and 221).

There was atrophy of cells in the central parts of the liver lobules. The atrophic hepatocytes exhibited a compact cytoplasm containing glycogen, enlarged mitochondria, scanty but dilated rough endoplasmic reticulum and several large microbodies. The hepatocytes were separated from one another, and bundles of collagen and reticulin fibers lay between the hepatocytes and in the spaces of Disse (Fig. 222). The canalicular borders of the hepatocytes were almost free of microvilli.

This excess connective tissue was clearly seen in sections examined by light microscopy. The central parts of the reticulin framework of the lobules were collapsed and there were more reticulin and collagen fibers than normal around the central veins (Fig. 223). Increased amounts of reticulin and collagen were also present in the portal tracts of five of the livers (OT 2, 5, 8, 11, and 12). This portal fibrosis was accompanied by slender bands of reticulin and collagen that linked some portal tracts and central veins one to the other, and by similar bands that subdivided lobules and joined together some of the central and portal areas of fibrosis. In one homograft (OT 11) the hepatic fibrosis had progressed to cirrhosis, with regeneration nodules and foci of hepatocyte necrosis.

In three of the eight liver homografts (OT 2, 5, and 8) there were still a small number of mononuclear cells lying in the tissues around the portal vein radicles in the portal triads. Few of these cells were large pyroninophilic lymphocytes; most were small lymphocytes.

In one graft (OT 25) there were small groups of plasma cells in the portal tracts. Immunofluorescent studies showed that these cells contained IgG in their cytoplasm (Fig. 224); few cells contained IgM. Immunoglobulin G, fibrino-

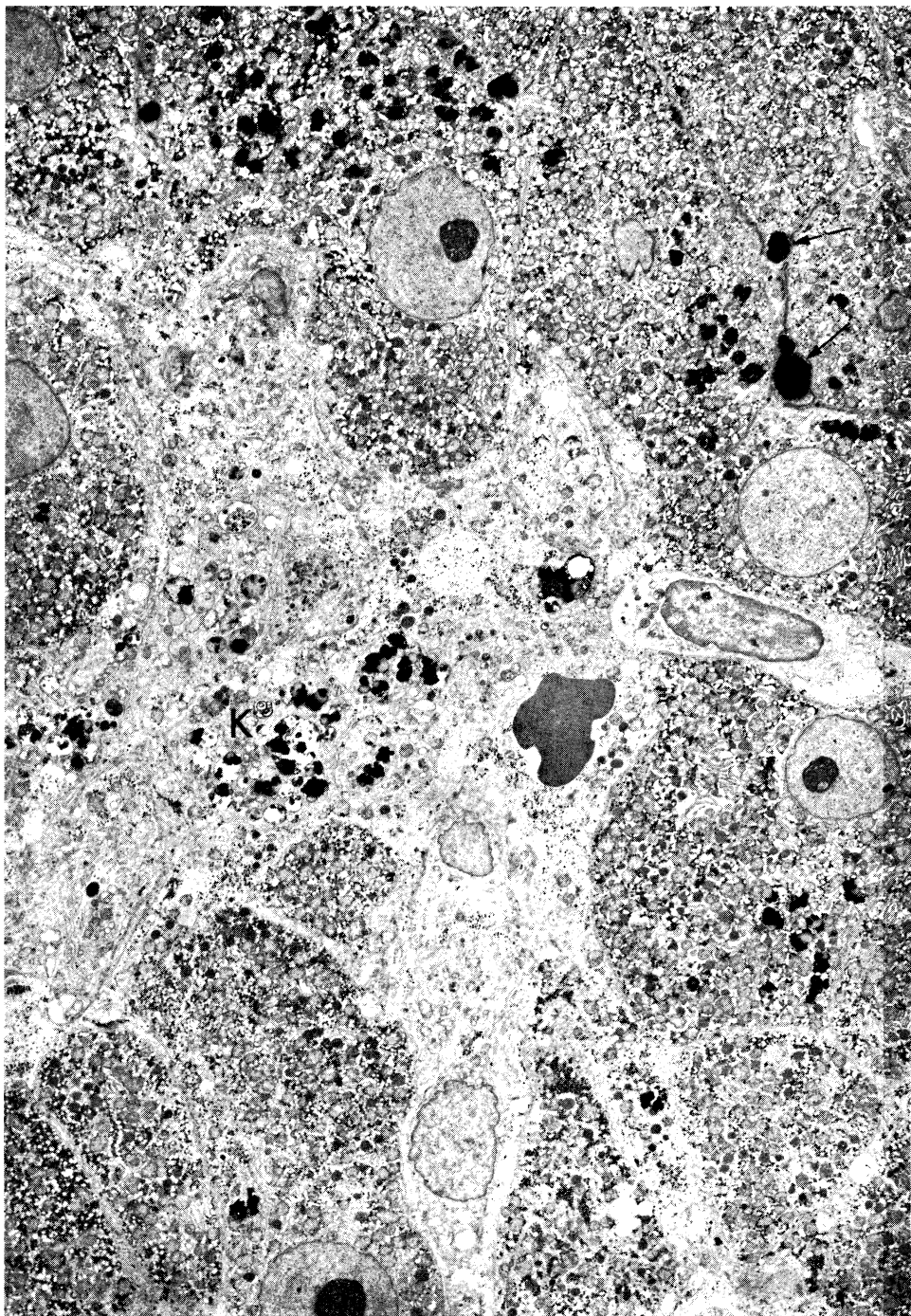


Figure 220. Central part of a liver lobule from a human hepatic homograft (OT 8) 100 days after transplantation. The hepatocytes are atrophic and several contain lipofuscin and bile pigment. There is intracanalicular cholestasis (arrows) although the patient was not overtly jaundiced at this time or for many subsequent months (see Figure 164, Chapter Seventeen). Much bile pigment is present in Kupffer cells (K). (Lead stain. $\times 820$.)

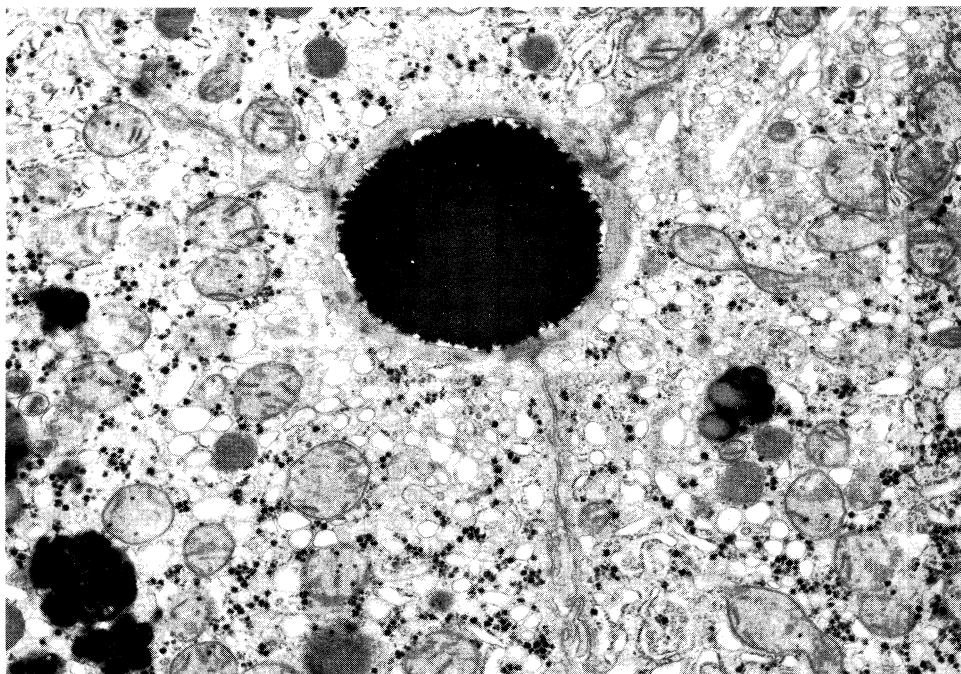


Figure 221. Higher power view of a bile canaliculus from hepatic homograft (OT 8) shown in Figure 220. The microvilli are flattened and the lumen obstructed by a mass of bile pigment. (Lead stain, $\times 12,000$.)

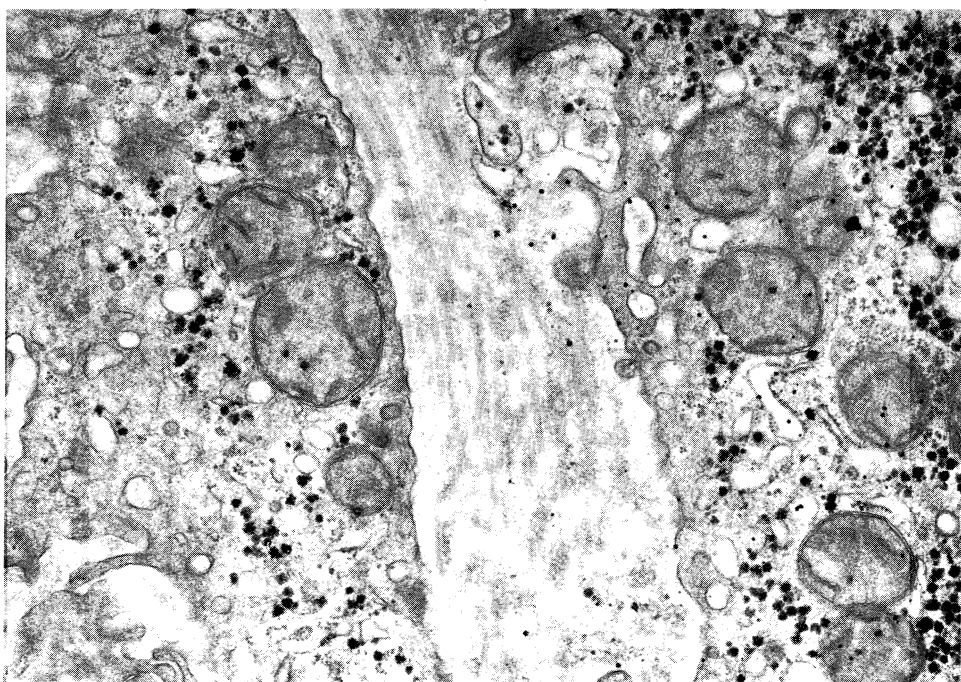


Figure 222. Higher power view of human hepatic homograft (OT 8) shown in Figure 220. A thin band of collagen subdivides a lobule. The characteristic periodicity of the fibers can just be seen. (Lead stain, $\times 12,550$.)



Figure 223. Human orthotopic liver homograft (OT 5). There is collapse of the supporting centrilobular reticulin where hepatocytes have undergone necrosis and atrophy. P=Portal tract. (Reticulin stain, $\times 30$.) (From *Advances in Surgery*, 1966, by permission of Year Book Medical Publishers, Inc.)

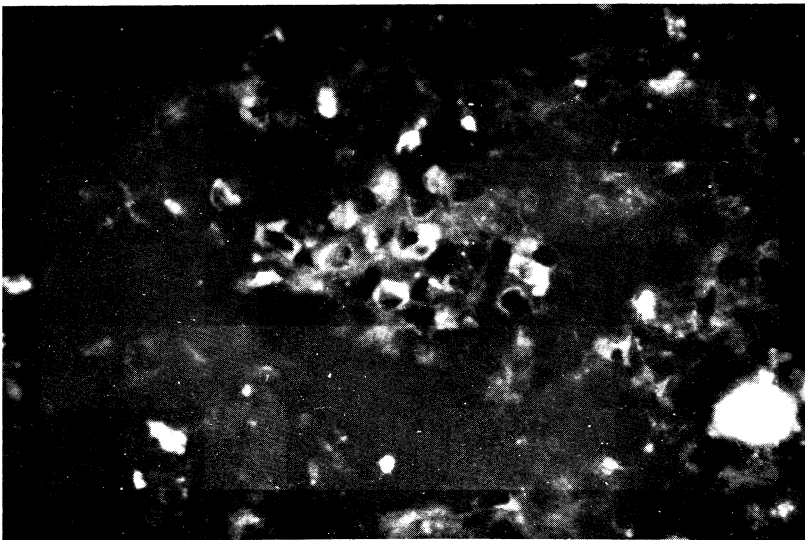


Figure 224. Human hepatic homograft (OT 25) 39 days after transplantation. A frozen section has been treated with fluoresceinated goat-antihuman IgG and then viewed by ultraviolet light. A group of mononuclear cells lie in a portal tract. Their cytoplasm stains positively for IgG. ($\times 150$.)

gen, and complement deposits were also found in the walls of the portal and central veins in this same liver (Figs. 225 and 226). Additional delicate linear deposits were present in the walls of the sinusoids and bile canaliculi.

In another transplanted liver (OT 15) the bile ductules were surrounded by neutrophilic and some eosinophilic polymorphonuclear leukocytes, some macrophages, and a few small lymphocytes. The leukocytes were probably mainly due to cholangitis (see later section).

Infiltrating cells were absent from the other three homografts (OT 11, 12 and 23).

Late Rejection with Vascular Changes. In human and canine renal homografts episodes of rejection occurring months or years after transplantation are often associated with intimal thickening of the intrarenal arteries.¹⁸ Similar changes have now been seen in four human liver homografts (OT 9, 10, 14A, and 16A) which were examined at 133, 186, 107 (and 380), and 68 days after transplantation. The arterial narrowing was accompanied in all instances by fibrosis and cholestasis, and in two patients by cirrhosis (OT 9 and 14).

All the patients had passed through a phase of early rejection. In one patient (OT 10) there was an "anicteric rejection" (Chapters Fourteen and Fifteen) which began during the first postoperative month. The other three patients had suffered the indolent and persistent variety of early rejection. Following this the high blood bilirubin levels fell in three of the patients and there intervened a jaundice-free period of 27 days to five months before a second bout of icterus occurred; the other patient (OT 16) had no remission of jaundice (Chapter Fourteen). In all four patients the hepatic function ultimately became progressively more abnormal (see Chapter Seventeen).

At the time of autopsy or retransplantation the four livers were shrunken

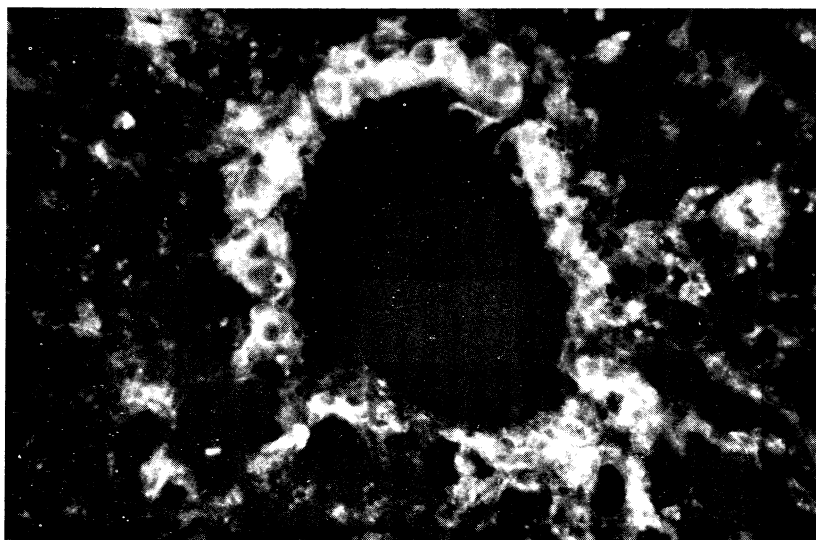


Figure 225. Human hepatic homograft (OT 25) 39 days after transplantation. Frozen section treated with fluoresceinated goat-antihuman IgG and then examined under ultraviolet light. The wall of a portal vein stains positively for IgG. ($\times 300$.)

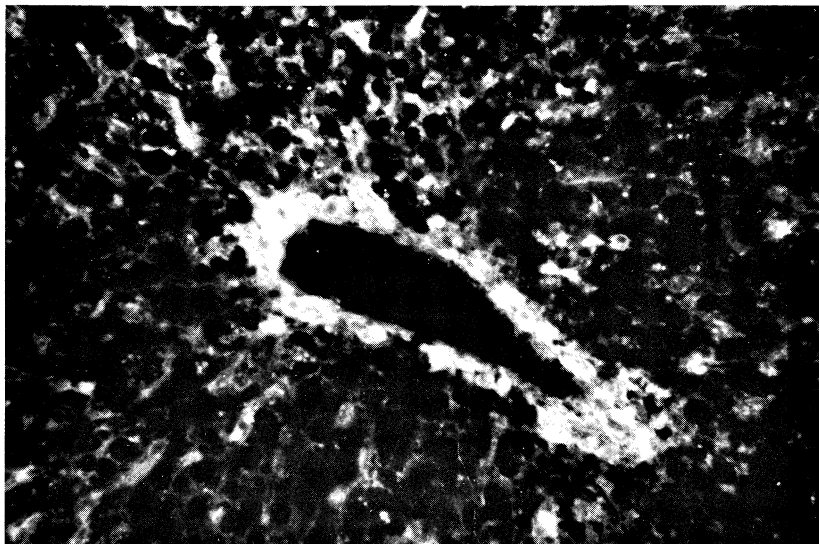


Figure 226. Human hepatic homograft (OT 25) 39 days after transplantation. Frozen section treated with fluoresceinated goat-antihuman fibrinogen and then viewed under ultraviolet light. The walls of a central vein and several sinusoids stain positively for fibrinogen. ($\times 150$.)

and firmer than normal. Two of the grafts (OT 9 and 14A) also showed a fine granularity of their surfaces and, on slicing, a cirrhotic pattern was apparent.

Microscopically in each liver homograft many of the small branches and some of the large branches of the hepatic artery were narrowed or even completely occluded by intimal thickening (Fig. 227). The changes were most widespread and severe in the grafts from patients OT 10 and 16. The thickened intima contained many large cells with abundant foamy cytoplasm that was full of neutral fat (Fig. 228). In many of the arteries the internal elastic lamina was ruptured (Fig. 229). In the homograft from patient OT 10 there was fibrous tissue in the thickened intima of many arteries.

Immunofluorescent studies showed the presence of IgG and the C'1q fraction of complement in the walls of the narrowed hepatic arteries. The deposits were on both sides of the internal elastic lamina.

Ultrastructurally the large foamy cells in the intimal wall consisted of altered and hypertrophied endothelial cells as well as some macrophages, all containing lipid deposits and other material in phagosomes. Deep to these cells were deposits of a finely granular homogeneous material compatible with antigen-antibody complex.

The pattern of fibrous and reticulin increase that was also present in all four livers did not differ from that seen in the eight homografts that were described under the heading "Repair Following Acute Rejection." The cirrhosis in the two most badly scarred livers (OT 9 and 14A) was of the portal type (Fig. 230). Broad bands of connective tissue subdivided the lobules. Regeneration nodules were small and frequent and there was some small bile duct proliferation.

Cellular infiltration was present in three of these grafts; in OT 10 and 14 it

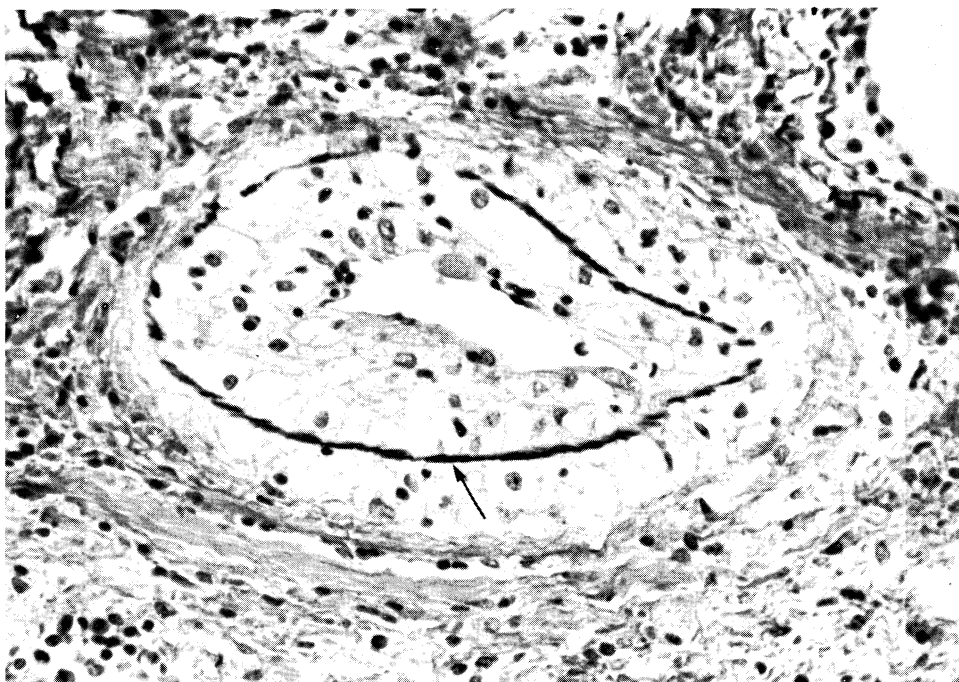


Figure 227. Human hepatic homograft removed 68 days after transplantation (OT 16). Persistent rejection followed early withdrawal of antilymphocyte globulin. In the portal tract a branch of the hepatic artery is greatly narrowed by large intimal cells with foamy cytoplasm. The internal elastic lamina of the vessel is marked with an arrow. (Elastic stain, $\times 300$.) (By permission of Surg. Gynec. Obstet. 128:327, 1969.)

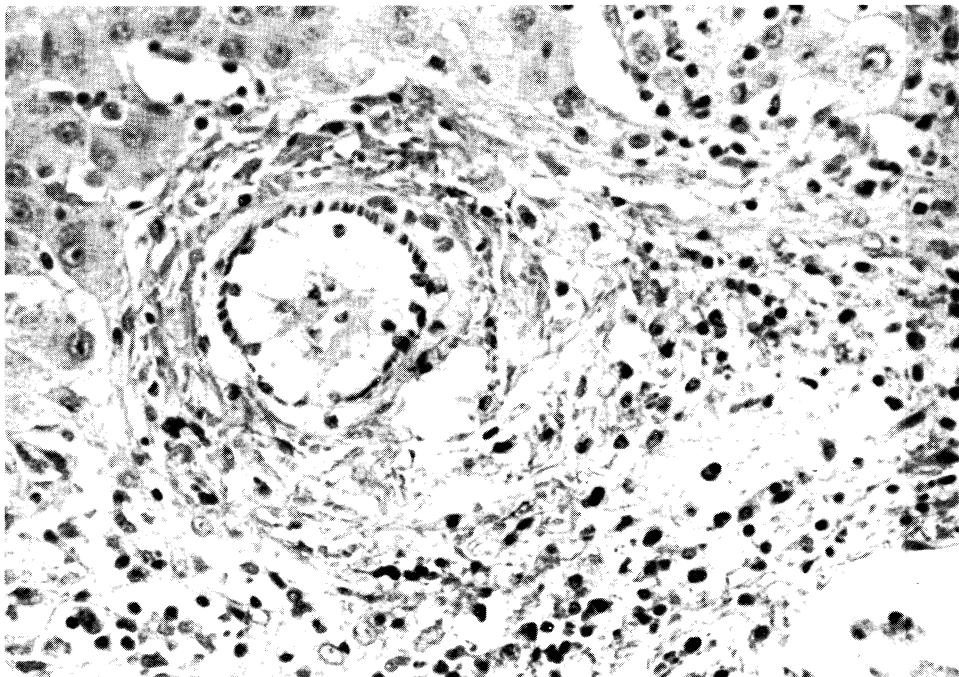


Figure 228. Human hepatic homograft removed 186 days after transplantation (OT 10). The portal tract contains excess fibrous tissue and is lightly infiltrated with lymphoid and plasma cells. A small hepatic artery branch is almost occluded by large intimal cells with foamy cytoplasm. (Elastic stain, $\times 300$.)

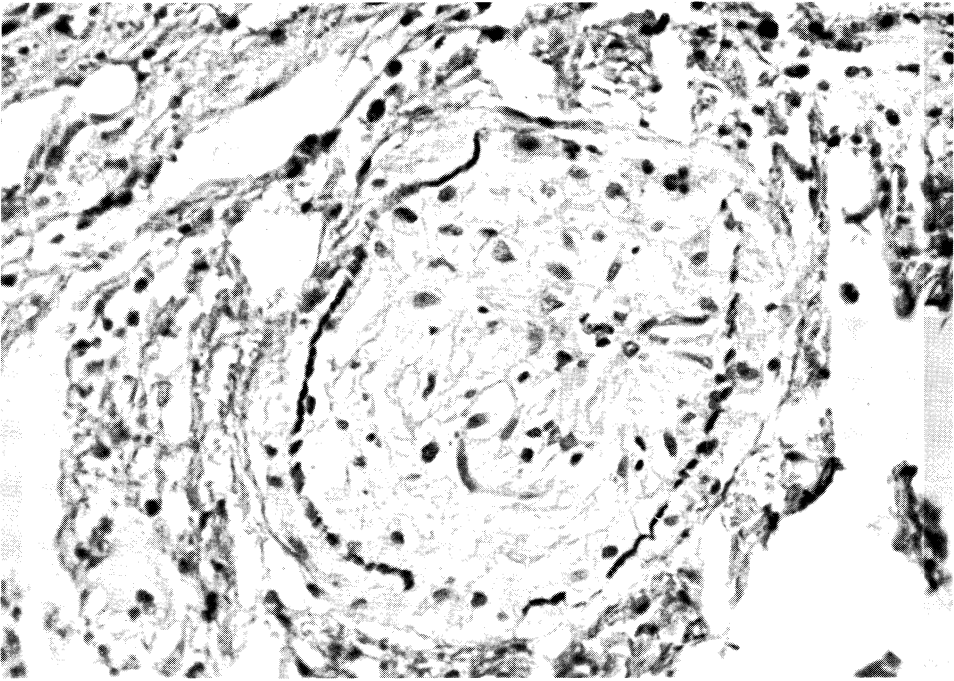


Figure 229. Same liver graft as in Figure 227, showing a branch of the hepatic artery in a portal tract. The vessel is completely occluded by intimal thickening and the internal elastic lamina is ruptured. (Elastic stain, $\times 300$.) (By permission of Surg. Gynec. Obstet. 128:327, 1969.)

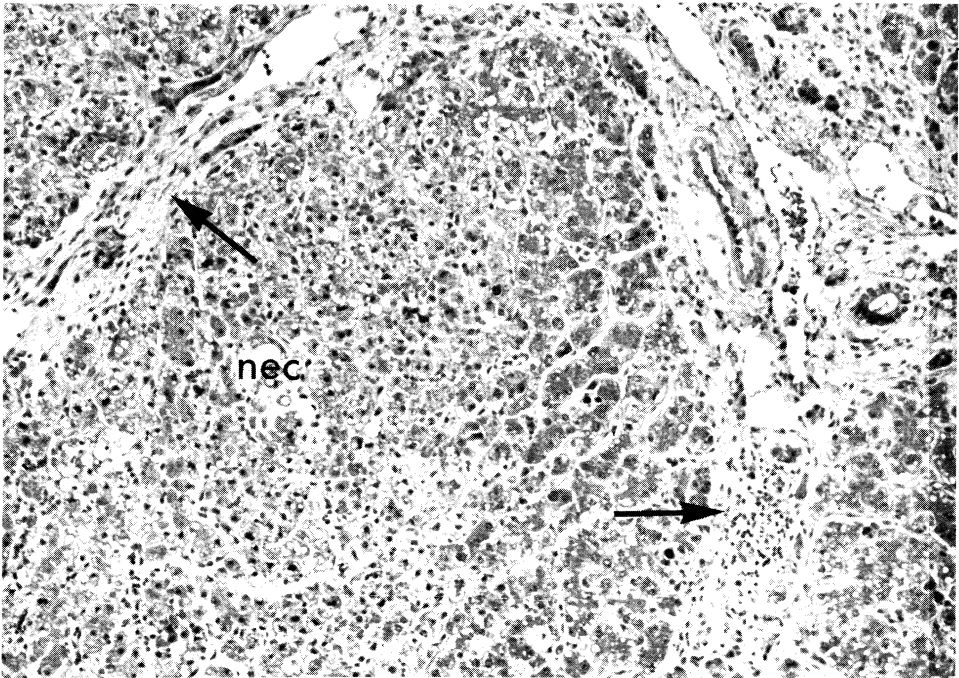


Figure 230. Human hepatic homograft removed at autopsy four and a half months after transplantation (OT 9). The normal lobular architecture is interrupted by bands of fibrous tissue (arrows), producing a portal type of cirrhosis. Part of the irregularly regenerating liver is necrotic (nec). (Hematoxylin and eosin stain, $\times 180$.) (By permission of Ann. Surg. 168:392, 1968.)

was mild, but in OT 16 dense collections of large pyroninophilic lymphoid cells were present. Intrahepatic bile stasis was present in all four livers, but was particularly severe and accompanied by extensive bile pigmentation of hepatocytes in the graft of patient OT 14.

