

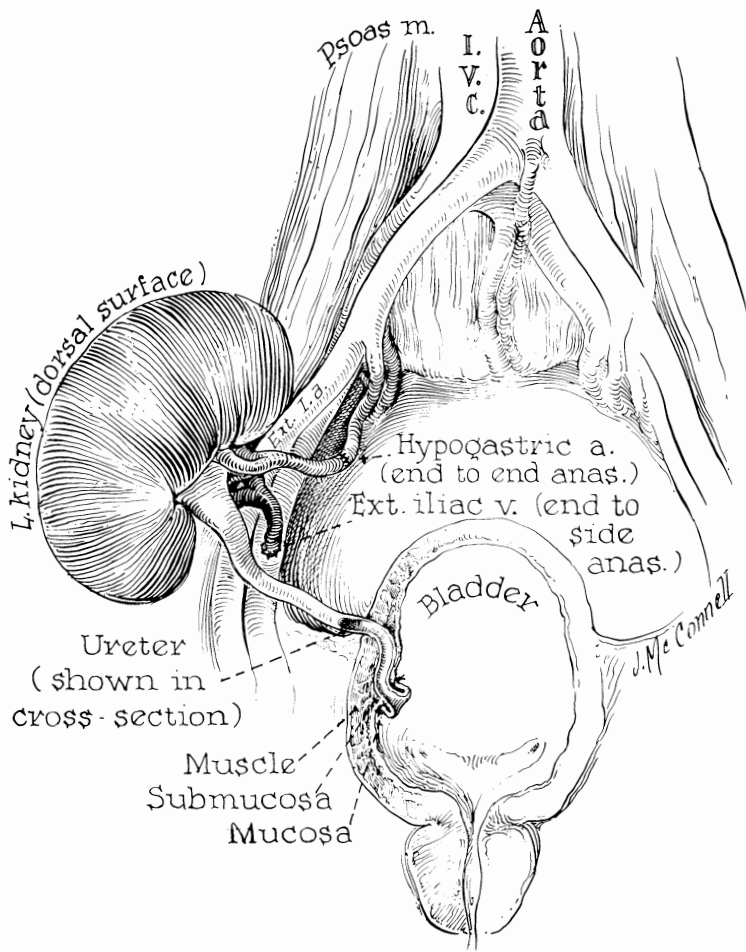
**Experience in RENAL
TRANSPLANTATION**

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Renal homograft. (By permission of JAMA 187:734, 1964.)

Experience in RENAL TRANSPLANTATION

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Experience in Renal Transplantation

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TO

My Mother, Father, and Wife

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FOREWORD

This volume is an account of a brief but intense experience with transplantation of kidneys into uremic patients. Despite the involvement of many individuals and the representation of many interests in a project of this sort, the motives have been simple and few, namely, to help the patients and – to the extent possible – accumulate data that would allow evaluation of current concepts and methods with an eye to the future. Although the procedure is an experimental one, there has been little experimentation in the usual sense, but simply an intense effort to care for the patients in the best possible manner followed by careful observation, above all, careful recording of data, and, finally, frequent summation and analysis of results. Certain dividends have accrued from this approach, and there are also disadvantages. The methods of management have varied little, making it possible to acquire a homogeneous body of data from which some conclusions have been reached with a degree of certainty that was not previously possible in evaluating renal transplantation as it exists in 1964.

The main and far-reaching conclusion is that organ transplantation has some clinical application which is not yet perfect but is moving in that direction. It seems of great importance that four of the first six patients in this Denver series are still well. Doctor Starzl and the numerous other people who have contributed to the project and to this volume have worked hard to carry out such transplantation research in addition to their usual clinical and teaching duties. They deserve our thanks.

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PREFACE

The past 20 years have been a time of unprecedented progress in the field of tissue transplantation, as attested by thousands of published experimental studies which treat of the events, mechanisms, and prevention of rejection as well as innumerable other facets of the problem. Throughout this period, there were sporadic attempts to apply renal homotransplantation to the treatment of human disease. During the past few years, such efforts have become more insistent despite the fact that the pooled results of cases from throughout the world present a dismal picture of repeated failures and only an occasional success. The ethical and moral issues involved in a clinical undertaking of this type have been subjected to critical scrutiny, especially by those involved in the actual care of the patients, and by others as well.

The place, if any, of renal hetero- or homotransplantation in the treatment of terminal renal disease is not yet clear. To say that the problem of homograft rejection has been satisfactorily solved is folly. The converse view that this means of therapy has no real value would, however, appear to be an equally limited attitude. An operation which can in the individual case provide five years of relatively healthful existence, as has been the case in Murray's first successful transplant between a nonidentical donor and recipient, cannot be said to be without significant worth at least in the isolated case.

The collections that have been made of total experience with human renal homotransplantation cannot be accepted as accurately reflecting the outlook of a patient to be treated in this way today. Such compilations often do not delineate the deficiencies of those therapeutic methods employed in the early era as compared to the modern one. In order to characterize the limitations and the spheres of usefulness of renal homotransplantation, it is desirable to analyze the results from series in which homogeneous care has been provided. Objective scrutiny of such series can serve as a better index for evaluation of the pro-

cedure than occasional cases treated in different ways, by different personnel, and with varying case selection and follow-up.

Thus, the primary objective of this book is not to recapitulate all previous experience with human renal transplantation. Rather, the purpose is to present a simple and practical analysis of those cases treated by this means at the University of Colorado Medical Center, in order to help define the indications, technical requirements, limitations, causes of failure, and results at one institution. The laboratory effort supporting the clinical program will not be treated in detail. It is sufficient to say, however, that no program of human renal homotransplantation can hope to succeed without a strong collateral investigative effort involving animal research.

Although primary attention will be focused upon cases treated in this series, it would be inappropriate not to state that most details of both the technical and nonsurgical care have had their origin in other centers. The pioneer contributions of Murray, Merrill, Hume, Calne, Küss, Goodwin, Woodruff, Hamburger, and numerous others have provided the base from which our efforts have begun. Furthermore, the personal encouragement, advice, and unifying exchange of information from most of these men have made it possible for us to pursue this work at a time when the effort might otherwise have been discontinued.

It is to be hoped that the publication of this work with the large number of cases involved will not be misconstrued as evidence that renal homotransplantation has now outstripped its infancy, or that the procedure is ready for general use. The employment of renal homografts is still a fundamentally experimental practice, which is attended by a distressingly high incidence of early failure even under the most nearly ideal circumstances. Moreover, the fate of homografts in terms of function for years or decades is not known, and can be learned only after the passage of time. There will inevitably be an increasing experience with human renal transplantation, but a stampede of uncontrolled activity at this time will serve no useful purpose, and may discredit the operation before its merits are fully determined.

A special note is warranted concerning the type of environment in which renal transplantation can be carried out with benefit to the patient and to the scientific community. In its present developmental state, homotransplantation requires an institutional effort, since even the studies which are necessary to document the course of a single patient are beyond the knowledge, skill, and time of any individual or small group of physicians.

Thus, the program at the University of Colorado Medical Center has had strong representation from almost every major preclinical and clinical department. In addition to those who have written chapters for the text, important support and independent effort have been contributed by Doctors Robert S. Brittain, Gilbert Hermann, Oliver G. Stonington, I.C.S. Knight, Martin P. Hutt, David W. Talmage, Matthew H. Block, David A. Ogden, Philip L. Dern, James L. Tong, Kurt N. von Kaulla, Ruheri A. Perez-Tamayo, Carlos E. Garciga, David S. Le Vine, John H. Githens, Conrad M. Riley, Joseph H. French, David T. Rowlands,

Jr., Catherine W. Anthony, Elizabeth H. Macintyre, David E. Starrett, Frederick H. Herring, James K. Weaver, Eugene Heller, and Arnold H. Greenhouse, as well as many pediatric, medical, and surgical interns and residents.

In addition, such a program could not be sustained without unwavering internal support from the administration of the involved hospitals. There are many vexing problems which concern the financing of hospital care, relocation of bed quotas, and establishment of special investigative facilities, to mention just a few. A deluge of unwanted lay publicity has proved to be unavoidable, and those officials who are entrusted with preserving the dignity and integrity of institutions both here and elsewhere have been confronted with many painful and unfamiliar dilemmas. Doctors Robert J. Glaser and John J. Conger, the past and present Deans of the University of Colorado Medical School, provided such support from the beginning; as did Doctors Paul M. Ireland and Joseph A. Hall, the Hospital Director and Chief of Staff, respectively, of the Denver Veterans Administration Hospital.

Finally, the development and growth of a meaningful transplantation endeavor is not possible without that unique dual personality—the able administrator who is also a working and inventive scientist. Doctor William R. Waddell, Chairman of the Department of Surgery of the University of Colorado Medical Center, has filled this role as few men could have. His influence has been felt from the initial planning stages to the present time in all aspects of transplantation research as well as in the performance of day-to-day patient care. In addition to helping formulate medical policy, he has subjected himself to personal sacrifice in order to raise funds for continuation of the work and to insure that the inevitable release of information for public consumption would be done with propriety and accuracy. The faults of this book I must bear alone. Its merits, if any, result from a collaboration with him which is based upon both mutual scientific interests and a warm personal friendship.

Denver, Colorado

THOMAS E. STARZL

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Chapter One

INTRODUCTION

Although the possibility of organ homotransplantation has intrigued surgeons since antiquity, major strides toward this end have been achieved only in the past few years. Initially, progress was limited by lack of understanding of the processes involved in homograft rejection. Prior to the beginning of the Second World War, many physicians believed that homograft rejection was the consequence of a primary technical failure. In 1944 and 1945, Medawar published the classic studies which not only defined the events that transpired after the placement of skin homografts, but provided a rational explanation for the rejection process. He demonstrated that homografts behaved initially in much the same manner as autografts, but that they were eventually sloughed after a varying period of time. The histologic alterations in the transplants consisted of mononuclear cell infiltrates, a variety of vascular lesions, and eventual distortion or destruction of the normal architecture.

IMMUNOLOGIC REACTION OF HOST

Medawar's studies provided strong evidence that these phenomena were due to an immunologic reaction of the host to the foreign tissue (Fig. 1). The key observation in support of this concept was that once a homograft had been placed, a second graft from the same donor was destroyed in an accelerated fashion (second set reaction), suggesting the acquisition of host immunity. The immunity conferred by contact with the first graft was of long duration, and applied to all tissues subsequently transplanted from the same donor. The sensitization was specific, inasmuch as homografts from other donors were not rejected in an accelerated manner. A feeble quantitative effect was noted by Medawar, with more rapid rejection of larger than of smaller grafts.

These important observations were soon extended by Dempster and by Simonsen to whole organ homografts of the kidney, in which the mode of revascularization is quite different. Free skin grafts survive without immediate restitution of blood supply, revascularization occurring from the graft bed over

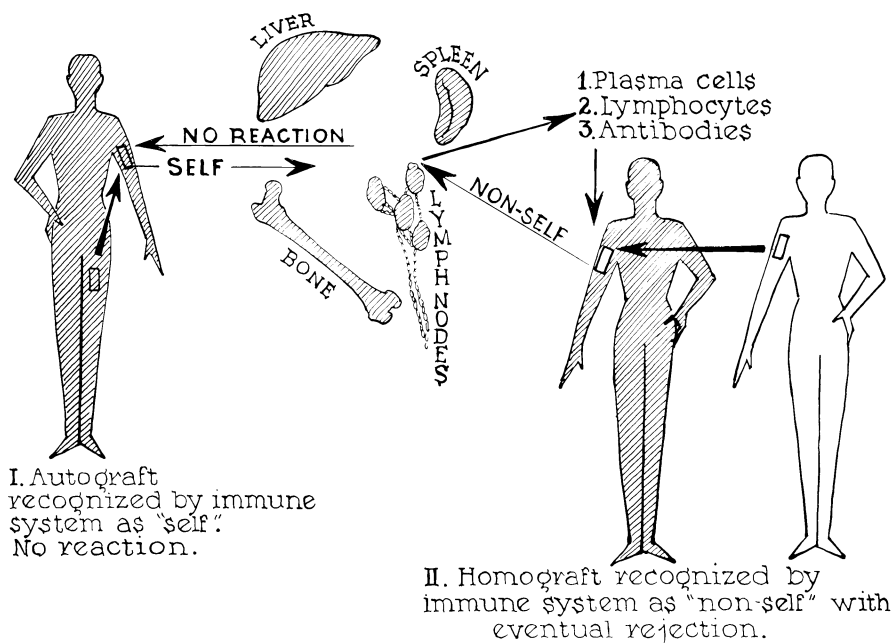


Figure 1. Response to autografts (left) and homografts (right). Tissues transferred between identical twins behave as autografts and are not rejected.

a period of days. With kidneys, a blood supply is promptly restored by means of surgically performed vascular anastomoses. Despite this difference the fate of renal homografts was comparable in most respects to that previously described for skin.

The delay between exposure to a foreign graft and rejection has prompted comparisons between homograft immunity and the delayed tuberculin type sensitivity. However, the precise details of homograft immunity are not known. The nature and location of the antigen and of the resultant antibodies are matters on which there are conflicting opinions. There is abundant evidence that the reticuloendothelial system plays an important role in rejection (Fig. 1). After the placement of skin grafts, the regional lymph nodes become enlarged and packed with large lymphoid, reticulum, and plasma cells, while a similar reaction is seen in the subjacent graft bed. All vascularized homografts are ultimately invaded by lymphocytes and plasma cells during rejection. Work by Weaver, Algire, and Prehn has focused attention upon the small lymphocyte as the ultimate cellular agent of destruction (Fig. 2).

Although it has been customary to think of homograft rejection primarily in terms of a host cellular response, recent evidence has pointed to the possibility that humoral factors may also be of great practical importance. Najarian and Feldman's investigations, which demonstrated the invocation of a presumed host humoral rejection factor by specifically sensitized tissues isolated in millipore chambers, is particularly pertinent in this respect. Clini-

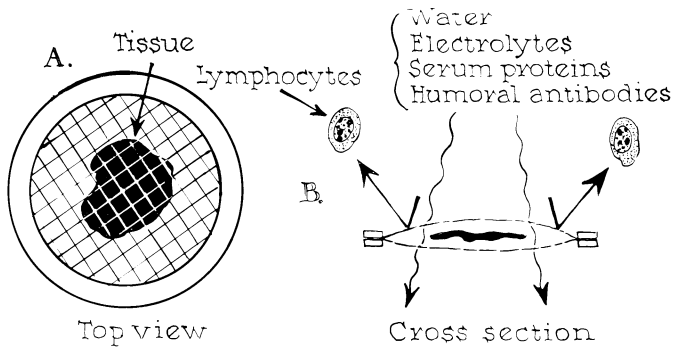


Figure 2. Diffusion chamber experiment of Algire. The enclosed homograft which is protected from physical contact with lymphocytes can survive for protracted periods.

cally, Murray and his associates and Hamburger have also drawn attention to the probability that sudden homograft failures could be due to humoral factors only, not only because of the rapidity of destruction of some grafts, but because histologic evidence of cellular infiltration has often not been present under these circumstances. Finally, evidence to be described within our own experience has suggested that some deleterious effects upon the homografted kidney must be explicable by mechanisms other than cellular invasion. These various lines of inferential evidence have led to considerable speculation that an important, if not vital, element of the rejection process may be an immunologically mediated process which involves the blood vessels, leading to a vascular catastrophe which either occurs independently or concomitantly with cellular invasion.

Methods of Altering Host's Reaction

Because of the evidence indicting an immunologic etiology for homograft rejection, it was natural that attention should be focused upon means of altering the immunologic capacity of the prospective host.

Total Body Irradiation. Host conditioning with total body irradiation was extensively evaluated for this purpose in the early 1950's. Successes in experimental animals were rare because irradiation so injured the treated animal that survival in a normal environment was impossible. The method of total body irradiation was given a clinical trial by Murray and his associates at the Peter Bent Brigham Hospital in the mid 1950's. Although only one success was attained, this was a signal event since the recipient of this homograft from his nonidentical twin brother is still alive and is the first example of a chronically successful human homotransplantation between a donor and recipient who were genetically different. The most common cause of failure was bone marrow depression, agranulocytosis, and overwhelming infection. Subsequent efforts by European investigators, notably Küss and Hamburger, were somewhat more successful. Nevertheless, the prospect of achieving consistent success with this approach has ultimately appeared to be virtually hopeless.

Drug Immunosuppression. A new chapter in immunosuppressive therapy was opened in 1959 by the work of Schwartz and Dameshek, who demonstrated an obtundation of immunologic response in animals which were exposed to foreign protein antigens while receiving 6-mercaptopurine. One year later, Schwartz and Dameshek, Calne, and Zukoski independently demonstrated that this drug prolonged homograft survival. Still later, Calne and Murray demonstrated an improved effect with a chemical compound closely related to 6-mercaptopurine, termed azathioprine, especially if it were combined with weaker antirejection agents such as actinomycin C and azaserine. These drugs, alone or in combination, were superior to irradiation in that their effects were more incisively directed. It was possible with these agents to inhibit homograft rejection without the production of agranulocytosis. Thus, for the first time, rejection could be prevented or delayed without rendering the host totally nonreactive against other environmental antigens.

CURRENT STATUS OF RENAL HOMOTRANSPLANTATION

Despite these encouraging findings, it was not yet possible to obtain consistent success with homotransplantation procedures, either in experimental animals or in man. Like the elusive jigsaw puzzle, in which many of the pieces had been fitted into their appropriate slots, the picture was not yet complete. The pioneer efforts of Murray, Küss, Hamburger, and Hume had all demonstrated that a renal homotransplant was capable of protracted function in the occasional case. If this could be achieved sporadically, it seemed reasonable to expect that the proper manipulation of a number of small details might provide a consistently successful solution. Despite this expectation, almost all renal homotransplants had failed when, in the spring of 1963, Goodwin and Martin compiled the known renal transplants from various centers throughout the world. Less than 10 per cent of those cases treated to that time had survived for as long as three months.

The courageous and often tragically unsuccessful attempts of the early pioneers provided a vast, although frequently uncatalogued, background of valuable information upon which future progress might be built. Quite apart from the information obtained concerning the prevention of homograft rejection, important strides were taken of a purely technical nature. The surgical technique described by Küss in 1951 was popularized as a result of its use by Murray and Harrison for the transplantation of kidneys between pairs of identical twins (isografts) in 1954. Subsequent repeated successful experiences with identical twins have contributed much to the definition of effects of ischemia upon subsequent renal function, the response of the host patient to sudden alleviation of his complex metabolic disorder, and the unique problems of pre- and postoperative care presented by these chronically and terminally ill

patients. As will be suggested, it is possible that extrapolation of these lessons to the field of true homotransplantation has been too free.

Despite the great advances made by the aforementioned pioneer workers, as well as many other investigators, renal homotransplantation is not yet regarded as a standard surgical operation, because it has failed to conform to certain criteria. These requirements are: first, that the operation be performed with an acceptably low mortality; second, that the patient be restored to a reasonable state of health for a significant period of time; and finally, that the financial burden of care should be within the reach of the patient, the hospital, and the community. If these practical objectives could be achieved, it would be possible to extend the use of kidney transplantation to a more general utility in which such care could be offered in an increasing number of hospitals.

Experience at the University of Colorado Medical Center within the past year has led to a growing hope that these objectives may be within reach, at least within a highly selected group of recipients. The purpose of this book is to document experience with all the patients treated at the University of Colorado Medical Center from March 27, 1962, to March 30, 1964. During this interval, 75 renal transplants were performed, 73 involving the transfer of kidneys from donors other than identical twins. In the course of this experience, a vast amount of information has been obtained relating to the procurement and preservation of the grafted tissue during its transfer, details of the implantation of the organ, the relationship of ABO blood group incompatibility between donor and recipient to success or failure, the influence of consanguinity on rejection, the relation of early function to the ultimate outcome, the identification and the reversal of the rejection process, the development of host tolerance to the homograft, the ability of the surviving patients to resume normal activity while receiving immunosuppressive therapy, the procurement of cadaveric organs, the influence of age and associated disease upon prognosis, and the potential future value of heterografts.

The chapters which follow contain an account of the procedures employed at this center in the treatment of patients receiving renal homo- and heterotransplants. Although some of the details may seem trifling, they are presented in full for two reasons. First, the problem of patient care is so complex that the omission of a single precautionary facet has on occasion led to an inexorable chain of adverse events and death. Second, the steps which are important, and those which are inconsequential, have not yet been clearly delineated. For evaluation of the methods used, it has, therefore, been necessary to describe each detail, in the hope that eventual simplification of overall procedure will be possible.

Throughout the text, the courses of individual patients have been frequently used to graphically illustrate certain features. In addition, tables have been included which summarize the influence of various factors upon results. Such tables, which were brought up to date on June 1, 1964, have been drawn chiefly from those earlier cases in which longer follow-up makes analysis more meaningful. An extended summary of all patients operated

upon prior to March 31, 1964, is included in the appendix, brought up to date to June 1, 1964. In this tabular summary (Chap. 28), the patients are listed in four groups depending on the source of the donated organs. The categories are: 1, living donors (LD); 2, cadaveric donors (CD); 3, simian (baboon) donors (SD); 4, identical twin donors (ITD). Each illustration in the book is coded so that by cross-referencing to the appendix, the current status of an individual patient can be readily determined. Thus, further details of the patient identified as LD 2 in Chapter 2 (Fig. 3) can be quickly obtained by consulting the same number in the appendix.

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Chapter Two

PROCUREMENT AND ORGANIZATION OF DATA

The care of patients receiving renal homotransplants crosses all specialty barriers, involving as it does the services of the internist, hematologist, urologist, pediatrician, immunologist, neurologist, psychiatrist, and surgeon. The interdigitation of effort is so complex and the quantity of data so voluminous that a comprehensive grasp of the course of even a single patient may become difficult or virtually impossible.

In caring for the early patients in this series, dependence was placed upon collection of data within standard hospital charts. This practice proved to be inefficient. A macroscopic view of the entire course of any individual patient was frequently impossible without a daily exhaustive review of the chart. In addition, valuable data were lost because of failure of secretarial recording of laboratory examinations known to have been ordered, but from which the results were misplaced. Complete retrospective documentation of such vital matters as pharmacologic therapy was sometimes unreliable. When representatives of different specialty groups recommended therapy for a patient, the significance of the suggestions frequently was not understood by other members of the team. Because of failure in the centralization of data collection, a breakdown was observed in management of the cases in which diffusion of responsibility deprived the patient of the privilege of having a single physician who had sole final responsibility for all details of care.

At this institution, the evolution of a master flow sheet has solved many if not most of these problems (Table 1). The flow sheets are 21 by 38 inches in size, dimensions which allow attachment to a wall of the hospital room. Each item of information is recorded by the nurses on duty or by the physicians involved in the case. The surgeon in charge of the case is responsible for the daily upkeep and accuracy of each detail of the record. A quick check can and must be performed on all the data for each day. The progress in the preceding

Table 1. Master Flow Sheet

Patient -
Date Splen -

Date Rt. Neph -
Date Lt. Neph -

Pt. Blood Type -
Pt. Age -

Date	Instru- mentation	Acti- C	Pred	Imuran	Other Drugs		Anti- biotic	Intake	Output	Wt	Temp			BP	Hct	WBC	DIFFERENTIAL					
																	Stab	Seg	L	M	E	B
3-5																						
3-6																						
3-7																						
3-8																						
3-9																						
3-10																						
3-11																						
3-12																						
3-13																						
3-14																						
3-15																						
3-16																						
3-17																						
3-18																						
3-19																						
3-20																						
3-21																						
3-22																						
3-23																						
3-24																						

Table 1. Master Flow Sheet (Continued)

Donor -
D. Blood Type -
D. Age -

Organ Preservation -
Ischemia Time

Platelet	BUN	Creat (B)	Ccr	Blood				URINALYSIS								Days Post-op	
				Na	K	Cl	CO ₂	S.G.	Sug	Prot	Urea	Na	Cl	Creat	Culture		
3-5																	
3-6																	
3-7																	
3-8																	
3-9																	
3-10																	
3-11																	
3-12																	
3-13																	
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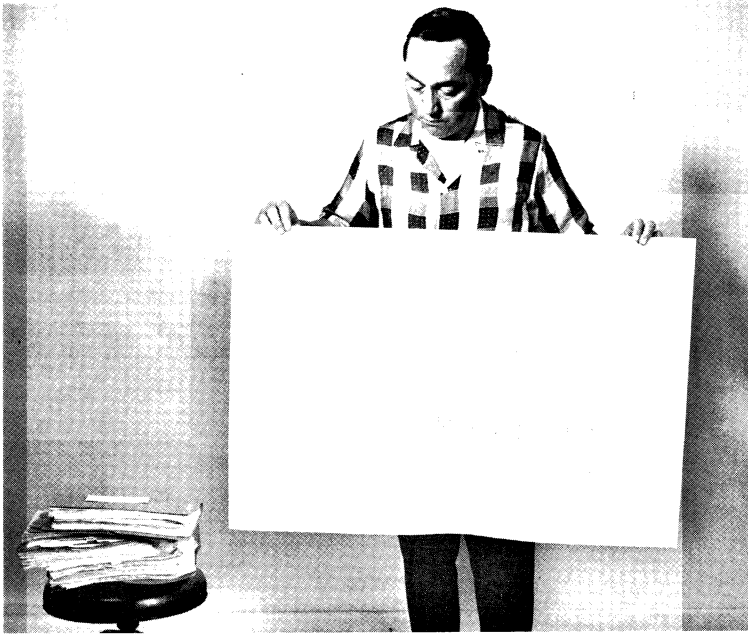


Figure 3. Comparison of hospital and outpatient records (on stool) with simplified flow sheet held by the recipient of a renal homograft. The patient (LD 2) was operated upon in January, 1963, and the photograph was taken 11 months later.

days or during the entire hospitalization can be readily reviewed within a few minutes. The simplification of record keeping can be appreciated by a comparison of the flow sheets with the hospital record of a patient operated upon in January, 1963 (Fig. 3). All relevant information is contained on two flow sheets, weighing 5 ounces. The official hospital records stand 12 inches high and weigh $7\frac{1}{4}$ pounds.

Aside from the convenience of the flow sheets in formulating daily therapy, they have contributed to easy and frequent analysis of the results for purposes of scientific communication. The use of the same format and the procurement of comparable information in all cases permits simultaneous study of some or all of the cases with a minimum of effort. Trends are easily observed, and recurrent patterns can be readily identified and quantitated which might otherwise be lost in a maze of detail.

An additional use of the flow sheets has assumed importance because of the peculiarly developmental nature of renal homotransplantation and the need for constant interinstitutional exchange of experience. Accurate and unbiased information is frequently requested by visitors engaged in or contemplating entry into this field. The system described permits prompt communication to interested parties of a completely up-to-date account in a compact and usable form.

Chapter Three

SELECTION OF CANDIDATES FOR HOMOTRANSPLANTATION

Proper selection of candidates for renal homotransplantation is of paramount importance if good results are to be obtained. The pressures which are applied in behalf of some of the prospective patients may be intense, despite the chances of a successful outcome being small because of a variety of complicating circumstances in a particular case. Referring physicians may be caught in the same emotionalism as that afflicting the family and be unable to accept with equanimity a decision against this form of therapy. In order to resist coercion, it may be convenient to escape behind a facade of committee action. Most of the members of such a selection group will be able to act objectively because of freedom from previous personal commitments to the patient or the family.

Clinical Status of Patient

From a medical point of view, the requirements for candidacy are relatively simple. The patient should have irreversible renal disease from which life expectancy is limited to a few weeks or months. He must not have a major element of infection at the time of transplantation. A normal lower urinary tract must be present. Other serious disease processes must be ruled out. Vascular, cardiac, or neurologic changes which are secondary to the renal failure should be judged to be reversible. Finally, it is necessary to have evidence that the prospective recipient can withstand the immediate trauma of a major surgical procedure after the maximal improvement of preoperative resuscitation. Although these requirements appear to be straightforward, they have not been met in all our patients. Violations have almost inevitably resulted in transplantation failure.

Table 2. Influence of Age on Survival*

Age in Years	No.	Nonrelated Donors	Alive	Dead
0-10	3	0	2	1
11-20	12	2	9	3
21-34	11	1	6	5
35-44	11	6	5	6
45-55	8	4	3	5
	<u>45</u>	<u>13</u>	<u>25</u>	<u>20</u>

*All patients were operated on before December 10, 1963. Survival is to June 1, 1964.

Age

Some of the most difficult decisions of patient selection concern the role of age as a disqualifying factor. In the first 45 patients receiving homografts from living donors, the results were better in that group who were less than 35 (Table 2), although these results were heavily influenced by the use of a large percentage of genetically nonrelated donors for the older recipients. Nevertheless, the need for individualizing each case is demonstrated by the number of socially useful middle-aged or even elderly citizens who have returned to responsible vocational activities after transplantation. In general, it has appeared that a reasonable prognosis can be offered to older patients only if operation can be performed at a somewhat earlier phase of their disease than is necessary for the young. If cardiac, vascular, or neurologic complications are severe, or if the older patient has been bedridden for a prolonged period, the chances of success are slim.

Disease

The diseases necessitating transplantation in all the patients at the University of Colorado are listed in Table 3. Chronic glomerulonephritis was the commonest renal disorder, accounting for 59 cases. End-stage pyelonephritis and polycystic disease were next in frequency. A few unusual indications were present in the rest of the cases (Table 3).

Those potential recipients who have an established infection in the upper renal tract fall into a special category in which transplantation should be offered only after extirpation of the diseased organs. Infected polycystic kidneys and active pyelonephritis, for example, require preliminary bilateral nephrec-

Table 3. Etiology of Uremia in First 75 Cases of Transplantation at the University of Colorado Medical Center

*Chronic glomerulonephritis	59
**Pyelonephritis	11
***Polycystic kidney	3
Congenital renal hypoplasia	1
Inadvertent removal of only kidney	1

*Several patients had coexisting pyelonephritis. One had superimposed gouty nephritis.

**One patient originally had congenital urethral valves. Another had congenital absence of one kidney and ureteropelvic obstruction of the remaining organ, which was previously treated with nephrostomy.

***One patient had multiple abscesses in the cysts and coexistent pyelonephritis.

tomies in a first stage, several weeks before immunosuppressive treatment is begun. Only when all wounds are healed and the sterility of the lower urinary tract has been demonstrated is it possible to safely proceed at a second stage with placement of the homograft (Fig. 4). Failure to obtain primary healing at the first operation almost certainly dooms the patient, whether or not transplantation is performed. In one of our cases, first-stage nephrectomy was carried out in a patient who had had a nephrostomy tube for 10 years in his only kidney. A massive wound infection delayed transplantation for two months, during which time he was maintained with dialyses (Fig. 5). Complete healing of the flank wound could never be obtained, despite which transplantation was carried out. Although the kidney functioned reasonably well, death occurred one month later from pneumonia, sepsis in the originally infected wound, and general paralysis resulting from polyneuritis.

The acceptance of patients with infected kidneys for two-stage therapy imposes a permanent moral obligation upon the physicians in charge of the case, inasmuch as creation of an anephric state is an integral part of the overall plan. It must be made clear to the family that it is possible that transplantation will never be feasible in the eventuality of a bacterial wound complication. It must also be accepted by the involved medical renal unit that such a patient may become a candidate for chronic hemodialysis for the rest of his life, or until the requirements for a second-stage operation can be fulfilled. If these conditions are unacceptable to the family or to the physicians who may have to assume the responsibility for chronic dialysis, surgical care of the case should not be undertaken.

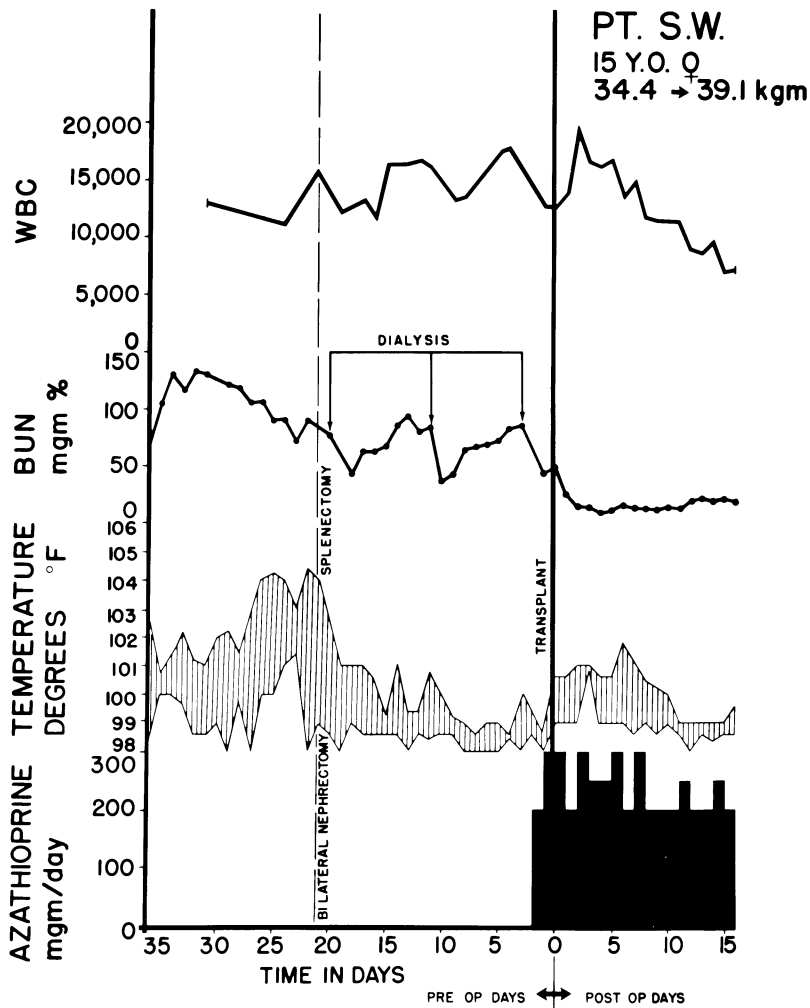


Figure 4. Course of a 15-year-old girl (LD 22) who received staged surgical care because of a urinary tract infection. She had polycystic kidney disease with coliform and fungal abscesses within multiple cysts of each kidney. Bilateral nephrectomy and splenectomy were carried out at a first stage, resulting in prompt relief of the pre-existing hyperpyrexia. Three weeks later, a maternal homograft was placed. (By permission of Arch. Surg. 89:87, 1964.)

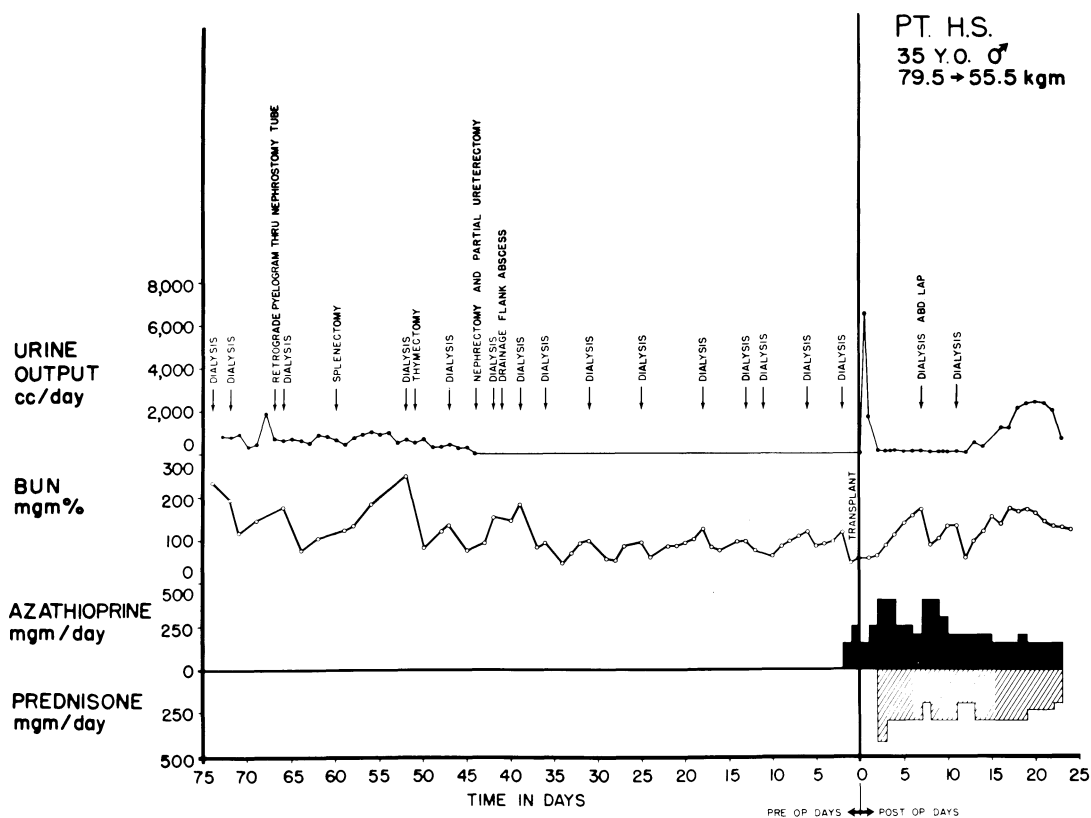


Figure 5. Course of a 35-year-old patient (LD 11) who had removal of his remaining kidney in which a nephrostomy tube had been present for 10 years. The flank wound developed a massive infection requiring long-term dialysis therapy. Wound healing did not occur. Transplantation was done in spite of this, and the patient eventually died of sepsis. Note that after transplantation, a massive diuresis occurred but was followed within 36 hours by anuria. The post-transplant renal shutdown which lasted for two weeks was reversible, and at the time of death there was improving kidney function.

Donor Availability

Finally, consideration of the availability of a donor may influence the overall evaluation of prognosis. Although this subject will be considered in detail in Chapter 5, it is appropriate to state that a prospective donation from a genetically favorable source such as a parent or sibling may significantly influence the decision for or against transplantation. Because the difficulties encountered in control of rejection are predictably fewer in this circumstance, it may be reasonable to rule favorably for a patient who might otherwise be considered too ill.

Lower Urinary Tract Involvement

In attempting to meet the criteria delineated at the beginning of this chapter, a special note should be made of the problem of ruling out mechanical defects of the lower urinary tract. It is important to obtain as much information as possible concerning the integrity of the bladder and urethra, without compromising the possibility of a transplantation. This can almost always be done without instrumentation by means of a careful history and physical examination, and by procuring records from other hospitals or from other physicians. A clear diagnosis nearly always results from perusal of old records and x-rays, without the necessity for imposing further potentially dangerous diagnostic procedures. Cystoscopy or retrograde pyelography has not been necessary in a single case in our series, and even preoperative introduction of a urinary catheter has been performed only on unusual occasions.

History

Detailed analysis of past records is also valuable in determining the inexorability of the patient's deterioration. If a pattern of consistently decreasing renal function can be demonstrated by retrospective study of serial biochemical studies, a decision for transplantation may be reached at an earlier time than would otherwise be possible.

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Chapter Four

PREPARATION OF RECIPIENT FOR TRANSPLANTATION

by J. H. Holmes, M.D.

The recipient's preparation for renal transplantation often starts in the outpatient clinic, occasionally when an elevated BUN is discovered for the first time. As a first step, it is important to establish that the etiology of the azotemia is primary renal disease rather than a mechanical defect of the lower urinary tract, or a systemic disorder such as lupus erythematosus, amyloidosis, or multiple myeloma. Urine cultures are obtained, and if an infection of the urinary tract is found, appropriate antibiotic therapy is instituted. If there are abnormalities of the bladder or urethra, these should be corrected surgically if transplantation is ever to be considered. A trial of conservative management is almost always attempted in order to ascertain the need for more aggressive measures. Regulation of fluid and salt intake, control of hypertension, and cardiac care are components of the regimen. Any sources of bleeding which might increase the invariably present anemia should be defined and treated.

At the beginning, it is important to discuss with the family the ultimate prognosis of the case, and it may be prudent to mention the potential role of renal transplantation in the future management. With some families, discussion of transplantation at this time may make easier the ultimate decision concerning this form of treatment, even in those instances in which a single dialysis may improve the patient's condition so much that proper conservative management may delay the need for other measures for many months.

In most cases, however, the patient is referred for transplantation in a state of advanced uremia. Here, the dialysis unit is an absolutely essential part of the transplantation program, since a major operative procedure cannot be considered until resuscitative measures are carried out. In addition to an elevated BUN, such patients frequently have fluid retention, heart failure, hyperkalemia, hyponatremia, or acidosis. All these complications are readily corrected by dialysis. Often the first treatment with the artificial kidney must

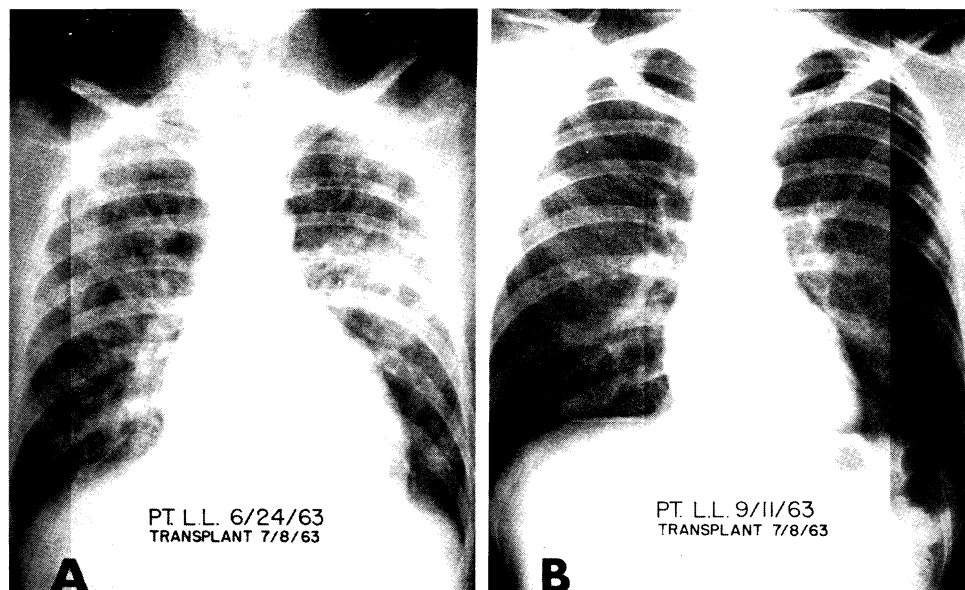


Figure 6. Chest x-rays before (A) and 2 months after (B) renal homotransplantation. The patient (LD 15), who was of A- blood type, received a kidney from an older brother of O+ blood type. Note resolution of cardiomegaly and pulmonary edema.

be done as an emergency procedure to treat pulmonary edema, hyperkalemia with cardiac arrhythmia, convulsions, or coma. Emergency pericardiocentesis or thoracentesis may be necessary.

Congestive heart failure is present in at least half the cases. A typical chest film shows an enlarged heart and pronounced pulmonary congestion (Fig. 6A). This is compared with an x-ray of the same patient taken two months later after a successful renal transplant with complete regression of these abnormalities (Fig. 6B). This patient had had several previous admissions to the hospital for treatment of pulmonary edema and congestive heart failure.

In contrast to the usual case in which water-logging is a primary problem, a few patients will be severely dehydrated either because of continued nausea and vomiting, or because of severe oral lesions which prevent food and fluid intake.

PROCEDURES IN DIALYSIS

All patients considered to be potential candidates for renal transplantation are treated by hemodialysis. The use of peritoneal dialysis can delay surgery, as occurred in several patients referred for transplantation from other hospitals. Septic complications at the abdominal sites of dialysis delayed transplantation in some cases and precluded it altogether in others.

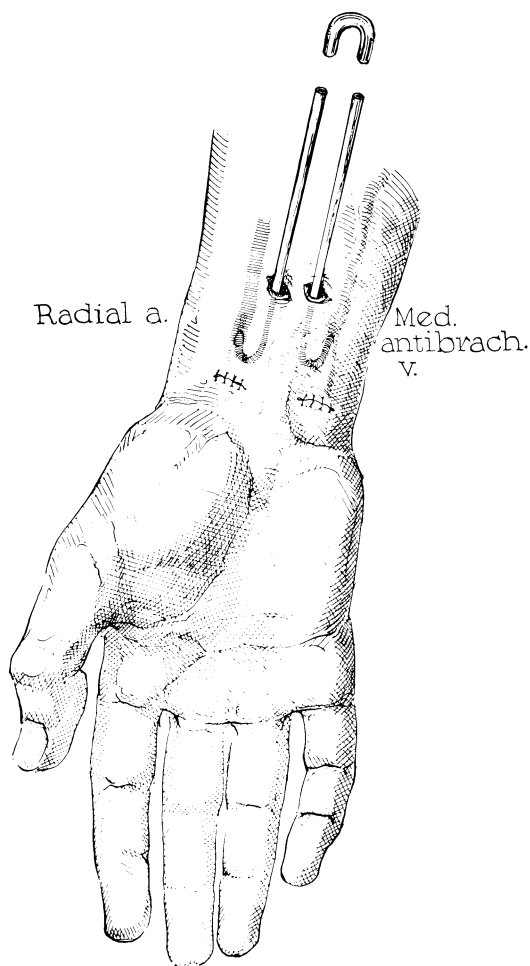


Figure 7. Chronic Scribner-type Teflon shunts used for chronic dialysis. Note that tubing is brought through the skin at a distance from the sites of their intravascular insertion.

Hemodialysis was performed with the conventional Kolff disposable twin-coil artificial kidney.* The Renal Service now does approximately 300 dialyses a year. The exact technique used at this institution is described in greater detail in references cited at the end of the chapter.

As soon as a decision is made for transplantation, an estimate is made of the total number of dialyses which will be necessary prior to operation. If only one or two are anticipated, catheters are placed in the artery and vein before each dialysis and removed at the conclusion. If a more protracted period of management is prognosticated, an indwelling Scribner-type arteriovenous Teflon shunt† is inserted, as shown in Figure 7, allowing continuous blood flow through the connecting plastic tube in the intervals between treatments. At

*Equipment obtained through Travenol Laboratories, Inc., Morton Grove, Ill.

†These can be made from commercially available Teflon tubing, or purchased ready-made from the U.S. Catheter and Instrument Co., Glens Falls, N.Y., or from the Swedish Freezer Co., Portland, Oregon.

the time of dialysis, the connecting loop is removed, and the arterial and venous cannulas are connected to the coil system. While there is an increased risk of infection inherent in the use of such a prosthesis, this is usually more than offset by the advantages of the ease of dialysis, the reduced trauma to the patient, and the increased frequency with which treatment can be conveniently provided. The majority of complications occur in the last two hours of a six-hour dialysis, so that the use of a shunt which permits shorter and more frequent runs is advantageous. When an arteriovenous shunt is used pre-operatively, the prosthesis is often kept in place until the time of transplantation. As soon as homograft function is demonstrated, the shunt is removed in order to avoid the possibility that it will serve as a source of bacteremia in the postoperative period. Removal is usually accomplished in such cases during closure of the transplant wound.

Several techniques of dialysis are particularly pertinent in preparation for transplantation. With a twin-coil unit, fluid can be removed both by increasing the osmolality of the bath (by increasing glucose concentration) and by constricting the outflow of the coil unit to raise the filtration pressure to a maximum of 240 mm Hg (monitored by an aneroid manometer). Using this high-pressure dialysis system, as much as 4 to 7 kgm has been removed during a single six-hour run. The patient is dialyzed on a weight scale so that an accurate record can be kept of the fluid changes. Regional heparinization is usually employed, introducing heparin into the extracorporeal circuit as the blood leaves the patient and neutralizing it with protamine as the blood is returned. In many uremic patients who have a hemorrhagic diathesis at the time dialysis is begun, it is possible to restore the blood coagulation time to normal during a six-hour dialysis. In these cases, anticoagulants may not be required, at least at the beginning. Von Kaulla has suggested that a circulating thrombin inhibitor is being removed in such instances.

After regional heparinization is started, Lee-White coagulation times are measured from both the coil system and the patient every 30 minutes. The only other routine laboratory test during dialysis is the hematocrit. A fall in hematocrit value usually indicates the need for transfusion at the end of a treatment, and this is commonly done with packed red cells. The blood can be removed from the extracorporeal circuit and used for immediate postdialysis transfusion after separation of the cells.

In some patients, the flow rate through the coil is kept low (less than 200 cc per minute) to minimize changes in osmolality and to prevent rises in intracranial pressure. Measurements may be made of intraocular tension (using a Schiøtz tonometer) and interpreted as an index of changes in intracranial pressure.

Hemodialysis is employed with one or more of the following objectives: to correct serum electrolyte abnormalities; to remove body fluid; to remove urea and other retention products; and to lower serum potassium in patients with hyperkalemia. The bath solutions are selected to achieve the specific alteration desired.

The frequency of dialyses varies in different preoperative patients, ranging from once every three or four days to once every 15 or 20 days. The primary objective is to get the patient into an acceptable condition for major surgery. No attempt is made to establish a completely normal metabolic state.

The number of dialyses required in preparation for transplantation can be reduced by conservative management. Water intake is restricted. Daily fluids are limited to the previous day's output plus an additional allowance for insensible water loss, which may vary from 400 to 1,000 cc per day. The insensible loss must be determined for each patient, based on weight changes and apparent fluid retention as estimated clinically. On such a regimen, patients complain of thirst and usually have a marked reduction in salivary flow. Gum chewing is encouraged as a prophylactic measure to prevent acute parotitis and to assist in mouth care. The oral lesions commonly observed in uremic patients are treated by a periodontist. Oral and pharyngeal cultures are obtained on all patients with ulcerative mouth or pharyngeal lesions.

The prescribed diet is usually limited to 20 to 40 gm of protein daily, the amount depending on the patient's ability to excrete urinary urea nitrogen as described in the following section. In badly debilitated patients, protein allowance may be increased even though this may necessitate extra dialyses. Drug administration is kept to a minimum, since many agents are unusually toxic in uremic patients.

Electrolyte Control

Although hyperkalemia is a serious problem in only about half the patients, it has been a frequent indication for emergency dialysis. There are three commonly accepted procedures for controlling a progressive rise in serum K without resorting to dialysis: the use of cation exchange resins either orally or by rectum; administration of sodium bicarbonate, particularly when the CO₂ combining power is markedly reduced; and administration of hypertonic solutions of glucose with insulin. The cation exchange resins have proved the most useful in our hands. When the serum sodium is low, sodium cycle resins are given. When the sodium is above normal, ammonia cycle resins are used. When the serum sodium approximates normal, a half-and-half mixture is employed. A dose of 15 gm is administered one to four times a day, the frequency depending on the severity of the hyperkalemia. When the patient cannot take the resins by mouth, retention enemas are used. Each gram of sodium cycle resin contains approximately 7 mEq of sodium. After passage through the intestine, each gram of resin will contain approximately 6 mEq of cation divided as 3 mEq of sodium, 2 mEq of potassium, and 1 mEq of mixed calcium and magnesium ions. The sodium retained in the body fluids after the exchange serves to correct the associated acidosis. When resins are administered, the serum sodium and CO₂ combining power as well as the potassium should be checked frequently, since prolonged therapy may produce hypernatremia and alkalosis.

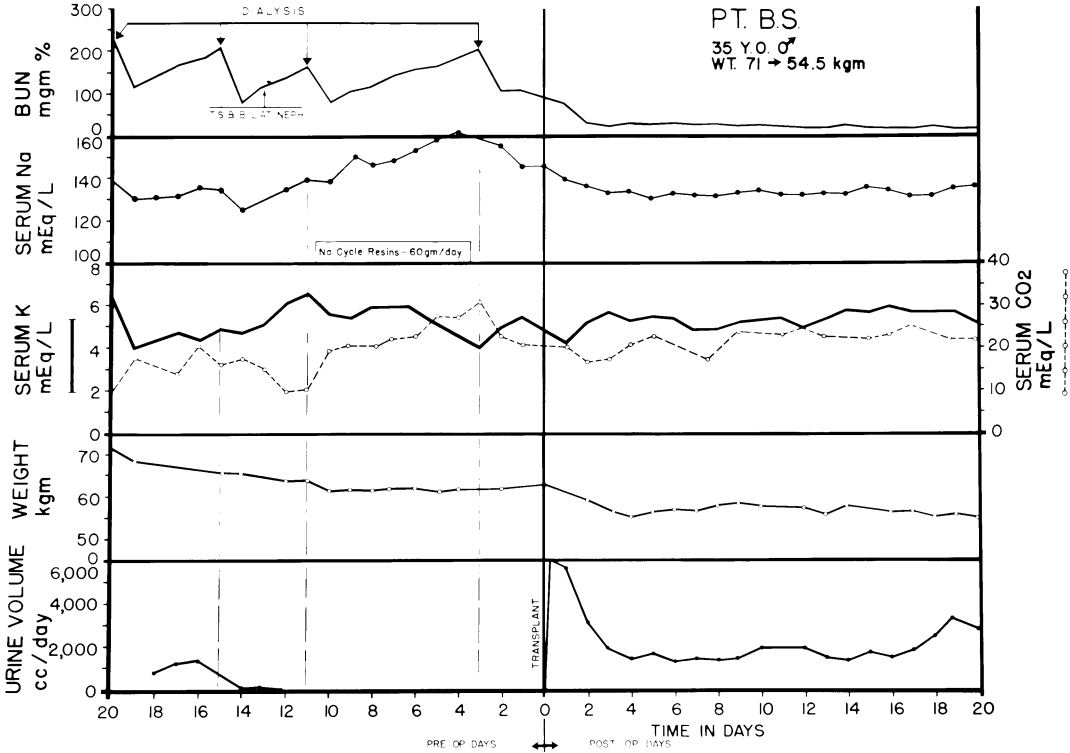


Figure 8. Combined preoperative use of dialysis and ion exchange resins (LD 4). Thymectomy, splenectomy, and bilateral nephrectomy (TS & Bilat. Neph.) were performed 13 days before transplantation. Note the alkalosis and hypernatremia caused by sodium cycle resins during the anephric interval. The homograft was given by the patient's wife.

Figure 8 is illustrative of the hypernatremia that can occur with use of sodium cycle resins. In this case, the resins were effective in reducing the serum potassium concentration, but there was an associated rise in serum sodium and an increase in CO₂ combining power with conversion of an acidotic state to one of alkalosis. Hypernatremia could have been prevented by decreasing the dosage of resins as the serum sodium rose and the CO₂ combining power increased. Figure 8 also illustrates the frequently observed inverse relationship between serum potassium values and CO₂ combining power. In some patients who have reduced serum calcium concentrations, tetany and muscular twitching may be the first clinical indication of alkalosis caused by resin overdosage.

Figure 8 also illustrates that in an anephric patient, total body fluids can be controlled and a steady decrease in weight achieved by fluid removal at dialysis, and by appropriate restriction of intake. It also shows that the interval between dialyses can be long without detriment, despite the absence of renal function. This may be due to depressed tissue catabolism, since the rise in BUN and serum phosphate concentration is sometimes surprisingly gradual.

Fecal impaction and ileus are frequent complications during the use of resins, and these problems may persist for many days after the transplantation. A mild laxative administered with the resins will often prevent these side effects. Since the resins avidly take up water, the assumption is made in calculating fluid intake that half the fluid administered with the resin is not available for dilution of the body fluids.

Prior to transplantation, the blood concentrations of sodium and potassium can be effectively controlled by limiting the sodium, potassium, and protein intake. In those patients who are excreting urine, measurements are made of the 24-hour urinary sodium and potassium, and the allowed intake is planned accordingly. The urinary urea nitrogen is also determined, and the results are used as a basis for restricting protein intake. Thus, a patient excreting 6 gm of urea nitrogen per day could be allowed a regular hospital diet, while the patient excreting 3 gm per day is usually restricted to a protein intake of 20 to 30 gm. Even with severe protein restriction, balance studies on three preoperative patients did not demonstrate a significant negative nitrogen balance. If the patient has lost large amounts of weight, a high protein diet may be required in order to improve the patient's condition for surgery as discussed before, and the frequency of dialysis increased accordingly.

Control of Pre-existing Conditions

In the management of cardiac failure, digitalis is avoided whenever possible, especially when the patient will require frequent dialyses. The fall in serum potassium and the rapid shifts in extracellular electrolytes occurring during dialysis may accentuate digitalis intoxication and produce cardiac arrhythmias. It is important that the EKG be monitored throughout dialysis so that any serious arrhythmia may be corrected by readjustment of the calcium, potassium, or magnesium concentrations in the bath. Prevention of fluid overload is the most important factor in managing cardiac failure in the uremic patient. In addition to weight changes, an increase in fluid load is best judged by evaluation of the neck vein distention and the presence of pulmonary congestion.

Hypertension is treated with the usual antihypertensive agents. It is not usually feasible to reduce the blood pressure to normal levels. The general policy is to obtain systolic blood pressures below 180 mm Hg. The doses of antihypertensive agents must be watched carefully, since less medication is often required in individuals with a reduced urinary volume. Sodium restriction is the most important means of controlling hypertension, rather than pharmacologic agents. On a salt-restricted diet, the serum sodium is brought down to a preselected level, usually 130 mEq liter. The sodium excretion is thereafter determined in the patient's urine, and precise sodium replacement is prescribed. Reserpine should be used sparingly, as it tends to make the patient lethargic. Ganglionic blockers have proved useful, but apresoline is usually tried first.

Bed rest may be used in refractory cases, although this is generally an undesirable practice in preoperative patients.

A special note should be made of the preoperative management of anemia, which is almost always present in these patients. Except for the packed red cell transfusions employed after hemodialysis, transfusions are avoided during the phase of resuscitation unless the hematocrit value drops below 20 per cent. Additional packed cell transfusions may be given just prior to surgery to increase the hematocrit value, although most patients go to the operating room in an anemic state. In those patients who have received multiple hemodialyses, it is frequently difficult to obtain cross-matched blood for purposes of pump priming, presumably because of multiple previous exposures to homologous blood.

Precautions with Dialysis

Although the minimum use of preoperative dialysis just described has its disadvantages, there are reasons for this policy. The financial and professional demands made by more frequent dialyses in any one patient would preclude its use for others who require this service just as urgently. In addition, the following complications and dangers may be associated with dialysis. The use of 2 units of blood to prime the coil system entails a risk of transfusion reaction. The patient is often fatigued or nauseated for some hours after completion of therapy. A febrile reaction lasting 24 to 36 hours is common, and sometimes is due to bacteremia. Hypotension, which is usually a result of too rapid a removal of fluid, may occur, although this can be alleviated by administering fluids or albumin. Conversely, sharp increases in blood pressure may occur during dialysis, and such episodes are often difficult to treat with pharmacologic agents. Severe headache sometimes occurs in the later portion of a dialysis. This may be related to increases in intracranial pressure secondary to rapid solute shifts. In several patients with this complication, measurements were made of intraocular pressure, and definite increases were observed. Finally, dialysis in patients receiving preoperative azathioprine carries an increased risk of septicemia, as will be discussed in Chapter 21.

An example of the use of dialysis during preoperative care is shown in Figure 9. A 15-year-old boy was referred with the diagnoses of glomerulonephritis and renal failure. He had been maintained at home on conservative management for six months with occasional hospitalization for dialysis. Deterioration was progressive, however, and on his last admission he had acute uremia with a BUN of 260 mgm per cent. He required three dialyses preoperatively. Serum potassium was controlled (Fig. 9). After the third dialysis, the BUN dropped to 50 mgm per cent, and although the urine volume was negligible, the BUN rose slowly to a value of only 100 mgm per cent just before operation, making a final dialysis unnecessary. The patient had a good result following transplantation with an early initial diuresis and a rapid drop in the BUN to normal range.

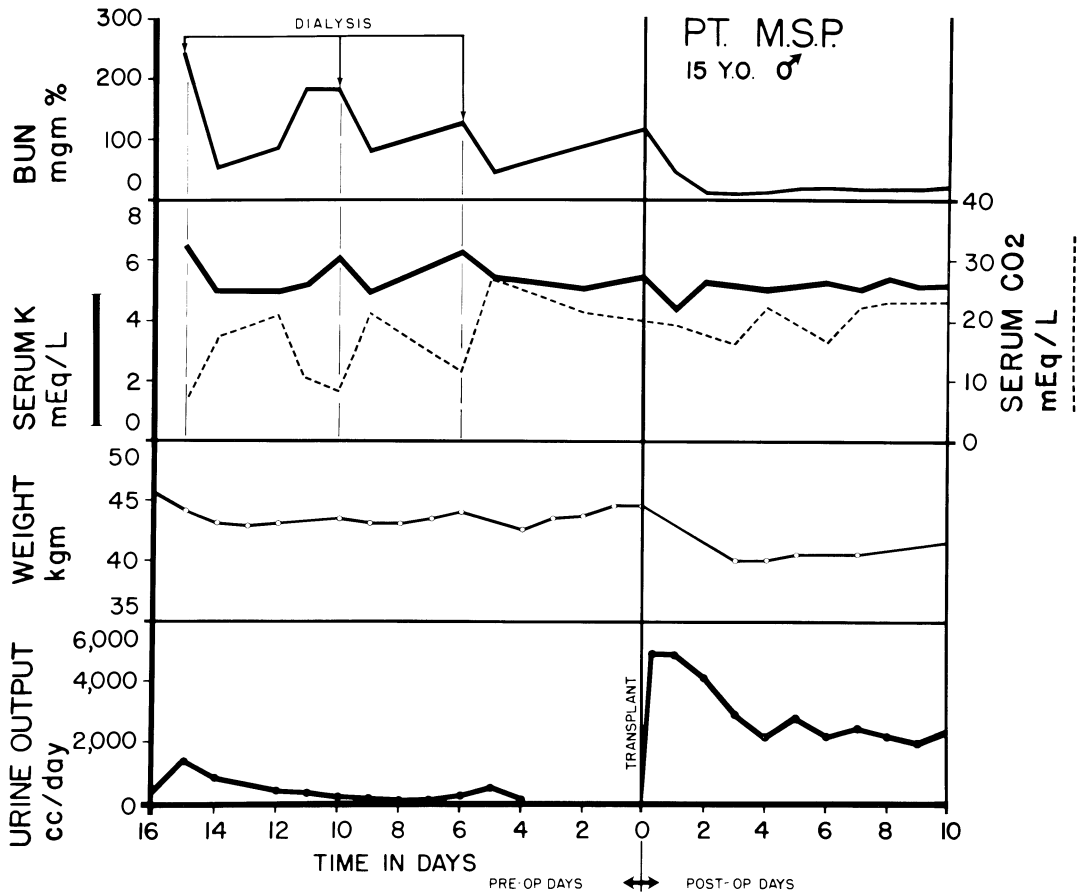


Figure 9. Typical preoperative resuscitation (LD 13). Note coincident correction of hyperkalemia and acidosis with each dialysis. Bilateral nephrectomy and splenectomy were performed at the time of transplantation. The boy's mother provided the homograft.

Final Preoperative Dialysis

Once the date for homotransplantation has been set, a decision must then be reached concerning the final preoperative dialysis. Usually the final run is carried out two or three days prior to transplantation, thus permitting the patient to recover completely from any immediate ill effects. The BUN and serum electrolyte concentrations are checked on the morning of surgery to make sure that no abnormal patterns have developed which would be dangerous during surgery. On a few occasions, it has been necessary to correct an elevated serum potassium by a short dialysis the night before or on the morning of operation. The presence of azotemia alone does not present any inherent hazards during anesthesia. Because of the policy of scheduling the last dialysis several days before homotransplantation, almost all the patients go to the operating room with significant azotemia, the blood urea nitrogen being in excess of 100 mgm per cent in the majority of cases. In only one case has an

operative complication occurred which might be attributed to this nonaggressive preoperative regimen. A 50-year-old man developed a cardiac arrest during the transplantation procedure, which was the consequence of an unrecognized pericardial tamponade. Although the patient survived the procedure, death followed 10 days later from infection in the wound required for open cardiac massage.

In some cases, it may be necessary to stage the surgical care of the recipient as described in Chapter 3. The surgically anephric patient must then be maintained by dialysis for 10 to 20 days or until the first-stage operative wounds are completely healed. The most common indication for staging is the presence of an established infection in the kidneys which requires their preliminary removal. Another indication for bilateral nephrectomy may be hypertension which cannot be controlled by medical treatment. The first-stage operation reduces the patient to a stage of renoprival hypertension, which is less severe and which can be readily controlled by medication. Another indication for staged surgery is the presence of an active peptic ulcer, which in two cases necessitated emergency gastrectomy and vagotomy prior to transplanta-

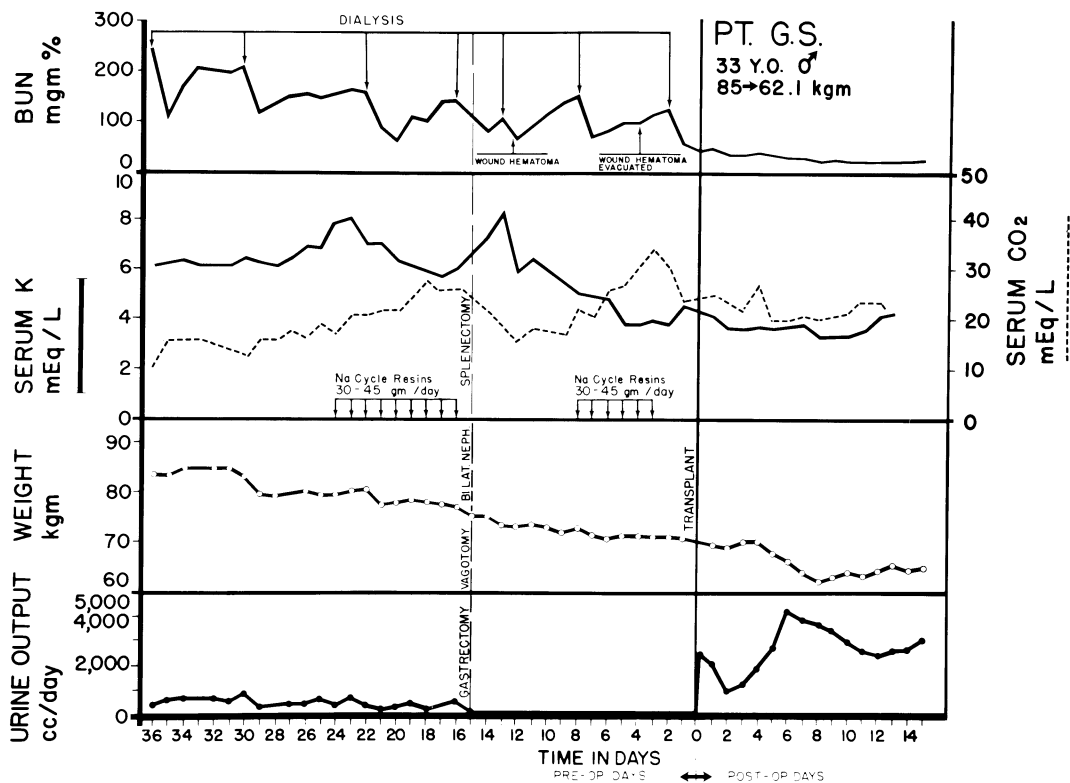


Figure 10. Preoperative management of a patient (LD 25) in whom a bleeding duodenal ulcer complicated the preoperative azotemia. Gastrectomy, vagotomy, bilateral nephrectomy, and splenectomy were carried out at a preliminary operation 13 days before transplantation. Note concomitant correction of hyperkalemia and acidosis with sodium cycle resins. Dialysis, performed too quickly after the first stage operation, apparently was responsible for the formation of a wound hematoma. The homograft was donated by the patient's younger brother.

tion. Concomitant bilateral nephrectomy and splenectomy were carried out, and the patients received renal homografts 13 to 28 days later.

The hospital course of one of these patients is illustrated in Figure 10. Three dialyses were required to prepare the patient for the preliminary procedure. Sodium cycle resins were used as a supplementary means of controlling hyperkalemia. There was a rise in CO_2 combining power during resin therapy, at which time the patient's daily urinary volume ranged from 200 to 600 cc.

On the third day after the first-stage operation, dialysis was repeated (Fig. 10). Previously well-healing wounds developed extensive hematomas, which were probably the result of inadvertent systemic heparinization during dialysis. As a result of this and a few other similar experiences, procrastination for as long as possible after major surgery before performing hemodialysis is now practiced. To do this, it is necessary to carefully plan the last preoperative dialysis so that the patient will not require a postoperative treatment for at least four to five days.

TREATMENT OF INFECTION

Care of any infectious complication is an important problem during the preoperative period. The patient is increasingly at risk during this time because of the use of preoperative azathioprine (see Chapters 14 and 21). All wounds are cultured, and intensive local care is given to the cut-down sites used for dialysis. Cultures are obtained from the throat, skin, urine, and blood. In all cases, cultures are obtained from the crevices of the umbilicus. Because of the subnormal temperatures which are characteristic of uremic patients, fever may be absent despite an established infection. A rise in white cell count, which is ordinarily a good index of infection in uremic patients, may also be depressed inasmuch as pretreatment with azathioprine is usually used (see Chapter 14).

The patient who has been rendered anephric by a first-stage operation constitutes a special problem for prophylaxis of infection. In this case, an antibiotic solution selected on the basis of sensitivity studies is inserted into the bladder every two days with a fine catheter. The patient is asked to retain the fluid for as long as possible. Usually, he will be able to hold the solution until just before the time of the next instillation when he is asked to void. In this way, the bladder is never free of local antibiotic. This use of catheterization is the only one permitted prior to transplantation, and then only for the few seconds necessary to place the fluid into the bladder. All ward personnel are instructed not to instrument the lower urinary tract for any other reason in patients being prepared for renal transplantation. If the patient is anuric on arrival, the ultrasonic compound scan is used to determine if the bladder contains urine, thereby avoiding the need for diagnostic catheterization.

The treatment of progressive uremia after transplantation is similar to

that just described. Of the first 48 patients, 10 required a total of 23 dialyses following operation. When possible, dialysis is avoided when the white count is low because of the increased hazards of infection. In patients who have already received homografts, shorter and more frequent dialyses are usually used. In several patients, dialysis has been a critical factor in maintenance of the patient until the transplanted kidney passed the rejection crisis and resumed function (see Chapter 16).

PSYCHOLOGICAL FACTORS

It is extremely important to prepare the patient and the patient's family psychologically for renal transplantation. As much understanding as possible must be provided of the many problems which will be encountered from the time of admission to the time of eventual discharge. Furthermore, the patient must plan to remain under close medical scrutiny for an indefinite time after operation, and agree to be seen regularly for studies in a follow-up clinic. In many instances, the patient has already accepted the immediate eventuality of death and must now reconsider a new life. The decisions involved may bring out hidden emotions and antagonisms within the family. When members of the family discuss the source of kidney donation, further emotional problems become evident, and situations of familial ostracism can be created unless care is exercised to avoid donor coercion. Overconcern and overprotection are often manifested by one or more members of the family which may disturb the patient and interfere with his hospital care. In most instances, these problems are the responsibility of the internist in charge of the case and of an assigned social worker. Occasionally, it is necessary to obtain psychiatric help. In some cases, the family's minister has been of great help. Association of the patient and his family with other patients undergoing this procedure is inevitable, and in most instances seems to be helpful in solving some of the difficulties mentioned.

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Chapter Five

SELECTION AND EVALUATION OF LIVING DONORS

In evaluating donors, priority is given to those volunteers having genetic relationships to the patient. A mass of experimental evidence can be cited in support of the position that homografts are tolerated better and for a longer period of time if the donor and recipient subjects are closely related. In humans, a similar tendency has been noted. For example, donations of mothers to offspring have been highly successful in our experience (Table 4). Donations from siblings fared less well (Table 4). Although it is possible to prevent rejection of homografts obtained from genetically nonrelated donors, the means necessary are more extreme, leading to a heightened incidence of drug toxicity reactions and frequent deaths from infection or bone marrow depression. In the last group, only four of the 13 recipients treated before December 10, 1963, were alive on June 1, 1964 (Table 4).

In discussing kidney donation with those who wish to volunteer, a perfectly objective account is given, not only as regards the risks to themselves, but concerning the prognosis of the recipient patient. The sacrifice of the donor may be so great that he must not commit himself to participation without realizing that there is a significant chance of early failure. Furthermore, he must understand that the results in the late stages of homotransplantation are not known with any degree of certainty. Undoubtedly, the eagerness with which family members or friends offer themselves is related to the results being obtained in any particular clinic. With a mounting hopeful experience, the incidence of unsolicited offers increases accordingly.

Determination of Blood Groups

After availability of donors for any given patient has been assessed, samples are drawn for the determination of major blood groups. Although conformity of the donor and recipient major blood types is not always necessary, it is more desirable than not to work with pairs who have major group compatibilities. When blood type dissimilarities exist between the donor and recipient patients, they must meet the requirements outlined in Chapter 6. Any other similarities are taken into consideration if the donor is genetically related. Physical resemblance, for example, would be considered a favorable sign.

Table 4. Effect of Consanguinity in First 45 Patients in Whom Living Volunteer Donors Were Used (LD Series)*

	No.	Alive	Dead
**Mother to son	8	5	3
Mother to daughter	5	5	0
Father to daughter	2	1	1
***Brother to brother	10	6	4
Brother to sister	1	0	1
**Sister to brother	6	4	2
Unrelated			
**Same sex	8	2	6
Female to male	4	1	3
Male to female	1	1	0
Total	45	25	20

*All operations were done between November 24, 1962, and December 10, 1963. Follow-up is to June 1, 1964.

**In each group, one additional homograft was donated and removed either immediately or early in the postoperative course. The failures were due to either breaches of ABO incompatibility (two cases) or the imposition of excessive ischemia (two cases). Second homografts were placed, and in the above tabulation, only the final donors are considered.

***Includes two sets of fraternal twins. One patient is alive after more than a year. The other died of a fungus brain abscess and gastrointestinal hemorrhage after 208 days.

Medical Evaluation

Once an expression of willingness from one or more potential donors has been given, a general medical evaluation is conducted to ascertain the constitutional suitability of the volunteer. Under special circumstances, as with maternal donors, kidneys have been taken from volunteers as old as 57 years. In general, however, selection is confined to those less than 45 years of age. A careful history is taken to detect past cardiovascular, pulmonary, or chronic infectious diseases. Complete physical examination is performed. In addition to the studies of renal function described below, an electrocardiogram and a chest x-ray are obtained.

Unsuspected pathologic findings of importance are sometimes uncovered. One 39-year-old woman was found to have a serous cystadenoma of the ovary which was so large that it had been interpreted by her as obesity (Fig. 11). Another patient was found to have a coin lesion of the right lower lobe (Fig. 12). This was removed by pulmonary wedge resection and found to be a histoplasmosis. The latter patient subsequently underwent donor nephrectomy.

The rest of the studies are designed for the mutual protection of the donor patient and the prospective recipient. The information sought concerns the quality of renal function of both donor kidneys and the clarification of any anatomic abnormalities in either organ. Particular care is taken to be sure that the kidney which is to be left in the volunteer is normal. At the same time, the technical difficulties presented by the organ to be removed are analyzed.

Determination of Renal Function

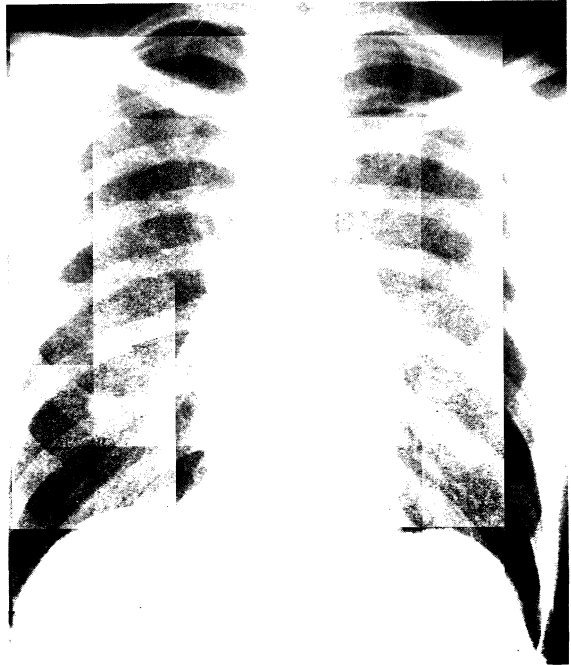
General determinations of renal function are first obtained with analysis of blood urea nitrogen (BUN) and creatinine (Ccr). If these are normal, multiple urine cultures are procured, using a noncatheter method of specimen collection. An intravenous pyelogram is obtained. If this and the other tests are normal, retrograde aortography is done with transfemoral insertion of the catheter under fluoroscopic control (Figs. 13, 14).

Some clinics do not believe that aortography is warranted, because of the slight but definite risk to the donor patient. Those holding this point of view believe that the operative penalties imposed by the necessity for reconstituting a multiple arterial supply to the donor organ are not sufficiently great to justify this diagnostic procedure. Nevertheless, in our hands, the longer time necessary to construct more than one arterial anastomosis has been a serious deterrent to success, as will be discussed in an ensuing chapter. For us, the aortogram has been the most useful single determinant in deciding which kidney is to be excised. Aortograms have been obtained in all but four of the living volunteers. In many cases, this examination has directly influenced the selection of the kidney to be used, most frequently because there is a single vessel

Figure 11. Large serous cystadenoma of the ovary discovered in a prospective donor. The abdominal x-ray was obtained prior to an intravenous pyelogram. The mass was of such large size that it had been interpreted by the patient as obesity. The patient was treated with hysterectomy and bilateral salpingo-oophorectomy, and was not further considered for donation of a homograft. (By permission of *Arch. Surg.* 88:711, 1964.)