No Evidence of Rejection. Only one of the human hepatic homografts (OT 17) examined showed no evidence of active or past rejection. This patient had good liver function and died after 35 days from pneumonia caused by *Pseudomonas* (see Chapter Sixteen). Her terminal bilirubin level was less than 1 mg/100 ml. It is of interest that a violent and nearly fatal rejection had been diagnosed only a few weeks earlier (Fig. 126, Chapter Fourteen).

At autopsy a few small lymphocytes and occasional macrophages were present in the portal tracts of the graft, but the numbers were within normal limits. There were no large pyroninophilic lymphoid cells. Collagen was absent. Immunofluorescence showed no immunoglobulin deposits.

Cholangitis. As mentioned earlier, cholangitis has been a major complication in some canine hepatic autografts and homografts, and in porcine homografts. In the 13 human hepatic homografts available for examination three weeks or longer after transplantation, it was present in only three (OT 8, 14A, and 15) that were examined 400, 380, and 339 days postoperatively.

Two of the three patients with cholangitis had developed obstruction to their biliary tract by metastatic carcinoma. In patient OT 8 a huge tumor mass in the right upper quadrant of the abdomen compressed the cholecystoduodenostomy (see Chapter Seventeen). In the second patient (OT 15) a large recurrent carcinoma developed in the hilum of the liver and pressed upon and eroded the common hepatic duct. In both homografts the biliary radicles within the liver were dilated and contained a few stones and extensive sludge. In the third patient with cholangitis (OT 14A) the biliary duct system appeared unobstructed at the time the homograft was removed and replaced 380 days after its original insertion.

Microscopically the large and small bile ducts and bile ductules were surrounded by neutrophilic and eosinophilic polymorphonuclear leukocytes, and some small lymphocytes. In many places in the three grafts the bile duct epithelium was necrotic. A granulomatous reaction was present around a few ducts (Fig. 231).

Biliary Duct Obstruction Without Cholangitis. In another liver homograft (OT 12) a kink at the junction of the cystic and common duct produced dilated intrahepatic biliary ducts filled with inspissated bile and soft stones, but without cholangitis.

Metastasis of Tumor to Graft. Eleven hepatic homografts in the Denver series were transplanted into patients suffering from carcinoma of the liver. Four of the recipients survived for 143 days or longer. Of these four grafts, all became the seat of metastases (Chapter Seventeen). The invading tumors were all hepatomas. The secondary deposits were more anaplastic than the primary growths.

Regional Hepatic Infarction. In seven patients undergoing rejection of their hepatic homografts (OT 8 to 12, 15 and 16), the results of serial liver scans using ^{99m}Tc -technetium suggested that areas of the transplants had become

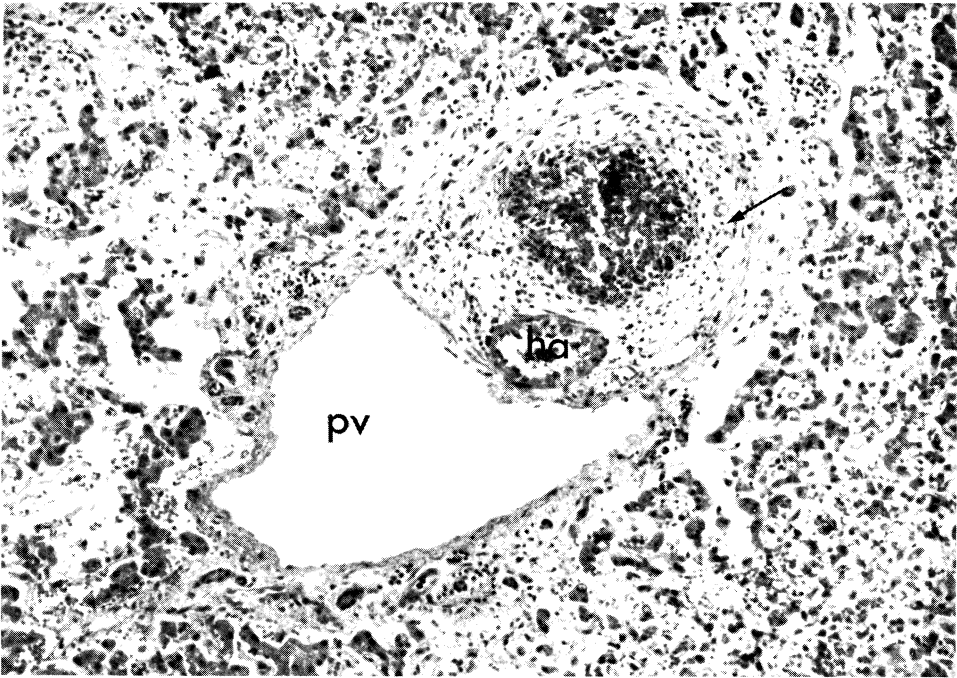


Figure 231. Cholangitis in a human hepatic homograft more than one year after transplantation (OT 14). The wall of the small bile duct is thickened (arrow) and infiltrated with polymorphonuclear leukocytes. The biliary lumen contains polymorphonuclear leukocytes and inspissated bile. The hepatic artery branch (ha) and portal vein (pv) are normal. (Hematoxylin and eosin stain, $\times 300$.)

ischemic (Chapters Fourteen and Fifteen). The process appeared to reverse spontaneously in one case (OT 15), and in another patient (OT 16) the homograft was replaced with a second organ.

There were multiple small infarcts in the tissue removed from the five homografts (OT 8 to 12) in which frank gangrene had developed. Each of the necrotic areas was rimmed with abundant neutrophilic polymorphonuclear leukocytes, many eosinophils, and some lymphoid cells, but no fibrous tissue. The necrotic centers of most, but not all, of the infarcts contained gram negative bacilli.

As described in previous sections, the residual viable parenchyma in these five livers had evidence either of acute rejection (OT 8 to 10) or of repair following acute rejection (OT 11 and 12). In the latter two patients the homograft gangrene led to death within a few days, and the right hepatic artery was found at autopsy to be freshly thrombosed in the hilum; the terminal intrahepatic branches did not seem nearly so badly damaged by rejection as the veins. The three patients whose lives were prolonged by the debridement procedures (OT 8 to 10) had mononuclear cell infiltration and injury of the portal and central veins and sinusoidal lining in the viable parenchyma,^{19, 20} but the small arteries were then relatively unaffected. However, by the time of death many months later, old thrombosis of the common (OT 8) or right hepatic artery (OT 9 and 10) was demonstrated. In the intervening period widespread narrowing of the patent intrahepatic arteries to the left lobe had occurred in patients OT 9 and 10.

The homografts of the two recipients who apparently narrowly escaped the complication of partial hepatic gangrene were also examined. The liver which had suffered rapidly reversible ischemic changes in the second and third post-operative weeks (OT 15) contained neither infarcts nor arterial lesions at autopsy 11 months later. In contrast, the homograft which was removed and replaced after 68 days (OT 16) had multiple old and new infarctions. Furthermore, there was widespread arterial narrowing (Fig. 227).

The pathogenesis of early necrosis in liver homografts was discussed in Chapters Fourteen and Fifteen. Although mechanical considerations were apparently important in determining the localization of the acute ischemic changes, an even more important underlying factor was probably a reduced blood flow secondary to damage by rejection of the veins and sinusoidal bed of the homografts, as has been demonstrated in dogs.⁸ It has become clear that the resulting sudden hemodynamic alterations can be decisively reversed under the proper circumstances. Later, however, the development of arterial narrowing as the consequence of chronic rejection can be responsible for continuous homograft ischemia.

Correlation of Histopathologic Findings with the Results of Tissue Typing .

With the small amount of histocompatibility data at present available (Table 5, Chapter Three) no correlation between HLA group mismatches and the presence or absence of histologic changes is yet discernible.

The liver homografts from the two patients (OT 8 and 16A) who showed no HLA group mismatches with their donors were densely infiltrated with lymphoid cells. In one of the grafts (OT 16A) there was also severe arterial intimal thickening.

Origin of Kupffer Cells and Endothelial Cells in Long-surviving Human Hepatic Homografts

Using the technique first described by Barr et al² it is possible to sex the nuclei of many cells. The nuclei of female cells contain a distinctive chromatin mass.

Among the 23 Denver patients with liver homografts in whom samples of tissue from the transplant were available for examination, there were nine instances (OT 5, 8, 12, 14, 17, 18, 20, 21, 24) in which a liver from a male donor was transplanted into a female recipient. A modification of Barr's method was used to determine the sex of the Kupffer cells and the hepatic arterial and portal venous endothelial cells in the graft. The modification was to examine azure II-stained serial 0.5 μ thick sections from Epoxy-embedded tissue.

The sex of the vascular endothelial cells remained male in all nine instances (Table 35). However, the Kupffer cells became female and, therefore, of host origin in the three patients (OT 8, 12, 14) whose livers were examined

Table 35. Analysis of Sex of Cells*

PATIENT OT NUMBER	DAYS GRAFT RESIDENCE	SEX CHANGE IN GRAFT (MALE TO FEMALE)	
		<i>Kupffer Cells</i>	<i>Endothelial Cells</i>
5	23	No	No
8	400	Yes	No
12	105	Yes	No
14	380	Yes	No
17	35	No	No
18	4	No	No
20	1/2	No	No
21	1/2	No	No
24	11	No	No

*Analysis of the sex of the Kupffer and vascular endothelial cells in nine patients in whom orthotopic livers were transplanted from male donors to female recipients.

400, 105, and 380 days, respectively, after transplantation (Table 35). The same sex transition had not occurred in the other six homografts that had been in residence for one half to 35 days (Table 35).

Lymphoid Tissue Transplanted with Graft

With each liver graft a number of donor lymph nodes and cellular aggregations of lymphoid cells are transplanted. This lymphoid tissue was examined in 12 of the 23 Denver human hepatic homografts. In each instance there were lymphoid follicles with prominent germinal centers and plenty of plasma cells in the medulla. These latter cells may have been the source of the donor type IgG that was found in some of the patients after hepatic transplantation (see Chapter Eighteen).

CHANGES IN HOST LYMPHOID TISSUES AFTER ORTHOTOPIC LIVER TRANSPLANTATION

In dogs not treated with immunosuppressive agents there is generalized enlargement of the lymph nodes. Microscopically there is a proliferation of large lymphoid cells with pyroninophilic cytoplasm around the postcapillary venules in the paracortical area of the nodes. These cells are larger than those

that invade the portal tracts of the graft but are otherwise morphologically very similar.

In the first week after transplantation more and more of the large lymphoid cells accumulate in the paracortical zones of the lymph nodes. By seven to 10 days, when most untreated animals die from hepatic failure, plasma cells are appearing in large numbers in the medulla and in the prominent germinal centers of lymphoid follicles in the superficial cortex. A similar proliferation of large pyroninophilic lymphoid cells occurs soon after liver transplantation in the periarteriolar sheaths in the white pulp of the spleen. Later, multitudes of plasma cells can be seen in the splenic red pulp.

Those animals which are treated with azathioprine or antilymphocyte globulin, but which still reject the transplant, show lymphoid changes that may be delayed but that are essentially the same as those occurring in the untreated animal. Observations made at autopsy on patients with liver grafts suggest that a similar series of events transpires in the lymph nodes of man if the graft undergoes rejection.

CHIMPANZEE TO HUMAN HETEROGRAFT

In Chapter Nineteen the clinical course was described of a child who received an orthotopic chimpanzee liver. At autopsy nine days after transplantation the graft was not swollen and all the vessels were patent. The only gross abnormalities were many small hemorrhagic foci scattered throughout the substance of the liver and a number of subcapsular focal necroses.

Microscopically there was a dense infiltration of lymphoid cells in all the portal tracts (Fig. 232). About 30 per cent of these cells possessed pyronine-positive cytoplasm. Rare polymorphonuclear leukocytes were also present. A few lymphoid cells were seen around the central veins. The hepatocytes adjacent to the central veins were atrophic and there was marked centrilobular cholestasis with bile "thrombi" (Fig. 233). The majority of the hepatocytes appeared normal. The lobular reticulin pattern was normal. Portal vein tributaries, hepatic artery branches, and small bile ducts were normal.

In the hemorrhagic foci there was necrosis of the centrilobular hepatocytes with destruction of sinusoids and extravasation of blood. In the paler subcapsular foci there was necrosis of the hepatocytes in the middle and central zones of the lobules but no central hemorrhage (Fig. 234).

The histologic changes in this liver heterograft were very like those that occur in hepatic homografts. The lymphoid cell infiltration in the portal tracts was dense, but no more so than in the grafts from patients OT 7 to 10 (see Figure 215 for comparison) and in many treated canine hepatic homografts. There were no lesions of large blood vessels. Fibrinoid necrosis of arterial walls was conspicuously absent. It was difficult to believe that this child's death had been the direct result of hepatic failure produced by rejection. The analysis of the postoperative clinical events given in Chapter Nineteen tended to support the conclusion that considerable liver function was maintained until almost the end of life. *KA Ponten 1969*

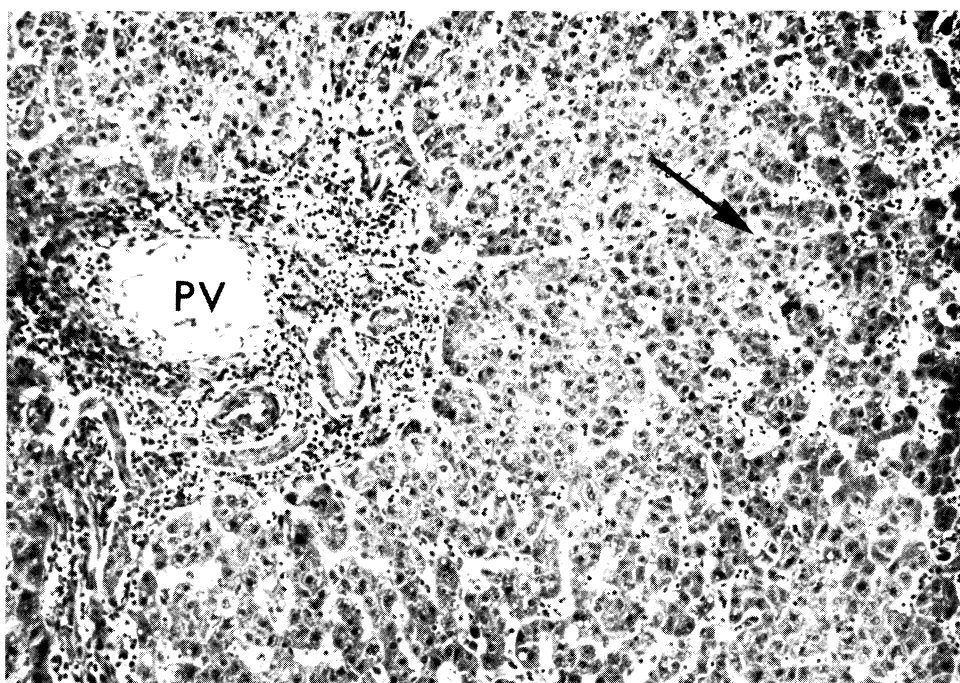


Figure 232. Human orthotopic hepatic heterograft nine days after transplantation. Sample from grossly normal part of chimpanzee liver. There is dense cellular infiltration in the portal tract, particularly around the portal vein (PV). The peripheral and midzonal hepatocytes contain a few droplets of fat but are essentially normal. The central hepatocytes (arrow) are atrophic. (Hematoxylin and eosin stain, $\times 80$.)

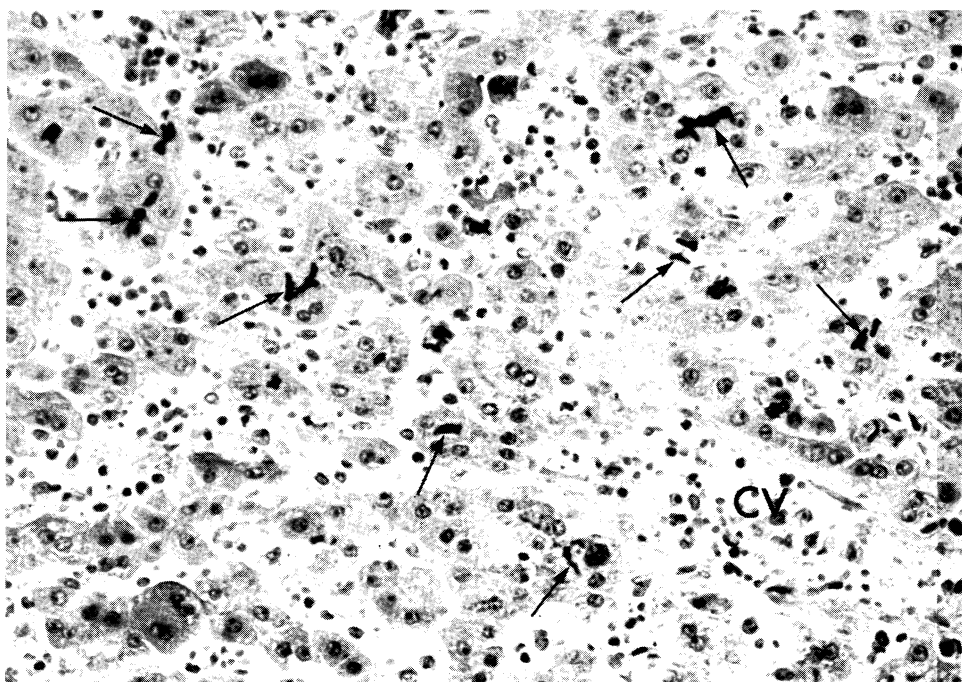


Figure 233. Same chimpanzee to man liver heterograft as in Figures 232 and 234. High power view of central part of one of the liver lobules showing inspissated bile in the canaliculi (arrows). CV=central vein. (Van Gieson and elastic stain, $\times 300$.)

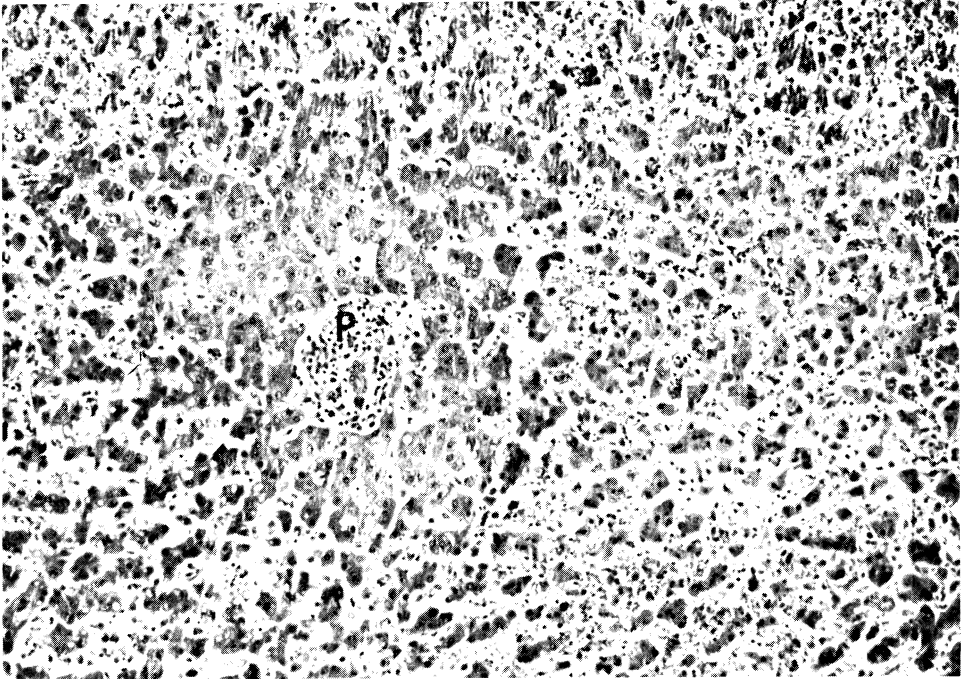


Figure 234. Sample from a subcapsular necrotic focus of the chimpanzee to man liver heterograft. Only a rim of hepatocytes survive immediately adjacent to the portal tract (P). Many of the mid-zonal and centrilobular hepatocytes are necrotic. (Hematoxylin and eosin stain, $\times 80$.)

CONCLUSIONS

From these studies of orthotopic whole liver homografts in dogs, pigs, and man a fairly clear picture emerges of the sequence of pathologic events which occur when the recipient rejects the graft. As will be mentioned in connection with auxiliary liver transplantation (Chapters Twenty-one and Twenty-three), the same conclusions probably also apply in rats.

In untreated members of all species there seems to be a quiescent phase of at least two or three days during which only rare small lymphocytes are found in the tissue spaces of the liver. Other changes can occur in the graft at this time, such as widening of the space of Disse and some damage to the centrilobular hepatocytes, but such alterations are nonspecific and are commonly found in autografts. However, during this period, large pyroninophilic cells start proliferating in the paracortical zones of the host lymph nodes.

About the third day after transplantation, lymphoid cells, many of them with pyroninophilic cytoplasm containing abundant free ribosomes, begin to leave the portal vein tributaries in a random way throughout the graft. The venous endothelium becomes lifted away from the basement membrane and fibrin collects in the subendothelial space. After passing through the vessel wall the lymphocytes accumulate in the portal tracts. Smaller numbers of similar lymphoid cells immigrate through the walls of the central veins and through the endothelial lining of the sinusoids. These cells invade the space of

Disse and some squeeze between hepatocytes. Immunoglobulins are rare in the cytoplasm of these infiltrating cells (see also Chapter Twenty-three).

Associated with the cellular infiltration, the walls of many of the sinusoids disintegrate, the blood flow through the liver begins to decrease, and some centrilobular hepatocytes die. As the centrilobular necrosis progresses to mid-zonal necrosis, the liver function becomes affected. Inspissated bile appears in surviving bile canaliculi, and lipid droplets accumulate in the hepatocytes around the portal tract. Shortly before the death of the recipient, foci of fibrinoid necrosis may occur in the walls of the small branches of the hepatic artery and these are associated with the appearance of IgG and complement in the intima and media.

The reduction in blood flow which destroys the graft is probably the result of damage to the venous and sinusoidal parts of the vasculature. The lymphoid cells seem to produce this damage, but how is not known. Cell-bound antibody is suspected but has not yet been demonstrated.

Lee and Edgington's experiments with rats (see Chapter Twenty-three) have shown that these events are accelerated with increased histocompatibility differences between donor and recipient; presumably this situation pertained in some of the human homotransplantations (as in Case OT 7) and also in the heterotransplantation. The changes are delayed and often attenuated either if the histocompatibility differences are slight or if the recipient is treated with immunosuppressive agents. The host response evoked by the liver may be inherently less vigorous or at least less lethal than that induced by a number of other grafted tissues. The strongest indication that this may be the case has come from the pig experiments cited earlier in this chapter and in Chapter Eleven. However, the same may be true in the dog (Chapter Twelve) and in other species as well.

When rejection is minimal (as in pigs) or if it is modified by treatment (in dogs and humans), the destruction of hepatocytes stops, but there is often collapse of the central part of the lobular reticulin framework. This event is frequently accompanied by the marked accumulation of inspissated bile in the centrilobular bile canaliculi, but whether the centrilobular changes cause the cholestasis is not known. An alternative possibility is that the bile stasis is secondary to the widespread loss or distortion of the canalicular microvilli which occurs at about the same time.

Later, connecting bands of reticulin may be laid down between the central areas, subdividing the lobules. What triggers this progression to hepatic fibrosis in some grafts is unknown. In a few recipients a true cirrhosis may even eventually be produced.

Great intimal thickening with obstruction of the lumen occurs in many of the branches of the hepatic artery in some long-surviving human liver homografts. The accumulation of IgG and complement in the altered vessel wall raises the possibility that this damage is a late manifestation of rejection brought about by circulating antibody, but this is at present pure speculation.

Some of the hepatocyte damage in treated human grafts may have been due to the immunosuppressive drugs used, particularly azathioprine (see Chapter Twelve). Viral hepatitis may also have been responsible for a few of the

histopathologic changes that were encountered in liver grafts in patients. However, comparison of the clinical specimens with those obtained from a variety of animal experiments has made it clear that the major alterations were immunologic in etiology.

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PART IV

AUXILIARY TRANSPLANTATION



Chapter Twenty-one

METABOLIC CONSIDERATIONS IN ANIMALS

There have been dozens of reports of heterotopic hepatic transplantation in dogs. The studies all had a clinical orientation since, at first thought, the concept of using an auxiliary hepatic homograft for the treatment of patients with benign liver disease has a special attractiveness. First, sacrifice of the remaining limited function of the failing recipient liver can be avoided. Thus, in the event of poor initial performance by the homograft due to ischemia or to a severe but reversible rejection, it might be hoped that some assistance would be provided by the diseased host liver during a transition period. This would be predicted to be a particularly significant advantage in patients with biliary atresia, since the synthesizing functions of the liver are often retained until the terminal stages of this disease. Second, it was initially assumed that the placement of an extra liver would be safer and technically less demanding than the orthotopic procedure.

The appeal of auxiliary hepatic transplantation has lost a good deal of its luster in view of some of the mechanical difficulties which have been encountered (Chapter Twenty-two) and in light of the special physiologic problems to be discussed later which are posed by dual liver systems. Nevertheless, there may be a limited role (see Chapters One and Twenty-two) for carefully planned and executed auxiliary transplantations.

THE PROBLEM OF GRAFT ATROPHY

The first experiments with whole organ liver homografts were carried out in dogs by Welch.^{9, 39} The operation as originally described, or slightly modified by later authors, involved the transplantation of an extra canine liver in the right paravertebral gutter or pelvis of a nonrelated mongrel recipient. The hepatic arterial supply was derived from the aorta or iliac artery (Fig. 235). Venous inflow was reconstituted by anastomosing the distal iliac vein or inferior vena cava to the homograft portal vein; outflow was into the proximal iliac vein or

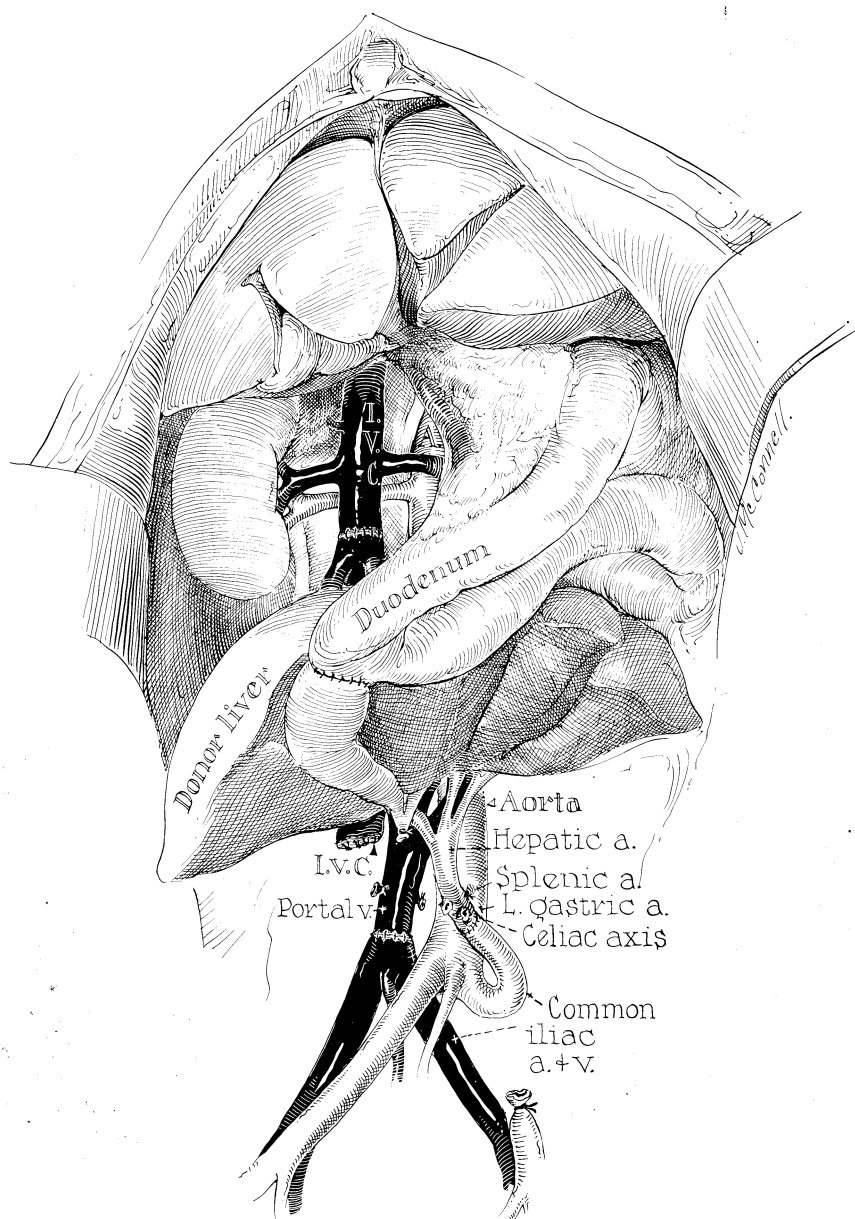


Figure 235. Auxiliary liver transplantation in dogs by a modification of Welch's original technique. Note that the reconstituted portal blood supply is from the distal inferior vena cava. Cholecystoduodenostomy is performed. (By permission of *Ann. Surg.* 160:411, 1964.)

vena cava. Welch⁵⁹ with Goodrich⁹ and those who followed^{30, 37, 38} proved that such livers produced bile for several days after transplantation and then ceased to function. The organs had histopathologic evidence of rejection. This was to be expected since immunosuppressive therapy was not employed.

It was 10 years after Welch's first publication before auxiliary transplantation was attempted in immunosuppressed canine recipients. A curious and disquieting observation was soon made;^{44, 47} It was found that auxiliary homografts, inserted by a modification of Welch's technique into dogs being

treated with azathioprine, were much more severely damaged than orthotopically placed livers. There were moderate and sustained increases in the serum transaminases and alkaline phosphatases. However, the most striking event was rapid shrinkage of the extra organ, which was usually evident within two weeks and which was very advanced at all times after one month (Fig. 236).

The gross appearance and lobar proportions of the now diminutive homografts remained relatively unaltered except for size. The duct system did not participate in the shrinkage and often was more or less completely spared from rejection. Within the parenchyma there was a massive loss of hepatocytes from focal or widespread necrosis, reticulin collapse, and consequent crowding together of intrahepatic portal tracts. Later, Daloze et al¹ showed that an inherent vascular insufficiency was not responsible for these changes. In their studies the total blood flow through auxiliary Welch homografts was about equal to that of the hosts' own livers.

These small transplants were incapable of sustaining life. In four dogs, the host livers were removed four weeks after transplantation.⁴⁴ All four animals died within 12 to 48 hours. At autopsy no explanation for death could be found, and it was concluded that the animals had died of hepatic insufficiency. Hemorrhage such as that which invariably terminates experiments after simple total hepatectomy⁴³ had not occurred.



Figure 236. The auxiliary homograft (right) and the recipient dog's own liver (left) in an experiment in which the host and transplanted organs had originally been about the same size. The method of revascularization was as illustrated in Figure 235. Postoperative immunosuppression was with azathioprine. Note the well preserved but dimensionally reduced general structure of the homograft. The gallbladder did not shrink proportionately. The specimens were obtained 45 days after transplantation. (By permission of *Ann. Surg.* 160:411, 1964.)

Three possible explanations were advanced to account for these findings.⁴⁷ The least likely was that immunosuppressive therapy, which is partly dependent on accurate day-to-day monitoring of homograft function, had been indecisively delivered because of the inability to differentiate between the metabolism of the homograft and the host liver; this was later proved not to be the case. A second proposal was that the host's own liver "may have contributed substantially to the immunologic reaction [against the auxiliary homograft], a factor which would be eliminated in the orthotopic preparation." This proved not to be the essential explanation for the graft shrinkage.

The third suggested possibility proved to be the correct one: "The abnormal revascularization may have contributed even though... dogs with portacaval transposition do not have loss of hepatic mass.³ Competition [of the homograft] with the dog's own liver for nutritional substrate may have been an unfavorable condition."⁴⁷ Subsequent work has established beyond doubt that auxiliary liver homografts which are placed in various ectopic locations can undergo involution by this mechanism.

Competition and Graft Atrophy

Evidence of competition between coexisting livers was subsequently provided from our laboratories by Marchioro et al.²² In these experiments canine homografts were placed in the right paravertebral gutter in the usual way except that the portal vein of the homograft was connected to the superior mesenteric vein. Splanchnic flow through the auxiliary liver was then promoted by ligating the portal vein at the hilum of the host liver (Fig. 237B).

The animals, which were treated with azathioprine, now usually had atrophy of their own livers but not of the homografts. In commenting on these results⁴⁵ it was remarked: "Apparently, there is a competition between coexisting livers for some metabolite or other substance in the portal venous blood. That organ which has first access to the portal flow retains its functional and morphologic integrity. The other organ, whether it be the homograft or the autologous liver, undergoes atrophy predominantly affecting the centrizonular area." The validity of this point of view and the fact that the competitive relationship can be unbalanced in ways other than by the presence or absence of splanchnic flow will now be considered.

The Quantitative Effect of Splanchnic Venous Blood. During the first half of the twentieth century conflicting opinions and evidence were presented concerning the influence of splanchnic venous blood on hepatic morphology and function. After Eck's initial observation that portal venous diversion in dogs did not result in death,⁶ the importance of splanchnic venous inflow to the liver was minimized. Within a few years, however, Hahn,¹¹ working with Pavlov, demonstrated that animals with this operation had liver atrophy, serious weight loss, and neurologic aberrations that could be triggered by protein ingestion. Since animals with Eck's fistula have a reduced total hepatic blood flow,^{13, 15, 49} the unresolved question was whether these adverse consequences were simply due to the quantitative flow change or whether they resulted from depriving

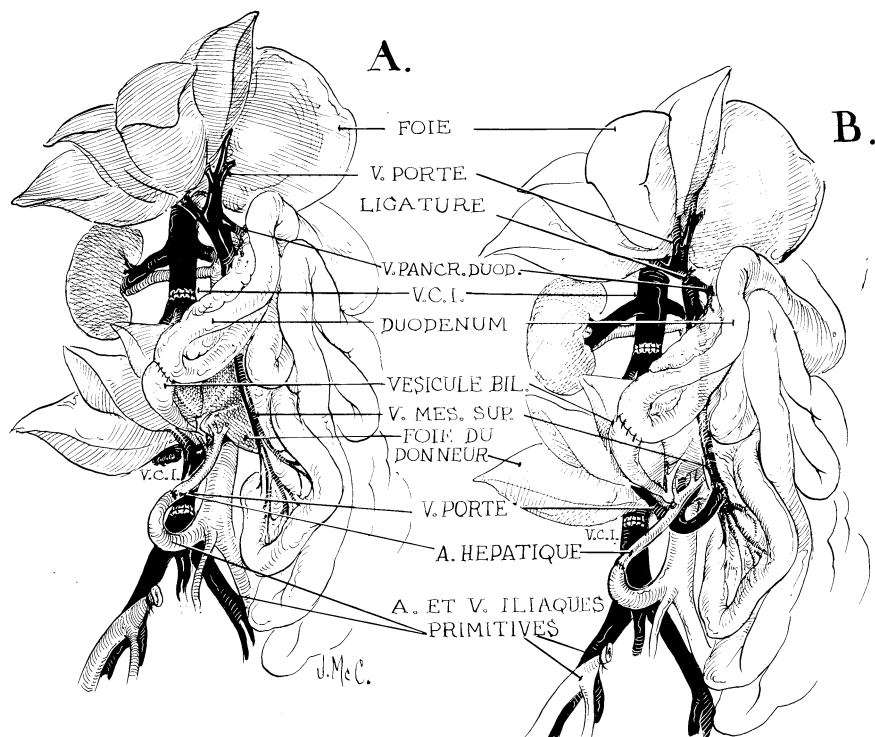


Figure 237. Marchioro's first experiment with auxiliary hepatic homotransplantation, which suggested competition between coexisting livers. A, Modification of Welch-Goodrich technique; the portal blood flow to the transplant was from the systemic venous system. The homograft underwent rapid atrophy. B, The portal venous inflow was obtained from the nonhepatic splanchnic bed. The host liver received only an arterial supply. With these changes, the homograft atrophy was prevented and in some experiments the shrinkage now involved host livers. (By permission of Surg. Gynec. Obstet. 121:17, 1965.)

the liver of some substance or substances present in high concentration in the intestinal venous effluent from which the portal system is normally supplied.

The issue seemed to have been settled in 1953 with the observations on canine portacaval transposition by Child,³ which were later confirmed and extended by Silen⁴² and many others.^{16, 48, 49} With this operation the portal venous blood was diverted from the liver as with an Eck's fistula but it was replaced by a systemic venous inflow from the inferior vena cava (Fig. 238); total hepatic blood flow was unchanged or increased,^{16, 49} and the illness caused by Eck's fistula was avoided. Furthermore, dogs with transposition were thought by Child³ to have a normal capacity for liver regeneration and by others to have normal hepatic function except for a reduced ability to eliminate an intravenous ammonia load.⁴²

These findings indicated that the quantity of portal venous inflow is a vital element in the maintenance of hepatic integrity in normal dogs. The same conclusion applies equally or even more so for heterotopic hepatic homografts as will subsequently be made clear, especially in discussing how to prevent atrophy of these auxiliary canine organs. For the moment it will only be pointed out that the research with portacaval transposition did not rule out the possi-

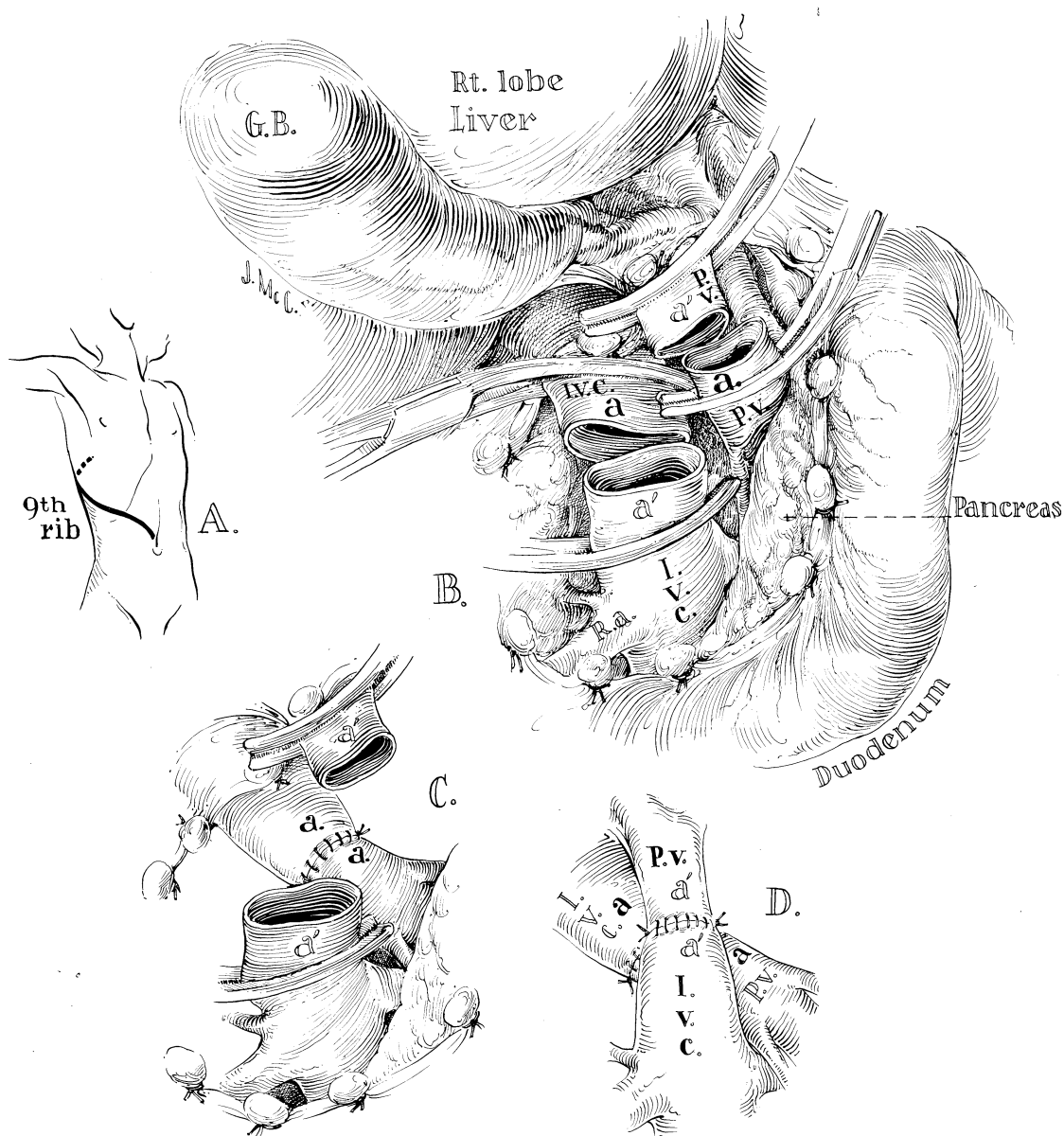


Figure 238. The technique of portacaval transposition as it has been carried out in patients with glycogen storage disease. In canine experiments the safety of the operation is greatly increased if it is performed under total body hypothermia. (By permission of *Surgery* 57:687, 1965.)

bility that the composition of splanchnic venous blood might be an important additional factor.

With transposition, the blood which is initially bypassed around the hepatic tissue is processed through the mixing chambers of the heart and eventually delivered back to the liver in diluted form. In spite of this recirculating effect, severe deglycogenation of hepatic tissue has been described^{36, 48} within six or eight weeks after transposition, suggesting that subtle metabolic changes had been produced and that the livers, although compatible with good health and long life, were not completely normal.

The Qualitative Effect of Splanchnic Venous Blood. The concept that the presence of two livers altered the just described metabolite recirculation was implicit in Marchioro's interpretation of his results after auxiliary transplantation. He suggested that the organ which was perfused first by splanchnic blood extracted a disproportionate share of unspecified substances, and that the other liver atrophied because of its disadvantaged competitive situation.²² This view was soon supported by Thomford,⁵¹ Halgrimson,¹² and Tretbar.^{52, 53} Thomford showed that the atrophy in Welch auxiliary homografts could be prevented in immunosuppressed recipients if the host livers were removed within a few days after transplantation, and Tretbar and Halgrimson demonstrated that the shrinkage could be reduced by diversion of portal blood away from the host liver.

The value of various double liver techniques for making apparent this otherwise well masked physiologic effect of interliver competition could hardly be overemphasized. However, the transplant preparations originally used had two serious flaws which prevented definitive conclusions about the exact pathogenesis of the atrophy. First, the total flows delivered to the two coexisting livers were often different. Second, there was by definition an inherent inequality of the two organs since the homograft was under immunologic attack despite host immunosuppression whereas the animal's own liver was not. Consequently, another experiment was undertaken which was designed to circumvent both deficiencies.

The preparation used was termed a "split transposition."^{21, 23} In normal dogs either the right or left portal trunk was detached from the main portal vein and revascularized by anastomosing it to the suprarenal inferior vena cava (Fig. 239). Thus, the portal inflow of one fraction of the liver came from the hind quarter and kidneys. The portal perfusion for the other fraction came from the nonhepatic splanchnic bed. The total hepatic flow to the respective sides

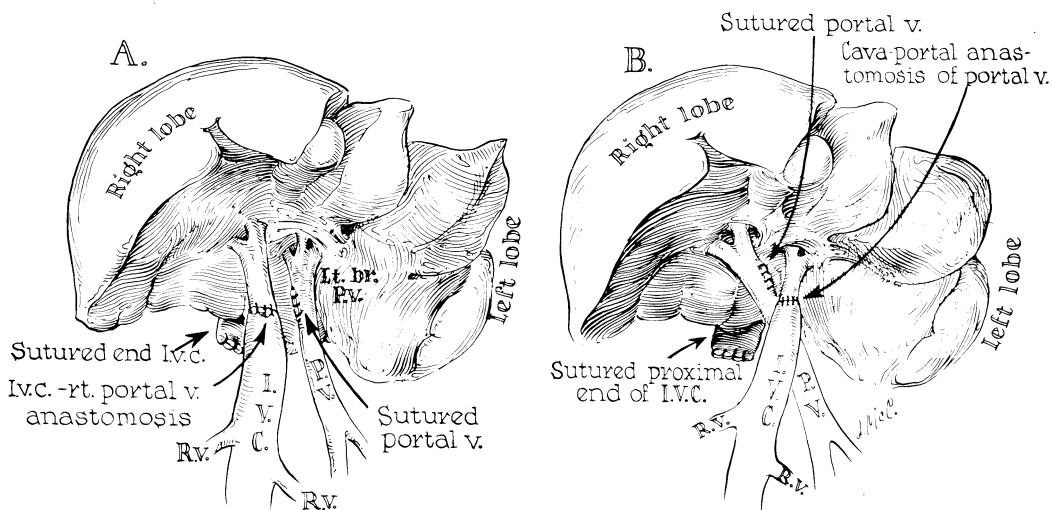


Figure 239. An experiment designed to show the effect of splanchnic venous blood upon the liver (partial portacaval transposition). Note that one portion of the liver received venous blood from the intestinal effluent, but that the other part derived its portal inflow from the inferior vena cava. (By permission of Surgery 61:723, 1967.)

was unchanged.^{21, 23} Oxygen content in the two sources of venous supply was not significantly different.¹⁷ The arterial supply and biliary tract drainage were not disturbed.

Under these conditions the portion of livers receiving splanchnic venous flow thrived and often hypertrophied, whereas the fragment nourished with equal or even greater volumes of systemic venous blood atrophied (Fig. 240). Biochemical analyses of the two sides showed marked deglycogenation of the shrunken hepatic tissue,^{21, 23} a finding which supported the possibility that the peculiar "hepatotrophic" effect of splanchnic blood was due to metabolites such as sugar, which it contains in high concentration after meals. However, measures of proteins and various enzymes (including phosphorylase, glucose-6-phosphatase, acid phosphatase, and phosphoglucomutase) were equal on both sides.

The advantage (and hypertrophy) enjoyed by the tissue which was perfused with splanchnic blood and the disadvantage (and atrophy) of the other fragments were quickly confirmed in dogs by the studies of Price³¹ and Sigel⁴⁰ and their associates, who transplanted pieces of autologous liver to ectopic locations, varying the methods of vascularization of the residual and transferred portions. More recently, Lee and Edgington¹⁹ have reported similar findings in

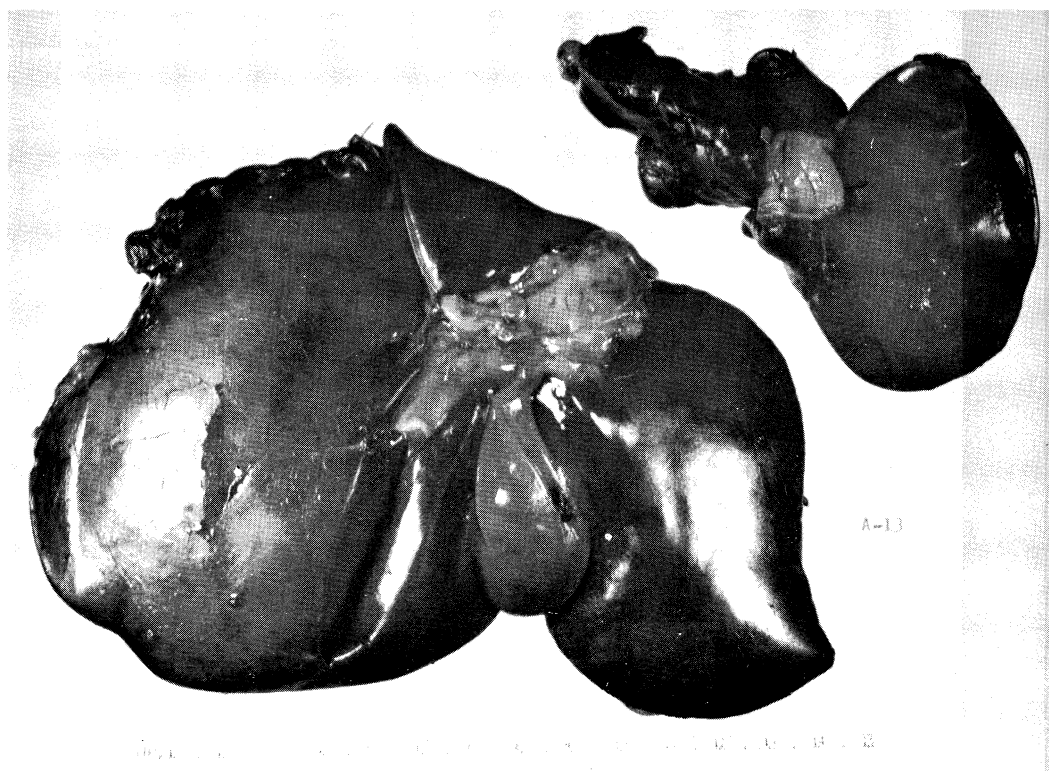


Figure 240. Marked atrophy after partial transposition to the left main portal trunk (Fig. 239B). The autopsy specimen was obtained 73 days after operation. The atrophic left portion weighed 52 gm. The hypertrophied right fraction weighed 234 gm. (By permission of Surgery 61:723, 1967.)

rats. The application of these principles in auxiliary homotransplantation will be considered subsequently.

Other Factors in the Competitive Balance. Factors other than the quality and quantity of blood supply can influence the outcome of such an experiment. This clinically significant fact was acknowledged in many of our early publications by comments such as the following: "Whether substrate competition will prove to be of important clinical significance is not known. In the benign diseases for which liver transplantation might be contemplated, there would be pre-existing failure of the recipient patient's liver, so that it might be incapable of efficient metabolic extraction. Should this prove to be the case, the exact method of auxiliary homograft revascularization will be less critical."⁴⁵

There are a variety of experimental ways in which liver tissue can be injured. One of the simplest is to ligate the duct system. It has been long known that when a lobar or segmental duct is occluded in rabbits,^{32, 34} cats,^{14, 50} dogs,²⁶ pigs,³⁵ and monkeys,²⁶ the hepatic tissue which it drains may ultimately atrophy, whereas the remaining undisturbed parenchyma undergoes hypertrophy. Completion of the sequence of events is rapid in the rabbit, requiring only four to six weeks, and slower in the other animals, taking as long as 12 to 15 months in some species. To account for this effect several of the authors cited above speculated about a reciprocal mechanism which has obvious analogies to that proposed to explain the findings after "split transposition."

The hypothesis was very clearly developed by Schalm et al,³⁴ who wrote in 1956: "These observations would seem to support the theory that any relative difference in functional possibilities between both hepatic parts is associated with (differential) atrophy and hypertrophy. . . . If part of the liver is deprived of its possibility of bile drainage or its supply of portal blood, atrophy of this hepatic part occurs, associated with simultaneous hypertrophy of the other hepatic part which has retained its functions. It is likely that these phenomena always occur when one part is in a relatively more favorable functional condition than the other. The rapidity of development of this phenomenon is determined by the extent of the functional difference." Ten years later Schalm briefly reviewed this subject again, drawing attention to its relevance in auxiliary transplantation.³³

Host common duct ligation in dogs in conjunction with auxiliary transplantation was first reported by Hagihara and Absolon,¹⁰ originally as a means to permit better tracking of the bilirubin-clearing capacity of the extra liver. As an incidental finding they observed that the function of the homograft, at least in terms of bile excretion, was thereby prolonged. Later, Gliedman of New York,⁸ Faris in our laboratories,⁷ and van der Heyde of Leyden University⁵⁶⁻⁵⁸ did the same thing for the purpose of favoring the auxiliary liver. The precise degree to which this objective was met was not analyzable in any of these experiments since other variables were present, including inequalities of both the kind and quantity of hepatic blood flow to the two livers, and the unpredictable effect of immunologic repudiation on one of the two organs. Far more credible confirmation of the physiologic handicap imposed by biliary tract obstruction in a trans-

plant situation was later provided by the experiments of Price³¹ and Lee^{18, 19}, in which the element of rejection was eliminated by the employment of autologous and/or syngeneic grafts.

In Lee's studies in rats he also noted that the combination of biliary obstruction plus portal deprivation had a more profoundly injurious effect than either insult alone.¹⁹ The same conditions were probably present in the much earlier canine autotransplantations performed by Sigel, who free-grafted small pieces of hepatic tissue to defunctionalized loops of small intestine. The transplants underwent rapid shrinkage⁴¹ which, however, could be slowed by partial host hepatectomy or by Eck's fistula.³⁹

There is little reason to further belabor the point that one of two co-existing livers can be rendered relatively noncompetitive in many more ways than those mentioned above. One that so far has been mentioned only in passing is to submit it to the injury of immunologic rejection. The crucial importance of this factor in affecting the relation of a hepatic homograft to the host liver will be returned to later.

In this and the preceding sections interliver competition has been considered as a physiologic concept which was first fully appreciated in the course of research with ectopically placed homografts but which could then be most accurately studied outside the sometimes bewildering context of homotransplantation. The topic of competition was considered in detail because of the need to have a completely crystallized view of the problem before discussing the prevention of auxiliary homograft atrophy. In so doing, the first publications on the subject were directly quoted more freely than usual because of the desire to clarify the terminology initially used by us in respect to that later employed by the research team at Leyden, Holland.^{54, 56-58} The latter workers have used slightly different terms to describe the identical phenomenon. Since such semantic distinctions could be confusing, they will not be considered further.

THE PREVENTION OF HOMOGRAFT ATROPHY

The first step in minimizing or preventing auxiliary homograft atrophy in canine experiments is to avoid situations in which the transplanted liver is placed at an absolute physiologic disadvantage, as in Welch's original operation^{9, 59} and in several modifications of this procedure.^{20, 24, 27, 30, 37, 38, 44, 47} Following Marchioro's enunciation of this principle,²² many subsequent investigators have attempted to restore the competitive balance or to tilt it in favor of the transplanted organ either by giving the homograft a quantitatively or qualitatively superior blood supply,^{2, 5, 7, 12, 19, 25, 27-29, 46, 52, 53} by more or less defunctionalizing the host's own liver in a variety of ways that have ranged from its complete removal through common duct ligation,^{1, 7, 8, 10, 19, 51, 54, 55, 57, 58} or by a combination of these approaches.

The second step is to appreciate fully that the homograft requires a better than equal environmental opportunity to counteract the immunologic duress which it alone must suffer if there is not a perfect histocompatibility match or

else totally effective immunosuppression. The problem was recognized by Marchioro²² and most decisively studied by Halgrimson.¹² The dogs in the latter investigation¹² were subjected to Eck's fistula and treated with azathioprine. Auxiliary homografts were placed in the right paravertebral gutter and provided with a double blood supply (Fig. 241). In several instances the portal venous anastomosis clotted (Fig. 241B), leaving both organs comparably arterialized. There was profound and rapid shrinkage of the transplants.