Figure 12. A 22-year-old volunteer convict donor who was found to have a coin lesion in the right lower lobe. Wedge resection was performed for what proved histologically to be a histoplasmoma. Donor nephrectomy was subsequently performed (for LD 23).



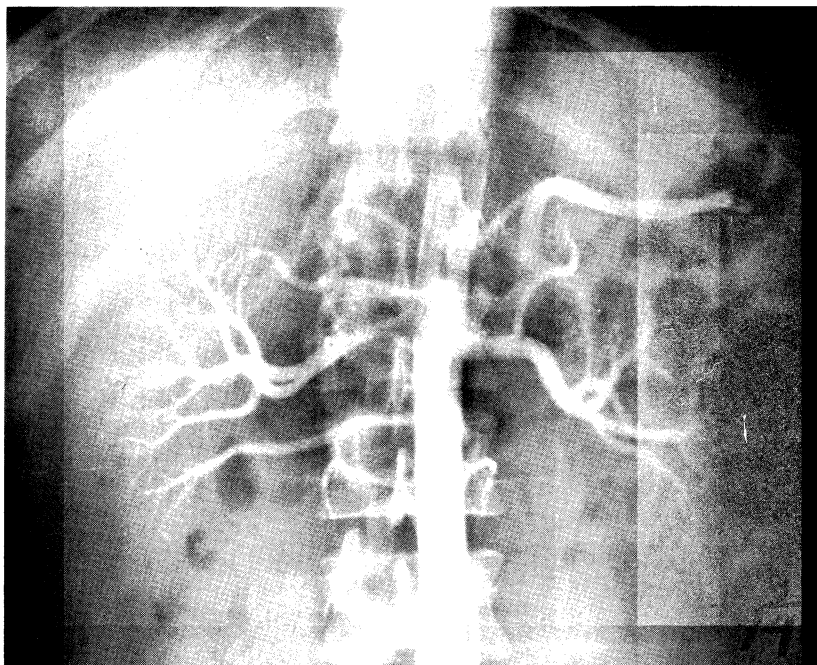


Figure 13. A common vascular pattern noted with aortography. In this case, there are two renal arteries to the right kidney and a single vessel to the left. The left kidney was used for a homograft (for LD 30). (By permission of *Arch. Surg.* 88:711, 1964.)

on one side and a double arterial supply on the other, an arrangement which has been found in 30 per cent of the cases (Fig. 13).

Aside from allowing selection of the kidney which can be transplanted with the greatest facility, aortography has on occasion offered protection to the donor patient as well. In two cases, long renal arteries were detected which appeared to have significant segmental occlusive disease. Both donors were women with moderately ptotic right kidneys (Fig. 14). Excision of the more commonly used donor left kidney would have left them with a predictably high future morbidity. If any equivocal abnormality is detected in either of the donor kidneys, the less perfect organ is used for the transplant. The first consideration of safety is always given to the donor.

On two occasions, violations of these general rules were perpetrated. In one case a mother scheduled to provide a kidney for her 15-year-old daughter (LD 17) was known to have had a past episode of pyelonephritis. At the time of removal of her right kidney, it was noticed that there were multiple pitted areas in the cortex typical of chronic pyelonephritis. On the day following operation, a positive urine culture of *E. coli* was returned from a previously submitted specimen. Surprisingly, the pyelonephritis in the transplanted kidney was brought under rapid control with antibiotic therapy. Effective treatment of the mother's urinary tract infection was also carried out. The case is more completely discussed in Chapter 21.

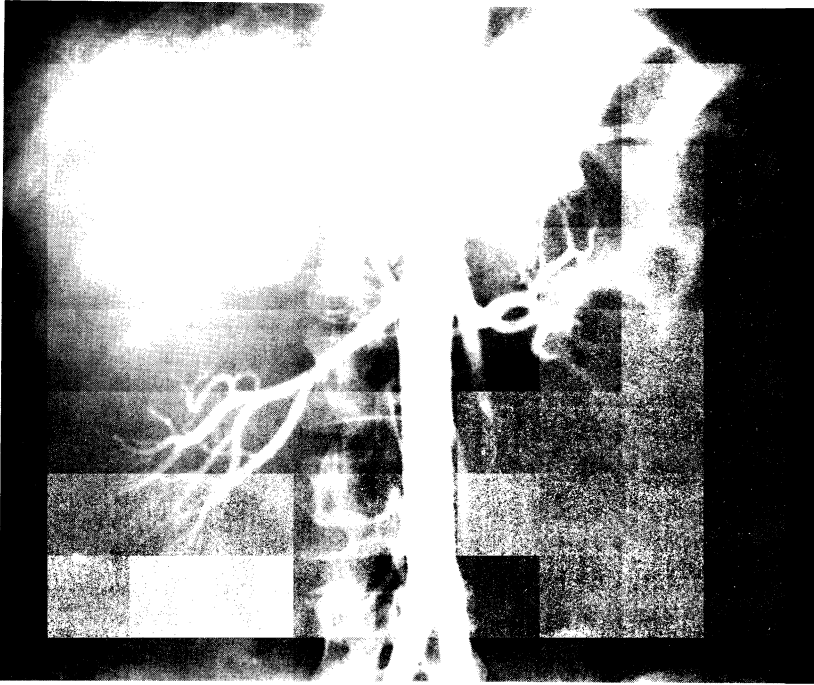


Figure 14. A partially occluding vascular lesion was noted in the nephroptotic right kidney in a 42-year-old woman, who was under consideration as a donor for her daughter. Use of the more commonly employed left kidney would have left her with a diseased renal vascular supply in her remaining organ. Ultimately, the right kidney was used for a homograft, transecting the renal artery as far distally as possible. An excellent result was obtained (LD 33). (By permission of *Arch. Surg.* 88:711, 1964.)

In another case, a 55-year-old war veteran who had sustained a shrapnel wound near his left kidney insisted upon donation of his right kidney for his 51-year-old brother (LD 12). No other donors were available. After protracted discussions, the operation was performed in May, 1962. Both brothers have returned to work and both are well, although the recipient had a mild delayed rejection 10 months after operation which was easily controlled.

RECENT CLINICAL EXPERIENCE

During the evolution of the experience at the University of Colorado, a slight but definite change in philosophy has evolved in relation to donor selection. From the beginning, the primary issue has been ensurance of the donor's safety. If this could be achieved, kidneys were used despite predictable technical difficulties of transplantation involving the use of kidneys with multiple small arteries. More recently, there has been a firmer insistence upon not only leaving, but also removing an ideal organ. If a kidney cannot be found which has a single renal artery of adequate length and diameter, donor nephrectomy is not thought to be justified. The donor patient must endure an operation of major proportions. It is as unfair to him as to the recipient to subject him to a

nephrectomy, knowing that the chances of success are materially and predictably reduced. Only in the case of identical twins is the use of kidneys with multiple renal arteries thought to be a justifiable practice.

The experience in the Colorado series in the selection of donor kidneys is quite different from that which might have been predicted on the basis of a previous anatomic analysis by Ross and his associates, which concerned the random usability of kidneys for homografting procedures. For various reasons, these authors concluded that the left kidney would be anatomically satisfactory in only 33 per cent of the cases and that the right kidney would be suitable for transplantation in only 3 per cent of the cases. In practical experience, it has been found that all kidneys, no matter what their side of origin, can be inserted without difficulty. In the first 42 cases of the Colorado series, 25 left kidneys from living donors were placed into the right iliac fossa, and 18 right kidneys into the left iliac fossa. In three other cases, donated right kidneys were transplanted to the right side of the recipient.

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Chapter Six

PATTERNS OF PERMISSIBLE DONOR-RECIPIENT TISSUE TRANSFER IN RELATION TO ABO BLOOD GROUPS

It has often been stated that renal homografts should be employed only when the major blood groups of the donor and recipient patient are identical. Although blood group antigens are not generally thought to be involved in the immunologic process of rejection, it has been feared that hemagglutination would occur within the grafts. Adherence to this widely accepted view has restricted the donor pool, and is known to have resulted in the denial of therapy to otherwise acceptable candidates for homotransplantation.

It is of historic interest that the first attempt at human renal homotransplantation by Voronoy involved the transfer of a B+ kidney to a patient of O+ blood type. Initial urinary excretion was observed, but death followed in 48 hours after reaction to a postoperative blood transfusion. Since that time, there has been a handful of reports, summarized by Hume, concerning similar violations of the dictum that donor-recipient blood group matching is essential. The significance of these pioneering experiences is difficult to evaluate for two reasons. First, many of the cases were done before 1955 when effective antirejection therapy was not available. More importantly, the quality of the kidneys used was poor in almost all cases. Most of the homografts were obtained from cadavers, usually with very prolonged periods of ischemia, and frequently without any protective device such as cooling or perfusion.

Table 5. Blood Groups of Donors and Recipients in the First 45 Patients Treated with Living Donor Kidneys*

	No.	Alive 6-1-64	Dead
**O Recipient with:			
Related O donor	12	9	3
Unrelated O donor	5	3	2
Related A donor	1	0	1
A Recipient with:			
Related A donor	13	7	6
Unrelated A donor	4	1	3
Related O donor	3	2	1
Unrelated O donor	2	0	2
Related B donor	1	1	0
B Recipient with:			
Related B donor	1	1	0
Unrelated O donor	1	1	0
AB Recipient with:			
Unrelated AB donor	1	0	1
Unrelated A donor	1	0	1
Total	45	25	20

*In the four patients receiving two homografts (LD 19, 23, 29, 35), only the second or definitive donor is tabulated. All patients were operated upon before December 10, 1963.

**In two of these O recipients (LD 19 and 23), A and B homografts were placed. The transplants were destroyed immediately after revascularization by an apparent hemagglutinative process, and removed; second homografts from O donors were placed 10 and 14 days later (see text). In one of the cadaveric cases (CD 1), an A to O transfer was made. The result is described in this chapter and in Chapter Eight.

More recently, reports from Hume and from our own clinic have documented the successful use of renal homografts provided by donors of different major blood groups from those of the recipient patients. In our experience, good function was obtained with various combinations of mismatches, and it was initially thought that indiscriminate crossing of blood groups was possible. This conclusion was supported by the fact that initially satisfactory renal function was obtained with virtually every combination (Table 5). As more experience accumulated, it became apparent that the transfer of mismatched tissue was safe only under specific circumstances, and that an increased incidence of early failure would be encountered if these conditions were not met.

In defining the circumstances in which blood group barriers may be safely crossed, principal credence will be given to that practical experience acquired at the University of Colorado Medical Center, rather than to that described in

the literature. The case material studied at this center has the advantage of homogeneity. In all but one of the first 12 cases in which ABO blood type incompatibilities existed, the homograft was donated by a living volunteer. The ischemic periods were reasonably short in all but the cadaveric kidney, ensuring a generally good quality of tissue. All organs were perfused with cold lactated Ringer's solution in order to remove residual blood before revascularization (see Chapter 10). The immunosuppressive therapy was similar in each case. There were no mechanical failures to alter the statistics.

CASES WITH MISMATCHED KIDNEYS

In 12 cases, kidneys were obtained from donors with different ABO blood groups from those of the recipients. The donor-recipient transfers were O to A in five patients, O to B in one patient, A to O in three patients (including one cadaveric kidney), B to O in one patient, B to A in one patient, and A to AB in one patient. In three of these ABO mismatches, there was a coexistent Rh incompatibility.

With the cadaveric kidney, in which transplantation was from an A+ donor to an O+ recipient, the time from death to revascularization in the new host was 120 minutes. Urinary excretion was delayed for eight hours. For the first 10 days a slow reduction in the BUN was seen, but after this there was progressive uremia until death 25 days after transplantation (see Figure 23, page 58). Histologically, the homografted kidney was extensively damaged. There was minimal cellular infiltrate, but there was considerable hemorrhage and moderate tubular necrosis. Severe generalized arteriolitis was noted. In this type of case, the influence of the blood group mismatch upon the unfavorable outcome is difficult to assess, because of the introduction of other factors attendant upon the use of severely ischemic tissue.

In the other 11 cases, the homografts were obtained from living donors under uniformly favorable conditions. All kidneys were devascularized for less than 40 minutes, and all were cooled by intra-arterial perfusion. The greatest experience was with kidneys donated by O donors to A recipients. Five such combinations were used, as well as an O to B donation (Table 5). Immediate gratifying diuresis was observed in all six cases. The difficulty in controlling the rejection process was comparable to that encountered in patients receiving matched kidneys. The rejection crises occurred one to 19 days after operation, and were reversed (see Chapter 15). Three late deaths due to sepsis occurred after 77, 79, and 206 days (see appendix).

In another case, an A+ kidney was donated by the wife of an AB recipient. Excellent renal function persisted until the death of the patient from sepsis after 113 days. The homograft was relatively undisturbed by a series of calamitous complications, including multiple wound infections, intestinal obstruction, and a massive pulmonary embolus treated by pulmonary embolectomy and subsequent plication of the inferior vena cava above the transplantation site (Fig. 15). Histologic sections of the homograft obtained at autopsy

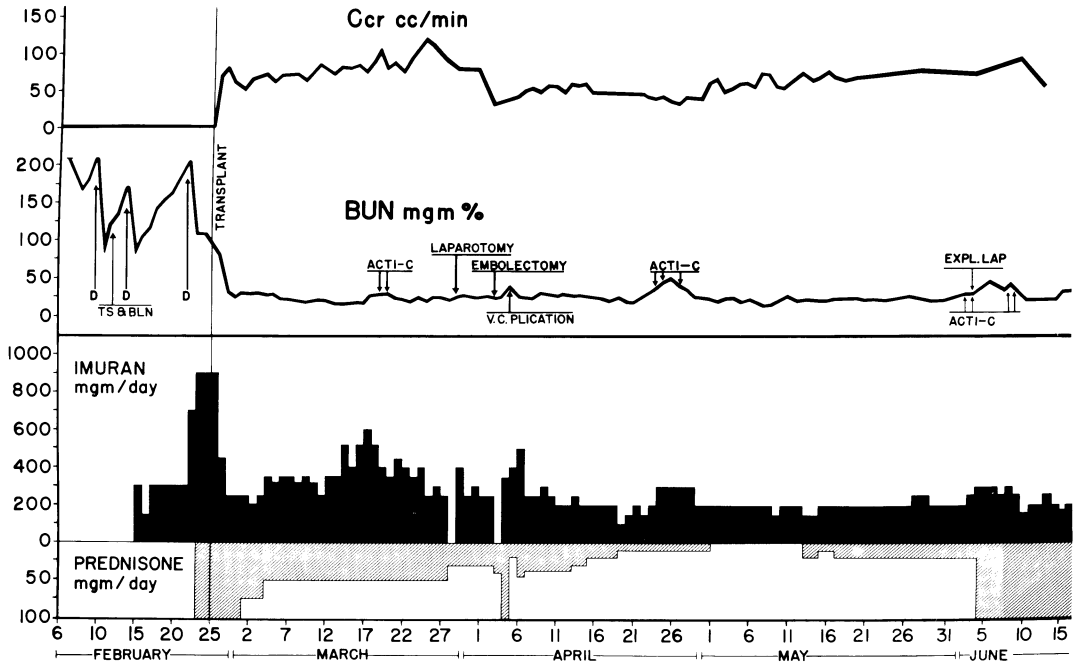


Figure 15. Course of patient (LD 4) with AB+ blood type who received an A+ kidney from his wife. A definite rejection crisis did not occur, possibly because of the prophylactic use of prednisone (see Chapter 18). D—Dialysis; TS & BLN—Thymectomy, splenectomy, and bilateral nephrectomy; Acti C—Actinomycin C, each arrow equaling 200 μ g actinomycin C administered intravenously. Patient ultimately died of unrecognized intra-abdominal abscess. (By permission of *Surg. Gynec. Obstet.* 117:385, 1963.)

showed good preservation of architecture with only a few foci of round cell infiltration.

An additional patient with A+ blood type received a kidney from his sister who belonged to B+ blood group. This was the second renal homotransplantation performed at this center. The immediate and late result was excellent, although the patient had a very severe rejection crisis 25 days after the operation (Fig. 16).

In numerous patients, the Rh factors were different in the donors and recipients, the transfers being both from Rh— to Rh+ and in the reverse direction (see appendix). Good early renal function was obtained in all.

Complications Encountered with Mismatched Kidneys

Three additional patients are considered separately inasmuch as their courses appear to have been adversely affected by problems of blood group incompatibility. In these cases, A or B kidneys were transplanted to hosts of O blood type.

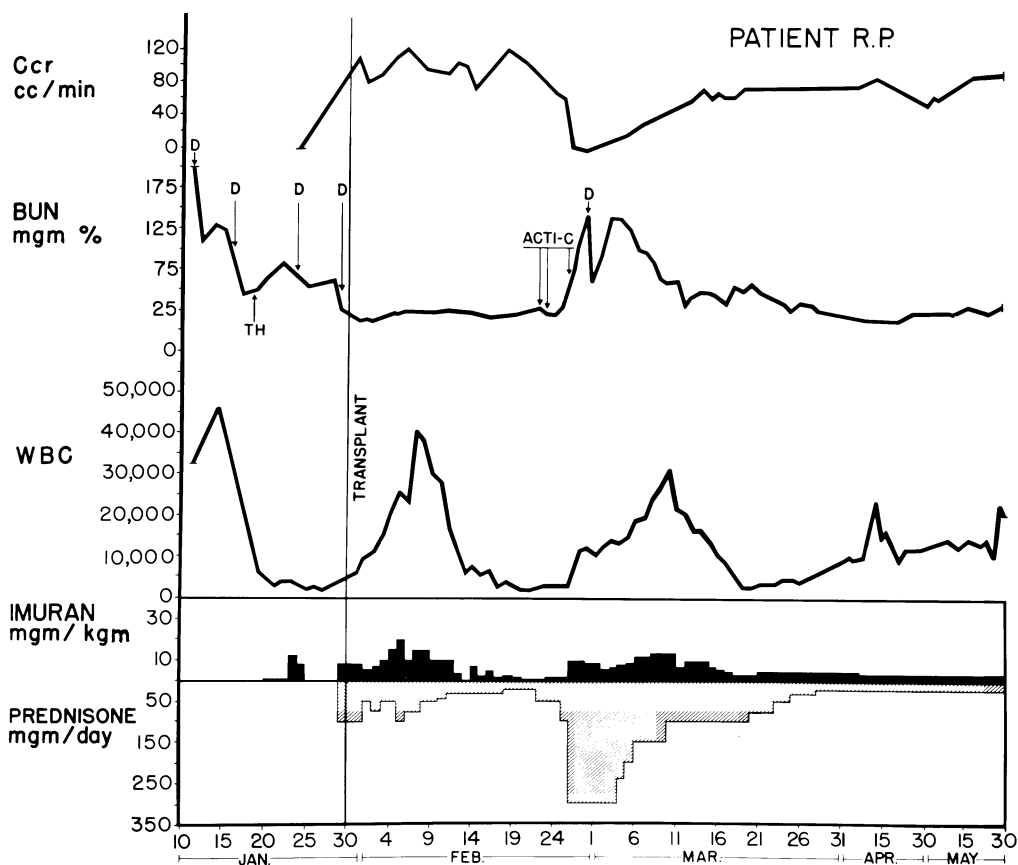


Figure 16. Patient of A+ blood type who received a kidney from his sister who was of B+ blood type. The patient is the same one as shown in Figure 3 (LD 2). A rejection crisis which began on the twenty-fifth postoperative day was reversed with standard measures. Transplantation was performed on January 30, 1963, and the patient has had normal renal function more than one year later. Despite this, this type of donor-recipient mismatch would no longer be employed. D—Dialysis; Th—Thymectomy; Acti C—Each arrow equals 200 gamma actinomycin C, I.V. Left nephrectomy and splenectomy were performed at the time of transplantation. Right nephrectomy has never been performed. (By permission of JAMA 187:734, 1964.)

The most dramatic complications occurred with two patients who were O- blood type. One received his homograft from an A- donor and the other from a B+ donor. In both cases, the homografts became cyanotic within a few minutes after revascularization. The circulation could not be improved despite intra-arterial injections of procaine, prednisolone, heparin, and papaverine. Incision into the parenchyma resulted in venous hemorrhage but little or no arterial bleeding. More deeply within the medulla, sluggish arterial bleeding was present. Both kidneys remained soft without restoration of the firmness and turgor normally seen. The two homografts were removed within three hours of the time of their insertion. After transection of the specimens, areas



Figure 17. Renal homograft from patient with A+ donor and transplanted to O+ recipient (LD 19). The organ was removed three hours after revascularization. Note dark color and hemorrhagic zone at corticomedullary junction. (By permission of *Surg. Gynec. Obstet.* 118: 819, 1964.)

of hemorrhagic discoloration were observed at the corticomedullary junction (Fig. 17).

Arteriograms of the surgical specimens showed filling of the major arterial ramifications, with absent (Fig. 18A) or poor (Fig. 18B) vascularization of the cortex. Histologic sections of the two kidneys were interpreted as normal except for areas of congestion and aggregation of red cells, especially in the glomeruli and small arterioles (Fig. 19). No thrombi were present. In one patient, sharp rises in the titer of the specific hemagglutinins were observed within a few days after operation (see Chapter 22). Second homografts from blood-type compatible donors were placed 14 and 10 days later.

The course of a third patient of O+ blood type is thought to have been adversely affected as the result of nonconformity of the blood groups. The recipient, a six-year-old child, received a kidney from his A- mother. Diuresis began almost immediately, but was succeeded by oliguria within 30 hours. The process of renal destruction was halted and controlled with the therapeutic regimen described in Chapters 14 and 16, but only with the greatest difficulty. Reversal of the secondary acute renal failure which started after one and a half days required more time than any other case treated by us (see Figure 60, Chapter 14). In addition, ultimate renal function was impaired in several ways despite the fact that the BUN had returned to normal. The patient's creatinine clearance remained low, proteinuria persisted, and he had low grade hypertension. Five and one-half months postoperatively, he developed convulsions and coma, presumably as the result of a cerebrovascular accident. He died 202 days after operation. Permission for autopsy was denied.

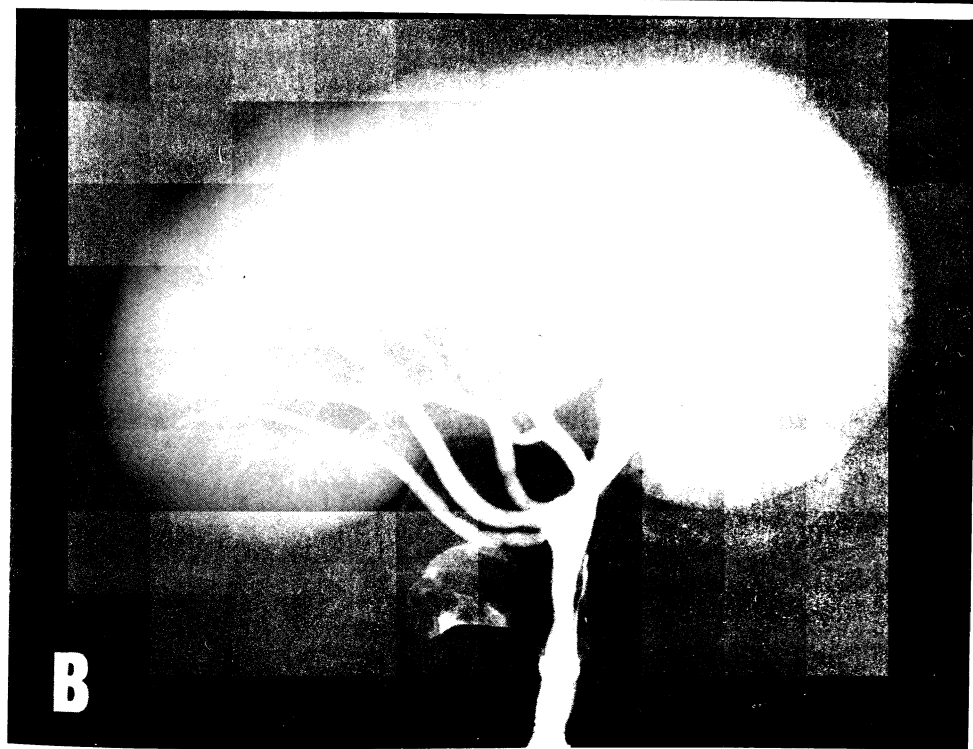
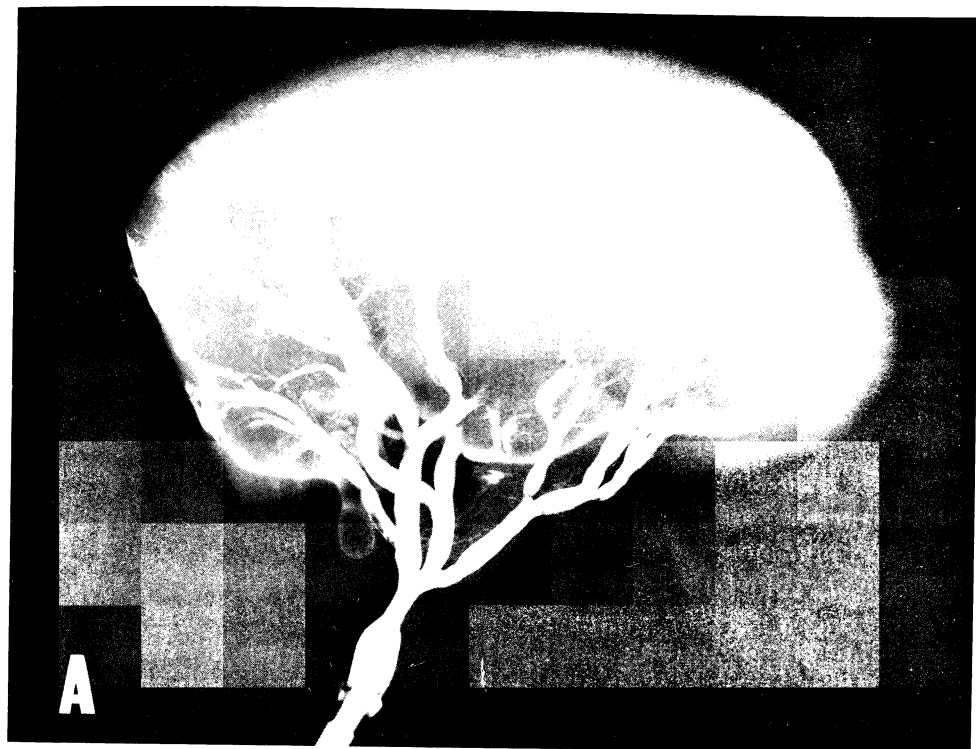


Figure 18. Homografts removed a few hours after revascularization. Both recipients were O+ blood type (LD 19 and LD 23), and the donors were A- and B-, respectively. The arteriogram in (A) is from the same specimen as shown in Figure 17, and the dye staining at the corticomedullary junction corresponds to the hemorrhagic area seen in the gross specimen. The cortical devascularization in (B) is not so extreme. (By permission of *Surg. Gynec. Obstet.* 118:819, 1964.)

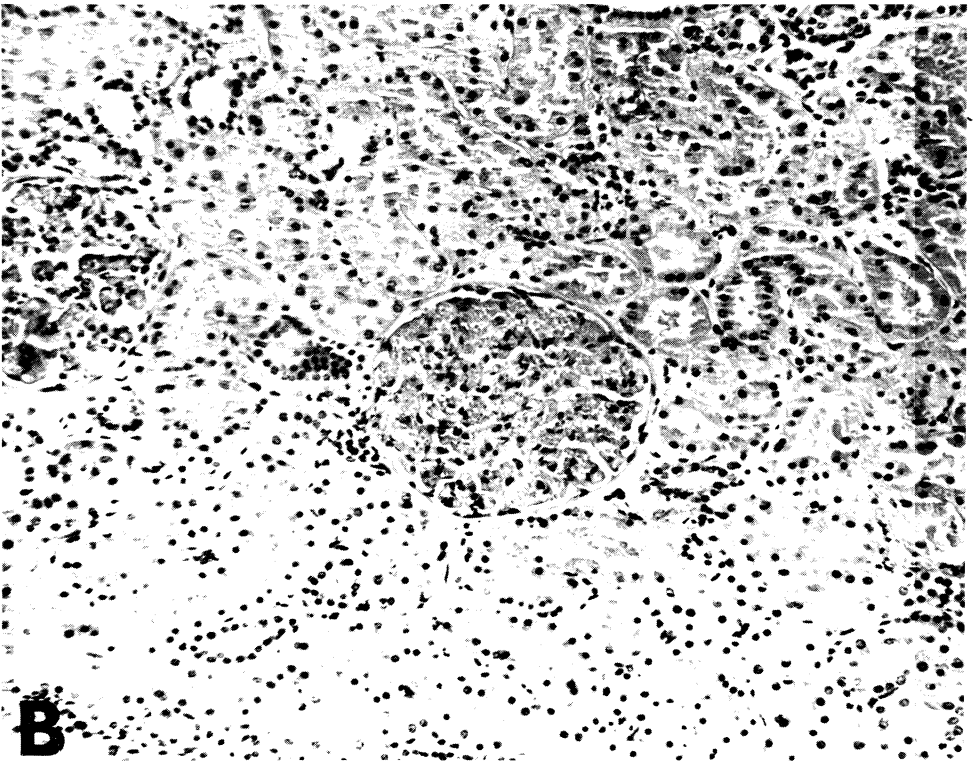
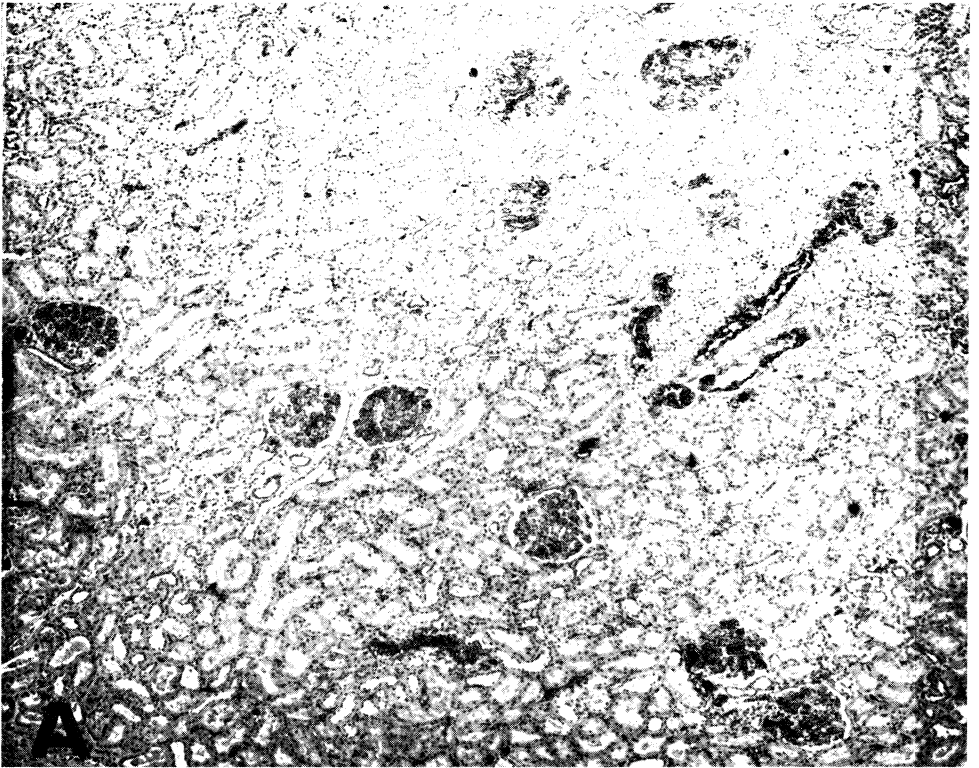


Figure 19. Histologic appearance of the two unsuccessful homografts seen in Figure 18. (A) and (B) are in the same order. Note aggregation of red cells, particularly in glomeruli and in small arteries. A—H and E (X 32). (By permission of *Surg. Gynec. Obstet.* 118:819, 1964.) B—H and E (X 80).

CONCLUSIONS CONCERNING BLOOD GROUP INCOMPATIBILITY

A brief consideration of the immunologic factors involved in the use of mismatched tissues is pertinent, especially as they relate to the presence of preformed host antibodies. If the recipient belongs to O blood group, circulating anti-A and anti-B hemagglutinins are predictably present. If the recipient is of A or B type, anti-B and anti-A hemagglutinins, respectively, are present, although these are likely to be of lower titer. Patients of AB group will not have preformed hemagglutinins of either type. A critical issue in predicting the recipient's immediate reaction thus involves evaluation of the probable status of the hemagglutinating humoral antibodies in the host.

With the donor tissue, the converse is true. The antibody content of the retained blood in the homograft is of little importance, since the kidney can be flushed (as described in Chapter 10) before it is exposed to its new circulation. Although this simple technique rids the kidney of most of its unbound antibodies, it does not alter the blood group-specific antigenicity of the tissue, since Hogman and Szulman have demonstrated that renal parenchymal cells possess the same blood types as erythrocytes. Consequently, it would be anticipated that an O kidney would not react with host hemagglutinins since it does not have A or B antigens. A and B kidneys could react with host anti-A and anti-B antibodies if these were present. An AB kidney could react with both types of host hemagglutinins.

Comparison of these theoretical considerations with the practical experience already described has led to rules governing the use of mismatched combinations. These rules are based primarily on the avoidance of situations in which preformed host hemagglutinins are present which could react acutely against the homograft being provided. It has been concluded that the donor to recipient incompatibilities which are relatively safe are O to non-O, Rh- to

Table 6. Direction of Acceptable Mismatched Tissue Transfer*

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.

Rh+, and Rh+ to Rh- except in the unusual and predictable circumstances in which recipient presensitization has occurred (Table 6). The combinations which involve a heightened risk are: A to non-A, B to non-B, and AB to non-AB (Table 6). Thus, the pattern of acceptable tissue transfer within the ABO system is comparable to that already defined for blood transfusions in that O patients are probably universal donors and AB patients are universal recipients.

OTHER BLOOD GROUP CONSIDERATIONS

In the preceding portions of this chapter, those combinations were described of donor-recipient blood group incompatibilities which were and which were not relatively safe. Within the larger categories of patients having identical donor-recipient blood types, there was a subtler difference in late results which is of unknown significance but which warrants description. In the first 45 cases of the living donor series, there were 17 homotransplants of A kidneys to A recipients (Table 5). Nine of the patients died at a relatively early time in the postoperative period, a failure rate which was exorbitantly high with the use of both related and unrelated donors (Table 5). In contrast, there were only five deaths in the comparable series of 17 patients with O blood type (Table 5). In both groups, excellent early homograft function was obtained, but the subsequent rejection episodes in A-type matches tended to be more severe and protracted, suggesting that a specific sensitization to the red cell antigens in the homograft may have occurred and contributed to the difficulty of maintaining homograft viability. Slight differences in the A substance of the donor and recipient which are commonly present despite the absence of agglutination with direct cross-matching could presumably lead to the delayed production of hemagglutinins and other circulating antibodies. Further observations will be necessary before it can be determined if the difficulties in transplanting A kidneys to A recipients are real or are coincidental.

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Chapter Seven

THE IMPORTANCE OF HOMOGRAFT QUALITY

One of the most significant factors in obtaining ultimate success is the attainment of good immediate homograft function. When this is achieved, there is a massive postoperative diuresis with consequent early improvement in the patient's general condition. Later, diagnosis of the rejection crisis is greatly facilitated if a change in the pattern of renal excretion can be demonstrated against a background of initial good function (see Chapter 15).

FACTORS INFLUENCING INITIAL HOMOGRAFT FUNCTION

Incidence of Anuria

Because of the great importance of obtaining prompt and adequate homotransplant function, it is desirable to know the prerequisites for achieving this state and to know with accuracy the incidence to be expected of immediate anuria. Immediate oliguria or anuria after human renal transplantation has been noted by a number of authorities. Although Egdahl and Hume believed initial anuria to be a rarity in dogs, Dempster's collected data indicated that a substantial number of human renal homografts never excreted urine in adequate quantities, and he suggested that the factors responsible for some of these sudden failures could be immunologic as well as technical.

Data analyzed from the first 42 cases studied at the University of Colorado, involving the use of 46 homografts from living donors, demonstrate that immediate postoperative anuria is an uncommon occurrence, and define as well the circumstances which appear to have been responsible for those instant organ failures encountered in this series. In three instances, the infliction of excessive ischemia upon the transplanted tissue accounted for the absent or sluggish early function. In these three cases, the intervals necessary

for transplantation were 61, 71, and 85 minutes. The only other cause for immediate postoperative excretory failure was the presence of highly unfavorable donor and recipient blood group incompatibilities in two cases, as described in Chapter 6.

Ischemia

The degree to which excessive ischemic injury of homografts impairs early function has not been known with certainty. It has been assumed by many authors that the margin of safety is a large one. Those holding this point of view allude to the almost uniform success of renal transplantation between identical twins, despite great differences in the trauma inflicted upon the donated kidney in terms of ischemia and in terms of organ temperature at the time of its devascularization.

A different point of view can be easily supported, based upon the contention that the margin of safety in identical twins (isografts) is incomparably greater than with true homografts. Even though severe but reversible ischemic injury occurs, steady improvement may be expected, since a secondary parenchymal insult does not occur when isografts are used. In contrast, the recovery of homograft function is almost inevitably interrupted by a rejection phase. The ability of the graft to remain viable is almost surely related to some extent to the quality of the tissue at the time of the rejection crisis.

In addition, it is equally possible that the immediate function of the homograft is also more dependent than genetically identical tissue upon minimization of ischemia, particularly in view of the classic studies of Dempster which showed a more severe acute edematous reaction in renal homografts than in comparable autografts. It has been our growing conviction that improvement in the technical aspects of tissue transfer, including homograft cooling and heparinization, is an important element in the attainment of the prompt function without which the possibility of long-term success is greatly diminished.

An effort has been made to define the limit of ischemia within which it has been safe to work in our own experience. The following analysis concerns the function obtained from 44 homografts provided in the first 42 patients for whom living donors were used, excluding the two additional homografts which had to be removed immediately after revascularization because of acute hemagglutination reactions (Chap. 6).

Analysis of Ischemic Intervals. With 24 of the homografts, time from occlusion of the donor renal artery to restoration of the blood flow in the recipient ranged from 17 to 30 minutes, with a mean of 25.4 ± 0.7 (SE) minutes (Table 7). Homograft function was generally prompt in these cases, and a massive diu-

Table 7. Effect of Duration of Ischemia on Function in 44 Homografts

	<u>Less than 30 minutes</u>			
	No.	Mean	SD	SE
1. Time urine onset (min)	24	33.7	36.9	7.6
2. Diuresis per hour for first 12 hours (ml)	24	511.0	275.0	56
3. Preop BUN (mgm%)	24	71.2	23.7	4.8
4. Lowest BUN within two days (mgm%)	22	24.5	14.3	3.0
5. Highest Ccr (ml/min) within two days	22	97.9	36.2	7.7
	<u>31 to 43 minutes</u>			
1. Time urine onset (min)	17	30.7	34.2	8.3
2. Diuresis per hour for first 12 hours (ml)	17	393	183	47
3. Preop BUN (mgm%)	17	97.9	49.4	11.9
4. Lowest BUN within two days (mgm%)	17	38.4	27.7	6.7
5. Highest Ccr (ml/min) within two days	15	53.4	21.6	5.6
	<u>61, 71, and 84 minutes (three cases)—see text</u>			

resis ensued of 511 ± 56 (SE) ml per hour for the first 12 hours. Within 48 hours, the mean BUN fell from 71.2 ± 4.8 (SE) mgm per cent to 24.5 ± 3.0 (SE) mgm per cent. Creatinine clearance rose to 97.9 ± 7.7 (SE) ml per minute. The mean interval between revascularization and the beginning of urine excretion was 33.7 ± 7.6 (SE) minutes.

Ischemic periods of 31 to 43 minutes were inflicted upon 17 homografts with a mean of 38.1 ± 0.8 (SE) minutes. Early urine excretion was also obtained with this group after an interval of 30.7 ± 8.3 (SE) minutes. Within 48 hours, BUN's fell from 97.9 ± 11.9 (SE) mgm per cent to 38.4 ± 6.7 (SE) mgm per cent. The mean creatinine clearance was 53.4 ± 5.6 (SE) ml per minute.

Three additional homografts required 61, 71, and 84 minutes of ischemia for transfer. Times from revascularization to notation of first urine excretion were 30 and 600 minutes in the first two patients and in the last case the homograft was anuric until its removal. Within 48 hours, the BUN went from 63.5 to 60 mgm per cent, 84 to 87 mgm per cent, and 100 to 118 mgm per cent, respectively. Creatinine clearances were 22, 15.6, and 0 ml per minute. The course of the second of these cases is shown in Figure 61, Chapter 14.

It is obvious from these results that ischemic periods approaching or exceeding one hour are unsatisfactory. Analysis was also conducted* of the significance of differences in those homografts ischemic for less than 30 minutes compared to those ischemic for 31 to 43 minutes. On the average, it will be noted (Table 7) that the function of kidneys with lesser degrees of ischemia was superior in all aspects except for the time of onset of urine flow which was equal in both groups. Statistically, the most easily demonstrable effect of ischemia was observed in the measurements of creatinine clearance in which the difference in the two groups was highly significant ($p < 0.001$). Even within the group with ischemic periods of 30 minutes or less, rank order analysis demonstrated a positive correlation between ischemic period and the magnitude of early postoperative creatinine clearance ($R=0.494$), the confidence level being 5 per cent.

These findings emphasize the need to develop facility in performance of vascular anastomoses before embarking on clinical homotransplantation. The data support the view that good function can be consistently obtained if the period of ischemia can be kept to less than 40 minutes, but they also suggest that even within this interval the results will be roughly related to the time expended in revascularization. Unlike many other operative procedures, which can be learned in the clinical operating room, the ability to perform renal homotransplantation with controlled speed must first be mastered by extensive practice in the laboratory if good results are to be expected.

Hypothermia. Before such figures are accepted as guides for the permissible periods of ischemia, it must be emphasized that one form or another of homograft hypothermia has been consistently applied in the series from which

*By Doctor Allen Sexton.

these values were derived (see Chapter 10). The degree to which cooling has lengthened the period of acceptable ischemia in this type of case is not known. However, it seems inevitable that the degree of trauma which is compatible with good early function would be considerably less if transplantation were performed with normothermic kidneys. In planning a transplant procedure, the need for organ cooling must therefore be related to an objective and honest evaluation of the projected rapidity with which revascularization can be performed.

Aortographic Evaluation. Inasmuch as the speed with which transplantation can be carried out appears to have an important influence on the prognosis, aortographic evaluation of the donor assumes a special significance. The use of donor organs which have two or more arteries inevitably leads to prolongation of the time necessary for completion of the vascular anastomoses. Re-establishment of circulation to these smaller vessels has, in our experience, doubled or tripled the duration of the ischemic interval. It is noteworthy that the organs most severely injured, in which reconstitution of blood flow was not obtained until 61, 71, and 85 minutes, all had a double arterial blood supply. It has become our policy not to consider donors with multiple renal vessels, no matter how favorable the genetic relationship of the donor to the recipient, with the sole exception of identical twins.

Other Elements

Aside from efforts to minimize the ischemia during transfer of the graft, other measures are taken to protect the donated tissue. Heparinization, either by local infusion or by total body administration to the donor, may be of value in preventing intraparenchymal clotting. In experimental studies on dogs, Mims has demonstrated that homograft arterial flushing occurs more promptly with the use of this drug. Care is exercised in mobilization and dissection of the kidney in the donor patient to avoid inadvertent occlusion of the vessels. Hypotensive episodes in the donor patient are studiously avoided, not only to protect the kidney which is being removed, but to insure maximum security as well for the organ which is to be left.

In the final analysis of the reasons for success or failure in renal homotransplantation, there is often an irresistible urge to ascribe failures to poor control over the biologic processes of rejection. It is necessary to explicitly state at this time that there may be an important interrelationship between purely technical performance and the ease with which these processes can be controlled. The clarity with which complications can be tabulated as technical or nontechnical is not so well defined as with some other kinds of surgery. Without question, many homotransplantation failures have resulted from rejection, in which a complex chain of events was initiated and rendered unmanageable by the use of less than optimal homografts. Acceptance of these

failures as essentially nontechnical must be rigidly resisted by surgeons if overall results are to be improved.

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Chapter Eight

THE ROLE OF CADAVERIC DONORS IN HOMOTRANSPLANTATION

by Thomas L. Marchioro, M.D.

It is possible that in the future cadavers will be used with increasing frequency as a source of renal homografts. The concept of obtaining needed organs without the necessity of placing living donors in jeopardy is so attractive as to be irresistible in the long view. In order to effectively utilize cadaveric sources, considerable developmental work will be necessary in both the social and scientific spheres. An increased understanding and willingness by the public and by physicians to cooperate in the procurement of permission to remove postmortem tissue promptly will be necessary. In addition, more effective ways of processing and preserving autopsy specimens will have to be devised.

Much of the early work with human renal transplantation was performed with cadaveric kidneys. Although occasional temporary successes were obtained, these were rare. The use of cadaveric kidneys, which almost invariably were seriously damaged, made interpretation of the results virtually impossible for the reasons stated in the preceding chapter. It was necessary to attempt to separate those events which were a direct consequence of the ischemic injury to the tissue from those which resulted from rejection. An unclear picture emerged concerning many vital details. Such important concepts as the combinations of blood type mismatches between donor and recipient patients which could be accepted, the incidence of immediate post-transplantation anuria, the degree of ischemic injury which could be tolerated with consistent recovery, the time of onset of rejection, the reversibility of rejection, and the function which could be anticipated from the chronically functioning homograft were all obscure.

One of the most important consequences of the use of living donors has been clarification of most of these previously unanswered questions. An in-

creasingly lucid understanding of the problems which are to be expected has resulted, and a number of at least partially satisfactory solutions have evolved. It is to be hoped that this information can be ultimately transferred to the more difficult problems involved in the use of cadaveric organs in which postoperative therapy must usually be provided in the absence of good homograft function. In this way, empirical regimens can eventually be outlined which are not so dependent upon function of the renal homograft for formulation of day-to-day therapy.

TECHNIQUES USED TO OVERCOME ISCHEMIC DAMAGE

The principal deterrent to the successful employment of cadaveric homografts is the ischemic injury imposed during the agonal and postmortem period. A number of techniques have been described to protect the prospective transplant from anoxia.

Hypothermia. This technique has been most commonly used. The elegance with which homograft cooling has been carried out has ranged from simple chilling of the corpse in a refrigerated room, through immediate removal of the kidney and placement into an ice bath or ice water, to intra-arterial infusion of cold fluids of various compositions, to removal of the kidney and pump perfusion with blood which is oxygenated with a mechanical or autogenous lung.

Hypothermia and Perfusion. More recently, we have described a simple technique which utilizes the advantages of both hypothermia and perfusion. The method employs an extracorporeal apparatus using a glucose or electrolyte primed system into which is incorporated a heat exchanger (Fig. 20). As soon as death is certified, cannulas are inserted into the aorta and inferior vena cava through the easily accessible femoral artery and vein. In this way, total body perfusion and hypothermia can be instituted within minutes after death. Harvesting of the kidney or kidneys can then be carried out in a relatively leisurely manner with the knowledge that artificial circulation is being maintained. In order to obtain selective perfusion of the inferior half of the cadaver, the lower thoracic or upper abdominal aorta is cross-clamped as soon as this is feasible (Fig. 20).

Several observations were made in the course of this investigation. The use of vascular pressures to guide perfusion rates is worthless. Pressures obtained from the arterial tree of the perfused cadaver are usually near zero, presumably as the result of loss of vascular tone. To increase the blood pressure, even to 20 to 40 mm Hg, such exorbitant flow rates are required that organ rupture often results from overperfusion. The safest flow rates for cadaver perfusion appeared to be 30 to 60 ml/kgm per minute. Rapid cooling to 15° could usually be obtained within 30 minutes after the institution of such low flow perfusion (Fig. 21). It also appeared that prolonged cooling at low temperatures might be harmful to the prospective homograft. The trans-

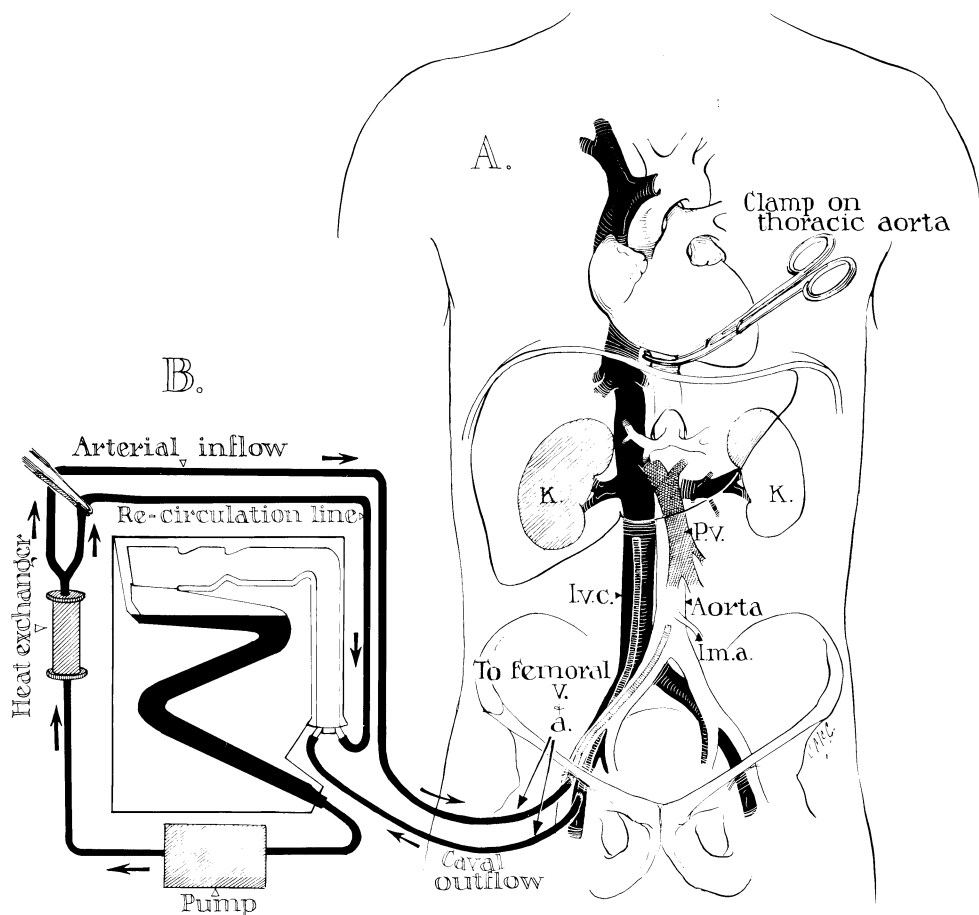


Figure 20. Technique of extracorporeal cadaver perfusion. Catheters are inserted via the femoral vessels into the aorta and vena cava as soon as possible after death. The extracorporeal circuit is primed with heparinized glucose or electrolyte solution to which procaine is added. The cadaver is anticoagulated with the first surge of the pump. Temperature control is provided by the heat exchanger.

planted organ often appeared to have been frostbitten, if the cadaveric perfusion was carried out below 10°C for protracted periods. Finally, the need for provision of procaine in the perfusate to prevent renal afferent arteriolar constriction at the low temperatures was evident. The necessity for this pharmacologic adjuvant in hypothermic perfusion had been previously demonstrated by Couch, Cassie, and Murray, and by Kiser and Hitchcock and their associates under different experimental conditions.

Using extracorporeal cadaveric perfusion in dogs, it is possible to consistently obtain functional kidneys from 10 to 16 hours after death of a previously healthy animal. Nevertheless, there are limitations to the method. Animals perfused for longer than six hours show temporarily depressed renal function. Excretion of urine is sometimes delayed for as long as 24 hours after transplantation. Acute azotemia is commonly observed which requires several days for reversal (Fig. 22).

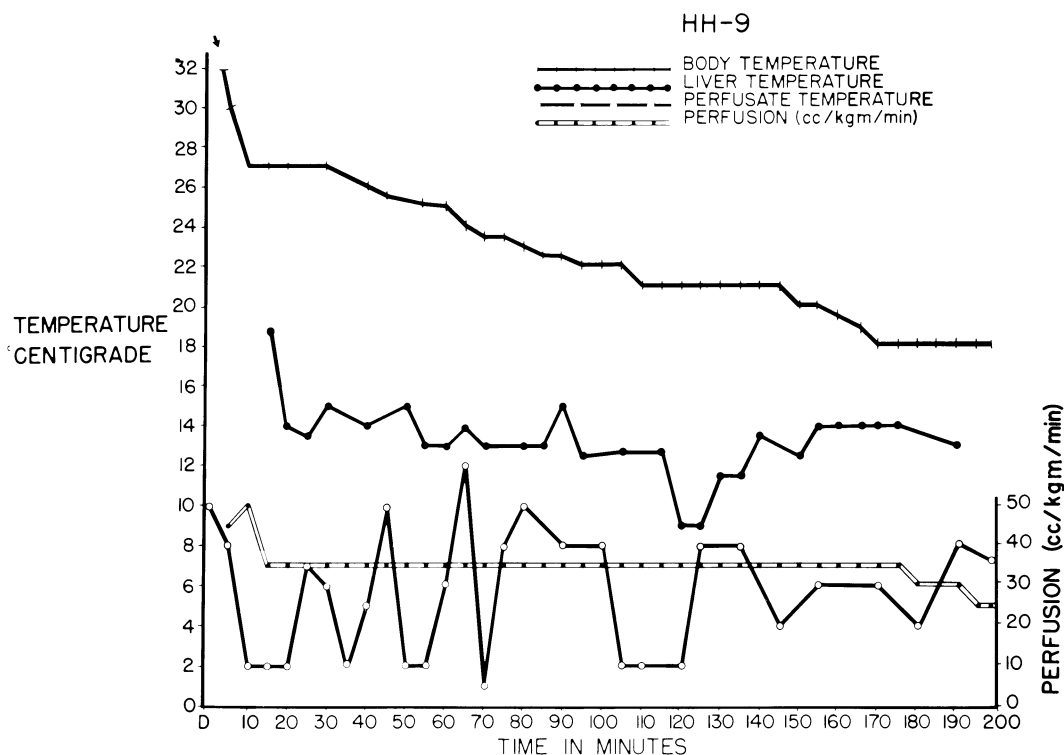


Figure 21. Cooling curves obtained during cadaver perfusion for a canine liver homograft. Note the rapid response of the internal organs (liver temperature measured in this experiment) to changes in perfusate temperature. (By permission of *Surgery* 54:900, 1963.)

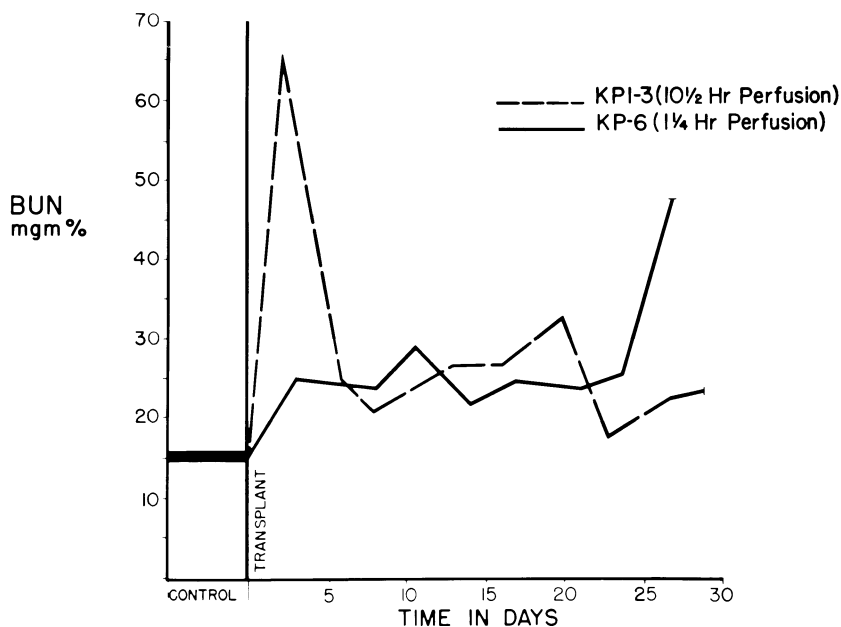


Figure 22. BUN's in two dogs receiving renal homografts. Note the acute azotemia observed in the recipient of the homograft which was obtained from a canine cadaver perfused for 10½ hours. By contrast, early azotemia is minimal in the animal which received a homograft from a cadaver perfused for one and one-quarter hours. (By permission of *Surgery* 54:900, 1963.)

DEFICIENCIES IN USE OF CADAVERIC HOMOGRAPHS

Despite the demonstration that useful cadaveric homografts can be obtained with extracorporeal cadaveric perfusion, our clinical experience with three cases has been disappointing. The first cadaveric homograft used at this center was obtained from a patient who was thought to have a brain tumor, but who was subsequently shown at autopsy to have died of acute bacterial endocarditis. His blood pressure was 60 mm Hg or less for four hours prior to death. Cadaveric perfusion was instituted within five minutes after death, but the maximum flow rates that could be obtained were 15 to 20 ml/kgm per minute. Extracorporeal perfusion was continued for an hour while the kidney was removed, and revascularization in the recipient bed was accomplished 106 minutes after death. The homograft apparently functioned temporarily, although at reduced efficiency. The BUN fell from 176 to 119 mgm per cent, but then rose again after 10 days (Fig. 23). Death of the host occurred 24 days after transplantation from a combination of sepsis and progression of uremia.

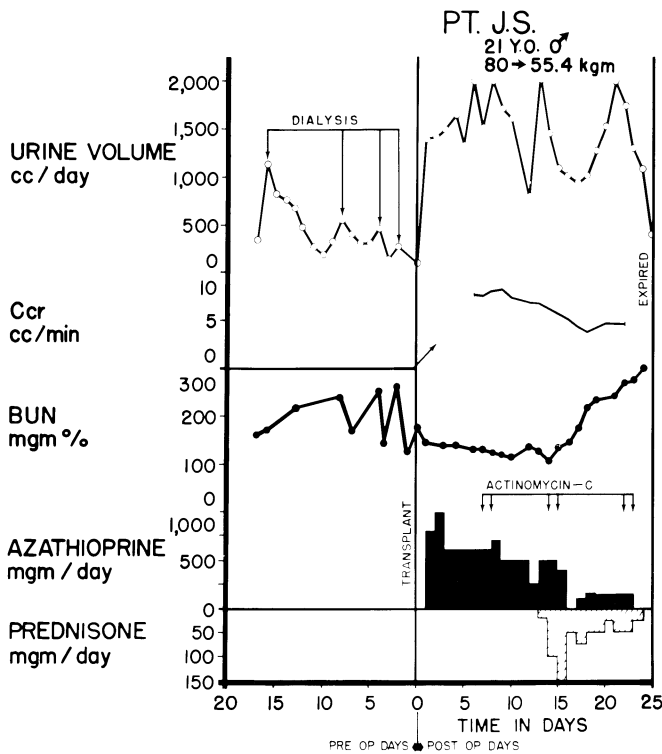


Figure 23. Course of a 21-year-old male who received a cadaveric kidney (CD 1). The donor blood type was A+ and the recipient O+. The recipient's own kidneys were not removed. However, some homograft function is evident from the increased urine volumes and the early fall in BUN. Improvement ceased after 10 days, and the patient died of a combination of sepsis and renal failure after 25 days.

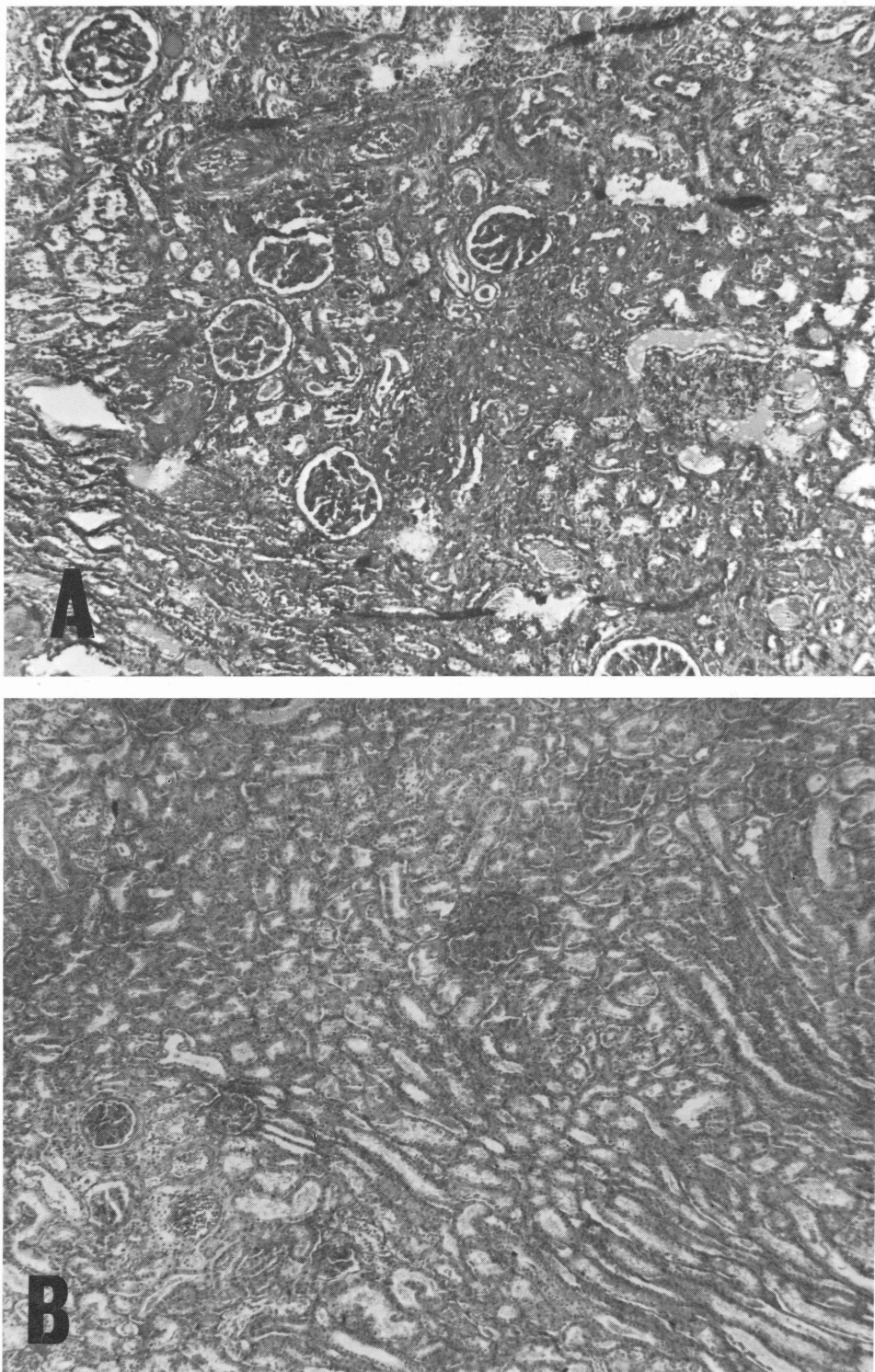
Histologic sections of the transplanted kidney showed severe interstitial hemorrhage and the presence of proteinaceous material in the tubules. There was minimal mononuclear infiltration (Fig. 24). The cadaveric donor was of A blood type, and the recipient was O. In subsequent experience with the use of donors having different blood types from those of the recipients, it has been found that this combination of blood groups is a dangerous one (Chap. 6). In retrospect, this factor may have materially contributed to the poor outcome.

In the second case, postmortem hypothermic perfusion failed within a few minutes after its inception. The donor who died of subarachnoid hemorrhage was severely dehydrated terminally, and probably had a low blood volume. Adequate venous return could not be obtained. The kidney was revascularized in the recipient bed 124 minutes after death. Renal function did not return. Twelve days later the graft was removed, after it had ruptured following relatively minor trauma incurred when the patient fell out of bed. Histologic sections showed evidence of cellular rejection (Fig. 25). The patient died one month later.

A third patient, a 42-year-old woman, received both kidneys from a 42-year-old donor who died four hours after suffering an acute myocardial infarction, the interval between death and the institution of extracorporeal perfusion being 20 minutes. The times from death to revascularization of the homografts were 137 and 215 minutes. Both individuals were A blood type. The kidneys did not function. The patient died four days later shortly after a postoperative dialysis, but no discrete cause of death was found at autopsy. The homografts had minimal cellular infiltrate.

The discouraging experience in these three patients, as well as in numerous other reported cases, has served as a deterrent to employment of cadaveric kidneys, and provides a basis for the belief that much of the damage to the homograft may occur before death except under the most exceptional circumstances.

The good results that can be obtained with cadaveric kidneys in experimental animals have no present counterpart in human use. Although the efficiency of the extracorporeal postmortem perfusion was clearly demonstrated in dogs by the fact that uniform resumption of renal function could be expected for many hours after death, these results have not been reproduced in clinical experience. With use of human cadaveric tissues, the degree of injury has appeared to be much greater, not only in our hands but in most other clinics using various forms of postmortem preservation. This fact suggests that proper donor selection is as important a factor as the method used for preservation. Kidneys obtained from patients who have had a protracted terminal course are undoubtedly variably damaged before death, resulting in unpredictable later function. Criteria are needed for the evaluation of desired organs in patients who are thought to have a hopeless prognosis, in order to allow greater selectivity of the situations in which autopsy tissue will be acceptable.



*Figure 24. Same patient as in Figure 23. A—Appearance of homografted cadaveric kidney 24 days after transplantation, compared to B—Cadaveric contralateral kidney immediately after cessation of perfusion. H and E (X 25). (By permission of *Surgery* 54:900, 1963.)*

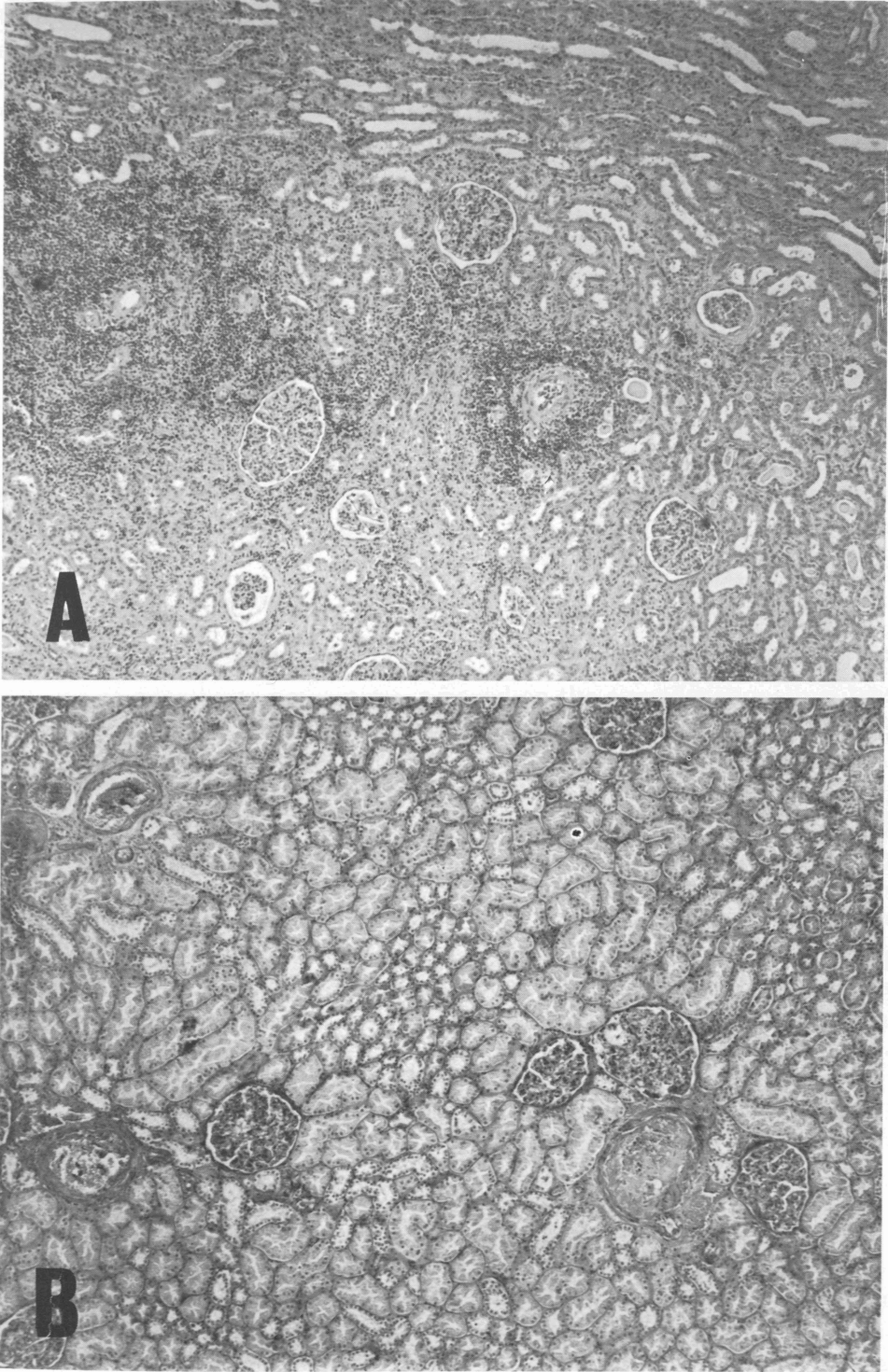


Figure 25. A—Homografted cadaveric right kidney removed 12 days after transplantation (CD 2). Note evidence of rejection. B—Contralateral donor kidney obtained at conclusion of perfusion. H and E (X 25). (By permission of *Surgery* 54:900, 1963.)

A special note should be made of the importance of refined hemodialysis techniques in the care of patients receiving cadaveric homografts. Kolff and his associates have shown that return of renal function can occur in those transplants which initially pass through a phase of acute tubular necrosis if aggressive supportive care is provided, an observation previously made by Hume. The degree to which improvements in dialysis will permit success with cadaveric donors remains to be determined, but this type of investigative effort is certain to influence future developments in clinical transplantation.

The ultimate in exploitation of cadaveric organs will occur when finely machined and controlled extracorporeal perfusion equipment is developed which will allow the long-term maintenance of kidneys by perfusion directly into the renal artery after removal from the body. Until now, perfusion circuits have not permitted this kind of use, inasmuch as maintenance of the organs in an optimum state has been feasible for only a few hours. After a short time, parenchymal damage, edema, and functional deterioration have resulted. With more effective perfusion techniques, it may become possible to evaluate the function of the isolated organs and eliminate from further consideration those which do not have a reasonable excretory capacity.

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Chapter Nine

ANESTHESIA FOR PATIENTS INVOLVED IN RENAL HOMOTRANSPLANTATION

by Robert W. Virtue, M.D.

Choice of anesthetic procedures is based on the considerations involved in any such decision, namely, the safety of the patient, the needs of the surgeon, and the comfort of the patient, in that order. The safety of the patient requires flexible methods of management because three types of people come to surgery in renal homotransplantation. The needs of the surgeon are for a quiet patient and adequate muscular relaxation. The conditions desirable for the patient are analgesia, sedation, and a lack of reflex effects. These prerequisites cannot be met with the same methods in all cases. A few patients are cooperative and sufficiently oriented so they understand the necessity for being quiet and can do so voluntarily. Regional anesthesia may be desirable in such cases. A few patients have been disoriented but cooperative, and regional anesthesia may be adequate for these as well. Uncooperative subjects must be rendered quiet. Consequently, general anesthesia is usually employed for them.

Healthy Volunteer Donors

The first of the three types of patients is the kidney donor. All donors are healthy. Three factors may affect the choice of procedure. The first is that the operations are of considerable duration, so a "one-shot" spinal anesthetic does not ordinarily afford a sufficient operating period. The second factor involves the frequent request by the surgeon for availability of electrocautery, thereby preventing the use of cyclopropane, which would otherwise be a most useful agent. The third factor is that many donors early in the series had general hypothermia, although this method has since been discontinued.

Hypothermia. Surface cooling has been safe in our hands in more than 1,000 nonrenal cases when two specific precautions were taken. The first of these is to establish good muscle relaxation before immersing the patient in the cooling mixture. Before immersion, we have usually produced second plane of third-stage anesthesia and then administered in addition about 9 mgm of d-tubocurarine (to an adult). The second precaution is that of producing a two-step change from room temperature to ice water. When the patient has been relaxed he is first placed in a bath containing water at 15 to 20° C. The head and arms are held out of water. Sufficient time is allowed (about three minutes) to ascertain that the patient shows no signs of light anesthesia or lack of relaxation. If any spontaneous respiration, motions of limbs or fingers, "goose flesh," coughing, stiffness, or eyeball activity appears, either more anesthetic agent or more relaxant or both are administered. When the anesthesiologist is assured that the patient is stable and well relaxed, ice is added to the water bath, and the patient is permitted to cool to an esophageal temperature of about 33°, from which level a drift usually occurs to 30 to 32° C. He is removed from the ice water and dried, and preparation for surgery begun. A preplaced warming blanket on the operating table is useful for rewarming as incisions are being closed, allowing earlier resumption of spontaneous respirations which will generally not return until the temperature rises to or above 32.5° C. Warming can commence as soon as the kidney is removed. Agents used for this anesthetic procedure may be whatever the anesthesiologist desires, for the subjects will all be in good condition.

In more recent cases, total body hypothermia has not been used for living donors (Chap. 10). The method is still employed, however, for procurement of baboon heterografts and the precautions mentioned should also be observed for the care of these animals (see Chapter 23).

Patients Whose Poor Renal Function Will Not Be Improved by Operation

The second type of patient is the ill person who is having surgery for a nontransplant procedure such as preliminary splenectomy, nephrectomy, drainage of an abscess, or an emergency procedure for control of gastric hemorrhage. When "splenectomy and bilateral nephrectomy" were first placed on our surgical schedule without simultaneous transplantation, the operating room personnel expressed amazement. It was gratifying, however, to follow the course of our patient following removal of his diseased kidneys. He had been hypertensive with progressive blindness, and had severe congestive heart failure before transplantation. Two weeks later, his blood pressure had dropped to normal levels. Heart failure was no longer a problem, and his vision was improving. The role of renal dialysis in the anephric interval is discussed in Chapter 4.

On several subsequent occasions, nephrectomy and splenectomy have also

been done at a preliminary stage prior to the transplantation. When it appeared that a spinal anesthetic alone might be of sufficient duration, this was employed. In some instances supplementation was required. On other occasions continuous epidural anesthesia with or without a general agent afforded satisfactory operating conditions.

Since all the recipient patients are severely ill, a thorough preoperative evaluation of cerebral, cardiac, and pulmonary status is essential. When congestive heart failure is or has been severe, extreme caution must be taken to avoid depression of heart action more than is absolutely essential. Under these circumstances, spinal anesthesia can be utilized as a bloodless phlebotomy. This technique provides excellent relaxation for the surgeon, but its duration is inadequate for prolonged operations. On a few occasions it has been used and then supplemented with general anesthesia toward the end of the surgical operation. Continuous spinal anesthesia would also be a good choice, although we have not used it.

Choice of Agent. When general anesthesia is required for patients in incipient pulmonary edema, the use of positive pressure with oxygen has specific value, combined with minimal anesthetic agent and preferably in combination with spinal or epidural anesthesia. Halothane has been a useful anesthetic agent in this setting, especially with severely hypertensive patients, since this agent tends to lower the blood pressure to easily controlled levels.

Poor renal function has been present in all the transplant candidates and in a few of the post-transplant subjects who required reoperation for a variety of reasons. Relaxing agents have been used cautiously in such cases, since renal excretion is the principal means of elimination of some of these compounds. When it is thought that renal excretion will be no better after than before operation, relaxation is best obtained with an inhalation agent rather than with an agent that the kidney must eliminate.

Other reasons can be cited for caution in the use of muscle relaxants for patients with renal disease, and especially those who have been recently dialyzed. A pertinent example was observed a few years ago in a patient who developed severe muscle spasms and painful cramps in his legs while being dialyzed. To relax the leg muscles, 10 mgm of succinylcholine was given intravenously. This stopped not only his respirations but also his heart. He was resuscitated, and a sample of blood was drawn for analysis. The plasma cholinesterase content had diminished to zero. For this reason, any patient who has recently been dialyzed is one in whom the use of succinylcholine is avoided.

A few patients have come to the operating room with severe gastric hemorrhage either before or after transplantation. The choice of agent here has been cyclopropane, since induction must be done rapidly. Furthermore, cyclopropane does not depress renal function, and it does not cause hypotension. As with nonrenal patients with similar hemorrhages, depth of anesthesia should be kept light. It is mandatory to provide large venous cannulas for administration of fluids and blood.