Unfortunately, the "lead" which is required for protection of a canine homograft, or indeed if any advantage will be sufficient, is not analyzable in advance in any given experiment. The reasons are that histocompatibility matching cannot be accurately done between mongrel dogs and that the efficiency of immunosuppression may be variable from case to case. Using azathioprine as the sole method of immunosuppression, the privilege extended to the homograft should ideally be of the magnitude of that provided by Marchioro²² when he deprived the host liver of its portal flow and diverted the nonhepatic splanchnic venous blood through the homograft (Fig. 237B), thereby causing atrophy of the recipient's own organ and avoiding it nearly completely in the transplant. The penalty for a less complete approach, using

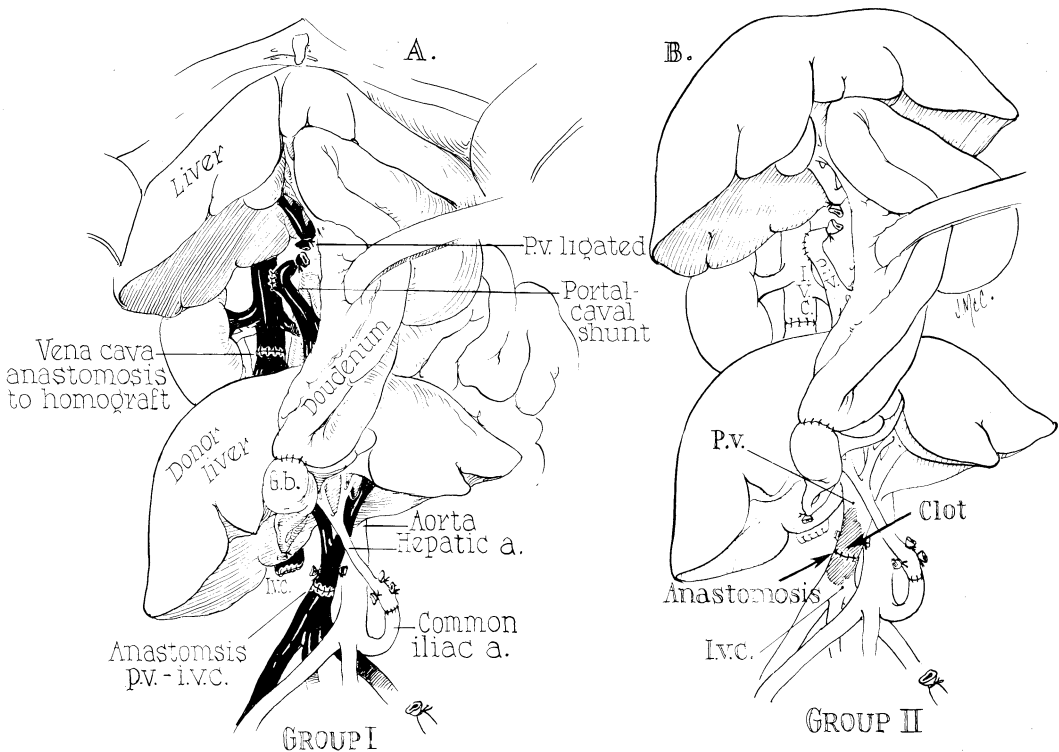


Figure 241. Experimental design of auxiliary canine homotransplantation of the liver, in conjunction with host portacaval shunt. A. Note that the homograft received a double blood supply, the portal inflow being of systemic venous origin. Except for the host portacaval shunt, the procedure was essentially that described by Welch.^{19,20} Moderate atrophy of both the homograft and host liver were observed. B. In these dogs the portal venous anastomosis clotted; thus, both the host liver and homograft received only an arterial blood supply. Transplant atrophy was profound. (By permission of Arch. Surg. 93:107, 1966.)

this form of immunosuppression, was demonstrated by Halgrimson¹² and Daloze⁵ in our laboratories and by Tretbar.⁵² Halgrimson and Tretbar performed an Eck's fistula on the host liver and revascularized the homografts as with portacaval transposition, thereby giving the auxiliary liver a double blood supply as opposed to an arterial supply alone for the autologous organ (Fig. 241A). The transplant atrophy was considerably reduced but not prevented after an average follow-up of 58 days.

In an extension of this experiment Faris⁷ and van der Heyde^{57, 58} added ligation of the host common duct. There were apparently 11 dogs in van der Heyde's series that survived for at least three weeks, but only one that had lived for more than 46 days. Atrophy affected the transplant in only 3 (or 4) of the 11 cases. The shrinkage that can occur even under these relatively favorable circumstances was also remarked upon by Faris⁷ and is illustrated in Figure 242.

The ultimate criterion of success after auxiliary hepatic transplantation is the ability to have life sustained by the homograft after delayed removal of the host liver. This has rarely been achieved even for short periods and it has never been accomplished with really long survival. Three dogs in Marchioro's series²² had transplants of normal size in conjunction with atrophy of their own livers 77, 61, and 73 days after the procedure shown in Figure 237B. Autologous hepatectomy was then performed. The animals lived for 49, 8, and 27 more days, respectively, although they had abnormal hepatic function (Fig. 243). To date, these experiments and those of Thomford⁵¹ have been the only ones in which total recipient dependence upon auxiliary homografts has been compatible with life for more than a few days. The hepatectomies in Thomford's animals were carried out seven days after transplantation. The longest subsequent survival was seven and a half weeks.

RELEVANCE TO CLINICAL TRIALS

It is virtually certain that the principles outlined in this chapter apply in a general way to auxiliary hepatic transplantation in humans. What is not known, since there have not yet been any opportunities to study long-term human survivors after such operations, is to what degree chronic interliver competition will occur in patients. All that can be said now is that the evidence of homograft atrophy has been suggestive but quite inconclusive in recipients observed for as long as 34 days (Chapters Twenty-two and Twenty-three). All these patients had Laennec's cirrhosis. On the other hand, it is conceivable that the host liver in victims of biliary atresia could be capable of promptly and seriously compromising the welfare of the new organ since the hepatic parenchymal function in such patients is often surprisingly well maintained until just before death. There have not yet been any clinical observations with which to rule out this possibility.

Be that as it may, the host liver in all legitimate candidates for auxiliary transplantation will have been rendered relatively noncompetitive by the disease for which treatment is indicated, the question being only one of degree. Under these circumstances the advantage which must be created for the homo-

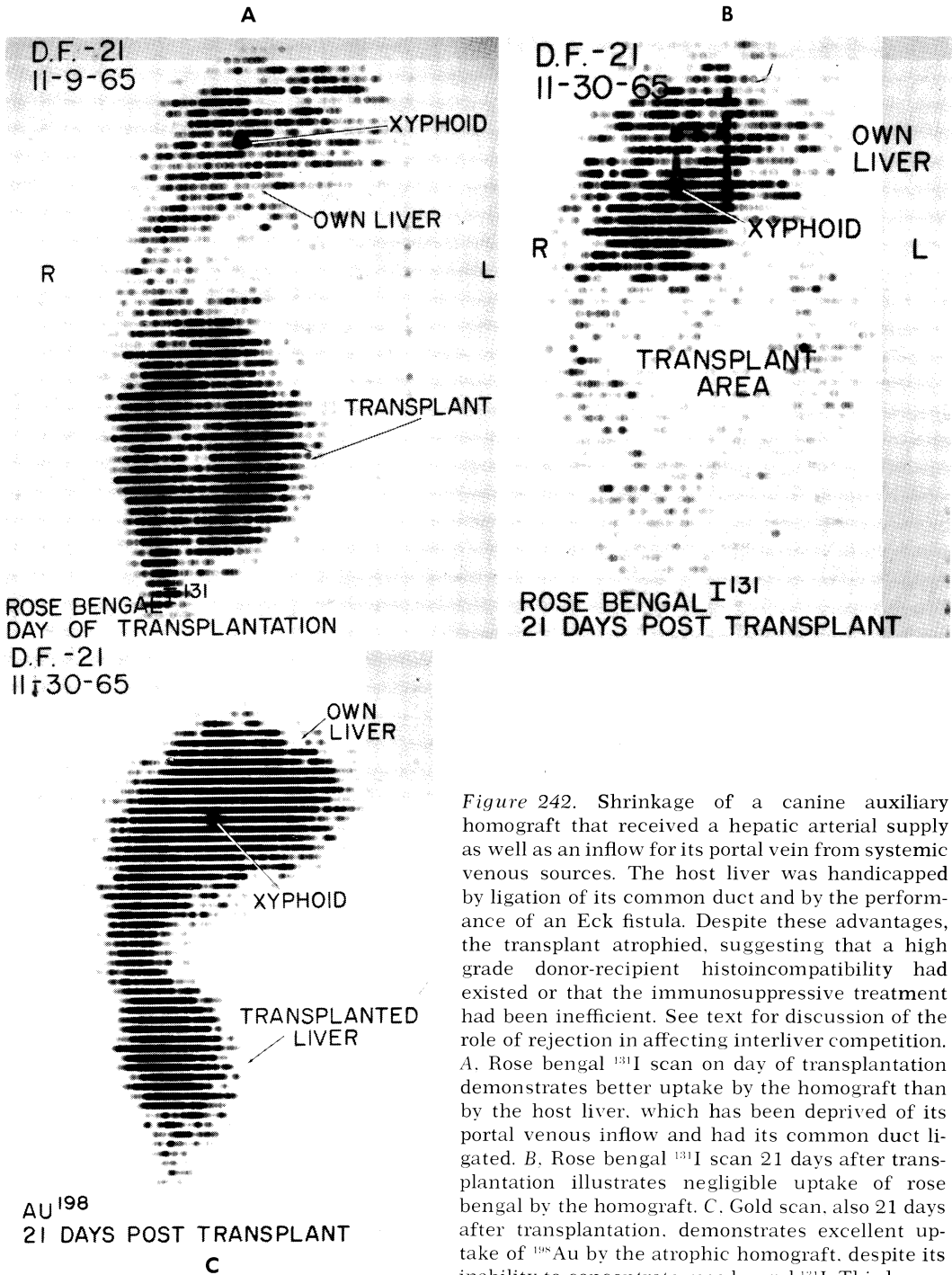


Figure 242. Shrinkage of a canine auxiliary homograft that received a hepatic arterial supply as well as an inflow for its portal vein from systemic venous sources. The host liver was handicapped by ligation of its common duct and by the performance of an Eck fistula. Despite these advantages, the transplant atrophied, suggesting that a high grade donor-recipient histoincompatibility had existed or that the immunosuppressive treatment had been inefficient. See text for discussion of the role of rejection in affecting interliver competition. A. Rose bengal ^{131}I scan on day of transplantation demonstrates better uptake by the homograft than by the host liver, which has been deprived of its portal venous inflow and had its common duct ligated. B. Rose bengal ^{131}I scan 21 days after transplantation illustrates negligible uptake of rose bengal by the homograft. C. Gold scan, also 21 days after transplantation, demonstrates excellent uptake of ^{198}Au by the atrophic homograft, despite its inability to concentrate rose bengal ^{131}I . This homo-

graft, which weighed 464 gm when transplanted, was only 80 gm at autopsy on the thirty-second postoperative day. (By permission of Surg. Gynec. Obstet. 123:1261, 1966.)

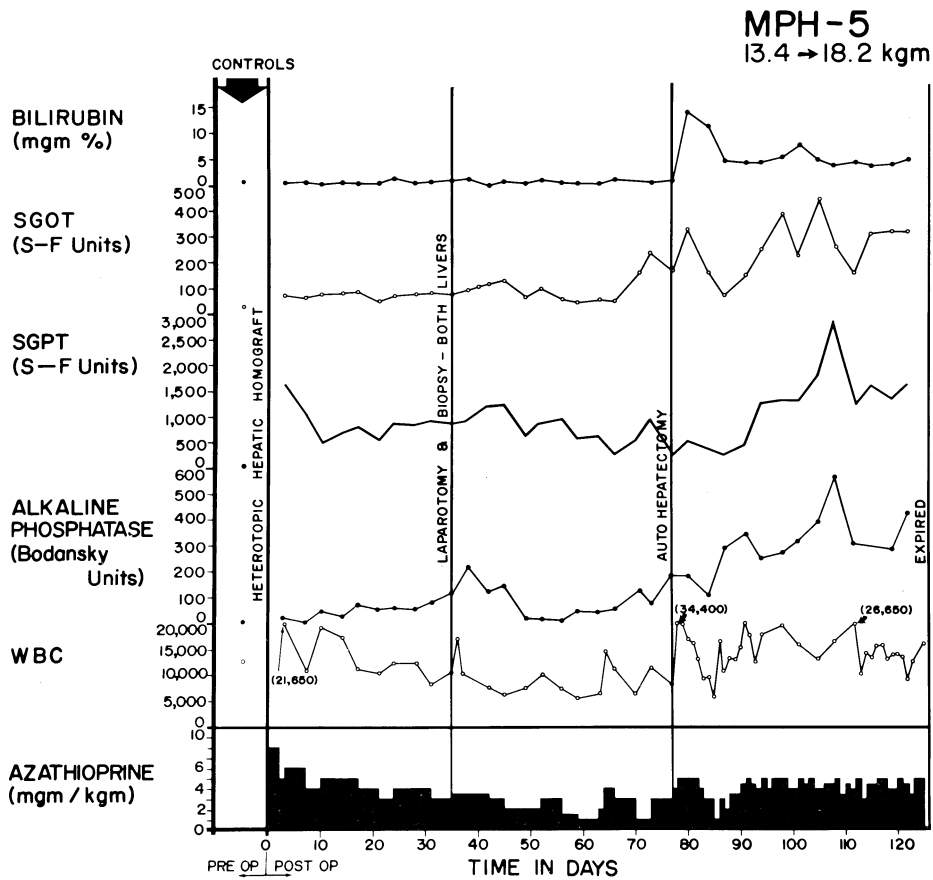


Figure 243. The clinical course of a dog which received an auxiliary liver homograft by the technique shown in Figure 237B. Note the abrupt hyperbilirubinemia that followed removal of the dog's own liver on the seventy-seventh post-transplantation day. After autologous hepatectomy, the dog lived for 49 days with sole dependence upon the homograft, ultimately dying as the result of wound dehiscence and evisceration which followed biopsy. The gain in body weight was due to ascites. (By permission of Surg. Gynec. Obstet. 121:17, 1965.)

graft by the surgical procedure would be predicted to be less, a point that was taken into consideration in planning the operations to be described in the next chapter.

Another feature which may make the precise physiologic environment less critical in humans is the greater effectiveness with which complex and chronic immunosuppressive treatment can be given to patients as compared to experimental animals for reasons described in Chapter Thirteen. As a consequence rejection, which is probably the most important of all the factors described earlier in inhibiting the competitive potential of an auxiliary homograft, can at least be minimized. The use of donor-recipient histocompatibility matching should have the same effect.

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Chapter Twenty-two

CLINICAL AUXILIARY TRANSPLANTATION

This chapter will be concerned primarily with a total of nine auxiliary liver transplantations in man that have appeared in the world literature, four from our own center^{14, 20, 21} and five from other institutions.^{1, 6, 10, 16, 19} In all instances the attempts ultimately failed, the longest postoperative survival being only 34 days.^{14, 21} These documented efforts represent only a small fraction of the actual experience compiled to date, since it is known by personal communication, by newspaper reports, and by word of mouth that many other unsuccessful trials have been made, probably as many as 25. However, inasmuch as the latter cases cannot be carefully reviewed they will be referred to only in the event of some unique or especially interesting feature which has been verified by personal contact with the responsible surgeon.

INDICATIONS FOR OPERATION

The first auxiliary hepatic transplantation in a human was performed on November 3, 1964, by Dr. Karel B. Absolon and his associates at the University of Minnesota.¹ The patient was a 13 month old child with extrahepatic biliary atresia, the same diagnosis as in one of our recipients (AT 3). In the other formally reported cases the patients were adults with alcoholic or postnecrotic cirrhosis,^{6, 14, 19-21} hepatitis,¹⁶ or hepatoma.¹⁰

All the patients suffering from non-neoplastic liver disease (Table 36) were in very poor condition at the time of transplantation, and most were moribund. For example, the three adults with cirrhosis treated at our institutions were either stuporous or in frank hepatic coma. Their serum bilirubin concentrations were 27 to 38 mg per cent and the prothrombin times were 20 to 23 per cent of normal (Table 36). One patient (AT 4) had been essentially anuric for several days with the poorly understood^{2, 4, 17, 18} "hepatorenal syndrome" and required intensive hemodialysis before he could be taken to the operating room. Another had received an emergency portacaval shunt three days previously because of

Table 36. Profiles of Hepatic Transplant Recipients*

CASE	DATE	AGE (Yrs)	SEX	LIVER DISEASE	ASCITES	BILI- RUBIN (mg%)	SGOT	TOTAL PROTEIN	ALBUMIN (gm%)	PRO- THROMBIN TIME (% normal)	DONOR		DONOR- RECIP. BLOOD GROUP	SUR- VIVAL (Days)
											Age	Sex		
Denver cases (Refs. 9, 14, 20, 21)														
AT 1	2-20-65	50	M	Alcoholic cirrhosis	Yes	38	240	5.2	2.8	23	79	M	A-A	22
AT 2	7-5-65	47	M	Alcoholic cirrhosis	Yes	27	190	5.2	2.1	22	12	M	O-O	34
AT 3	11-3-65	1-1/3	F	Extrahepatic biliary atresia	Yes	21	158	6.8	3.7	70	7/12	F	A-A	0
AT 4	6-20-68	48	M	Alcoholic cirrhosis	Yes	31	94	5.2	2.4	20	Newborn	F	A-A	24
Other reported cases (Refs. 1, 6, 10, 16, 19)														
Absolon	11-3-64	1	M	Extrahepatic biliary atresia	Yes	21	200	6.2	2.7	50	2-1/2	F	A-O	13
Cree	10-27-66	17	M	Subacute hepatitis	No	21	216	—	—	10	Elderly	F	O-?	2
Calne	Summer 1967	47	M	Alcoholic cirrhosis	Yes	2	—	—	1.9	50	50	F	Not stated	1/2
Fonkalsrud	3-2-67	46	M	Hepatoma	Yes	1.2	76	6.5	3.5	82	9	M	Not stated	12
Sheil	4-12-68	45	M	Cirrhosis	Yes	12	—	—	—	—	44	F	Not stated	3

*Clinical and biochemical profiles of the recipients in the formally reported cases of auxiliary hepatic transplantation. Survival and donor data are also given. The ischemic intervals of the transplanted livers in the Denver cases are in the captions of Figures 244, 246, 247, and 248.

major hemorrhage from esophageal varices. All three had massive ascites as well as peripheral edema. Even the 16 month old child with biliary atresia was a bad risk since ascites formation and rapid clinical deterioration had started. Perusal of the reports from the other authors^{1, 6, 16, 19} suggests that the same unfavorable conditions pertained in their cases as well (Table 36). The limited and relatively nonspecific resuscitative measures that could be taken were discussed in Chapter Four.

THE DIFFERENT OPERATIONS

Unlike the orthotopic procedure, which has a relatively fixed design, auxiliary transplantation may be performed in several ways and with placement of the new organ in any part of the abdomen except the right upper quadrant. Before deciding on one of the variations, thought should be given to the condition of drainage from the host's nonhepatic splanchnic venous bed, which is present at the beginning and which will eventually exist after completion of the procedure. There are physiologic reasons, discussed at length in the preceding chapter, why such considerations may be important, particularly if the patient's diseased liver retains enough function so it is able to engage in competition with the homograft.

Of even greater practical significance is the fact that the state of the splanchnic venous circulation can profoundly influence the difficulty of the transplantation according to the presence or absence of portal hypertension. This factor may even determine whether it is technically feasible to do the operation at all without an excessive risk of producing uncontrollable venous hemorrhage from high pressure intra-abdominal varices. At least in adults with end stage cirrhosis, it is probably highly desirable if only for the latter reason to decompress the portal system of the recipient, either before or at the same time as the transplantation, by one of the techniques to be described later.

It would be reasonable to place the cases of clinical auxiliary transplantation into categories according to the homograft location, the diseases treated, or other criteria. However, in view of the emphasis in the foregoing remarks and in those of the preceding chapter about the importance of the details of both the host and the transplant vascular systems, the classification used below will be based upon the homograft blood supply.

Arterial Supply Only

In one of our cases (AT 4) and in Absolon's patient¹ only a hepatic arterial supply was restored. Outflow from the homograft was through its distal inferior vena cava which, in turn, was anastomosed to the host systemic venous system. The organs were placed in the left upper quadrant after splenectomy. In our recipient, it had been planned in addition to anastomose the distal splenic vein to the transplant portal vein for two reasons: the double objective was to nourish the new liver by perfusing its sinusoidal bed with splanchnic

venous blood and to coincidentally provide by this route a means for decompression of the patient's portal hypertension.

The effort to restore portal flow to the homograft failed. The recipient was a 48 year old male with Laennec's cirrhosis who was given the liver of a newborn anencephalic monster. The weights of the recipient and donor were 84.0 and 3.1 kg, respectively. A splenectomy was performed through a thoracoabdominal incision. The splenic artery was attached to the distal end of the infant's aorta, which had been removed in continuity with the celiac axis and hepatic artery (Fig. 244). The homograft portal vein was less than a millimeter in diameter and was ligated. Vascular outflow passed through the full length of the donor infrahepatic inferior vena cava, the distal end of which was anastomosed to one of the renal vein divisions in the recipient (Fig. 244); the suprahepatic vena cava was closed by suture.

Until this stage of the procedure, the severe portal hypertension secondary to the advanced cirrhosis had made it impossible to obtain adequate hemostasis

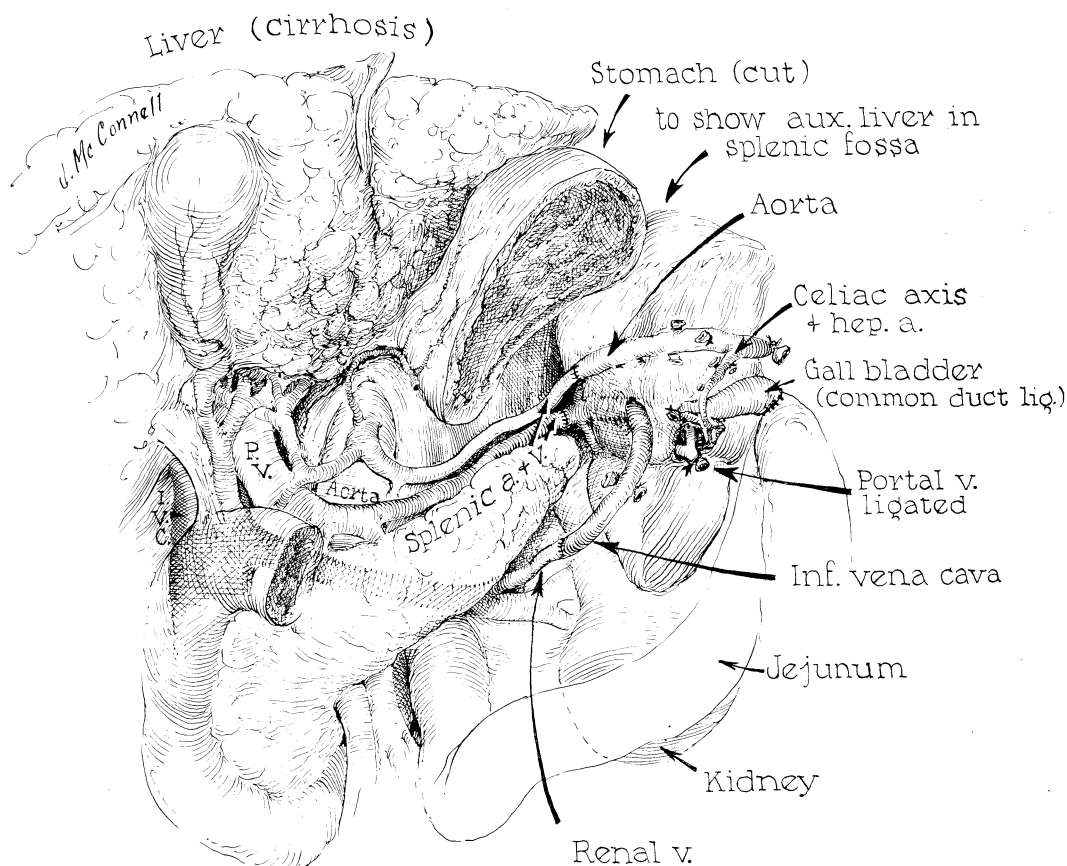


Figure 244. Auxiliary liver transplantation carried out in a 48 year old man with Laennec's cirrhosis (AT 4). The homograft, which was taken from a 3.1 kg. newborn anencephalic monster, weighed 80 gm. Its interval of ischemia, all hypothermic, was 88 minutes. For technical reasons it was possible only to provide an arterial blood supply. Note that the vena cava of the transplanted organ was used as a homovital prosthesis through which to perform a splenorenal shunt. It is probable that the extra flow thereby directed through the homograft vena cava protected it from clotting, to which it might otherwise have been prone if it transmitted only the hepatic venous effluent.

of the operative wound. As a last resort it was decided to perform a splenorenal shunt through the homograft. This was accomplished by reopening the suprahepatic vena cava of the transplant and anastomosing it to the enlarged recipient splenic vein. The host splanchnic blood could then pass through the graft vena cava and directly into the left renal vein (Fig. 244). The venous bleeding ceased immediately, and the procedure was completed by anastomosing the gallbladder to the proximal jejunum through a window in the left mesocolon (Fig. 244). The operation required 14 hours and blood replacement of 8000 ml.

The technique used by Absolon was different in several ways (Fig. 245). Host splanchnic decompression was not attempted. In addition, the connections for the arterial supply and the venous outflow were to the external iliac vessels. Finally, the homograft portal vein and vena cava were anastomosed together (Fig. 245) "in order to decompress the transplant in case. . . prolonged ischemia. . . led to high resistance to blood inflow."¹ In retrospect the addition of this technical detail to the operation was probably unnecessary and, since it could promote shunting of the blood supply away from the sinusoidal bed, it may not have been advisable.

Arterial and Systemic Venous Inflow

Although it is desirable to provide a portal inflow from splanchnic venous sources (Chapter Twenty-one), this may be technically more difficult to achieve in people than in dogs because of species differences in anatomy. The human portal vein and its major tributaries are not only less accessible because of a more complete fusion of the colonic and duodenal mesenteries to the posterior body wall, but they are also less mobile even after being exposed.

Consequently, a compromise procedure was performed in two of our patients (Figs. 246 and 247) aged 50 and 47 years (AT 1 and 2). The auxiliary homografts were obtained from 79 and 12 year old donors of the same blood type, placed in the right paravertebral gutter, and rearterialized from the host aorta or hypogastric artery. Venous blood from the lower extremities was used to perfuse the portal system of the new organ. In both patients standard portacaval anastomoses had been performed three days (AT 1) and one day (AT 2) previously. In the first recipient the preliminary shunt was for the control of a massive variceal hemorrhage, but in the second it was carried out with eventual placement of an auxiliary organ in mind. After transplantation the hemodynamic conditions simulated those (Fig. 241A, Chapter Twenty-one) reported from our laboratories by Halgrimson¹⁴ and from the Cleveland Clinic by Tretbar.²²

The techniques of these two human operations are shown in Figures 246 and 247. They were the same in principle but differed in some details having to do with the level of homograft insertion and the exact source of the arterial and venous blood supply (compare Figures 246 and 247). In both instances outflow from the organ was through its suprahepatic vena cava into the abdominal vena cava of the host. The graft infrahepatic vena cava was closed by suture. To make room for the new organ the cecum and ascending colon were reflected

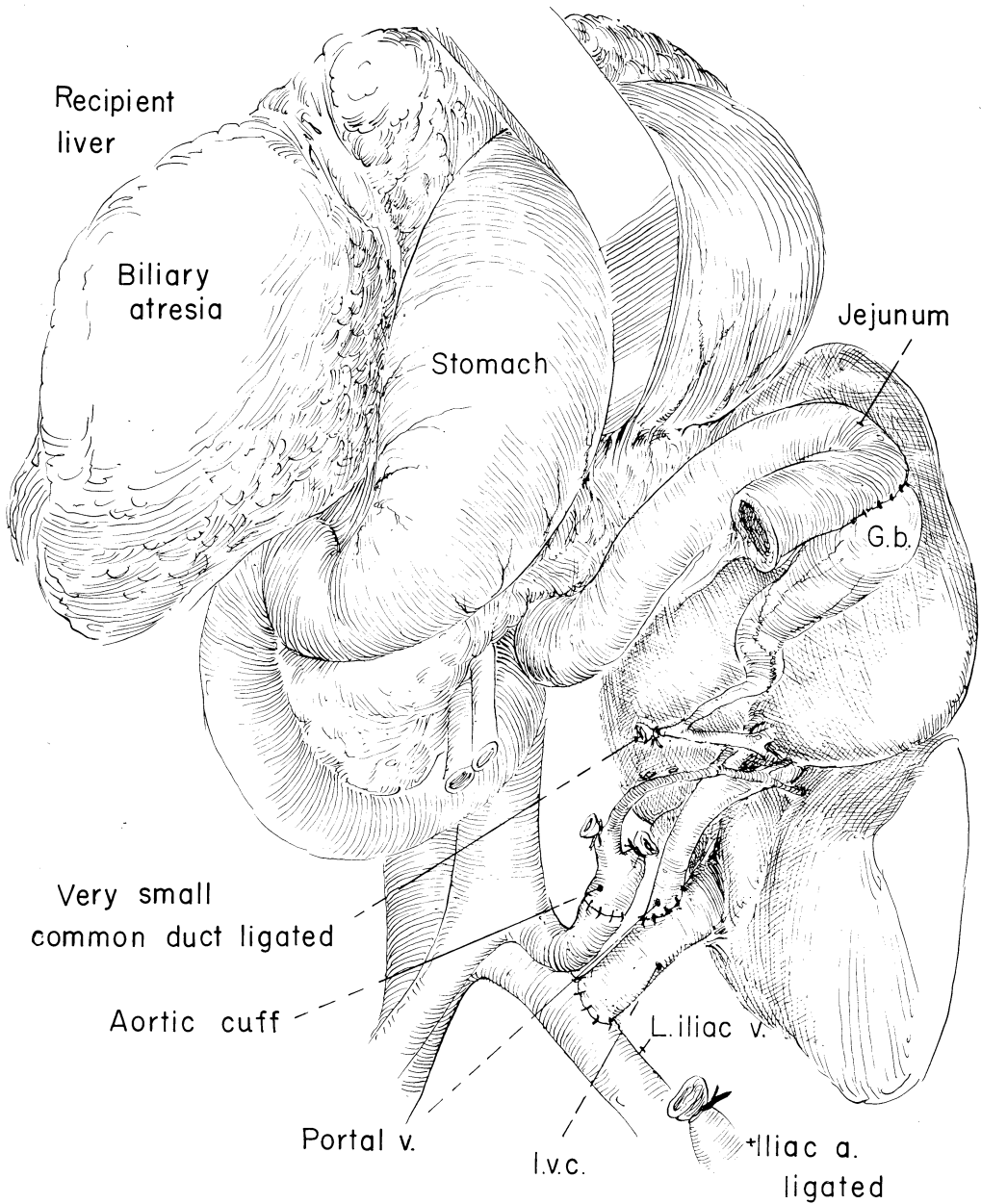


Figure 245. The technique used by Absolon¹ in the first auxiliary hepatic transplantation in a human; only an arterial supply was provided. Note that the portal vein and vena cava of the homograft were joined by anastomosis to ensure an adequate run off in the event of an outflow block.

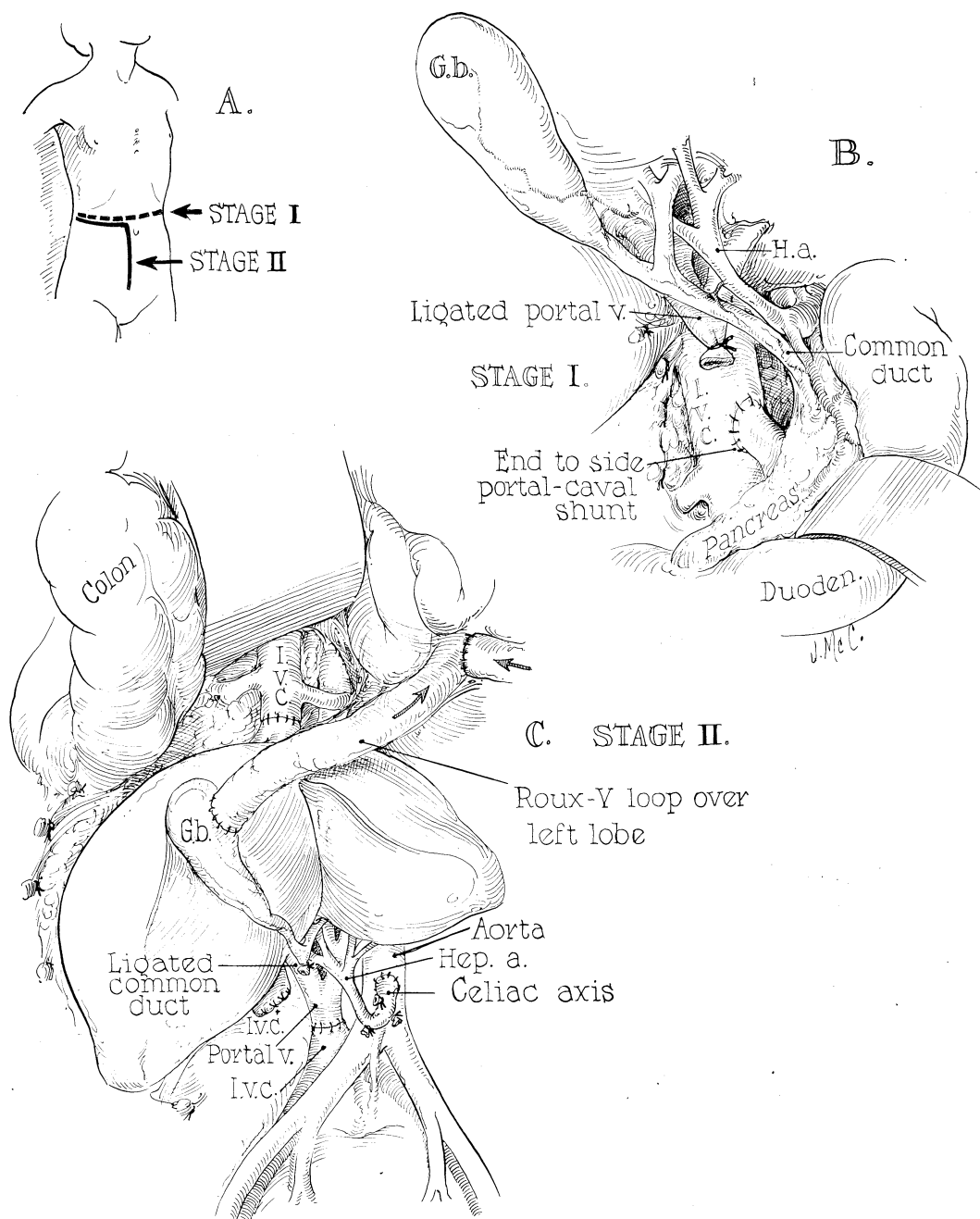


Figure 246. Auxiliary liver transplantation from a 79 year old cadaveric donor to a 50 year old recipient (AT 1) who was dying of Laennec's cirrhosis; both patients had A blood type. The donor had a cardiac arrest on February 20, 1965, which was treated with external and internal cardiac massage for 105 minutes. When a heart beat could not be restored, he was placed on hypothermia-inducing extracorporeal bypass for the next 110 minutes (see Chapter Five), during which time the liver was removed. It was revascularized 49 minutes later in the recipient. **A.** Incisions used for the two operations. **B.** Stage I. An emergency end-to-side portacaval shunt was carried out three days before the transplantation for the control of massive hemorrhage from esophageal varices. **C.** Completed transplantation. Note that revascularization of the homograft is in principle the same as that after portacaval transpositions inasmuch as the portal flow comes from a systemic venous source. In spite of the preliminary portacaval shunt, large venous collaterals in the right retroperitoneal space made reflection of the ascending colon very difficult. The control of hemorrhage required 11 hours and necessitated the transfusion of 5000 ml blood. (By permission of Arch. Surg. 93:107, 1966.)

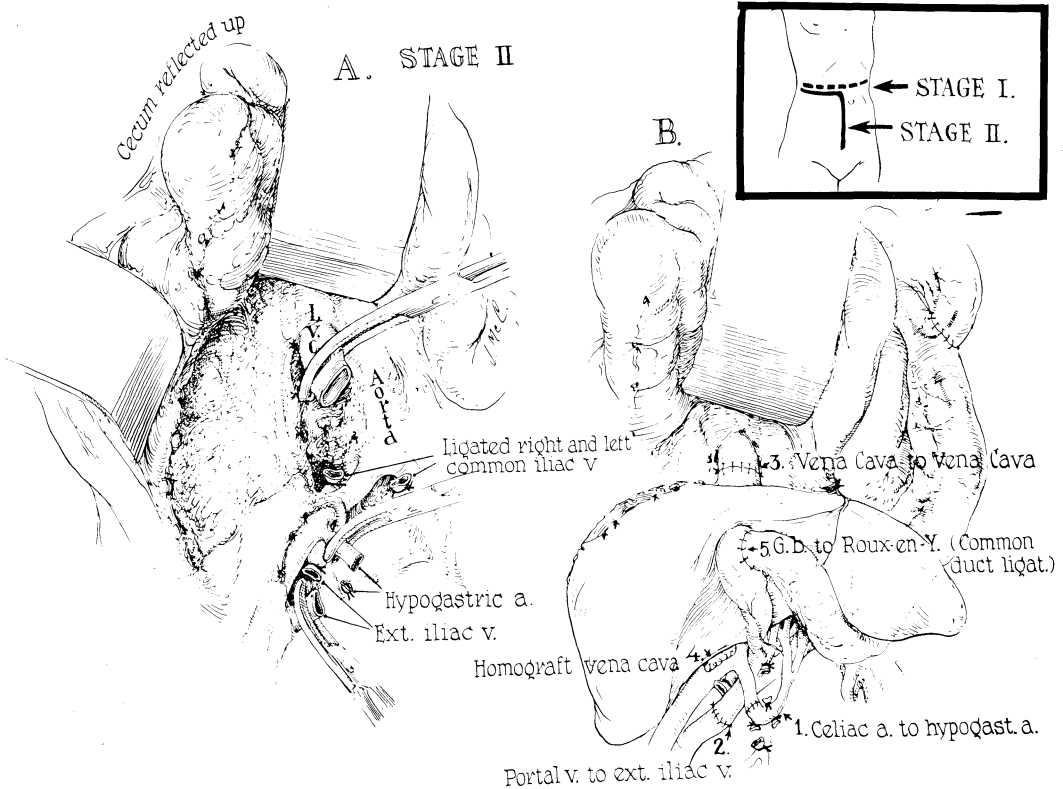


Figure 247. The method of auxiliary liver transplantation used in a 47 year old patient (AT 2) on July 5, 1965; both donor and recipient had O blood type. The donor, a 12 year old boy, died from head trauma a few minutes after arrival at the hospital. Circulation was maintained for 45 minutes by external cardiac massage until the liver could be cooled by intraportal infusion of a chilled electrolyte solution. The subsequent hypothermic period required to remove the liver and revascularize it in the recipient was 120 minutes. A, A segment of vena cava below the anastomosis has been removed, as well as all the right common iliac vein. The field is ready for receipt of the homograft. B, The outflow of the new organ was directed into the transected inferior vena cava. The portal blood supply was obtained from the external iliac vein. The hypogastric artery easily reached the celiac axis for end-to-end anastomosis. The liver was small enough to readily fit behind the reflected cecum. The operation was completed in five hours, with a blood loss of 2500 ml. Note the Roux-en-Y cholecystojejunostomy. (By permission of Arch Surg. 93:107, 1966.)

upward and to the left. The opening of the retroperitoneal plane might have been technically impossible had it not been for the prior relief of the portal hypertension. In spite of this advantage the fact that the venous collaterals had not yet undergone involution complicated the dissection, particularly in patient AT 1, who required intraoperative blood replacement of 5000 ml.

After revascularization had been completed no effort was made to close the retroperitoneal space. Biliary drainage was with a Roux-en-Y cholecystojejunostomy after ligation of the homograft common duct. The abdominal incisions were closed without drainage.

Arterial plus Splanchnic Venous Inflow

There have been five well documented attempts to divert both arterial and splanchnic venous blood through auxiliary homografts. Each of the recipients

died a short time later, although not because of any special problems caused by the method of portal revascularization. The least successful effort was in our institution.^{14, 21} The patient was a 16 month old child with extrahepatic biliary atresia into whose splenic fossa was transplanted the liver of a seven month old infant with Krabbe's disease. The technical difficulties encountered in this case have been published and need only be summarized here. First, the small size of the recipient splenic artery made it necessary to eventually use the host common iliac artery for end-to-end anastomosis to the transplant celiac axis (Fig. 248), as originally described by Absolon.¹ Second, the retrograde flow from

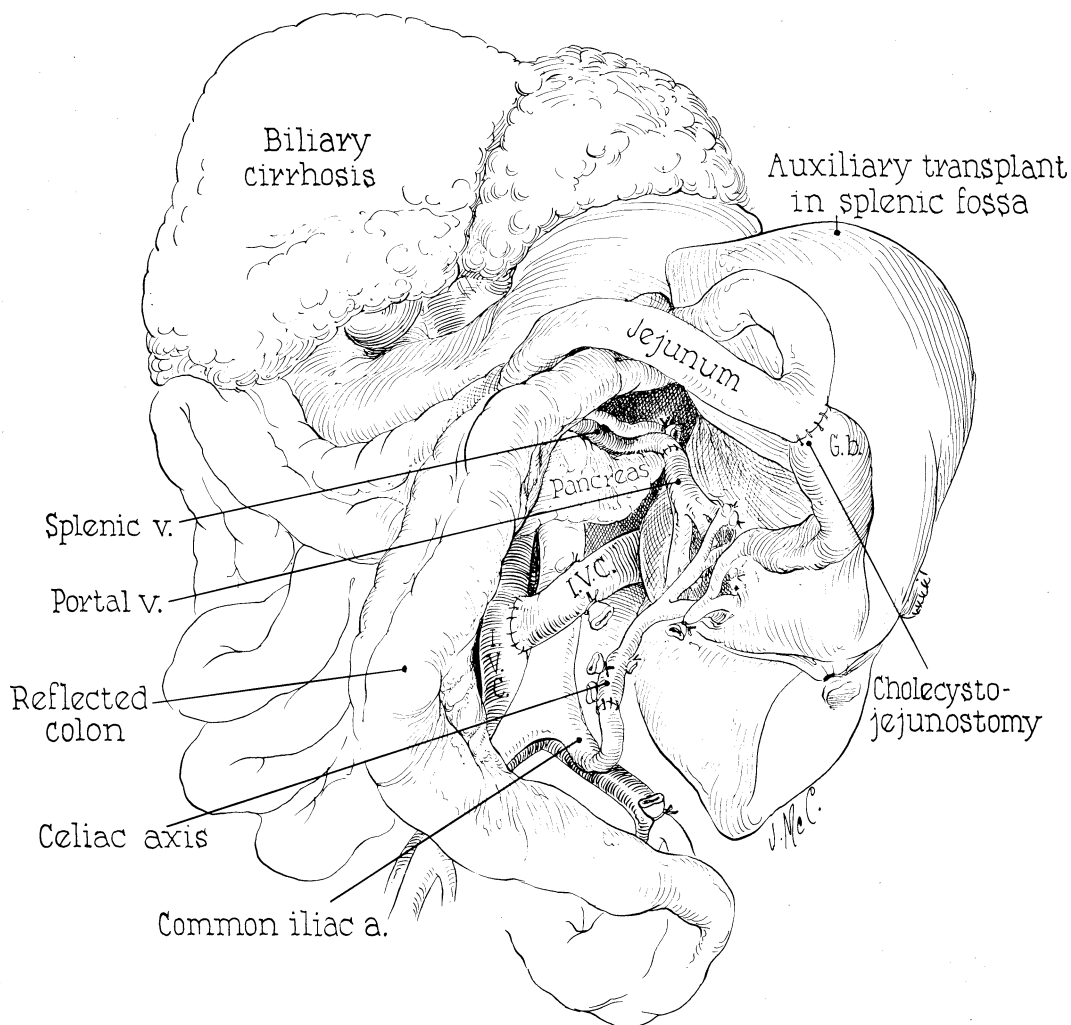


Figure 248. The technique used for attempted auxiliary transplantation in patient AT 3. The donor's temperature was 24°C at the time of death; the liver was further cooled by an infusion technique, removed and revascularized in the recipient 152 minutes later. The homograft faced medially, permitting anastomosis of the host splenic vein and common iliac artery to the homograft portal vein and celiac axis, respectively. Venous outflow was provided by anastomosing the graft abdominal vena cava to the vena cava of the host. Note the angulation near the origin of the left common iliac artery. This was responsible for the technical failure of the transplant. (By permission of Arch. Surg. 93:107, 1966.)

the host splenic vein into the auxiliary organ was sluggish, since portal hypertension was not very severe; consequently, good portal perfusion could not have been expected.

Finally, a serious technical accident occurred during operation. After revascularization the homograft was soft, pink and elaborated bile almost immediately. However, when the abdominal incision was allowed to reapproximate to cover the overcrowded viscera, the host aorto-iliac junction became kinked (Fig. 248). The hepatic arterial supply promptly clotted. The arterial suture line was excised, thrombectomy was carried out, the anastomosis was reformed, and the folding of the iliac artery was relieved by ligation and division of the two most inferior left lumbar arteries.

An arterial supply was partially restored, but there were multiple residual infarcts in the hepatic parenchyma. A liver scan with ^{198}Au was obtained as soon as the wound was closed (Fig. 249). This showed no isotope concentration in the auxiliary liver. Consequently the homograft was immediately removed and the host iliac artery repaired by end-to-end anastomosis. The child had a cardiac arrest and died one hour later. Acute acidosis caused by metabolites

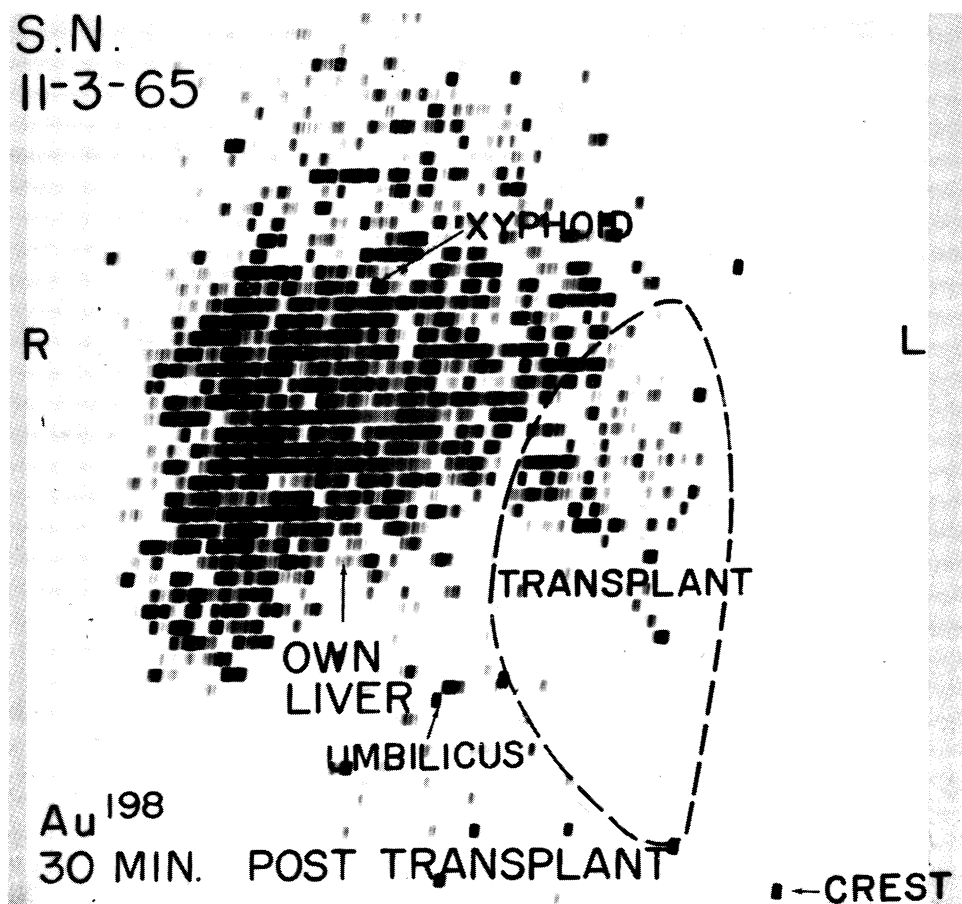


Figure 249. A liver scan obtained immediately in the 16 month old child (AT 3) whose operation is shown in Figure 248. The negligible uptake of ^{198}Au is apparent. The superior portion of the homograft occupies the bed of the excised spleen. (By permission of Surg. Gynec. Obstet. 123:1261, 1966.)

from the transplanted liver and possibly from the recipient's ischemic left lower extremity was suspected to have been responsible for the sudden death.

Calne⁶ has also described a similar attempt at transplantation to the splenic fossa. The donor and recipient were both adults. The patient bled to death a few hours after operation.

In the three other cases reported by Cree,¹⁶ Sheil,¹⁹ and Fonkalsrud¹⁰ the auxiliary livers were placed on the right side of the abdomen. Cree anastomosed the host inferior mesenteric vein to the graft portal vein. Sheil performed the operation shown in Figure 250. These two recipients died after 46 and 76 hours, respectively. All the vascular anastomoses were patent. The probable reasons for the unfavorable course in these patients will be discussed later. Fonkalsrud¹⁰ employed essentially the same operation as Sheil for a 46 year old man with hepatoma who still had good liver function. The hepatic artery, which was revascularized from the host renal artery after right nephrectomy, was found to be clotted at the time of autopsy 12 days later. The transplanted organ was necrotic.

SPECIFIC PROBLEMS

The many difficulties that can be encountered during or immediately after operation are evident from the foregoing accounts. Some of the problems are not specific to auxiliary transplantation since they have also been noted after orthotopic operation. Included are bleeding, intravascular clotting, and acute life-threatening metabolic aberrations. However, there are also some special complications which may result from placing an extra organ into the abdominal cavity.

As already suggested, it is probable that the immediate technical failure from arterial thrombosis in one of our cases (AT 3) was related to this unique factor. Absolon also drew attention to the same aspect of the procedure. To make room for the homograft in the splenic fossa it was necessary in his case to remove the recipient's left kidney as well as the spleen. In spite of the added measure, a photograph of the child in the early postoperative period, published in the report of the case,¹ testified that the closure of the wounds had been with considerable tension.

A more extreme example of intra-abdominal overcrowding was related to us by Dr. David Hume of Richmond, Virginia.¹⁵ In 1964, he attempted to place an auxiliary homograft in the splenic fossa of a teen-aged girl after splenectomy; the donor was an adult. After revascularizing the extra organ, it was impossible to close the incision until after the patient's own diseased liver had been removed.

The existence of such great disparities between the space which is available and that which is needed undoubtedly predisposes to serious pulmonary complications. The same may be true even under more favorable anatomic conditions. The three patients in our series with Laennec's cirrhosis (AT 1, 2, and 4) had abdominal cavities that had been so stretched by massive ascites that adult organs could easily have been placed in each instance, although, in

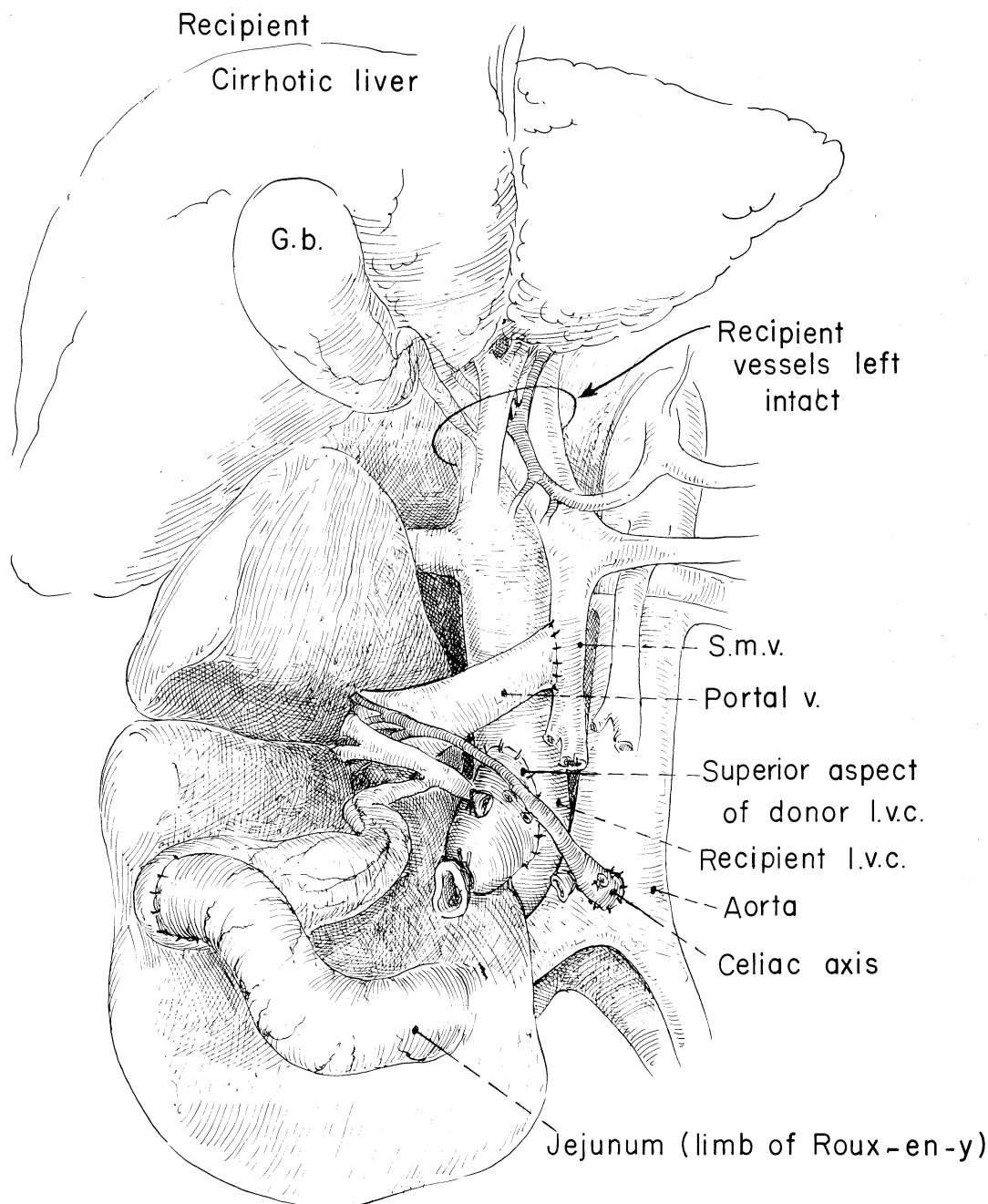


Figure 250. Auxiliary liver transplantation as carried out by A. G. R. Sheil of Australia from a 44 year old woman to a 45 year old man with Laennec's cirrhosis. Note that the transplant was given a double blood supply and that the venous component was from the nonhepatic splanchnic bed. The technique was almost identical to that developed in dogs by Bengoechea-Gonzalez.³ Biliary drainage was with a Roux-en-Y cholecystojejunostomy. Unfortunately the patient died 76 hours post-operatively. There was no evidence of function from the homograft.

actuality, two of these auxiliary livers were obtained from one day (AT 4) and 12 year old (AT 2) donors. Consequently it was not expected that diaphragmatic movement would be impaired. It is disquieting to note that all three patients had difficulty breathing postoperatively and that all had widespread pneumonitis at the time of their death three to five weeks later.