*Patients Whose Poor Renal Function Will
Be Improved by Operation*

The third type of patient is the one who receives a transplant. These subjects have almost invariably been kept alive for varying periods by means of the artificial kidney, and many have already had preliminary operations as discussed earlier. Despite preparation with dialysis, the patient is neither normal nor stable in respect to internal fluid or electrolyte environment. Most patients are water-logged. As soon as the homograft is placed into circulation,

Table 8. Anesthesia Used for First 55 Patients
Treated with Homotransplantation

Transplant operations (usually also splenectomy and bilateral nephrectomy):	
Spinal alone	1
Continuous epidural alone	1
Halothane alone	21
Fluroxene alone	2
Cyclopropane alone	2
Spinal supplemented with halothane	11
Spinal supplemented with fluroxene	4
Spinal supplemented with cyclopropane	1
Continuous epidural supplemented with halothane	7
Continuous epidural supplemented with fluroxene	4
Continuous epidural supplemented with meperidine and nitrous oxide	1
Surgery for recipient patients other than the actual homografts:	
Spinal alone	3
Continuous epidural alone	1
Halothane alone	8
Fluroxene alone	8
Cyclopropane alone	11
Nitrous oxide alone	1
Spinal supplemented with halothane	4
Continuous epidural supplemented with halothane	2
Continuous epidural supplemented with fluroxene	1

urine is formed in copious amounts. As much as 16 liters has been excreted within 12 hours following transplantation. When brisk diuresis starts on the operating table, the urinary fluid loss is partially replaced (usually 75 per cent) with lactated Ringer's solution, a general plan which is carried into the post-operative period as discussed in Chapter 12. Urine excretion may be so great that two large bore venous catheters may be required for efficient replacement. Loss and replacement of blood is not usually a difficult problem. If renal transplantation is the sole procedure, transfusion is not ordinarily needed. An average of 1,000 cc blood is required for simultaneous bilateral nephrectomy, splenectomy, and transplantation. As with the second type of patient, many of the recipients have hypertension. This may be satisfactorily regulated with halothane. If the patient's blood pressure tends to be low, we have used fluroxene on several occasions to provide safe analgesia without drop of blood pressure.

In the first cases, 12.5 gm of mannitol was infused into the patient just before circulation was begun through the new kidneys. If diuresis commenced, approximately 38 gm of mannitol was then infused additionally. The present practice, based on continuing experience, involves administration of only the first 12.5 gm of mannitol. Although there is no clinical evidence that this is essential, studies in dogs suggest that immediate renal blood flow is thereby increased.

Little has been said concerning the actual anesthetic agents employed. Operating procedures can be done with safety if the drugs and procedures are chosen on the basis of the condition and requirements of the individual patient, taking into account the surgical procedure to be performed. It would be a mistake to outline a procedure for anyone to follow. Rather, such decisions are made after careful study of the individual situation. A summary of the various procedures we have utilized for recipient patients is shown in Table 8. Other anesthesiologists may prefer to use other techniques for their patients, the decisions for the methods used being based upon their own past experience and immediate judgment.

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Chapter Ten

DONOR NEPHRECTOMY

by Thomas L. Marchioro, M.D.

The donor operation must satisfy two requirements. It must be safe for the patient who is sacrificing the kidney. In addition, it must guarantee delivery of a minimally traumatized organ, inasmuch as a most important factor in the ultimate outcome of a transplant procedure is the quality of the tissue that is transferred. The procedures used appear to have satisfied these objectives.

A general comment is in order concerning the surgical exposure employed as it relates to the full needs of donor nephrectomy. In contrast to many extirpative renal operations, complete vision is needed for each step. Sharp dissection is required in many areas that are ordinarily mobilized by blunt means. Because of the long vessel segments required, and the consequent necessity for freeing them to their origins, perfect exposure is mandatory near the aorta and inferior vena cava. Finally, the ureteric blood supply from the renal pedicle can be preserved with certainty only under ideal operating conditions (Fig. 26). The generous incision that is used allows exposure for such meticulous dissection, thereby providing protection not only for the patient but for the specimen as well.

HOMOGRAFT COOLING AND HEPARINIZATION

All kidneys are delivered in a cooled, heparinized state. This has been achieved in one of two ways. If the donor and recipient were of the same ABO blood type, as in 32 of the early cases, total body hypothermia (30 to 32° C) was used for the donor patients. Five minutes before removal of the kidney, 2 mgm/kgm of heparin was given intravenously. As soon as the specimen was removed, protamine sulfate, 2 mgm/kgm, was given in 100 cc of 5 per cent dextrose in water over a 10-minute period. Although both heparinization and hypothermia might be expected to increase problems with hemorrhage, careful hemostasis

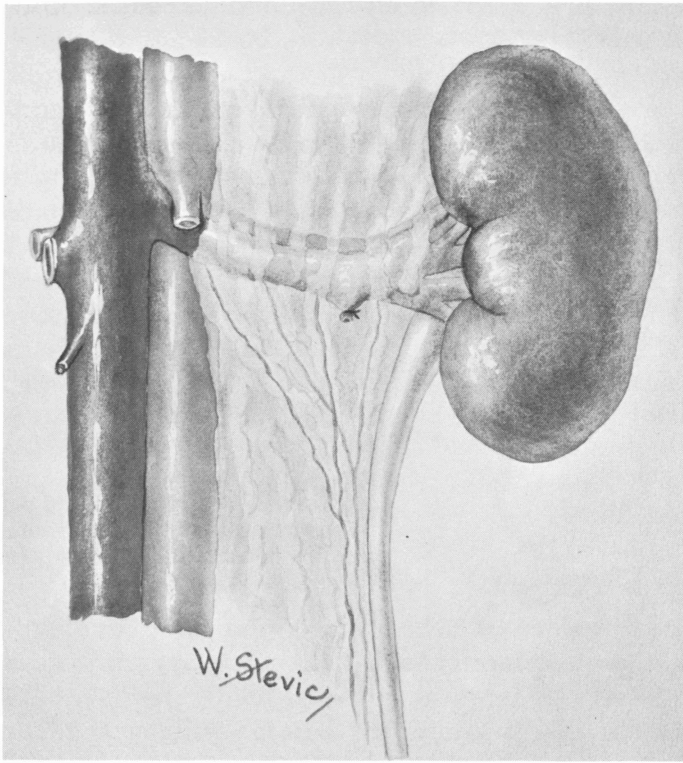


Figure 26. Blood supply of pelvis and ureter occasionally encountered in autopsy studies, in which multiple fine vessels are distributed from the renal pedicle. The fatty-areolar area enclosed by the aorta, renal artery and vein and ureter is removed *en bloc* in order to avoid injury to the ureteric vessels. (By permission of *Arch. Surg.* 86:600, 1963.)

throughout the entire procedure prevented this difficulty. Among all the donors at the University of Colorado, none has received transfusions. Nevertheless, the practice of donor cooling and heparinization has been discontinued, as will be noted, in favor of a cold perfusion technique.

Total body hypothermia and systemic heparinization have never been used in the presence of an ABO blood group incompatibility between donor and recipient patients. Instead, the donor nephrectomy was performed at normal body temperature. As soon as the kidney was removed, a plastic tubing or a blunt needle of appropriate size was inserted into the renal artery, and the kidney was perfused with lactated Ringer's solution or with 10 per cent low molecular weight dextran in normal saline* (precooled to 15° C) each liter of which contained 50 mgm (5,000 units) of heparin and 1 gm of procaine chloride (Fig. 27).

In performing the renal arterial perfusion, certain precautions are necessary. All antiseptic must be washed from the tubing, and air should be flushed out before insertion of the tip into the renal artery. It is important to avoid traumatizing the intima of the renal artery with the nozzle, since this might later necessitate sacrifice of vessel length. After the perfusion is started, the

*Supplied as Rheomacrodex by Pharmacia Laboratories, Inc., New York, New York.

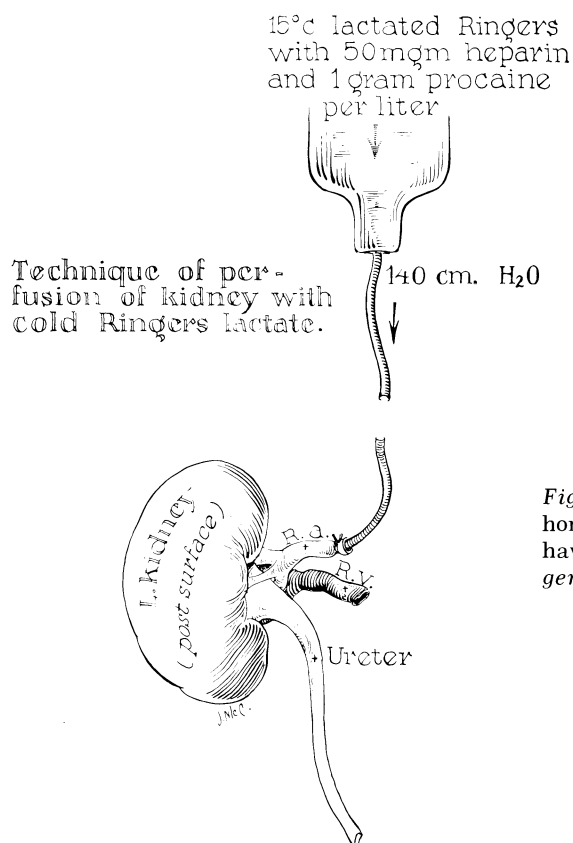


Figure 27. Method of cold perfusion of renal homograft. Various other solutions (see text) have been evaluated. (By permission of *Surgery* 55:195, 1964.)

hydrostatic pressure can be controlled either by the elevation or lowering of the stand from which the solution is administered or by a pressure bulb attached to the air inlet of the perfusate fluid container. The pressure employed is approximately 70 mm Hg (90 to 100 Cm H₂O). In the usual case, approximately 200 cc of fluid is required before the kidney has been thoroughly washed of blood and before the effluent from the renal vein returns clear.

A special note should be made concerning the use of procaine in the perfusing fluid. Experimentally, the use of cold electrolyte solutions that do not contain this substance or some other vasodilator results in unsatisfactory perfusion. Flow ceases within a few minutes, apparently owing to afferent arteriolar vasospasm. The addition of procaine avoids this vasospasm and uninterrupted flow can be obtained for as long as desired.

A controversial point concerns the choice of fluid to be used with this method. Kiser and Hitchcock and their associates believe that 10 per cent low molecular weight dextran in normal saline is superior to pure electrolyte solutions, but Knight could not demonstrate such an advantage. In our experience, both have been satisfactory.

In our institution the technique of cold perfusion was used at first only for those donors whose major blood groups were different from those of the recipients, and for the specific purpose of preventing intravascular hemagglutination at the time of the subsequent revascularization. Use of cold perfusion was limited to such cases for several reasons. First, an additional three to 10-minute period of ischemia is imposed by the use of this technique, since this is the time necessary for adequate perfusion. Second, there is a risk of inflicting damage upon the renal artery at the time of insertion of the perfusion tip. Finally, the effect upon ultimate renal function of instilling cold electrolyte solutions is not precisely known, although no harmful sequelae have been seen in our experience. After the perfusion technique proved to be an entirely safe method, it became the preferable method for all cases, since the slight but definite additional risks of donor heparinization and total body hypothermia were thereby avoided.

RIGHT NEPHRECTOMY

Right nephrectomy is usually performed if transplantation is planned to the left iliac fossa of the recipient. A modified lateral position is employed (Fig. 28A), using a kidney rest, which is raised in order to extend the presenting flank. The patient is slightly rotated posteriorly to provide exposure of the lower lateral abdomen (Fig. 28B). The underlying left leg is kept relatively straight, and the overlying right lower extremity is flexed at the knee. It is important to protect the fibular head with padding in order to prevent peroneal palsy. The patient is maintained in position with strips of tape passed across the pelvic girdle and fastened on either side of the operating room table. If desired, exposure may be enhanced by extending the head or foot of the table.

The posterior part of the incision is made directly over the eleventh rib (Fig. 28A), and the incision is extended across the lateral and anterior abdomen to a point midway between the umbilicus and pubis (Fig. 28B). The medial end of the incision is frequently curved inferiorly, parallel to the lateral border of the rectus sheath, for 2 or 3 inches (Fig. 28B). After incising the skin and subcutaneous tissue, one encounters the first muscle layer, consisting of the latissimus dorsi posteriorly and the external oblique muscle and aponeurosis anteriorly (Fig. 28C). After these muscles are divided, the periosteum of the eleventh rib can be visualized. The periosteum is incised and the eleventh rib is resected subperiosteally (Fig. 28C). The plane of incision of the periosteal bed is then followed anteriorly, dividing the internal oblique and transversus abdominis muscles (Fig. 28C). Finally, the inner layer, which consists anteriorly of the transversalis fascia, is divided through the entire length of the incision (Fig. 29D). With the last step, the retroperitoneal space is entered.

Special care must be exercised in the posterior end of the wound at the

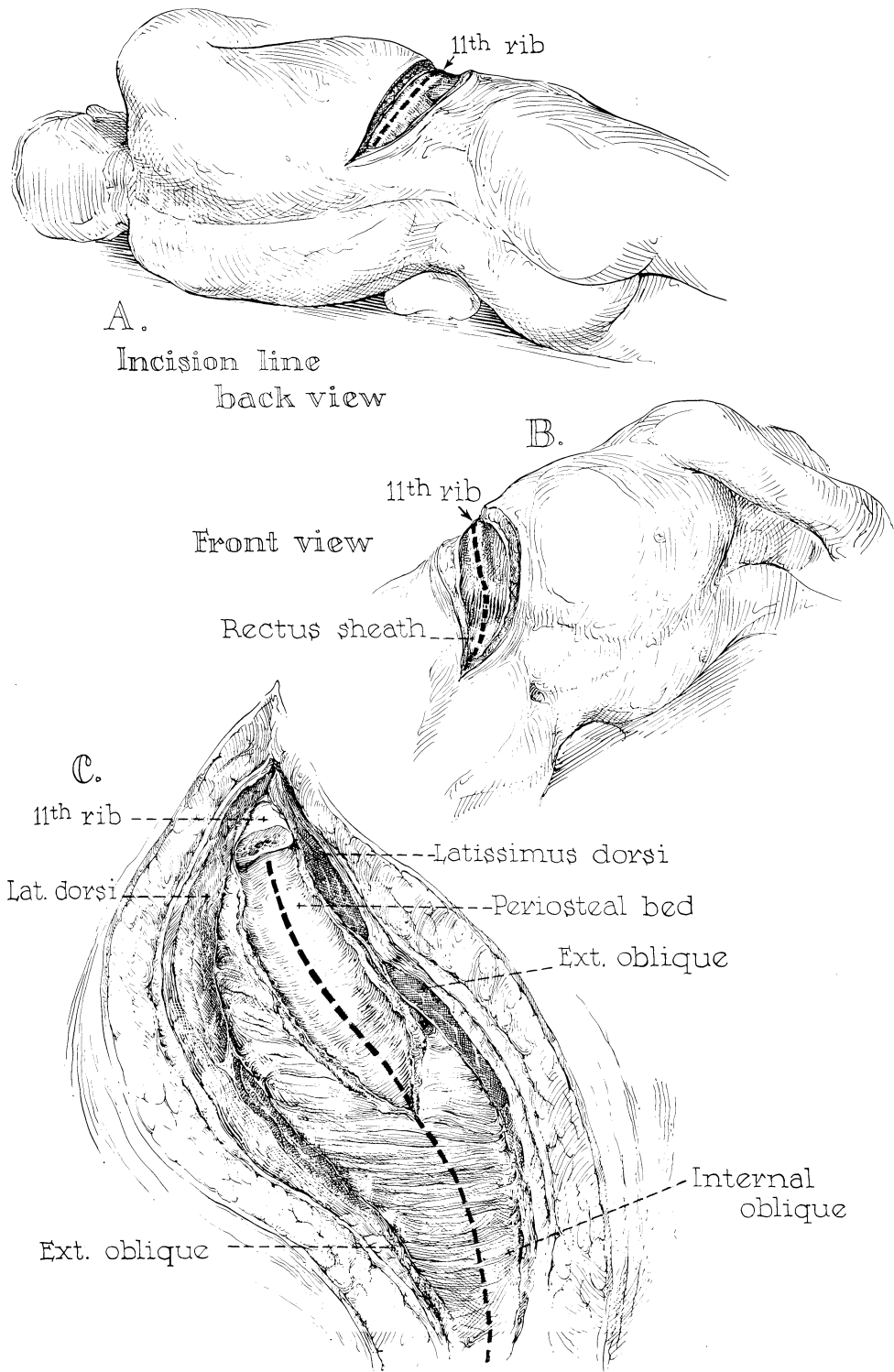


Figure 28. Operative exposure for donor right nephrectomy. A and B—Position and extent of incision. C—Eleventh rib has been excised. (By permission of *Arch. Surg.* 88:711, 1964.)

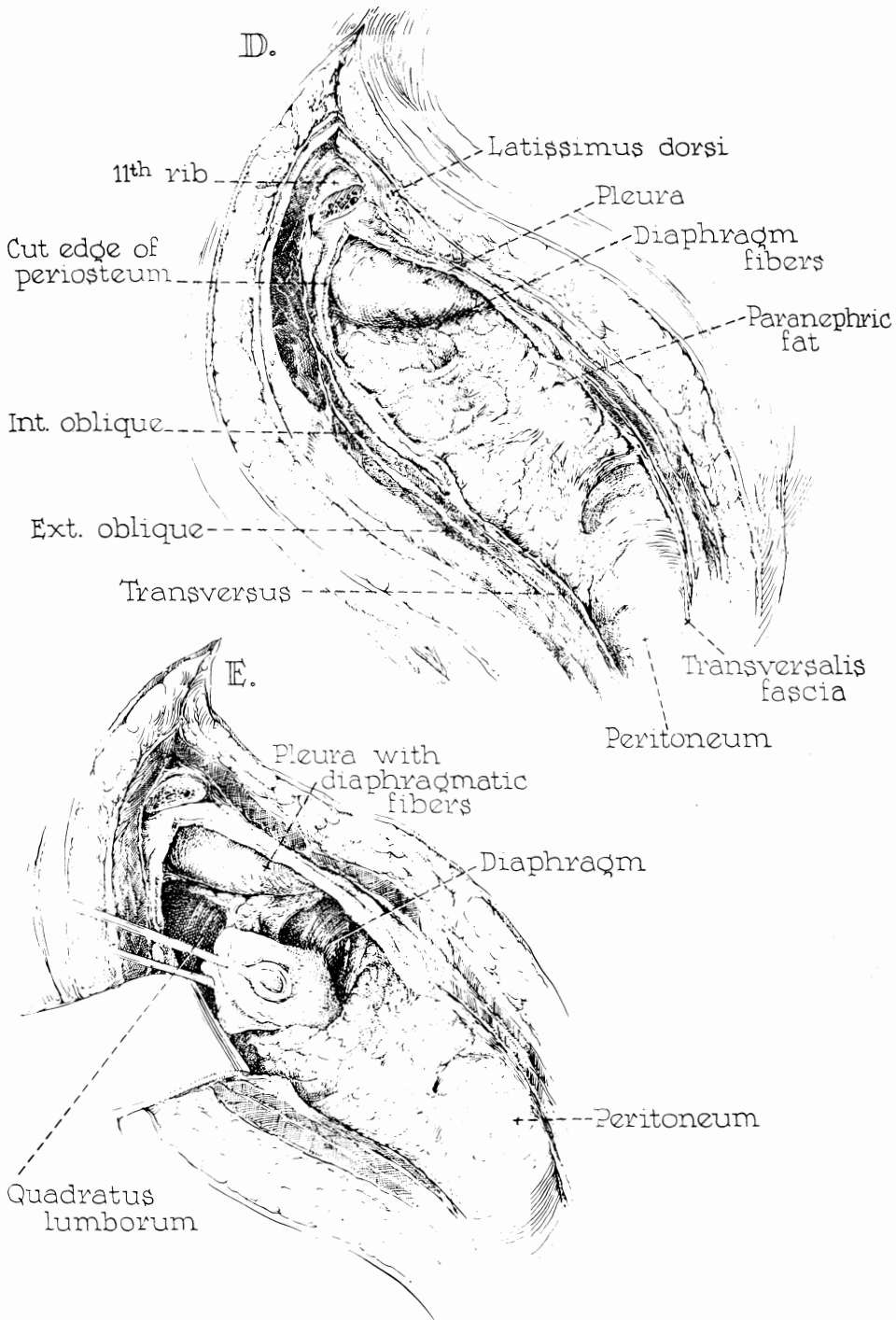


Figure 29. Donor right nephrectomy (cont.). Exposure after incision of the periosteal bed and transversalis fascia. D—Anatomy as encountered before manipulation. Note pleura (posterior) and peritoneum (anterior). E—Mobilization of the kidney and its investments posteriorly. (By permission of *Arch. Surg.* 88:711, 1964.)

junction of the thorax and abdomen. In this area, pleura is almost always seen protruding beneath the stump of the excised rib (Fig. 29D, E). Just below this, diaphragmatic fibers are frequently encountered (Fig. 29E). Both the pleura and the diaphragm are gently swept superiorly, obtaining adequate mobilization with the placement of a chest retractor so that they are not torn. The wound is then opened widely. The kidney and its investing layers are pushed anteriorly off the quadratus lumborum and the psoas muscles (Fig. 29E).

The paranephric fat is now in the central part of the wound, bracketed by the pleura laterally and the peritoneum medially (Fig. 29D, E). The fat layer is entered and pushed off the underlying Gerota's fascia by gentle blunt dissection. The numerous small vessels running in this tissue should be carefully electrocoagulated or ligated prior to division.

Once the superficial surface of Gerota's fascia has been exposed, this structure is incised superoinferiorly (Fig. 30F). The underlying perirenal fat must be detached from the true renal capsule to which it is attached by numerous areolar bands containing vessels. These vessels must be ligated or electrocoagulated (Fig. 30F), not only to insure hemostasis in the donor patient but to provide a dry homograft for subsequent use in the recipient. Dissection is continued until the entire presenting surface of the kidney has been cleaned (Fig. 30F).

In proceeding with mobilization of the kidney, it is advisable to free the upper pole as a first step. Having done this, it is possible to extract the kidney more completely into the wound, making the remaining dissection easier. In carrying out this step, caution is exercised not to divide an anomalous superior polar branch of the renal artery. This danger is largely averted if preoperative aortograms have been obtained with which such anomalies can be detected in advance.

Next, the renal convexity is freed in a similar manner (Fig. 30G). When the inferior pole is reached, dissection and mobilization are discontinued temporarily.

The kidney is placed back into its normal anatomic position and attention is turned to the identification and dissection of the proximal renal vein (Fig. 30G). The renal vein is the most anterior structure in the renal pedicle. On the right side, its junction with the inferior vena cava is seen almost immediately and cleared of all areolar tissue for approximately 1 cm distally. Within this segment of the right renal vein, there are usually no entering tributaries which need to be ligated and divided.

After mobilization of the major portion of the kidney is completed, the rest of the operation allows eventual removal but avoids as far as possible the central hilar structures. First, the groove between the renal vein and the more superiorly located adrenal gland is separated, ligating and dividing in continuity the bridges of tissue between these structures (Fig. 30G). Following this, the groove between the inferior vena cava and the ureteropelvic junction is separated with a similar technique (Fig. 30G).

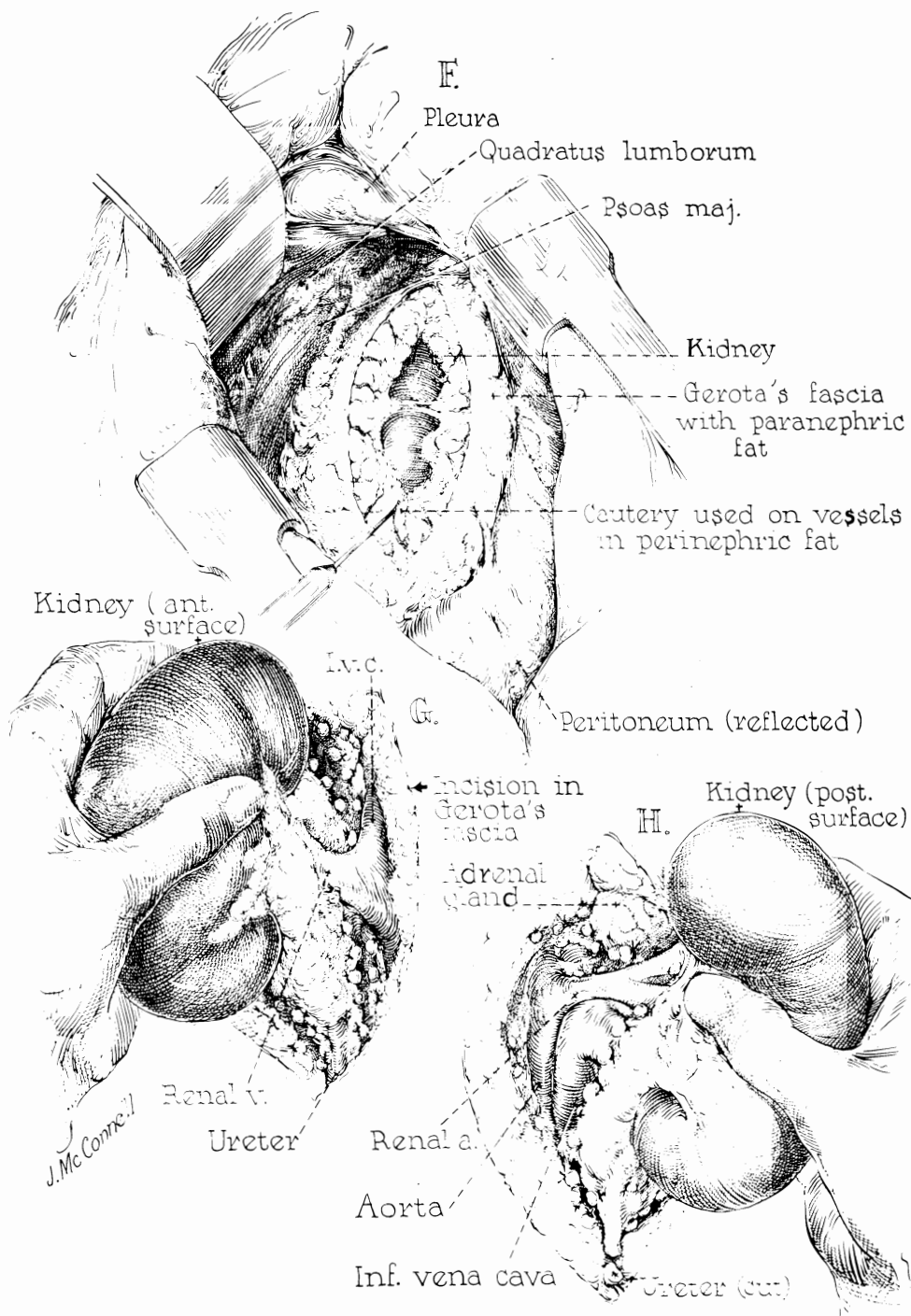


Figure 30. Donor right nephrectomy (cont.) F—Incision of Gerota's fascia and perirenal fat. Note careful hemostasis of capsular vessels. G—Kidney extracted from its bed. Block excision has been started with separation of the kidney medially and laterally and from the adrenal gland, without disturbing the central hilar structures. The anteriorly located renal venous junction with the vena cava is visualized and cleared. H—The Kidney displaced anteriorly and the proximal renal artery dissected free. If the ureter is cut, it is allowed to drain freely until subsequent removal. (By permission of Arch. Surg. 88:711, 1964.)

Finally, the kidney is lifted from its bed and rotated anteriorly (Fig. 30H). The renal artery is identified as far proximally as possible, usually at the point at which it passes beneath the inferior vena cava (Fig. 30H). The artery is skeletonized to or toward its origin from the aorta. Occasionally the field of dissection beneath the inferior vena cava may be obscured by one or more large lumbar veins. These should be ligated and divided after it is ascertained that they do not drain part of the eventual homograft specimen. After completion of this dissection, any filamentous bits of tissue connecting the contiguous renal artery and vein are divided and ligated.

The right kidney is now completely free except for its vascular and ureteral connections. If the recipient operating room is nearly ready for receipt of the kidney, the ureter is divided as far inferiorly as possible with a single distal ligature of 2-0 silk. The site of transection is at or beyond the common iliac vessels in order to provide adequate length for implantation. When the proximal end of the divided ureter is lifted into the wound, a few filmy areolar connections which bind it to the posterior wall are encountered. These are divided between ligatures. Upon signal from the recipient operating room that all is ready, the final steps preparatory to removal of the kidney are carried out. The necessary equipment for hypothermic perfusion is checked (see preceding section on "Homograft Cooling and Heparinization"). In the actual removal, right-angled Pott's clamps are used to grasp the artery and vein individually (Fig. 32E). For transection of the artery, a single clean cut is made, leaving a cuff of 2 or 3 mm for closure of the stump (Fig. 32F). Division of the vein is best accomplished by making a small incision in the inferior border of the vessel, distal to the vascular clamp. By compressing the vein between the index finger and the thumb, the incision can be kept bloodless and the anterior and posterior walls of the vein incised separately. This procedure permits more accurate incision of the vein and prevents intimal stripping which has occurred on occasion when the vein was transected by one cut of the scissors. The kidney is then carried directly into the recipient operating room and perfused with cold solution.

The vessel stumps are closed with continuous 5-0 vascular silk which is reinforced with a second layer (Fig. 32F). The wound is extensively irrigated, and all loose fat and other debris is removed. Occasionally a pneumothorax is encountered due to a rent in the pleura at the upper angle of the wound. Such pleural defects are closed with interrupted figure-of-eight sutures after evacuation of the pleural air with a No. 20 French catheter. Only two patients have required the subsequent insertion of a chest tube during the postoperative period.

The wound is closed in layers, using interrupted silk technique throughout. Drainage is not used.

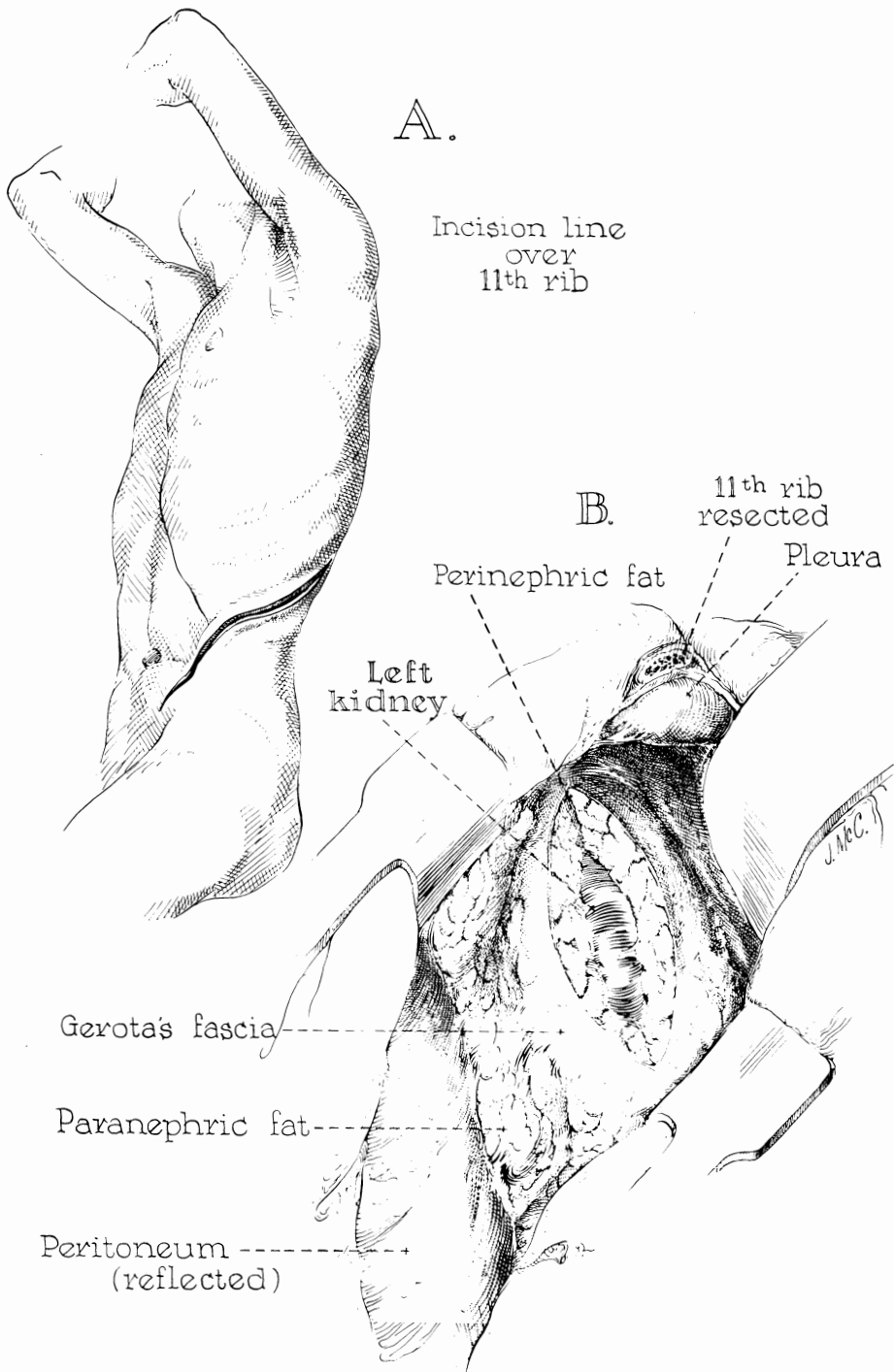


Figure 31. Incision and exposure for donor left nephrectomy.

LEFT NEPHRECTOMY

Left nephrectomy is usually performed if transplantation is planned to the recipient right iliac fossa. The position is a mirror image of that used for right nephrectomy. The general technique and all of the steps of exposure are comparable (Fig. 31A, B). There are, however, important differences in the dissection in and around the hilum which should be thoroughly appreciated.

After mobilization of its convexity and poles, the kidney is placed back into its normal anatomic position. As on the right side, the renal vein is the most anterior structure (Fig. 32C). Its junction with the inferior vena cava cannot easily be seen since this is deeply located within the wound to the right of the aorta (Fig. 32C). In contrast to the right renal vein which receives no tributaries in the area cleaned off for transection, the left renal vein regularly admits the testicular or ovarian vein and the adrenal veins in this location (Fig. 32C). The adrenal vein enters superiorly, and the ovarian or testicular veins enter inferiorly. These vessels are ligated in continuity and divided. Occasionally, the testicular or ovarian vein is absent and may be replaced by ureteral veins in the same location (Fig. 33D). The obvious hazard of venous ligation in the latter circumstance makes it imperative that all inferiorly oriented veins be accurately identified before they are sacrificed.

As on the right side, left nephrectomy is designed to allow block removal of the hilar structures with a minimum of dissection except at the sites of transection. After the renal vein is prepared the kidney is rotated anteriorly, and the renal artery is located at or near its origin from the aorta. In contrast to the situation on the right side, this is easily accomplished because the aorta is not obscured by the inferior vena cava (Fig. 32D). As soon as a suitable length of artery is obtained, the grooves separating the aorta from the ureteropelvic junction and the left renal vein from the left adrenal gland are separated (Fig. 32C, D), and the bits of tissue are tied in continuity. When one opens the space between the adrenal gland and the renal artery, a small adrenal branch is frequently encountered which must be ligated. This must be done with care since a small branch to the superior pole of the kidney also may take its origin from this segment of the renal artery. Inadvertent ligation of such a vessel has resulted in a small superior polar infarct in one case (Fig. 47, Chapter 11).

The subsequent manipulations prior to and after removal of the left kidney are exactly the same as those employed on the right (Fig. 32E, F). In closing the stumps of the transected renal vessels, it will once again be noted that in contrast to the anatomic arrangement of the other side, the aorta is most accessibly located, and the inferior vena cava is deeply recessed in the depths of the wound.

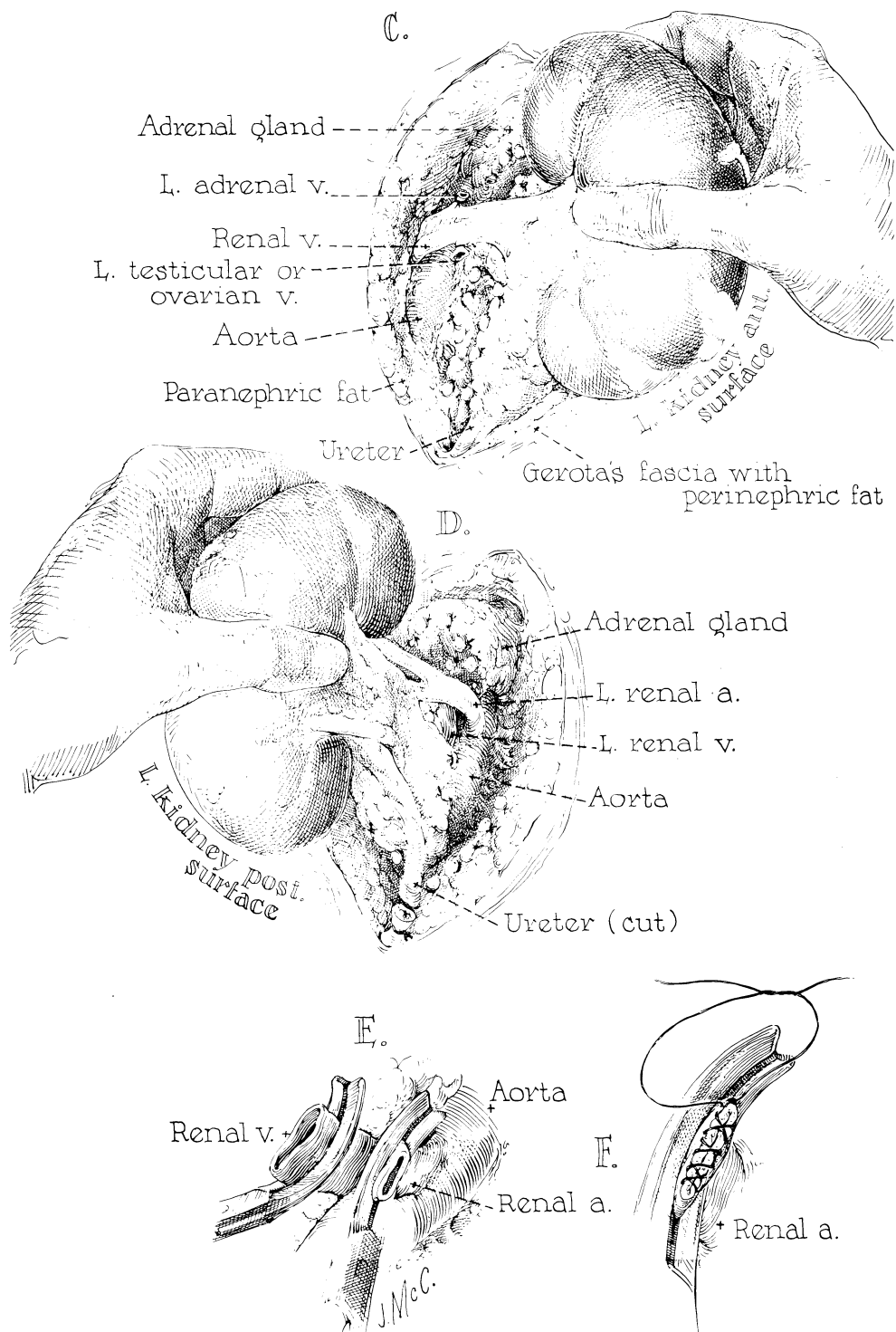


Figure 32. Donor left nephrectomy (cont.) C—Dissection of left renal vein. Note inaccessibility of its junction with the vena cava, and the testicular (or ovarian) and adrenal tributaries which must be ligated. D—Exposure and dissection of left renal artery. Note that all dissection is at the greatest possible distance from the central hilar structures. E—Potts clamps on stumps of vessels after specimen is removed. F—Double-suture closure of cuffs. (By permission of *Arch. Surg.* 88: 711, 1964.)

VARIATIONS IN ANATOMY OF THE RENAL VEINS

In approximately 15 per cent of cases, large lumbar veins are found entering the posterior surface of the renal vein proximal to the site of proposed transection (Fig. 33A). These vessels are ordinarily of no consequence, although they must be ligated before removal of the specimen. Their surgical significance is that blind dissection of the back wall of the renal vein could lead to serious hemorrhage if their presence was not suspected.

As has been previously described, donor patients are not accepted for surgery without complete preoperative evaluation of the arterial blood supply to the proposed homograft. If previously unsuspected anomalous arteries are encountered, it is mandatory to attempt vascular reconstruction, since occlusion of even tiny branches leads to renal parenchymal infarcts. With the renal veins, the situation is quite different. There is no practical means of determining the pattern of renal venous drainage prior to operation. In addition, it has been demonstrated by Smith and his associates that large auxiliary renal veins can be ligated, with the expectation that return can occur through widely anastomosing collateral channels.

Multiple renal veins were encountered in five cases. In four of these, there were two vessels, a large upper and a small lower one (Fig. 33B). In a third case, two medium-sized vessels occupied the upper part of the hilum, and a large third vessel was found inferiorly (Fig. 33C). In these cases the smaller vessels were ligated, leaving only one renal vein for anastomosis in the recipient patient. Homograft function was prompt, and no sequelae were observed that were attributable to the sacrifice of a portion of the venous drainage.

A final potentially dangerous venous anomaly was observed in one case. During performance of left nephrectomy, two venous channels were observed entering the renal vein inferiorly. Because these did not look like the customary testicular or ovarian veins, a more distal dissection was carried out which disclosed an anomalously draining ureteric vein (Fig. 33D). With occlusion of the abnormal vessel, the distal ureter became intensely cyanotic. Failure to recognize this anatomic variation would probably have resulted in venous infarction of the distal ureter.

POSTOPERATIVE CARE

Levine tubes and urinary catheters are not used unless they are specifically required. Resumption of a graded diet and ambulation are begun on the morning after operation. The patients are usually discharged in six or seven days. Repeat urine cultures and renal function tests are obtained at increasing intervals. It is planned to examine donors periodically for the rest of their lives.

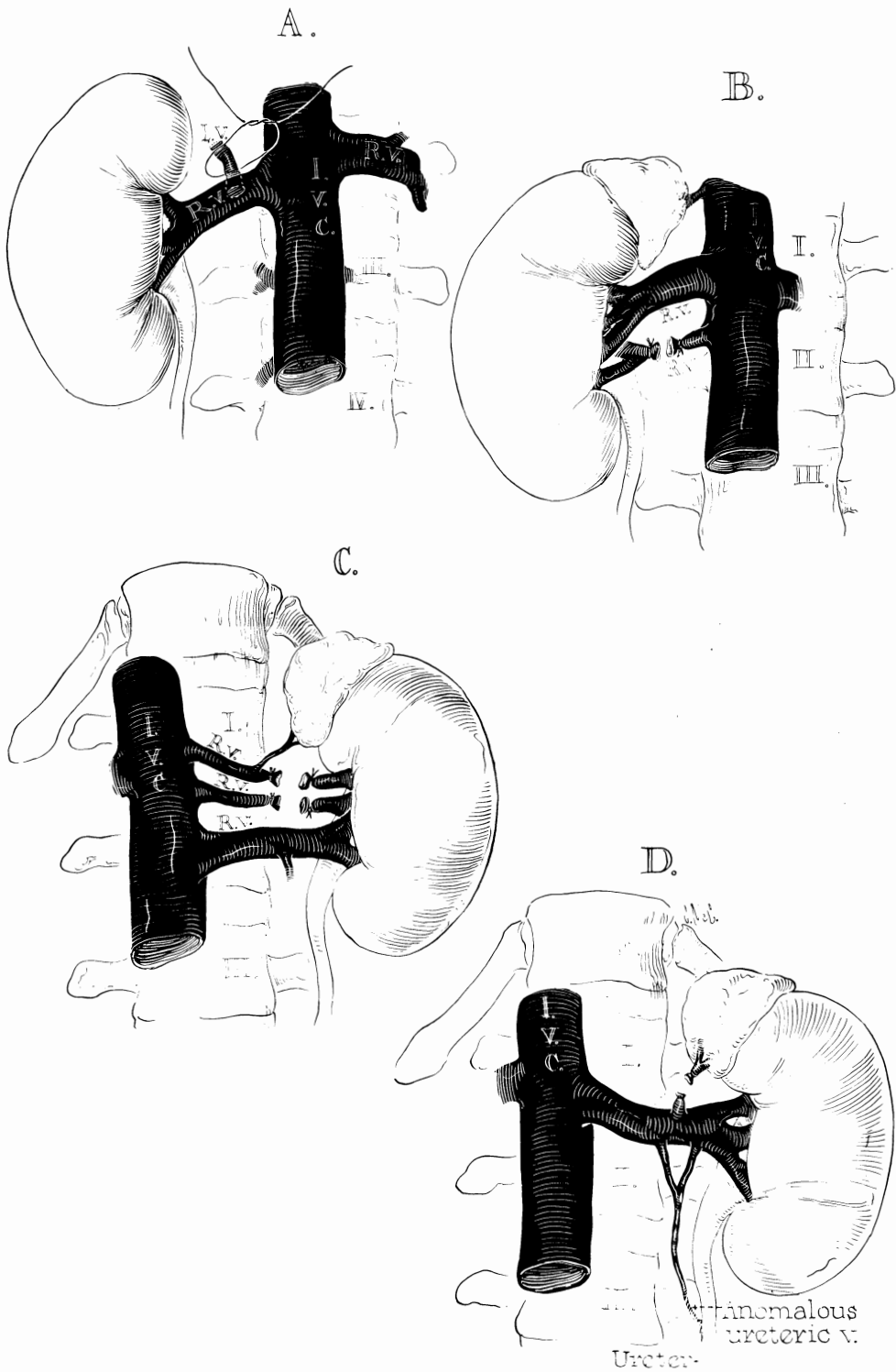


Figure 33. Venous anomalies encountered. A—Posteriorly entering lumbar vein. B—Double renal vein. The small vessel is sacrificed. C—Triple renal vein. Only the largest vessel is used for anastomosis. D—Anomalous ureteric vein which must be preserved.

Table 9. Complications in the First 74 Volunteer Donors

Subcutaneous wound infection	2
Deep wound infection	1
Pneumothorax	2
Atelectasis	1
Pulmonary embolus (?)	1
Transient peroneal nerve palsy	1

Renal insufficiency has never been observed. With statistical analysis the mean differences in pre- and postoperative BUN and serum creatinine were not significantly different. In the first 40 donors, postoperative creatinine clearance studies within the first month after operation showed an average decrease of 21.6 ml per minute, but return to previous values usually occurred within six months.

COMPLICATIONS

There have been no donor deaths. The complications in all 74 living donors are listed in Table 9. The only life-threatening complication was a possible pulmonary embolus in a 48-year-old mother who donated a kidney to her 17-year-old son (LD 39). Three weeks after discharge, she was re-admitted for right pleuritic pain, anemia, fever, and calf tenderness. An infiltrate in the right lower lobe was compatible with the diagnosis of pulmonary infarction. She was given a two-week course of heparin therapy with improvement and with resolution of the pulmonary density.

The deep wound infection in another case involved the retroperitoneal space, but responded immediately to drainage and antibiotics. The pneumothoraces were treated with closed catheter drainage of the pleural space.

TRANSABDOMINAL NEPHRECTOMY

Heterografts, obtained from baboons, and cadaveric human homografts have been removed from an anterior approach employing a total midline abdominal incision with or without a thoracic extension. The principles and technical details are the same as those described with the flank incision, with emphasis on block excision and preservation of ureteric blood supply.

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Chapter Eleven

RECIPIENT OPERATIONS

A description of the surgical techniques employed in homotransplantation involves more than the homografting procedure, since recipient right and left nephrectomy and splenectomy are also integral parts of the plan of treatment at this institution, and are usually performed at the same time as homotransplantation. The additional adjuvant procedure of thymectomy, which was used in nine of the patients early in the series, will not be described since its use has been discontinued, at least until later evaluation of these earliest patients demonstrates a superiority in long-term survival.

From a general technical point of view the problems are not different from those encountered in any major surgical procedure. Nevertheless, the predictably poor healing caused by the metabolically depleted state of the patients and by the necessity for immunosuppressive therapy reduces the margin of safety which may permit success in other operations in spite of the commission of small errors. Specifically, constant vigilance is necessary to avoid breaches in aseptic technique. Operating fields are treated with a double skin prep, and such areas are heavily draped. Attainment of absolute hemostasis should be an obsession, in order to avoid later foci of infection caused by hematomas. This may be difficult, since many of the patients have complex coagulation deficits. Interrupted fine silk sutures are used for the closure of all incisions. Cystostomy tubes or drains are never used, in order to avoid retrograde contamination of the wounds or of the lower urinary tract.

One of the greatest potential sources of wound contamination is the bladder, which must be opened for the ureteral implantation. Meticulous care is therefore exercised to prevent introduction of bacteria during catheterization. A separate 10-minute perineal prep is performed before placement of the Foley catheter. Fifty cubic centimeters saline, to which 1 gm neomycin and 50,000 units bacitracin have been added, is inserted into the bladder. To assure that the solution will be retained, the catheter is clamped until just before ureteroneocystostomy is begun, at which time it is allowed to drain.

Table 10. Staging of Recipient Nephrectomies, Splenectomy, and Homotransplantation in First 42 Patients (LD Series)*

	No.	Alive 6-1-64	Dead
One stage (splenectomy, bilateral nephrectomy, and transplantation)	31	18	13
Staged recipient operations	11	5	6

*All patients were treated prior to November 27, 1963.

INTERRELATIONSHIP OF OPERATIONS

Originally, it was thought that multiple operations would be required because of the critical condition of patients with terminal uremia. Subsequent experience has shown that this is not the case. Bilateral nephrectomy, splenectomy, and homotransplantation have been better tolerated at one stage than at separate stages (Table 10). There appear to be several reasons for this. If splenectomy is to be of maximal value, it should be done—at least on theoretical grounds—before or at the time of transplantation (see Chapter 13). Yet splenectomy, if performed in advance, does nothing to improve the patient's already serious condition. If the nephrectomies are done as a preliminary operation, the metabolic disorder is temporarily further complicated by removal of whatever renal function remains, necessitating an interim period of intensified dialysis therapy. Alternatively, if one or both nephrectomies are performed at a later time, after transplantation, there are at least four disadvantages. Function of the homograft is not easily assessed because of the residual function of the patient's own kidneys. The delayed operation or operations must be performed while the patient is receiving immunosuppressive therapy to protect the recently acquired homograft. Retention of the kidneys may aggravate a pre-existing hypertensive state, to the detriment of the transplant. Finally, there is at least a theoretical risk that in the patient with glomerulonephritis his own renal tissue will contribute to the development of the same disease in the homograft ("transplant disease").

Despite the advantages of an all-inclusive single operation, there are occasions when staging is still carried out for specific indications, such as the presence of localized sepsis in the recipient's own kidneys. For example, if infected polycystic or pyelonephritic kidneys are known to be present, it is mandatory to remove these, maintain the patient on dialysis, and obtain cleanly healed wounds before proceeding at a later date with homotransplantation (see Figure 4, Chapter 3). Under these circumstances splenectomy would also be performed at the time of the nephrectomies. In other situations in which preliminary laparotomy becomes necessary for some other reason, it may be convenient to add bilateral nephrectomy and splenectomy to the operation for which the exploration was primarily performed. This was done in two patients who required gastrectomy and vagotomy for a bleeding duodenal ulcer, which had developed while they were being evaluated for kidney transplantation (see Figure 10, Chapter 4).

Coordination of Operations

A variety of exposures have been used for patients receiving one-stage therapy, depending to some extent upon the location planned for the homograft. Most versatile and most commonly used at the present time is an upper midline incision through which both kidneys and the spleen are removed transperitoneally, in combination with an oblique left or right lower abdominal incision through which extraperitoneal transplantation is performed (Fig. 34 C).

If transplantation of a donor left kidney to the right iliac fossa is planned, both transplantation and right nephrectomy can be done extraperitoneally through an oblique right lower abdominal incision, and left nephrectomy and splenectomy can be performed transperitoneally through a separate left subcostal incision (Fig. 34A). In transplanting a donor right kidney to the left iliac fossa, three incisions may be used—one for extraperitoneal transplantation, another for transperitoneal left nephrectomy and splenectomy, and a third for either extra- or intraperitoneal right nephrectomy (Fig. 34B). In four children of three to eight years, the entire operation was performed transperitoneally through a total midline abdominal incision (Fig. 34D), using relatively oversized homografts from adult donors.

Although the donated kidney is customarily placed on the contralateral side of the recipient, this is not an essential feature of the operation. As will be described in the section on “Special Problems of Transplantation” (p. 106), it is no more difficult to transplant the organ to the ipsilateral side, and this has been done without difficulty in both children and adults.

In order to coordinate activities in the two operating rooms, it is necessary to have a well-planned sequence of the various operative manipulations and a rough estimate of the time required to complete each step. The bilateral nephrectomies and splenectomy are done first. Completion of these preliminary procedures, closure of the upper abdominal wound or wounds, and preparation of the transplantation site for reception of the homograft require 90 to 150 minutes. If the operations on donor and recipient are started simultaneously, both patients are ready for transfer of the kidney at approximately the same time. If homotransplantation is the only procedure to be done, operation on the donor is begun 60 to 90 minutes before operation on the recipient.

PROCEDURES IN HOMOTRANSPLANTATION

As previously mentioned, the donated kidney is usually placed in the contralateral iliac fossa after the method described by Küss and by Murray and Harrison. The anteroposterior relationships of the hilar structures are thereby reversed, placing the pelvis anteriorly and the renal vein posteriorly in the new

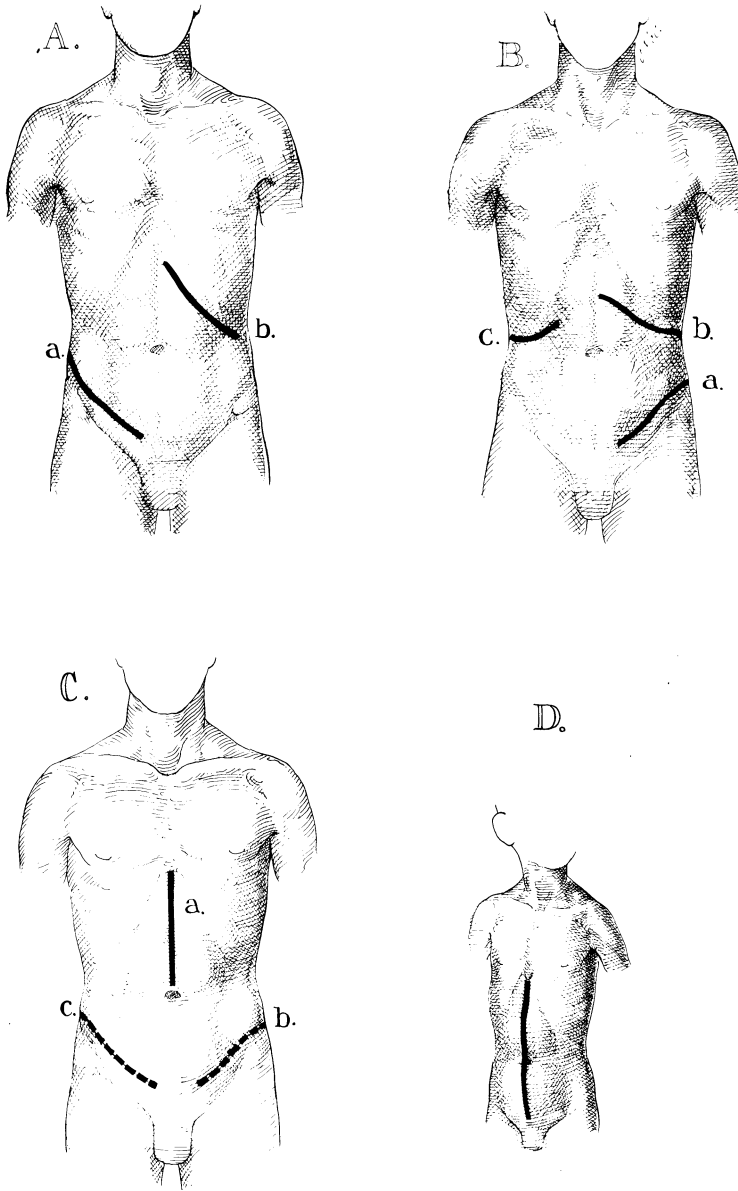


Figure 34. Combinations of incisions used for bilateral nephrectomy, splenectomy, and renal transplantation. The various procedures can be done in stages or preferably at a single operation. At present, the incisions shown in C are most often used.

A—Transplantation to the right iliac fossa. Right nephrectomy may be done through the transplant incision. The left kidney and spleen are removed through the left subcostal exposure.

B—Transplantation to the left iliac fossa. Splenectomy and left nephrectomy are performed through the left subcostal incision, and right nephrectomy through a right subcostal incision.

C—Most versatile exposure. Nephrectomies and splenectomy are performed through the upper midline incision. Transplantation is to either iliac fossa.

D—Total abdominal incision used in children for one-stage transperitoneal operation.

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location. There are no important differences in technique with transplantation to one side as opposed to the other, and the following description, in which the homograft is placed on the right, applies equally to the comparable operation on the left. Usually, the splenectomy and nephrectomies have already been completed in those patients receiving a complete one-stage operation, and the upper abdominal incision or incisions have been closed.

Preparation of Homograft Bed

An oblique incision is made from the lateral border of the rectus abdominis muscle into the flank, 1 to 1.5 cm above and parallel to the inguinal ligament (Fig. 35A). Subcuticular bleeding usually occurs, and is best controlled with electrocautery. After Camper's fascia is incised, the external oblique muscle and fascia are split to the lateral extent of the wound and 1 cm onto the rectus sheath medially (Fig. 35B). The subjacent internal oblique and transversalis muscles are fused, and are best incised as a unit, rather than separately (Fig. 35B). The medial fascial continuation of these muscles which forms part of the anterior rectus sheath is also partially cut, and the lateral edge of the rectus abdominis muscle exposed (Fig. 36C). The transversalis fascia is opened, care being taken not to cut the underlying peritoneum. This fully exposes the inferior epigastric artery and vein for ligation and division near the medial end of the incision (Fig. 36C). Just lateral to these vessels, the spermatic cord (in males) or the round ligament (in females) is skeletonized, ligated in continuity, and divided (Fig. 36D).

The retroperitoneal space is exposed by rostral blunt dissection of the peritoneum off the iliac vessels and the iliac fascia (Fig. 37E). If the operation is on the right side, mobilization can be carried up to the inferior pole of the kidney, and that organ immediately removed from below (described on page 107 in a subsequent section on right nephrectomy).

Attention is next directed to cleaning off the fatty areolar and lymphatic tissues overlying the external iliac vein, just medial to the external iliac artery (Fig. 37E). It is particularly important to ligate and divide in continuity all tissue in this region in order to prevent lymph leaks and the postoperative formation of lymphoceles. The dissection is carried superiorly from the inguinal ligament until the hypogastric artery is encountered running across the vein into the pelvis (Fig. 37F). Throughout this length, the external iliac vein is completely freed so that it can be conveniently controlled during the subsequent venous anastomosis. It is usually necessary to ligate one or two small posterior tributaries.

An effort is made at this time to detect any venous valves which might be present in this segment. These have been found in almost half the cases, and can be readily identified by the oblique white impression made on the surface of the vein by the valve attachments and by the slight bulges more inferiorly which conform to the cusps. Valves have been found at all levels of the

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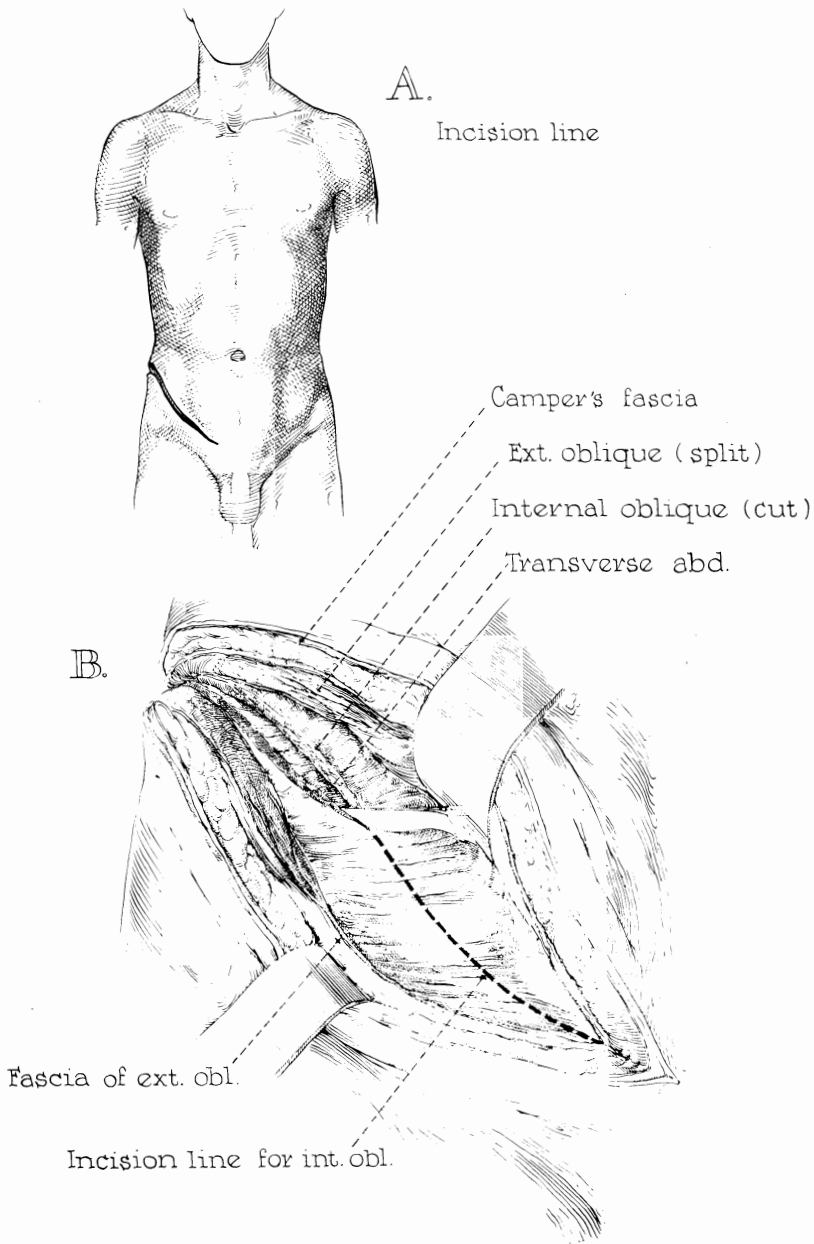


Figure 35. Exposure for renal transplantation to the right iliac fossa.

A—Line of incision.

B—Incision of muscle layers.

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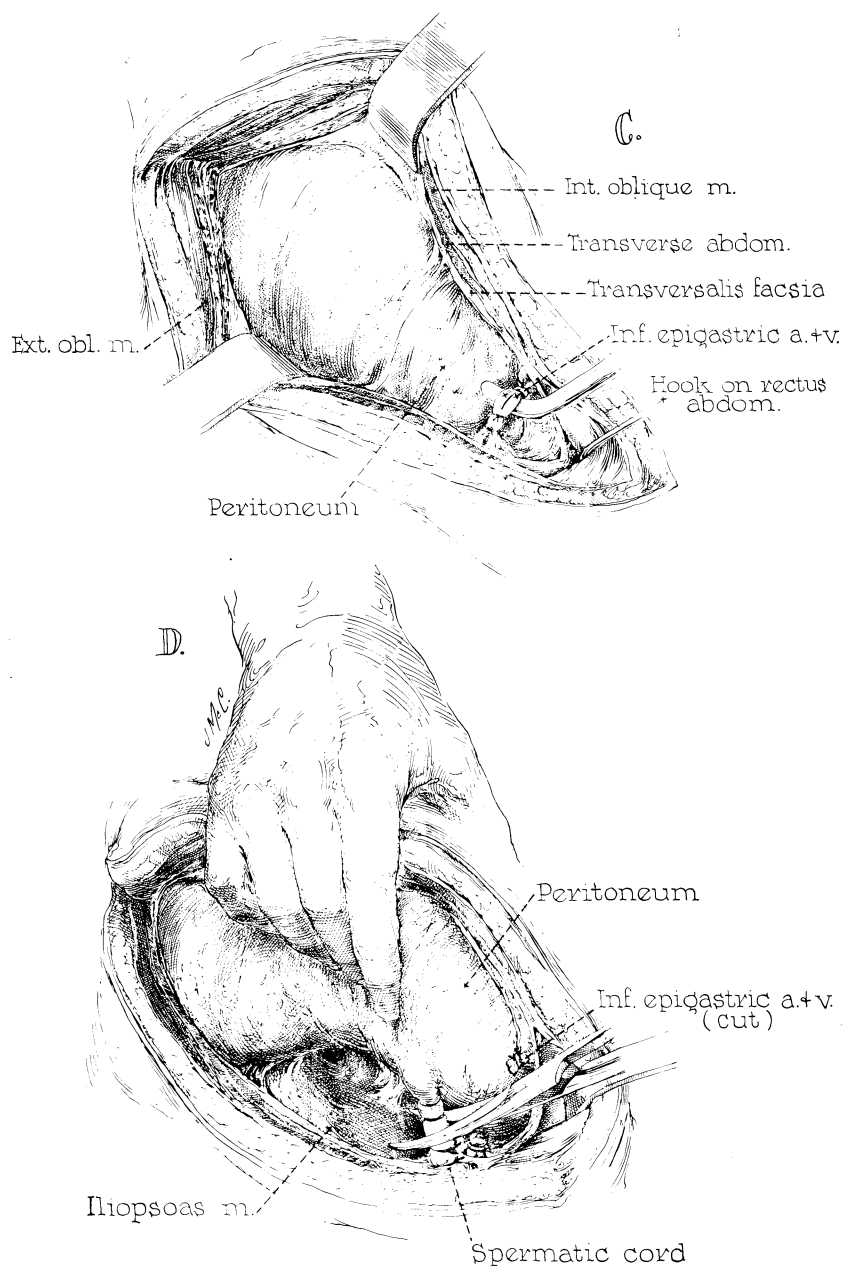


Figure 36. Exposure of retroperitoneal space.

C—Ligation and division of inferior epigastric artery and vein.
D—Quadruple ligation and division of the spermatic cord or round ligament. (By permission of Arch. Surg. 89:87, 1964.)

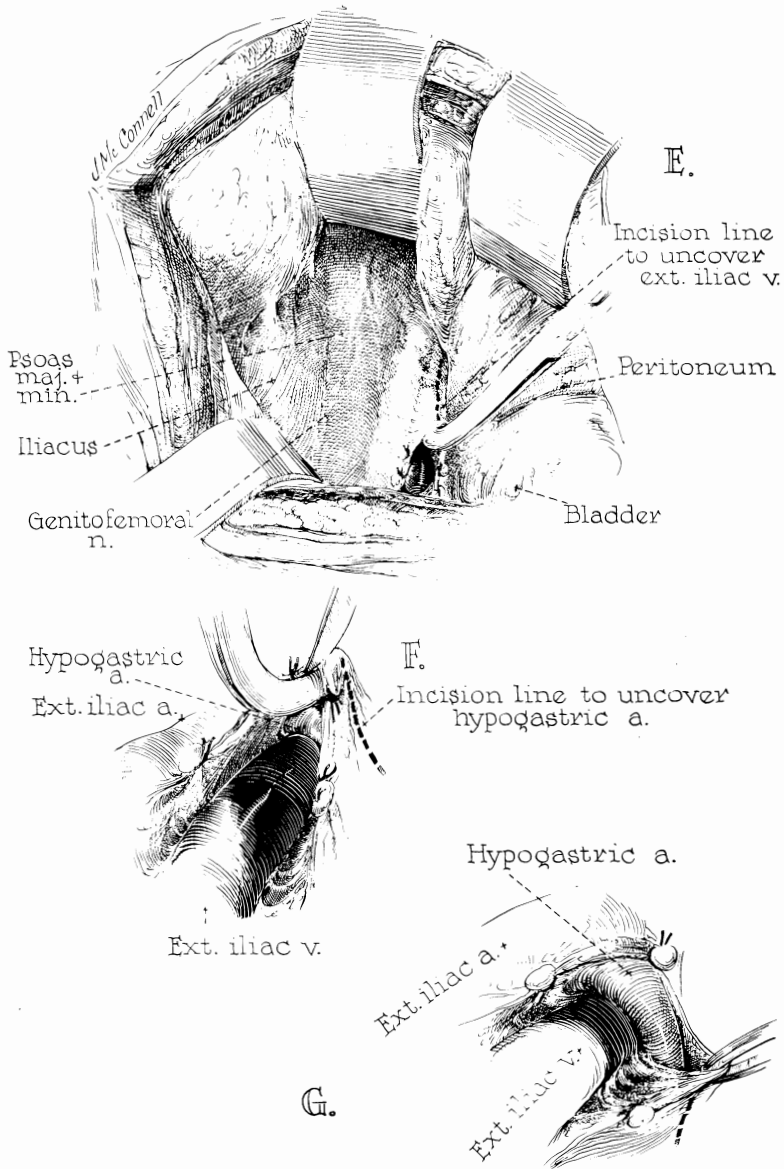


Figure 37. Exposure of the iliac vessels.

E—Dissection of the external iliac vein. Note that all tissue in this area is doubly ligated before division in order to prevent the subsequent development of lymphatic leak.

F—The external iliac vein is freed to the point at which it is crossed by the hypogastric artery.

G—The hypogastric artery is denuded distally to its major division.

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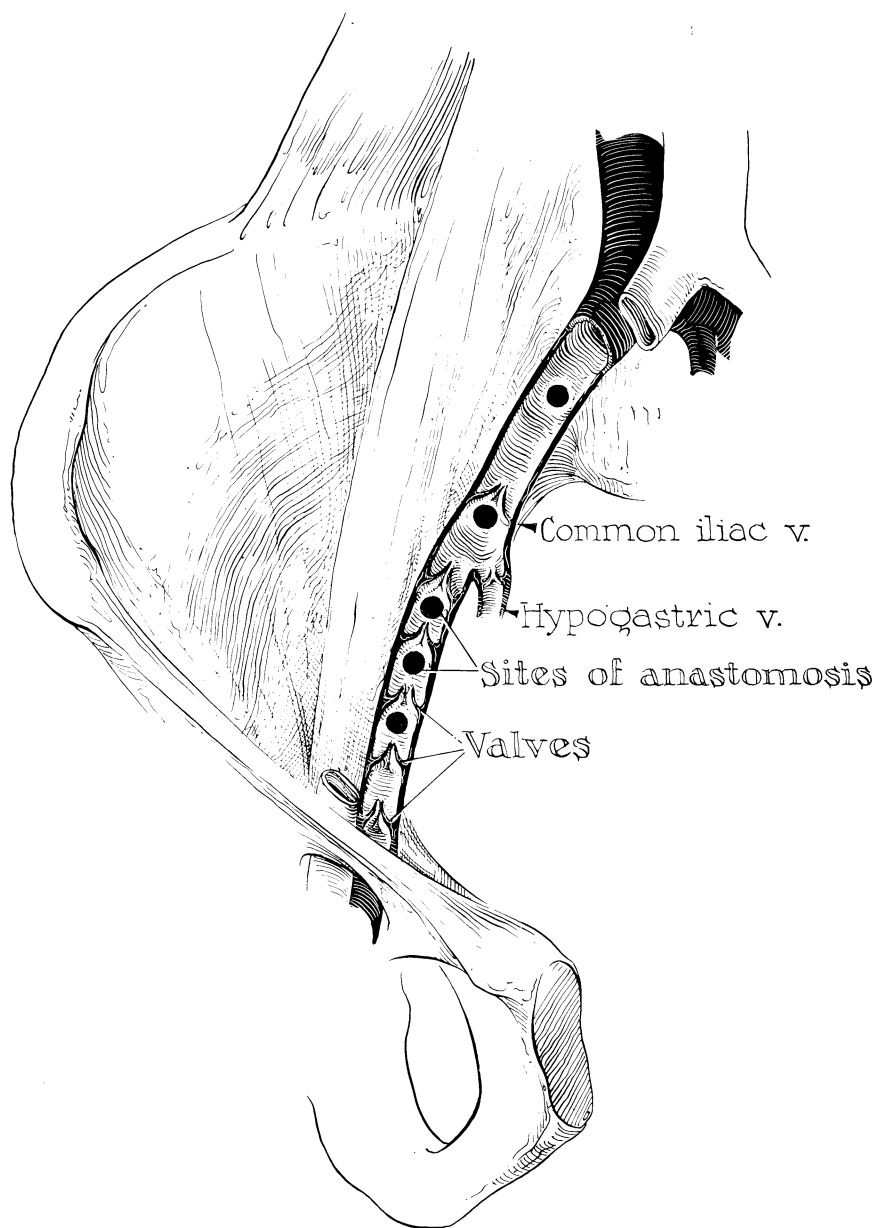


Figure 38. Location of venous valves which were encountered in almost half the cases. Sites of venous anastomoses are indicated by solid circles. When possible, anastomosis is placed to avoid instrumentation of valves.

external iliac vein, and their presence may influence the location of the renal venous implantation (Fig. 38). A decision is made concerning the site of the subsequent venous anastomosis: if a valve is present in the general area selected, the suture line is planned for placement above or below this point, if possible. If this is not possible, the valve leaflets are excised when the vein is opened.

Next, the hypogastric artery is freed and mobilized, starting at its origin (Fig. 37G). The angle formed by the junction of the hypogastric and external iliac arteries is cleaned with special care to allow mobility of this area, but no other portion of either the external or common iliac arteries is freed. The hypogastric artery is dissected distally to its bifurcation into major anterior and posterior branches (Fig. 39A). Frequently one or two small branches are encountered, ligated, and divided proximal to this division. At the site of eventual transection and anastomosis, the vessel is denuded of adventitia to facilitate later suturing. In all cases the number of renal arteries to the homograft has been determined by donor aortographic studies, and in almost all cases the kidney selected has a single blood supply. In the exceptional situation in which two or more renal arterial anastomoses are necessary, the dissection is continued more deeply, freeing the terminal branches for anastomosis to the multiple homograft vessels. After preparation of the hypogastric artery, it is not transected or further disturbed until the renal arterial anastomosis is to be performed. In this way, blood flow through the vessel continues until the last possible moment—a detail of technique which may be of importance in preventing intimal trauma, drying, or undetected clot formation within the lumen.

After these steps have been completed, an assistant coordinates the final steps in the operating rooms. This is important, not only to allow perfect timing of events, but to provide last-minute information for the recipient's surgical team concerning any anatomic peculiarities in the kidney which is shortly to be delivered. In every case, the most important information to be transmitted at this time is the size of the renal vein, since the aperture for its anastomosis into the external iliac vein is fashioned before the kidney is delivered.

After it has been ascertained that the kidney will be available within a few minutes, as much as possible of the external iliac vein is isolated between noncrushing sponge rubber clamps (Fig. 39A, B). These instruments are particularly well suited to this function, not only because their size and shape allow an easy fit into the wound, but because the sponge covering ensures minimal trauma to the vessel.* The anterolateral surface of the vein wall is then grasped with a fine pickup, and by cutting beneath this an ellipse is removed from the wall (Fig. 39B). If a venous valve is present, the leaflets are excised at their attachments. The defect in the vessel is divided into quadrants by the placement of arterial sutures of 5-0 silk, including one at each end (Fig. 39C). Exposure is maintained, and all activity is suspended until the homograft is delivered from the donor room.

*The clamps, described by Brown, are manufactured by Edward Weck and Co., Brooklyn, New York.