For the indication of biliary atresia there has not been enough experience with auxiliary transplantation to arbitrarily dismiss such a therapeutic approach. However, in view of the lethal pulmonary complications that have developed even under the favorable circumstances mentioned above, it is probable that children with atresia should usually be considered for orthotopic rather than for auxiliary transplantation. With the former procedure, removal of the bulky host liver (and preferably also the spleen) can permit rapid reversal of the respiratory embarrassment that is often present preoperatively. The most important reason is that the amount of tissue removed is invariably several times greater than that which is added. Another advantage of the replacement operation which should not be overlooked is that the abnormal vascular communications feeding pulmonary veno-arterial shunts are functionally almost immediately closed (Chapters Four and Nine). This prompt benefit could not be expected after auxiliary transplantation.

One other extraordinary complication of clinical auxiliary hepatic transplantation has been described to us by Dr. Marvin Gliedman¹¹ of Brooklyn, New York. In his patient the extra liver was placed in the right paravertebral space by a modification of the technique shown in Figure 247. Within a few hours ascites began to form at such a rapid rate that more than a hundred liters of fluid were aspirated in the few days of postoperative life. At autopsy it was found that the intrahepatic and suprarenal vena cava of the recipient had been almost completely closed by compression from the diseased liver. The same problem has been encountered by Fonkalsrud.¹⁰

In a later angiographic and autopsy study,¹⁷ Gliedman showed that this anatomic change is not uncommon in patients with Laennec's cirrhosis; furthermore, the same findings can be produced in dogs by causing liver injury in a variety of ways.¹² The implication of these investigations is evident; if such an abnormality were present, the outflow of the new liver would be placed below the site of an obstruction with all the techniques described in this chapter. Consequently Gliedman¹¹ has recommended that a venacavogram be considered a mandatory step in the evaluation of any patient for auxiliary hepatic transplantation.

IMMUNOSUPPRESSION

The first two patients who survived operation in our institutions (AT 1 and 2) were treated with the double drug regimen of azathioprine and prednisone that was described earlier in the book. The average daily doses administered were summarized in Table 17, Chapter Thirteen. The complete courses of these patients have also been shown graphically in Figure 109, Chapter Thirteen, and

Figures 251 and 252. The attempts at therapy failed, both because of drug toxicity and because of the inability to control rejection.

The third recipient who survived operation (AT 4) was treated with the triple drug protocol of azathioprine, prednisone, and ALG (Fig. 253). The development of thrombocytopenia after two weeks necessitated discontinuance of the immune globulin. The doses of azathioprine were very small (Fig. 253), averaging only 0.36 mg/kg per day for the 24 days of post-transplantation life. It is not surprising that he was unable to support larger quantities since the presence of both hepatic and renal failure compromised the two most important pathways of detoxification of this drug (see Chapter Thirteen). Leukopenia was not produced at any time, despite which the patient developed multiple infectious complications (Chapter Sixteen).

COURSE AND LIVER FUNCTION

The survival after all reported attempts at auxiliary liver transplantation is given in Table 36. Absolon's patient¹ unquestionably obtained function from the new organ during 13 days of survival, even though the direction of tissue transfer was across a strong red cell group incompatibility (A to O). The bilirubin fell from about 20 mg per cent to almost normal, coincident with the appearance of bile in the stools; prothrombin times were improved.

In contrast there was no objective evidence of supplementary hepatic function in the cases of Cree,¹⁶ Calne,⁶ Sheil,¹⁹ and Fonkalsrud.¹⁰ The bilirubin concentrations in the sera of their recipients fell during operation, an effect which was probably explicable by the infusions of fluid and blood. Two of the patients with non-neoplastic liver disease^{16, 19} were comatose before the procedures were begun and did not wake up afterward; Calne's recipient, who was rational before operation, did not recover from anesthesia.⁶ Refractory hypotension developed shortly after the three auxiliary organs were revascularized and this persisted until death 14, 46, and 76 hours later, respectively. Although hepatocellular necrosis of the homografts was not a particularly prominent autopsy finding, it is probable that very serious ischemic injury had occurred during the last stages of donor death and in the course of the transplantation. As mentioned earlier, the homograft underwent necrosis in Fonkalsrud's case¹⁰ because of thrombosis of the hepatic artery. The patient survived for 12 days, probably because hepatic failure was not present preoperatively; the indication for operation was hepatoma. For reasons discussed in Chapter Seventeen, auxiliary transplantation for hepatic malignancy will probably not be considered advisable in future cases.

Patient AT 4. Organ damage from ischemia was probably not the explanation for failure to demonstrate a metabolic contribution by the homograft in the adult patient in our series who received the liver of an anencephalic monster and who was treated with triple drug immunosuppressive therapy. The donor was placed on a mechanical ventilator immediately after birth, was cooled to 30° C before hepatectomy, and was known to have had an effective circulation

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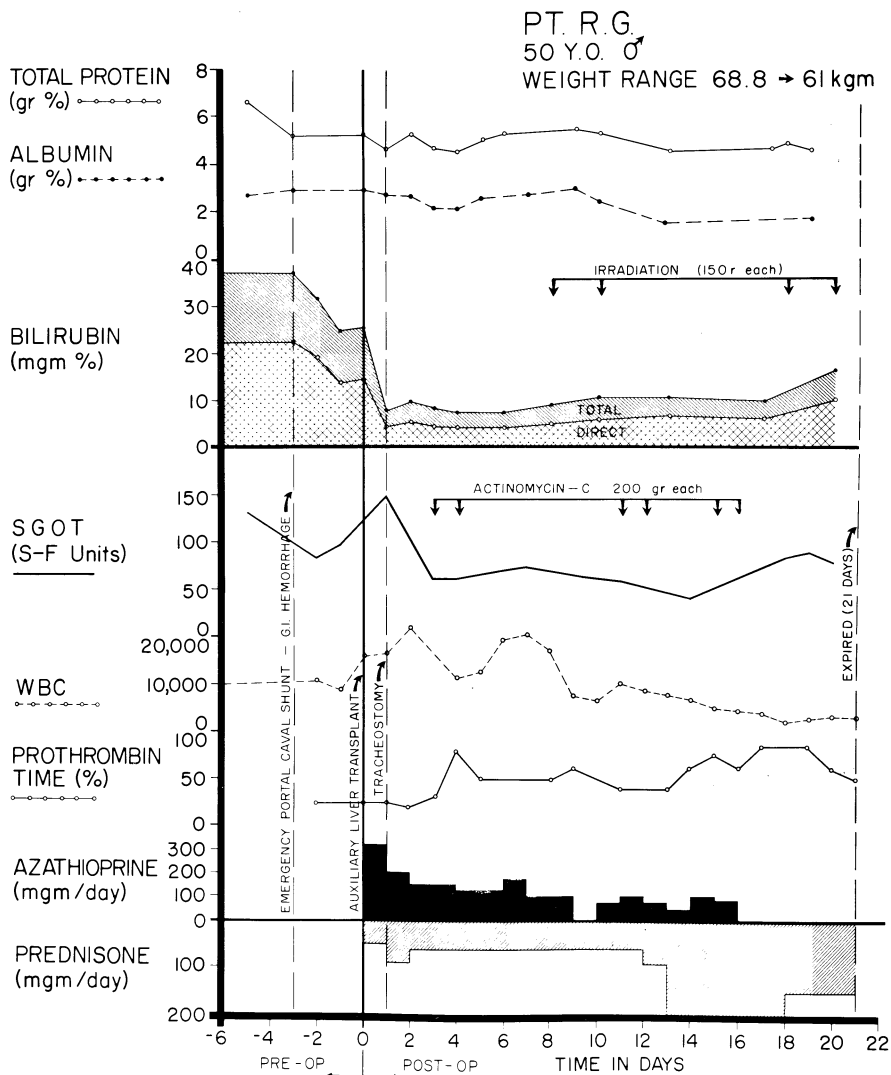


Figure 251. The clinical course of a patient (AT 1) with Laennec's cirrhosis who received an auxiliary liver homograft; the operation is depicted in Figure 246. The preliminary portacaval shunt controlled massive hemorrhage from esophageal varices. Note the evidence of function of the transplanted liver, with a fall in the serum bilirubin and a sustained improvement in the prothrombin time. The rejection that followed was not well controlled. During the last several days of life there was profound leukopenia. The irradiation dosages were to the center of the homograft. (By permission of Arch. Surg. 93:107, 1966.)

AT 2 47 y.o. ♂
68.5 → 62.6 kg.

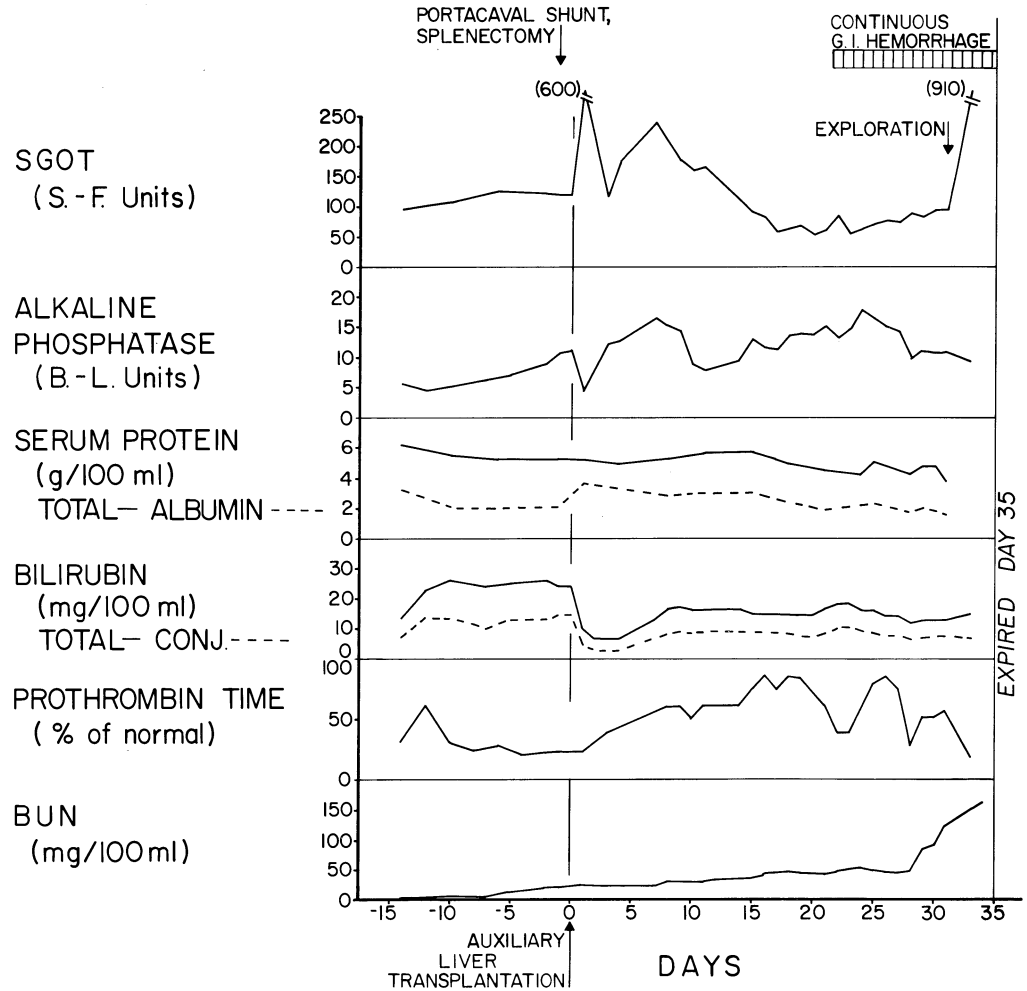


Figure 252. The course of the patient (AT 2) whose operation is depicted in Figure 247. There was apparently good initial function which later deteriorated when rejection was not controlled. Note the rapid development of azotemia late in the course. The gastrointestinal bleeding which was the immediate cause of death was due to widespread intestinal moniliasis; the exploratory laparotomy was made in an effort to control the bleeding. The details of the immunosuppression used in this case as well as the consequent effects upon the peripheral hematologic findings are shown graphically in Figure 109, Chapter Thirteen. Profound leukopenia developed during the fourth post-operative week.

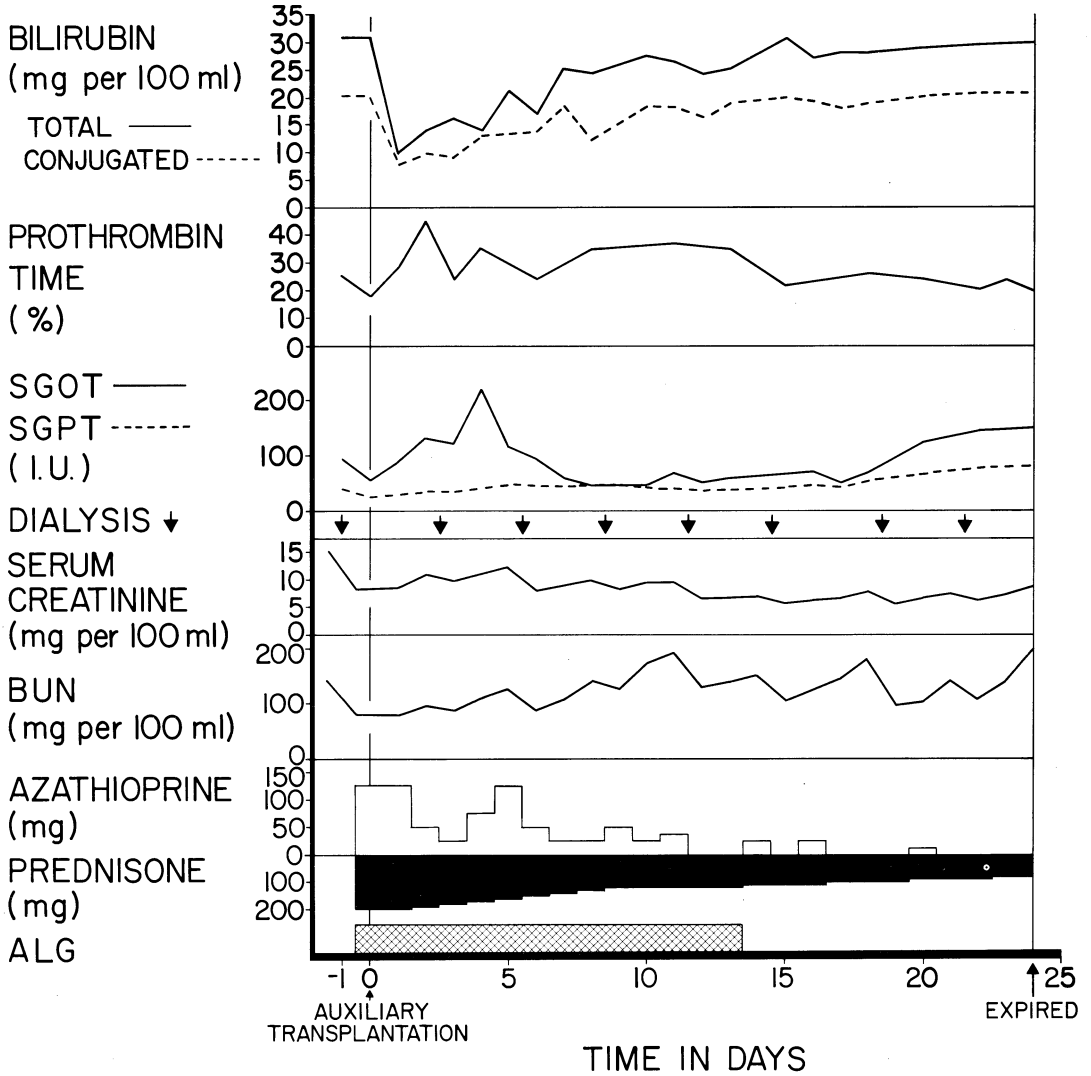


Figure 253. The course of a patient (AT 4) with Laennec's cirrhosis and hepatorenal syndrome who received a newborn auxiliary homograft. The transient reduction of jaundice was probably due to the blood transfusions during operation. The patient required emergency hemodialysis before operation and at frequent intervals thereafter. Death on the twenty-fourth day was due to hepatic and renal failure and to pneumonitis.

until almost the moment of organ removal and hypothermic perfusion. The subsequent interval of cold ischemia was 88 minutes.

Instead, the difficulty in detecting function of the auxiliary organ may have been the consequence both of its tiny size (80 gm) and its physiologic immaturity. Newborn livers are well known to have striking limitations of function due at least in part to deficiencies of microsomal enzymes required for drug detoxification and excretion and for the metabolism of naturally occurring substrates, including bilirubin.⁷ As specific examples, the activities of UDPG dehydrogenase and glucuronyl transferase, which are necessary for the conjugation of bilirubin and other compounds, are notably reduced at birth.^{5, 8}

The course of the patient is summarized in Figure 253. Before operation he was stuporous. Afterward and for the next few days he was perfectly oriented and cooperative, but this may have been due to the massive transfusions of fresh blood. Within a few days his clinical condition began to deteriorate slowly. The intermittent hemodialysis that had been commenced preoperatively was continued, since his own kidneys never resumed life-sustaining function. The immediate cause of death on the 24th postoperative day was pneumonitis, the underlying causes being hepatic and renal failure. He also had a severe infection of his thoraco-abdominal wound.

As with the clearing of sensorium, the changes in liver function tests could have been due to the intraoperative exchange transfusion. There was an acute fall in the serum bilirubin (Fig. 253). Thereafter the jaundice gradually deepened to about its previous degree. There were slight variations in the other liver function tests, but these were neither great enough nor sufficiently sustained to ascribe them to the presence of the new liver. The most suggestive finding was an improvement in the prothrombin time, which was maintained for slightly more than two weeks; at the same time other clotting factors (especially V) also increased (Fig. 254). Total serum proteins were essentially unaltered. In spite of the lack of unequivocal evidence for its function, the transplant was proved with liver scans to be viable throughout the entire postoperative period. The results of the scans will be returned to later.

Patients AT 1 and 2. The other two recipients who survived auxiliary transplantation in our institution had adequate to excellent hepatic function during the first postoperative days (Figs. 251 and 252). The early transaminase changes were moderate, suggesting that a severe ischemic injury had not occurred. Both patients had prompt lowering of the pre-existing hyperbilirubinemia. At about the same time, or shortly thereafter, they developed watery diarrhea which was green and had an obviously high bile content. However, the most convincing changes were of the prothrombin times, which were badly depressed prior to operation, but which were strikingly restored toward normal afterward. The total serum proteins were less affected, either remaining at about the preoperative level (Fig. 251) or being slightly improved (Fig. 252). The significance of the early protein measurements was obscured by the fact that albumin infusions were given to both patients during operation.

Clinically there was a prompt clearing of sensorium from the preoperative stuporous or comatose state. The patients began to eat within a few days and one (AT 2) took an adequate diet for more than three weeks. The latter recipient

AT 4

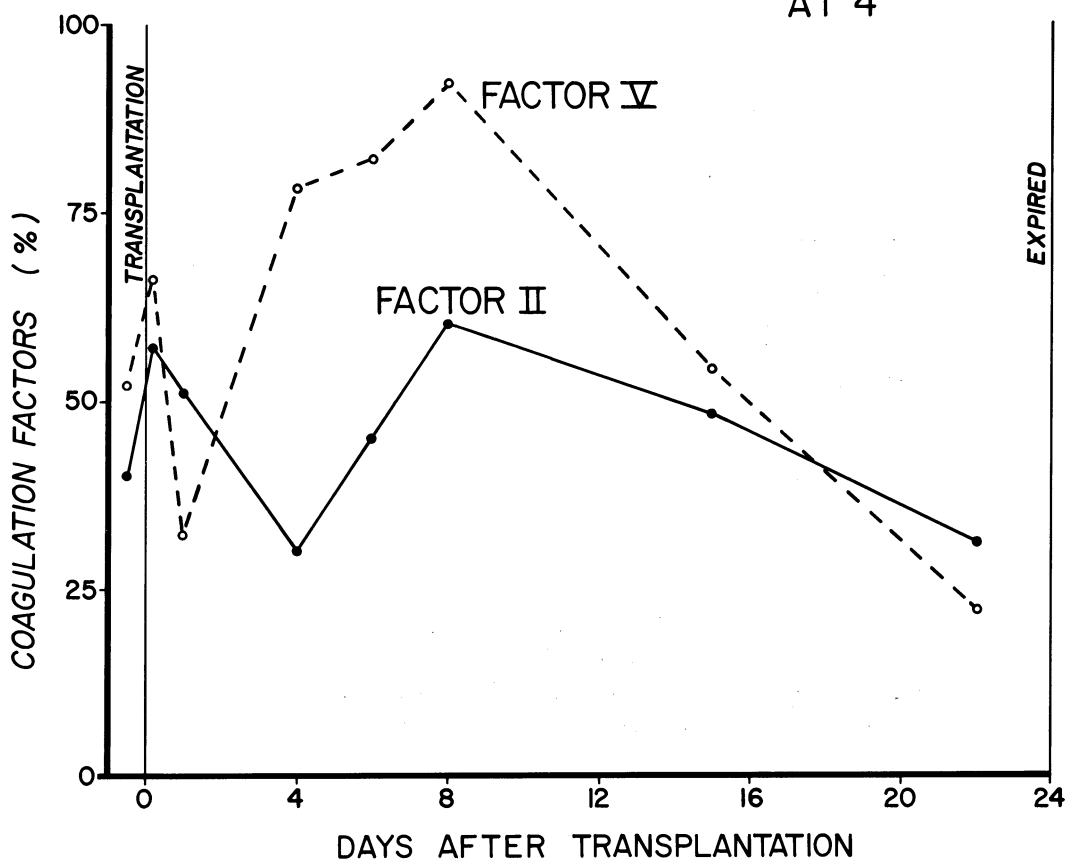


Figure 254. Clotting factors in a 48 year old man with Laennec's cirrhosis (AT 4) who received the liver of a newborn anencephalic monster. The immediate improvements in the clotting Factors II (prothrombin) and V (accelerator globulin) were probably due to massive intraoperative transfusions. However, the increase in the coagulation factors from days 4 to 16 did not seem explicable on this basis. The research studies were carried out by Dr. Liberto Pechet. Note that Dr. Pechet's analyses of prothrombin activity did not reveal exactly the same results as were obtained in the hematology laboratories (compare with Figure 253); however, the general conclusions were the same.

appeared to be quite healthy at first. The other man (AT 1), who had required a tracheostomy on the first postoperative day, was clinically very ill throughout the remainder of his life.

The double drug therapy with azathioprine and prednisone did not prevent the onset of moderately severe rejection. Within a week the bilirubin concentration began to increase secondarily more slowly in the first (Fig. 251) than in the second patient (Fig. 252). The latter recipient also had rises in the alkaline phosphatase to levels far exceeding those present before the transplantation (Fig. 252). The other measurements of liver function were not affected so early. It will be recalled (Chapter Fourteen) that the manifestations of homograft repudiation in cases of orthotopic homotransplantation have also often had an initial component strongly suggestive of biliary obstruction.

Unfortunately the process was not reversed (Figs. 251 and 252) despite the administration of the immunosuppressive drugs in doses that proved to be

lethal. Eventually there was deterioration of all measures of liver function. Death was contributed to by recurrence of the hepatic failure, but was caused primarily by the complex of infections which was considered separately in Chapter Sixteen (see Table 21). Both patients had widespread pneumonitis. One (AT 2) ultimately bled to death from multiple gastrointestinal ulcerations, apparently caused by monilia (Fig. 153, Chapter Sixteen). Survival was for three (AT 1) and five (AT 2) weeks.

Before the auxiliary transplantations both these patients had subnormal kidney function; although their blood urea nitrogens (BUN) were low, the creatinine clearances were less than 35 ml/min. After operation but before the beginning of the liver homograft rejection, urine excretion was well maintained or even improved. With the onset of this process, renal function deteriorated, most severely in patient AT 2, whose last BUN and serum creatinine were 156 and 9.0 mg per cent, respectively (Fig. 252).

THE QUESTION OF ATROPHY

In patient AT 1 there was minimal evidence at autopsy 22 days after operation that atrophy had affected the auxiliary liver. The homograft was somewhat smaller (1400 gm) than normal adult size but it had the same gross appearance as at the time of its transplantation. Moreover, there were few histopathologic features to suggest that shrinkage had occurred (see Chapter Twenty-three).

An attempt was made in the next case (AT 2) to measure the size of the auxiliary organ with serial liver scans. Two techniques were used.⁹ With one, rose bengal ¹³¹I was administered intravenously in a dose of 150 μ c. Since the elimination of this isotope is through the hepatocytes, it could also be used as a crude test of the relative parenchymal function of the two coexisting livers. Alternatively, radioactive gold ¹⁹⁸Au, which is picked up by the reticuloendothelial system, was given. With the latter method the size and configuration of the liver can be assessed in analogous canine experiments long after the ability to define the organ with rose bengal has been lost.⁹

One day postoperatively in patient AT 2 a gold scan revealed intense concentration in the homograft, but none in the cirrhotic autologous liver (Fig. 255). On the eleventh postoperative day after most of the gold had cleared from the transplant, a rose bengal scan was performed and it also showed a greater concentration in the homograft; this examination was carried out after the clinical rejection had started. Rose bengal scans on the fourteenth, twenty-first and twenty-eighth days continued to show the homograft uptake to be greater than in the patient's own liver (Fig. 256). At no time did the visually estimated ratio of the rose bengal specific activity seem to change in favor of one organ or the other, although ultimately the concentrating ability of both organs appeared to have diminished in parallel. On the thirty-second day a second gold scan again visualized the homograft exclusively, with no detectable activity found in the autologous liver (Fig. 255). With both kinds of scans there appeared to be a diminution in the size of the transplanted organ; however, the changes could have been due to the loss of isotope concentrating ability rather than a real

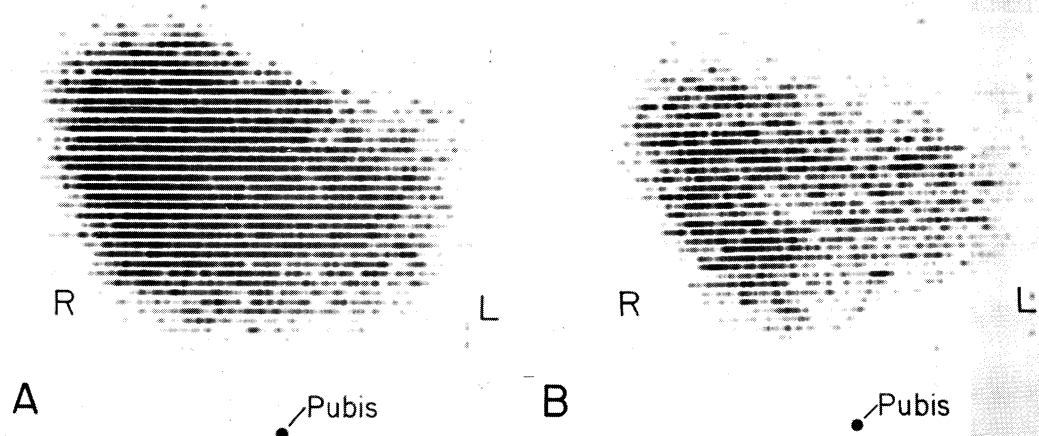


Figure 255. Gold scans (^{198}Au) obtained on the first (A) and thirty-second (B) days after auxiliary liver transplantation (AT 2). The field shown is the lower abdomen; the patient's own liver is not included since it did not pick up any isotope on either occasion. Note that the homograft seems smaller after residence in the new host for one month. However, the apparent change in dimensions may have been due to the poor concentrating ability, rather than to any real reduction in size. The photographic techniques were exactly the same for both of the scans.

reduction in size. The impression of atrophy could not be supported with certainty from the histopathologic studies of the homograft (Chapter Twenty-three). However, both hepatocyte loss and collapse of the supporting reticulin were features of the autopsy specimen.

The third patient (AT 4) who survived the operative procedure of auxiliary transplantation at our institution also had several liver scans. The first one was with ^{198}Au on the first postoperative day. For subsequent examinations $^{99\text{m}}\text{Tc}$ -technetium was used.¹³ There was a progressive increase in the size of the homograft and a decrease in its concentration of isotope (Fig. 257), presumably as it was suffering a rejection. At autopsy the homograft was found to be swollen. Its weight was 370 gm compared to 80 gm 24 days previously; the edges were rounded. The histologic findings are described in Chapter Twenty-three.

FUTURE PROSPECTS OF AUXILIARY TRANSPLANTATION

Until now life has not been prolonged for more than a few days in any recipient by means of auxiliary liver transplantation. The disappointing results may be explained partly by the generally very poor condition of the patients treated, by overly toxic immunosuppression in most of the early cases, and by a high incidence of technical difficulties. However, it is probably more than coincidence that the results with such operations in dogs have also been extremely disappointing (see Chapter Twenty-one).

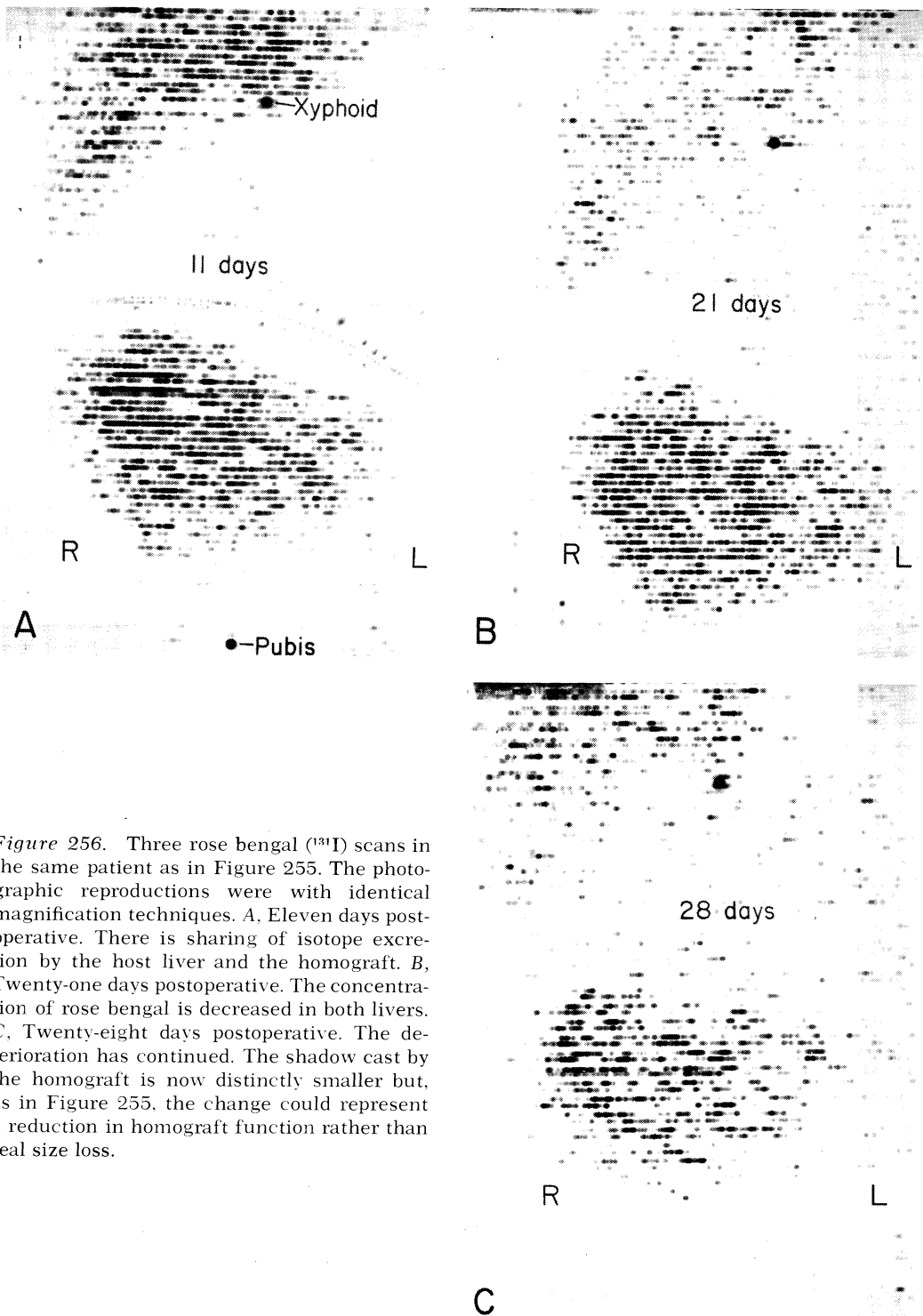


Figure 256. Three rose bengal (^{131}I) scans in the same patient as in Figure 255. The photographic reproductions were with identical magnification techniques. A, Eleven days postoperative. There is sharing of isotope excretion by the host liver and the homograft. B, Twenty-one days postoperative. The concentration of rose bengal is decreased in both livers. C, Twenty-eight days postoperative. The deterioration has continued. The shadow cast by the homograft is now distinctly smaller but, as in Figure 255, the change could represent a reduction in homograft function rather than real size loss.

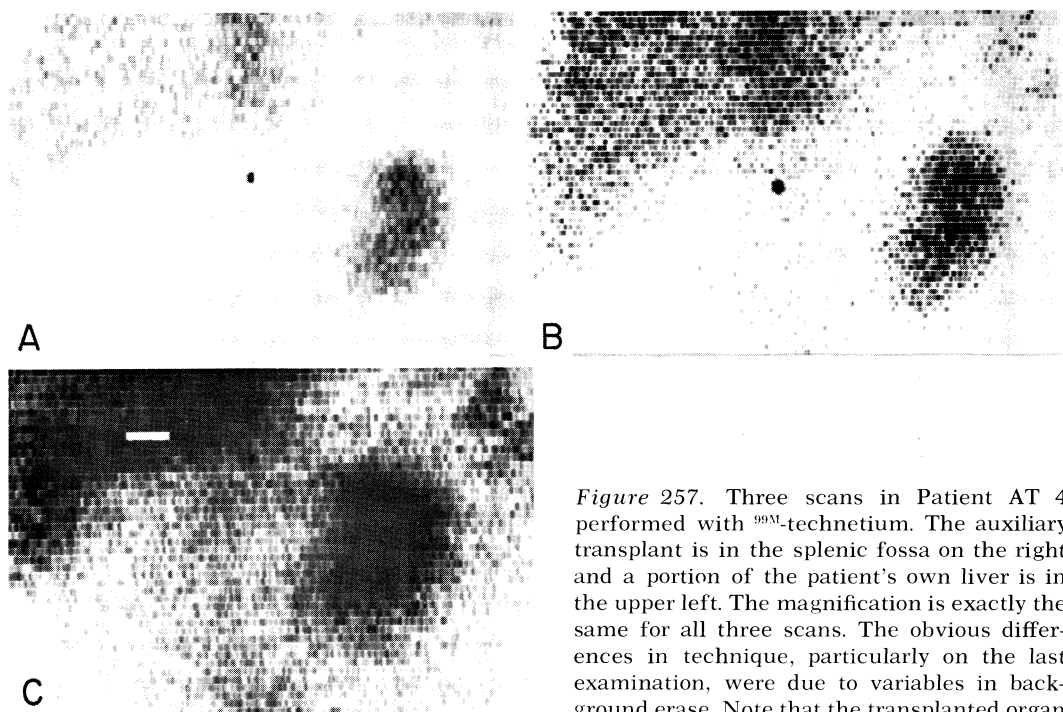


Figure 257. Three scans in Patient AT 4 performed with ^{99m}Tc -technetium. The auxiliary transplant is in the splenic fossa on the right and a portion of the patient's own liver is in the upper left. The magnification is exactly the same for all three scans. The obvious differences in technique, particularly on the last examination, were due to variables in background erase. Note that the transplanted organ

retains its size and, if anything, becomes bigger. A, Four days post-transplantation; B, seven days post-transplantation; C, 19 days post-transplantation.

Nevertheless, it may ultimately be found that auxiliary transplantation can be used with benefit for victims of non-neoplastic liver disease under the appropriate circumstances. Because of the space problems described earlier and in view of the frequency of pulmonary complications to which abdominal overcrowding may have contributed, it would seem prudent to give special thought to the relative size of the homograft which is available. Our present belief is that the organ would ideally come from a smaller donor and be placed in a recipient whose abdomen had been prepared by stretching with long-standing ascites. Such a perfect anatomic situation was present in one of our cases (AT 2).

If the operation is not technically simple, many of its most important advantages are lost. For this reason we would plan in future trials to do the compromise operation depicted in Figures 246 and 247, in which the homograft is revascularized as with portacaval transposition in the right lower abdomen; preoperative venacavograms would be highly desirable to establish the patency of outflow channels. Furthermore, we would probably think it mandatory for reasons discussed earlier to first perform a host portacaval shunt even if this meant storing the organ (Chapter Five) for several hours while the portal decompression was accomplished. We would no longer consider auxiliary transplantation to the splenic fossa since it seemed to be a more traumatic and technically demanding operation.

The clinical observations made so far about interliver competition and atrophy are by no means conclusive. Although homograft shrinkage did not

occur at a rapid rate within the follow-up intervals of three to five weeks, there is still no assurance that this would not have occurred later. In the patient with the longest survival (AT 2) a kind of competition could be monitored throughout the postoperative course, at least in terms of the division of rose bengal excretion. The proportions seized by the two livers did not change drastically but the size of the homograft, at least as measured by scans, seemed to gradually become smaller. To what extent a sharing of other metabolic processes might have influenced the long term welfare of either organ can only be speculated upon in the absence of more information.

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Chapter Twenty-three

PATHOLOGY OF THE AUXILIARY HOMOGRAFT

by K. A. Porter, M.D., D.Sc.

As reviewed in Chapter Twenty-one, the fate of the auxiliary hepatic transplant is influenced to an extraordinary degree in animals by competition between the recipient's own liver and the homograft. Atrophy of one or the other coexisting livers may occur according to their relative physiologic advantages, including the quantity and quality of their blood supply and the state of their biliary drainage. Moreover, the functional interrelationship of the organs can be unpredictably unbalanced in outbred animals by the magnitude of the immunologic attack upon the transplant. Under these circumstances it is necessary in examining an auxiliary homograft to differentiate the histopathologic changes that are secondary to a nonimmunologic etiology from those that are due to rejection. The distinction may be difficult in any given case.

THE MORPHOLOGIC CONSEQUENCES OF LIVER COMPETITION

Consequently the most precise information about interliver competition has come from the study of preparations in which immunologic factors were avoided. In some of these experiments the blood supply of a portion of the canine liver was altered *in situ*⁷ by the performance of "split transposition" (Chapter Twenty-one); in others, parts of the canine liver were relocated as autografts,^{11, 13, 18} or partial liver isografts were transplanted to ectopic sites in rats.⁶

In Marchioro's investigations⁷ of split transposition in seven dogs (Fig. 239, Chapter Twenty-one), the hepatic arterial supply and biliary drainage were not disturbed. Both portions of the divided liver were thought to have received initially about the same quantity of venous inflow to the portal vein; however, this was derived from the inferior vena cava on one side and from the normal

splanchnic venous source on the other. The part of the liver receiving caval blood atrophied after 70 to 94 days in every case (Fig. 240, Chapter Twenty-one). Histologically it contained shrunken lobules with centrizonal atrophy, depletion of glycogen, and irregularity of cell size, shape, and staining. There were often central collapse, reticulin condensation, and sinusoidal irregularities. Capsular thickening, increased subcapsular lymphatics, centrizonal cholestasis, and an increased number of Kupffer cells were present in a few of the animals. The blood vessels and intrahepatic bile ducts were normal. Ultrastructurally many cells showed marked reduction of rough and smooth endoplasmic reticulum; the remaining cisterns were often dilated. Glycogen was scarce and there were moderate numbers of small fat vacuoles. In some cells the electron density of the hyaloplasm was greatly reduced. Mitochondria were sparse, were often vacuolated, and occasionally contained fibrillar inclusions.

The liver tissue receiving splanchnic venous inflow was grossly hypertrophied. Microscopically this portion of the liver appeared to have larger lobules and hepatocytes than were present in the pretransposition biopsy. There were also binucleate and trinucleate liver cells, mitoses, and proliferating bile ducts. Ultrastructurally the enlarged cells were essentially normal, although the profiles of endoplasmic reticulum were more irregularly arranged than usual.

The other studies cited on page 516 in which portions of livers were revascularized in heterotopic locations^{6, 11, 13, 18} have agreed about the value of providing a splanchnic venous inflow to the liver for the purpose of preventing transplant atrophy. In Lee and Edgington's investigations with rat isografts,⁶ 30 per cent of the host liver was left in place, with an obstructed duct system but with an intact splanchnic venous and arterial blood supply. The heterotopically placed partial hepatic isografts (30 per cent of a donor liver) also had bile duct obstruction. In some of their experiments in which the transplants were given only an arterial inflow, an injury pattern could be seen within the first day. The livers developed edema of the portal regions and space of Disse, and mild hydroptic changes occurred in the hepatocytes. By day three the pericholangiolar region had become infiltrated by polymorphonuclear leukocytes together with a few lymphocytes and monocytes. After day three bile duct proliferation in the portal tracts became prominent. Subsequent atrophy was profound at the same time the host liver hypertrophied. In contrast, when the host hepatic remnant was deportalized and the isograft given a normal double blood supply, a progressive gain in the graft mass occurred which was most rapid during the first seven days after transplantation and which was completed by day 21.

The numerous other ways in which interliver competition may be unbalanced in favor of one organ or other were described in Chapter Twenty-one.

AUXILIARY LIVER HOMOGRAFTS IN UNTREATED DOGS

There have been several descriptions of the pathologic and immunopathologic changes that occur in whole auxiliary liver transplants in unmodified dogs.^{3, 5, 9, 10, 12, 16, 17}

A particularly careful study was made by the Mount Sinai group of New York City.^{5, 10, 12} In the first two days they found only some nonspecific centrilobular ischemic necrosis and an increase in prominence of the Kupffer cells. About the fourth day after transplantation lymphoid cells with basophilic cytoplasm appeared in the portal tracts and these cells steadily increased in numbers over the next seven days. Foci of necrosis of hepatocytes were first noted five days after transplantation. These were particularly common at the periphery of the lobules, and as they progressed the liver cells adjacent to the portal tracts disappeared. With graft destruction the composition of the cellular infiltrate changed and macrophages, polymorphonuclear leukocytes, and plasma cells began to predominate. Two livers removed at 20 days were shrunken and wrinkled, and the liver cells were entirely replaced by either pools of blood or infiltrating cells; the latter were mostly plasma cells. The arteries remained unchanged throughout; the veins were narrowed by 20 days.¹²

Ultrastructurally many of the infiltrating mononuclear cells contained few profiles of rough endoplasmic reticulum but had numerous polyribosomes. These cells were found in the tissue spaces adjacent to and indenting the hepatocytes. The indented hepatocytes were smaller than normal and lacked surface microvilli. Macrophages and fibrin deposits were present in the spaces of Disse and the sinusoids were compressed by the large number of infiltrating cells.⁵

Immunofluorescent studies revealed that in the first week after transplantation only a few of the infiltrating, pyroninophilic, mononuclear cells contained IgG and there was no binding of immunoglobulins or complement to the vessels and bile ducts. During the second week IgG was found in many more of the infiltrating cells and was now localized in the cytoplasm of the bile duct cells and in the intima and media of the vessels. Complement was fixed in vitro by the same structures.¹⁰

When interpreting these findings, it must be borne in mind that all these transplants were deprived of portal vein blood and that this complicating element alone would be sufficient to cause necrosis of hepatocytes and shrinkage of the graft.⁸

AUXILIARY LIVER HOMOGRAFTS IN UNTREATED RATS

Exactly the same reservation applies to the observations made by Lee and Edgington.⁶ They studied the sequence of the rejection of Sprague-Dawley to Lewis rat homografts, using one of the partial transplant models mentioned in the first section of this chapter in connection with their work with isografts. Both the host and auxiliary organs had bile duct obstruction. The recipient's own liver had a double blood supply, whereas the transplant was nourished only by an artery.

During the first two days the homografts did not differ histologically from isografts. Then abruptly on day three a cellular infiltration appeared in the

portal zones. This infiltrate was quantitatively greater than that observed in the isografts and consisted predominantly of large lymphoid cells. Numerous mononuclear cells were found within portal venules and frequently appeared to be in intimate contact with the endothelial surface of these vessels. Rare lymphoid cells were found in the region of the central veins. The pericholangiolar infiltrate of polymorphonuclear leukocytes seen in the isografts was also found. In the next three days mitotic figures were frequent in the portal infiltrate.

On day seven mononuclear cells appeared within the lobules in association with endothelial cells and the surface of hepatocytes. At the same time there was focal necrosis of hepatocytes, and mononuclear cells were frequently found in immediate contact with the injured liver cells. The number of mononuclear cells in the portal regions also diminished and portal edema increased. Arteritic lesions were not seen. The cellular infiltrate and hepatocellular injury, initiated on day seven, rapidly progressed and the graft was destroyed by day 10 or 11.

Immunofluorescence showed that at all stages only a few of the mononuclear cells contained IgG. A fine linear deposit of fibrin appeared along the sinusoids on day seven.

When livers were transplanted from Brown-Norway strain rats to Lewis recipients the same events occurred but more rapidly, indicating that the degree of histocompatibility was a significant factor.

AUXILIARY LIVER HOMOGRAFTS IN TREATED DOGS

When whole auxiliary livers are heterotopically transplanted to normal dogs that are receiving immunosuppressive treatment with azathioprine, it is found that the auxiliary grafts markedly diminish in size within a few weeks after operation.^{15, 16} This fate is in contrast to that of orthotopic hepatic homografts in similarly treated animals (Chapter Twenty). The difference in behavior of the two types of homograft is not immunologic; shrinkage of the auxiliary homograft is due to competition with the unhandicapped host liver, as explained earlier in this chapter and in Chapter Twenty-one. If the situation is reversed so that the nonhepatic splanchnic blood flow is taken from the recipient's own liver and directed through the homograft, the dog's own organ shrinks.⁸ In the host liver the centrilobular hepatocytes undergo necrosis and there is collapse and condensation of the reticulin in the centers of the lobules (Figs. 258 and 259).

When the homograft is given a physiologic advantage of this magnitude in the azathioprine-treated canine recipient, the pathologic features of modified rejection are not especially different from similarly immunosuppressed orthotopic transplants (Chapter Twenty). Under these circumstances⁸ the majority of the auxiliary livers become infiltrated by lymphoid cells around the small branches of the portal vein and around the central hepatic veins (Fig. 260), but sequential biopsies show that the cellular infiltration tends to decrease with time. Later, centrilobular fibrosis (Fig. 261) and, to a much less extent, periportal fibrosis occur. In the longest survivors these changes progress to the

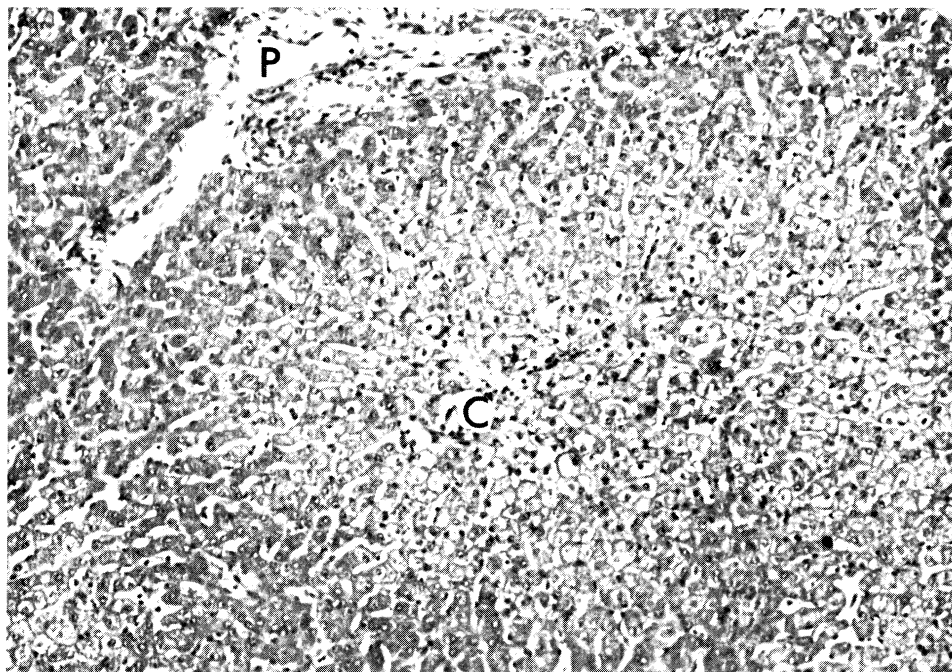


Figure 258. Biopsy of host liver from a dog with an auxiliary homograft. Thirty-five days previously an operation had been performed on the animal, directing the splanchnic blood flow in a retrograde fashion through the homograft. The dog's own liver was supplied only by the hepatic artery. There is necrosis of the centrilobular hepatocytes. P=portal tract; C=central vein. (Hematoxylin and eosin stain, $\times 65$.)

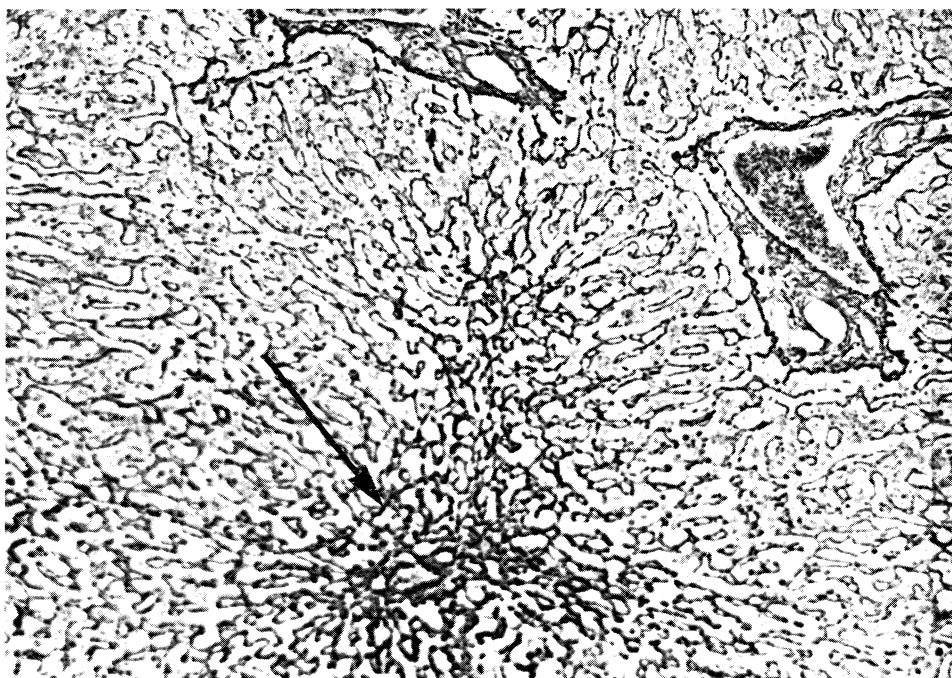


Figure 259. Biopsy of host liver from a dog with an auxiliary homograft. Fifty-one days previously the splanchnic blood flow had been diverted through the graft and the dog's own liver was left with only a hepatic arterial supply. The liver shows marked collapse of the centrilobular reticulin (arrow). (Reticulin stain, $\times 90$.)

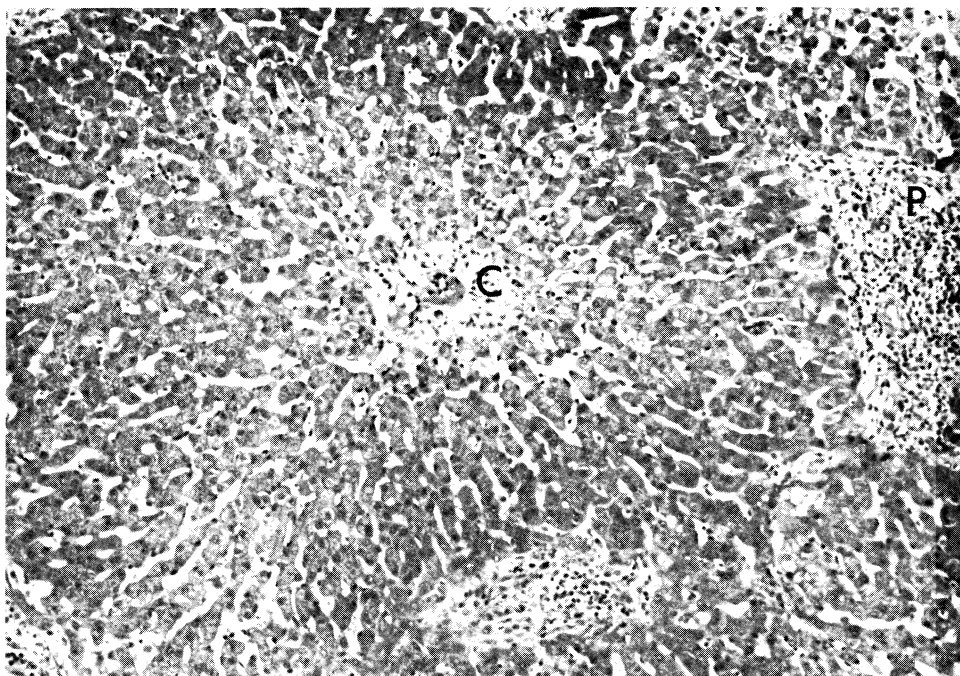


Figure 260. Biopsy of canine auxiliary hepatic homograft that was provided with a splanchnic blood flow at the time of transplantation 35 days previously. The portal tracts (P) contain many infiltrating lymphoid cells. There is an accumulation of fibrous tissue around the central vein (C) and this area is lightly infiltrated with lymphocytes. This dog's own liver is shown in Figure 258. (Hematoxylin and eosin stain, $\times 65$.)