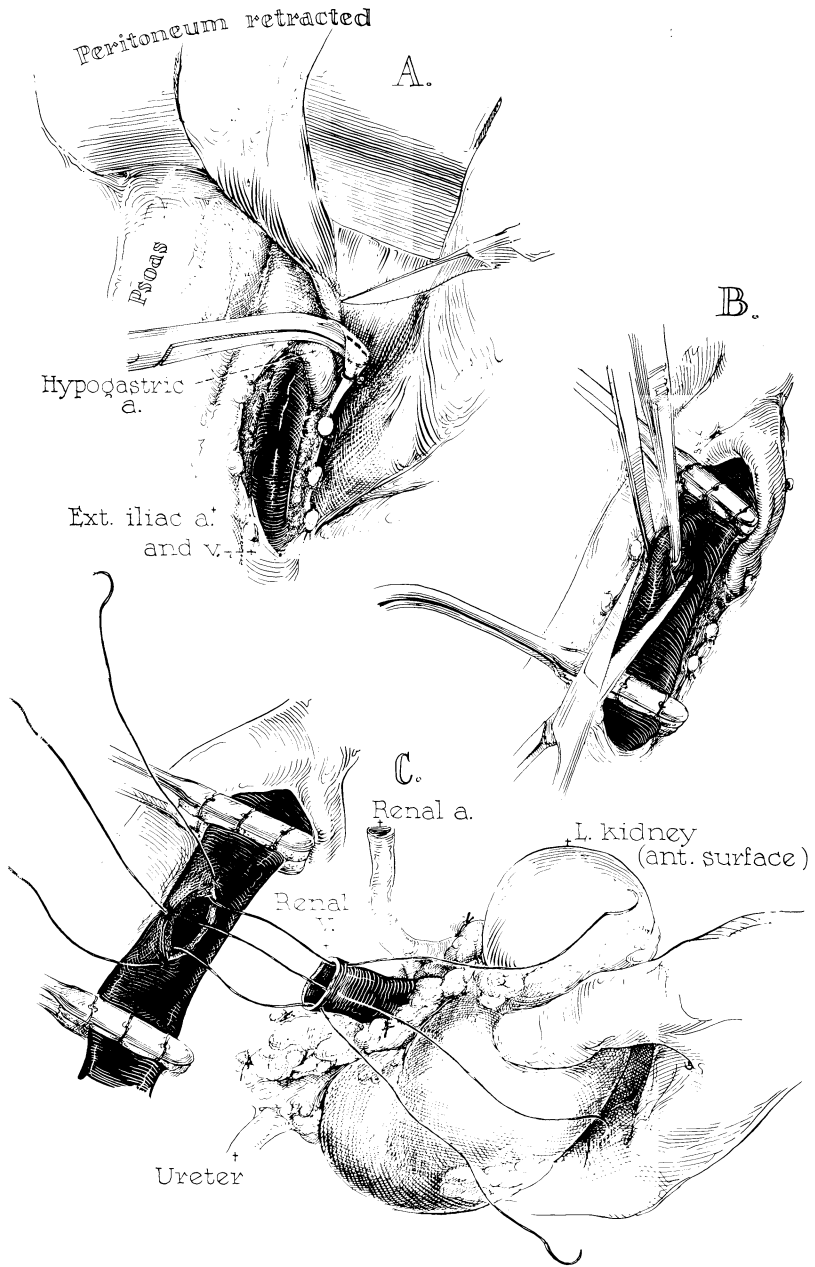


Figure 39. Venous anastomosis.

A—General operative field.

B—Excision of an ellipse of the anterolateral wall of the external iliac vein.

C—Placement of guide sutures connecting the renal and external iliac veins.

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Ureteroneocystostomy

The person who delivers the kidney is asked to place it in the position it would have occupied in the donor patient in the supine position. The landmarks are checked, and the kidney is placed on the drapes adjacent to the wound, in its normal anatomic position. Thus, the vein, which is anastomosed first, is anterior and easily exposed (Fig. 39C). The sutures previously placed in the ends of the oval defect in the external iliac vein are then attached, passing from the lumen to the outside in the superior and inferior corners of the renal vein. The lateral suture in the iliac venous defect is similarly placed in the appropriate position (Fig. 39C). The kidney is then guided down the guy sutures until the intima of the two veins is coapted. The sutures are tied (Fig. 40D). The homograft is then rolled laterally and the fourth of the quadrantic guide sutures is passed through the renal vein (Fig. 40E). The medial and lateral stay sutures are of extreme importance in preventing accidental grasping of the opposite wall while the venous anastomosis is formed, since the vein can be flattened and spread by gentle traction (Fig. 40E, F). The venous anastomosis is then fashioned, with the use of one end suture for sewing. Continuous over-and-over suturing is used, taking care to evert the lips, and passing around the entire 360 degrees. It is usually somewhat easier to suture the anterior row first and then reflect the kidney medially to obtain access to the posterior surface (Fig. 40F).

After the venous anastomosis is completed, the kidney is placed in what appears to be the most comfortable position in the iliac fossa (Fig. 41A). A ligature of 2-0 silk is passed around the distal hypogastric artery (Fig. 41A). This is tied, a noncrushing vascular clamp is placed across the origin of the hypogastric artery, and the vessel is transected. Because of the curve which must be negotiated by the arterial trunk, the hypogastric artery is incised with a 45 degree bevel (Fig. 41A). Anchoring sutures of 6-0 arterial silk are placed at the tip and base of the beveled cut end and attached to the comparable positions on the renal artery (Fig. 41B). It is preferable to use silk with a needle mounted on each end, since this allows placement of the sutures from within the lumen of each vessel. Disparities in size of the hypogastric and renal arteries frequently make it necessary to dilate one or the other vessel with a smooth hemostat (Fig. 41C).

After the stay sutures are tied, the arterial anastomosis is formed with continuous over-and-over everting techniques, using the same suture for the entire 360 degrees. The anterior row is completed first and the vessel then rotated (Fig. 41D, E). The kidney is then revascularized. Certain details of technique are scrupulously observed at this time. Both arterial and venous clamps are removed at exactly the same time. The clamps are released abruptly rather than in a graduated fashion. The suture lines are scrutinized for leaks between or at the sutures, and these are systematically repaired. Under no circumstances is the blood supply to the kidney ever interrupted again. It is always possible to repair the small suture line leaks without re-occluding the circulation.

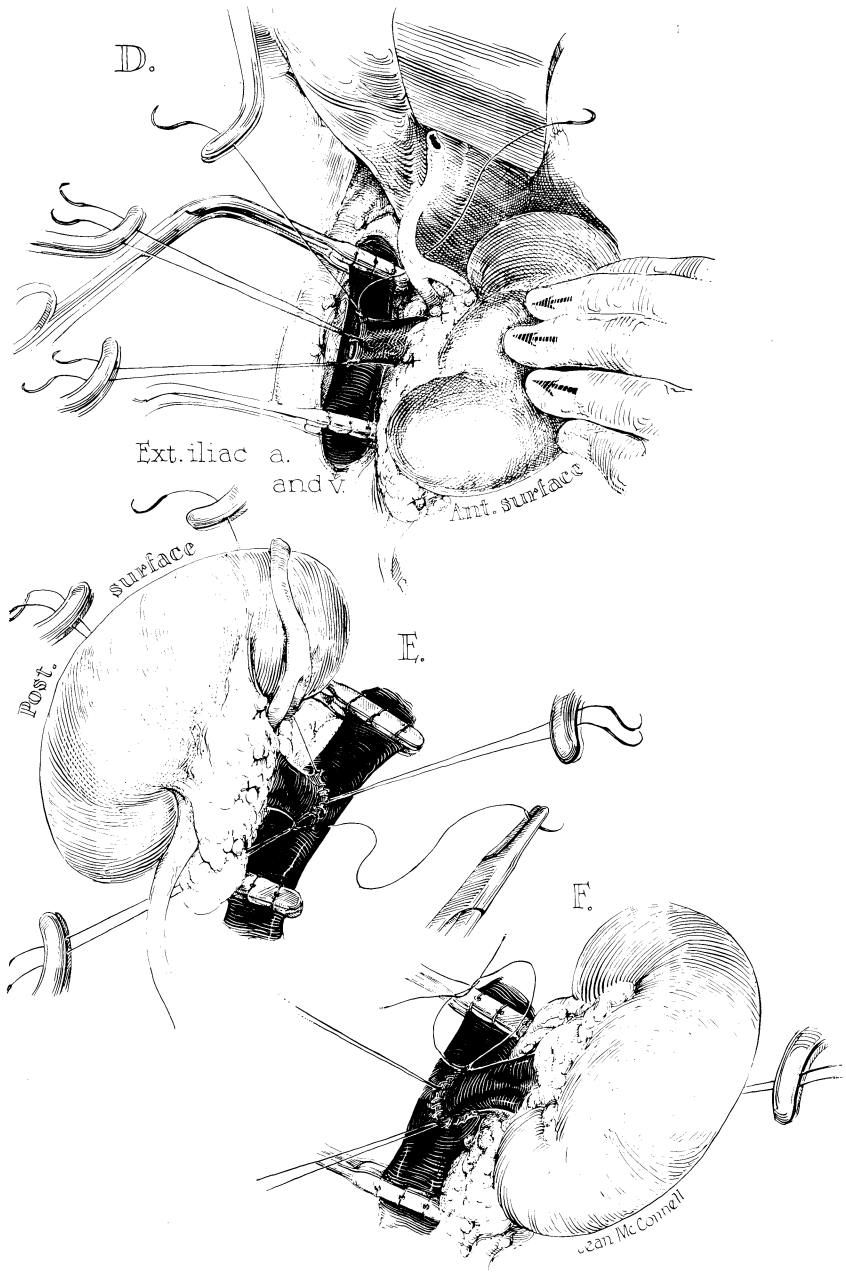


Figure 40. Venous anastomosis (cont.)

- D—Fixation of the venous anastomosis by tying the guide sutures.
 - E—Performance of circumferential anastomosis using continuous silk suture which is carried around the entire 360 degrees.
 - F—Completion of anastomosis.
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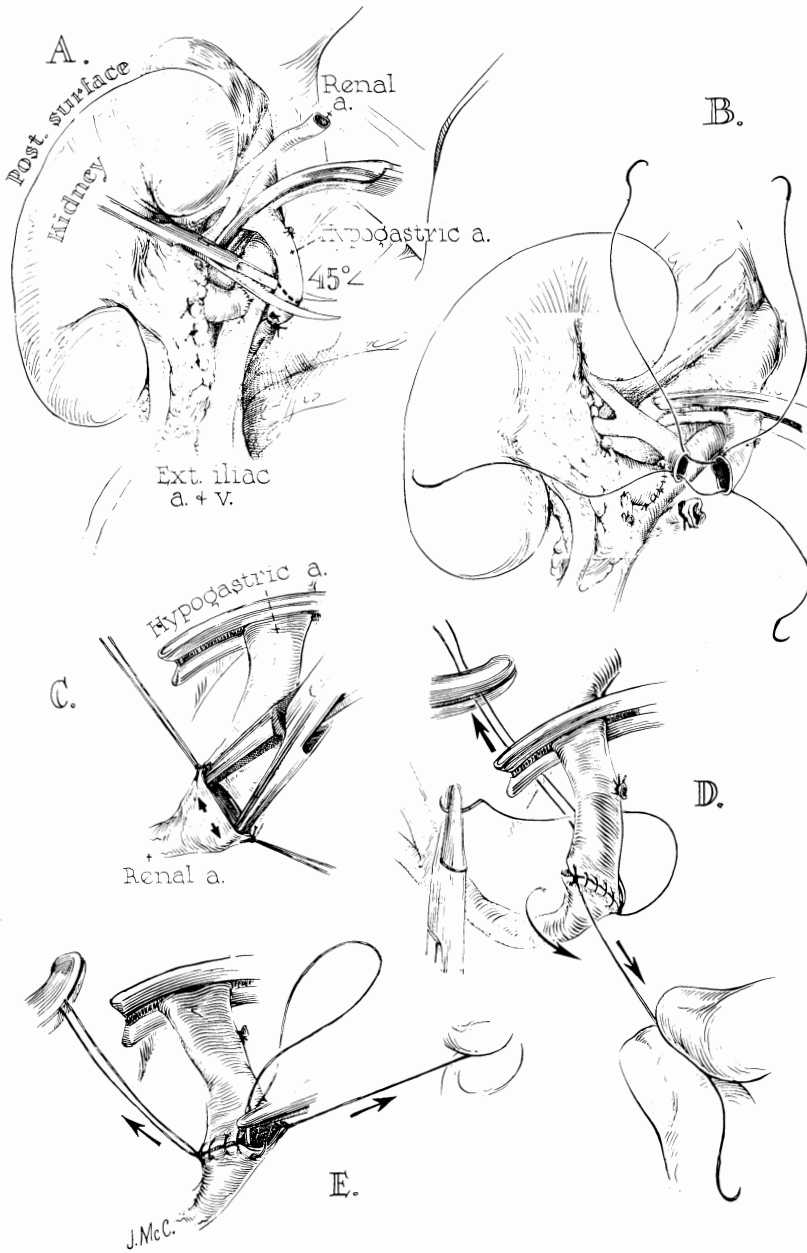


Figure 41. Arterial anastomosis.

A—The hypogastric artery has been ligated distally and is cut. Note bevel of incision line which makes a smoother curve of the reconstructed vessels.

B—Fixation sutures connecting two vessels.

C—Dilatation of the smaller of the two vessels, the renal in this instance.

D—Completion of the anterior row and beginning rotation of the anastomotic line.

E—Continuous suture of the posterior suture line.

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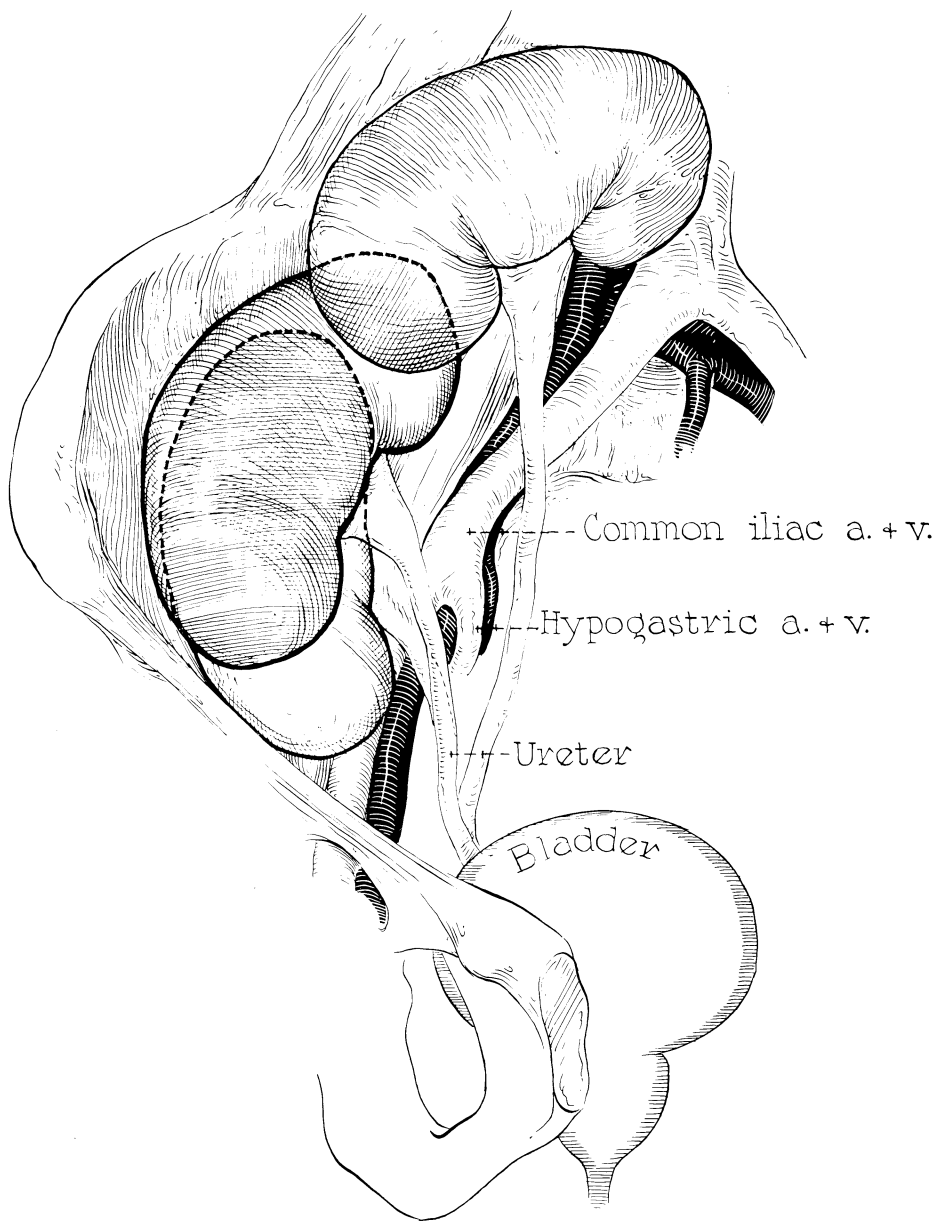


Figure 42. Various positions assumed by the homografted kidney. The placement selected is the one which appears at the time of operation to be the most comfortably accommodated. (By permission of *Arch. Surg.* 89:87, 1964.)

Before the ureteroneocystostomy is undertaken, experimentation is again conducted to determine the most comfortable position for the homograft. The most useful rule is that the kidney will usually seek the position which is most suitable. The integrity of the vessels and their anastomoses should be checked, after which the decision regarding position can be made. The ultimate location of the kidney has varied considerably (Fig. 42). In some patients the hollow of the ileum is adequate to accommodate the organ, but in others, particularly recipients of small stature, all or part of the homograft is extrapelvic.

Once the homograft is positioned, the ureter is anastomosed to the bladder by a modification of the method of Paquin and Marshall (Figs. 43, 44). In the previous description of the operative exposure, no mention was made of mobilization of the bladder. Mobilization is also avoided now. Although ureteral implantation is thereby made more difficult, the retropubic space is not entered, so as to prevent the formation of a retropubic dead space which would need to be drained. A rather small incision is made in the dome of the bladder near the peritoneal reflection (Fig. 43A). By appropriate manipulation of thin-bladed retractors, an adequate view can be obtained of the interior of the bladder.

A site is selected 2 or 3 mm above the ipsilateral ureteral orifice for the location of the new ureteral opening. A small mucosal incision is made at this point, and a similar counterincision is placed 1 to 2 cm higher on the lateral wall (Fig. 43B). The two defects are joined by blunt submucosal dissection (Fig. 43C). A right-angled clamp is thrust through the upper mucosal incision and out through the lateral bladder wall (Fig. 43D). The tip of the ureter is grasped and brought into the bladder (Fig. 43D) and guided through the previously formed tunnel (Fig. 43E). The upper of the two mucosal incisions is closed with a continuous 6-0 plain catgut suture (Fig. 44F). The proper ureteral length is determined and the ureter fixed in its definitive position by placement of one or two sutures at the point at which it enters the bladder (Fig. 44F).

Attention is then directed to formation of a ureteral nipple and a mucosa-to-mucosa anastomosis of the tip. The excess ureter is drawn up with a clamp, and the ureter is partially transected at the site of the eventual nipple (Fig. 44G). The cut is made on the deep relatively inaccessible portion of the ureter, and the residual distal connection is used for retraction (Fig. 44G). The principal ureteric blood vessels are identified and ligated with 6-0 plain catgut a few millimeters from the cut end (Fig. 44G). The partially transected ureter is split 3 or 4 mm in its long axis, forming two posteriorly placed fish-mouth flaps (Fig. 44G). Three 6-0 plain catgut sutures are placed, catching first the bladder mucosa, then the ureteral adventitia 3 or 4 mm proximal to the cut end, and finally grasping the edges of the ureteral mucosa (Fig. 44H, I). When these three sutures are tied, a partial nipple is immediately formed with good mucosal coaptation of the part of the anastomosis which is most difficult to see (Fig. 44J). The rest of the nipple is formed after the transection of the ureter has been completed. In doing this, a flap of tissue is preserved (Fig. 44J, K) so that the upper part of the nipple is eccentric with more tissue than the lower aspect (Fig. 44L). This flap of tissue is sutured into position with several fine 6-0 plain catgut sutures (Fig. 44L).

The cystotomy is closed in three layers. The mucosa is sutured with continuous 4-0 chromic catgut (Fig. 44M, N). Slight eversion is deliberately obtained to prevent intravesical bleeding from the raw mucosal surface. Continuous 4-0 chromic catgut is also used for the second layer, grasping the detrusor muscle in firm bites (Fig. 44O). The superficial tissues are approximated with interrupted fine silk (Fig. 44P).

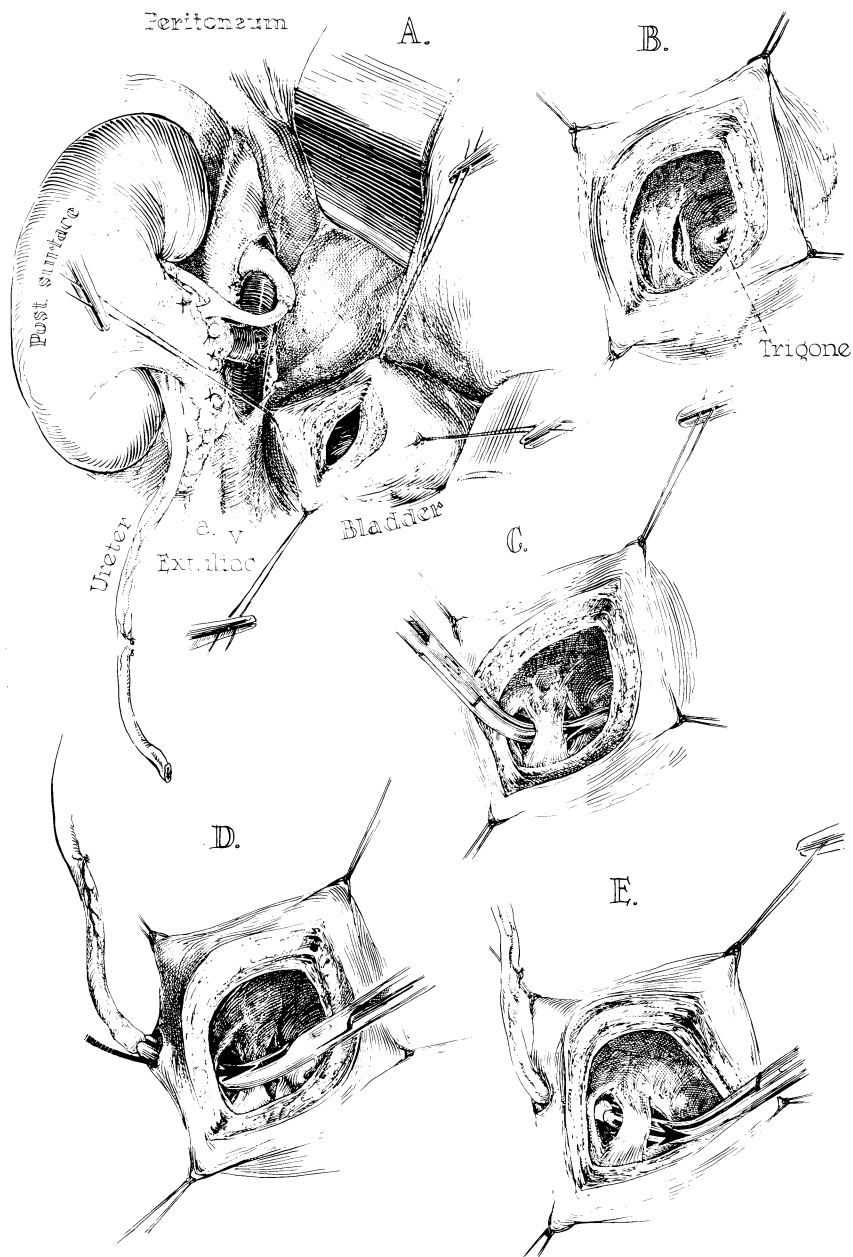


Figure 43. Ureteroneocystostomy.

A—Bladder cystotomy performed.

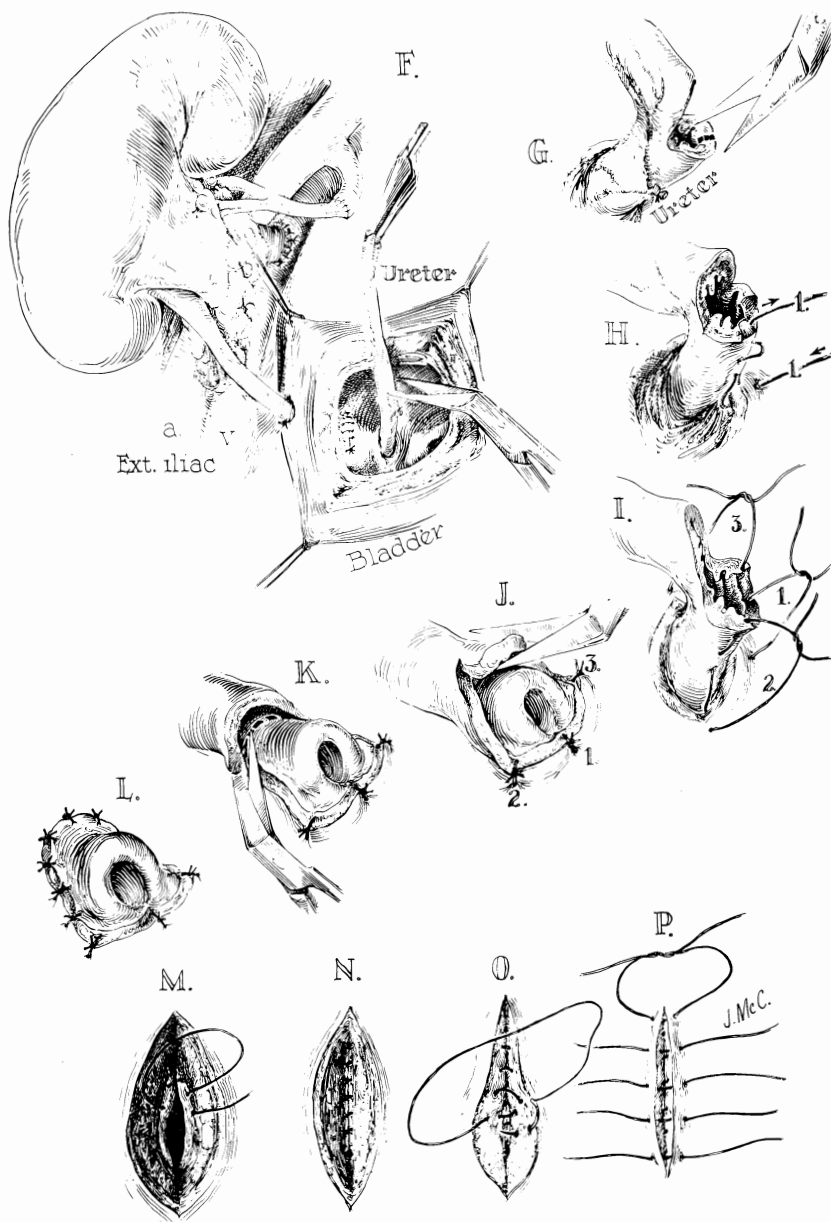
B—Beginning of creation of the submucosal tunnel by performance of two mucosal incisions. Note proximity of lower incision to trigone.

C—Completion of tunnel with blunt hemostat dissection.

D—The ureter is pulled through the bladder wall at an angle so that an additional tunnel effect is created.

E—Extraction of the intravesical ureter through the preformed submucosal tunnel.

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Incision in bladder closed

Figure 44. Ureteroneocystostomy (cont.).

F—The upper mucosal incision has been closed and the ureteral length decided upon. Note that the vascular bundle of the ureter has been ligated.

G—The ureter has been hemitranssected and a fish-mouth is being created. Note that the latter cut is made at the most recessed and inaccessible portion of the ureter.

H—A 5-0 or 6-0 catgut suture is placed from the bladder mucosa to the urethral adventitia and to the apex of the fish-mouth incision.

I—Placement of similar corner sutures catching the tips of the fish-mouth flaps.

J—The three anchoring sutures have been tied, and a flap of ureteral mucosa is fashioned for the ultimate formation of a hood.

K—Continuation of flap incision.

L—The eccentric flap is completed and sutured in place.

M-P—Bladder closure. Note that the inner two layers are of continuous catgut, insuring a water-tight closure. The external layer is of interrupted black silk.

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Many of the details of the bladder and the ureteral manipulation seem to be inconsequential. Yet they are designed to achieve two specific objectives: first, the construction of a wound which can be closed without drainage and, second, the establishment of a situation in which the urethral catheter can be safely removed within 12 to 24 hours after operation. The precautions exercised to provide complete and reliable closure of all mucosal rents, to protect the blood supply of the ureter, to provide the flap valve action of a long submucosal tunnel, and to assure watertight closure of the cystotomy are all insurance factors which circumvent the need for prolonged postoperative decompression of the bladder.

Before closure, a final check is made for hemostasis. The wound is irrigated with 1,000 to 2,000 cc of saline solution. All residual fluid is withdrawn, after which an antibiotic solution of 0.5 gm neomycin and 50,000 units bacitracin, suspended in 50 cc normal saline, is instilled into the wound. Closure is carried out in layers with interrupted 3-0 or 4-0 silk sutures, each layer being irrigated with antibiotic or saline solution.

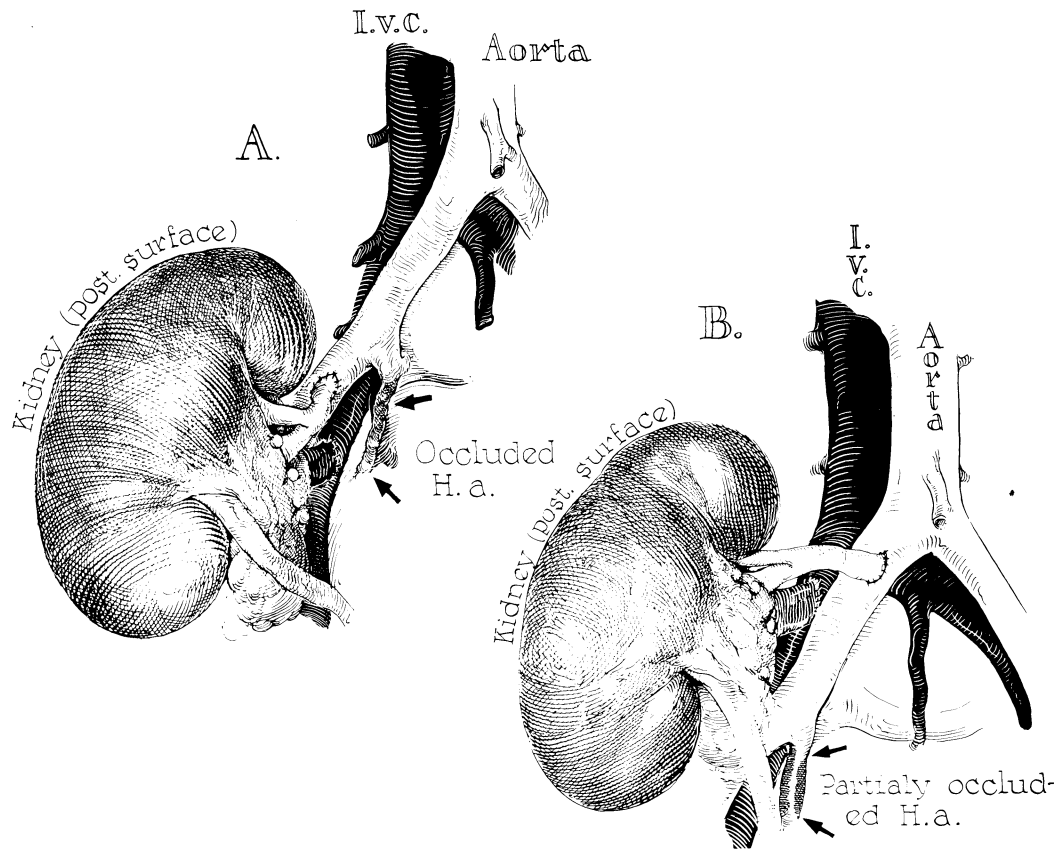


Figure 45. Special problems in arterial reconstruction. Occlusion of the hypogastric artery, necessitating end-to-side anastomosis to the external (A) or common iliac (B) artery.

SPECIAL PROBLEMS OF TRANSPLANTATION IN ADULTS

Deviations from the operative plan just described have been necessary, frequently as a consequence of abnormalities of the vessels of the donated kidney, or of the recipient patient. In five cases the hypogastric artery was partially or completely occluded with atheromata. The arterial anastomosis was placed end-to-side to the common or external iliac artery (Fig. 45A,B). When this is necessary, the attachment of the renal artery lies most satisfactorily if it is 1 to 2 cm superior to that of the renal vein. A quartering technique similar to that already described for the renal vein is employed.

In four instances, it was necessary to restore the arterial blood supply through two renal vessels. Although this can easily be done by using more distal branches of the hypogastric artery to connect to the renal arteries, the problem of obtaining smooth and unknicked curves may be trying. Solutions to some of the mechanical problems are depicted in Figure 46.

It has been stressed throughout this chapter that the donor kidney is usually transplanted to the contralateral side of the recipient. This has been a traditional technique, justified by the theoretical advantage that the pelvis and ureter are thereby placed anteriorly, in which aspect they are less likely to be compressed by the iliac vessels or iliopsoas muscle.

In practice, it has been demonstrated that placing the kidney contralaterally is not necessary. In three cases, donor kidneys have been transferred to the ipsilateral iliac fossa or paravertebral gutter with satisfactory results (Fig. 48).

Other interesting departures from standard techniques are possible with the use of cadaveric organs. Hume has dealt with multiple arteries by suturing a disc of aorta containing the origins of the vessels into the side of the external iliac artery. Goodwin has described block excision of both kidneys with revascularization by insertion of a segment of attached aorta and vena cava into the recipient's vascular system, a technique which has been applied with modifications to heterotransplantation (see Chapter 23).

In two cases, small infarcts resulted from the operative procedure following occlusion of arteries too small to be reconstructed. In one case, a small polar artery originated in the line of transection of the renal artery. In forming the anastomosis, this artery was closed by the suture line (Fig. 47A). In the other case, ligation of the same vessel occurred accidentally during operation on the donor (Fig. 47B). The area of involvement of renal parenchyma was almost identical in both cases. There were no detectable immediate or late sequelae.

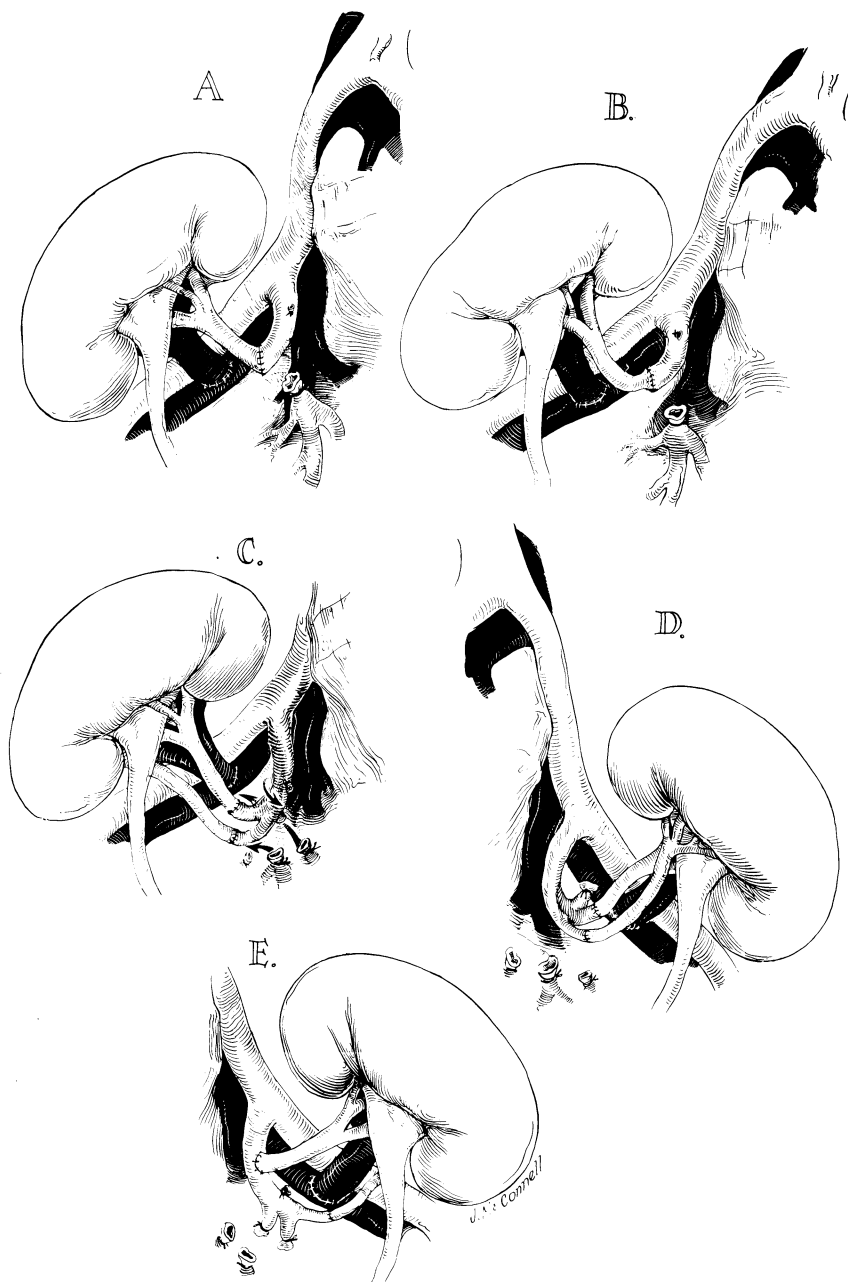


Figure 46. Special problems in arterial reconstruction.

A—Usual arrangement of artery. Note bevel of cut hypogastric artery which promotes proper curvature of anastomotic site.

B—Telescoped anastomosis, necessary when major disparity exists between the size of the two vessels.

C—Connection of double renal arteries to the anterior and posterior trunks of the hypogastric artery. Note the sharp rotation of the posterior trunk which is necessary.

D—Similar problem in which the posterior trunk is rolled in the opposite direction.

E—Double renal arteries connected to the side and to the terminal branch of the hypogastric artery.

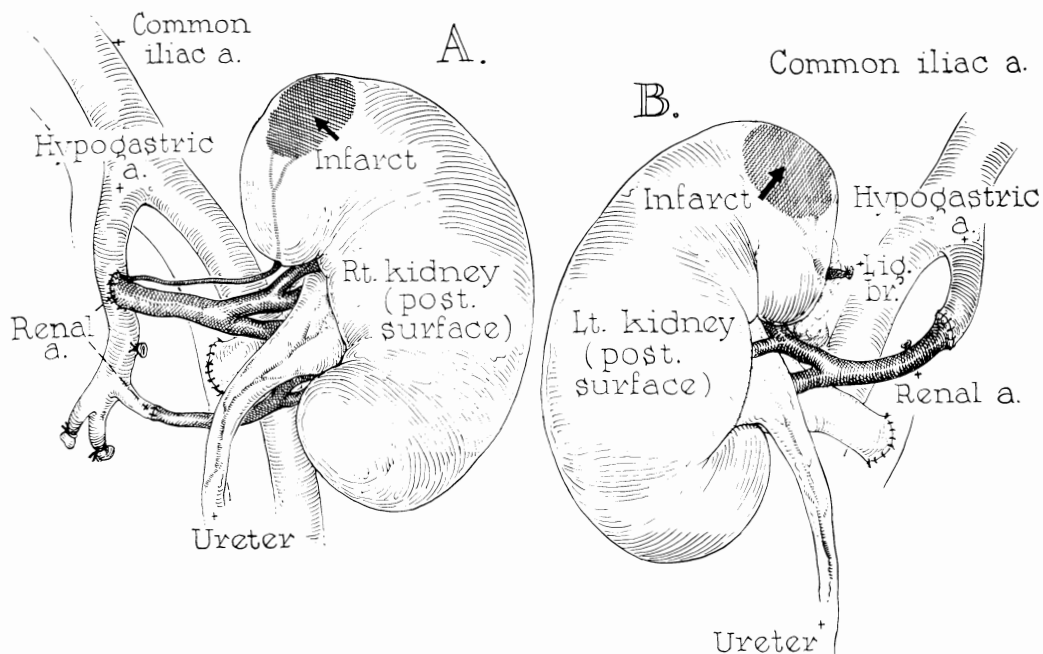


Figure 47. Superior polar infarcts encountered in two cases, due to inadvertent occlusion of small branches of the renal artery.

A—Polar branch included in suture line (LD 8).

B—Polar branch tied during donor operation (LD 19).

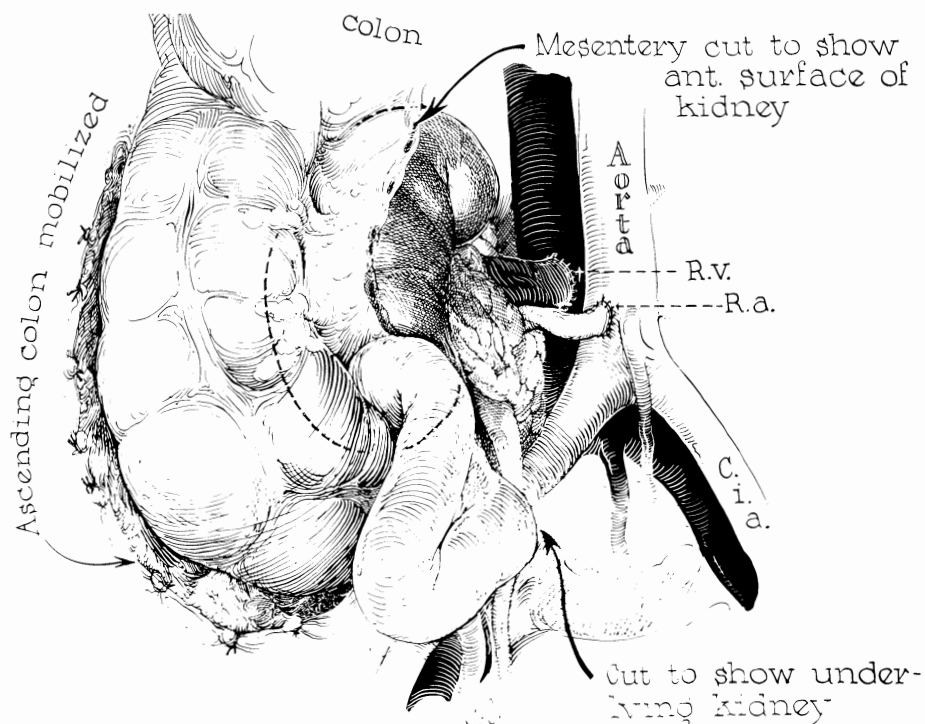


Figure 48. Technique used for transplantation of adult homografts to infants or small children (LD 20, 34, 41, 61). Entire operation is performed transabdominally (see incision in Figure 34D). (By permission of *Surg. Gynec. Obstet.* 119:106, 1964.)

SPECIAL PROBLEMS OF TRANSPLANTATION IN INFANTS AND SMALL CHILDREN

Use of the extraperitoneal operation is inconvenient or impossible in a child if an adult kidney is used, because the large homograft may not fit into the relatively diminutive iliac fossa. In addition, the pelvic vessels may be so small compared to those of the homograft that the unfavorable disparity can create additional hazards.

These problems have been considered in four children whose mothers provided the renal homografts. The patients were eight, six, five, and three years old, and weighed 22.5, 20, 15, and 15 kgm, respectively. A previously undescribed method was employed for transplantation.

A midline abdominal incision is used, extending from the xiphoid to the pubis (Fig. 34D). Splenectomy and left nephrectomy are performed. An incision is then made in the posterior peritoneum to the right of the ascending colon. The ascending colon is reflected to the left as in the preparatory stages of colectomy (Fig. 48), and the right kidney is removed.

The terminal inferior vena cava is freed circumferentially for a distance of 3 or 4 cm. Posteriorly, two or three tributary lumbar veins require ligation and division. The junction of the aorta with the right common iliac artery is similarly denuded. The bifurcation of the aorta occurs at a slightly higher level than that of the inferior vena cava, consequently, the right common iliac artery crosses the lower end of the vena cava en route to its location lateral to the right common iliac vein. Just above this point, an anatomic window is present in which lies the mobilized portion of the vena cava (Fig. 48).

The technique is essentially the same with use of either the left or right donor kidney. After the homograft is brought, a segment of the distal inferior vena cava is isolated between noncrushing clamps, and an end-to-side renal venous-inferior vena caval anastomosis is formed with 5-0 silk. The end of the renal artery is attached with 6-0 silk to the side of the common iliac artery just below or at the origin of the latter vessel from the aorta (Fig. 48). It is not necessary to completely occlude the terminal aorta at any time, since a proximal vascular clamp can be placed in such a way that flow to the left common iliac artery is not interrupted. Distal control is obtained by cross-clamping the common iliac artery just above its division. In three cases, the renal artery was brought in front of the terminal vena cava. In the other case, it was more convenient to bring the renal artery behind. During and immediately after restoration of homograft circulation, particular attention was paid to the recipient's hemodynamic response. No evidence of high-output cardiac overload was encountered in the cases in which the method was used.

The adult organ almost completely fills a child's right paravertebral gutter, extending from the undersurface of the liver to the pelvis. Since revascularization of the homograft is provided from a level which is lower than normal, but which is higher than that in the customary retroperitoneal operation, the anatomic position of the new kidney is mechanically perfect. The ascending

colon is dropped back onto the anterior surface of the transplant, and no other fixation for the new kidney is provided.

The ureter is brought inferiorly, crossing the common iliac artery midway along its course, and is tunneled retroperitoneally to the base of the bladder. It is brought through the vesical wall just superior to the normal entrance of the ureter and implanted, as described on page 95.

After operation, the patient is confined to bed for 48 hours, during which time he is allowed to lie only on his back or right side. Ambulation is then commenced.

Good renal function was obtained in the four cases outlined, and no adverse consequences of any sort have been encountered which could be attributed to the operative procedure per se.

In two of the four cases, donor right kidneys were transferred to the right paravertebral gutter of the recipient. With this arrangement, structures of the renal pedicle occupy a normal anteroposterior relationship in the transplant site, in contrast to the more traditional retroperitoneal technique. However, it was just as easy in the other cases to transplant the donor left kidney to the same location in the recipient's right side, since the technical details were essentially unchanged.

Although the operation has thus far been employed only in the children described, the space it provides for the homograft is such that an adult organ could probably be fitted into a recipient of even smaller size. Because of the small total body dimensions, the length of ureter that is necessary to reach the bladder is no more than usual, even though the kidney is placed at a higher anatomic position. There is little likelihood that relative stenosis of the three anastomoses will result from growth of the recipient patient, since the lumina created are as large as will ever be required.

RECIPIENT RIGHT NEPHRECTOMY THROUGH TRANSPLANT WOUND

Right nephrectomy through the transplant wound is sometimes performed when the transplantation is to the right iliac fossa, but it is feasible only when the recipient's kidney is small. The right nephrectomy is ordinarily performed before the homograft is installed. After the basic exposure for the transplantation has been provided, the posterior peritoneum is mobilized superiorly until the right kidney can be palpated. After placement of deep retractors, two sutures are placed in the inferior tip of Gerota's fascia, and an incision is made in this structure (Fig. 49A,B). A finger can then be inserted through the perirenal fat and the inferior tip of the kidney exposed (Fig. 49B). A large figure-of-eight O silk suture is then placed through the renal parenchyma (Fig. 49C). It is possible, with gentle traction, to pull more of the organ into the operative field. With blunt dissection, in which the perirenal structures are moved aside, more and more of the kidney is mobilized until the hilar structures can be

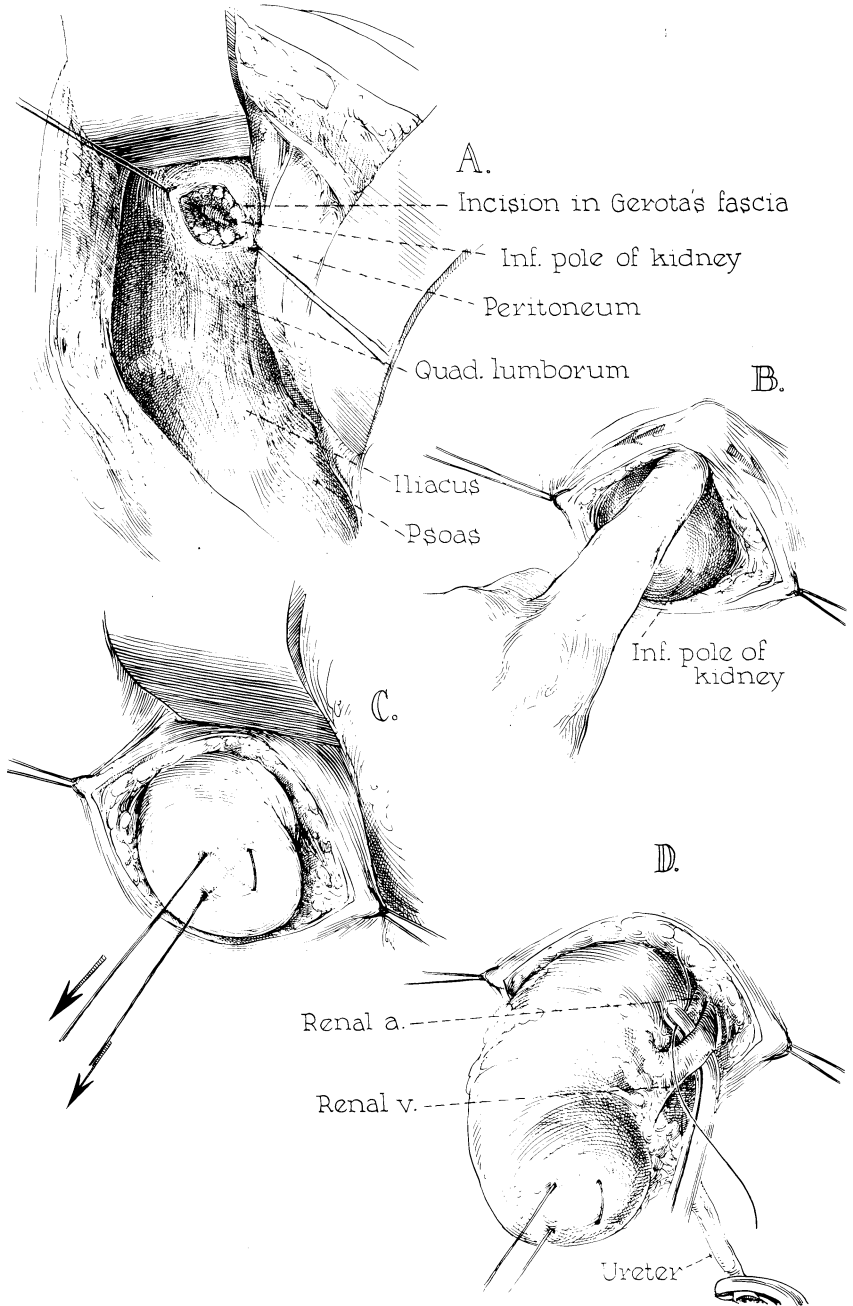


Figure 49. Right nephrectomy through the transplant wound.

- A—The retroperitoneal space is opened until the inferior pole of the right kidney is visualized.
- B—Gerota's fascia is incised and the kidney mobilized with blunt finger dissection.
- C—The kidney is extracted inferiorly with a large figure-of-eight suture.
- D—Ligation of hilar structures.

visualized and ligated. Because of the difficult exposure, it is important that the hilar structures be ligated in continuity, inasmuch as it would be extremely difficult to find and control the renal vessels if a ligature were to slip (Fig. 49D). The performance of nephrectomy with this awkward exposure is sometimes trying, but it usually can be completed within 15 to 30 minutes.

RIGHT NEPHRECTOMY THROUGH A RIGHT SUBCOSTAL INCISION

Right nephrectomy through a right subcostal incision is occasionally performed when the transplantation is to the left iliac fossa. A short right subcostal incision is made, starting at the lateral border of the rectus sheath. The external oblique muscle is cut, and the two subjacent muscle layers are split. The retroperitoneal space is entered, and mobilized. The lateral portion of Gerota's fascia is identified, and incised between two stay sutures. The kidney is shelled out of its bed with blunt dissection, and the hilar structures are individually ligated. The wound is closed without drainage.

Alternatively, the right nephrectomy can be performed transperitoneally through a subcostal or midline incision. The technique is similar to that described in the following section.

SPLENECTOMY AND LEFT NEPHRECTOMY

The abdomen is entered, the splenorenal ligament is incised, and the splenocolic and splenophrenic attachments are freed. The raw surfaces are immediately examined for hemorrhage, and the resultant fine capillary bleeders electrocoagulated. All splenic hilar tissues are ligated and divided in continuity. In obtaining adequate exposure for this purpose, the most important initial step is ligation and division of the upper one or two short gastric vessels. Special attention is paid to prevention of pancreatic injury. The best way to insure this is to separate the splenic pedicle into the two layers from which it was embryologically derived. The anterior layer, containing the short gastric and the gastroepiploic vessels, is defined with a finger placed into the lesser omental sac. After ligation and division of this layer in small bites, the posterior layer, containing the splenic vessels, is similarly treated.

After completion of the splenectomy, the stomach and the splenic flexure and its mesocolon are retracted (Fig. 50), and left nephrectomy is performed. A small incision is made in the posterior peritoneum overlying the kidney. The kidney is then extracted through this rent, and the hilar structures are identified, doubly ligated, and divided (Fig. 50B).

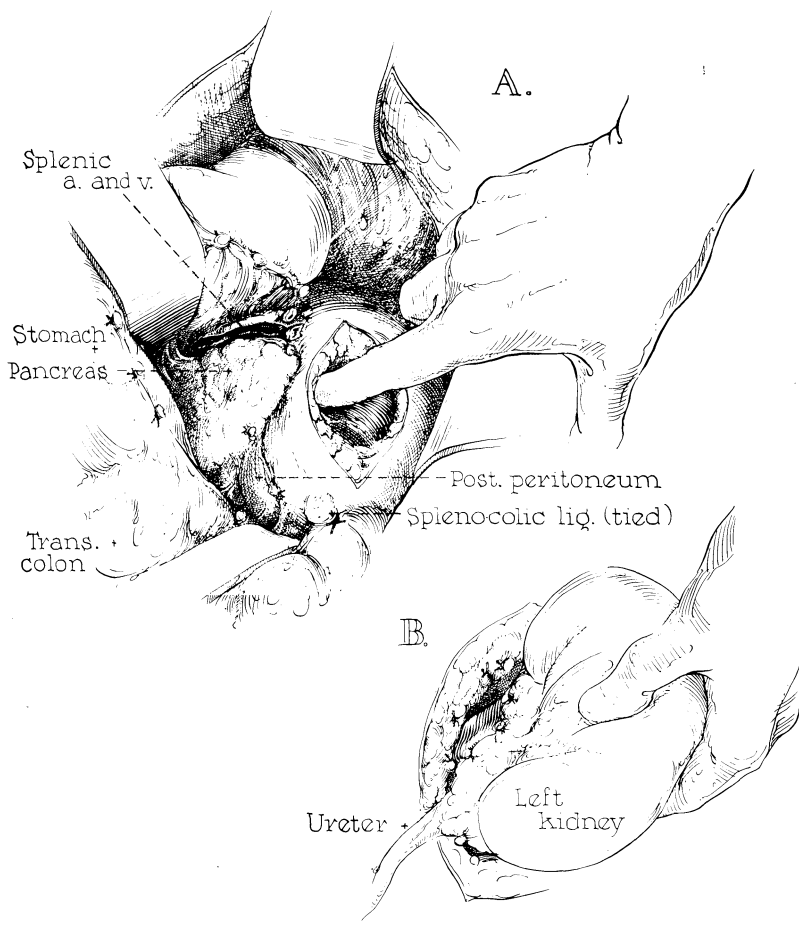


Figure 50. Transabdominal left nephrectomy. Splenectomy has been completed. A—Kidney mobilized; B—Completion of nephrectomy.

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Chapter Twelve

POSTOPERATIVE CARE AND SURGICAL COMPLICATIONS

The unique aspects of postoperative therapy are concerned with prevention of homograft rejection; the measures which are necessary for this purpose are detailed in Chapters 14 and 16. Other more general facets of post-transplantation care are also vitally important.

Virtually all patients considered for homotransplantation are in the terminal phase of their disease. Despite resuscitation, it is often not possible to transform them into good operative risks. Many are profoundly anemic, and restoration of an acceptable hematocrit level by transfusion may be impossible because of the danger of causing acute heart failure. Muscle wasting is invariably present. Almost two-thirds of the patients have nervous system disorders, including neuropathies, coma, convulsions, and psychoses. Many come to surgery with gastrointestinal disorders such as hypomotility syndromes, frank ileus or diarrhea, or with a recent history of gastrointestinal bleeding.

With successful transplantation, many of these events are reversed with such abruptness that the consequent homeostatic alterations may be a potential threat to life. A massive diuresis almost invariably begins on the operating table (Fig. 51). In the first 42 patients who received kidneys from living donors, the mean interval between revascularization and the detection of the first flow of urine was 32 ± 7.8 (SE) minutes, ranging from five to 600 minutes. In these 42 patients, the hourly urine output averaged 444 ± 39 (SE) ml for the first 12 hours (Table 11), being as high as 2,000 ml per hour in one patient who died from an acute electrolyte imbalance 12 hours after operation.

During the diuretic phase, good management of fluid balance is of the utmost importance, and in planning therapy, accurate knowledge of the urinary composition is mandatory (Table 11). The urinary sodium and chloride content is most predictable. The mean concentrations in these 42 patients were 97 ± 3.5 (SE) mEq liter and 70 ± 3.0 (SE) mEq liter, while specific gravity was 1.011 ± 0.0005 (SE). Urine urea concentration was 290 ± 19.4 (SE) mgm

Figure 51. Postoperative management of a 15-year-old girl (LD 17) who received a renal homograft from her mother. During the first 48 hours after operation, the child lost more than 30 pounds of weight. During the acute diuretic phase, intake was provided to replace approximately two-thirds of the fluid loss. The diuresis immediately after surgery was as much as 1,000 cc per hour. Bilateral nephrectomy and splenectomy were carried out at the same time as transplantation.

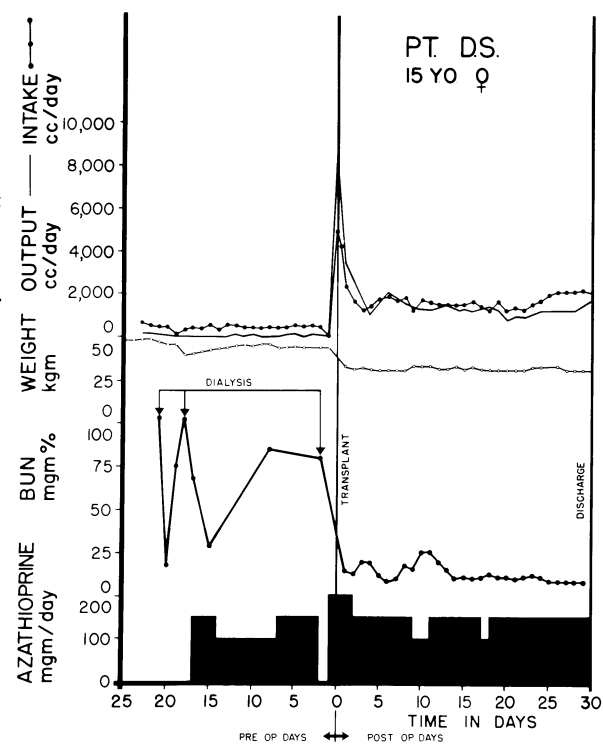


Table 11. Mean Urine Volumes and Composition Observed during Acute Postoperative Diuresis in 44 Homografts Obtained from Living Donors for Treatment of 42 Recipients*

	No.	Mean	SD	SE
Diuresis (ml/hr)	44	444	258	39
Na (mEq/liter)	44	97	23.4	3.5
Cl (mEq/liter)	44	70	19.6	3.0
K (mEq/liter)	40	16.4	8.5	1.3
Creat (mgm%)	19	57.9	29.3	6.8
Urea (mgm%)	39	290	121	19.4
Protein (gm/24hr)	30	4.19	2.62	0.48
Specific gravity	38	1.011	0.0032	0.0005

*In all but three cases, the diseased kidneys of the recipient patient were removed before or at the time of transplantation. All determinations were not available for each patient; hence the difference in numbers.

per cent and creatinine was 57.9 ± 6.8 (SE) mgm per cent. Significant proteinuria was common initially with 24-hour excretion of 4.19 ± 0.48 (SE) gm during the first day, but this finding usually disappeared within 48 hours. Potassium was 16.4 ± 1.3 (SE) mEq/liter (Table 11).

When the general range of electrolyte loss is known, it is possible to use a simple empirical regimen of intravenous replacement. The patients are usually given 5 per cent glucose in 0.45 per cent saline in an amount equal to two-thirds to three-fourths of the urinary output of each preceding hour or the same fractional replacement is carried out with lactated Ringer's solution. Under ordinary circumstances, potassium is not replaced, but if the diuresis is massive, 10 to 20 mEq/liter potassium chloride is added to the fluid. Despite the usual predictability of these electrolyte values, it is necessary in all cases to perform "spot checks" of both serum and urinary electrolytes, at least every four or eight hours, to be certain that the expected composition is present.

The tragic consequence of mismanagement during the diuretic phase is illustrated by the death of a 16-year-old girl (LD 26), who succumbed 12 hours after operation as the result of hyponatremia and hyperkalemia. Diuresis had averaged 1,310 ml per hour, and was as high as 2,000 ml per hour. During this critical period, sodium losses were not fully replaced. She was accidentally given excessive quantities of potassium chloride. Because of the large volumes, relatively small deviations from the proper composition of the intravenous infusions greatly magnified the errors. Cardiac arrest was the cause of death.

The urine volumes described usually begin to diminish after eight to 24 hours (Fig. 51), but remain so high for the ensuing day or so that it is advisable to continue intravenous therapy, even though the patient resumes alimentation. The nasogastric tube is ordinarily removed on the morning following surgery, and a liquid diet instituted. On the following day a bland diet can be given. After two or three days, fluid intake becomes greater than the urinary output (Fig. 51).

Weight loss during the first few days after operation has ranged from 5 to 35 pounds. The change in mental responsiveness is frequently astonishing; a state of hopelessness and mental apathy changes to one of euphoria. Ambulation is encouraged, beginning on the second postoperative day. A high calorie diet is ordered, and no fluid or salt restriction is imposed until the onset of a rejection crisis as described in Chapters 15 and 16. If pre-existing hypertension persists during the phase of recovery, as occurs in approximately one-third of the cases, it is managed with antihypertensive drugs.

During the entire hospitalization, the patient is kept in as sterile an environment as can be attained with reasonable precautions. One to four postoperative patients are quartered in a room. Nurses and physicians don masks, gowns, and gloves before entering for the administration of treatment or for examination. The care with which these procedures are followed is comparable to that employed in the standard treatment of burns. It is probable, as

will be discussed in Chapter 21, that the chief accomplishment of these precautions is protection of the patient from the indigenous strains of bacteria which are commonly present in most hospitals.

After three or four days, or as soon as the patient is physically able, he is encouraged to leave the sickroom and spend the greater part of the day pursuing whatever activities he prefers outside the hospital. A member of the family provides the necessary care during such absences. By this time, no dressings or other manipulations of the wounds are required, inasmuch as drains are not inserted at the time of surgery. This outpatient activity is continued, in most cases, even during treatment of the almost inevitable rejection crisis. With the advent of rejection, adjustments are made in the drug and dietary regimens as described in Chapters 14, 15, and 16. Antibiotic therapy during both early and late phases of convalescence is treated in detail in Chapter 21.

SURGICAL COMPLICATIONS

The events described pertain to the majority of patients. The appearance of any deviation from this sequence of recovery is a serious omen. The greatest risks are relatively specific consequences of the use of immunosuppressive agents, as described in subsequent chapters; most deaths resulted from drug toxicity and generalized sepsis which were induced during efforts to reverse unusually vigorous rejection crises. Those deaths which were the direct result of drug toxicity are discussed separately in Chapter 19.

In addition, certain complications have been observed frequently which, while not unique to renal homotransplantation, appeared to occur far more often after this than after other major surgical procedures. The operation itself and the therapy necessary to achieve chronic function of the homograft impose an increased danger in several pathophysiologic areas, as will be discussed on the basis of analysis of the first 42 cases in which homografts were obtained from living donors.

The cyclic course which follows renal homotransplantation is fully described in Chapters 15 and 16. Briefly, a technically successful operation is followed by a temporary period of good renal function. At a varying interval postoperatively, ranging from one to 42 days, virtually all patients pass through a crisis, during which homograft rejection is threatened. The acute illness which occurs at this time with fever and with evidence of acute functional deterioration of the homograft can usually be reversed with the addition of actinomycin C and massive doses of steroids to the azathioprine therapy (see Chapter 14). Many of the complications which have had their inception at this critical time are probably due, at least partially, to the presence in the retroperitoneal space of foreign tissue which is under immunologic attack, as well as to the pharmacologic agents which must be used in high doses at this time.

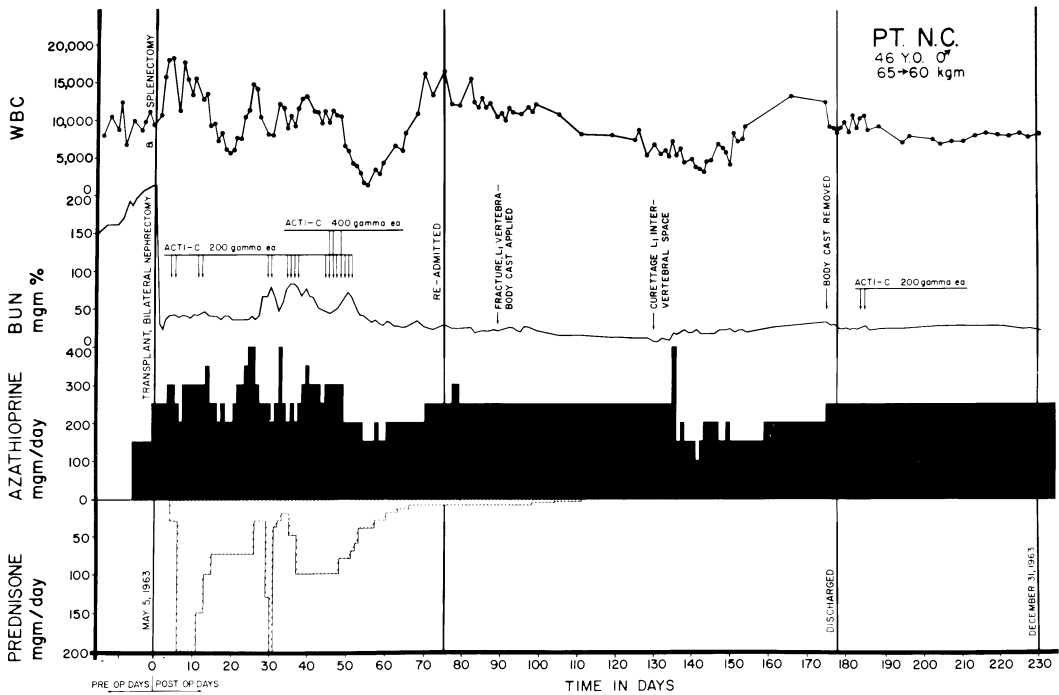


Figure 52. Course of patient (LD 10) who had multiple postoperative complications. His blood type was O-. The homograft was donated by a convict of O+ blood type. Massive gastrointestinal hemorrhages occurred on the twenty-eighth and forty-ninth postoperative days. On the first of these occasions, eight transfusions were required in a 12-hour period. The patient's course was further complicated by a myocardial infarction at this time. The rapid changes in steroid therapy were due to the complexities of management during the bleeding episodes. Three months after operation, aseptic necrosis of the first lumbar vertebra was diagnosed and later treated with curettage and spinal fusion. The patient died 295 days after operation of uncontrolled late rejection. Acti C—Intravenous actinomycin C.

Gastrointestinal Hemorrhage

A high incidence of gastrointestinal bleeding occurred in the early postoperative period in close temporal relation to the rejection crisis. During maximum steroid therapy, 35 of the first 42 patients treated with homografts from living donors developed guaiac-positive stools. In seven cases the hemorrhage was of sufficient magnitude to require transfusion (Table 12). The most massive bleeding occurred in a 46-year-old man who required 8 units of blood in one 12-hour period (Fig. 52). Unfortunately, he sustained a coronary occlusion during this time, but recovered. He died of an uncontrolled late rejection 295 days after operation (see Chapter 20). Another patient (LD 9) died six months and 23 days after transplantation (see Figure 70, Chapter 15): 10 days before death, he underwent suture ligation of a bleeding duodenal ulcer, combined with pyloroplasty and vagotomy. At autopsy, multiple fungal brain abscesses were also found.

Table 12. Noninfectious Complications in First 42 Patients Treated with Homografts from Living Donors (LD Series)

Urinary fistula	2
Hydronephrosis	2
Testicular necrosis	1
Bladder hemorrhage	4
Renal hemorrhage (on heparin treatment)	2
Paralytic ileus	6
Intestinal obstruction	2
Operative cardiac arrest	1
Thrombophlebitis	9
with pulmonary emboli	6 (5 proved)
without pulmonary emboli	3
Diabetes mellitus	13
Pancreatitis	5
Hepatitis	2
Gastrointestinal hemorrhage	7
Coronary occlusion	1
Aseptic bone necrosis	1
Neurologic complications	
Neuropathy, myopathy, or both	*18
Coma	3
Convulsions	4
Stroke	1
Brain abscess	3
Psychosis	4
Arthralgia	5

*The complication was present before operation in all 18 patients.

Other Complications of Steroid Therapy

Other complications were directly or indirectly attributable to the temporary high-dosage steroid therapy. All patients treated with prednisone developed some evidence of steroid-induced diabetes mellitus. The tendency to hyperglycemia or glycosuria was progressive, usually requiring several weeks to develop. In the most severe examples, polydipsia, polyuria, weight loss, acidosis, and ketosis were present. In the majority of cases, therapy with insulin was not necessary, and the alterations in carbohydrate metabolism were quickly reversed as the doses of prednisone were subsequently reduced. In 13 of the first 42 cases, however, the diabetes was severe and required active management (Table 12). As much as 120 units daily of NPH insulin was administered, although the usual maintenance doses were 30 to 50 units per day. In all but two patients the need for insulin was eliminated when the dose of prednisone was reduced to less than 30 mgm per day. In these two exceptional patients, who were possible latent diabetics, there has been a continuing need for insulin despite reduction of prednisone to less than 10 mgm per day.

Another complication which may be related to steroid therapy is pancreatitis. Biochemical evidence for this diagnosis was present in five patients, and in three, confirmation was obtained at autopsy. In one case pancreatitis, which has been noted both in animals and in man after prolonged steroid administration, was the only postmortem finding that might have accounted for death (Fig. 53). The patients, who died 83 days after operation, had passed through two rejection episodes and had good renal function until she died (Fig. 53).

As would be expected, physical appearance has been affected by the prednisone therapy. Virtually all patients developed moon facies and abnormal fat distribution, which were temporally related to the period of such therapy. One patient developed aseptic bone necrosis of the first lumbar vertebra, apparently as a result of steroid-induced osteoporosis, as has been described by Boksenbaum and Mendelson (Fig. 54), and two others developed spontaneous femoral fractures (see Chapter 20).

Thromboembolic Complications

Nine of the first 42 patients had clinical evidence of thrombophlebitis during convalescence, and in five of these the presence of one or more pulmonary emboli was proved either at the time of pulmonary embolectomy or at autopsy (Figs. 55, 56). Pulmonary embolization was not the direct cause of death in any of these five patients. The diagnosis was made clinically in only two instances. In two of the nine patients, heparin therapy was begun, but had to be discontinued because of serious hematuria (Table 12).

The high incidence of thrombotic complications is of great interest, and may have been caused by a combination of factors. Virtually all the recipients were in the terminal phase of uremia when homotransplantation was first

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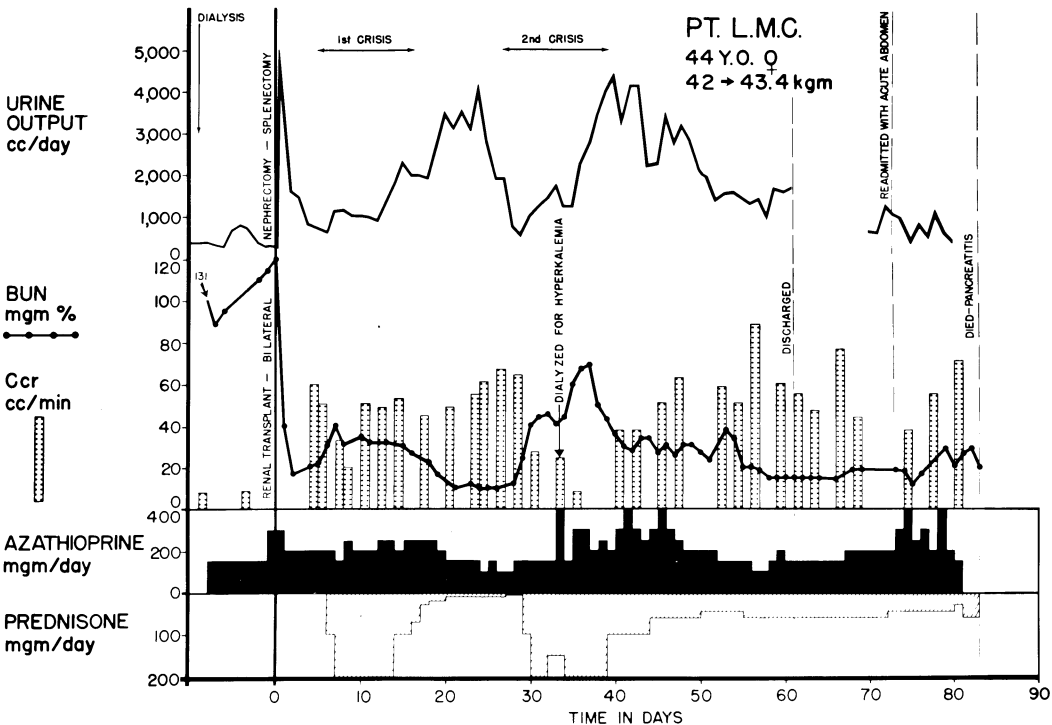


Figure 53. Course of a 44-year-old woman (LD 16) who died of acute and chronic pancreatitis, 83 days after homotransplantation. Prednisone schedule was unusually protracted because of two rejection episodes. The pancreatitis was thought to have been steroid induced. (By permission of Arch. Surg. 89:87, 1964.)

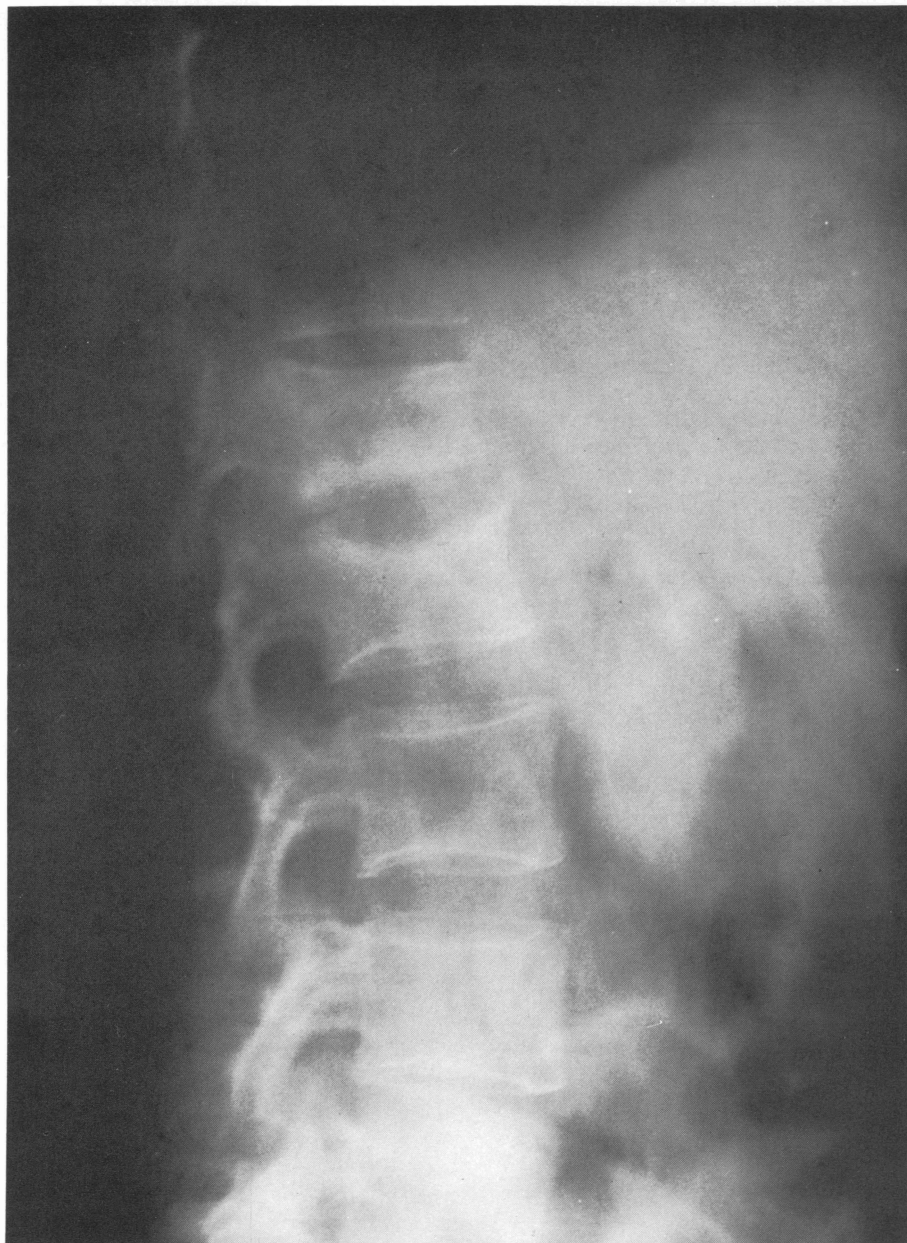


Figure 54. Lateral x-ray showing fracture of L1-L2 lumbar vertebrae (LD 10). Case is the same as in Figure 52. Spinal fusion was performed. (By permission of JAMA 189:397, 1964.)

Figure 55. Distribution of pulmonary emboli in case (LD 4) treated as shown with cardiopulmonary bypass and pulmonary embolectomy. The principal clots were in the right pulmonary artery. Forty-eight hours later a vena caval plication was performed in the indicated location. The patient died of sepsis two and a half months after the embolectomy. At autopsy, the distal vena cava, ileofemoral systems, and transplanted renal vein were completely filled with clot. Despite this, renal function remained good (see Figure 15, Chapter 6), adequate capsular venous collaterals having evidently developed. (By permission of *Surgery* 55: 505, 1964.)

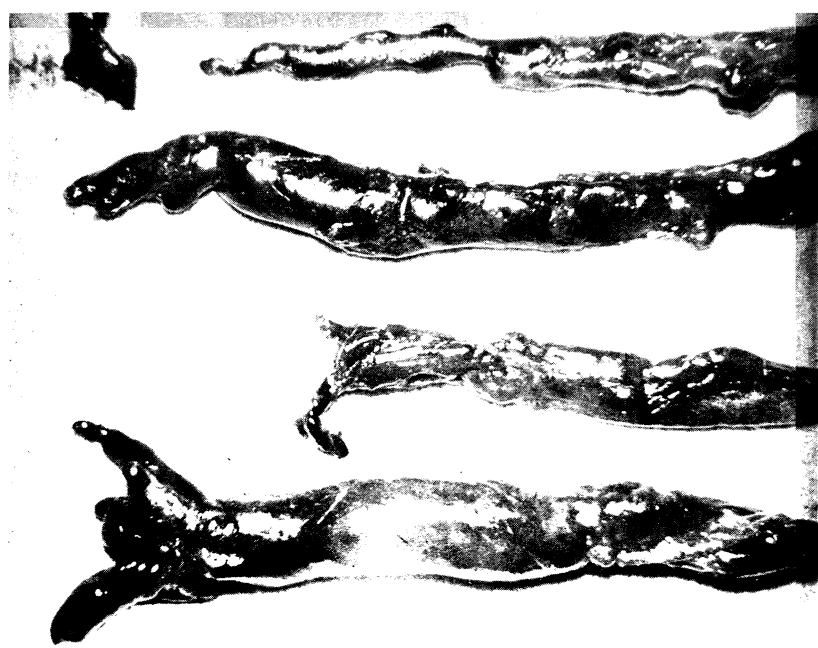
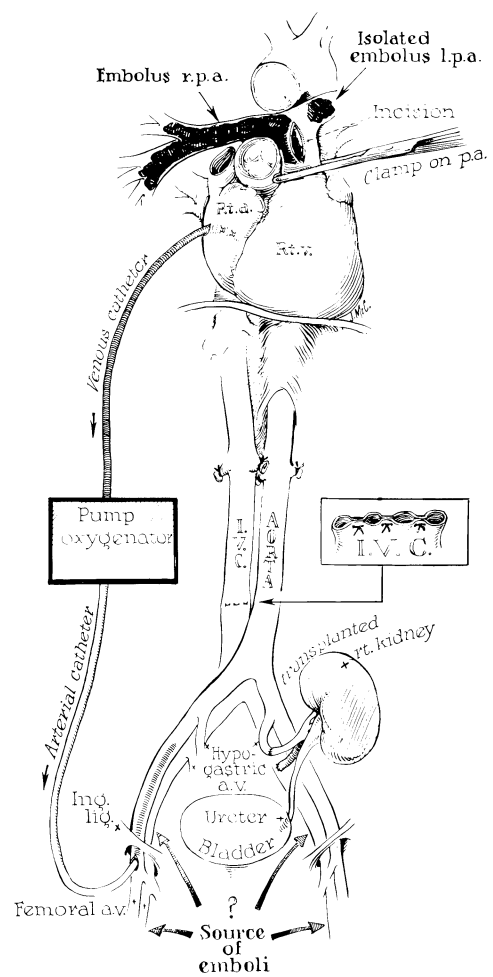


Figure 56. Pulmonary emboli removed during pulmonary embolectomy (LD 4). (By permission of *Surgery* 55:505, 1964.)

considered. Their degree of muscle wasting, debility, and inactivity was usually extreme. During the early postoperative period, accentuation of weight loss was frequently seen, some patients losing as much as 50 or 60 pounds. Such patients would be expected to have a heightened incidence of thrombophlebitis after any surgical operation.

With renal transplantation, specific dangers are added. There is the necessity for direct manipulation and anastomosis of the external iliac vein. Postoperatively, there are major changes in body fluid composition during the diuretic phase, with a sudden relative dehydration. Finally, the adjuvant procedure, splenectomy, is known to cause thrombocytosis, and may accentuate a clotting tendency.

Vascular Anastomoses

In the care of the first 42 patients treated with living donors, 46 homografts were employed. There were 97 vascular anastomoses, five homografts requiring reconstruction of a double arterial renal blood supply. No known anastomotic failures occurred. Although closure of an arterial anastomosis would certainly become clinically evident, there is no assurance that a venous occlusion would be detectable without examination of the specimen, as illustrated by the course of the patient described who had a pulmonary embolectomy (Figs. 55 and 56).

This 35-year-old man, who had a massive pulmonary embolus five weeks after homotransplantation, was treated by cardiopulmonary bypass and pulmonary embolectomy. Two days later a Spencer vena caval plication was performed. The patient recovered from these operations, and had good renal function until his death from sepsis more than two months later. At autopsy, it was found that the entire ileocaval system was completely thrombosed, including that segment into which the renal vein was anastomosed. Furthermore, the renal vein itself was occluded out to its smaller branches. There were extensive venous collaterals in the surrounding tissues which had allowed adequate drainage and continuation of good function (see Figure 15, Chapter 6).

Ureteral Anastomoses

Ureteral implantation was also relatively free of early technical complications (Table 12). In one case, a ureteral fistula at the ureteroneocystostomy site developed six days after operation. Reimplantation was carried out immediately with an apparently satisfactory early result. Nevertheless, the patient (LD 7) ultimately died of a perinephric abscess surrounding the homograft, as will be more completely described in Chapter 20.

In a second case, a small area of necrosis developed on the anterior surface of the pelvis of a renal homograft which had functioned satisfactorily for

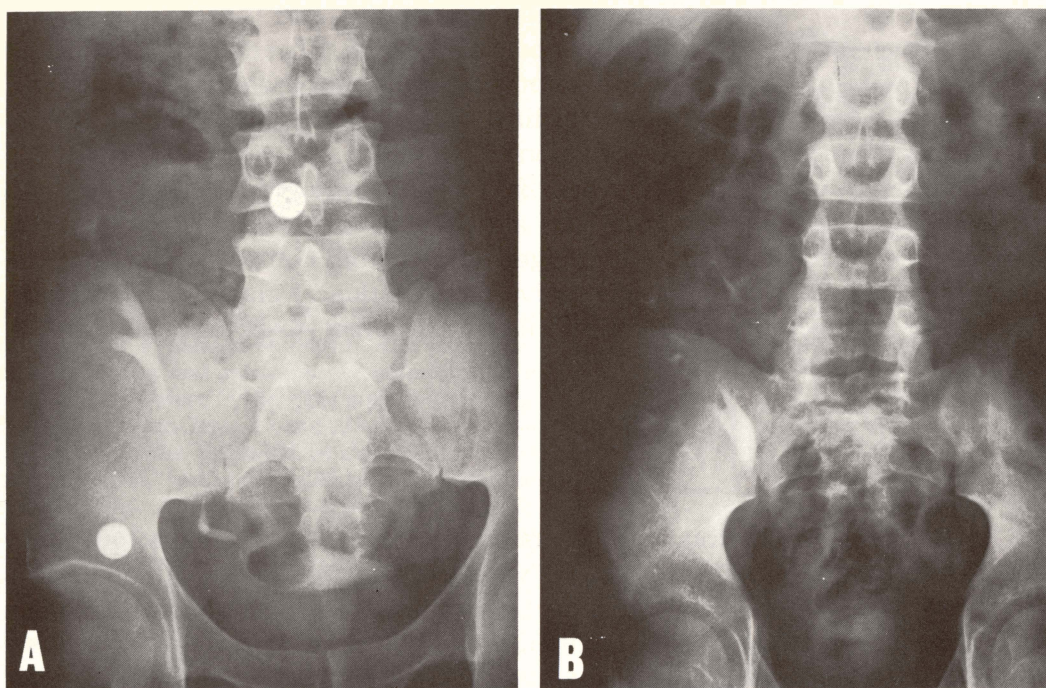


Figure 57. A—Four-minute intravenous pyelogram (LD 2) 24 days after renal transplantation to the right iliac fossa. Prompt and concentrated dye excretion is observed. Within 24 hours after this roentgenogram was taken, the patient experienced a severe rejection crisis and became essentially anuric. Recovery was ultimately complete. Note area of ureteral tunnel. B—Three-minute intravenous pyelogram (LD 1) nine and a half weeks after transplantation and four weeks after complete recovery from a rejection crisis. Note prompt and concentrated dye excretion in the kidney which is located in the right iliac fossa.

almost three weeks after operation. The patient (LD 35) had entered a rejection crisis a few days previously. At re-operation the interior of the ureteropelvic junction had patchy necrosis, and the extrarenal collecting system was filled with debris and tissue which appeared to have blocked the distal ureter. It was not possible to be certain whether this complication represented thrombosis of the blood supply to the ureter and pelvis, was secondary to a technical error resulting in obstruction of the ureter, or was the consequence of the tissue injury incurred during the rejection crisis. The homograft was removed and a second transplantation carried out 19 days later. The patient died of sepsis 10 days after the second homotransplantation.

Intravenous pyelograms were obtained in all cases either before rejection or after its reversal. In the entire experience at the University of Colorado, hydronephrosis was encountered in four patients to be considered separately in Chapters 19 and 20. In some cases, the area of the submucosal tunnel could be seen (Fig. 57A). In others, drainage was so rapid that delineation of the fine details of pelvic or ureteral configuration was not possible (Fig. 57B).

Genitourinary Hemorrhage

Serious hemorrhage was encountered from the lower urinary tract in four of the first 42 patients (Table 12). In each instance the hemorrhage began a few hours after operation following an early period of relatively clear urinary excretion. Once initiated, the tempo of the bleeding increased progressively; transfusions were required in two cases. The urethral catheter was removed, and the hemorrhage ceased within minutes. In some subsequent cases, it was noted at the time of cystotomy that in the area of the trigone a beefy area of hemorrhagic cystitis developed near the point of contact with the Foley catheter. This finding, and the observation that removal of the catheter resulted in cessation of bleeding, indicate that such a focus of hemorrhagic cystitis was responsible for the disquieting complication.

As mentioned, genitourinary hemorrhage was induced in two additional patients during treatment with heparin, but it subsided at once with discontinuation of anticoagulant therapy (Table 12).

Ileus

In 36 of the first 42 patients treated with homografts from living donors, it was possible to remove the gastric tube and resume alimentation within 24 hours after operation. In the other six, gastric suction was required for two to eight days (Table 12). During this time, therapy with azathioprine was provided in an intravenous solution (see Chapter 14), and steroid therapy, if required, was given with intramuscular prednisolone. Two patients had surgery for intestinal obstruction 29 and 32 days after transplantation, both requiring adhesiolysis. One (LD 34) had an uneventful recovery. The other (LD 4) died several months later of a left paravertebral gutter abscess which probably formed following laparotomy.

Neurologic Complications

The most frequent complication involved the central or peripheral nervous system, and usually began in the preoperative period (Table 12). Advanced neuropathy or myopathy, both of which were much more common in older patients, indicated a poor prognosis. If such changes were severe enough to prevent effective ambulation during the postoperative period, prolonged survival was not attained in a single case. Such bedridden patients frequently developed decubitus ulcers, could not eat well, and were unusually susceptible to drug toxicity.

The relative irreversibility of uremic neuropathy is of interest. Two patients with advanced foot drop have now been followed for 12 and 18 months, but have shown almost no improvement. Correction of the original metabolic abnormality therefore cannot restore to normalcy the patient who is crippled with uremic neuropathy.

Acute neuropsychiatric disorders such as convulsions, behavioral disorders, or coma occurred in both the pre- and postoperative periods, but these did not have a grave prognostic significance except when the underlying cause was brain abscess (three cases) or a massive stroke (one case) (Table 12). In two patients with postoperative convulsions, the seizures occurred during the acute post-transplant diuresis, and were probably due to rapid shifts in body water composition.

Infectious Complications

Failure to control infectious complications has been the leading factor in mortality; this special problem is analyzed in detail in Chapters 19 and 21. It is encouraging to note here that an uncontrollable infection in either the splenectomy-nephrectomy or transplant wound occurred in only one of the first 42 cases and that superficial infections could be effectively treated (Table 13).

Table 13. Clinical Wound Infections in 42 Patients (LD Series)

	No.	Controlled	Uncontrolled
Transplant wound			
Superficial	5	5	0
Deep	1	0	1
Thymectomy wound			
Superficial	1	1	0
Deep	1	0	1
Splenectomy-nephrectomy			
Superficial	3	3	0
Deep	0	0	0
Other wounds (deep)	1	0	1
Cardiac arrest, thoracotomy wound			
Previous flank nephrectomy	1	0	1
Intestinal obstruction	1	0	1
Total	14	9	5

Miscellaneous Complications

One patient had a cardiac arrest during transplantation, and died 10 days later (see Figure 61, Chapter 14). Another developed testicular necrosis postoperatively necessitating orchiectomy (see Figure 67, Chapter 15). There were two nonfatal cases of hepatitis. Five patients developed arthralgia postoperatively which seemed to be related to the use of antihypertensive drugs.

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Chapter Thirteen

ROLE OF EXCISION OF LYMPHOID MASSES IN ATTENUATING THE REJECTION PROCESS

Excision of two lymphoid masses, the thymus and the spleen, has been carried out at the University of Colorado. At first, both thymectomy and splenectomy were performed, but the former procedure was discontinued after it had been done in nine cases because of the excessive number of surgical complications encountered and because there was no early evidence that the operation was of significant value in mitigating the rejection process. Four patients who had their thymuses removed are still alive, with normal renal function, after 14 to 20 months (LD 1, 2, 3, and 6), and comparison of the long-term fate of this small group with those later patients who did not receive thymectomy may eventually be of value in defining subtle effects of the procedure which are not obvious with short-term follow-up. In the subsequent cases, the use of splenectomy alone has been continued, to some extent because it has appeared to be a safe and easily executed procedure.

Justification of the excision of these or other lymphoid masses depends to a large extent upon projecting the results of research in lower animal forms to the homotransplantation problem in man. Frequently, the findings which might be interpreted as favoring the use of the adjuvant operations are inferential. Lest the following remarks appear to indicate overenthusiasm, it should be stated at once that the value of thymectomy or splenectomy in promoting homograft acceptance is entirely unproven. In our studies, and in those done in other laboratories, attempts to demonstrate increased homograft survival in dogs that received one or both of the ancillary operations have been unsuccessful.

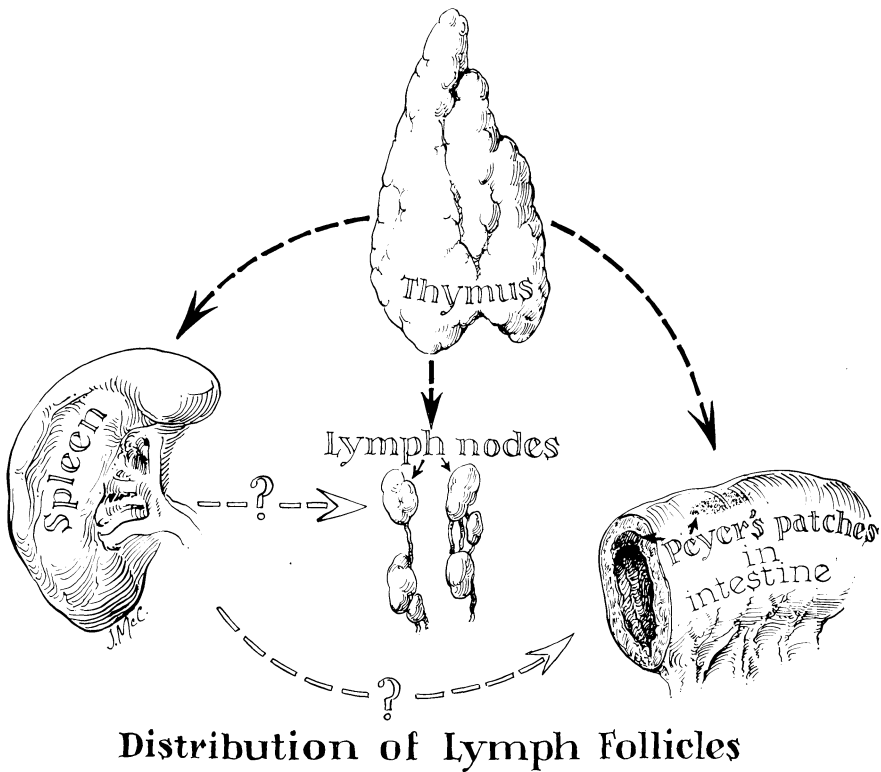
ful. The following discussion should, therefore, be construed as an argument for clinical experiment, not as a recommendation for the use of either thymectomy or splenectomy, or both.

The most immediate and obvious consequence of both operations—especially splenectomy—is reduction of the total mass of lymphoid tissue. There is abundant evidence that lymphoid tissue plays an important role in rejection, as described in Chapter 1. Nevertheless, the degree—if any—to which reduction of the total lymphoid mass influences the rejection process is unknown. If the effect is related to the amount of immunologically active tissue removed, splenectomy might be expected to be of some value. The thymus, in contrast, has undergone atrophy in all but young patients. All surgical specimens in our nine patients prepared with thymectomy weighed less than 20 grams. Histologically, the glands consisted chiefly of fibrous tissue infiltrated with fat; morphologically, it is almost inconceivable that such an inconsequential remnant could contribute vigorously to rejection, at least in a direct way.

Since removal of lymphoid tissue may temper the force of the host's immediate immunologic attack, it could also be reasoned that removal of the thymus, the spleen, or both could alter the organization of the host response. For example, the role of the thymus in promoting immunologic reactivity in the newborn state appears to be unquestioned, as a result of Miller's investigations. In rodents, thymectomy performed shortly after birth results in immunologic crippling and subsequent inability to reject homografts in a normal manner. Removal of the thymus at this stage of development results in generalized lymphoid hypoplasia, due either to the absence of a thymic organizer substance which promotes lymphoid development, or to the absence of thymocytes which migrate to distant areas of the lymphoid system and populate them. In adult life, thymectomy has no demonstrable effect on antibody response or homograft survival. However, it has been shown in adult mice that thymectomy combined with total body irradiation can result in homograft tolerance of a high degree. This finding, reported by Miller, suggests that the thymus gland may resume its preceptor function in adult life under circumstances in which there is temporary suppression of the lymphopoietic system.

It has already been stated that the morphologic features of thymic tissue make it difficult to believe that this organ could resume an important role in determining the immunologic defenses of adults; nevertheless, judgment must be reserved concerning the worth of thymectomy in human homotransplantation. The chronic courses of the four patients who had this adjuvant operation have been without any evidence of delayed rejection, in contrast to those of later recipients who received kidneys from equally favorable familial donors.

The spleen may also play a significant role in the development of total body response. It has been known for decades that the antibody response to many antigens is obtunded after splenectomy. Wissler and his colleagues have recently reported histologic evidence that the spleen is the most active organ



Distribution of Lymph Follicles

Figure 58. Theoretical considerations in the use of adjuvant thymectomy and splenectomy for the conditioning of recipient patients. The concept of organizer function of the thymus in establishing fetal and neonatal immunologic reactivity is based largely on the work of Miller, and may have no relevance in the adult human. The role of the spleen in controlling response to antigenic stimuli in adult life is suggested by the work of Wissler and his colleagues. The contribution of these ancillary procedures to homograft survival is highly speculative at present. (By permission of *Surgery* 56:296, 1964.)

of the adult lymphoid system in responding to intravenous antigens. On the basis of findings in rats immunized to various antigens, Wissler has suggested that migrant cells from the sensitized spleen can populate other lymphoid tissue and establish specific clones in their new location. The moderator role of the spleen in determining antibody response in the adult rat could thus be envisioned as roughly comparable to that described at an earlier stage for the thymus (Fig. 58).

With splenectomy, a restraining influence upon the peripheral white blood count is removed. In the normal subject, relatively long-lasting thrombocytosis and leukocytosis result from this operation. In the patient who is receiving immunosuppressive therapy following transplantation, the same effect is noted as will be described in Chapter 14. As a consequence, it might be expected that larger doses of azathioprine could be administered without causing leukopenia, at least early in the postoperative course. Whether or not splenectomy

allows larger drug doses to be used is not known, and will be determined only with controlled studies.

Recently, a somewhat different justification for splenectomy at the time of transplantation has been defined as the result of reports from Hume and Hamburger and their colleagues. Both authors reported late sequelae of hypersplenism necessitating delayed splenectomy, sometimes under unfavorable circumstances, in several otherwise successfully treated cases. This eventuality is avoided if the spleen is removed initially.

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Chapter Fourteen

ANTI-REJECTION THERAPY

While the effectiveness of splenectomy or thymectomy in mitigating the vigor or persistence of the rejection process has not been proved, as was noted in the preceding chapter, that of certain pharmacologic agents has been clearly demonstrated. These drugs are crucial in preventing destruction of the foreign tissue. That this could be achieved by means which did not destroy the host seemed an impossibility prior to the experimental reports of Schwartz and Dameshek and of Calne and Murray on the immunosuppressive qualities of some of the thiopurine compounds, notably 6-mercaptopurine and azathioprine.

AZATHIOPRINE

The most important single drug in the management of rejection is azathioprine (Burroughs Wellcome 57-322, "Imuran"). This compound is an imidazole derivative of 6-mercaptopurine. It was developed by Doctor George H. Hitchings and his colleagues at Burroughs Wellcome and Company, Inc., Tuckahoe, New York, and found by them to inhibit hemagglutinin formation in mice challenged with heterologous red blood cells. The efficacy of azathioprine in modifying or preventing homograft rejection in experimental animals was later demonstrated by Calne and Murray. The precise mode of action of this drug is not known, although the main effect is thought to be by interference with nucleic acid synthesis.

In our institution, azathioprine was used until December 10, 1963, in a way fundamentally different from that of the other pharmacologic agents to be described subsequently, in that it was administered continuously rather than in response to specific acute indications of rejection (Fig. 59). It was used before, during, and after rejection and was, therefore, prophylactic during the first and last of these periods. Azathioprine was the basic agent in immunosuppressive therapy to which other drugs were temporarily added. Use of it has not been discontinued in any of the surviving patients, even those followed postoperatively for more than a year.

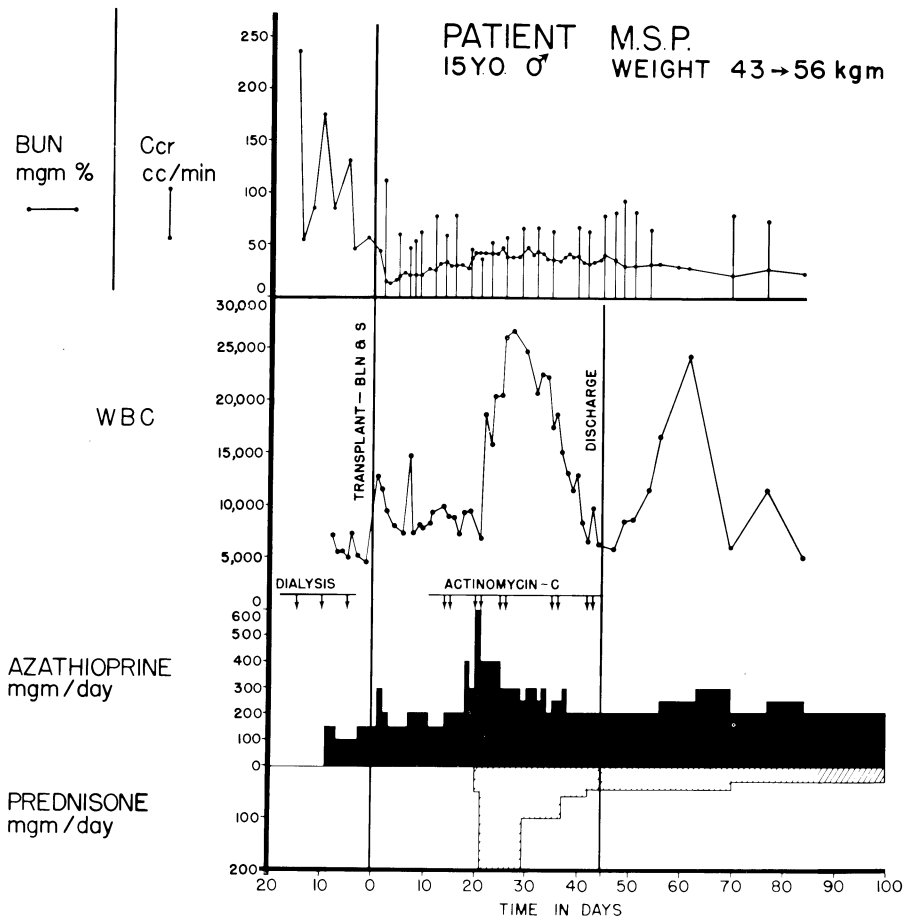


Figure 59. Typical use of immunosuppressive agents in first 45 patients treated with homografts obtained from living donors. The azathioprine was started several days before operation (usually 10) and continued throughout the entire course. Massive doses of prednisone were added during the rejection crisis. The steroids were then withdrawn as quickly as possible thereafter. Actinomycin C was employed only during the rejection crisis. Ultimately, if possible, the patient was returned to therapy with a single agent, azathioprine. This 15-year-old boy (LD 13) received a transplant on July 3, 1963. By January 1, 1964, prednisone had been discontinued, and his maintenance therapy was solely with azathioprine. Resumption of maintenance steroid therapy later became necessary.

For reasons which will be discussed in Chapters 18 and 19, this one-sided approach to drug therapy was subsequently modified. All patients treated since December 10, 1963, have also received prophylactic prednisone which was started before operation and continued into the postoperative period. Nevertheless, the following discussion is based primarily upon information obtained during the earlier period, when the practice was to add one drug at a time, since conclusions about pharmacologic action can be interpreted more clearly when the agents are added sequentially.

Pretreatment with azathioprine has been employed in most patients both before and after December 10, 1963, beginning eight to 10 days before operation, in a dose of 1.5 to 4 mgm/kgm per day (Figs. 59, 60). On the last day

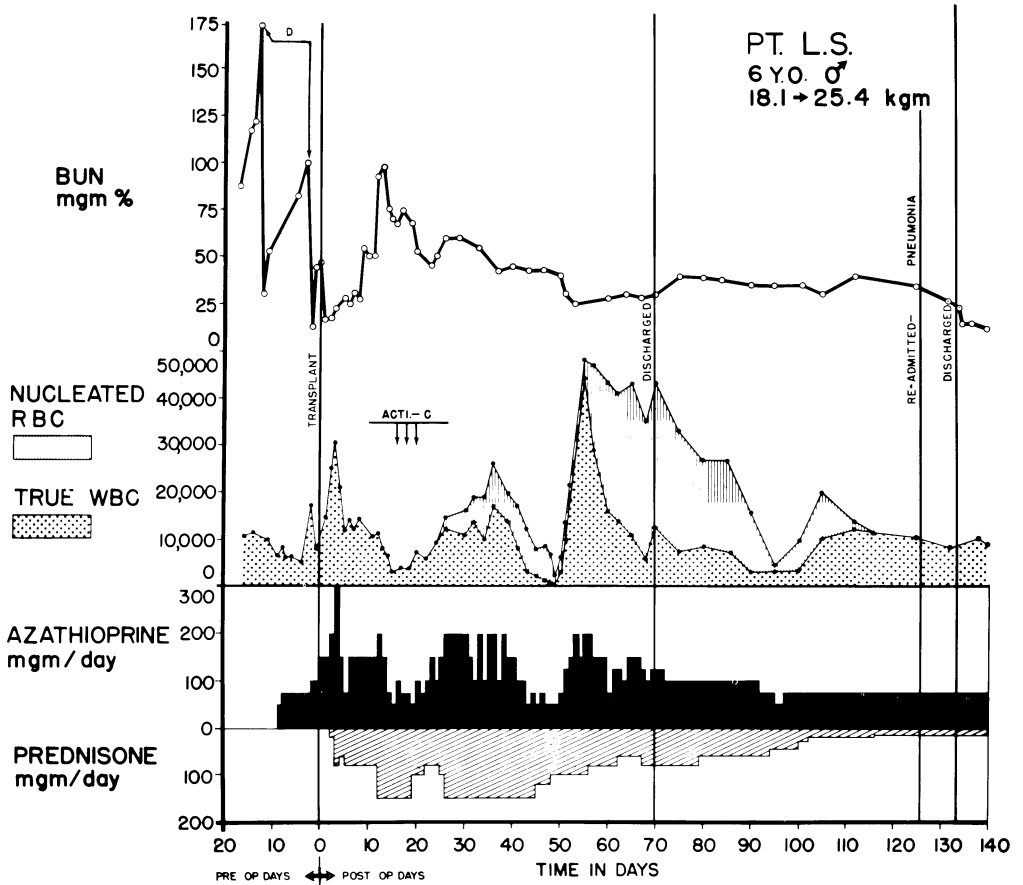


Figure 60. Course of a six-year-old boy (LD 20) of O+ blood type who received a kidney from his A-mother. Bilateral nephrectomy, splenectomy, and transplantation were done at a one-stage operation. Note early rejection crisis which started 30 hours after operation. The total count of nucleated cells in the peripheral blood is seen, as well as the true white blood count which is indicated by the lower stippled area. Each arrow indicates 200 gamma intravenous actinomycin C. The shower of normoblasts persisted for three months and was associated with fever. (By permission of *Surgery* 56:296, 1964.)

or two preoperatively, the dose is temporarily increased to 5 to 8 mgm/kgm. During this interval, resuscitative measures, including dialysis, are continued as described in Chapter 4. Although this use of the drug adds the additional hazard of bacterial complications during the preoperative period, only two patients have developed potentially lethal infections. In both cases, staphylococcal septicemia resulted from infected dialysis cut-down sites. With discontinuance of azathioprine therapy, and institution of appropriate antibiotic therapy, the infections were controlled and homotransplantation was done in the first case 10 days later, and in the second, 14 days later.

The rationale for the practice of pretreatment with azathioprine is based on the 60 dog experiments summarized in Table 14, in which homotransplantation and bilateral nephrectomy were performed. Untreated animals lived for

Table 14. Influence of Pretreatment with Azathioprine upon Post-transplant Longevity in Dogs

	No.	Mean Survival Days	SD	SE
Controls	21	10.8	2.75	.06
Acute azathioprine	21	36	13.7	3.0
Azathioprine pretreatment	18	69.1	68.1	16.1

Comparison of groups 2 and 3 ($p < 0.001$).

10.8 \pm 0.06 (SE) days. Animals which received treatment with azathioprine, started on the day of operation, lived 36 \pm 3.0 (SE) days. Animals which received preoperative treatment with azathioprine for seven to 30 days, as well as postoperative therapy, lived for 69 \pm 16.1 (SE) days. The difference between the two groups was highly significant ($p < 0.001$).

Importance of White Cell Count

From the beginning of azathioprine therapy, close scrutiny is accorded the hematologic picture. White counts are obtained on each subsequent day during hospitalization. This laboratory measurement makes a uniquely important contribution to the determination of the dose schedule. Although the white counts give no indication of the effectiveness of azathioprine in preventing or treating rejection, they provide an index of the toxicity of the drug and a direct clear warning when overdosage has occurred. From the beginning, the desirable dose is fixed at whatever level is possible without causing leukopenia. The white counts thus determine the doses beyond which further increases are dangerous. These limits have been purposefully exceeded in the past only in a few patients whose rejection was unusually violent or prolonged, as described in Chapter 16, and it is our present policy never to induce leukopenia deliberately.

Because of the importance of the white blood count in determination of azathioprine dosage, some note should be made of the delay between administration of toxic doses of the drug and the response of the formed elements of the blood. This is difficult to determine with accuracy, but it has commonly seemed that a depressed white count could be ascribed to the therapy provided five to seven days previously, as exemplified by one case in which excessive doses of azathioprine, first given on the day of operation and continued for several more days, resulted in leukopenia in six days and fatal agranulocytosis

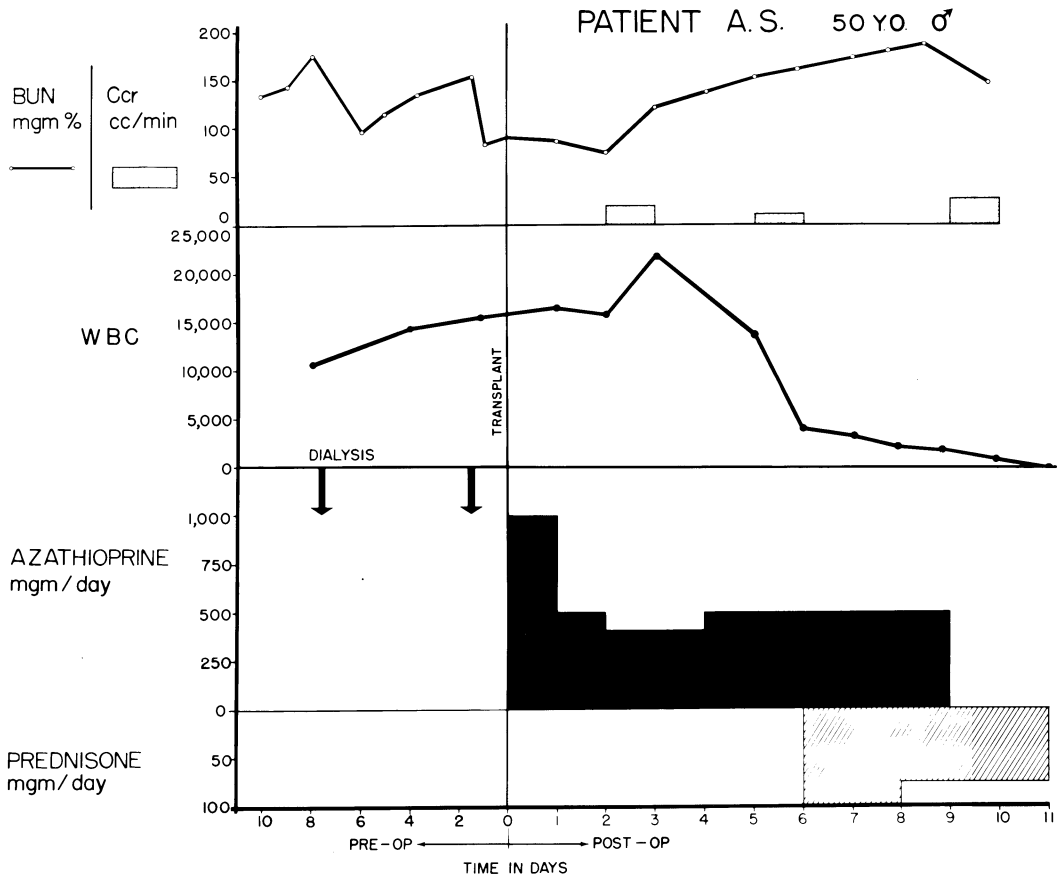


Figure 61. Azathioprine toxicity in patient (LD 5) who received excessively large doses at the time of transplantation and shortly thereafter. Note the leukopenia which was already developing within five days after the first large dose of the drug. The patient who had a cardiac arrest at the time of transplantation died of fulminating sepsis in the chest wound through which cardiac massage was performed. Terminally, there were almost no platelets or white cells in the peripheral blood.

in 10 days (Fig. 61). Conversely, it has been noted that established leukopenia, or even agranulocytopenia, fortunately has not persisted for more than a few days in those patients who did not die of acute sepsis, provided the drug dose was immediately reduced.

In prescribing azathioprine, it is also important to know that variations in responsiveness of the white count to the therapy are regularly observed throughout the course of an individual patient. Preoperatively, relatively small doses (1 to 3 mgm/kgm per day) may cause a rapidly developing leukopenia, particularly in those patients who already have the relatively low white counts which are commonly observed in uremia (Fig. 62). Immediately after operation, this sensitivity to the drug is replaced by a resistant state. In many patients who are operated upon despite low white blood counts, an immediate leukocytosis is observed (Figs. 62, 63). In almost all patients, the substantial

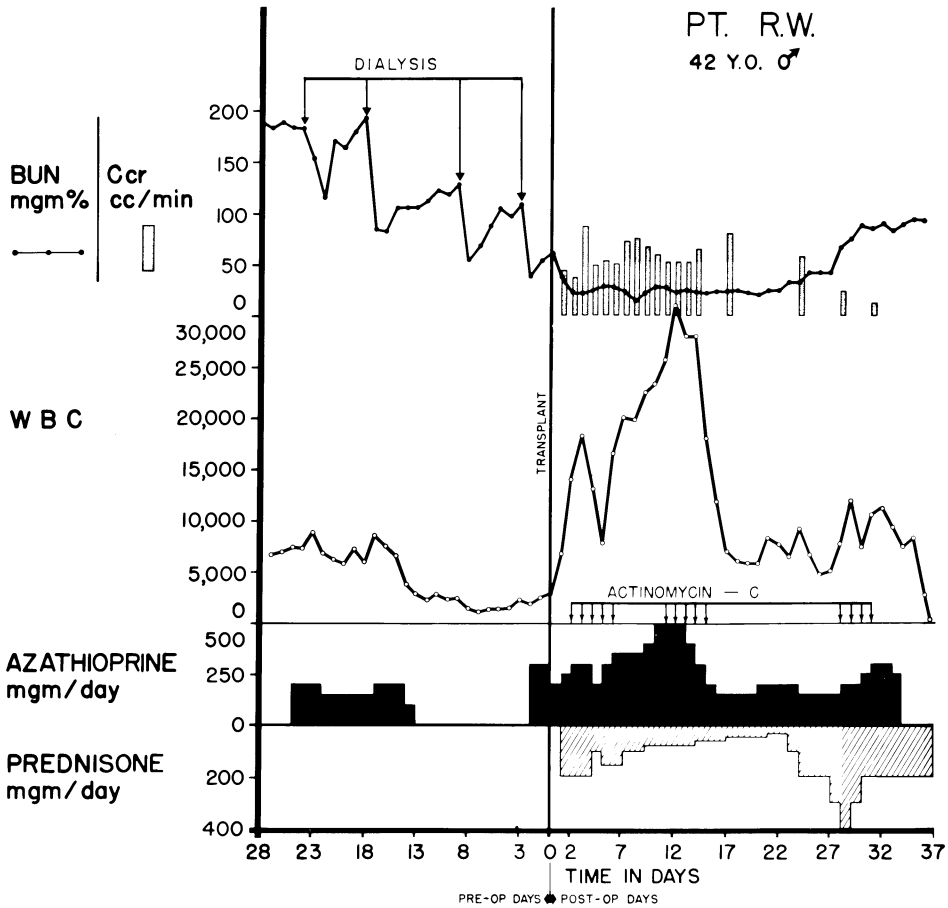


Figure 62. Death of patient from combination of rejection crisis and drug toxicity (LD 24). Note the abrupt development of agranulocytosis on the last day of life. Platelets at this time were less than 20,000. The combination of uremia during rejection, plus drug toxicity, was the most common circumstance leading to death. Note also the leukopenia induced preoperatively with small doses of azathioprine and the marked leukocytosis which followed surgery despite a preoperative white blood count of only 1,200. Bilateral nephrectomy and splenectomy were carried out at the same time as transplantation. Each arrow is equal to 200 μ g actinomycin C intravenously. (By permission of *Surgery* 56:296, 1964.)

leukocytosis seen in the immediate postoperative period permits an increase in azathioprine dosage for several days. The phenomenon of a sudden rise in white count is undoubtedly contributed to both by the nonspecific trauma of surgery and by removal of the specific inhibitory influence of the spleen.

A second phase of nonresponsiveness of the white count is inconstantly observed during the rejection episode (Fig. 59), especially in those cases in which secondary deterioration of renal function is not very severe. If previously satisfactory doses are continued in such cases, white counts may rise to 40,000 to 50,000. During this critical period, as much as 10 or 15 mgm/kgm per day of azathioprine may be required temporarily to return the white count to a normal or slightly elevated level (Fig. 59). After the rejection threat has passed in such

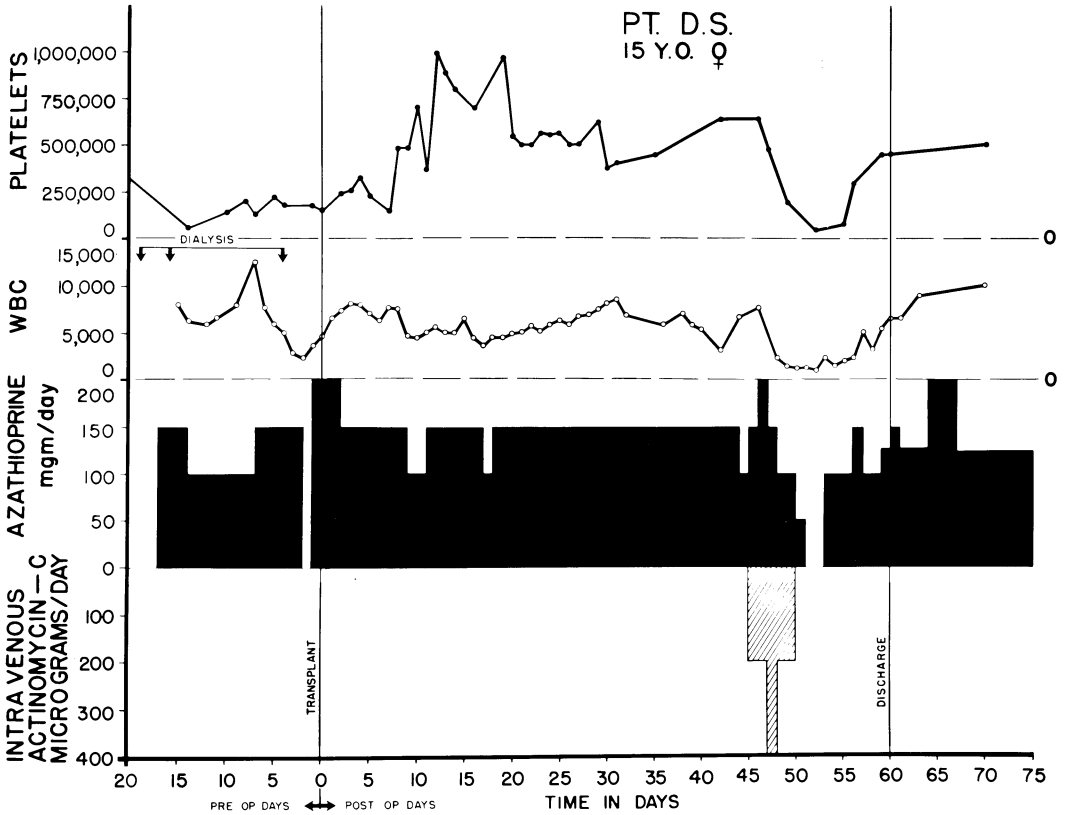


Figure 63. Demonstration of effect of actinomycin C upon the white blood and the platelet count in a patient (LD 17) who had previously been on a long course of continuous azathioprine. Note the striking leukopenia and thrombocytopenia which appeared three days after institution of a course of intravenous actinomycin C. After discontinuing actinomycin C, there was prompt recovery, and it was possible to resume therapy with azathioprine. The transplantation, bilateral nephrectomy, and splenectomy were performed on July 19, 1963, and the patient has been well with normal renal function. (By permission of *Surgery* 56:296, 1964.)

cases, a safe and stable maintenance dose can quickly be established at 2.5 to 5 mgm/kgm per day. Only after the maintenance dose is established can the patient be permanently discharged from the hospital.

Paradoxically, other patients, particularly those who have an extremely severe rejection crisis with marked impairment or even cessation of renal function, have the opposite change in sensitivity to azathioprine. It is in such cases that drug toxicity is the greatest threat, as will be discussed in Chapter 19, since continuation of moderate doses of azathioprine may unpredictably cause profound leukopenia (Fig. 62). The altered susceptibility to bone marrow depression has seemed to be directly related to the degree of secondary renal failure. Thus, patients with mild rejection can be treated vigorously, since they usually exhibit leukocytosis as described in the foregoing paragraphs. Patients with severe rejection episodes cannot be treated aggressively, since even small doses of azathioprine (2 to 4 mgm/kgm per day) may be excessive, leading to

sudden agranulocytosis (Fig. 62). The relation of the degree of renal failure during rejection to azathioprine therapy is of interest, since this drug is not thought to have a major renal pathway of detoxification.

In following daily white counts, it is important to know differential values. In some patients, particularly in the pediatric age groups, the peripheral smear sometimes has four or five times as many normoblasts as white cells (Fig. 60). Failure to differentiate these nucleated red cell forms from white cells could lead to misinterpretation of the white count values, with potentially tragic consequences. The shower of normoblasts, when observed, is usually of transient duration, but on occasion it has persisted as long as three months (Fig. 60). Doses of azathioprine are guided by the corrected white counts during this time.

The continuity of azathioprine therapy is of great importance. Ideally, the dose should not be omitted for even a single day. For this reason, intravenous administration has been temporarily used in some patients who were incapable of alimentation, the day's dose being infused slowly over a 12 to 14-hour period. It has been our impression that the potency is somewhat increased with the intravenous route. A powder form of the drug is added to isotonic saline which has been alkalized by the addition of 1 gm molecular equivalent of sodium hydroxide. Two milligrams of azathioprine are added per ml of saline. The resulting yellow-tinged solution is sterilized by passage through a Seitz filter and either used or discarded within 24 hours. Some typical constituents of the infusate are as follows:

400 mgm azathioprine
1.4 ml normal NaOH
200 ml isotonic saline

300 mgm azathioprine
1.1 ml normal NaOH
150 ml isotonic saline

200 mgm azathioprine
0.7 ml normal NaOH
100 ml isotonic saline

No matter what the route of administration, an order for azathioprine is written each day at 6 PM. The entire day's dose is immediately ingested by those patients who are able to eat. Divided doses are avoided, to prevent confusion. During hospitalization, this timing allows analysis and collection of all the morning hematologic and biochemical data, before that day's therapy is determined.

Despite the value of azathioprine, it cannot in most cases prevent rejection

of the homograft when used as the sole immunosuppressive agent. In only two patients has uninterrupted recovery occurred without the necessity for adding the other drugs to be described. In the first of these exceptional cases, the kidney was donated by a fraternal twin in whom all of the major and 21 of the 23 minor blood subgroups were identical with those of the recipient. In the other case, the kidney was provided by a nontwin brother who also had a high degree of blood group identity.

PREDNISONE

The unique value of prednisone when used as an adjunct to cytotoxic drugs was first appreciated clinically by Goodwin and his associates. They described a case in which severe rejection occurred, despite therapy with nitrogen mustard and cyclophosphamide, after renal homotransplantation from a mother to her daughter. Administration of large doses of prednisone resulted in startling alleviation of symptoms. A similar experience was reported by Merrill and his associates in treating late rejection in a kidney donated by one fraternal twin to his brother. They employed supplementary total body irradiation, plus intermittent steroid therapy. An attack of rejection was aborted, but the roles of irradiation and steroid therapy were difficult to differentiate. The consistent reproducibility of this beneficial effect has subsequently been demonstrated in our investigations and those of others, both in dogs (Fig. 64) and in man.

In almost all the cases treated by us before December 10, 1963, prednisone was added to the basic azathioprine therapy for treatment of an established rejection reaction and slowly withdrawn when the symptoms had been alleviated. The value of prednisone and the principles governing its use were most clearly defined in these earlier cases (see Chapter 16). The benefit of steroid therapy, not only in restoring rapidly failing renal function, but in reversing the systemic complaints found at this time, is frequently evident within a few hours after it has been started. The more recent use of steroids as a prophylactic measure is considered separately in Chapter 18.

The massive quantities of prednisone (150 to 400 mgm per day) administered for the treatment of a rejection crisis are usually required for only a few days, after which the dosage is slowly reduced (see Figure 59). In successfully treated cases, the control and reversal of rejection is usually relatively complete within one month (see Chapter 16). By this time, the dose of prednisone has usually been reduced to 30 to 60 mgm per day, at which time the patient can be discharged from the hospital. During the ensuing months, administration of prednisone is further reduced and, if possible, stopped altogether. When steroids are used prophylactically, a similar withdrawal program is followed (see Chapter 18).

Necessary as they are for success, these large doses of prednisone are not without continuous danger. Seven of the first 42 patients so treated developed

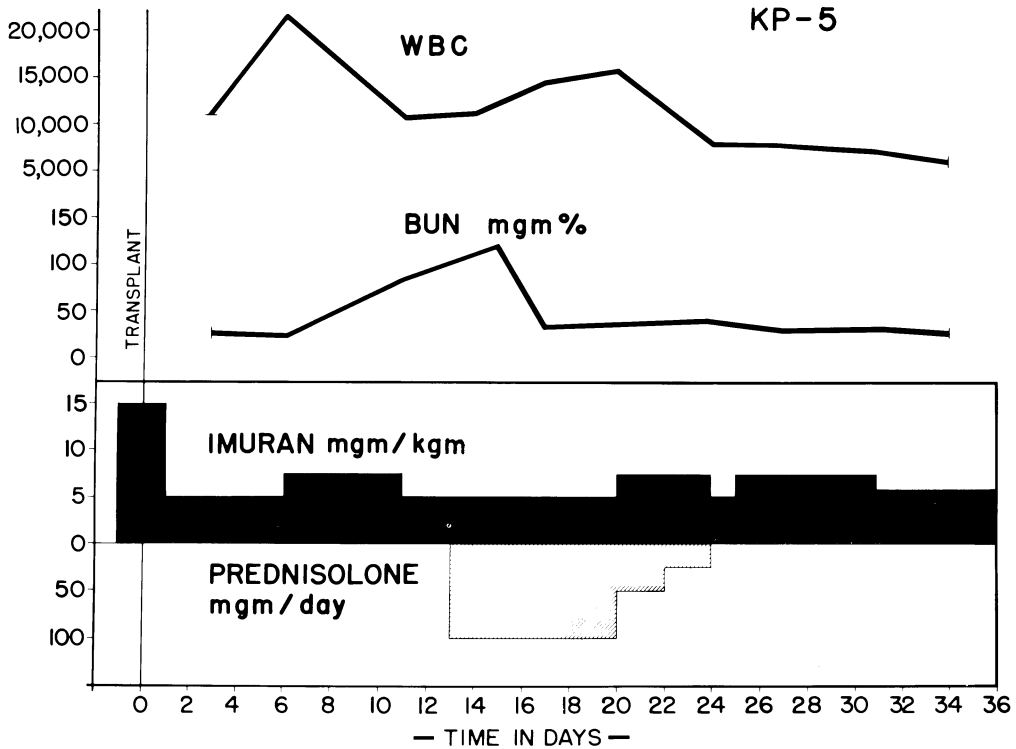


Figure 64. Effect of prednisolone on BUN in dog which developed rejection despite therapy with azathioprine. Note reversal of azotemia. BUN did not rise again when prednisolone was discontinued, a chain of events frequently observed in clinical cases. (By permission of *Surgery* 55:412, 1964.)

acute upper gastrointestinal bleeding (see Chapter 12). Consequently, all patients treated with steroids are immediately placed on a strict program of ulcer management, with antacid and milk and cream administration every hour during both night and day.

In an occasional case, oral ingestion of prednisone has not been possible for various reasons. When this has occurred, intramuscular prednisolone has been used, following the same dosage schedule.

The mechanism of the action of prednisone in the reversal of rejection is not known with certainty. When used as the sole immunosuppressive measure, steroids have a very feeble potentiating effect on homograft survival which cannot even be demonstrated except under the most rigidly controlled experimental circumstances. Dempster, working with greyhound dogs, was unable to prolong the survival of renal homografts despite the administration of 150 to 200 mgm of cortisone acetate per day. However, his meticulous histologic and physiologic studies provided a rationale for the use of steroid therapy for renal homotransplants which are undergoing rejection. He showed that the vascular endothelial reaction in the rejecting tissue was materially reduced,

that the plasma cell and lymphocyte infiltration was moderated, and that the arteriolar constriction which characterized the active rejection process was reduced, thereby providing a better peripheral blood supply to the kidney while it was under immunologic attack.

As experience accumulates, it is possible that adrenocortical steroids other than prednisone will be found to be more advantageous, either because of greater potency or because of fewer undesirable side effects. In the search for better agents, general guidelines established by earlier work on the potentiation of skin homograft survival with steroid administration may be of value. These earlier studies indicated that cortisone and cortisol had the greatest efficacy, that ACTH had a feeble effect, and that corticosterone was without value.

ACTINOMYCIN C

Actinomycin C is used for the treatment of an established rejection (see Figures 59 and 62). This agent is a mixture of three related antibiotics, isolated by Brockman from *Streptomyces chrysomallus*. Actinomycin C has a cytostatic or a cytotoxic action on lymphoid tissue and upon certain tumors. It inhibits the synthesis of nucleic acids by intact organisms and enzyme preparations. It has been said that actinomycin C is effective in the dose range used (200 to 400 μg per day) without causing bone marrow depression, but this opinion does not appear to be justified. Two cases have been studied in which acute leukopenia and thrombocytopenia occurred in patients who had been receiving relatively fixed treatment with azathioprine. In one instance the patient died of sepsis and a diffuse hemorrhagic diathesis. In the other recovery followed promptly after discontinuance of treatment with actinomycin C (Fig. 63). The original maintenance dose of azathioprine was immediately reinstituted. Superficial ulcerations of the oral mucous membranes are an additional common annoyance after the use of this drug.

At the time of rejection, actinomycin C is given intermittently every three to five days in a dose range of 4 to 8 $\mu\text{g}/\text{kgm}$ per day (Fig. 59). The entire dose for a day can be given at one time intravenously over a five or 10-minute period, or it may be given as a divided dose, every 12 hours. As soon as the threat of rejection has passed, administration of the drug is discontinued.

The true value of actinomycin C for the treatment and reversal of a rejection crisis is difficult to assess in the clinical setting in which it is used at this institution, inasmuch as it is added to the regimen concomitantly with prednisone. Experimental studies reported by Calne, Alexandre, and Murray have established the synergistic value of actinomycin C under more exact experimental conditions.

Local irradiation by the method of Hume has been used at the time of rejection. The techniques and indications of this valuable adjunct are described in Chapter 16.

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Chapter Fifteen

HOMOGRAFT REJECTION IN PATIENTS RECEIVING IMMUNOSUPPRESSIVE THERAPY

In describing the untreated rejection process as it affects both the host and the renal homograft, it is necessary to rely heavily on information learned in experiments on animals, principally dogs. The classic studies of Dempster and Simonsen which have since been confirmed by numerous investigators clearly define the events that follow homotransplantation in the canine species. At first, urinary excretion occurs, but within three to six days there is oliguria and anuria, proteinuria, and progressive azotemia. Dempster was particularly lucid in his description of a "toxic syndrome" frequently observed at this time which was characterized by fever and apathy in the dogs and which was of indeterminate etiology. Once initiated, the rejection process was inexorable in untreated animals. Spontaneous resumption of renal function has never been observed under these experimental conditions. The pathologic features of the rejected kidneys were alluded to in Chapter 1, and are fully described in Chapter 25.

In man, comparable information is scanty, being found principally in the brilliant studies of Hume and his colleagues which assume a special significance since it is unlikely that opportunities will ever again be available to study the fate of untreated homografts in humans. The cases reported by Hume confirmed, at least in general terms, that homograft rejection in man followed the same clinical course as that in dogs, with the exception of two important features. The rejection process appeared not only to be slower in beginning, but was less violent, resulting in protracted homograft function in two cases; in one for five and a half months. Hume suggested that this could be due either to a species difference in the vigor of host immunologic activation, or to a suppression of the recipient's antibody mechanism secondary to the chronic uremia from which all his patients suffered. Subsequent

evidence suggests that both factors may have contributed to prolongation of the graft survival.

The importance of the foregoing studies in understanding the pathologic physiology of rejection can hardly be overestimated. Nevertheless, it is also necessary to describe the rejection process which occurs in the patient who is receiving immunosuppressive therapy. In this chapter the course of rejection will be characterized as it has been observed in clinical practice at the University of Colorado Medical Center. This series lends itself well to analysis, since virtually all the patients operated upon before December 10, 1963, were treated in a comparable manner. Most received kidneys from living donors. Azathioprine was generally used in these earlier cases as the sole chemotherapeutic agent until evidence of active rejection was firmly established (Chap. 14). Secondary drugs were added at this time in order to reverse the process (Chaps. 14 and 16). It is of interest that Küss, Murray, Shackman, Woodruff, Hamburger, Hume, and Goodwin have all made observations similar to those to be recounted, using various therapeutic approaches to the problem of immunosuppression. Furthermore, the one patient in the Colorado series who had primary treatment with total body irradiation experienced the same sequence of events as those patients treated with azathioprine (Fig. 65). Finally, the change in therapy initiated on December 10, 1963, after which date steroids were used prophylactically, caused quantitative but not qualitative changes in rejection (see Chapter 18). Thus the following description is not peculiar to any one method of management, but the various features are clearly delineated only when therapeutic agents are added in sequence. The comments are based on experience with the first 45 patients, and the influence of prophylactic steroids upon these events is described separately in Chapter 18.

TIME OF ONSET OF REJECTION

In the usual case, the homograft begins to excrete urine within 90 minutes after it is revascularized, provided it has not been excessively damaged by ischemia (see Chapter 7). A variable period of good renal function is then observed. Uremia is alleviated, excess body fluid is quickly eliminated, and the patient experiences a feeling of well-being. Despite continuous immunosuppressive treatment, this phase of improvement is interrupted in 80 to 90 per cent of patients by an abrupt secondary illness which is characterized by systemic manifestations and by multifaceted evidence of acute renal failure. This rejection crisis has manifested itself as early as 18 hours after operation and as late as 42 days, the average time of onset being approximately 13 days. All patients and their families should be warned of this eventuality in advance. Failure to do so caused an unsuccessful suicide attempt by one patient in our series who concluded that the onset of rejection meant that death was imminent.

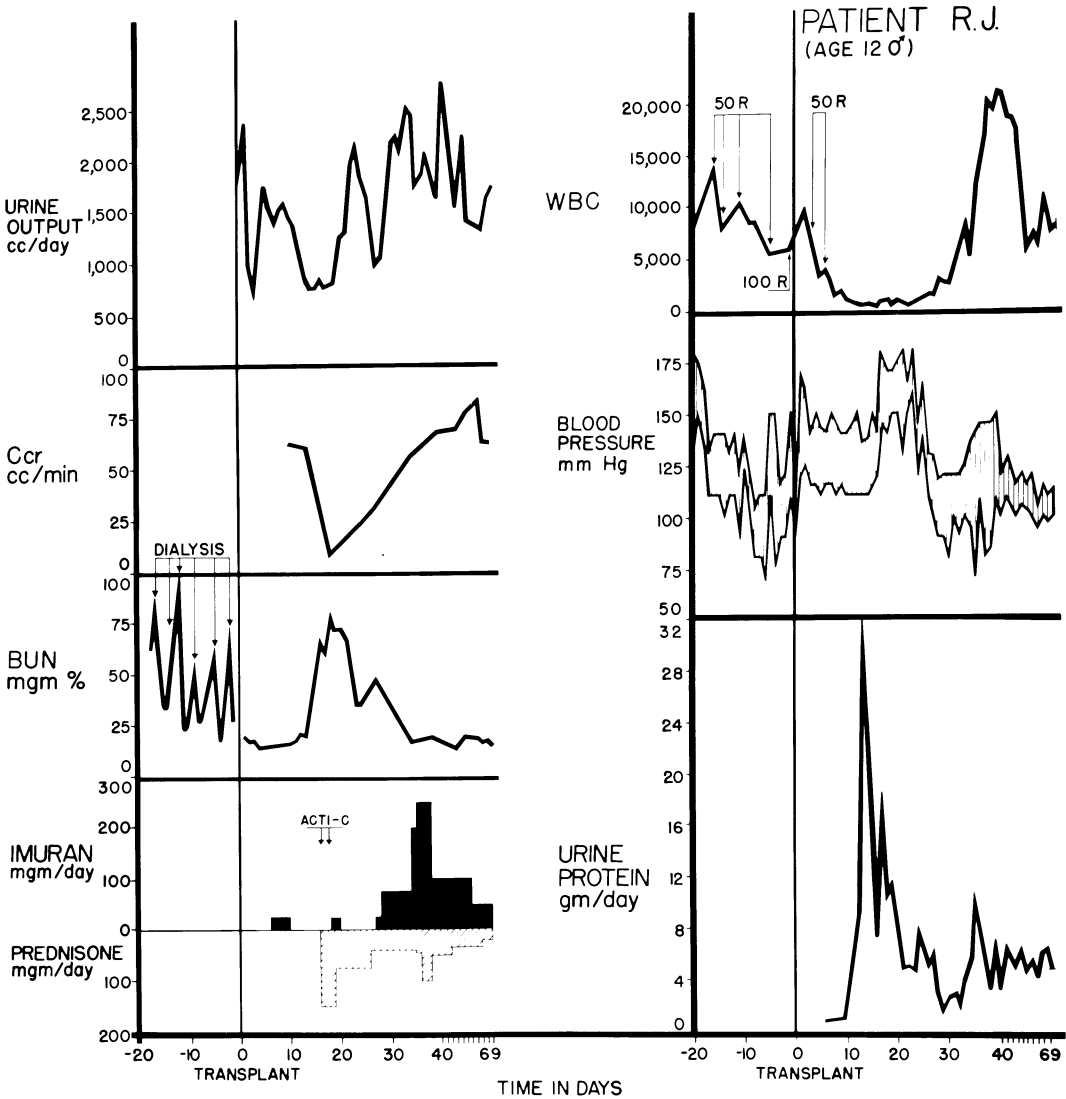


Figure 65. Rejection crisis in patient (LD 1) treated initially with total body irradiation (400 R). Note transient oliguria, depression of creatinine clearance, and elevation of blood urea nitrogen, blood pressure, and urinary protein excretion. The changes were all reversible. The patient previously had undergone bilateral nephrectomy, splenectomy, and thymectomy. R—Dose total body irradiation: Acti-C—Actinomycin C, each arrow equals 200 μ g of actinomycin C administered intravenously. Imuran is synonymous with azathioprine. These general events of rejection have occurred despite treatment with various therapeutic regimens used at this and at other centers. (By permission of *Surg. Gynec. Obstet.* 117:385, 1963.)

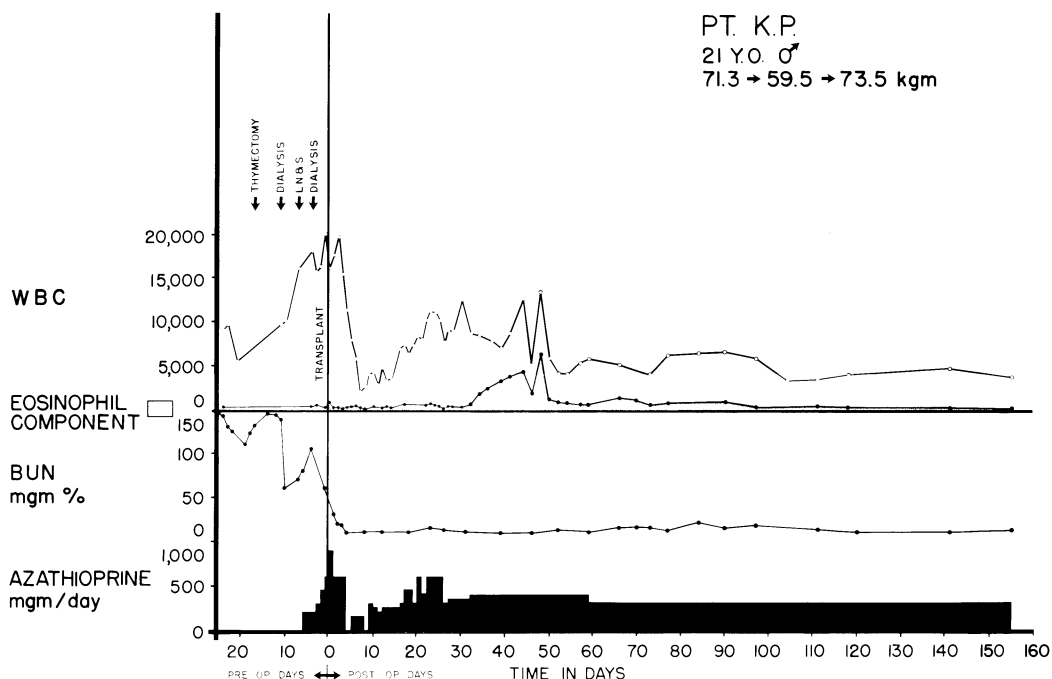


Figure 66. One of the exceptional patients (LD 3) in the series who did not have a clearly demonstrable rejection crisis. The patient, a 21-year-old male, received a kidney from his fraternal twin. Renal function has been excellent from the time of operation on February 9, 1963. However, a striking eosinophilia was observed in the second and third months after operation which probably was a manifestation of mild rejection. The patient did not have fever. LN&S—left nephrectomy and splenectomy. Right nephrectomy was done at the time of transplantation.

Three of the first 45 patients in this series never passed through a demonstrable rejection episode, and two of these three were never given any drug other than azathioprine. One received his homograft from a fraternal twin, the other from his younger brother. Although deterioration of renal function was not observed, there was other evidence of immunologic alteration during the postoperative course. Both patients had substantial eosinophilia, one from the thirty-second to the ninetyth postoperative day (Fig. 66), and the other from the eighteenth to the fifty-fifth postoperative day. No systemic manifestations were present in the first of these patients, but in the second a protracted fever was recorded (Fig. 67).

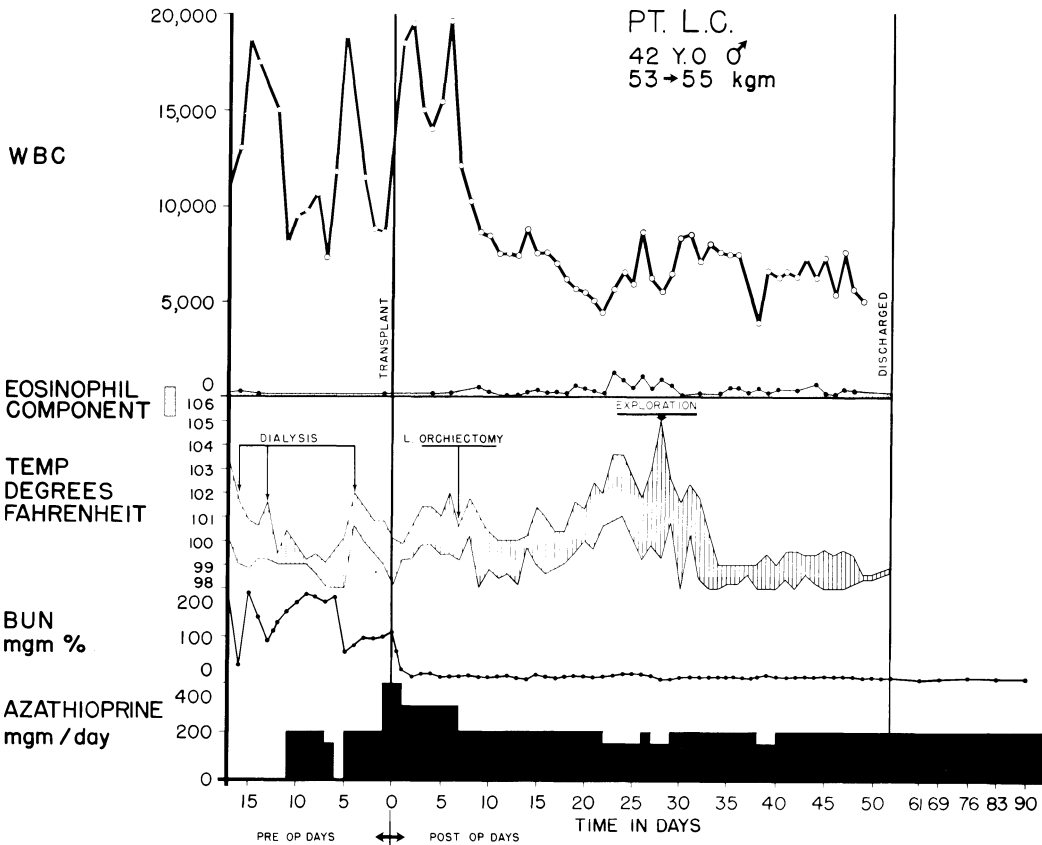


Figure 67. A patient (LD 14) similar to that shown in Figure 66, in whom a fully developed rejection episode did not occur. Protracted eosinophilia was observed, however, lasting for more than one month. In addition, the patient had a febrile crisis for which no explanation could be found. It was at first thought that the fever was due to infarction of the testis which had occurred on the side of transplantation, and later that a deep wound infection might be present. The patient was first subjected to left orchiectomy, and later to wound re-exploration. Ultimately, there was deferescence without provision of specific therapy. No drug other than azathioprine was ever employed. Bilateral nephrectomy and splenectomy were performed at the time of transplantation. (By permission of *Surgery* 56:296, 1964.)

A third patient of AB+ blood type received an A+ kidney from his wife. He was treated differently from most of the other earlier patients in that large doses of prednisone, in addition to azathioprine, were given from the time of operation (see Figure 15 in Chapter 6). Falls in his serum complement level were noted after 17 and 42 days, but no clear rejection attempt could be identified in the 113 days of his survival. He died eventually of sepsis. In retrospect, the absence of features of rejection was probably due to prophylactic treatment with steroids (see Chapter 18).

CLINICAL FEATURES OF THE REJECTION CRISIS

The rejection crisis is a complex illness which cannot be diagnosed on the basis of any single symptom, sign, or laboratory measurement. It is manifested by a systemic host reaction to the foreign tissue and by renal failure caused by an immunological assault upon the newly acquired organ. Any one of the features of the rejection crisis could be simulated by other etiologic factors. Consequently, the diagnosis may be difficult, and it is always inferential. Only the constant sifting of a mass of data will allow consistent and accurate identification of the onset and course of the rejection crisis. Some type of simplified but complete flow sheet, such as that described in Chapter 2, is essential for this purpose.

Importance of the Sequence

The single most important aid in accurate identification of a rejection episode derives from perusal of the entire course. The dramatic early improvement following transplantation has already been described. Deterioration at any time following this immediately beneficial phase is suspected of heralding rejection, and demonstration of such a pattern in which an acute secondary illness succeeds an interval of initial improvement is more important than any other criterion in making the diagnosis.

Diagnosis in the Incipient Phase

There is no reliable method as yet for accurately diagnosing a rejection episode in its premonitory asymptomatic phase. Goodwin and Hume believe that the appearance of lymphoid cells in the urine may occur early, but we have not observed this consistently, and others have expressed disappointment about the reliability of this test. At one time or another, it was thought that one or more of the features to be described below preceded the others. In general, however, the events have seemed to occur almost simultaneously, with the exception of fever, which may be present for several days before any other finding is noted and which may occur alone in occasional cases (Fig. 67).

Systemic Manifestations

Fever is the most prominent systemic manifestation in patients not receiving prophylactic prednisone. When the rejection crisis occurs early in such cases, within a few days after operation, hyperpyrexia is sudden and extreme. Temperatures of 105° F. are common (Fig. 68) and relatively unremitting until secondary drug therapy is started (Chaps. 14 and 16). With the more common later rejection, low-grade but progressively increasing fever often precedes other findings by several days.

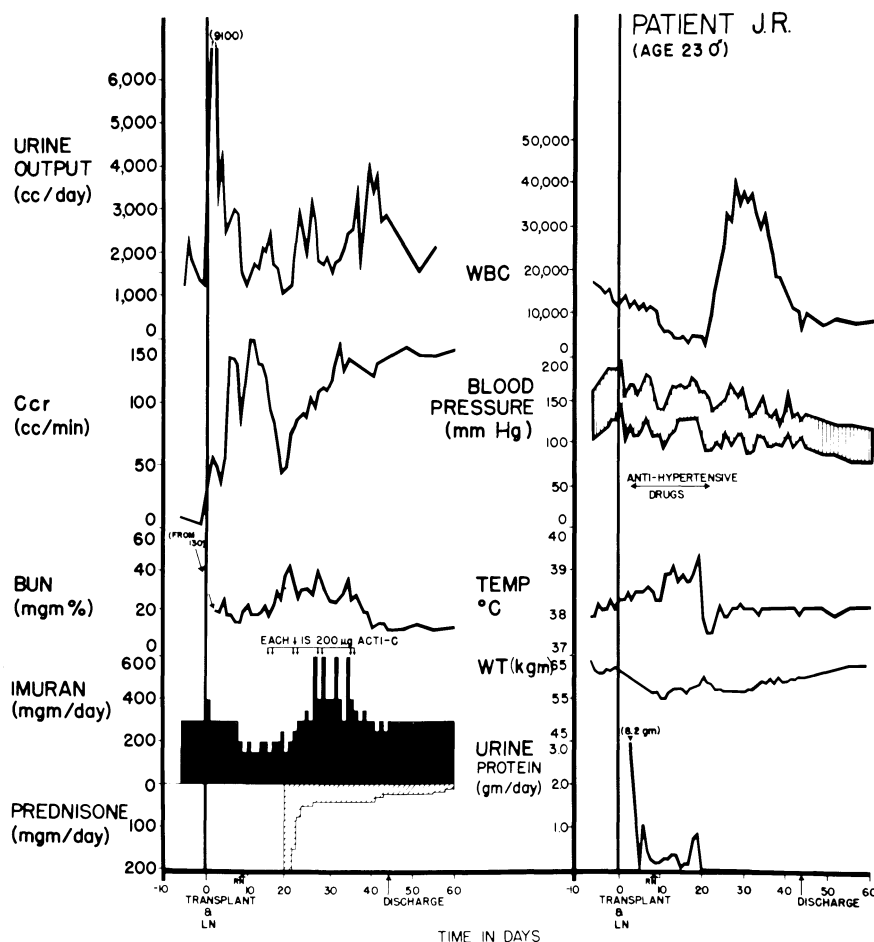


Figure 68. Classic rejection crisis in patient (LD 6) treated with drugs alone. Deterioration of renal function began 19 days after transplantation. All stigmata of rejection are present except for acute hypertension and weight gain, which were successfully prevented by medical treatment. Acti-C—Actinomycin C; LN—Left nephrectomy at time of transplantation; RN—Right nephrectomy. Imuran is synonymous with azathioprine. (By permission of *Surg. Gynec. Obstet.* 117:385, 1963.)

Differentiation of the hyperpyrexia of rejection from that of sepsis is an important aspect of management at this time. An exhaustive search is made for evidence of sepsis, since the therapy for rejection is counter to that which would be desirable for infection. It is partly for this reason that the rejection episode was not treated in our earlier series until definite evidence of homograft damage was obtained. In patients receiving prophylactic prednisone, fever is usually completely masked (see Chapter 18).

It has already been noted that important and often unpredictable alterations occur at the time of the rejection crisis in the responsiveness of the white blood count to immunosuppressive therapy (Chap. 14). The predominant cell type is usually the polymorphonuclear leukocyte, although relative lymphocytosis and eosinophilia may occur.

Certain less easily defined manifestations of systemic illness, including anxiety, apathy, and paranoid behavior, are also commonly observed. In some patients anorexia develops, and unless transient it may develop into a peculiar wasting disease which outlasts the rejection crisis and which has brought some patients to the brink of death. This nutritional disorder may be due primarily to the chemotherapy employed to reverse rejection.

Hypertension, which is frequently present in a mild form immediately after transplantation, tends to become much more severe during a rejection episode (see Figure 65), although control is possible with the appropriate anti-hypertensive drug therapy (Fig. 68). Evidence to be presented suggests that the hypertensive crises observed at this time are based upon the Goldblatt mechanism.

Changes in Homograft Function

Most investigators have documented several consistent alterations observed at the time of rejection which are dependent upon local processes within or around the graft. These include progressive azotemia, decline in creatinine clearance, proteinuria, oliguria or anuria, tenderness over the transplant site, and the appearance of urinary casts (Figs. 65, 68, and 69).