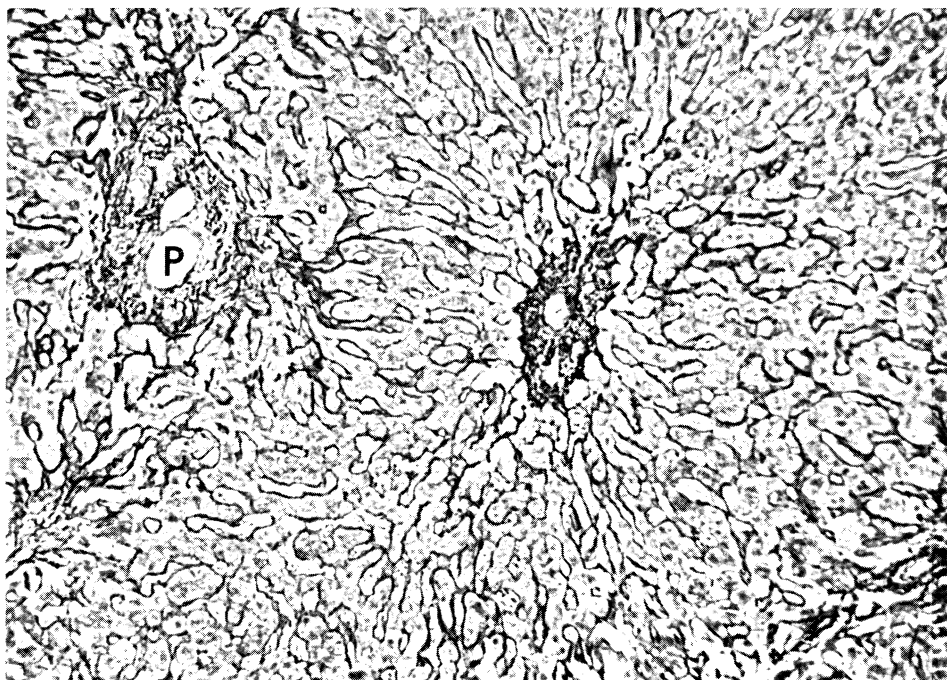


Figure 261. Biopsy of canine auxiliary hepatic homograft that was provided with a splanchnic blood flow at the time of transplantation 61 days previously. There is a dense accumulation of reticulin and collagen fibers around and within the wall of the central hepatic vein. P=portal tract. (Reticulin stain, $\times 65$.)

development of fibrous and reticulin bands which link some of the affected central zones (Fig. 262). In association with this hepatic fibrosis there is marked accumulation of inspissated bile in the centrilobular canaliculi. Fibrinoid necrosis of the branches of the hepatic artery is sometimes seen, but not commonly.¹⁻⁸ Fibrous intimal thickening of either arteries or veins has not been described.

The protection of auxiliary homografts from "competition atrophy" is not an all or none effect (Chapter Twenty-one); if the transplant is favored but to a lesser extent than described above, atrophy may be reduced but not prevented under comparable conditions of immunosuppression. Then the pathologic changes may represent the consequences of rejection injury combined with those of nonimmunologic damage.^{2, 4, 8, 16, 17}

Another example of such a compromise procedure was reported by Van der Heyde et al in dogs treated with azathioprine.¹⁹ They transplanted the left lobe of the liver with only an arterial supply and handicapped the host liver by depriving it of portal blood and by obstructing its bile drainage. Nine of the 11 recipients died after three weeks to 46 days; two other animals were still alive after 35 days and three months, respectively. Only three (or four) of these 11 transplanted liver fragments dropped from their pretransplantation weight during residence in the new hosts. The others either weighed the same or more at autopsy or else looked bigger at surgical re-exploration than at the time of the original transplantation. No details were given about the histologic changes in the grafts of the long survivors.

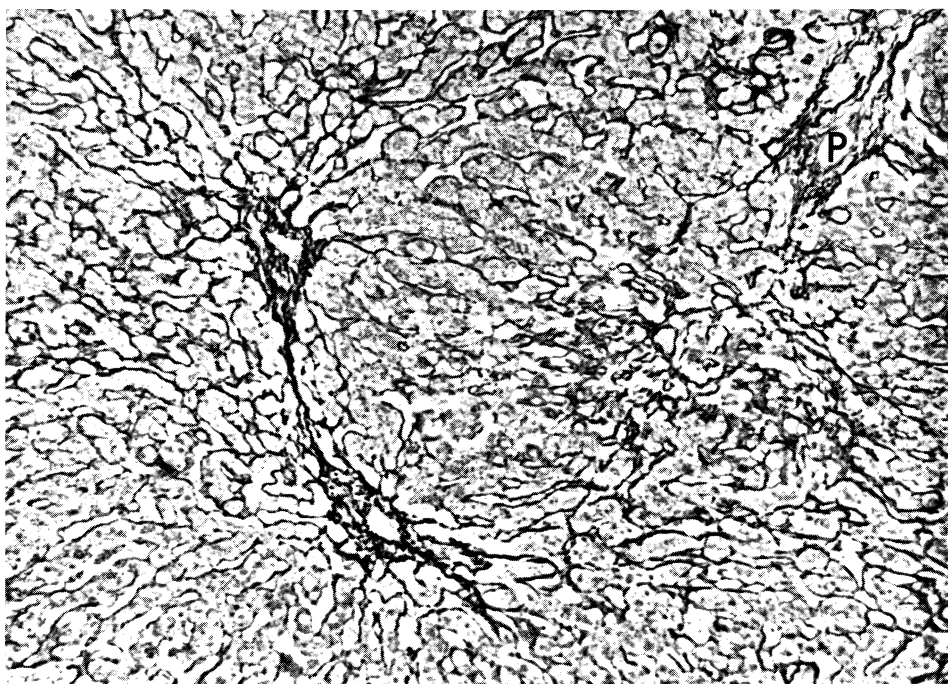


Figure 262. Canine auxiliary hepatic homograft 120 days after transplantation. The graft was provided with a splanchnic blood flow. A band of reticulin fibers links two central veins. P=portal tract. (Reticulin stain, $\times 65$.)

HUMAN AUXILIARY HOMOGRAFTS

The four Denver recipients of auxiliary hepatic homografts died a few hours to 34 days after operation. The pathologic changes in three of these organs were previously reported.^{4, 14}

Graft Removed Five Hours After Transplantation

The hepatic arterial supply to a transplant in a 16 month old child (AT 3) became thrombosed on the operating table. Although reanastomosis was performed, a liver scan with ¹⁹⁸Au showed no isotope concentration in the liver; therefore, the graft was removed (Chapter Twenty-two).

The liver contained many areas of hemorrhagic infarction. In the intervening liver there was necrosis of the central and midzonal hepatocytes with preservation of a thin rim of liver cells around the portal tracts. Several hepatic artery branches and portal vein tributaries were thrombosed. Hemorrhages were present in the portal tracts, but there was no cellular infiltration.

Grafts Examined 22 to 34 Days After Transplantation

Three auxiliary hepatic homografts were examined at 22, 24, and 34 days after transplantation. Two of the livers (AT 1 and 2) had a double blood supply restored, the portal inflow being from systemic venous sources; the third liver (AT 4) received only an arterial inflow.

In the shortest survivor (AT 1) the portal tracts were densely infiltrated with mononuclear cells, between 5 and 10 per cent of which were large lymphocytes with pyroninophilic cytoplasm (Fig. 263). The portal tracts of the other two grafts (AT 2 and 4) contained very few lymphocytes, with several macrophages and eosinophils. Less than 5 per cent of the lymphoid cells had pyroninophilic cytoplasm.

Intracanalicular bile stasis was a feature of all three homografts; it was associated with dilated bile ductules that contained casts of inspissated bile (Fig. 264). Atrophy of the centrilobular hepatocytes was also present. It was most severe in the longest survivor and was accompanied by collapse and condensation of the central part of the lobular reticulin framework (Figs. 265 and 266). Fat deposits were present in the midzonal and peripheral hepatocytes of two of the grafts (AT 2 and 4). In the graft that received only a hepatic arterial supply (AT 4) there was also necrosis of many of the hepatocytes in the central and middle zones of the lobules. There was no hepatic fibrosis or cirrhosis.

In the graft with marked cellular infiltration (AT 1) several of the smaller hepatic artery branches showed foci of fibrinoid necrosis in their walls, and in many of the larger arterial branches there was fibroelastic intimal thickening and reduplication of the internal elastic lamina. The lesions in the larger branches were thought to have been present in the liver before transplantation;

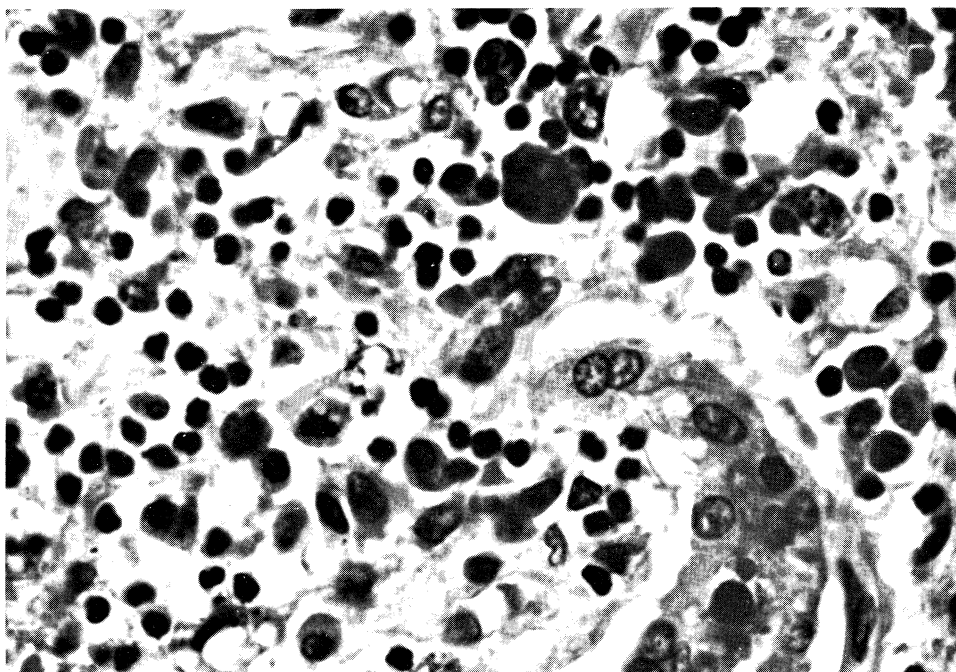


Figure 263. Human auxiliary hepatic homograft (AT 1) 22 days after transplantation. High power view of a portal tract which is infiltrated with mononuclear cells. (Hematoxylin and eosin stain, $\times 600$.)

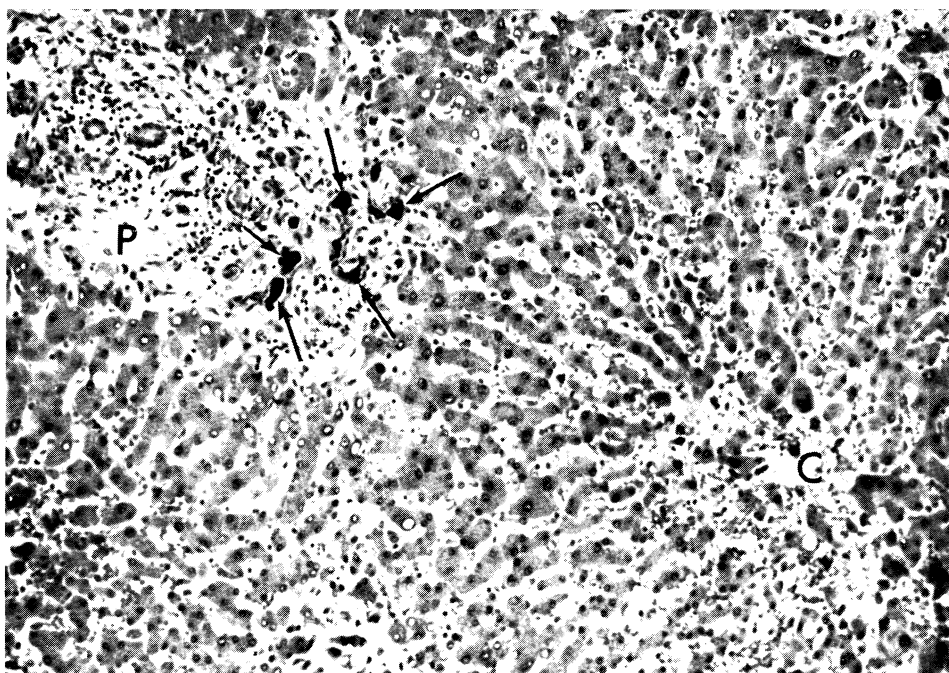


Figure 264. Human auxiliary hepatic homograft (AT 1) 22 days after transplantation. The portal tract (P) is infiltrated by mononuclear cells and several of the smaller bile ducts contain casts of inspissated bile (arrows). There is cholestasis in the centrilobular bile canaliculi, excess of lipofuscin in the central hepatocytes and atrophy of the liver cells adjacent to the central vein (C). (Hematoxylin and eosin stain, $\times 80$.)

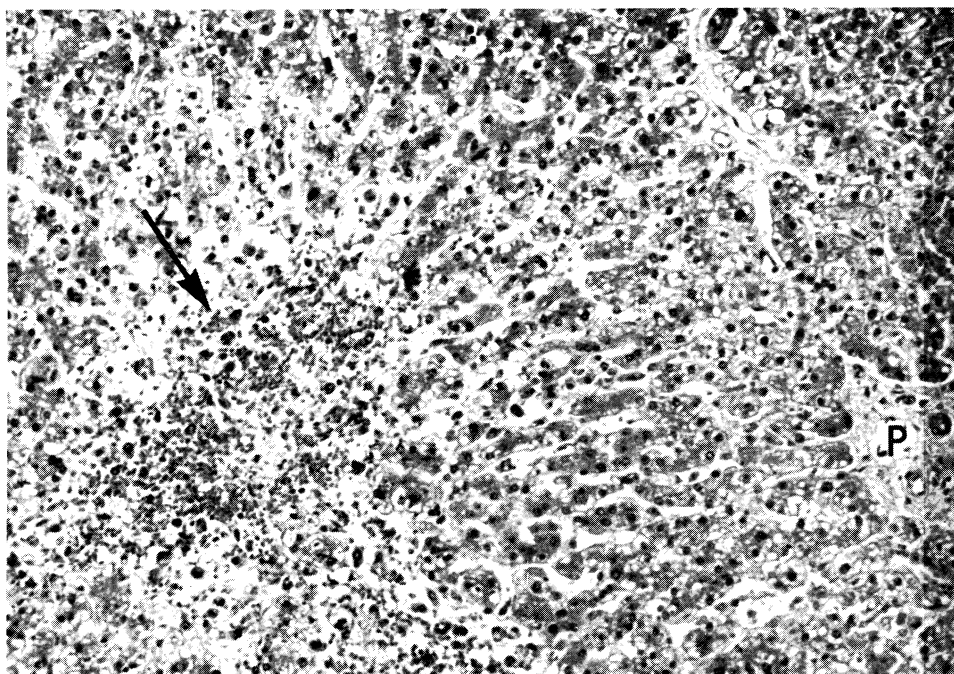


Figure 265. Human auxiliary hepatic homograft (AT 2) 34 days after transplantation. The centrilobular hepatocytes are atrophic (arrow) and there are fat droplets in the liver cells in the middle and peripheral zones of the lobules. There is no cellular infiltration in the portal tract (P). (Hematoxylin and eosin stain, $\times 80$.)

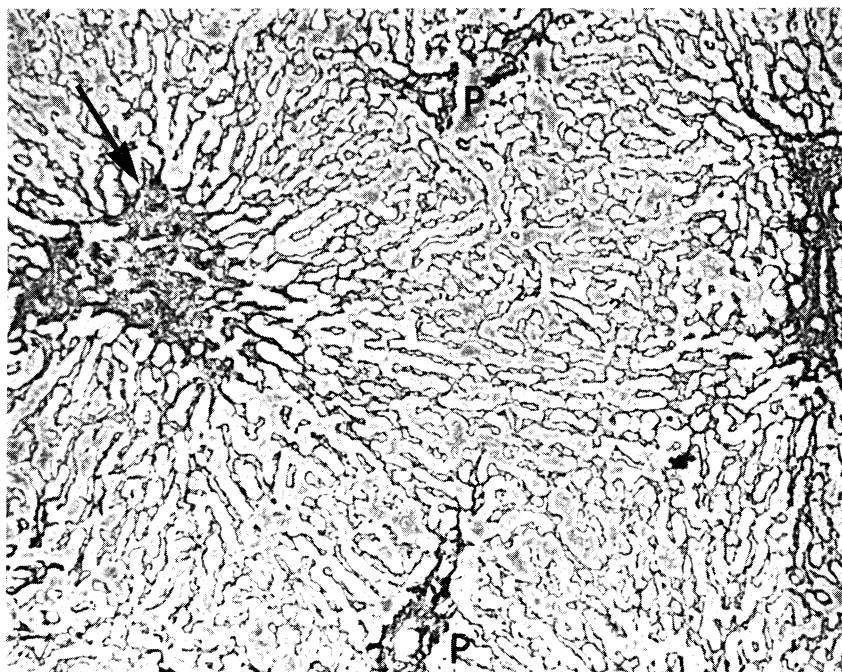


Figure 266. The same human auxiliary hepatic homograft as shown in Figure 265. There is collapse of the central part of the reticulin framework of the liver lobules (arrow). P=portal tract. (Reticulin stain, $\times 80$.)

the fibrinoid necrotic foci probably occurred after transplantation. The arteries and veins of the other two grafts were normal. Cytomegalic virus inclusions were present in many of the biliary duct epithelial cells in one of the livers (AT 2) (Fig. 154, Chapter Sixteen).

These findings suggest that only one of the grafts (AT 1) was undergoing active rejection. Because the cholestasis that was present in three of the grafts was accompanied by dilatation of the biliary ducts, it was suspected on histopathologic grounds that there had been an element of extrahepatic bile duct obstruction in all these livers. However, this had been carefully looked for by those performing the autopsies and it had not been found.

CONCLUSIONS

The experimental studies have shown that the features of auxiliary homograft rejection are not fundamentally different than in orthotopic transplants. They have also demonstrated that the auxiliary liver can be submitted to an additional injury caused by its necessity to compete with the host liver, presumably for nutritional substances in the circulating blood.

The degree to which the latter factor contributed to the morphologic alterations found in the three human auxiliary livers after 22 to 34 days could not be determined with any degree of certainty. Atrophic changes were found in all three of the transplants, most severely in the two organs that had been in place for the longest periods and that did not have much evidence of active rejection. These consisted of hepatocyte atrophy or loss, particularly in the centrilobular areas, and of reticulin collapse. Similar abnormalities have been seen in orthotopic livers (Chapter Twenty), but not usually to this extent so early. Nevertheless, the question of whether auxiliary homograft atrophy will be a special and troublesome problem in patients subjected to this kind of procedure cannot be decisively answered at the present time.

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Chapter Twenty-four

APPENDIX OF CASE MATERIAL AND BIBLIOGRAPHY

The following pages list the liver transplantations at the Colorado General Hospital and the Denver Veterans Administration Hospital. Other data on these cases are summarized in the various chapters. In particular, the reader may wish to cross refer to the information about histocompatibility (Table 5, Chapter Three), the circumstances of donor death (Table 6, Chapter Six), the infectious disease complications (Tables 21 to 23, Chapter Sixteen), and the timing and character of early and late rejection (Table 26, Chapter Seventeen).

In addition, all cases of liver transplantation are included which were formally reported before April 1, 1969, from other institutions. Many additional unreported attempts have been made. For a recent symposium held in Cambridge, England, Dr. Carl Groth of Stockholm was able to collect 24 and 12 informal accounts of orthotopic and auxiliary operations, respectively, from centers other than the University of Colorado, in addition to the eight and nine cases tabulated from the formal literature in this chapter. The unpublished statistics have not been compiled because of the difficulty of assuring the accuracy of documentation. Nevertheless, it is worth noting that survival of more than a month was achieved in a number of the unreported cases of orthotopic liver replacement, including one each of Calne (Cambridge), Fortner (New York City), Garnier (Paris), Kestens (Louvain), and Najarian (Minneapolis).

In the second part of the chapter an alphabetical bibliography is given of all the publications about liver transplantation that have appeared in the world literature prior to April 1, 1969.

Auxiliary Homotransplantations in Denver*

PATIENT NUMBER	DISEASE	DATE OF OPERATION	SURVIVAL (days)	AGE (years)	DONOR			WEIGHT (kg)	BLOOD TYPE	Age (years)	Blood Type		CAUSE OF DEATH
					SEX	SEX	Sex				Weight (kg)	Type	
AT 1	Alcoholic cirrhosis	2-20-65	22	50	M	M	68.0	A	79	M	60.0	A	Sepsis, hepatic failure
AT 2	Alcoholic cirrhosis	7-5-65	34	47	M	M	68.6	O	12	M	42.0	O	Sepsis, hepatic failure, GI bleeding
AT 3	Extrahepatic biliary atresia	11-3-65	0	1-4/12	F	F	8.0	A	7/12	F	6.0	A	Cardiac arrest
AT 4	Alcoholic cirrhosis	6-20-68	24	48	M	M	84.0	A	Newborn	F	3.1	A	Sepsis, hepatic and renal failure

*All 4 of these cases have been reported in past publications; the numbered references are in the bibliography in the second part of the chapter: 61, 71, 88, 97, 237, 249, 257-260.

Other Reported Auxiliary Homotransplantations

INVESTIGATOR	DISEASE	DATE OF OPERATION	SURVIVAL (days)	AGE (years)	DONOR						CAUSE OF DEATH	REFERENCES *	
					SEX	WEIGHT (kg)	BLOOD TYPE	Age (years)	Sex	Weight (kg)			Blood Type
Abelson	Extrahepatic biliary atresia	11-3-64	13	11/12	M	7.0	O	2-1/2	F	9.0	A	Bile peritonitis, sepsis	3
Cree	Subacute hepatitis	10-27-66	2	17	M	—	—	Elderly	F	—	O	Hepatic failure	153
Fonkalsrud	Hepatoma	3-2-67	12	46	M	—	—	9	M	—	—	Thrombosis hepatic artery, homograft necrosis	65
Calne	Alcoholic cirrhosis	Summer 1967 ?	1/2	47	M	—	—	50	F	—	—	Hemorrhage	36
Sheil	Cirrhosis	4-12-68	3	45	M	—	—	44	F	—	—	Hepatic failure	216

*The numbered references are in the bibliography in the second part of the chapter.

Orthotopic Homotransplantations in Denver

PATIENT NUMBER	DISEASE	DATE OF OPERATION	SURVIVAL (days)	AGE (years)	DONOR							CAUSE OF DEATH *
					SEX	WEIGHT (kg)	BLOOD TYPE	Age (years)	Sex	Weight (kg)	Blood Type	
OT 1	Extrahepatic biliary atresia	3-1-63	0	3	M	9.1	A	3	M	12	A	Hemorrhage
OT 2	Hepatoma, cirrhosis	5-5-63	22	48	M	68	A	55	M	60	A	Pulmonary emboli, sepsis
OT 3	Cholangiocar- cinoma	6-24-63	7-1/2	68	M	55	O	69	M	70	O	Sepsis, pulmonary emboli, GI bleeding
OT 4	Hepatoma, cirrhosis	7-16-63	6-1/2	52	M	53	A	73	M	57	O	Pulmonary emboli, ? hepatic failure, pulmonary edema
OT 5	Hepatoma, cirrhosis	10-4-63	23	29	F	62	O	64	M	65	O	Sepsis, bile peritonitis, hepatic failure
OT 6	Hepatoma	11-9-66	7	29	M	65	B	73	M	56	B	Hepatic failure, sepsis
OT 7	Extrahepatic biliary atresia	5-21-67	10	11/12	M	5.2	O	1	F	7	O	Hepatic failure, sepsis
OT 8	Hepatoma	7-23-67	400	1-7/12	F	9.6	O	1-6/12	M	6.4	O	Carcinomatosis
OT 9	Extrahepatic biliary atresia	7-31-67	133	1-9/12	F	8.7	O	4	F	13.0	O	Septic hepatic infarction, hepatic failure
OT 10	Extrahepatic biliary atresia	9-5-67	186	1-1/12	F	9.4	A	1-6/12	F	8.2	A	Septic hepatic infarction, hepatic failure
OT 11	Extrahepatic biliary atresia	10-8-67	61	1-2/12	F	7.8	A	1-8/12	F	7.3	A	Septic hepatic infarction
OT 12	Extrahepatic biliary atresia	11-24-67	105	1-4/12	F	7.5	A	1-2/12	M	8.5	A	Septic hepatic infarction
OT 13	Extrahepatic biliary atresia	2-9-68	431†	2	M	11.2	O	3	M	11.4	O	Alive
OT 14	Hepatoma	3-17-68	394††	16	F	49 57	O	27 17	M	82.0 60.0	O	Alive

OT 15	Hepatoma, cirrhosis	4-14-68	339	44	M	62	AB	20	F	59.1	A	Carcinomatosis
OT 16	Extrahepatic biliary atresia	5-26-68	324†§	1-11/12	M	11.0 11.2	O	3	M	15.3 25.0	O	Alive
OT 17	Hepatoma	6-18-68	35	24	F	54.1	O	22	M	86.0	O	Pneumonitis
OT 18	Extrahepatic biliary atresia	6-29-68	4	1	F	5.7	B	1-9/12	M	10.5	B	Hepatic artery thrombosis
OT 19	Intrahepatic biliary atresia	7-20-68	270†	4	M	14.3	B	10	M	32.0	O	Alive
OT 20	Posthepatic cirrhosis and cholangiectasis	8-13-68	1/2	8	F	15.3	A	10	M	27.7	A	Nonthrombotic occlusion of hepatic artery
OT 21	Extrahepatic biliary atresia	8-20-68	1/2	2	F	12.5	A	5	M	20.0	A	Portal vein thrombosis
OT 22	Laennec's cirrhosis	10-24-68	10	33	M	60.0	A	25	M	77.0	A	Biliary duct obstruction, hepatic and renal failure
OT 23	Hepatoma	10-26-68	143	15	M	41.0	AB	6	M	27.0	A	Carcinomatosis
OT 24	Extrahepatic biliary atresia	11-10-68	11	3	F	11.0	A	2	M	9.0	A	? Hepatic arterial insufficiency, hepatic failure
OT 25	Hepatoma	2-11-69	39	45	M	80.0	A	20	M	70.0	A	Bile peritonitis, sepsis, hepatic failure

†In actuality, most of these patients had other potentially lethal complications besides those listed. These are recorded in detail throughout the book. The term "sepsis" is used loosely. The specific infections are discussed in Chapters Fifteen and Sixteen.

‡Alive April 15, 1969.

§The patient has received two homografts. The first was removed after 380 days and replaced. The survival noted is from the time of the initial operation.

§The child has received two homografts. The first was removed after 68 days and replaced. The survival noted is from the date of the initial operation.

Most of the cases have been reported in past publications; the numbered references are in the bibliography in the second part of the chapter: 21, 71, 83, 84, 92, 93, 97, 105, 152, 193, 227-230, 232-237, 240-243, 249-252, 255, 257-260, 263-267.

Other Reported Orthotopic Homotransplantations

INVESTIGATOR	DISEASE	DATE OF OPERATION	SURVIVAL (days)	AGE (years)	DONOR			WEIGHT (kg)	BLOOD TYPE	Age (years)	Sex	Weight (kg)	Blood Type	CAUSE OF DEATH	REFERENCES*
					SEX	AGE (years)	Sex								
Moore, Birch	Metastatic colon carcinoma	9-16-63	11	58	M	—	M	—	A	35	M	—	B	Pneumonitis, liver abscesses, hepatic failure	183
Demis/leau	Metastatic colon carcinoma	1-64	0	75	M	—	M	—	A	71	M	—	O	Hemorrhage	57
Fonkalsrud	Biliary atresia	1-68?	16	2-1/2	M	8.6	F	—	—	1-1/2	F	—	—	Septic hepatic infarction	69
Calne	Bile duct carcinoma	5-2-68	76	46	F	—	—	—	A	5	—	—	O	Septic hepatic infarction, bile fistula	36, 37
Calne	Biliary atresia	Summer 1968?	0	1	—	—	—	—	—	2	—	—	—	Cardiac arrest	36
Birch, Moore	Hepatoma	Summer 1968	45	16	M	—	M	—	—	12	M	—	—	Pneumonitis, complete thrombosis of hepatic artery, septic hepatic infarction	7
Calne	Hepatoma	9-23-68	132	41	M	—	—	—	O	13	—	—	O	Cerebral abscess	37
Calne	Cholangiocarcinoma	11-68?	21	46	M	—	M	—	—	64	M	—	—	GI hemorrhage, hepatic failure, sepsis	36

*The numbered references are in the bibliography in the second part of the chapter. Supplementary data about the first Boston case and the longest surviving patient in the Cambridge series were provided by Dr. F. D. Moore and Professor R. Calne, respectively.

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