In addition, important alterations in urine composition occur at this time. In many patients, urinary constituents were serially measured before, during, and after a rejection episode. Similar changes were observed in almost all these cases. Sharp declines in sodium concentration were most characteristic, sometimes to as low as 0 to 10 mEq/liter, despite a continuing although reduced urinary output (Fig. 69). Alterations in chloride values tended to be similar. Potassium values were variable, while urea and—especially—creatinine concentrations usually rose. The effect of prednisone upon urine composition was paradoxical in that this drug, which ordinarily promotes salt retention, appeared to promote sodium excretion (Fig. 69). In one patient who had three distinct rejection episodes, such alterations were noted on all three occasions (Fig. 70). Even in those patients having a sudden early rejection with profound oliguria or anuria, similar but more rapidly evolving changes have been documented with the analysis of four-hour urinary aliquots.¹² The marked salt retention is undoubtedly an important factor in the development of the fluid retention and acute hypertension which are often seen at the time of rejection.

Blood urea nitrogen (BUN) is more frequently determined than any other measurement, primarily because it can be easily and accurately analyzed. Determinations are obtained each day before, during, and after the rejection crisis. The development or remission of azotemia can thereby be followed grossly. With a rejection episode elevation of the BUN is usually prompt.

The BUN is a relatively insensitive index of total renal function, however,

¹²By Doctor David A. Ogden.

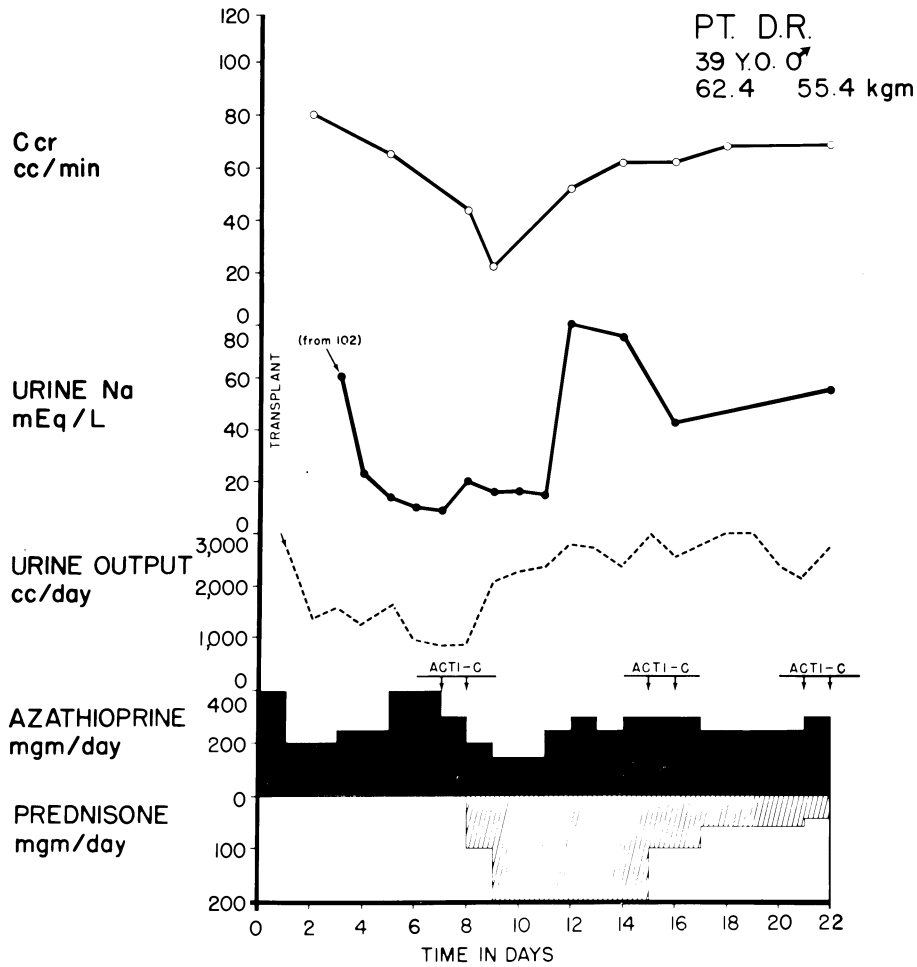


Figure 69. Changes in urine sodium (LD 18) commonly seen in patients with a rejection crisis of moderate intensity. Note the fall in urine sodium concentration at the time of the rejection crisis occurring concomitantly with relative oliguria and a sharp drop in creatinine clearance. The urine sodium excretion is influenced paradoxically by the addition of prednisone, a drug which ordinarily causes sodium retention. Instead, the addition of this agent to the regimen appeared to have increased sodium excretion. Other stigmata of rejection were present during the indicated interval, including fever, hypertension, local renal tenderness, proteinuria, and azotemia. The BUN which had dropped from 130 mgm per cent preoperatively to 31 mgm per cent by the fourth postoperative day rose to a secondary peak of 71 mgm per cent on the ninth postoperative day and declined thereafter with resumption of good function. (By permission of *Surgery* 56:296, 1964.)

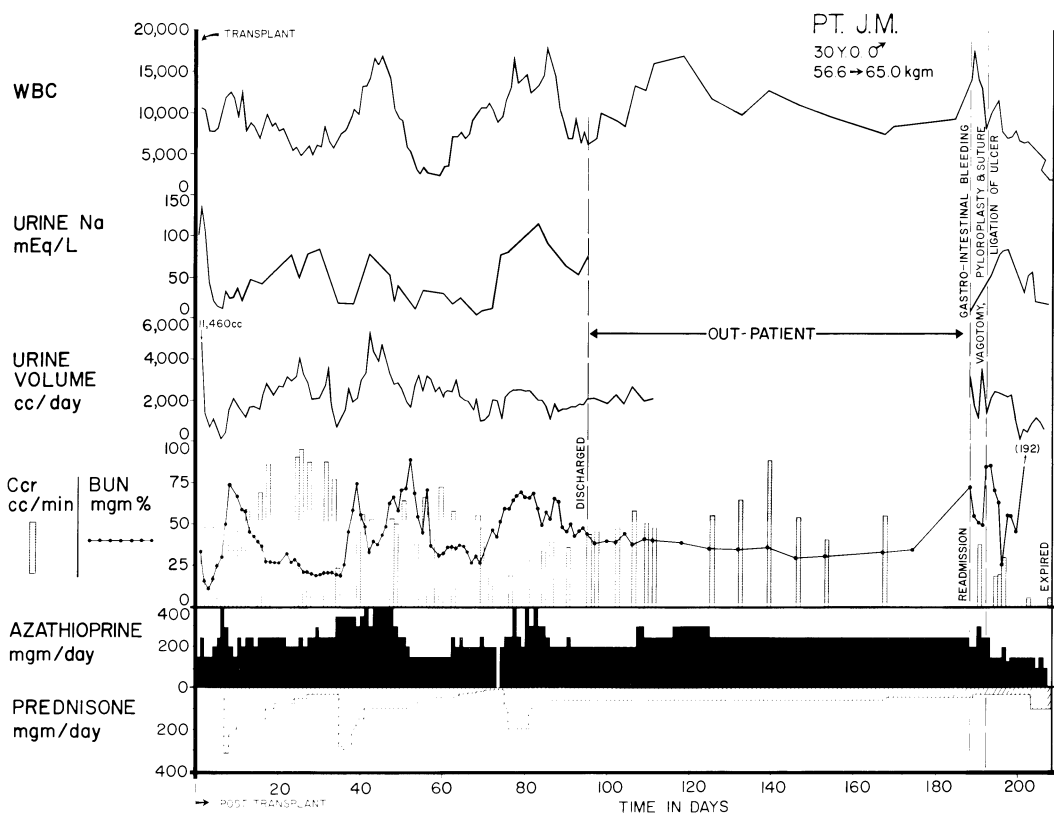


Figure 70. Course of patient (LD 9) who had three separate rejection episodes. Note decline of sodium excretion on each occasion, and reversal of pattern with prednisone dose increases. He died 10 days after emergency operative treatment of a bleeding duodenal ulcer. At autopsy, multiple fungal brain abscesses were also found. (By permission of *Ann. Int. Med.* 61: September, 1964.)

making it desirable to follow other expressions such as creatinine clearance. This is particularly true in patients in whom rejection occurs in a late and indolent manner. In this type of case, the BUN level may remain normal for prolonged periods despite a persistent low-grade fever and a progressive fall in creatinine clearance to a fraction of the normal value. Failure to recognize this as a delayed and attenuated rejection crisis has resulted in withholding appropriate therapy during the slow development of severe renal damage.

In those patients who develop an early rejection crisis (one to four days) measurement of the urine volume provides the only information which can be obtained quickly enough to be of value. Progression from the first evidence of fever and systemic toxicity to a state of absolute anuria may require only a few hours (see Figure 5, Chapter 3, and Figures 72 and 73, Chapter 16). This is perhaps the most overriding emergency in clinical transplantation and one which can be effectively treated only if immediate and vigorous action is taken (Chap. 16).

RECOGNITION OF A REJECTION CRISIS IN A NONFUNCTIONING KIDNEY

One of the most difficult problems encountered in renal transplantation is the recognition of a rejection crisis in the patient who has not had good post-operative homograft function. Historically, this has been a recurrent problem when cadaveric kidneys have been used, and it is probably an important reason for the very high failure rate under these circumstances. Inasmuch as the diagnosis of rejection depends in large part upon accurate evaluation of renal function, it becomes impossible to provide decisive therapy. It then becomes necessary to depend upon systemic evidence of rejection, but, unfortunately, even such signs may be obscured in the presence of unrelenting uremia. The serious condition of the patient will not be immediately improved by the installation of the graft, and it is usually temporarily aggravated. A new systemic disease—rejection—has been added to the one already present in which the nonspecific finding of fever may be virtually the only sign; if steroids have been administered prophylactically as described in Chapter 18, even this sign is eliminated. The diagnosis of rejection therefore must be made on very tenuous grounds.

If such blind therapy is effective, the organ, which most commonly has been damaged by acute tubular necrosis, may recover. Hume had one case in which the first urinary excretion from a cadaveric homograft was observed one month after transplantation.

DIAGNOSTIC INSTRUMENTATION

Diagnostic procedures are often considered at the time of rejection, usually for the purpose of proving the mechanical integrity of the anastomotic connections. Re-exploration of the wound, arteriography, cystoscopy, retrograde ureteral catheterization, and open or closed biopsy of the kidney are all possibilities.

Much information has been gained from such procedures, especially from serial needle biopsies. In spite of this, it is our policy to carry out absolutely no instrumentation at this time, unless there is a highly specific indication for it. If the kidney has previously been functioning well, it is assumed that the transplant is mechanically satisfactory, and that a rejection crisis is at hand. Appropriate therapy is then started (Chap. 16).

PATHOPHYSIOLOGIC MECHANISMS

For a number of years, it has been considered that focal and diffuse mononuclear cell invasion was the most typical and ubiquitous lesion in homograft rejection. More recently, there has been renewed interest in vascular lesions which were clearly described by Dempster and Simonsen. The vasculitis is characterized by the presence of edema in and between the layers of

the vessel wall, deposition of an eosinophilic fibrinoid material in these locations, endothelial proliferation, and infiltration with pyroninophilic mononuclear cells. The lesions are not dissimilar to those found in collagen vascular disorders.

Porter has drawn attention to the more chronic proliferative arterial and arteriolar changes which occur at a later stage in human renal homografts that have been transplanted weeks or months earlier. There was frequent narrowing and complete closure of the lumina of these vessels, or disruption of components of the medial layer. Both Porter and Dammin have demonstrated that these are reversible changes, even late in the course. The relation of these late changes to those found at an earlier time has not been proved, but it is possible that they are different stages of the same process (see Chapter 25).

The relationship of the vascular lesions to classic cellular infiltration and the possibility that the two types of changes occur independently or even by a different mechanism are not established, although an attractive hypothesis is that the vasculitis is related to an independent humoral response of the host, as has recently been suggested by Ramos on the basis of observations with cardiac homografts. What is clear is that in actual clinical experience with renal homografts, many cases have been observed in which functional deterioration has occurred with little or no evidence of classic cellular rejection (see Chapter 25).

With the demonstration of multiple small vessel disease, it becomes feasible to suggest that one of the first and most important events in rejection is acute ischemia, varying in intensity from that of an acute vascular calamity to a more indolent process. Edgerton's studies of vascular changes in skin grafts support this reasoning, as do numerous other observations which are discussed in detail by Porter in Chapter 25.

There is also some physiological evidence that such a mechanism is at least partly responsible for the rejection crisis in transplanted kidneys. As described earlier in this chapter, changes are seen in urinary electrolyte excretion when the rejection crisis is of moderate intensity, the most common findings being reduction or even cessation of sodium excretion at a time when urinary volume is declining and increases in creatinine concentration are seen. The concomitant development of hypertension suggests the presence of renal ischemia, since the aforementioned water and electrolyte alterations are comparable to those which are observed in the experimental Goldblatt preparation and which form the basis of the clinical Howard test and Stamey test for renal ischemia.

In violent early rejection in which anuria occurs rapidly, similar changes in sodium excretion have also been measured recently. If reversal is not quickly achieved, the consequences are like those seen in partial or complete acute tubular necrosis. Acute ischemia of a more severe nature may also be the explanation in this type of exaggerated rejection, the difference being only that of degree. It is noteworthy that restoration of function in this type of case can occur, but sometimes not until anuria has been present for many days, a time sequence similar to that seen in recovery from acute tubular necrosis (see Figure 5 in Chapter 3 and Figures 72 and 73 in Chapter 16).

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Chapter Sixteen

REVERSAL OF REJECTION

It was pointed out in the previous chapter that complete prevention of the body's attempt at homograft rejection is usually not possible. This fact has been the cause of much pessimism concerning renal homotransplantation, since rejection has historically been considered to be an event of finality. The immunologic forces subserving this process are generally thought to be so powerful and persevering that, once initiated, they lead inexorably to destruction of the transplanted tissue.

Recent observations indicate that the rejection process is by no means such a decisive event. The clinical features of rejection can be reversed regularly and completely, even when the degree of acute renal damage is profound. The functional capacity of kidney transplants after reversal of rejection has eventually returned to essentially normal standards in many of our cases, even in those patients undergoing the most vigorous crises.

That rejection is not an all-or-nothing phenomenon was first suggested by the canine retransplantation experiments of Balankura and his associates. They found that renal homografts which were undergoing active rejection resumed function and normal histologic appearance if they were returned to the original donor within several days. Subsequent isolated observations by Calne, Murray, Merrill, Goodwin, Shackman, Hume, Woodruff, Hamburger, and Küss have all suggested the possibility of reversing the homograft rejection, either in dogs or in man. In the University of Colorado series, it has been demonstrated that most rejection episodes can be reversed, no matter if these are vigorous or mild. In this experience, the key to success has proved to be the proper management and reversal of the rejection crisis, rather than its prevention. This has proved to be so in both the earlier cases in which steroids were given only after rejection was established, and in the more recently treated patients who were given prophylactic prednisone.

SALVAGE OF THE HOMOGRAFT

At the time of rejection, immunosuppressive measures are added to those already in use. In patients treated initially with azathioprine alone, prednisone is added (Figs. 59, 60, 68-73) as well as intravenous actinomycin C and local irradiation to the homograft. If prophylactic prednisone was begun previously (see Chapter 18), the steroid dose may be increased, actinomycin C added, and local irradiation employed, as discussed later in this chapter. When practicable, doses of azathioprine are increased, although this is not without danger (see Chapters 14 and 19). Rejection being subject to remission, the value of any detail of added therapy cannot be proven in a given case, since recovery may have occurred without it. Nevertheless, the value of each adjuvant measure has been demonstrated more or less clearly in animal experiments and in different clinics.

With these measures, it has been possible in most cases to reverse the features of rejection (see Figures 5, 59, 60, 65, and 68-73). The fever responds most quickly to the secondary drug therapy, hyperpyrexia usually being relieved within a few hours after the beginning of prednisone therapy. Frequently there is a short-lived progression of deterioration of renal function, lasting for several days, before the BUN and creatinine clearance are restored toward normal. In other cases, the rising BUN becomes temporarily fixed at an abnormally high level, sometimes despite a brisk secondary diuresis (Fig. 71). In approximately 18 per cent of cases, a period of complete anuria is noted that lasts one to 14 days before excretion is resumed (see Figures 5, and 71-73). In most instances, however, complete cessation of function does not occur (see Figure 68).

The term "rejection crisis" implies the development and resolution of graft rejection within a relatively short time. This has been true in a few patients in whom rejection began, reached its zenith, and regressed at least partially within a few days. More commonly, the crisis has been protracted. The most extreme example was in a six-year-old child who received a kidney from his mother. Rejection began approximately 30 hours after operation, and a significant reversal of the process was not obtained until almost two months later. The child, who weighed 20 kgm, was in fair health throughout this entire period, despite the fact that he received between 80 and 150 mgm per day of prednisone and very large quantities of azathioprine and actinomycin C (Fig. 60). The most important factor of success in a protracted episode of rejection is the avoidance of either over or undertreatment. The consequences of undertreatment are obvious. If the patient is given too much azathioprine, a fatal agranulocytosis or leukopenia may develop.

As soon as it has been clearly demonstrated that renal function is once again improving, the doses of steroid are progressively reduced to a maintenance dose which is usually 30 to 60 mgm per day (Figs. 71-73). Rapid reductions below this level are sometimes possible, but one of the most serious errors in management in our experience has been to reduce the dose of

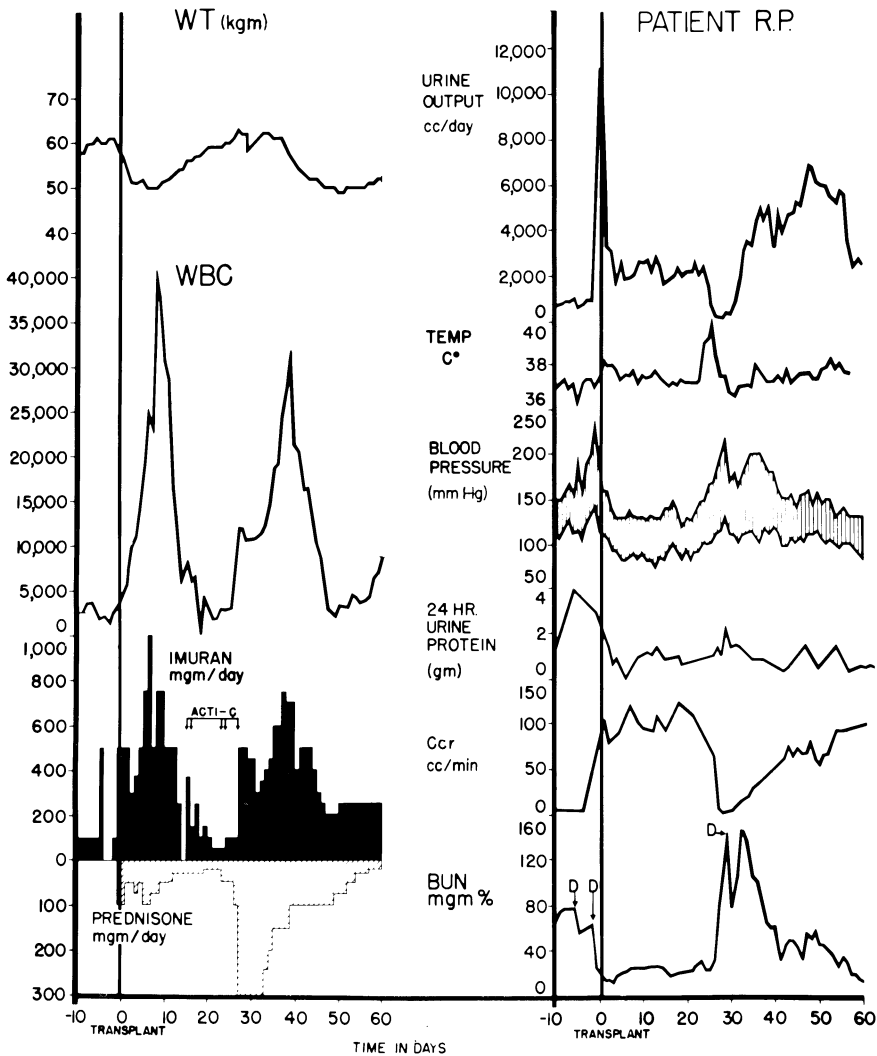


Figure 71. Rejection crisis (LD 2). Note temporary deterioration of renal function, coincident with fever and weight gain starting on the twenty-fifth day after transplantation. The patient was anuric for three days, and even after resumption of urine excretion, azotemia persisted for several weeks. Note reversal of all parameters including acute hypertension, which is commonly observed at this time. Acti-C – Actinomycin C, each arrow equals 200 μ g; D – Dialysis. Imuran is synonymous with azathioprine. Operation was on January 31, 1963. The patient has had normal renal function for more than one year. (By permission of *Surg. Gynec. Obstet.* 117:385, 1963.)

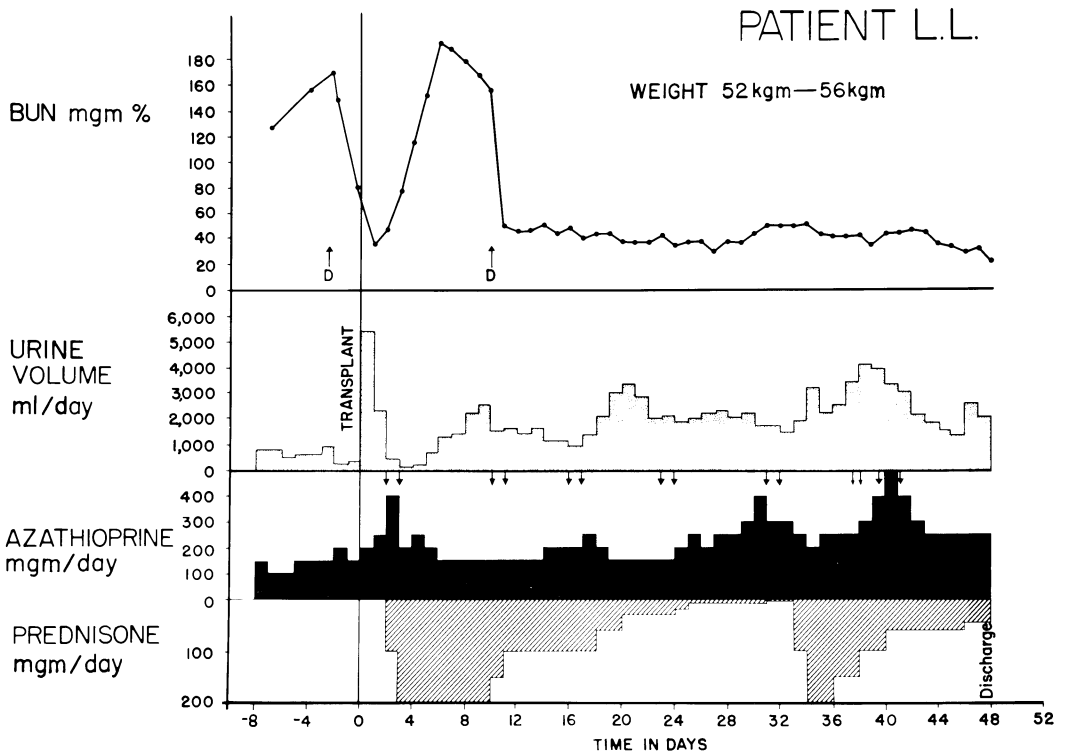


Figure 72. Development of early rejection crisis after 36 hours (LD 15). Although temporary anuria resulted, the crisis was reversed. Dialysis has been required during the rejection episode in 18 per cent of the cases. Splenectomy and bilateral nephrectomy were performed on the same day as transplantation. D—Dialysis. Each arrow is 200 gamma actinomycin C, I.V. (By permission of *Surg. Gynec. Obstet.* 118:819, 1964.)

prednisone too quickly. This has resulted in early secondary rejection crises in three cases (Figs. 53, 70).

During reversal of the rejection crisis, it is important to follow several indicators of renal function. After initial improvement in the serum biochemical values, the decline in BUN may stop at 30 to 40 mgm per cent, despite the fact that the patients have normal creatinine clearance and urea excretion (Fig. 71). The inability to immediately restore the BUN to normal is probably due to the excessive catabolism known to be induced by the large doses of prednisone. As the doses of steroids are further reduced, at a later date, the BUN ordinarily returns to normal. Completion of reversal, defined as the time necessary to restore normal or relatively normal renal function, has required five to 55 days.

In addition to the treatment described, local irradiation to the homograft has been employed in many patients, most having complete anuria at the time of the rejection episode. The technique used was adapted from that described by Hume and his associates, using doses of 150 R every other day for three or four treatments. Instead of local irradiation being given prophylactically, it was used only at the peak of the rejection crisis (Fig. 73). Urinary excretion was

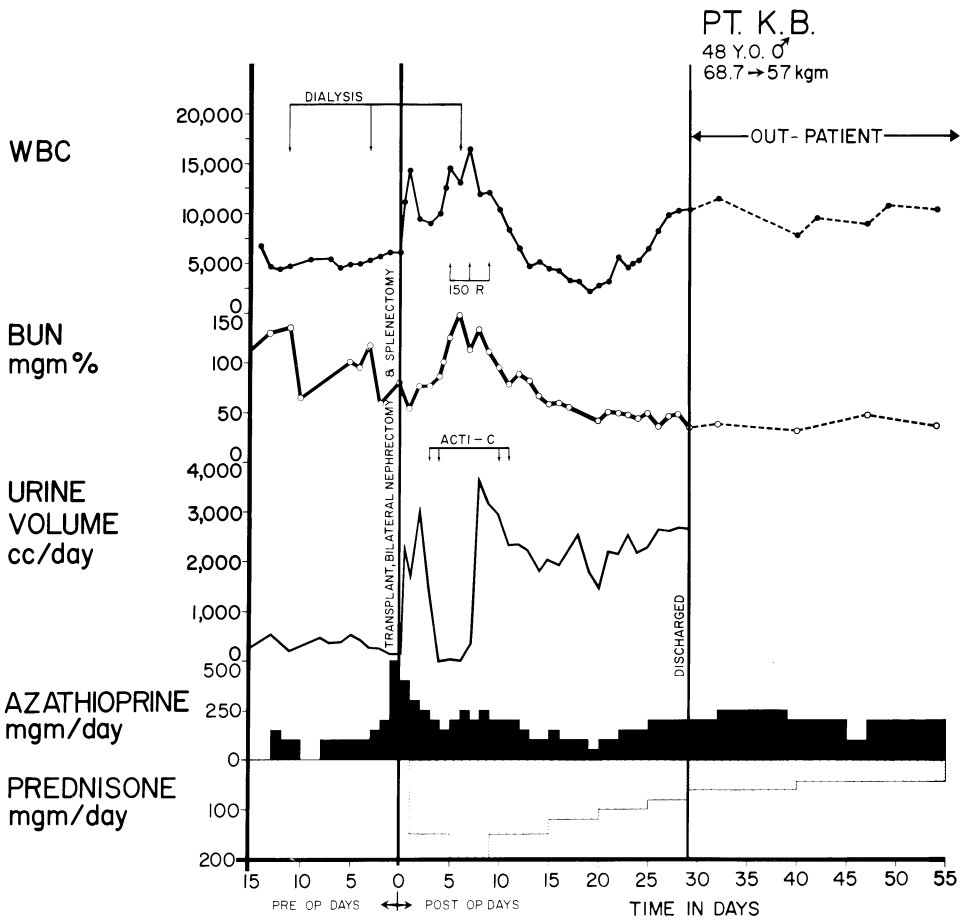


Figure 73. Use of local irradiation to the homograft during rejection crisis (LD 44). Three doses of 150 R (at depth) were given with a 250 KV unit. Homotransplantation was done December 4, 1963. Renal function has been normal. Note anuric interval. (By permission of *Surgery* 56:296, 1964.)

resumed in all but one of the patients treated in this way, but five patients subsequently died of later complications. Hume has demonstrated the value of his method in experimental animals, and it is possible that it deserves routine application in all cases, as he has recommended.

GENERAL MEDICAL CARE

It is important not only to reverse the rejection crisis, but to provide supportive care as well. The hypertension which is a common event during the rejection crisis (Chap. 15) is treated with antihypertensive drugs, including apresoline, reserpine, and Diuril. It has been suggested by Parsons that failure to do so may result in irreversible changes in the kidney which are similar to those seen in malignant hypertension. In addition, it is conceivable that the

use of these agents may have a more specific effect in counteracting the physiologic effects of acute vascular changes which were discussed in the preceding chapters as a possible specific initiating or potentiating factor in rejection.

It has also been noted that salt retention is a prominent feature of this period, urinary sodium excretion virtually ceasing in some cases. Consequently, the dietary control of electrolyte intake is of the utmost importance.

The patients are usually managed on a 200 mgm sodium-restricted diet during the peak of the crisis. Fluid therapy is also a significant facet of general care. Many patients are oliguric, and some are anuric, so that failure to restrict fluid intake may result—as has been observed in some cases—in vascular overloading and acute heart failure.

In the more violent rejection crises in which oliguria or anuria has developed, it has been our policy to perform postoperative dialysis promptly (Figs. 71-73). By doing this, the dietary intake can be liberalized, thereby preventing muscle wasting during the period of diminished renal function. Of the first 64 patients who received renal homografts from living donors (LD series), 13 were dialyzed after operation, usually only once, but in one case, eight times. Six of these survived the immediate effects of rejection, and five were alive on June 1, 1964.

As will be described in more detail in Chapter 21, this is the portion of the postoperative period in which the patient incurs the greatest risk of sepsis. The proper management of antibiotic therapy during this time is critical. Early in our experience it was considered mandatory that our patients be confined to a relatively aseptic room. While this may be desirable in theory, it has also been found that profound psychic depression has often followed, leading to anorexia and general physical deterioration. For this reason, the recent patients have been allowed to leave the hospital during rejection crises, even though they were receiving maximum immunosuppressive therapy. Such patients were escorted to the door of the hospital with a minimum of contact with hospital personnel or other patients and were allowed to remain away during the day, returning only at night. No serious infections have occurred which were thought to have had their inception during such passes.

Much information is still unavailable concerning systemic response during treatment of homograft rejection. A peculiar wasting disease is noted on many occasions during that part of the postoperative period in which a rejection crisis is evolving. Almost all patients lose weight. Profound muscle atrophy is sometimes observed. Some patients appear to be dying of malnutrition, despite a dietary intake which might otherwise be considered adequate. Pre-existing neuropathies may be accentuated. Diarrhea may alternate with gastrointestinal hypomotility in which pancolonic fecal impaction may become an almost insoluble problem. Dermatitis is observed in various areas. In three patients in our series, peculiar lesions appeared in the antecubital or popliteal spaces which looked like second degree thermal burns and which were similar to those found in pellagra. Cheilosis and multiple oropharyngeal ulcerations are common.

How this wasting disorder is corrected is not known. When recovery has ensued, it has seemed to be unrelated to any specific therapy. Because of the similarity of the condition to multiple deficiency avitaminosis, therapy with large doses of the B vitamins and vitamin C is carried out. That some patients recover from this phase and that others do not has not been satisfactorily explained. Equally confusing are the etiologic factors leading to malnutrition. It is conceivable that the wasting is simply a continuation of pre-existing debilitation, accentuated by the addition of large doses of prednisone. It is equally possible that interference with the metabolism of vitamins, or of other nutritional elements, is a specific consequence of the large doses of azathioprine prescribed at this time. Solution to these problems will require intensive study by experts in nutrition who are concerned not only with the immediate events of the rejection crisis, but with the long-term effects of immunosuppressive drugs upon nutritional processes.

A specific metabolic derangement which may require active therapy is diabetes mellitus, caused by the high doses of steroids. Wide variations have been noted in the development of side effects of prednisone therapy. In a few patients, moon facies, abnormal fat deposition, hypertension, and glycosuria develop at an early time. In these patients insulin therapy has been necessary on a number of occasions. The primary objective is prevention of ketosis, rather than rigid control of the urinary sugar loss. Doses as high as 120 units per day NPH insulin have been required in a few patients. In others, significant glycosuria has not been a problem even after protracted periods of high-dose steroid therapy. It is important not to attempt control of the induced diabetes mellitus by dietary restriction. Most patients who have successfully passed a rejection crisis are extremely debilitated. It is more important to re-establish good nutrition at this time than to control the diabetic state through limitation of diet.

In most patients who pass the rejection crisis, there is a remarkable secondary reconstitution of body mass. The appetite becomes voracious. Some patients have gained as much as 30 or 40 pounds in one month, and it has not been uncommon to note a 40-to-80-pound weight gain in the ensuing three or four months. In general, this gluttony is slowly replaced by a more temperate dietary approach when the optimal weight which had prevailed before the onset of the original illness has been reached, but in some patients obesity may be a serious late complication (Chap. 20).

RECURRENCE OF REJECTION CRISIS

It was pointed out that early recurrences of the rejection crisis are usually due to faulty manipulation of drug therapy, the most common error being too rapid a reduction of prednisone dosage. Early recurrence of rejection has been noted in three patients. Recovery from the previous assault is interrupted by all the features of primary rejection, with fever and acute renal failure. Although

reversal of recurrent rejection episodes can be effectively accomplished, this is frequently more difficult (Fig. 70), and, in addition, the patient requires large doses of prednisone for a much longer period, and is subject to all the attendant risks. One patient in our series had a very benign and easily controlled primary rejection crisis (see Figure 53). With rapid reduction of steroids, a secondary crisis developed requiring large doses of steroids for many days. After a temporary improvement, she began to lose weight, and ultimately developed jaundice and a paralytic ileus. At autopsy, 83 days after operation, she had diffuse hemorrhagic pancreatitis, probably caused by excessive amounts of prednisone, as has been demonstrated in animals given steroids for prolonged periods.

Another patient had three distinct rejection episodes each reversed with progressive difficulty with increased steroid dosages (Fig. 70). The patient, who was known to have a duodenal ulcer, ultimately required emergency vagotomy and pyloroplasty for hemorrhage, six and a half months after transplantation. He died 10 days after this operation. Terminally, he became anuric.

With recurrence of a rejection crisis, it becomes mandatory that an adequate dose of prednisone be established and maintained for a long time after discharge. The necessary maintenance dose was somewhat higher in these three cases than in those patients who had a single well-controlled crisis. After the recurrent rejection, the maintenance level of prednisone was 45 to 80 mgm per day. In contrast, a dose level of 30 mgm per day or less is usually reached within two months in well-managed patients.

Quite aside from the dangers evoked by protracted high-dose immunosuppressive therapy, the obvious additional hazard of secondary crisis is that further injury to the homograft parenchyma is inevitable. In the patient who had three rejection crises, the BUN never completely returned to normal (Fig. 70). The homograft, recovered at autopsy more than six and a half months after operation, had numerous histologic abnormalities which were explicable by the repeated immunologic insults (see Chapter 25).

The problem of late rejection, four months or longer after homotransplantation, is considered in Chapter 20.

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Chapter Seventeen

HOST-GRAFT ADAPTATION

The dangers of the therapy in current use for reversal of rejection have been repeatedly stressed. Hope for long survival with the drug combinations and dosages used during the rejection crisis would be remote indeed if such stringent measures were required permanently for protection of the homograft. Fortunately, it has become increasingly clear that a state of host-graft nonre-activity is an integral accompaniment of the successfully treated rejection crisis.

The mechanism by which adaptation is brought about is not clear; nevertheless, evidence that this occurs is now unequivocal, both in experimental animals and in man. At first, and for a long time, the only faint hope that adaptation in adult life might be possible was provided by the observations of Woodruff which demonstrated that guinea pigs developed tolerance to thyroid homografts implanted in the anterior chamber of the eye. Adaptation was so complete that the transplanted tissue could then be removed from this favored site to a sub-cutaneous location, with subsequent chronic survival. Woodruff pointed out that the necessary prerequisite for clinical application was the development of means to prevent graft destruction during the adaptive phase.

Decisive evidence that adaptation could occur in dogs with renal homografts was later obtained by Zukoski and Pierce and Varco. Their animals were treated with 6-mercaptopurine or steroids for prolonged periods postoperatively. After eight months or more, it was possible to withdraw antirejection therapy in some of the animals without cessation of renal function for as long as one and a half years. In our laboratories, it has been demonstrated that partial or even apparently complete tolerance can occasionally develop as early as four months after canine renal homotransplantation. At this early time, rejection usually occurs after withdrawal of drug therapy, but often in an indolent and delayed fashion, requiring many weeks or even several months (Figs. 74, 75). In one animal, normal renal function has continued for eight months after discontinuance of azathioprine therapy (Fig. 76).

In the experiments just alluded to, complete adaptation was a sporadic and unpredictable accomplishment, the few ultimate successes contrasting with a

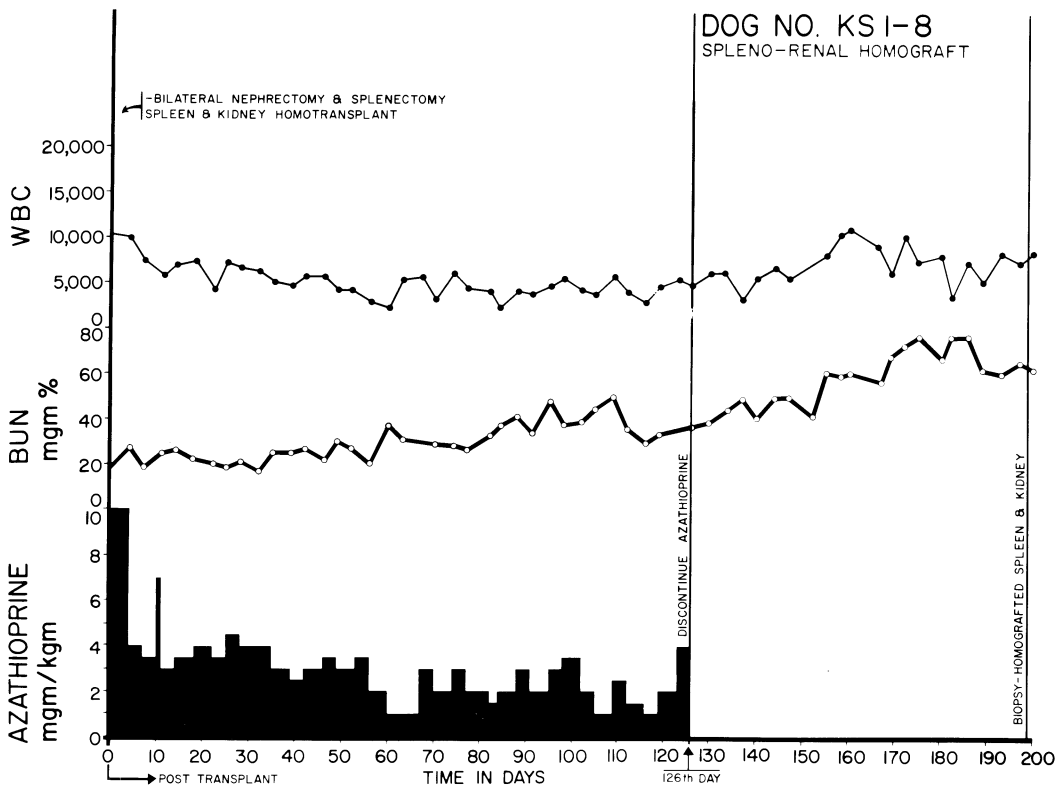


Figure 74. The development of partial tolerance in a dog after postoperative azathioprine administration for four months. The animal received both a whole organ spleen and a renal homograft at the same time as bilateral nephrectomy and splenectomy. Note the gradual development of azotemia after discontinuance of immunosuppressive therapy. The animal is still alive almost 15 months after operation.

larger group of failures. In our clinical series, the development of adaptation can also be demonstrated, but it is undoubtedly incomplete, except, perhaps, in those cases followed for the longest periods. In the latter group, the final test of complete adaptation has not been attempted, inasmuch as the attendant risk of discontinuing maintenance doses of azathioprine has seemed unwarranted.

In this chapter, the term "adaptation" has been used in an ambiguous sense; there has been no attempt to determine whether the host, the homograft, or both have undergone a change. It seems most reasonable to believe that an alteration in the recipient's immune mechanism has occurred, as suggested by Billingham, Medawar, and McGavic and their associates, but Woodruff has pointed out that a change in the antigenic structure of the transplanted cells may also occur. The principal experimental evidence in favor of the latter possibility is that a second transplant from the donor of a well-established homograft is often rejected despite continued survival of the first one.

In humans, it is possible to demonstrate partial adaptation at a surprisingly early time, frequently in four to six weeks. This is usually first seen just at the time when a clear-cut reversal of rejection is obtained. As the rejection crisis

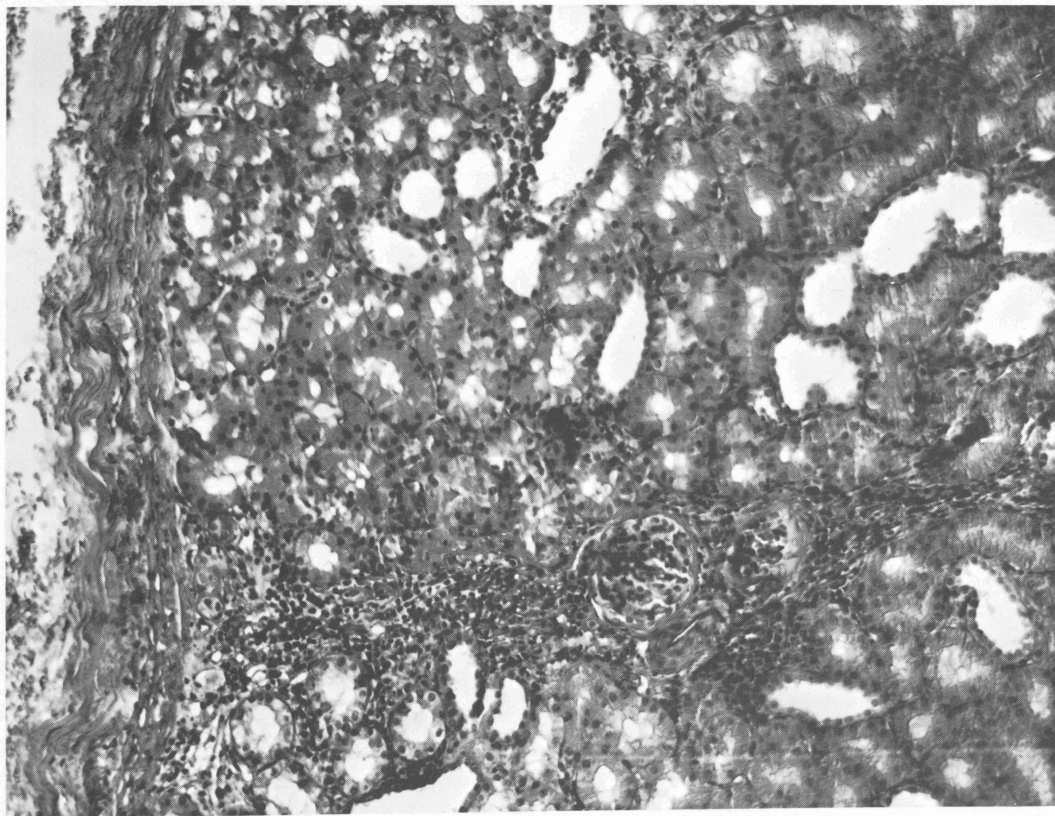


Figure 75. Renal homograft biopsy from animal shown in Figure 74, 75 days after discontinuance of azathioprine. Note moderate focal cellular infiltrate. H and E (X 80).

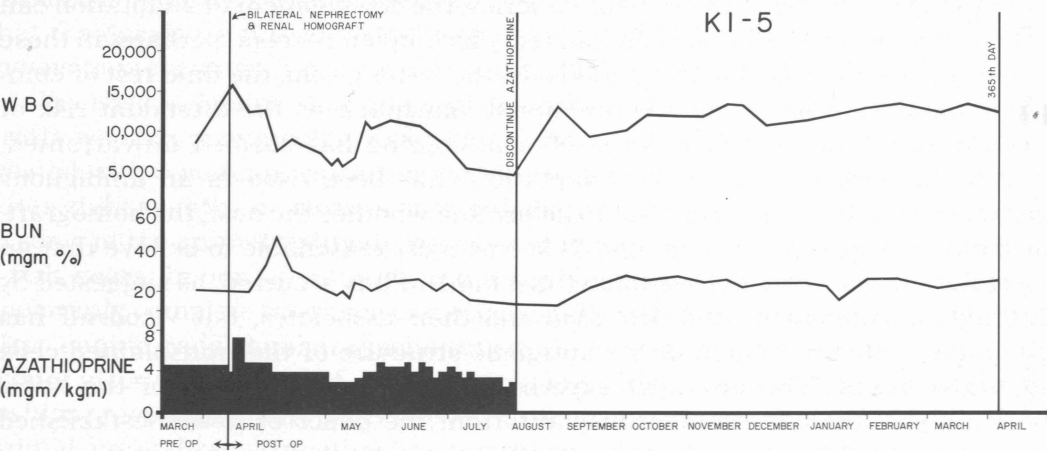


Figure 76. Demonstration of a high degree of tolerance in a dog treated with azathioprine for four months after homotransplantation and bilateral nephrectomy. Good renal function has continued for more than a year after stopping immunosuppressive therapy.

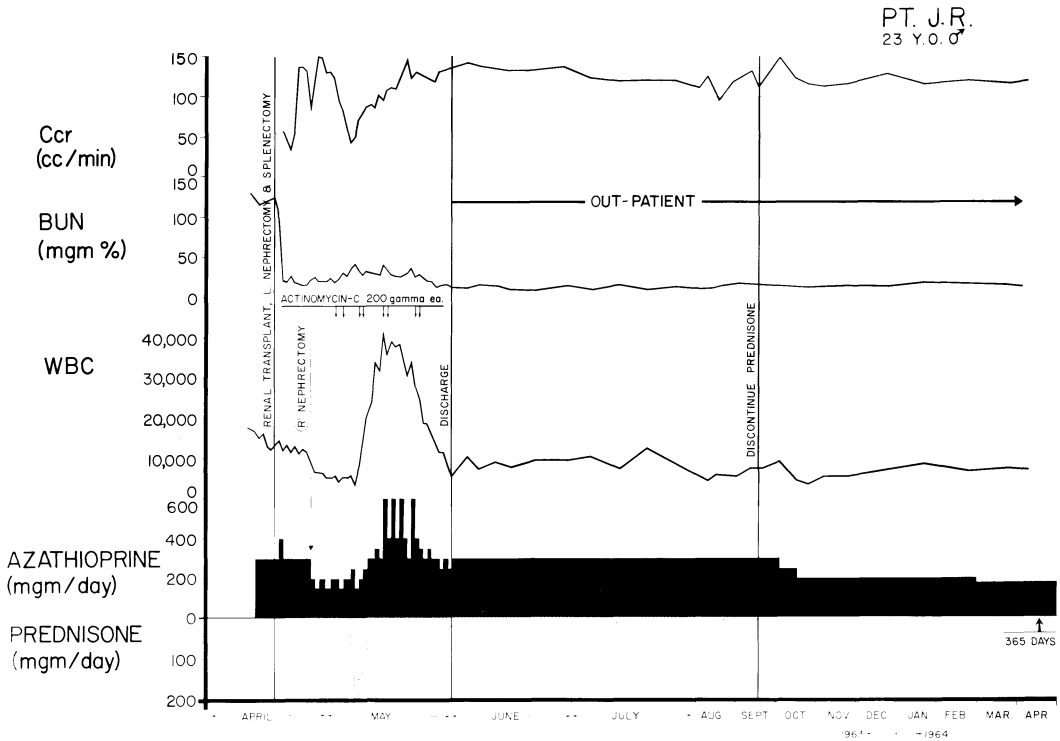


Figure 77. Course of a patient (LD 6) who received a homograft from his younger brother. Onset of rejection occurred despite treatment with azathioprine, and was reversed after the addition of prednisone and actinomycin C. Note subsequent return to maintenance therapy solely with azathioprine in gradually decreasing doses. (By permission of *Ann. Int. Med.* 61: September, 1964.)

is brought under control, the therapeutic regimen can be relaxed, and improvement in renal function continues despite a progressive reduction in immunosuppressive therapy (Fig. 77). The amount of azathioprine necessary usually decreases to a level which is near or slightly higher than the ultimate maintenance dose (Fig. 77). Gradual reduction in the amount of prednisone and discontinuance of actinomycin C therapy become possible. The pattern of recovery, in which management becomes progressively easier, and the demonstration that an initially ineffective regimen is ultimately adequate provide the strongest testimony that adaptation has occurred and that it has occurred at an early time.

In the few patients in whom an unequivocal rejection crisis did not occur, there also was evidence of adaptation. For those patients in whom azathioprine was the only treatment provided, the dosage has been periodically adjusted downward because of the increasing ease with which white blood count depression could be produced. In addition, the two patients of this type who had

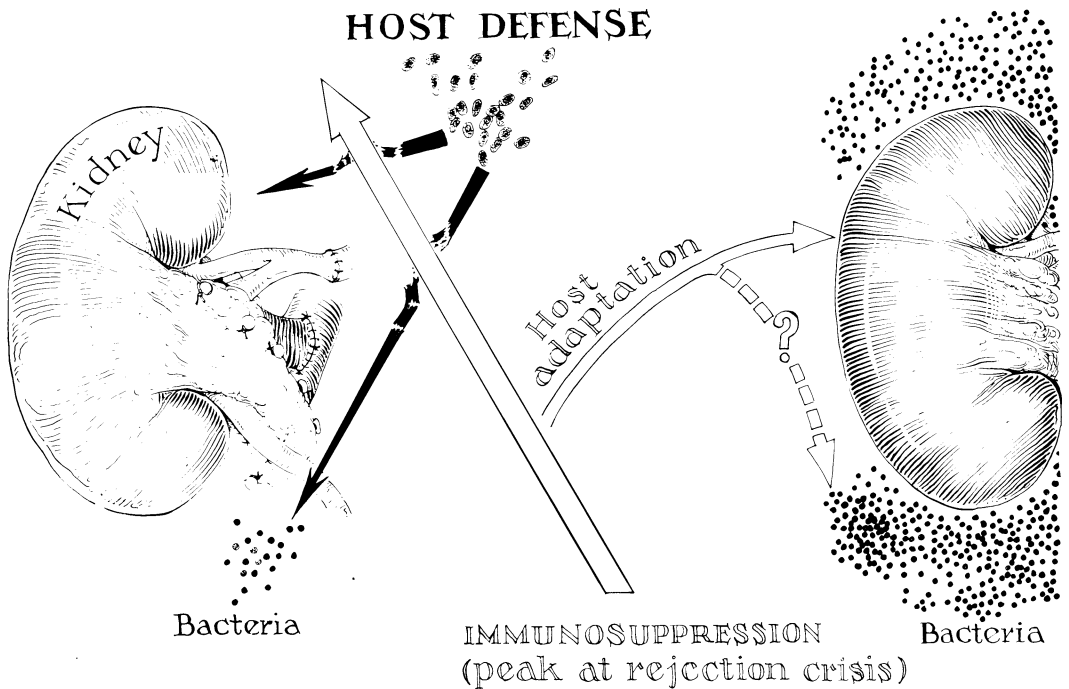


Figure 78. Possible mechanism of simultaneous loss of host reactivity to specific strains of endogenous bacteria, as well as to the alien renal tissue (see text). (By permission of *Surgery* 56:296, 1964.)

transient eosinophilia (see Figures 66 and 67 in Chapter 15) seemed to have passed through a phase of temporarily heightened immunologic activity, although renal function continued unimpaired.

Temporally, the first evidence of adaptation is 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 (Fig. 77). 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. The same adaptive phase occurred in those patients in whom temporary suppression of the white blood count was accidentally or deliberately produced. In both 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 competent against the homograft antigen and that the replacement cells had an absent or reduced memory of the alien tissue.

Because adaptation is incomplete in the early postoperative period, early reduction in immunosuppressive treatment, particularly of prednisone, may lead to recurrent rejection. When this has occurred, the second episode may be much more violent than the first. In each case in which more than one rejection

was observed, the recurrent crises were more violent than the original ones (see Figures 53 and 70). It appeared that interruption of the adaptive process caused activation of the remaining competent cell population in the manner of recall or booster immunization.

The demonstrations that adaptation occurs and that homograft protection becomes progressively more attainable later in the postoperative period are among the most important recent developments in homotransplantation. These findings indicate that the central problems of adaptation are those which occur early after operation, rather than at a later date. Prevention of rejection is not entirely dependent upon immunosuppressive agents; a key factor involves a dynamic biologic process in which the immunologic relationship between the host and graft changes rapidly. Undoubtedly, better drugs for antirejection treatment will become available, but these will be needed at maximum dosage only at the beginning of convalescence. In addition, through pharmacologic or other means it may become possible to augment the biologic changes subserving adaptation. An example of this last possibility is the current research in many laboratories which is directed toward achieving enhancement by inoculating the recipient with spleen, liver, or peripheral white cells from one or more donors.

It is probable that the state of "tolerance" which is so necessary for long-term survival of the homograft may prove to be a mixed blessing. It would be surprising if memory for other antigens were not eliminated during reversal of the rejection crisis. Thus, pockets of pathogenic bacteria could be established in a privileged and unrestrained position, unchecked by body defenses which no longer recognize them as inimical (Fig. 78)—a concept that has already received experimental support from the work of Forsen and Condie. Such specific clonal defects may constitute an immediate or delayed threat to life. As will be discussed in Chapter 19, the fatal course of some of the unsuccessfully treated cases may be explained by such a chain of events.

In addition, it must be emphasized that an early state of apparent relative tolerance is no assurance against delayed rejection. Several cases have been observed in which serious indolent rejection has appeared six to 12 months after operation (see Chapter 20).

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Chapter Eighteen

PRETREATMENT WITH PREDNISONE

In Chapter 14, the experimental evidence was considered favoring the employment of azathioprine in a pretreatment regimen. In almost all the first 45 cases in the living donor (LD) series we attempted to use only this pharmacologic agent in the early postoperative period as well, reserving administration of prednisone, actinomycin C, and local irradiation for the specific indication of rejection. There were several reasons for this practice. First, it was desirable to obtain information concerning the efficacy of azathioprine without the superimposition of other pharmacologic factors. Second, the benefit of the secondary drugs could be assessed only in a situation in which primary therapy with azathioprine had already proved inadequate. Third, only by the graded use of drugs was it possible to demonstrate clearly the presence and timing, first of rejection and then of host-graft adaptation. Fourth, it was not at first evident if adaptation would occur if completely successful efforts were made to prevent the rejection crisis. Finally, the use of potentially dangerous doses of steroids for periods during the early convalescent phase when they did not appear to be needed did not seem warranted. The last argument seemed especially pertinent inasmuch as the time of onset of the rejection crisis was so variable, and because an occasional patient never required any treatment with prednisone.

Many of the most forceful of the foregoing reasons for withholding steroid therapy until graft repudiation had clearly started were for the purpose of delineating the features of rejection and adaptation, and defining the influence of drug therapy upon these processes. From a clinical point of view, this information is now nearly complete, as has been described in the preceding four chapters.

Attention is now directed to the prophylactic use of steroids in a pretreatment regimen comparable to that already described for azathioprine. With this modification of management prednisone is started one to three days before operation in large doses and continued thereafter in slowly decreasing amounts. The comments concerning this use of steroids are based upon a clinical study of 19 cases at the University of Colorado which began after De-

Table 15. Genetic Relationship of Donors in Steroid-Pretreated Series*

	Living 6-1-64	Died
Mother	2	1
Father	2	0
Sibling	6	0
Aunt	1	0
Uncle	1	0
Cousin	1	0
Nonrelated	2	3
Total	15	4

*Operations were performed from December 10, 1963, to March 30, 1964.

In two patients, first homografts failed within one week for technical reasons, one because of a problem which was due to anomalous venous drainage and the other because of secondary wound hemorrhage. In the above tabulation, the second or definitive donor is listed. See appendix for details.

cember 10, 1963. Since that time other aspects of therapy, including pretreatment with azathioprine, have remained the same as before. Furthermore, the genetic relationships of the donors were quite comparable (Table 15) to those of the prior series. Preliminary results have shown a slight improvement of survival within the available period of follow-up, due to reduction in that early mortality which has plagued efforts at renal homotransplantation. Fifteen of these 19 patients are still alive on June 1, 1964, from 61 to 148 days after operation. Four (LD 46, 57, 59, and 61) died after 43, 76, 45, and 36 days, respectively (see appendix, Chapter 28).

Pretreatment with azathioprine was started first. Twenty-four to 72 hours preoperatively, a course of 3 to 5 mgm/kgm per day prednisone (or intramuscular prednisolone) was instituted (Figs. 79 to 82), divided in six-hourly fractional amounts. Care was taken to avoid the inadvertent omission of doses on the day of operation. Fifteen to 30 minutes before revascularization of the homograft, an additional intravenous infusion of 50 mgm prednisolone was given.

After operation, steroids were continued and reduced in amount every five or 10 days so that a dose level of 45 to 60 mgm per day had been reached in most adults after 30 to 40 days (Figs. 79 to 82). After this time, the dose schedule was carefully reduced to maintenance levels in the same general way as in the earlier series (see Chapter 16).

Local irradiation of the homograft and intravenous actinomycin C were

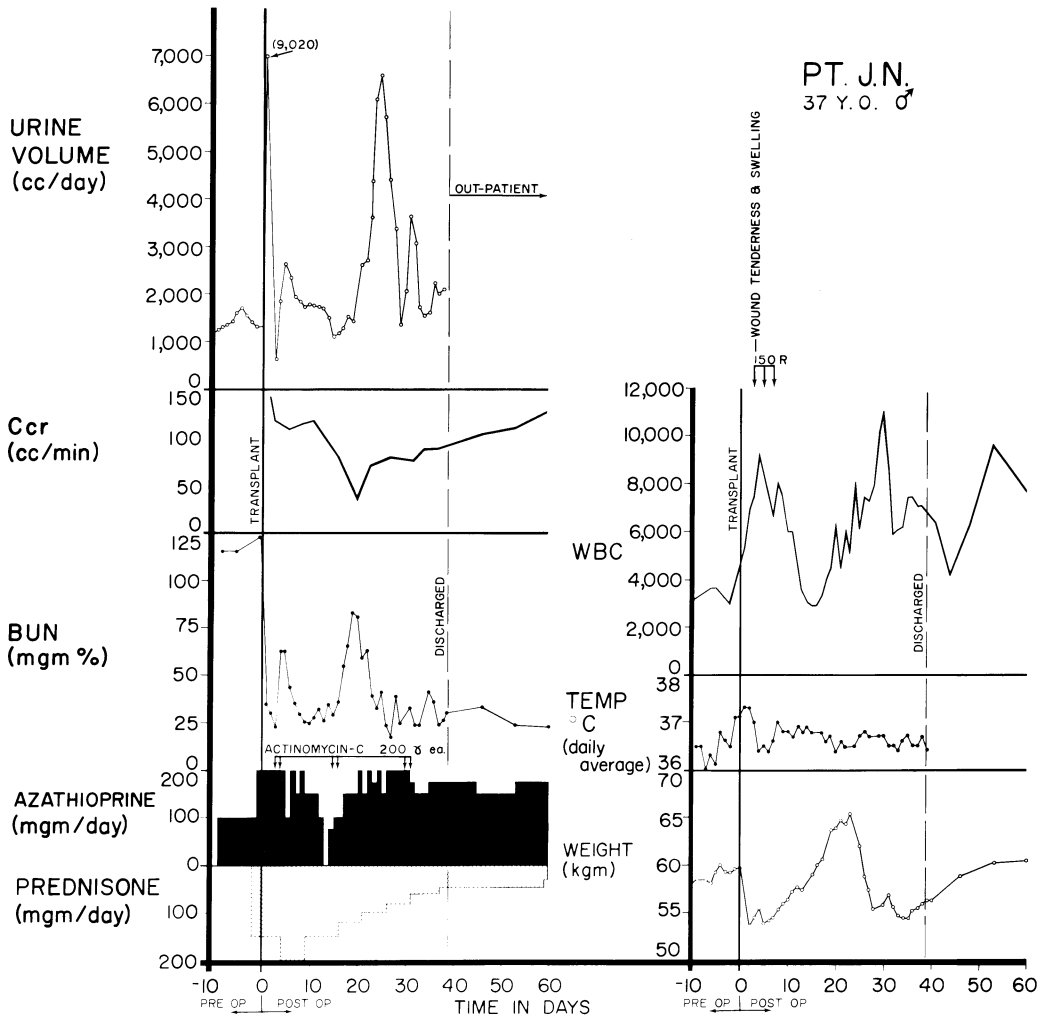


Figure 79. Use of steroid pretreatment in patient (LD 47) who received a homograft from his brother. Both were A+. Three days after operation, acute wound tenderness, hematuria, and anuria (for six hours) developed. Immediate relief followed the first dose of local irradiation. Restoration of function was so rapid that creatinine clearances based on 24-hour urine collection had only slight depression, although azotemia is evident. Second rejection was at 14 days with recurrence of renal failure, weight gain, and temporary depression of white count. The steroid dose was not increased to the previous high level, despite which reversal followed with a brisk secondary diuresis. Note complete absence of fever. (By permission of *Ann. Int. Med.* 61: September, 1964.)

added to the azathioprine-steroid base therapy when rejection was suspected. It was usually unnecessary to secondarily increase the steroids at this time, even though the dose had frequently been reduced considerably from the high levels employed before and immediately after operation (Fig. 79).

An increased incidence of complications did not seem to follow this use of prednisone. There were no infections in the operative wounds, although one patient (LD 57) sustained a third degree burn to the entire lower half of her

abdomen from a hot water bottle. Infection of this area was probably the initiating factor in her death (Chap. 19). Three others (LD 46, 59, and 61) had nonsurgical infectious complications which either caused or contributed to their deaths. Another (LD 51) developed moderately severe gastrointestinal hemorrhage which did not require operation. The incidence of cosmetic deformity and steroid diabetes was comparable to that encountered with the delayed use of prednisone. The total quantity of prednisone administered was approximately the same as in the earlier cases.

EFFECT UPON THE REJECTION CRISIS

An unequivocal rejection episode occurred in nine patients who received steroid pretreatment. In one patient, this occurred after her discharge from the hospital, and resulted in the rapid development of anuria (Fig. 80). The patient died from the syndrome of acute secondary renal failure, drug toxicity, and sepsis which is described in the following chapter. Another, a six-year-old girl who received a maternal homograft (LD 61), had rejections after three, 14, and 25 days with terminal anuria and leukopenia. The severity of rejection varied in others (LD 47, 50, 56, 57, 59, 62, and 64), and was an important factor in the death of only one (LD 59) who succumbed with pneumonia and a lung abscess which developed before the onset of a rejection episode at 37 days. Fever was completely masked in all the patients except three who died with septicemia or pneumonia (LD 57, 59, and 61).

Rejection crises were indefinite in five of the remaining 10 patients. In these five cases, the evidence on which the diagnosis was made was flimsy, being based on very minor depressions in creatinine clearance, the temporary appearance of oliguria, small rises in BUN, slight tenderness in the transplant wound, or a persistent accentuated hypertension. If any of these findings were present in conjunction with a tendency to fluid retention or with a fall in urinary sodium concentration, a course of local transplant irradiation or intravenous actinomycin C was immediately administered. The high index of suspicion undoubtedly resulted in overtreatment in some instances, inasmuch as secondary therapy was given to some patients in the absence of any measurable change in creatinine clearance or BUN. An example is shown in Figure 81. Transplant tenderness, relative oliguria, and low urinary sodium excretion were present. The tenderness was immediately relieved, and the other findings were reversed.

In addition to clouding other features of rejection, the steroid pretreatment detracted somewhat from the diagnostic value of daily BUN determinations. Heightened catabolism usually resulted in incomplete resolution of the preoperative azotemia. Postoperative BUN levels tended to be stabilized at 25 to 50 mgm per cent despite high creatinine clearances (Figs. 79, 81, 82) and in the presence of daily urea excretion of as high as 60 gm. Later, as the steroid doses were reduced, BUN dropped to within normal ranges.

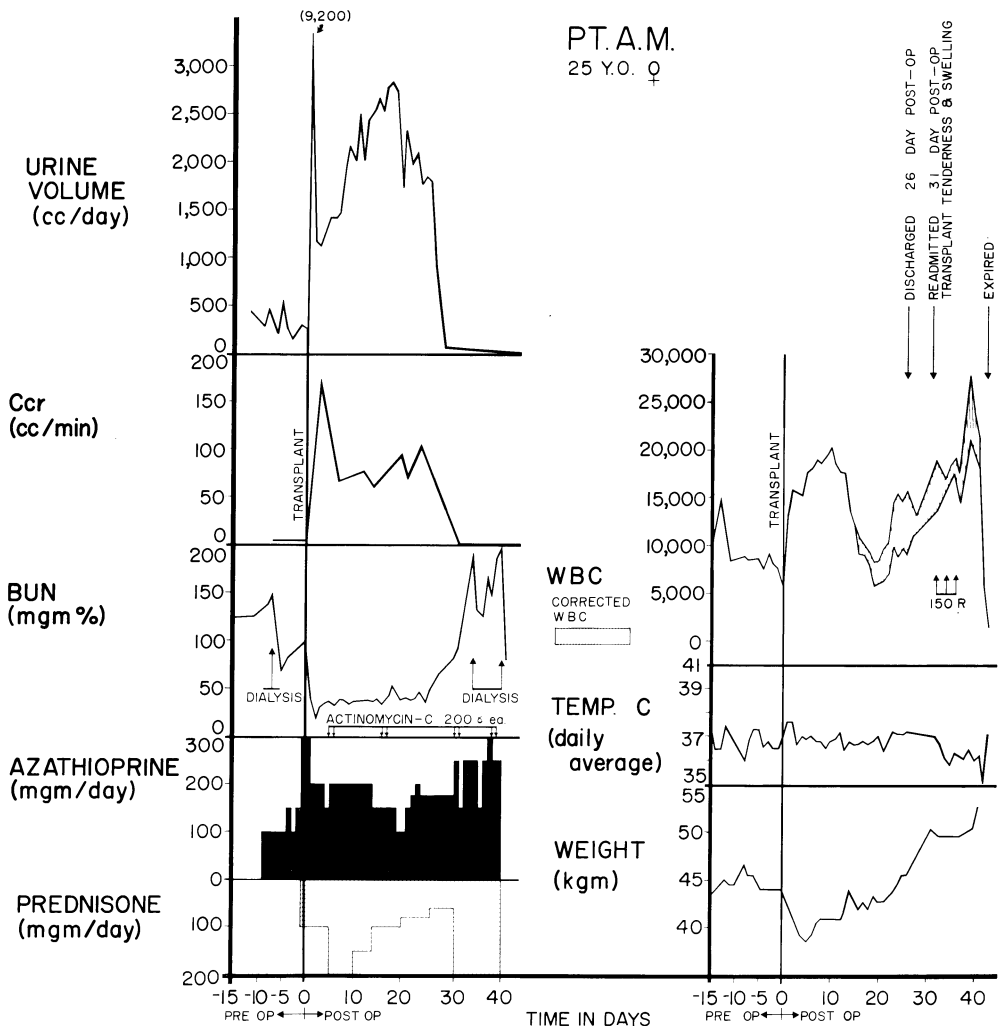


Figure 80. Abrupt rejection after 31 days in patient who had received steroid pretreatment. She died of sepsis after 10 days of anuria. She was never febrile. The recipient (LD 46) received the homograft from her husband. Both were O blood type.

In five other cases, no evidence whatever of rejection has ever appeared (LD 49, 52, 58, 60, and 63) during the follow-up (to June 1, 1964) of 61 to 104 days. The withdrawal of steroids has proceeded at the same rate (Fig. 82) as in those cases with modified or overt rejection described earlier.

INCIDENCE OF AZATHIOPRINE TOXICITY

It is pointed out elsewhere (see Chapters 14 and 19) that poor renal

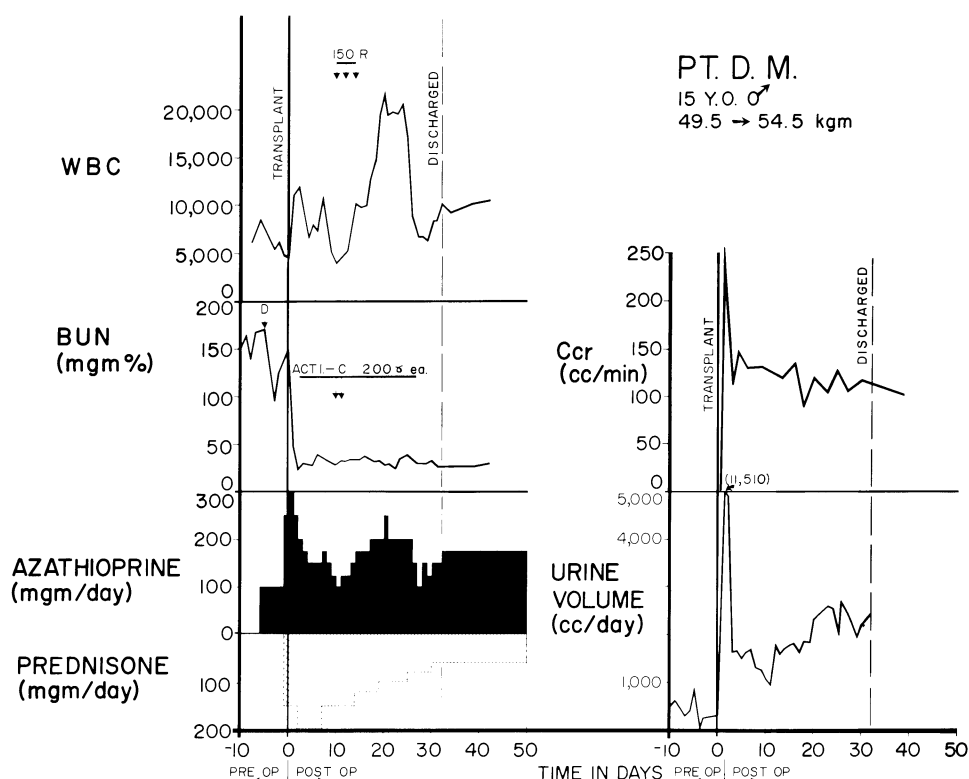


Figure 81. Use of secondary therapy in steroid pretreatment group, despite unequivocal evidence of rejection. The principal indication for actinomycin C (Acti C) and local irradiation was tenderness in the transplant site which developed at the same time as relative oliguria. Note that these findings were unaccompanied by changes in BUN and Ccr. The patient's (LD 53) discomfort was immediately relieved after x-ray therapy.

function is an important factor in predisposing patients to toxicity from azathioprine. Similar bone marrow depression and leukopenia were encountered in the steroid pretreatment series in the two patients (LD 46 and 61) who had the severest rejections. In those patients who did not have overt rejection and in those in whom the crises were easily manageable, precipitous drops of the white blood count were not seen.

MORTALITY

Four of the 19 patients are dead after follow-up of two to five months (Table 15). Three died during the first two postoperative months. In comparison, 11 of the first 45 patients, managed in all but two instances without steroid pretreatment, died within the first eight postoperative weeks. The alteration in use of immunosuppressive drugs does not appear, therefore, to have exerted a profoundly beneficial influence during the early phase of greatest risk. Despite the absence of convincing evidence, it has seemed to us that the

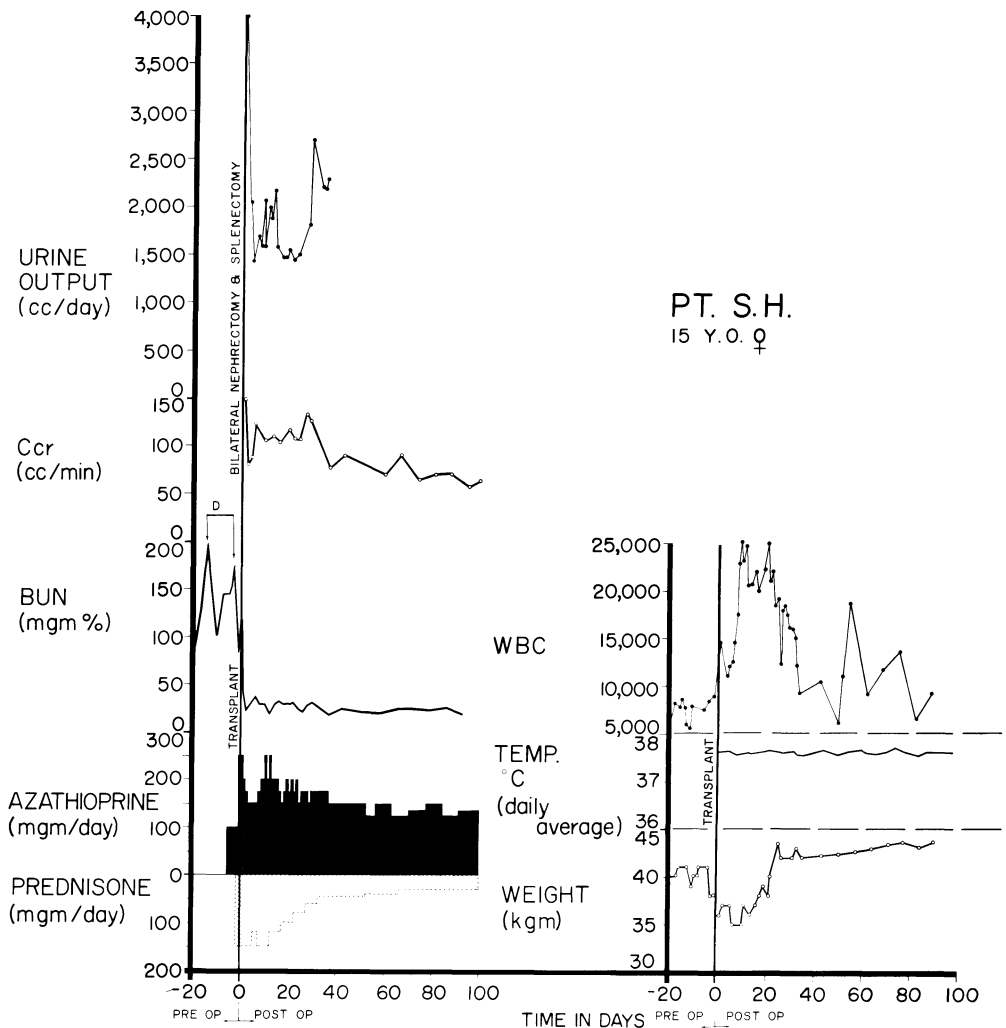


Figure 82. Absence of rejection in a 15-year-old girl (LD 52) in the group pretreated with steroids who received a homograft from her father. Both were O+ blood type. The operation was done on February 17, 1964. A slight but progressive diminution in creatinine clearance is evident, but the significance of this is not yet known.

difficulties of patient care have been lightened since this change in therapy was instituted. The worth of the prophylactic use of prednisone will, however, require much additional evaluation.

ADAPTATION

The establishment of host-graft nonreactivity cannot be documented with the clarity described in the earlier patients who were initially treated only with azathioprine. Nevertheless, there is hope that the same sequence of events will occur with the steroid pretreatment program. Withdrawal of prednisone

was accomplished as easily in the latter patients as had been possible in the earlier series, so that the total quantity administered during the first few months after operation proved to be no greater (Figs. 79, 81, 82). Furthermore, those patients followed for as long as five and a half months have not had an increased incidence of late complications.

Nevertheless, it is important to withhold judgment concerning the effect of prophylactic steroids upon host-graft adaptation. More than one rejection was recorded in six of the 19 patients in this series, compared to only two (or possibly three) in the earlier series of 45 (see appendix, Chapter 28). The explanation for this may be related to the ambiguity with which rejection presents in the patient receiving prophylactic steroids. Since many of its features are blurred by the therapy being used, there is a heightened sensitivity to small changes which previously might not have been considered sufficient to make the diagnosis. These minor alterations in renal function or in the patient's clinical state may not actually have been due to rejection, even though they were treated as such.

Chapter Nineteen

CAUSES OF FAILURE

In the analysis of failures, the essential information is given in tabular form for each patient treated before March 30, 1964 (Table 16), excluding those treated with cadaveric kidneys and heterografts. The latter two groups are accounted for separately in Chapters 8 and 23, respectively. All patients who died before June 1, 1964, are listed. Additional details of the individual cases can be obtained from the appendix (Chapter 28). In this chapter an attempt is made to identify in general terms recurrent clinicopathologic patterns which defeated attempts at treatment; therefore the principal emphasis in the following remarks is on those causes of death which were met repeatedly.

As has been made clear, the convalescence of patients who have received a renal homotransplant is not an easy one. The events of rejection and the heroic program of therapy necessary to prevent destruction of the homograft impose a period of great risk which lasts several months after operation. It has been pointed out that young patients (see Table 2, Chapter 3) and those receiving kidneys from familial donors (see Tables 4 and 15, Chapters 5 and 18) have a greater chance of surviving this dangerous interval than older people and those who have been donated homografts from genetically unrelated persons.

Although there are differences in the risk of certain classes of recipients, the steps to recovery are qualitatively similar in almost every case, there being variations only in degree. Thus maternal-to-offspring transplants usually have a more temperate rejection crisis which is more easily treated but is otherwise like that encountered with less favorable genetic combinations. In all groups, moreover, most of the deaths and complications either have occurred in this early postoperative period, or the events leading to later mortality apparently have had their inception at this time.

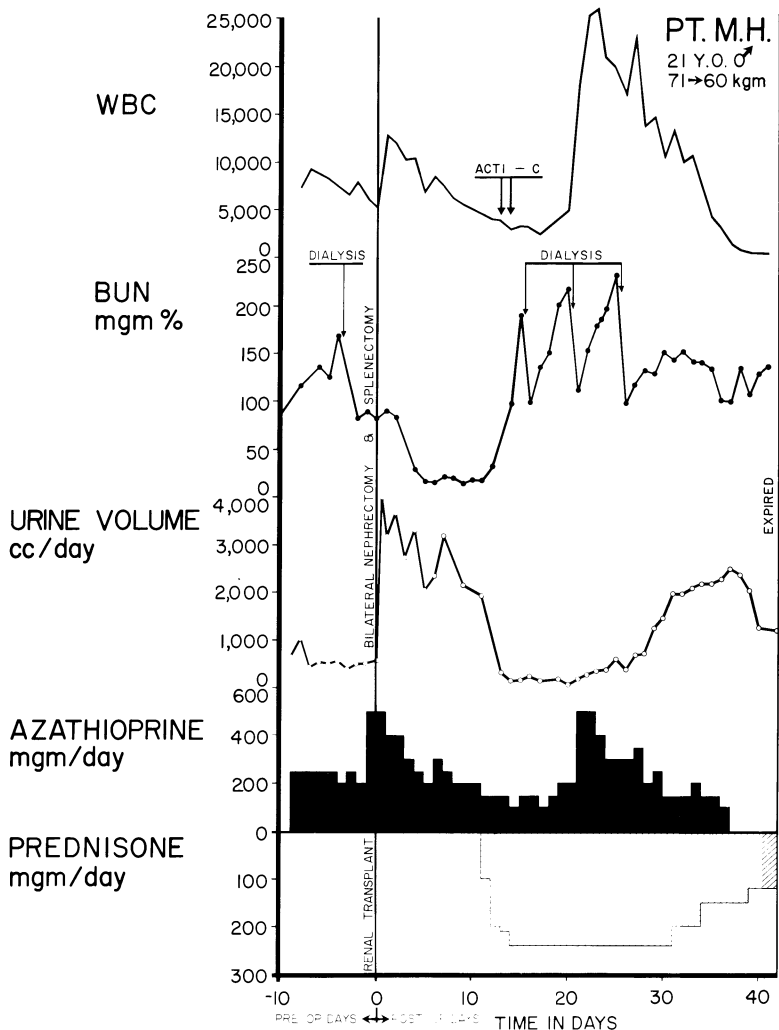


Figure 83. Typical unsuccessfully treated case (LD 32). The donor and recipient were brothers, both of A+ blood type. A violent rejection crisis followed good early function, and anuria developed which lasted two weeks. Although the rejection was reversed and a secondary diuresis began, the patient died from drug toxicity, leukopenia, and septicemia. Acti C—Each arrow is 200 μ g intravenous actinomycin C. (By permission of *Surgery* 56:296, 1964.)

EARLY MORTALITY FROM DRUG TOXICITY

The most frequent cause of failure was the induction of drug toxicity during the effort to treat a rejection crisis. Twelve deaths have been observed under these circumstances. The clinical course of one such patient is shown in Figure 83, and various other examples are depicted in other chapters. In all but one of these cases, the homografts provided excellent immediate function. The vigor of the subsequent rejection process was frequently greater than usual. In some instances, the disquieting picture of decreasing urinary volume, falling creatinine clearance, and mounting azotemia provoked ill-advised

increases in drug dosage to a dangerous level; in others, however, the therapeutic regimen employed was not thought to be predictably excessive, even upon retrospective analysis.

In such unsuccessfully managed cases, the acute renal failure of the rejection crisis was accompanied by rapidly developing leukopenia (Figs. 80, 83) and often by thrombocytopenia as if the patient had undergone a sudden change in sensitivity to azathioprine. Within a few hours or days, the onset of fever almost always signaled the beginning of inexorable deterioration which usually terminated with fatal pneumonia or septicemia or both (Table 16). Four patients who were dialyzed in the presence of white blood counts of 3,000 or lower developed septicemia almost immediately after treatment, and all died shortly thereafter. The infecting organisms in such cases are listed in Table 16.

In view of the intensity of the immunosuppressive therapy necessary for control and reversal of the rejection crisis, it is not difficult to appreciate the inherent danger of this phase of recovery. That homograft function could be retained at all is perhaps the strongest indication of the extent to which total body immunologic defenses have been weakened. In addition, loss of reactivity to various test antigens (see Chapter 22), the inconstant development of hypogammaglobulinemia (see Chapter 21), and the appearance of steroid diabetes are all indicators of the host's imperiled state.

The aforementioned general factors may be present to some degree in every case. Nevertheless, it was commonly the additional specific components of bone marrow depression and leukopenia which appeared to precipitate the beginning of a fatal infection. Although all such cases must be classified as errors of azathioprine dose control, it should be emphasized again, as was described in Chapter 14, that the effect of this agent becomes highly unpredictable in the presence of poor renal function which accompanies a rejection crisis, possibly because azathioprine has a partial renal pathway of detoxification. Whether this is the entire explanation is not certain, although it seems clear that the leukopenic effect of this drug is intensified at the time of acute renal failure. Thus, a patient with an unusually severe rejection crisis is most vulnerable to toxicity, even though this is the kind of case in which exact therapy with azathioprine is most needed. Consequently, the rejection crisis itself has often seemed to be the initiator of fatal drug toxicity. It might be reasoned that aggressive dialysis therapy under such circumstances would be remedial; in practice, however, hemodialysis at this time has, on at least four occasions, seemed to be the precipitating cause of terminal sepsis.

Exact classification of the cause of death in this kind of patient is often difficult because of the multiplicity of factors which invariably have been present. Thus, death cannot be accurately ascribed to rejection in many instances, since several patients had passed through the worst phase of renal failure and had improving homograft function (Fig. 83). Likewise, sepsis, which almost always supervened late in the course, appeared to be a complication of the drug toxicity. Finally, the development of drug toxicity seemed to be initially precipitated by the rejection.

Table 16. Principal Factors in Mortality in Living Donor (LD) Series

Case No.	Age	Drug Toxicity*	Died during Rejection Crisis	Died after Rejection Crisis	Infection	Organism	Time Survival (days)	Other Factors in Mortality
4	35			X	Subphrenic abscess, septicemia, pneumonia, mediastinitis	<u>Staphylococcus</u> , <u>Pseudomonas</u>	113	
5	50	X	X		Mediastinitis, septicemia	<u>Staphylococcus</u> , <u>Pseudomonas</u>	11	Cardiac arrest; thoracotomy during transplantation
7	25			X	Transplant wound, septicemia	<u>Staphylococcus</u> , <u>Pseudomonas</u>	79	
8	29				None		62	Cause of death not found
9	30			X	Brain abscesses, septicemia, pneumonia	<u>Pseudomonas</u> , <u>Candida albicans</u> , <u>Candida kruzei</u> , <u>Nocardia</u>	207	GI hemorrhage
10	47		X (Late)		Pneumonia	<u>Klebsiella-Aerobacter</u>	295	Old pulmonary emboli
**11	35	X	X		In old nephrectomy wound	<u>Staphylococcus</u> , <u>Pseudomonas</u> , <u>Proteus</u>	24	
16	45			X	None		83	Acute and chronic pancreatitis
19	17			X	Pneumonia	<u>Pneumocystis carinii</u>	****95	
20	6			X	None		202	Stroke two months before death; no autopsy
21	41			X	Brain abscesses, pneumonia	<u>Candida stellatoidea</u> , <u>E. coli</u>	76	
24	42	X	X		Septicemia	<u>Pseudomonas</u> , <u>Proteus</u>	37	
26	16				None		1	Died during diuresis of electrolyte imbalance
28	49	X	X		Septicemia, tuberculosis	<u>E. coli</u> , <u>M. tuberculosis</u>	25	Jejunal necrosis; pancreatic abscess; pulmonary emboli
29	40	X	X		Septicemia	<u>Paracolon</u> , <u>E. coli</u> , <u>Pr. mirabilis</u>	**18	

**31	54	X	X	Pneumonia	<u>Pseudomonas</u>	15	Pulmonary emboli
**32	20	X	X	Myocarditis, pyelonephritis, septicemia	<u>Candida albicans, E. coli</u>	41	
35	38	X	X	Pneumonia, septicemia	<u>Pseudomonas</u>	**48	
38	21		X	Pneumonia	Unknown	38	Superficial wound dehiscence: wasting; pulmonary edema
**	***43	25	X	Pneumonia, fungus pyelonephritis, septicemia	<u>Aspergillus fumigatus, Candida albicans, Streptococcus faecalis</u>	48	
**46	25	X	X	Septicemia	<u>Staphylococcus, Diplococcus</u>	43	
57	36		X	Pneumonia, third degree burn lower half of abdomen	<u>Pseudomonas</u>	****76	Extensive burn from hot water bottle
59	48	X	X	Pneumonia, lung abscess	<u>Klebsiella-Aerobacter</u>	45	
61	6	X	X	Septicemia	<u>E. coli</u>	36	Ureteral obstruction

*Bone marrow depression.

**Post-transplant dialysis required one or more times.

***This patient was reported as having tuberculosis (Surgery 56: 296, 1964). Re-examination of the cultures cast serious doubt on the diagnosis.

****These four patients each received two homografts. The time of survival is given from the second homotransplantation for LD 19 since the first homograft was immediately removed. For the others (LD 29, 35, and 57), survival is from the time of the first homotransplantation. Details are given in the appendix (Chapter 28).

Because immunosuppressive therapy was employed differently in the last 19 patients, it is important to note that the uncontrollable chain of events described above was also observed, although not so frequently. Two (LD 46 and 61) of the four deaths in the group who had pretreatment with steroids were accompanied by all the features of this syndrome (see Figure 80, Chapter 18). A third (LD 59) had all the features but leukopenia.

FAILURE FROM USE OF DAMAGED HOMOGRAFTS

The influence of adequate renal function upon the effectiveness of cytotoxic drugs was stressed in the preceding section. With the use of homografts that do not function well from the onset as a result of ischemic injury, the problems encountered with azotemia and unpredictability of drug action are present from the earliest postoperative period, rather than beginning later at the time of the rejection crisis. Four patients died after receiving homografts which functioned poorly or not at all. In three of these cases, cadaveric kidneys had been employed.

The fate of patients receiving cadaveric kidneys is fully described in Chapter 8. All died of a combination of unrelieved uremia, drug toxicity, and sepsis, the syndrome being similar to but earlier in development than that described in the preceding section. No urine excretion from the homograft was ever obtained in two cases, and function was poor in the third.

In one patient who received a homograft from a living donor, a 71-minute period of ischemia resulted from the necessity for treating a cardiac arrest which occurred during a double arterial anastomosis (see Figure 61, Chapter 14). Poor initial renal function, and later drug toxicity and sepsis, were the events leading to death. Another patient received a nonfunctioning homograft which had been subjected to 85 minutes of devascularization. A second well-functioning homograft was later placed (LD 29), despite which early death resulted from sepsis. In the latter case, the cause of death is classified according to the course after the second procedure (Table 16), although failure of the initial homotransplant was undoubtedly an important contributory factor.

DELAYED MORTALITY FROM EARLY COMPLICATIONS

In a second group of seven patients (LD 4, 7, 9, 16, 21, 38, 57), a rejection episode was successfully reversed despite which death occurred 48 to 207 days after operation (Table 16). In these cases, the ultimately fatal complications were thought to have originated during treatment of the preceding rejection crisis. An example is shown in Figure 84 of a 25-year-old man who received a homograft from his wife (LD 7). A ureteral fistula developed, and one week

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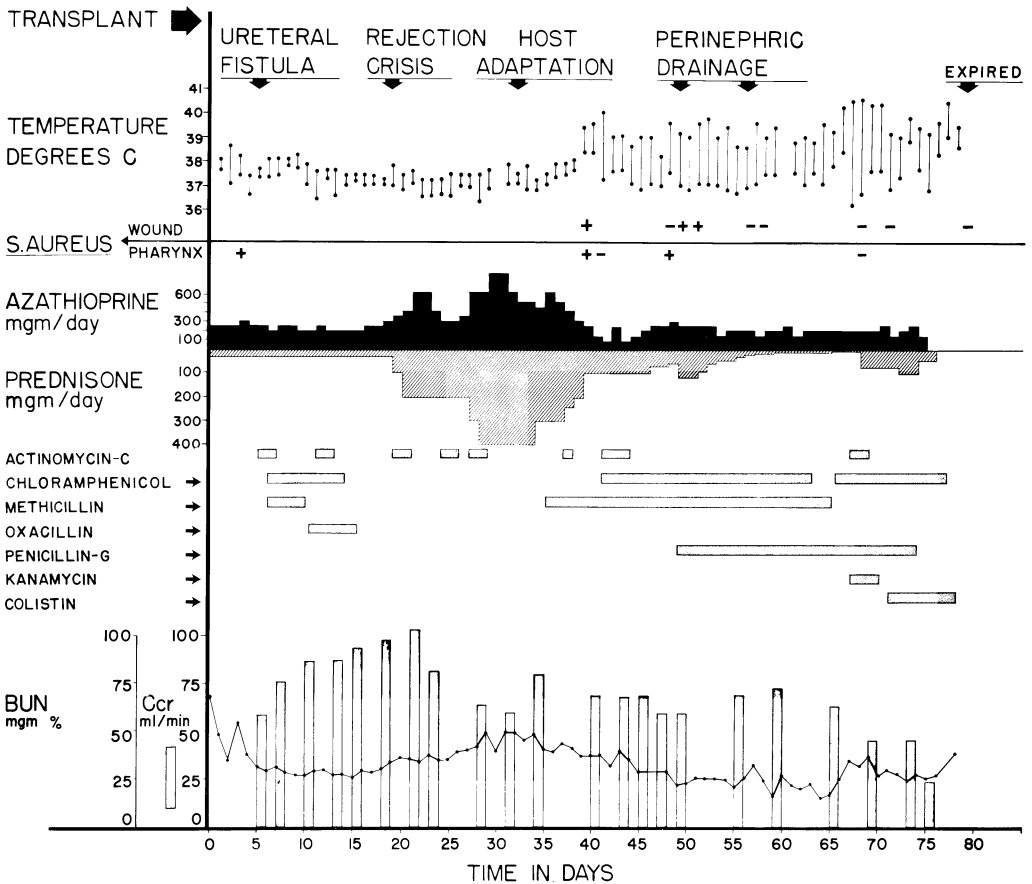
RENAL HOMOGRAFT STAPHYLOCOCCUS AUREUS
PERINEPHRIC ABSCESS

Figure 84. Delayed death from an early complication (LD 7). The ureteral reimplantation, which was necessitated by the development of a urinary fistula, seemed to have been successful until just after reversal of rejection. At this time, fever appeared due to staphylococcal infection around the homograft. The organisms were found throughout the tissues, but cellular response of host cells was lacking, resulting in an unrestrained cellulitis, which was uninfluenced by surgical drainage or antibiotics. Septicemia terminated the course. The simultaneous reversal of rejection and development of uncontrollable sepsis suggests that loss of reactivity to bacterial and renal tissue antigens may have occurred simultaneously. (By permission of *Surgery* 56:296, 1964.)

after operation ureteral reimplantation was required. After the second operation, the surgical wound apparently healed without infection. However, at the peak of the rejection crisis, fever and toxemia developed as a result of a peritransplant infection. The wound was widely drained. *Staphylococcus aureus* was found in the tissues, but there was no pus or other evidence of active cellular reaction against the pathogenic organisms. The patient ultimately died of septicemia. Another patient (LD 4) died almost four months after transplantation from a subphrenic abscess which had apparently had its inception at the time of laparotomy for intestinal obstruction three months previously (see Figure 15, Chapter 6).

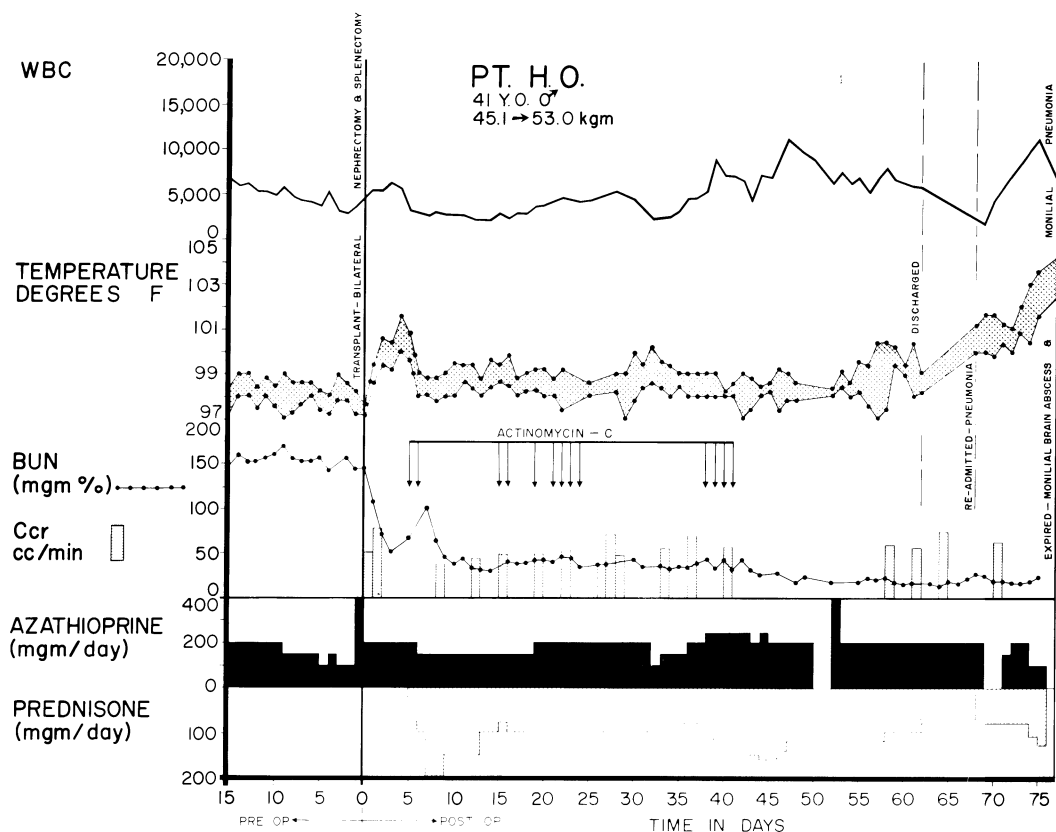


Figure 85. Course of patient (LD 21). A rejection crisis at four days was characterized by fever, azotemia, and a fall in Ccr. After reversal, good renal function was maintained until death, despite which bizarre behavior was manifested for almost the entire postoperative course. Terminally, the patient became febrile. At autopsy, fungus brain abscesses were present which probably had become established at a much earlier time.

Of particular interest were two cases (LD 9 and 21) in which death occurred 76 and 207 days after operation. Recovery had seemed to be without serious incident, although both patients developed headache, behavioral disturbances, and unexplained neurologic disability just after reversal of a rejection episode (Fig. 85). Fungus brain abscesses and a mixed fungal bacterial pneumonia were found at autopsy. These complications probably originated at the time of reversal of rejection, although they did not become overt until months later. A similar course was followed by a patient (LD 57) who sustained an extensive thermal burn of the lower abdomen three and a half weeks post-operatively. Although rejection was controlled, the granulating areas became infected with *Pseudomonas*. Ultimately, she died of *Pseudomonas* septicemia and pneumonia.

A possible explanation for these late septic deaths was suggested in Chapter 17 in the discussion of the concept of host-graft adaptation during and after the reversal of rejection. It was pointed out that loss of reactivity to other than homograft antigens was a distinct possibility. It is conceivable that de-

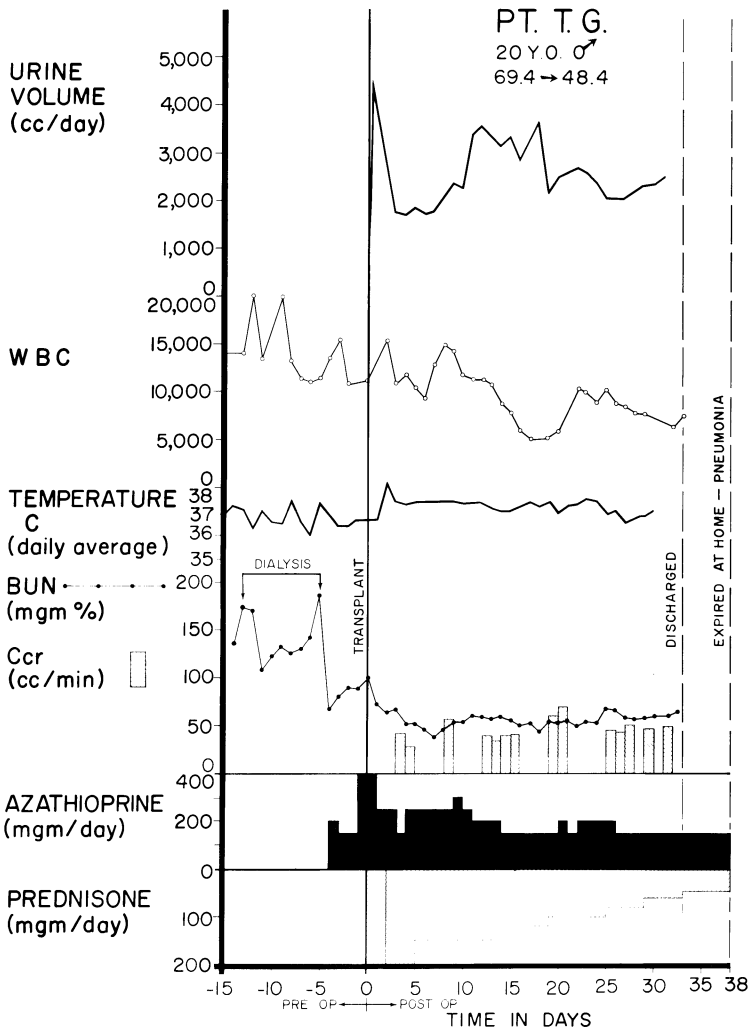


Figure 86. Course of patient (LD 38) who died of wasting after two-stage renal homotransplantation. Splenectomy and bilateral nephrectomy were performed three weeks before the homografting procedure. Steroids were started a few hours after homotransplantation because of fever and sluggish early diuresis. He lost 17 kgm (37.4 pounds) in the 38 days of survival. A superficial wound dehiscence had no tendency to heal. Pneumonitis was the terminal event.

layed infections, such as those described, were rendered uncontrollable by the development of host tolerance to the specific infecting microorganisms. It will be of great practical, as well as theoretical, interest to determine in future investigations whether such delayed infectious complications are due simply to the suppression of greatly weakened but balanced host defenses, or if there is the establishment of relatively specific clonal deficiencies which permit selective overgrowth of certain organisms.

A sixth patient (LD 16) died of acute and chronic pancreatitis 83 days after homotransplantation, a complication which was thought to have been steroid-induced during treatment of rejection (see Chapter 12).

A seventh patient, age 21, died of pneumonia 38 days after receipt of a homograft from his mother (LD 38). He had been treated in infancy with excision of congenital urethral valves, and later with bilateral ureterovesical reimplantation, but had been left with chronic pyelonephritis and hydronephrotic kidneys. At a first stage, bilateral nephrectomy, complete ureterectomies, and splenectomy were performed. Homotransplantation three weeks later was uncomplicated. He passed through a relatively mild rejection crisis but continued to lose weight (Fig. 86). After one month, superficial dehiscence of the transplant wound was noted, with no subsequent evidence of healing. Various measures to improve nutrition were unsuccessful. At autopsy, there were no distinctive features except profound wasting. There was slight pulmonary edema and nonspecific pneumonitis from which pathogenic organisms were not cultured. Intranuclear inclusion bodies were found in the alveolar cells, as commonly noted in the lungs of patients who have died after homotransplantation (see Chapter 21).

LATE REJECTION

One patient (LD 10) died 295 days after homotransplantation of a late and poorly controlled rejection. This problem is considered in detail in Chapter 20.

MISCELLANEOUS CAUSES OF DEATH

A 16-year-old girl died 18 hours after operation from electrolyte imbalance during the acute postoperative diuresis.

Three patients died of unknown causes. One was a six-year-old boy of O+ blood type (LD 20) who had received a kidney from his A-mother—a violation of blood type compatibility which usually has been unacceptable (see Chapter 6). After a stormy postoperative course, fair renal function was ultimately attained (see Figure 60, Chapter 14). Four months postoperatively, the patient developed convulsions and coma from which he never aroused. Death occurred 202 days after operation, but renal failure was not a factor. Permission for autopsy was denied.

Another unexplained death (LD 8) occurred in a 29-year-old man 62 days after he had received a kidney from his younger brother. He had passed a rejection episode without unusual difficulty. He spent the day before death in the mountains with his family; after returning, he abruptly developed convulsions and coma, and died 24 hours later. At autopsy, no cause could be established. Numerous diagnostic studies during life and postmortem toxicologic studies were nonrevealing.

An additional patient (LD 19) died of a pneumonic process which had a number of unique features and which is described fully in Chapter 21.

Chapter Twenty

LATE RESULTS AND COMPLICATIONS

Until very recently, opportunities to study the chronic function and fate of renal homografts were rare for the simple reason that survival of more than a few weeks after homotransplantation was truly exceptional. When Goodwin and Martin collected results of the world experience with this operation in the spring of 1963, it was found that 90 per cent of all patients treated with renal homografts died within the first two months. This unfavorable statistic had already improved when the results were reviewed in September, 1963, by Murray and the other participants at the National Academy of Sciences Transplantation Conference in Washington, D. C., at which time 30 patients were reported with survival exceeding six months.

Despite the bleakness of the collective figures, a remarkable change in the early postoperative outlook has taken place at several institutions during the last two years. By personal communication in May, 1964, it was learned that Murray, Hamburger, Hume, and Kolff each were following a half dozen or more patients who were living six or more months after transplantation. Of the first 64 patients at the University of Colorado Medical Center, treated from November, 1962, to March 30, 1964, with homografts obtained from living volunteer donors (LD series) 47, or 74.4 per cent, survived more than two months; and of this group, 40, or 62.5 per cent, were still alive on June 1, 1964 (see appendix, Chapter 28). As of June 1, 1964, 30 of these patients have survived transplantation more than four months—25 more than six months and five for a year or more. Thus the first hurdle of the transplantation problem has been overcome—at least partially—in that relatively protracted homograft function can be attained in considerably more than half the cases.

With this change has come a shift in responsibility for those institutions performing clinical investigation in renal homotransplantation. It has now become essential to systematically obtain data which will help those institutions to plan maintenance care and to establish a long-term prognosis for chronic survivors before a new series of homografting procedures is begun. For this reason, a six-month delay on new cases was decided upon at the University of Colorado, beginning in March, 1964, during which time addi-

tional information could be obtained about the adequacy of late homograft function; the frequency, severity, diagnosis, and treatment of delayed rejection; and the incidence of indolent urologic and other complications. In this chapter consideration is given to the late sequelae after transplantation, with special emphasis on those patients who lived or are still living as long as four months after operation. The two identical twin recipients who have normal renal function after 26 and 11 months are not included.

MORTALITY AFTER FOUR MONTHS

Thirty-three of the first 51 patients in the LD series survived at least four months. Subsequently, three of these died. The genetic relationships of all the donors in the group are given in Chapter 28. The best results were obtained when the donors and recipients were genetically related.

In one case (LD 9), death occurred after 207 days, following an emergency operation for control of a bleeding duodenal ulcer. At autopsy, fungal brain abscesses and pneumonia were found. In this patient, whose donor was a fraternal twin (blood groups O to A), it had been necessary to use large maintenance doses of prednisone (30 to 60 mgm per day) in order to maintain stable renal function (see Figure 70, Chapter 15). In the last few days of life there was deterioration of renal function which coincided with the development of fungal and bacterial septicemia. The pathologic findings in the homograft are described in Chapter 25. The kidney, which weighed 180 gm, grossly did not appear to be badly damaged. Microscopically, *Candida hyphae* and *Nocardia* were present. The most prominent features were diffuse tubular atrophy and recent necrosis, and diffuse interstitial fibrosis. Cellular infiltration was very mild, and the vascular system was quite well preserved except for destruction of a number of peritubular capillaries.

The second patient (LD 20), a five-year-old boy of O blood type who received an A group maternal homograft, survived 202 days. Two months before his death he suddenly developed convulsions and deep coma from which he never aroused. There was no evidence of late rejection. Permission for autopsy was denied.

A third patient who died at 295 days of uncontrolled late rejection is considered in detail subsequently.

LATE REJECTION

It was pointed out earlier (see Chapters 16, 17, and 18) that the intensity of immunosuppressive treatment can usually be relaxed after the critical post-operative period of one to three months. If this were not the case, there would be no hope for the useful clinical application of homografting procedures, since long-term use of maximum therapy results in immunologic crippling and

death from complications of the agents employed, a situation which has been consistently observed after heterotransplantation (see Chapter 23) and after the majority of unsuccessful homotransplantations (see Chapter 19).

Although some degree of host-graft adaptation seems to be common to almost all renal homotransplantations, the extent of this change in clinical cases is not known. It is conceivable that the apparently well tolerated homograft is in actuality under constant but imperceptible attack and that it will be slowly rejected over a period of months or years. In addition, the chronic lesions described by Porter in the vascular system of the homograft may prove to be important in limiting longevity after homotransplantation (see Chapter 25).

In those reported cases followed for the longest time, justification can be found for either a sense of futility or of hope. The first chronically successful renal homotransplantation was performed by Doctor Joseph Murray at the Peter Bent Brigham Hospital in Boston more than five years ago. Total body irradiation was the primary means of immunosuppression. Other early measures to control rejection were reported by Merrill in 1960. The patient, whose donor was a fraternal twin, was alive in May, 1964, with normal renal function. Murray in a personal communication reported the results of a recent complete re-evaluation of the case at that time as follows:

A dizygotic twin of 5 and a half years recently left the hospital after a checkup including a biopsy. He is perfectly normal in every way as far as renal function is concerned. There are some microscopic changes which we can't really interpret, not knowing whether they are scarring as a result of his abortive rejection crisis of 5 years ago or whether it is some new very slow drain on the kidney. However, if we hadn't done the biopsy we would consider him perfectly normal in every other way.

Although it may be true, as has been suggested by Goodwin and Martin, that some degree of natural chimerism might have existed in this case because of cross-placental circulation, there seems to be little reason to doubt that the homograft passed through a very mild immunologic insult. Histologic evidence of cellular rejection was present in a biopsy specimen obtained nine months after the original operation.

The only other homografts which had maintained life for more than two years at the time of the Washington Transplantation Conference in September, 1963, were reported by Antoine and Hamburger and by Shackman and Dempster and their associates. As with Murray's case, primary immunosuppressive therapy was with total body irradiation. In Hamburger's patient, who was operated upon a few months after Murray's, the kidney was also donated by a fraternal twin. By personal communication from Hamburger it was learned that the patient was still in good health in May, 1964.

The patient treated by Shackman and Dempster, alive two and a half years postoperatively in the fall of 1963, is of the utmost interest, since he has survived longest after homotransplantation from a nontwin donor. The patient, who was receiving virtually no immunosuppressive therapy, had had only fair function (Ccr 25 ml min), but there had been no recent tendency to deterioration. In a follow-up communication on May 8, 1964, Dempster has

informed us that the patient is still in good condition, now three years and two months after operation.

These three patients with exceptionally prolonged survival have not been subjected to late rejection, or, if this has occurred, it has been mild and relatively easily controlled. These facts may give a misleading impression of the late expectation of other patients who have been treated with homotransplantation. The donor-recipient relationship in this small group of cases was highly favorable. The homografts were provided by fraternal twins in two instances and by a nontwin sibling in the third—groupings which greatly enhance the chances of matching the antigenic constitution of the donor and prospective host. Furthermore, these patients represent the further refined product of a sensitive biologic test system which eliminated all but those who received the most genetically suitable homografts. The absence or mildness of late rejection in these valuable early cases is encouraging but it does not necessarily mean that delayed rejection will be a comparably trivial problem when the larger numbers of patients now receiving better early immunosuppressive treatment are brought to a similar chronic stage.

Indeed, there is sound reason to avoid overoptimism at this time. Delayed rejection has been reported long after operation, usually manifesting itself with gradual decline in renal function over many weeks or months. Woodruff and Robson, in a personal communication, have described two of the most disturbing examples, in which fatal rejections occurred 15 and 16 months after two parent-to-offspring homotransplantations. In the most completely documented example of late rejection in the literature, Küss (1962) described the same events in a patient who died 17 months after transplantation with progressive renal failure which was first recognized nine months postoperatively. In this case it is of interest that a needle biopsy 59 days after homotransplantation already showed interstitial edema and proliferative vascular changes.

In spite of the dearth of information concerning late rejection, it is known that this problem has been encountered in other centers. Hamburger (1964) has allowed us to read a manuscript which will appear in the *Annals of the New York Academy of Science* in 1964, in which he describes several variants of delayed homograft disease. These include two examples of acute rejection crises similar to those described in Chapter 15, one at six and the other at 10 months. Biopsy in both revealed interstitial edema and cellular infiltration, but interstitial fibrosis was not mentioned. The process was reversed in both cases. In another of Hamburger's patients, who died of slow rejection after 22 months, severe interstitial fibrosis was present.

Hamburger (1964) has also drawn attention to the late development of chronic glomerulonephritis in the homograft, a complication that led to the death of one of his patients after 23 months. Although Murray and Merrill (1958) had observed this in a patient with an identical twin isograft, it had not previously been seen after true homografting procedures except in the cases reviewed by Porter in Chapter 25. It is of great interest that glomerulonephritis

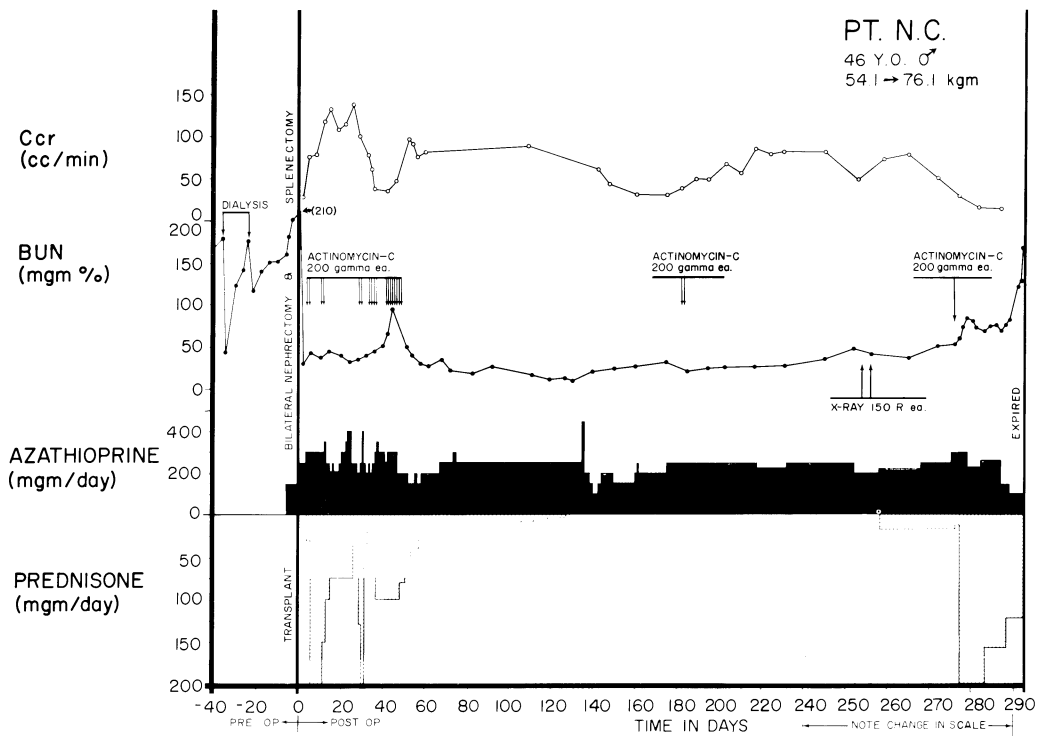


Figure 87. Course of a patient (LD 10) who died of late rejection 295 days after homotransplantation. The donor was unrelated. The diagnosis of rejection was first made after 229 days, but proper adjustments in therapy were not made. Note the transient fall in BUN after local irradiation of the transplant. Vigorous therapy was withheld for too long, and the recurrent uremia became progressive and uncontrolled. Terminally there were multiple pulmonary emboli. (By permission of *Ann. Int. Med.* (61: September, 1964.)

was also seen by Hamburger after transplantation to a patient whose original disease was congenital interstitial nephropathy, a surprising sequence also reported by Krieg to have developed within a few days in a patient who had originally had pyelonephritis. Whether this "glomerulonephritis" observed in homografts has the same pathogenesis as that commonly seen in clinical practice, or is some variant of rejection, remains to be clarified.

In the Colorado series, evidence of late rejection was observed in six patients four months or longer after operation. In one (LD 10), the process was not controlled. The patient, a 46-year old man of O+ blood type, received a homograft from a volunteer convict donor whose blood type was O-. Steroids were withdrawn unusually rapidly because of a series of early complications including myocardial infarction, gastrointestinal hemorrhage, and vertebral osteoporosis with fracture. Prednisone was discontinued 126 days after operation (Fig. 87). One hundred three days later, the BUN began to rise (Fig. 87). The homograft was locally irradiated with two doses of 150 R at depth, 254 and 256 days postoperatively with a subsequent brisk diuresis and rapid fall in the BUN. The improvement was short-lived. Within a few days progressive

Table 17. Timing of Late Rejections

LD No.	Days Postop.	*Days after Steroid Change
10	229	103
12	300	154
13	240	20
22	244	60
23	223	16
30	112	30

*In all but LD 30, steroid treatment had been stopped. For LD 30, the alteration consisted of reduction of prednisone from 45 to 30 mgm per day.

azotemia reappeared (Fig. 87). Prednisone therapy was restarted, first in doses of 20 mgm per day and later in doses of 200 mgm per day to which intravenous actinomycin C was added. Renal failure was not halted, and the patient died 295 days after homotransplantation. The last BUN was 165 mgm per cent. Terminally, multiple pulmonary embolization occurred. The patient became hypertensive for the last 40 days of life, with blood pressure readings in the range of 170/90 mm Hg.

Except for swelling and a few petechial hemorrhages, the homograft appeared grossly normal, although large (see Figure 150, Chapter 25). Histologically, there was a very light cellular infiltration. The most prominent features were interstitial edema and fibrosis. Evidence of old and recent tubular damage and acute and chronic vascular lesions was present. The features of this kidney are considered in detail in Chapter 25.

Five other patients (LD 12, 13, 22, 23, and 30) had more or less clearly documented late rejection occurring 112 to 300 days after operation (Table 17). The homografts were obtained from nonrelated donors in two cases (Figs. 88, 89), a sibling in one (Fig. 90), and from mothers in two (Fig. 91). The events preceding rejection were similar to those in the case described earlier. Good renal function had been maintained despite progressive decrease or complete discontinuance of steroid therapy. Sixteen to 154 days after steroid treatment was stopped (Figs. 87, 88, 90, 91) or reduced to below 45 mgm per day (Fig. 89) declines in creatinine clearance were noted, usually before any change had occurred in the BUN. In one asymptomatic patient a drop in routinely determined creatinine clearances was the only finding (Fig. 91). Orthostatic ankle edema was noticed by the others late in the day. Rapid loss of hair, low-grade fever, malaise, joint pains, and loss of appetite were all observed in more than one case but not in all. A sense of fullness and slight tenderness in the transplant site enabled two patients to suspect the diagnosis.

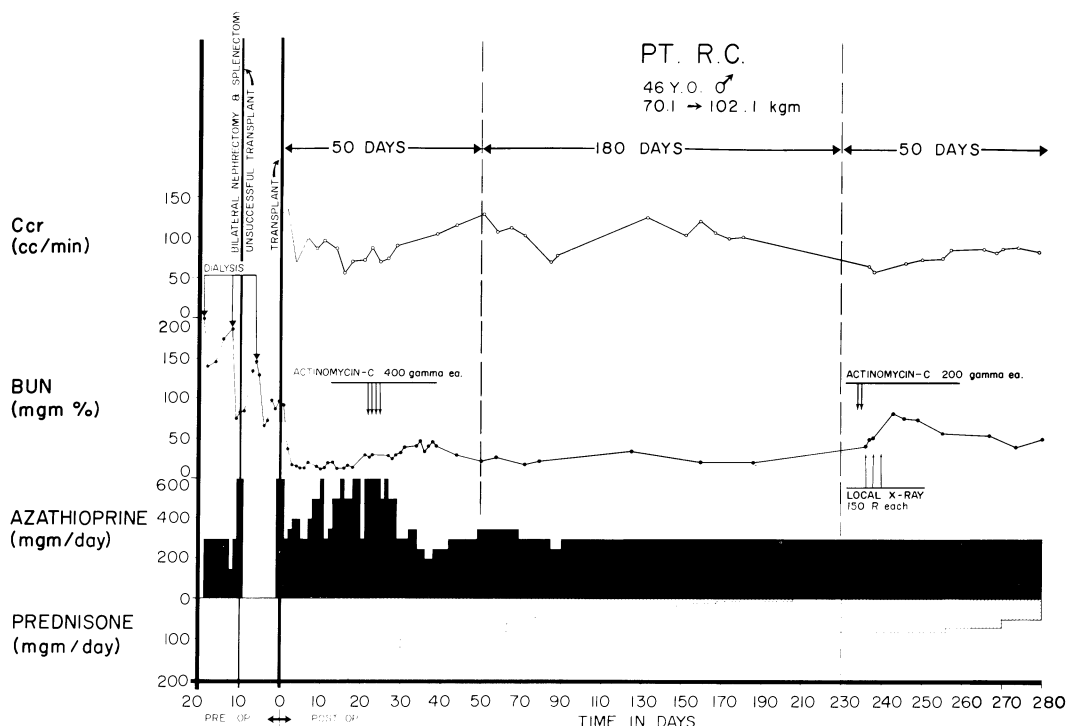


Figure 88. Severe delayed rejection in Patient LD 23 who received a homograft from an unrelated donor. Note rises in BUN and declines in creatinine clearance after discontinuance of steroid therapy. Creatinine clearance began to rise after institution of emergency therapy, although the azotemia became temporarily worse. Further improvement in renal function continued after preparation of this graph, and during the last two weeks of May, 1964, creatinine clearances averaged 85 ml per minute and BUN's averaged 32. (By permission of *Ann. Int. Med.* 61: September, 1964.)

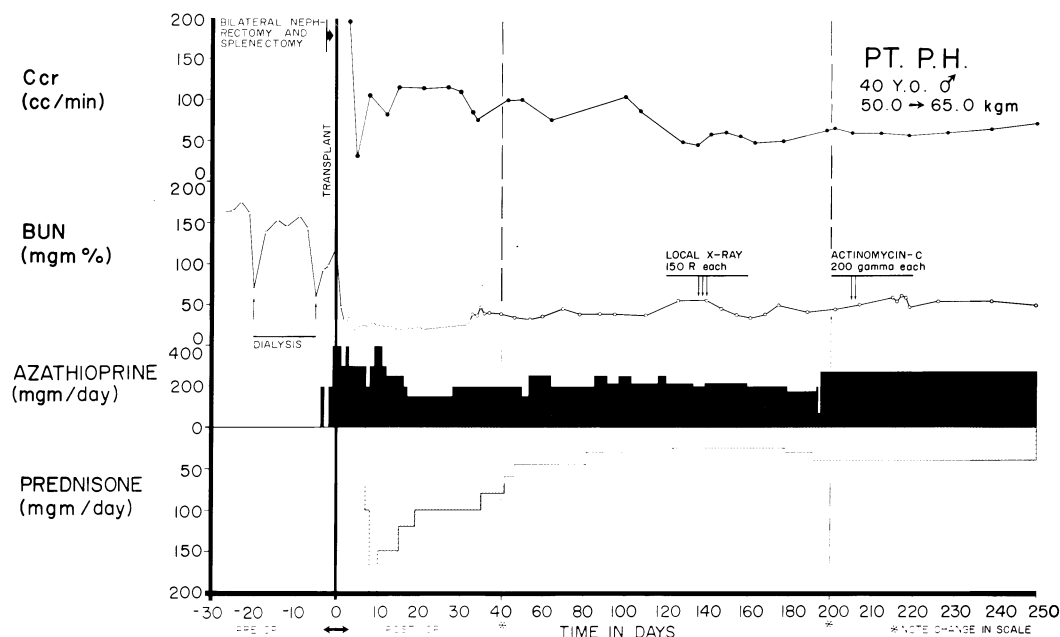


Figure 89. Delayed rejection in a patient (LD 30) who received a homograft from an unrelated donor. Deterioration of renal function occurred after reduction of prednisone from 45 to 30 mgm per day. Note transient improvement after a course of local transplant irradiation. Stable renal function was not obtained until the steroid doses had been returned to the previous higher levels. (By permission of *Ann. Int. Med.* 61: September, 1964.)

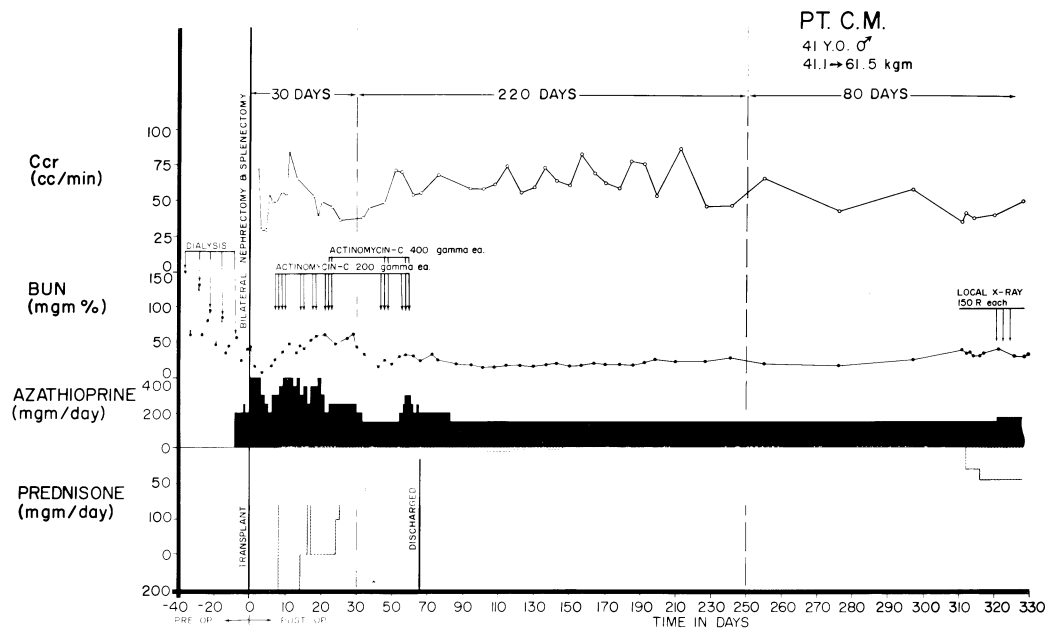


Figure 90. Late rejection, appearing 300 days after homotransplantation and six months after discontinuance of steroid therapy (LD 12). The homograft was provided by the patient's brother. (By permission of *Ann. Int. Med.* 61: September, 1964.)

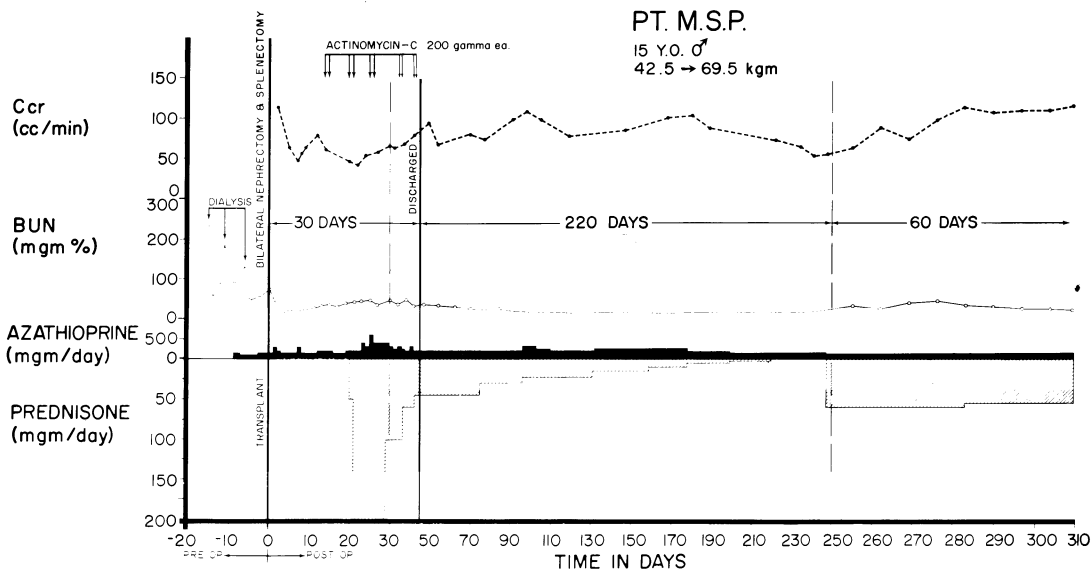


Figure 91. Delayed rejection in a 15-year-old boy (LD 13) who received a kidney from his mother. After steroid therapy was stopped, there was a gradual fall of creatinine clearance. The patient had no symptoms. Note gradual restoration of creatinine clearance after the institution of 60 mgm per day of prednisone. (By permission of *Ann. Int. Med.* 61: September, 1964.)

DIFFERENTIAL DIAGNOSIS OF LATE REJECTION

The diagnosis of late rejection must usually be made by exclusion of other causes for late renal failure including chronic pyelonephritis, occlusion of the arterial anastomosis, and ureteral obstruction. Examination of the tissues after needle biopsy has not been done because of the potential risks. Consequently, the principal emphasis was on detection of mechanical or infectious complications which were correctible. When these were not found, vigorous antirejection measures were instituted.

The physical findings of late rejection are sometimes almost pathognomonic, especially if careful examination was conducted and recorded by the same physician during preceding follow-up visits. The homograft which may have been nontender and almost impalpable may become painful and very prominent. Slight local edema of the transplant wound has been seen, in addition to the collection of fluid in the lower extremities just alluded to. Usually these changes evolve over a period of several weeks or even months. Rises in blood pressure above those previously recorded are almost always present.

The renal failure of delayed rejection is insidious in comparison to that which occurs in the immediate postoperative period—a fact which may add to the difficulty of establishing a certain diagnosis, but which also makes practical the systematic employment of certain procedures before the patient is subjected to an intensified course of immunosuppressive therapy. Multiple urine cultures are immediately obtained. Urine electrolyte concentrations are determined. A falling urinary sodium concentration such as that described in Chapter 15 has the same significance as in an acute rejection episode, although the diagnostic value is not so great since serial determinations usually have not been done while the patient was treated as an outpatient. The demonstration of increased proteinuria supports the diagnosis of rejection.

If rejection is suspected because of declines in renal function or because of the other symptoms or findings described, the first special examination is often a radioisotope renogram using Hippuran I¹³¹; Winter's technique, as applied to the care of transplantation patients by Collins and his associates, is employed. Inferential information concerning the renal circulation can usually be obtained quickly in this way. If a sharp vascular phase is demonstrated, the likelihood of an arterial anastomotic failure is greatly decreased. It has not been found necessary to perform dye arteriography in any case, and no occlusive lesions have been found subsequently. The radioisotope renogram is of limited value, however, in differentiating rejections from ureteral obstruction, since similar curves have been obtained in both situations.

Because the loss of renal function is gradual, pyelographic visualization of the extrarenal collecting system is usually possible if a reinforcing dose of contrast material is administered. A test dose of intravenous sodium diatrizoate*

*Hypaque, Winthrop Laboratories, New York 18, New York.

is given, followed by 30 ml of the contrast material. An additional 90 ml is then given by continuous infusion over a 30-minute period during which time x-ray exposures are made, developed, and reviewed.

LATE UROLOGIC COMPLICATIONS

Few examples of late urologic complications after transplantation have been reported, although it is known that one of Murray's identical twin recipients developed renal stones and one of the patients in the St. Mary's Hospital series developed urinary extravasation more than 100 days after transplantation (see Chapter 25).

In the Colorado series, late ureteric obstruction was seen in four patients. In three of these (LD 39, 44, 50) the stenosis was asymptomatic, being detected by routine intravenous pyelography; the narrowed area was at the site of implantation into the bladder (Fig. 92). The obstructive uropathy was relieved in each case by reoperation and anastomosis of the pelvis or ureteropelvic junction to the patient's own unilateral or contralateral ureter. The utility of this type of secondary procedure in treating complications at the bladder implantation site emphasizes the advisability of not performing ureterectomy except for specific indications at the time the patient's own diseased kidneys are excised (see Chapter 11).

In the fourth patient (LD 27) the cause of the obstructive uropathy could not be readily classified; this case is described separately because of the possibility that it represented a late complication of previous rejection. A 21-year-old woman received a homograft from a convict volunteer donor on September 3, 1963. There was only equivocal evidence of early rejection, following which she had stable renal function until March 18, 1964, by which time her prednisone dose had been reduced to 10 mgm per day. After a severe bout of diarrhea induced by castor oil, anuria developed which lasted five days (Fig. 93).

Emergency antirejection measures were taken with the administration of high doses of steroids, actinomycin C, and two courses (total 900 R) of local irradiation. During the next several weeks, anuria or oliguria alternated with diuresis (Fig. 93). An intravenous pyelogram obtained during a diuretic phase showed obstruction high in the ureter (Fig. 94). On April 18, 1964, the proximal homografted ureter was anastomosed to the distal portion of her own ipsilateral ureter. Recovery was uncomplicated, and good urinary drainage has since been demonstrated (Fig. 94).

That portion of the ureteric homograft which was removed at the time of reoperation had a high-grade internal stricture, extending over at least 1 inch. Microscopically, there was fibrous thickening of the resected segment with extensive muscle necrosis. The arteries were severely damaged with fibrous intimal thickening of the majority of the vessels and with rupture of the in-

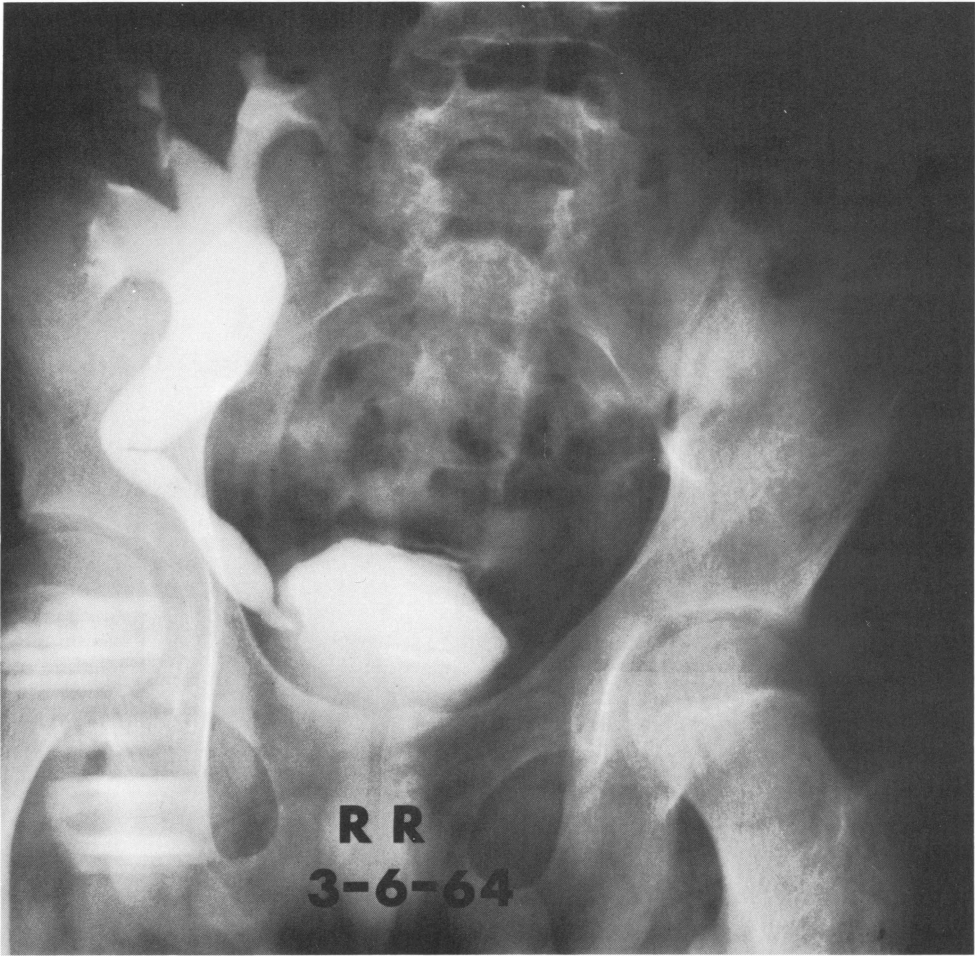


Figure 92. Intravenous pyelogram, four months after homotransplantation, in Patient LD 39. A contrast intensification technique was used. Note the partial obstruction at the ureteroneocystostomy. At a subsequent secondary operation, the proximal ureter of the homograft was anastomosed to the patient's own right ureter which had not been removed at the time of the original right nephrectomy. (By permission of *Ann. Int. Med.* 61: September, 1964.)

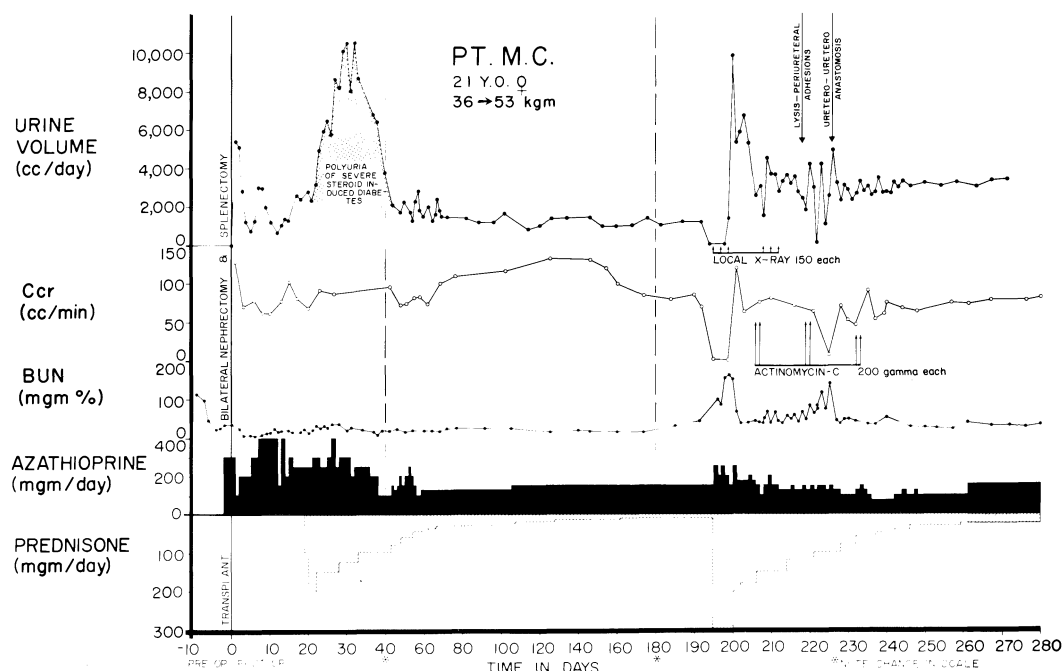


Figure 93. Course of a 21-year-old woman, LD 27, who received a homograft from an unrelated male donor. Both were of A blood type. Early postoperative rejection was very mild. After 190 days, she suddenly became anuric, subsequently alternating with bouts of brisk diuresis. Although she was thought to have had an acute rejection, it was subsequently demonstrated that she had severe midureteric stricture which was thought to be a consequence of late healing that followed a previous rejection. After correction of the mechanical difficulty, she has been well. (By permission of *Ann. Int. Med.* 61: September, 1964.)

ternal elastic lamina. A heavy cellular infiltrate was present, and virus inclusion bodies were noted. The last finding is of special interest, since this patient had previously passed through a serious bout of "transplantation pneumonia" (see Chapter 21). The evidence is strong that the complication resulted from healing of an earlier ureteric rejection.

TREATMENT OF DELAYED REJECTION

The principles of therapy are essentially the same as those described for reversal of rejection in Chapter 16. The relatively slow development of delayed rejection in the cases observed in the Colorado series has made it possible in some instances to evaluate the effect of individual agents. Thus, local transplant irradiation alone (Figs. 87, 89) and therapy solely with increased dosages of steroids (Fig. 91) have both been shown to be of therapeutic value in one or more isolated cases. Actinomycin C has also been used.

However, in one patient (LD 10) in whom these agents were used serially, remittent deterioration continued until the secondary renal failure became ir-

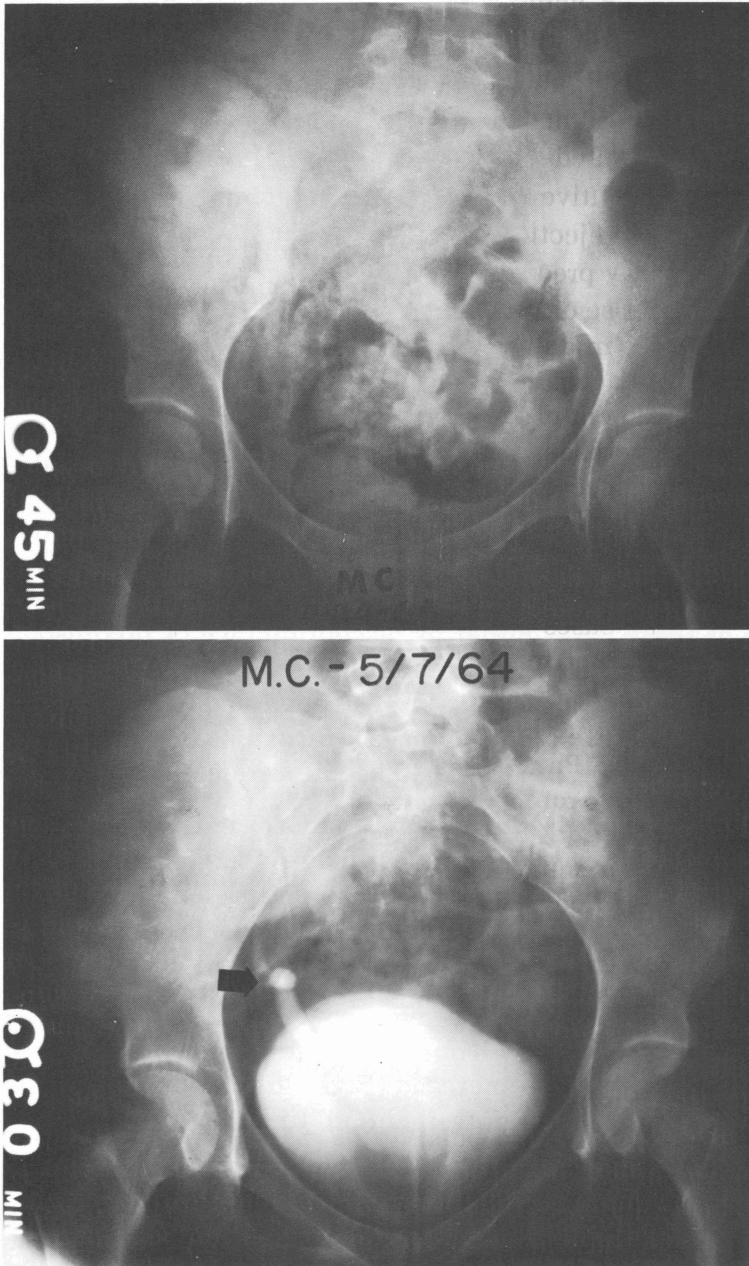


Figure 94. Intravenous pyelograms of same case (LD 27) as shown in Figure 93. Top—Intravenous pyelogram showing high-grade obstruction of the homograft midureter. Bottom—Appearance after resection of the stenosed ureter and re-anastomosis to the patient's own ipsilateral ureter. The anastomotic site is shown by an arrow. The somewhat dilated distal portion is the patient's own ureter, which had previously been demonstrated to have reflux. (By permission of *Ann. Int. Med.* 61: September, 1964.)

reversible despite the fact that urine excretion was never less than 1,000 ml per day. Although the homograft had serious histologic abnormalities (see Chapter 25), some of the findings probably would have been reversible, including the interstitial edema, had more aggressive immunosuppressive measures been taken immediately. Consequently, therapy with more than one agent is usually instituted.

An idea of the relative role of the various immunosuppressive agents in the treatment of late rejection can be obtained from a review of those events which have invariably preceded the onset of this serious complication. Since the maximum safe dose of azathioprine had been used in all these cases with only minor variations in daily quantity, inefficient administration of this drug was evidently not a factor in the initiation of delayed rejection. Furthermore, the previous employment of this agent to limits compatible with safety of the host made further increases in dosages for emergency therapy impractical. In actuality it may be necessary to temporarily lower the dose of azathioprine because of the increased drug sensitivity which commonly accompanies renal failure (see Chapter 14).

In contrast, it appeared as if stability of homograft function was disrupted in every one of these cases either by discontinuance of prednisone therapy or by reduction in dosage of this drug to a lower level than had previously been used for some time. Thus, steroid administration had been stopped for patients LD 10, 12, 13, 22, and 23; rejection was recognized 103, 154, 20, 60, and 16 days later, respectively. Patient LD 30 began to reject 30 days after reduction of the prednisone dose from 45 to 30 mgm per day.

Because of the demonstration of dependence upon continued steroids for stable homograft function in such cases, the most important immediate alteration is prompt resumption of prednisone administration in patients who have been taken off steroid therapy, or increase of dose in those patients who have had a recent reduction. The degree to which steroids must be implemented for treatment of late rejection is not definitely known. In view of the fatal outcome of one case in which small doses were first tried (Fig. 87), it may be advisable to begin with a drastic increase (Figs. 88, 91) and to continue this until unequivocal evidence of improvement in function has been sustained for several weeks. The most important objective measurement of excretory capacity to be followed is creatinine clearance, since misleading elevations in BUN can persist because of increased catabolism despite improvement in other measures of function (see Chapter 16). The greatest increased dose which was used, excluding that for patient LD 10 who ultimately died, was to 80 mgm per day (Fig. 88).

Later, steroid doses are slowly reduced in the same way as after early rejection (Figs. 88, 91). The success with which return to small daily doses can be achieved has yet to be determined, although it seems likely that continuation of prednisone in doses of 20 to 40 mgm per day will be required for many months and possibly even for the remainder of the patient's life.

Additional prompt immunosuppressive therapy is usually advisable at this

time of late rejection, particularly if the diagnosis was not made early and if there is more than slight impairment of renal function. Actinomycin C and repeated local irradiation of the homograft (Figs. 87 to 91) are used in the same way as for the treatment of an early postoperative rejection crisis (see Chapters 14 and 16), x-ray therapy being of the greatest immediate value. After the first application of 150 R, the sense of fullness or pain over the transplant site is almost immediately relieved. Reduction in the size of the homograft may be noted by palpation.

Although the value of these adjuvant procedures, especially local irradiation, is undoubted, they should not be used as the sole adjustment of treatment for several reasons. Although temporary improvement in renal function can be expected, it occurs against a background of that therapy which has already been proved to be insufficient. Furthermore, the chronic use of the added agents, which would presumably be necessary if steroid treatment were not intensified, has serious disadvantages since actinomycin C, which is probably only moderately effective, is toxic and therefore undesirable for protracted use (see Chapter 14). Alternatively, repeated courses of local x-ray would involve the risk of producing irradiation injury to the homograft. The use of local transplant irradiation and actinomycin C should, therefore, be viewed as emergency therapy and should coincide with a definitive alteration in steroid management.

It is evident from these remarks that the ease with which delayed rejection can be treated is directly related to the promptness with which the diagnosis is made. For this reason, all patients are followed in two special transplantation clinics after their discharge from the hospital. These clinics, which are staffed by internists, pediatricians, immunologists, and surgeons, have a relatively stable personnel so that patients can be repeatedly examined by the same physicians. A special effort is made to obtain accurate blood pressure measurements and weight, and to thoroughly evaluate any local features of the transplantation site. Hematologic studies and creatinine clearance determinations are performed by research laboratories, thus assuring accuracy in determining changes in the regimen.

Ordinarily, follow-up visits are scheduled once a week for the first month or two after the patient's discharge from the hospital. Subsequently he may be seen only once every two to eight weeks. At this phase of convalescence he may be allowed to return to his home, even if it is in a distant city, under the care of physicians who have a special interest in renal disease. Each patient is impressed with the necessity of immediately seeking medical care if fluid retention, oliguria, or transplant wound tenderness is noted. Between clinic visits, no restrictions are placed on activity except for avoidance of contact sports, such as football. The patients go to movies and other public gatherings. Virtually all recipients who are now living four months or more after operation have returned to some kind of work or to school.

At each clinic, blood urea nitrogen (BUN), creatinine clearance (Ccr), hematocrit value, white blood count and differential, urinalysis, and urine

cultures are ordered. Frequently, intravenous pyelograms are obtained early in the postoperative course, and these are always performed after three months. The importance of this test is evident when one considers that three asymptomatic patients have already been proved to have significant ureteral obstruction.

SYSTEMIC CHANGES

Perhaps it is natural in a text such as this to focus undue attention on the function and well-being of the homograft, sometimes almost to the exclusion of the beneficiary of this unnatural gift. In order to retain viability of the transplant, alterations in the host are inevitable. Some of these are obvious, others are subtle but measureable (Chap. 22), but most of the changes are probably not yet known. The ultimate toxicity of azathioprine, for example, in terms of a possible increased incidence of blood dyscrasias, will take years to determine. The influence of this drug on reproductive physiology requires additional study. One of the male recipients (LD 6) in the Colorado series recently became the father of a normal child who was conceived during the period of postoperative azathioprine and steroid therapy. Unpublished experimental studies by Doctor John A. Githens* indicate that spermatogenesis is not seriously disturbed by azathioprine but that its chronic administration to females induces a high incidence of anomalies in the offspring.

Precise measurement of immunologic capabilities is already possible on a limited scale; the results of such studies are reported in Chapter 22, in which it is shown that a significant loss occurs of responsiveness to various antigens. Despite this, the patients followed for more than four months have had a surprising lack of susceptibility to serious infection even though there has been no attempt at environmental control. Almost all have had minor infections from which recovery was prompt. In one patient (LD 1) herpes zoster first appeared 17 months postoperatively (Chap. 21). In only one patient (LD 9) was a fatal infection encountered after four months, and in this case the complication probably had its genesis at a much earlier time (Chap. 19).

Two of the patients (LD 15 and 36) developed symptoms and biochemical evidence of hepatitis six and three months after operation. In both cases, bilirubinemia (2 to 6 mgm per cent) and elevations in SGOT, SGPT, and alkaline phosphatase have been persistent. Whether the hepatic dysfunction is due to virus infection is not known since a high incidence of hepatotoxicity has been reported by McIlvanie and McCarthy, and by Einhorn and Davidsohn after chronic administration of 6-mercaptopurine to patients with leukemia.

There are thus many gaps in our knowledge of the long-term systemic effects of immunosuppression, particularly those of azathioprine. A much clearer view can already be expressed concerning the prognostic implications for those cases in which relatively high prednisone doses are needed for continued protection of the homograft. Those noninfectious complications which have attended chronic steroid administration for the treatment of various disease states have also been observed after renal homotransplantation. The

*Professor of Pediatrics, University of Colorado School of Medicine.

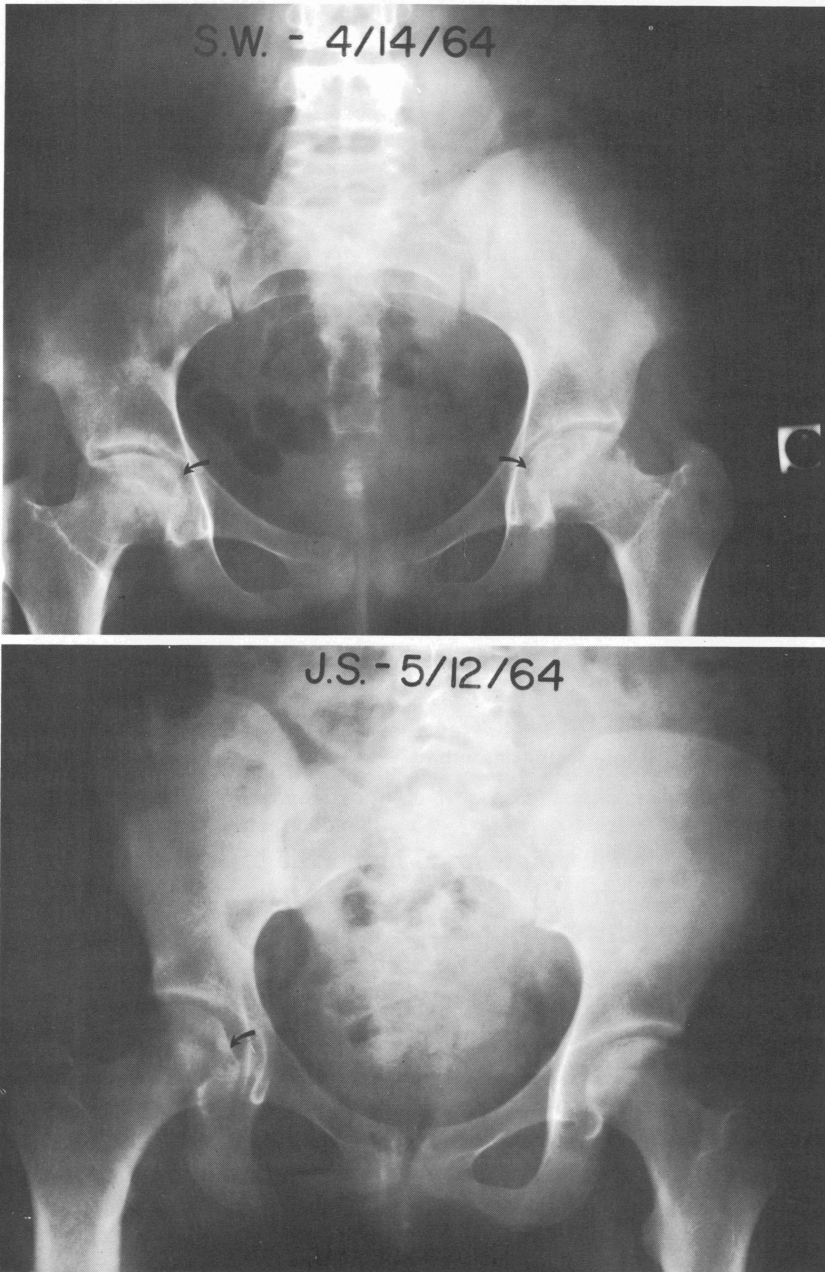


Figure 95. Appearance of aseptic necrosis of the femoral head in two young women after transplantation. Both were on prednisone therapy at the time of this delayed complication. Top—Patient LD 22. Bottom—Patient LD 50. (By permission of *Ann. Int. Med.* 61: September, 1964.)

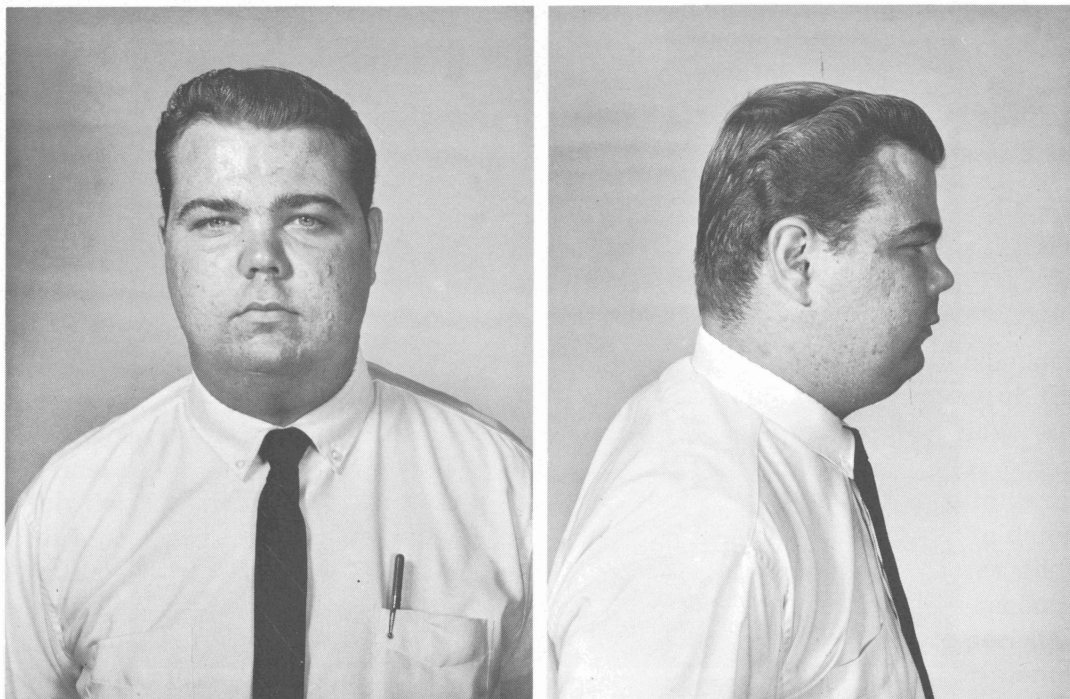


Figure 96. Moon facies in Patient LD 37, seven months after homotransplantation. Prednisone dose was 25 mgm per day. Rapid weight gain had been a severe problem.

high incidence of acute gastrointestinal hemorrhage during the first four postoperative months has already been described (see Chapter 12). After four months there have been two additional examples (LD 9 and 30) and in one of these bleeding was an important contributory cause of death.

Radiographic evidence of severe osteoporosis has been noted in many patients, but in only one (see Figure 54, Chapter 12) was there a fracture during early convalescence. Later, however, two young adolescent females (LD 22 and 50) developed aseptic necrosis of the femoral head (Fig. 95) five and a half and five months, respectively, after operation. The prednisone doses at the time of these complications were 10 and 30 mgm per day, respectively.

Earlier, the remarkable increases in body weight were described which are often observed in the first few months after transplantation (see Chapter 16). In some of the patients in whom it has been impossible to reduce prednisone administration below 20 to 30 mgm per day, appetites have continued to be insatiable, and obesity has followed almost to the degree seen in the pickwickian syndrome. Both girls with aseptic necrosis of the femoral head developed this complication only after a gain of 10.6 and 11.5 kgm over their preoperative weights. In Figure 96, another patient (LD 37) is shown seven months after transplantation. He had gained 25 kgm during the first 90 postoperative days, with stabilization only after prednisone administration was reduced to less than 25 mgm per day. Moon facies is still evident.



Figure 97. Patients LD 34 and 41, more than six months after homotransplantation. The maternal donors for these children are accompanying the respective recipients who are three years and eight years of age. Prednisone dose has been reduced to 5 mgm per day, but there is still evidence of hypercorticism. The technique of transplanting adult kidneys to children is described in Chapter 11.

In young children, the continuous use of large doses of steroids may have an especially ominous significance. The alarming weight gains already described can be life-threatening because of mechanical respiratory embarrassment. Bone growth may be limited or arrested if it is impossible to make substantial early reductions in dosage. Fortunately, the three youngest patients who are still alive have either had prednisone therapy discontinued (LD 1) or reduced to 5 mgm per day. Nevertheless, the last two patients have residual evidence of hypercorticism (Fig. 97).

A special note should be made of one consequence of steroid withdrawal that has been seen frequently. In Chapter 15, the hyperpyrexia that accompanies a frank rejection crisis was described in those patients whose initial therapy was solely with azathioprine. This fever is promptly controlled with the administration of prednisone.

In the absence of sepsis, hyperpyrexia does not subsequently return until the quantity of steroid is reduced to 20 to 60 mgm per day, from four to 20 weeks later. In more than one third of the patients, return of continuous high fever (103 to 105°F) is observed at this time, frequently starting just after reduction of prednisone dosage from 45 to 30 mgm or 30 to 20 mgm per day. In many cases, there is a protracted concomitant shower of peripheral normo-

blasts and other immature forms as depicted in Figure 60, Chapter 14. An exhaustive search for microbial complications is always conducted, but an explanation for the febrile illness is not usually found. With maintenance of the steroid dose at the new level, hyperpyrexia has subsided without treatment in the usual case in seven days to four weeks. Continued reduction of steroid dosage has usually been possible without further difficulty. In several exceptional cases, the fever was associated with a peculiar "transplant pneumonia" which is described fully in Chapter 21.

The cause of this late febrile syndrome is not known, but it may be a variant of the "late extrarenal syndrome" described by Hamburger, Crosnier, and Dormont. Several of their patients developed delayed fever, splenomegaly, hepatic dysfunction, and hypergammaglobulinemia. Hamburger has suggested that these findings may be due to the establishment of an imperfect chronic equilibrium between the host and the homotransplanted kidney.

LATE RENAL FUNCTION

Adequate, if not normal, renal function was obtained from virtually all homografts which were still functioning after four months. The last creatinine clearances and BUN's are listed in Chapter 28 for every patient who was still alive on June 1, 1964. Among those whose survival was four months or longer at that time, the lowest creatinine clearance was 27 ml/min (LD 41, a three-year-old child) and the highest BUN was 54 mgm per cent (LD 30). The mean creatinine clearance for those 30 cases after four months or longer was 88.1 ml/min, and the mean BUN was 23.7 mgm per cent.

Although these figures are important in making clear the fact that generally good homograft function can be obtained, it should be emphasized again that trends in function are of greater prognostic significance than any individual determination. Thus a patient whose creatinine clearance has dropped from 100 to 70 ml/min may pose a far more serious problem of late management than another who has a stable creatinine clearance of 50 ml/min.

In those patients followed for the longest times in whom delayed rejection has not adversely affected the late course, the function has tended to improve with the passage of time (Fig. 98; see Figure 77, Chapter 17) so that functional hypertrophy in the recipient has been almost parallel with that of the donor. Thus, in LD 1, 2, and 3, the creatinine clearances were 83, 91.8, and 129 ml/min. Determinations in the three respective donors were 112, 89.9, and 129 ml/min. Prior to delayed rejection, 146 days after operation, the comparative creatinine clearances in LD 12 were 70.5 ml for the donor and 73.3 ml min for the recipient. The tests have not been repeated since reversal of the subsequent delayed rejection shown in Figure 90.

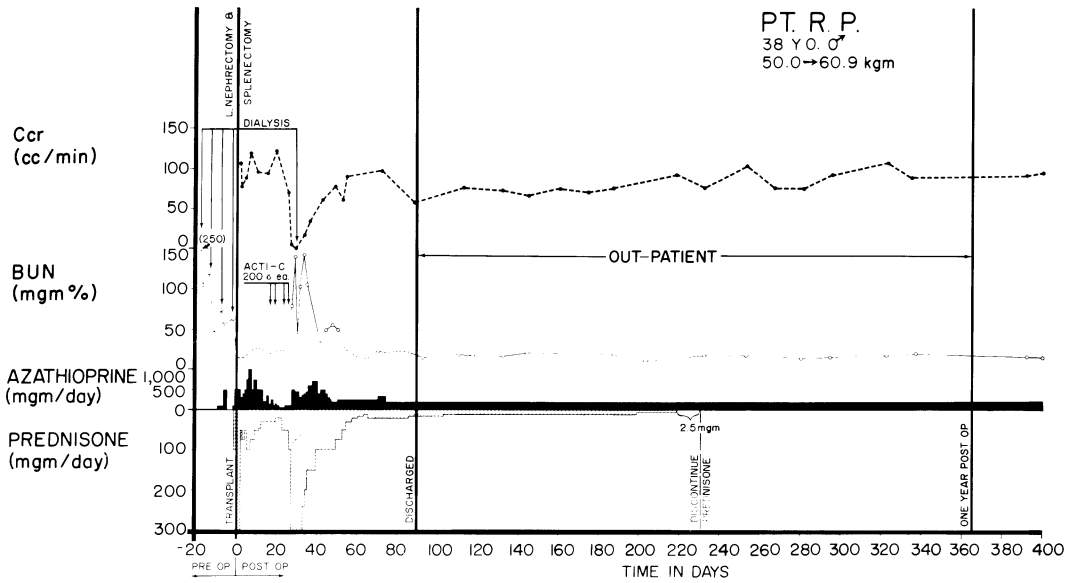


Figure 98. Chronic course of Patient LD 2 who received a homotransplantation from his sister in January, 1963. The patient was of A+ blood type. His sister was B+. Note progressive improvement in creatinine clearance, starting after three postoperative months. Prednisone therapy was discontinued after 230 days. The patient had a preliminary thymectomy. (By permission of *Ann. Int. Med.* 61: September, 1964.)

LATE BLOOD PRESSURE

In Chapters 15 and 16 emphasis was placed upon the cyclic appearance of hypertension which was often a troublesome problem during rejection and which tended to disappear after reversal of this process. Since very careful attention was exercised to maintain normal or near normal blood pressure during early convalescence, most patients were placed on one or more anti-hypertensive drugs during this period. The ultimate blood pressure in these patients and the need for later continuation of such pharmacologic agents assume an especial importance because of the vascular lesions which Porter has found in many homografts studied by needle biopsy or at autopsy (see Chapter 25).

Except in those cases in which convalescence was marred by delayed rejection, the tendency to hypertension and the requirements for antihypertensive drugs were less with the passage of time (see appendix, Chapter 28) and seemed to be directly related to the level of maintenance steroid therapy required. The average sitting blood pressure during May, 1964, is given for all patients in the Colorado series in Chapter 28, as well as an account of the anti-hypertensive drugs being used during this time. It will be noted that none of the patients followed for the longest intervals are receiving any medication of this type.

THE INFLUENCE OF THYMECTOMY ON LATE RESULTS

In Chapter 13, it was pointed out that nine of the first patients received preliminary thymectomy in preparation for transplantation. The rationale and experimental basis for the trial were described. This adjuvant procedure, performed through a sternum splitting incision in patients with terminal uremia, proved to have a high technical risk. In addition, the early course after homotransplantation did not seem to be different from that observed in patients who did not have the added operation. Consequently, use of this procedure was discontinued until long-term follow-up could be obtained in those patients for whom it had already been employed.

The efficacy of thymectomy in preventing or tempering early rejection does not, of course, rule out the possibility of later benefit. Theoretically, this might, in fact, be consistent with what has been learned of thymic function by Miller and others from experiments on lower animals (see Chapter 13). In his studies with adult rabbits, severe immunosuppression combined with thymectomy was required at the time of host exposure to the foreign tissue in order to obtain prolonged homograft survival. Presumably, thymectomy would not affect the reactivity of immunologically competent cells already present throughout the body.

Because of this it might be anticipated that the intensity and immediacy of early rejection would not be altered by this operation. If, however, the thymus is in some way necessary for continued replication of immunologically specific strains of host cells, these cell lines might be expected ultimately to undergo drastic attrition under the combined influence of unrelenting immunosuppressive therapy and the unremitting antigen overloading provided by the homograft.

Four (LD 1, 2, 3, and 6) of these original nine patients are still alive 14 to 18 months after homotransplantation. All have normal renal function. In three, maintenance therapy with prednisone was discontinued in 270 days or less and in one (LD 3) it was never required. Both in those who did (LD 1 and 2) and those who did not (LD 3 and 6) have a serious rejection crisis, there has been no evidence whatsoever of late deterioration of renal function (Figs. 77, 98).

Consideration of the fate of the other five patients who had removal of their thymus might be important in assessing the possible long-term role of this procedure. Unfortunately, this does not seem to be the case since all the patients either died in the very early postoperative period (LD 5 and 11; CD 2) or at a later time from complications other than renal failure (LD 4 and 9). The position could be taken that thymectomy did not receive a fair test in any of these patients.

There is at present insufficient evidence to permit even tentative opinions about the potential delayed value of thymus excision after homotransplanta-

tion. Most of the canine experimental work designed to test the influence of this operation has focused upon earlier postoperative events, partly out of necessity since the number of chronic survivors in dogs is much lower than after clinical renal homotransplantation. In our own laboratories, survival for as long as a year has not been achieved in any animal which was initially treated with thymectomy. The dogs with greatest longevity, including those which no longer required immunosuppressive therapy, did not have thymectomy (Chap. 17).

Nevertheless, the question of whether or not to remove the thymus gland, or to irradiate it as practiced by Hamburger, is by no means settled. Indeed the observations cited from experience with the earlier human cases emphasize the urgent need for further clinical evaluation of this procedure. With the small group of patients now under study, it will be impossible to determine if thymectomy contributed to the ease of late management of these patients and to the rapidity with which steroid therapy could be discontinued altogether, since in each of the four cases, the donor-recipient genetic relationships were highly favorable and the outcome may have been the same with or without removal of the thymus.

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Chapter Twenty-one

INFECTIOUS DISEASES ASSOCIATED WITH RENAL TRANSPLANTATION

by David Rifkind, Ph.D, M.D.

Infectious disease has contributed to 27 of the 33 deaths which have occurred to date among the 75 patients treated at this center by renal homo- or heterotransplantation (Tables 18, 19, and 20). In addition, infections of varying degrees of severity have complicated the postoperative course of virtually all the patients in this study group, except for those two who had identical twin donors and who did not require immunosuppressive therapy.

Septicemia, pneumonia, wound infections, and pyelonephritis have been the most frequent types of septic complications, although examples of myocarditis and abscesses of the lung, brain, and subphrenic space have been encountered. The majority of these have been due to the pyogenic and enteric groups of bacteria; but, in addition, clinically important infections have been associated with various fungi, acid-fast bacilli, viruses, and even a protozoan species.

PREDISPOSING FACTORS

Constitutional Alterations in the Host. Thirty-two of the 35 postoperative infections among the first 30 renal transplantation patients occurred following the appearance of the homograft rejection crisis (Fig. 99). All these cases were treated before December 10, 1963, initial therapy having been provided solely with azathioprine. At the time of rejection, prednisone and actinomycin C were added, and frequently the doses of azathioprine were increased. While the interval between surgery and rejection averaged almost 12 days in this

Table 18. Terminal Infections in Recipients Receiving Homografts from Living Donors (LD Series)

LD Case Number	Type of Infection	Etiologic Agent
4	Septicemia, pneumonia, subphrenic abscess, mediastinitis	<u>Staphylococcus aureus</u> <u>Pseudomonas aeruginosa</u>
5	Septicemia, mediastinitis, pyelonephritis	<u>Staphylococcus aureus</u> <u>Pseudomonas aeruginosa</u>
7	Septicemia, perinephric abscess	<u>Staphylococcus aureus</u> <u>Pseudomonas aeruginosa</u>
9	Septicemia, pneumonia, brain abscess, pyelonephritis	<u>Pseudomonas aeruginosa</u> <u>Candida albicans</u> <u>Candida krusei</u> <u>Nocardia asteroides</u>
10	Pneumonia	<u>Klebsiella-Aerobacter</u>
11	In old nephrectomy wound	<u>Staphylococcus aureus</u> <u>Pseudomonas aeruginosa</u> <u>Proteus species</u>
19	Pneumonia	<u>Pneumocystis carinii</u>
21	Pneumonia, brain abscess	<u>Escherichia coli</u> <u>Candida stellatoidea</u>
24	Septicemia	<u>Pseudomonas aeruginosa</u> <u>Proteus species</u>
28	Pneumonia, septicemia	<u>Escherichia coli</u> <u>Mycobacterium tuberculosis</u>
29	Septicemia	<u>Escherichia coli</u> <u>Proteus species</u> <u>Paracolon species</u>
31	Pneumonia	<u>Pseudomonas aeruginosa</u>
32	Septicemia, pyelonephritis, myocarditis	<u>Candida albicans</u> <u>Escherichia coli</u>
35	Septicemia, pneumonia	<u>Pseudomonas aeruginosa</u>
43	Pneumonia, septicemia, fungus pyelonephritis	<u>Aspergillus fumigatus</u> <u>Candida albicans</u> <u>Streptococcus faecalis</u>
46	Septicemia	<u>Staphylococcus aureus</u> <u>Diplococcus pneumoniae</u>
57	Pneumonia, third degree abdominal burn	<u>Pseudomonas aeruginosa</u>
59	Lung abscess, pneumonia	<u>Klebsiella-Aerobacter</u>
61	Septicemia	<u>Escherichia coli</u>

Table 19. Terminal Infections in Patients Receiving Cadaveric Renal Homografts (CD Series)

CD Case Number	Type of Infection	Etiologic Agent
1	Septicemia, pericarditis, empyema	<u>Staphylococcus aureus</u> <u>Pseudomonas aeruginosa</u>
2	Septicemia, lung abscess	<u>Staphylococcus aureus</u> <u>Pseudomonas aeruginosa</u> <u>Streptococcus faecalis</u>

Table 20. Terminal Infections in Recipients Receiving Baboon Heterografts (SD Series)

SD Case Number	Type of Infection	Etiologic Agent
1	**Pneumonia	<u>Escherichia coli</u> <u>Pseudomonas aeruginosa</u>
2	Septicemia, **pneumonia, empyema	<u>Escherichia coli</u> <u>Pseudomonas aeruginosa</u>
*3	Pneumonia, brain abscess, myocarditis, septicemia	<u>Pneumocystis carinii</u> <u>Aspergillus fumigatus</u>
*4	Pneumonia	Unknown, no autopsy
5	Esophagitis	<u>Candida albicans</u>
6	Pneumonia, pyelonephritis	Klebsiella-Aerobacter

*Subsequent homotransplantation.

**Pneumonitis was secondary to multiple pulmonary emboli.

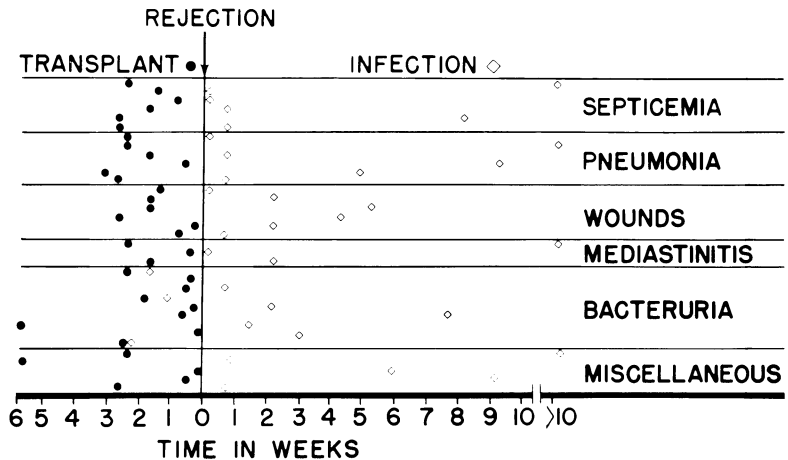


Figure 99. The time of appearance of infections (open diamonds) following renal homotransplantation (solid circles). The majority of infections occurred following the rejection crisis at which time immunosuppressive therapy is increased to maximal. (By permission of *JAMA* 189:397, 1964.)

group, less than 10 per cent of the postoperative infections occurred in this early postoperative period, during which only azathioprine was being given, suggesting that the intensification of immunosuppressive therapy which is necessary for control and reversal of rejection played a significant role in predisposing patients to infectious complications. Leukopenia, hypogammaglobulinemia, and steroid-induced diabetes were each noted to have resulted from such therapy.

Marked depression in the total white blood cell count occurred in 12 of the first 30 patients. In these cases, total leukocyte counts of less than 3,000/mm³ were observed and were accompanied by various degrees of granulocytopenia. This complication occurred in 60 per cent of all patients in whom death was caused or accompanied by overwhelming infectious diseases (see Chapter 19).

Total serum gamma globulin levels of less than 0.3 gm per cent (normal, 0.7 to 1.6 gm per cent) were detected by paper strip electrophoretic measurements in six of 22 patients. The deficiency was detected in four of 14 patients tested who subsequently died with infections. Hypogammaglobulinemia has been a feature unique to those patients receiving high doses of corticosteroids in addition to azathioprine, and the deficiency tended to be corrected to normal when the steroid dosages were decreased to low levels or were discontinued.

Steroid-induced diabetes was present in many patients tested who later succumbed with infectious complications. Blood sugar determinations indicated that the glycosuria resulted from hyperglycemia rather than from a lowered renal glucose threshold in the transplanted kidney. It should be emphasized that the diabetogenic effect of prednisone is only one of the many "anti-inflammatory" effects of steroids which may predispose subjects to infections and that it may have special clinical importance only because it is so easy to detect as an index to steroid toxicity.

The same could be said of both leukopenia and hypogammaglobulinemia. The several consequences of immunosuppression which are noted above demonstrate only isolated facets of the depressed resistance in these patients. Other aspects of loss of host resistance are not so readily apparent because of a lack of simple laboratory tests for their detection and quantitation, although varying degrees of loss of immunologic capability can be demonstrated with special analytic methods (Chap. 22).

Epidemiologic Factors. The presence of the staphylococcal carrier state prior to surgery was found to correlate with the development of postoperative infections caused by this organism. This relationship is apparent among the first 30 patients who were treated prior to the institution of routine antibiotic treatment of staphylococcal carriers. Survey cultures of the nasopharynx or skin indicated that 19 of this initial group were carriers of *Staphylococcus aureus* before operation. Of these 19 carriers, 17 developed a significant staphylococcal infection during hospitalization. In contrast, only one of the 11 noncarriers developed such an infection. Bacteriophage typing of the staphylococcal strains isolated from blood cultures and from the nasopharynx of two patients with staphylococcal septicemia suggested that the septic process was caused by the staphylococcal strain carried in the patients' own nasopharynxes.

At present, methods comparable to the staphylococcal bacteriophage scheme were not available for the typing of other infectious agents. Nevertheless, there is suggestive evidence in these patients that the infections caused by enteric gram-negative rods, fungi, and viruses were also of endogenous origin. The clinical experience, therefore, is compatible with the interpretation that infections are usually produced by endogenously carried organisms which invade and cause disease when the host defense mechanisms are sharply depressed by antirejection drug therapy.

DIAGNOSIS

As both infectious diseases and the rejection crisis may present initially as "fevers of unknown origin," the differentiation between the hyperpyrexias resulting from these two phenomena is of immediate importance in the management of transplantation patients. While the diagnosis of infectious complications is based primarily on the usual physical and laboratory criteria, certain distinguishing features are of confirmatory value in these patients.

The laboratory data of differential value in distinguishing rejection from infection may be compared in the same patient in which these two phenomena occurred sequentially (Fig. 100). In this patient (LD 15) an early rejection crisis was accompanied by fever and by marked renal failure as evidenced by oliguria, azotemia, and decreased creatinine clearance. With institution of high-dose steroid therapy, a prompt defervescence and return of adequate renal

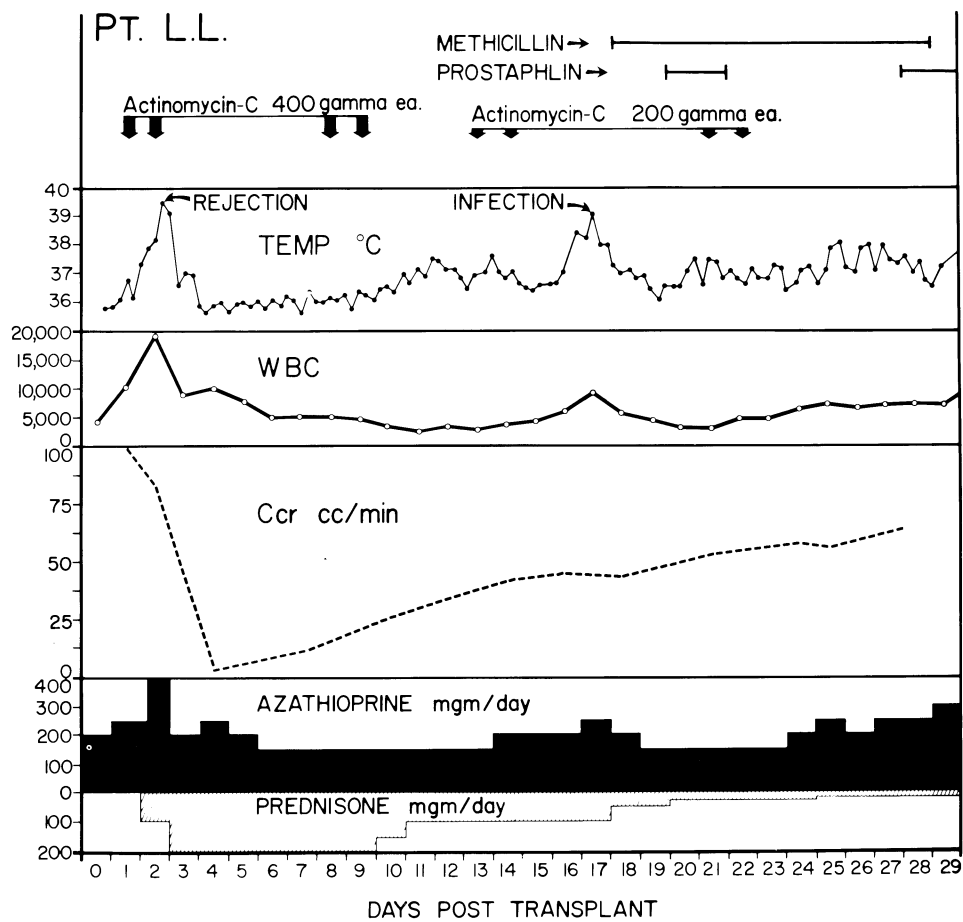


Figure 100. LD 15. Fever due to a homograft rejection attempt was associated with renal failure, and remitted with steroid therapy. In contrast, fever resulting from a staphylococcal wound infection appeared despite steroid therapy, and was not associated with impairment in renal function. (By permission of JAMA 189:402, 1964.)

function occurred. Two weeks later, fever reappeared which was due to a staphylococcal wound infection. The temperature rose to 39° C despite the fact that the patient was receiving 100 mgm of prednisone daily. There was no impairment in renal function during the infection. With institution of drainage and antibiotic therapy the temperature returned to normal and the wound healed. As in this case, the fever of rejection is invariably controlled by large doses of steroids. The fever of serious infection is usually not.

The white blood cell count has been found to be of little value in differentiating between infection and rejection since the degree of leukocytosis is under constant control by daily adjustments in azathioprine dosage. Consequently, the appearance of leukocytosis with either infection or rejection is probably more a reflection of bone marrow reserve after cytotoxic drug therapy than a response characteristic of either process. The peripheral white blood count is a resultant of the rates of production and destruction of white cells, and both

these processes at present defy quantitation. It is apparent that, with varying degrees of drug-depressed production and with differing rates of infection or rejection-stimulated utilization, the white count could rise, fall, or remain unchanged with either rejection or infection.

The differentiation of septic hyperpyrexia from that of rejection may be a simple problem as in the case cited above. Much more commonly, however, serious localized or blood stream infections have first been diagnosed at the same time as rejection. The coexistence of a rejection crisis and sepsis also leads to a characteristic clinical picture (Chap. 19) and one that often cannot be effectively treated since the requirements for control of the infection and reversal of rejection are mutually antagonistic. Thus, the clinical syndrome of severe rejection, immunosuppressive drug toxicity, leukopenia, and sepsis has accounted for the majority of deaths. In an effort to prevent this fatal series of events by tempering the severity of rejection, steroid therapy has been given prophylactically in more recent cases, with some improvement in results (Chap. 18). With this altered regimen, which involves the administration of massive doses before as well as after operation, the appearance of early post-operative fever is usually strong evidence of infection since the fever of rejection is almost always masked at this time.

GENERAL ASPECTS OF MANAGEMENT

Aseptic Precautions. The first cases in the Colorado series were treated under conditions of strict asepsis. The patients were placed in isolation at the time suppressive drug therapy was begun. Precautions included wearing of caps and masks, sterile gowns, and surgical gloves by all visitors or examiners. Everyone entering the room wore cloth shoe covers and walked over mats soaked with detergent. Even laboratory data slips, newspapers, books, and food were sterilized before being brought into the isolation units.

These expensive procedures were subsequently modified in the light of the findings described earlier which indicated that infections were largely of endogenous origin. Currently several patients are cared for in the same ward. Clean gowns are worn when the patient is being examined or dressings are changed. If an overt infection occurs, the patient is removed to an individual unit; but if recovery is uncomplicated, the patients are sent out on pass during the day, usually beginning a few days following surgery. When the patient enters and leaves the hospital, he wears a mask to protect him against the increased hazard of the nosocomial environment.

Both before and after operation, cultures are taken several times weekly of the nose, throat, skin, stool, and urine of the patients. Current information is thus continually available regarding the endogenous flora of the patients. In those circumstances in which an infection arises, it is frequently possible from these data to predict not only the type but also the antibiotic sensitivity of organism involved.

Antibiotic Treatment. The choice of antibiotics depends upon a number of considerations. These include the etiology and site of the infection, the general condition of the patient, the adequacy of renal function, and the severity of immunosuppressive drug effect which the patient manifests.

Staphylococcal infections are usually treated with either penicillin G or one of the penicillinase-resistant penicillins, depending upon the sensitivity of the particular strains. Oral or parenteral forms are given, and the dose is adjusted according to the renal function of the patient.

For serious gram-negative bacillary infections, chloramphenicol is frequently chosen because it is potent, possesses a broad antimicrobial spectrum, and has no increased toxicity in the presence of impaired renal function. Tetracycline is less commonly employed because of its antianabolic effect with resulting elevation of the blood urea nitrogen. Bactericidal agents, such as streptomycin, colistin, and kanamycin, are also rather frequently used. Dosage of these drugs must be carefully regulated in the presence of renal insufficiency because of the danger of jeopardizing the function of the transplanted kidney. In general, when renal function is impaired, one-half the usual daily dose is administered every third day. When necessary, kanamycin may be given intravenously in doses of 0.25 to 0.5 gm twice daily when renal function is normal, or 0.25 to 0.5 gm every third day when renal function is negligible as would be the case during a severe rejection crisis.

Pseudomonas infections are treated with colistin and, again because of the potential nephrotoxicity of this drug, dosage is carefully adjusted according to the renal function of the patient. Doses of this drug vary from 5 mgm/kgm daily to 2 mgm/kgm every third day. The administration of this antibiotic by the intravenous route is currently being investigated in cases in which edema, hypotension, or bleeding tendencies make intramuscular use undesirable.

Other Therapeutic Measures. Of equal importance with appropriate antibiotic therapy is the control of the side effects of immunosuppressive drug treatment. Agranulocytosis has come to be recognized as an extremely ominous sign as it may permit sudden, fulminant, and overwhelming sepsis. To date, neutropenia has been managed only by withdrawal of the suppressive therapy. However, other forms of treatment may merit consideration. Studies by Morse and his associates have suggested that white cell transfusions in leukemic patients are of value in counteracting infections. Blood from donors who have extremely high counts of mature neutrophils, such as those with chronic granulocytic leukemia, was particularly useful.

It has been shown by Rosaff that experimental animals will survive irradiation longer if they are treated with oral nonabsorbable antibiotics to sterilize the intestinal tract, and it may be that patients going through an episode of agranulocytosis should receive oral antibiotics on a prophylactic basis. The use of such agents as oral colistin has been suggested by Margaretten as a possible mode of prevention of *pseudomonas* infections in leukemic patients receiving cytotoxic therapy.

Hypogammaglobulinemia has been a frequent occurrence in patients during periods of high-dose corticosteroid therapy. When the gamma globulin level drops to 300 mgm per cent or less in the presence of an infection, gamma globulin in a dose of 20 to 40 ml intramuscularly is administered. It would seem reasonable also to administer gamma globulin on a prophylactic basis in the event of the simultaneous occurrence of agranulocytosis and hypogammaglobulinemia. There are insufficient data to evaluate the efficacy of this type of therapy.

Steroid-induced diabetes has been a frequent complication of immunosuppressive therapy. When marked glycosuria develops, patients are placed on a diabetic diet and insulin is administered. It has been noted that after the prednisone dosage is lowered to approximately 20 to 40 mgm daily or less, these control measures are usually no longer necessary.

SPECIFIC INFECTIONS AND TREATMENT

Staphylococcal Wound Infections. The importance of the staphylococcal carrier state in predisposing patients to infections by this organism has been emphasized. Because of the frequency of these infections, all patients shown to be staphylococcus carriers are given antibiotic therapy which is started before surgery and is usually continued five to seven days postoperatively. Ordinarily one of the penicillins is used, depending on the sensitivity of the staphylococcus. Since institution of this policy, some 30 patients have been treated by renal homotransplantation, with development of staphylococcal wound infection in only one. It is apparent, however, that this preventive use of antibiotics has not reduced the general incidence of infectious disease-related mortality (Tables 18 to 20). Deaths are still occurring from sepsis although somewhat later in the post-transplant period and from infections caused by gram-negative rods and fungi.

In the past, staphylococcal infections at the site of hemodialysis catheters have been frequently noted. In many instances, it is necessary to dialyze a number of times over several weeks prior to transplantation. In five such cases, serious staphylococcal infections occurred at the site of dialysis catheters with resulting bacteremia in two patients. When this complication occurs, the dialysis catheters should be removed and antistaphylococcal antibiotic therapy instituted. If the patient has been started on azathioprine pretreatment in preparation for transplantation, this drug is temporarily discontinued until the infection has been brought under control. It has been possible to perform transplantation in these patients within two weeks of the onset of their infections, without untoward complications.

In an additional case (SD 3), multiple septic pulmonary emboli occurred secondary to a staphylococcus-infected arteriovenous shunt catheter (Fig.

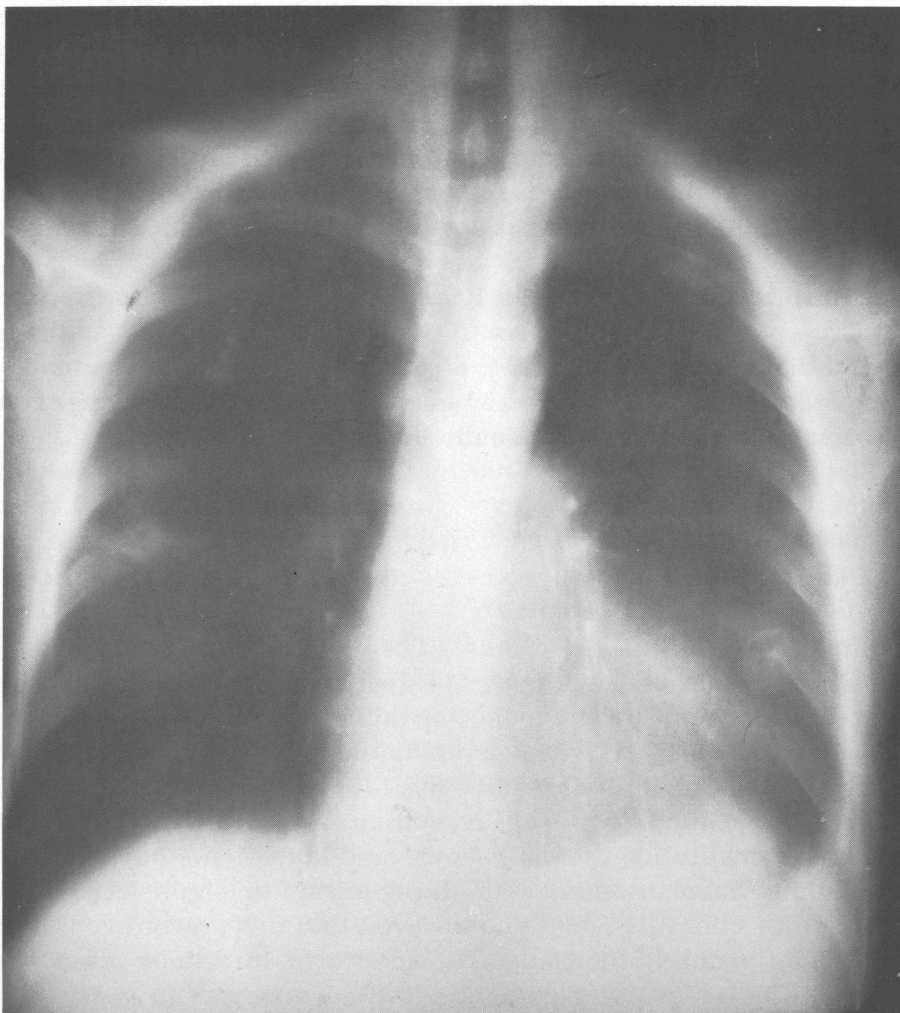


Figure 101. Patient SD 3. Tomogram of the chest at 7 cm. Note multiple cavitary staphylococcal emboli from an infected arteriovenous shunt catheter.

101). The catheter was removed and the patient treated for six weeks with methicillin and oxacillin. The lung lesions cleared and heterotransplantation was performed.

Clinically important wound infections occurred at the site of thymectomy, nephrectomy, splenectomy, renal homotransplantation, or other major wounds in six patients (Table 21). In all these cases staphylococci were involved, and in two cases were accompanied by gram-negative bacilli. These infections were cleared in two instances, and led to death of the patients in the other four.

Urinary Tract Infection. Bacteriuria has been a frequent postoperative finding, occurring in 19 of the first 40 patients. In four of these, the infections, which originated preoperatively, were cured or suppressed following surgery.

Table 21. Surgical Wound Infections: Clinical Diagnosis

LD Case Number	Site: Transplant	Nephrectomy-Splenectomy	Previous Nephrectomy	Splenectomy	Thymectomy	Intestinal Obstruction	Thoracotomy: Cardiac Arrest	Etiology
3	S	S						Staphylococcus albus No growth
4	S				D	D		Staphylococcus aureus Staphylococcus aureus Staphylococcus aureus
5							D	Staphylococcus aureus
7	D							Staphylococcus aureus
9					S			Staphylococcus aureus
11			D					Staphylococcus aureus Pseudomonas aeruginosa Proteus species
15	S							Staphylococcus aureus
18	S	S						Staphylococcus aureus Staphylococcus aureus
25		S						Staphylococcus aureus
28				S				No data
38	S							No growth

S — superficial.
D — deep.

LH-25 PT. G.S. 33 Y.O. ♂, CHRONIC GLOMERULONEPHRITIS, PNEUMONIA

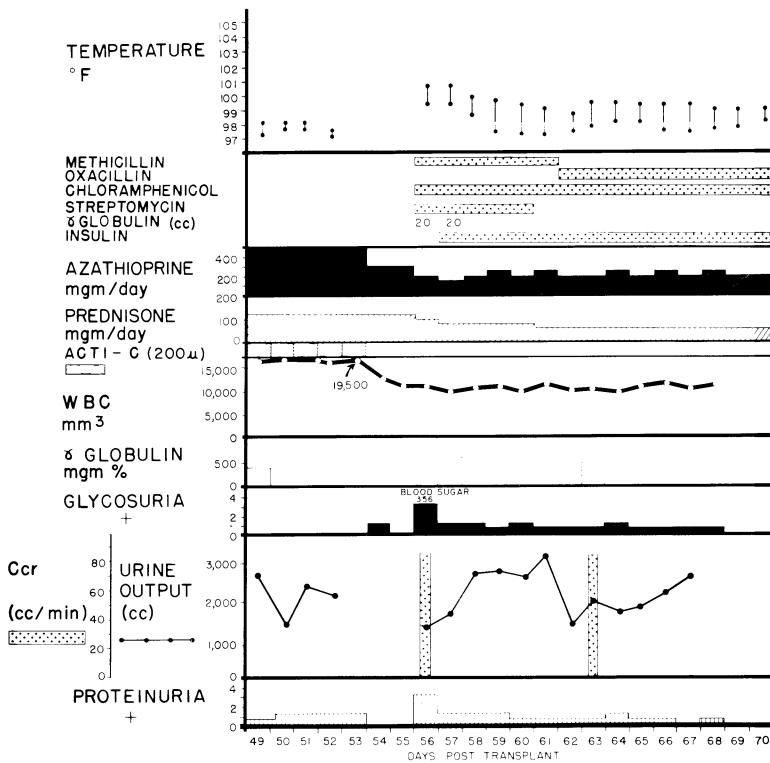


Figure 102. Patient LD 25. Clinical course of *E. coli* right middle lobe pneumonia which responded to therapy.

In such cases in which chronic pyelonephritis is known to exist preoperatively, intensive antibiotic therapy is given before surgery and continued for several weeks following bilateral nephrectomy, which may be advisable at a separate initial stage (see Chapters 3 and 11), and renal transplantation. The choice of therapy in these infections depends largely upon the results of cultures and, antibiotic disk sensitivity studies. Antibiotic therapy is usually followed by therapy for one to two months with an urinary antiseptic agent such as nitrofurantoin, methenamine mandelate, or nalidixic acid.

One patient (LD 22), a 15-year-old girl with polycystic kidney disease, had acute pyelonephritis and multiple fungal abscesses in the cysts. Because of continuing temperatures in excess of 40° C, bilateral nephrectomy and splenectomy were performed, following which temperatures fell promptly to normal (see Figure 4, Chapter 3). Antibiotics were subsequently discontinued. The patient was maintained by hemodialysis for three weeks until renal homotransplantation was performed. Her postoperative course was uneventful after each procedure. In cases such as this in which nephrectomies are performed at a first stage, the bladder has been kept bacteria-free by twice-weekly instillations of approximately 10 cc of normal saline containing 0.5 gm of neomycin and 50,000 units of bacitracin (see Chapter 4).

In 15 additional patients, urinary tract infections appeared for the first time postoperatively, possibly being introduced by the indwelling urethral catheter which was used for the first 24 hours following transplantation. Twelve of these infections were either cured or suppressed. These 19 urinary tract infections were caused primarily by the gram-negative enteric bacilli and pseudomonas species; however, in three instances staphylococci also occurred.

The long-term effects of bacteriuria following renal homotransplantation have yet to be assessed. It would seem reasonable to assume, however, that lower urinary tract infection in these patients with a single homograft kidney who are receiving cytotoxic drugs and corticosteroids could readily lead to ascending pyelonephritis and impaired renal function. For this reason, it is believed that the risk attendant upon the cautious administration of potentially nephrotoxic antibiotics in an attempt to eradicate these infections is warranted.

Pulmonary Infections. Pulmonary infections caused by bacteria or fungi have occurred in 17 patients following transplantation and have proved fatal in all but one. In at least four patients the infection has followed multiple pulmonary emboli. In one instance (LD 25) an *E. coli* pneumonia was cured (Fig. 102). In this case multiple antibiotics were given, the relative hypogammaglobulinemia was treated with 40 cc of gamma globulin, and the steroid-induced diabetes was controlled with insulin.

Fungal pneumonia has occurred in four patients following transplantation. The chest x-ray and microscopic section of a *Candida albicans* pneumonia are shown in Figures 103 and 104. Two cases of aspergillus pneumonia occurred (Figs. 105, 106). In three instances these fungal pneumonias were complicated by a brain abscess. In no case was amphotericin B administered, primarily because of failure to establish the diagnosis ante mortem. The nephrotoxicity of this drug, however, would make its use particularly hazardous for such patients. In addition, the antibiotic is only fungistatic and, therefore, eradication of systemic fungal infections would require the assistance of an adequate host response and resistance. Because of the impairment of these host factors by immunosuppression, it seems doubtful that cure could be effected with this antifungal agent.

Pulmonary tuberculosis occurred in one case following renal transplantation (Figs. 107, 108). In this patient a cavitory lesion in the right lower lobe became apparent following surgery, and two sputum cultures were positive for *Mycobacterium tuberculosis*. The intermediate strength PPD skin test was negative before surgery, a feature which is in keeping with the depressed delayed hypersensitivity that obtains in these uremic patients (Chap. 22). Active pulmonary tuberculosis has also been observed in one patient treated at this center with hepatic transplantation. In addition, this infection has been reported following renal transplantation by Hopewell and after bone marrow transplantation by Mathe. For these reasons transplantation patients are given

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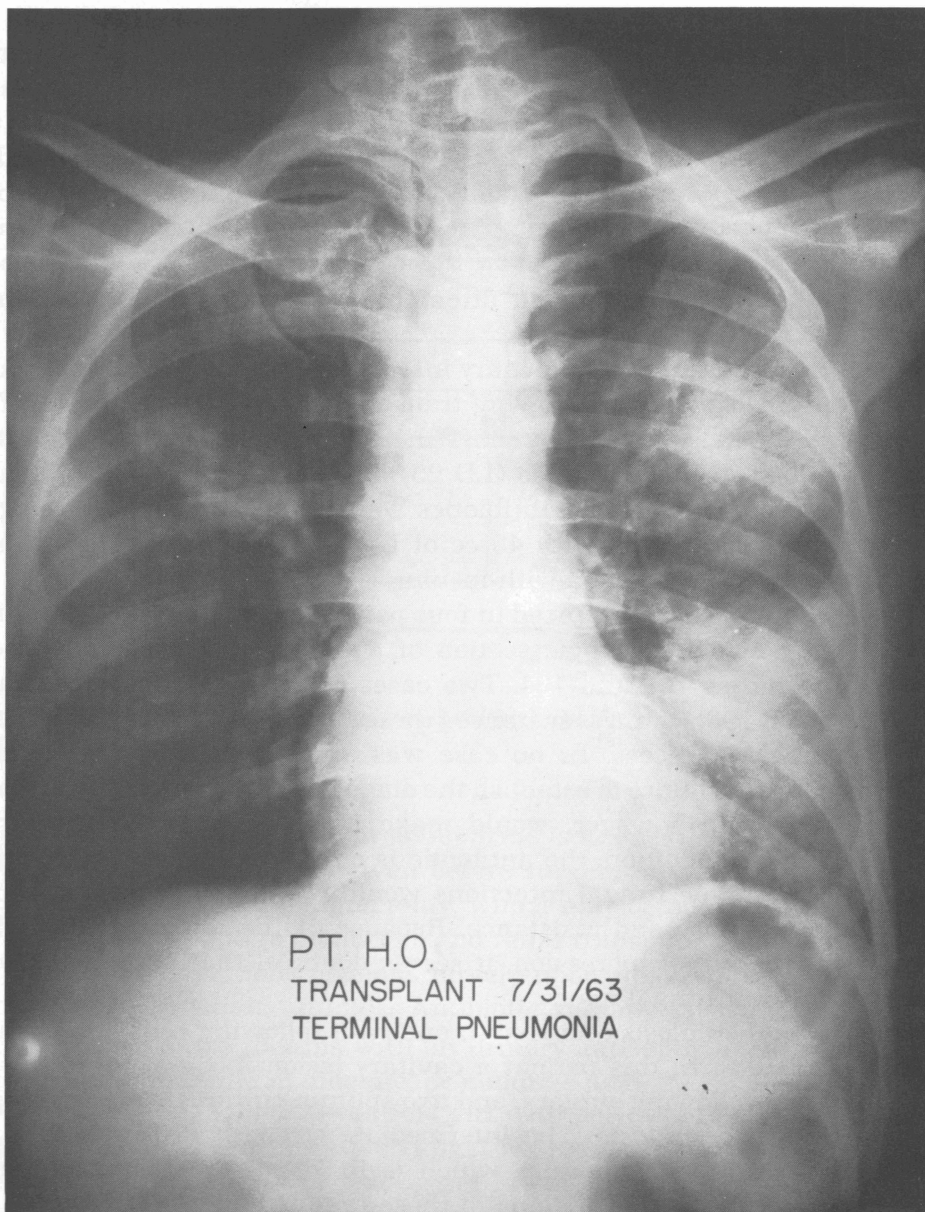


Figure 103. Patient LD 21. Chest x-ray of patient with fatal *Candida albicans* pneumonia.

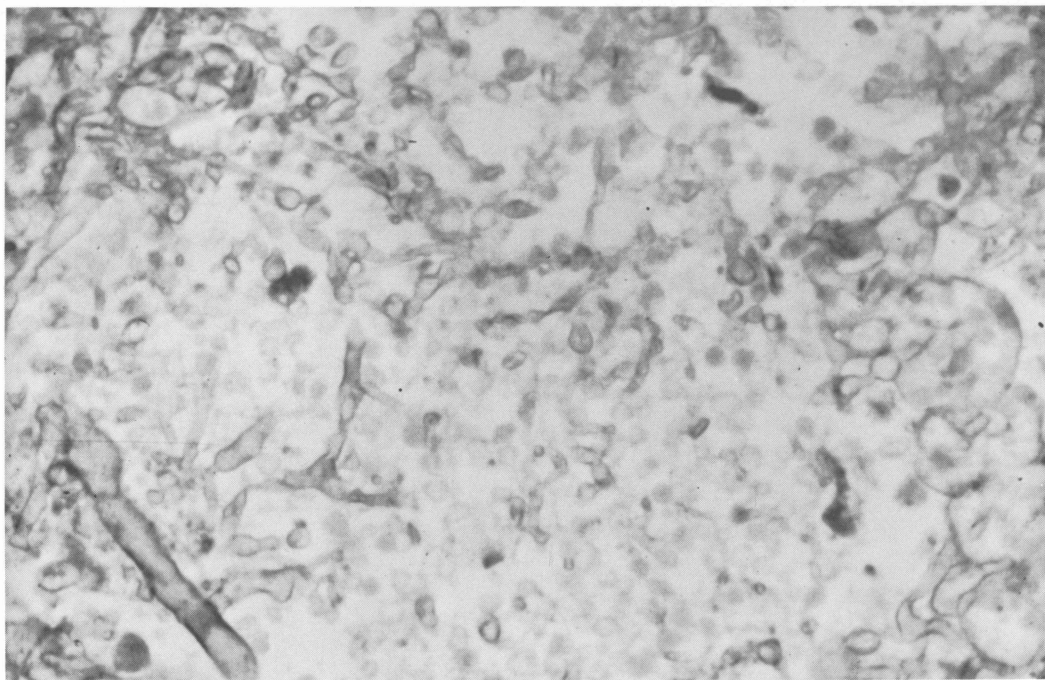


Figure 104. Microscopic section of lung showing necrosis due to *Candida albicans* infection. PAS (X 540). (Courtesy of Rolla B. Hill, Jr., M.D.)

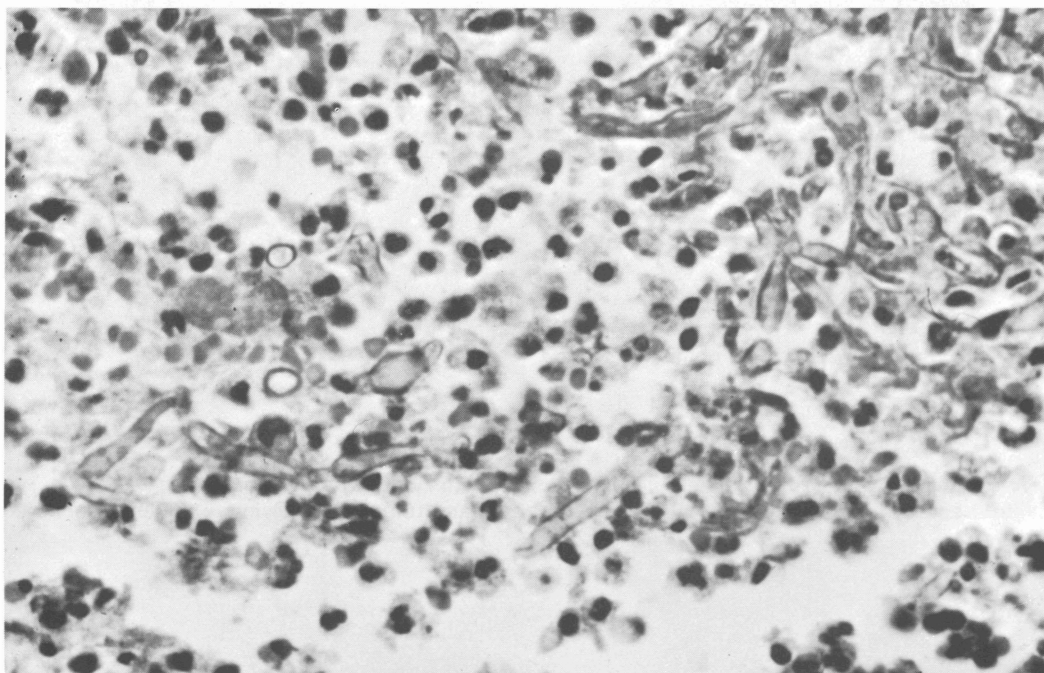


Figure 105. *Aspergillus* pneumonia. (Patient LD 43.) Hyphae elements are present within the focal abscess area. PAS (X 540). (Courtesy of Rolla B. Hill, Jr., M.D.)

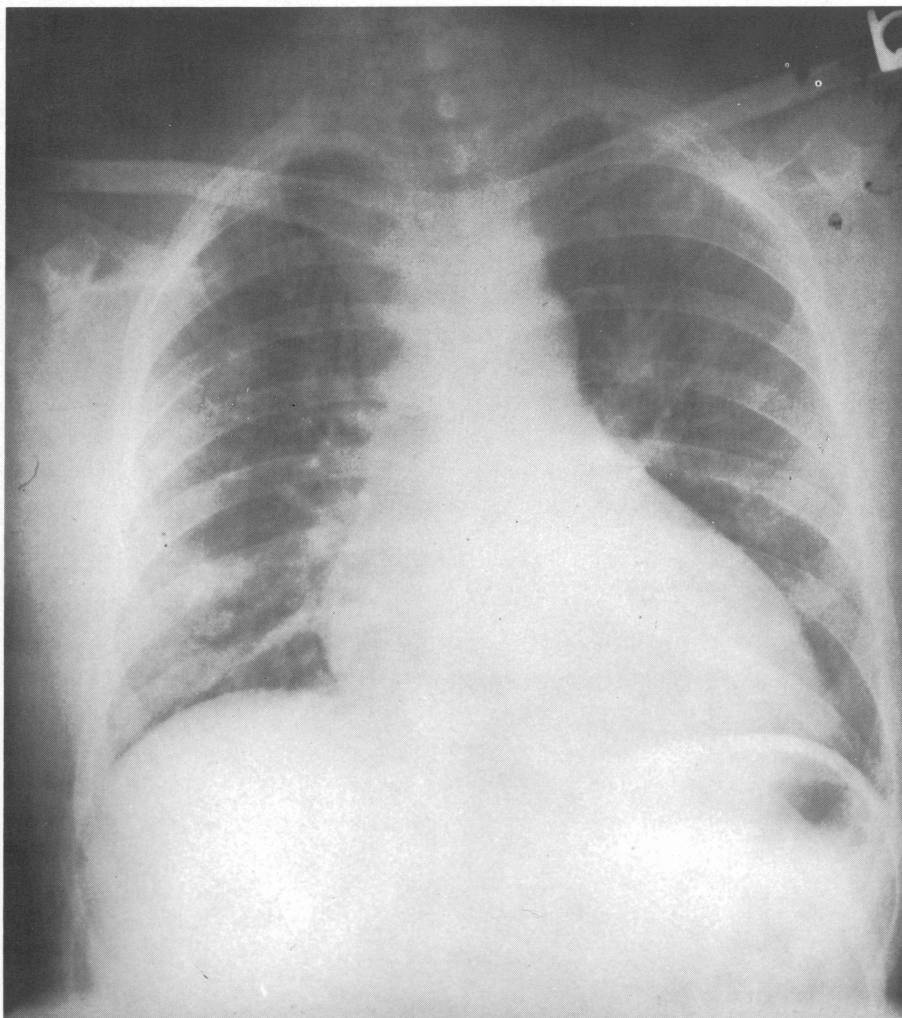


Figure 106. Patient LD 43. Chest x-ray of patient with fatal *Aspergillus fumigatus* pneumonia. Note the nodular lesions in the right lower lung field and the cavitory lesion in the left upper lobe.

LH-28 PT. J.L.H., ♂, CHRONIC GLOMERULONEPHRITIS, BRONCHOPNEUMONIA, TBC.

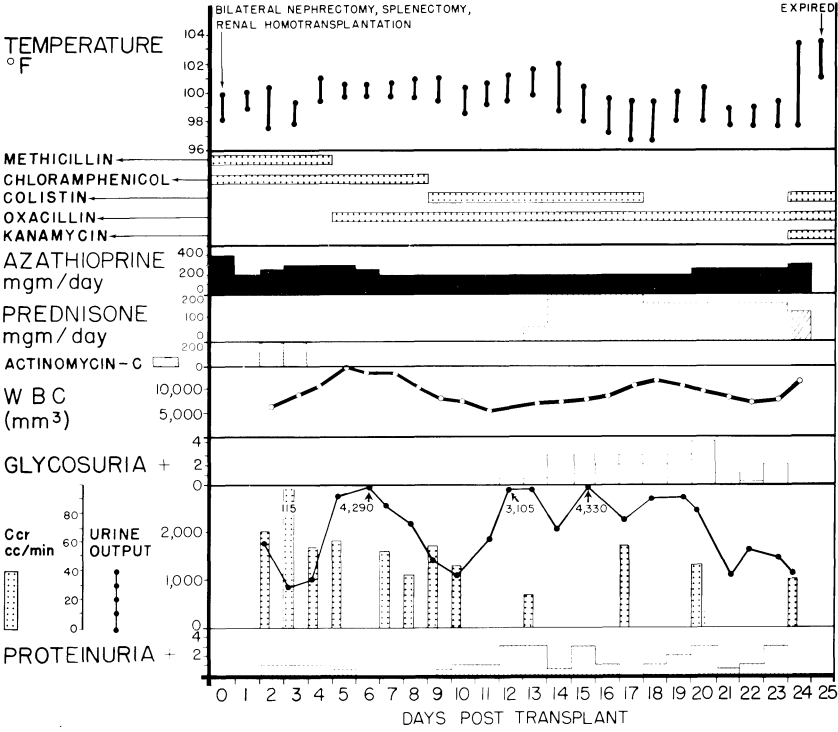


Figure 107. LD 28. Course of patient in whom tuberculosis was reactivated by immunosuppressive therapy.

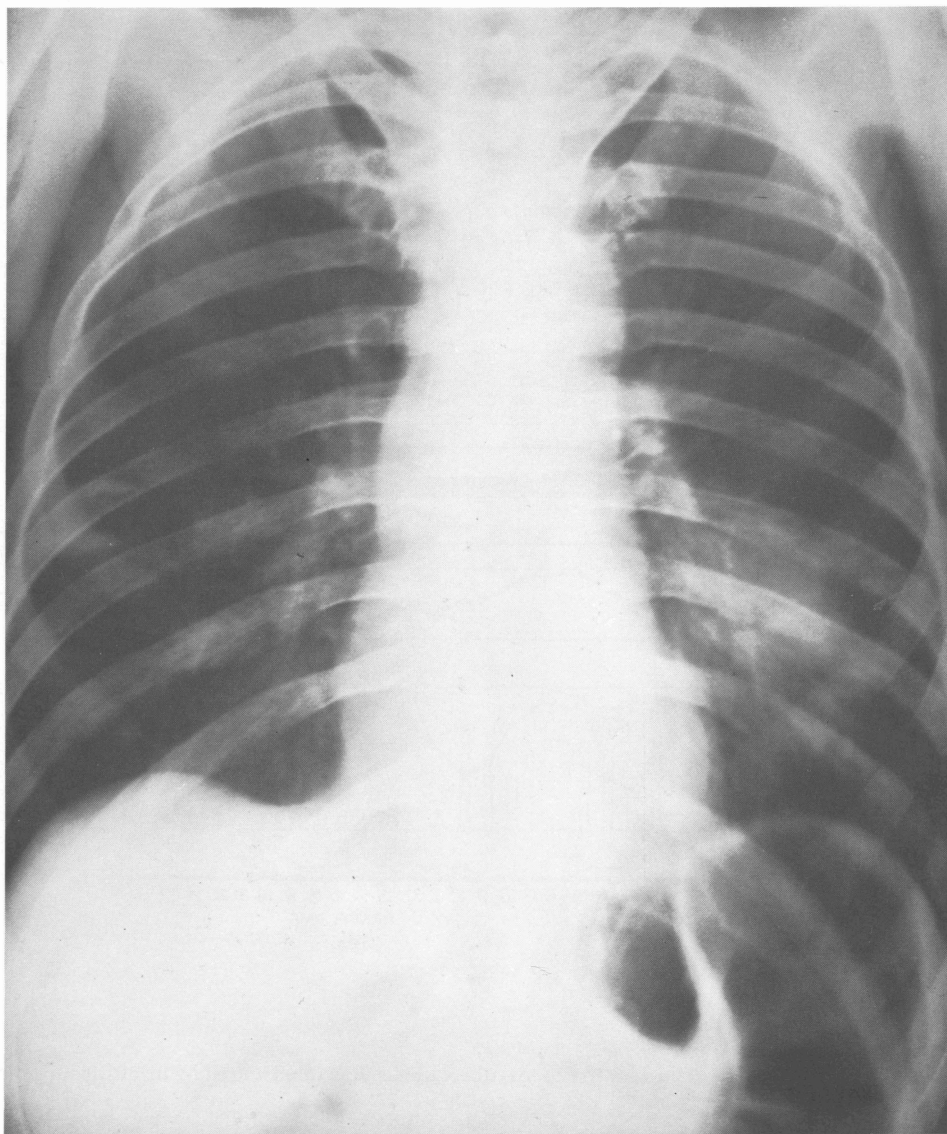


Figure 108. Chest x-ray (LD 28) taken on the day prior to death. Cavitary tuberculous lesion is seen in the right lower lobe and coliform pneumonia in the left lower lobe. (By permission of JAMA 189:402, 1964.)

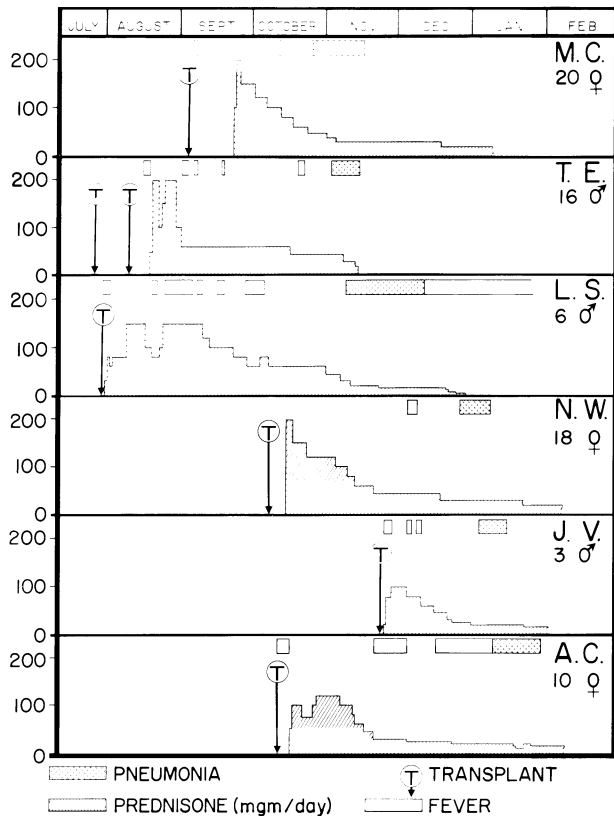


Figure 109. Time of onset of "transplantation pneumonia" in relation to steroid withdrawal in six patients. In each case the pneumonia appeared at a time when prednisone dosage was decreased below 1 mgm/kgm per day. (By permission of JAMA 189: September 14, 1964.)

prophylactic INH, 5 mgm/kgm daily, as soon as immunosuppressive therapy is initiated, and it is continued as long as prednisone is being administered.

"Transplantation Pneumonia." A unique type of pneumonia occurred in eight patients in which none of the usual bacterial or mycotic agents could be implicated etiologically. These "transplantation pneumonias" all occurred in patients under 21 years of age. They were characterized in general by an abrupt onset of fever and an insidious onset of pulmonary symptoms. These pneumonias occurred at the time of steroid withdrawal when the prednisone dosage was decreased to approximately 1 mgm/kgm daily (Fig. 109). Cough of a nonproductive nature, fever, cyanosis, and tachypnea were the predominating features. Physical examination of the chest was routinely unremarkable. The chest x-rays showed bilateral diffuse infiltrates without pleural reaction or hilar adenopathy (Fig. 110). Laboratory studies (Table 22) showed a white blood cell count which varied from 500 to 16,200 mm³. Six patients had a detectable cold agglutinin titer, and in two it was markedly elevated. One patient demonstrated an elevated heterophil titer and, in addition, one had a positive latex-fixation test for rheumatoid arthritis. In two patients lupus erythematosus preparations revealed tart cells.

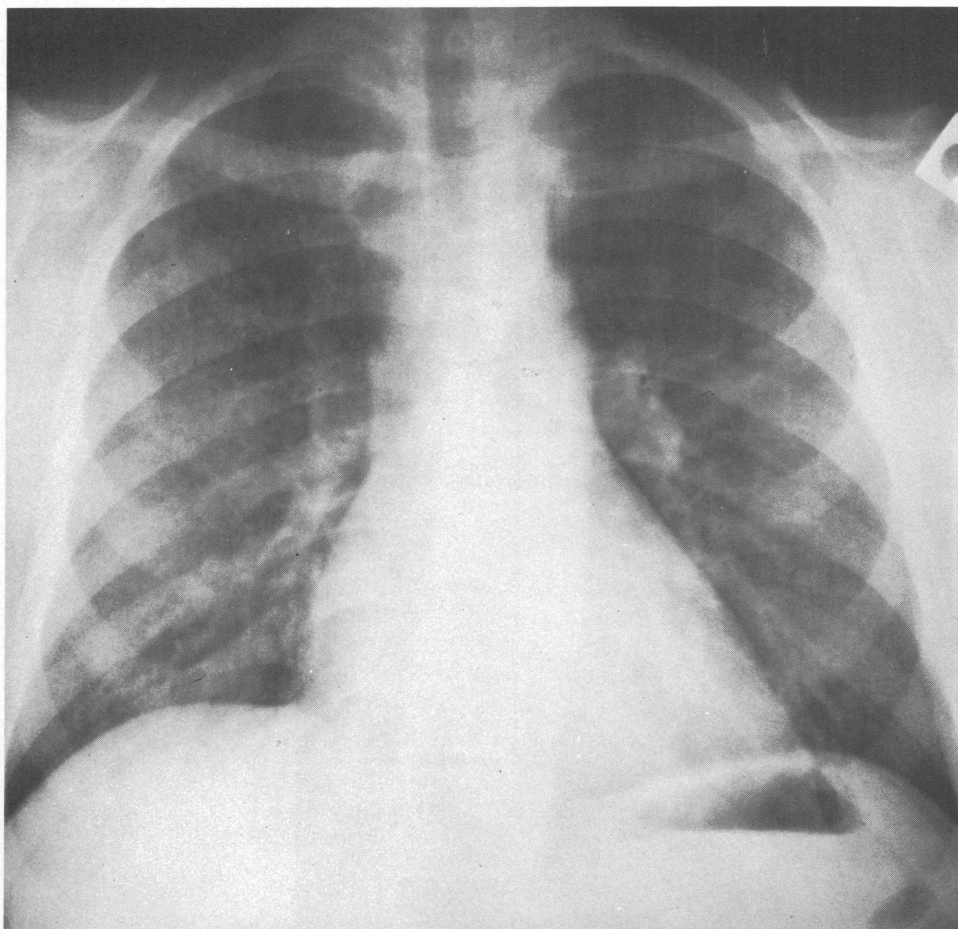


Figure 110. Patient LD 19. Chest x-ray one day prior to death from "transplantation pneumonia." Bilateral hazy infiltrates are present involving the entire lung fields.

Table 22. Laboratory Findings in Patients with "Transplantation Pneumonia"

Series and Number	WBC mm ³	Cold Agglutinin Titer	Heterophil Titer
LD 27	4,500	1:2,048	1:7
LD 19	8,000	1:64	1:14
LD 20	3,000	1:4	1:7
LD 33	14,000	1:1,024	1:896
LD 41	13,400	<1:4	1:14
LD 34	16,200	1:16	1:28
SD 4	500	1:32	1:7
SD 3	2,500	<1:4	1:7

Table 23. Pulmonary Function Studies in Patients with "Transplantation Pneumonia"

Test	Normal Values	LD 27	CASE LD 19	LD 20
Hbg O ₂ saturation, air	> 92%	43	71	46
Hbg O ₂ saturation, O ₂	100%	88	77	
CO ₂ content, air	40-45 vol %	42.2	44.6	39.5
CO ₂ content, O ₂	40-45 vol %	40.7	44.8	
DCO	15 cc/min/mm Hg			2.8
O ₂ V	25-35 liters/liter			46.2

Seven patients were cyanotic, and required oxygen administration. Blood gas studies on three demonstrated an alveolar capillary block syndrome (Table 23). Four patients were treated with antibiotics, although no effect was apparent from such therapy. Five patients recovered after rather protracted courses ranging from two to four weeks.

Examination of the lungs in one fatal case (LD 19) revealed enlarged alveolar cells with intranuclear inclusions characteristic of cytomegalovirus infection (Fig. 111). In addition, the alveoli were filled with a foamy eosinophilic exudate which contained numerous *Pneumocystis carinii* (Fig. 112). In another patient (SD 3) *Pneumocystis carinii* was found at autopsy in association with aspergillosis.

Pneumocystis carinii is believed to be a protozoan parasite which characteristically causes pneumonia in premature and debilitated infants—the so-called interstitial plasma cell pneumonia. More recently this disease has been recognized in adults with malignant disease of the hematopoietic system who are treated with cytotoxic agents and corticosteroids. According to Gajdusek, the coexistence of this agent with cytomegalovirus in pneumonia is not infrequent. The swollen alveolar cells containing intranuclear and occasionally intracytoplasmic inclusions are believed on morphologic grounds to represent cytomegalovirus infection. This agent is found in the salivary glands of approximately 10 per cent of infants at autopsy, and Weller and Hanshaw have demonstrated that antibodies to this agent are present in the serum of 80 per cent of normal adults. Intranuclear inclusions have been noted by Rolla B. Hill, Jr., in the lungs of approximately one-third of patients dying after renal transplantation (Figs. 113, 114). In a number of these cases no evidence of pulmonary disease was suspected clinically or could be demonstrated radiographically. The presence of these inclusions is believed to represent an essentially asymptomatic spread of latent cytomegalovirus under the influence of immunosuppressive therapy.

The frequency and role of *Pneumocystis carinii* and cytomegalovirus in transplantation pneumonia remains to be demonstrated conclusively. Both

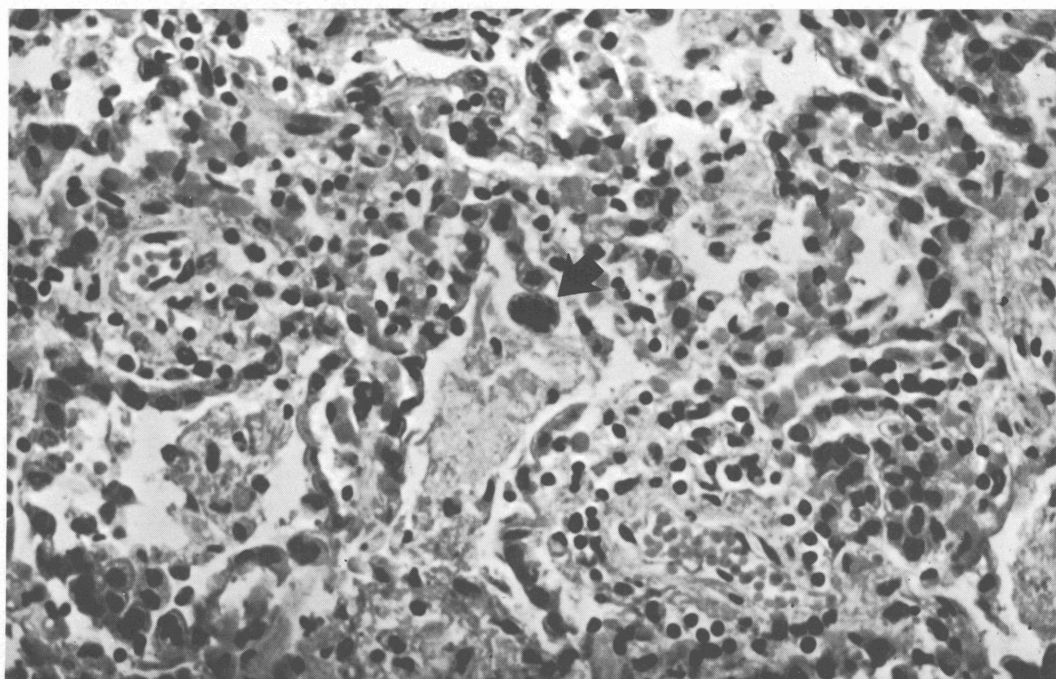


Figure 111. Microscopic section of lung of a fatal case of "transplantation pneumonia." Patient LD 19. Enlarged alveolar cells and macrophages (arrow) with intranuclear inclusions are present. The alveolar spaces are filled with a foamy eosinophilic exudate containing *Pneumocystis carinii*. H and E (X 460). (Courtesy of Rolla B. Hill, Jr., M.D.)

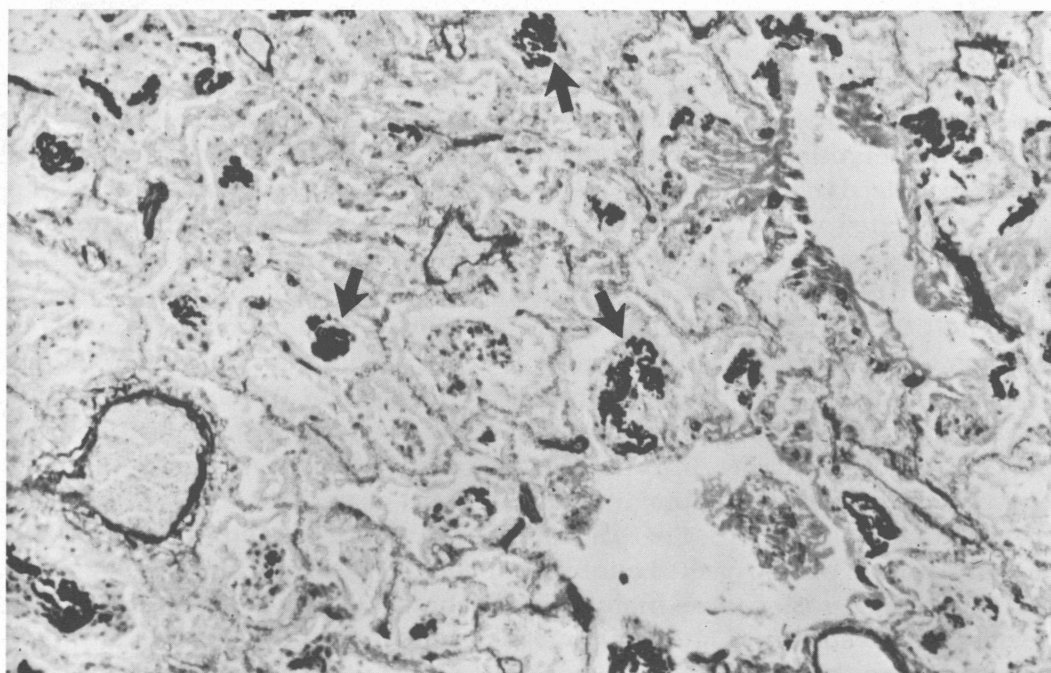


Figure 112. Patient LD 19. Dark staining cysts of *Pneumocystis carinii* are seen within the alveolar spaces (arrows). Methenamine silver (X 115). (Courtesy of Rolla B. Hill, Jr., M.D.)

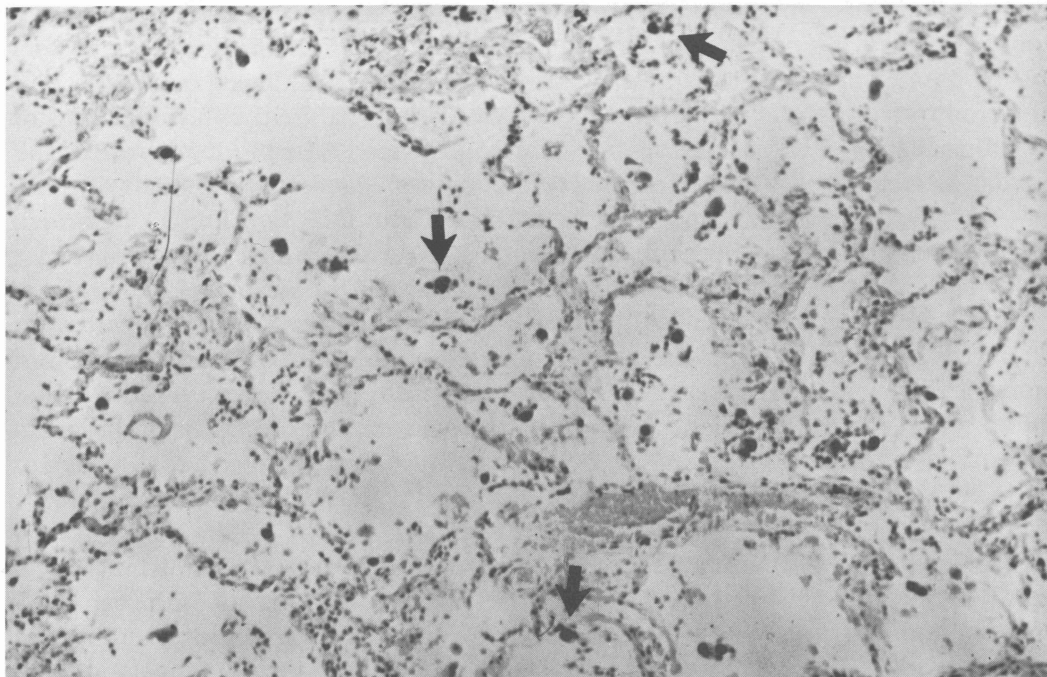


Figure 113. Microscopic section of lung of Patient LD 16. Many enlarged alveolar cells and macrophages are seen containing intranuclear inclusion bodies (arrows). H and E (X 95). (Courtesy of Rolla B. Hill, Jr., M.D.)

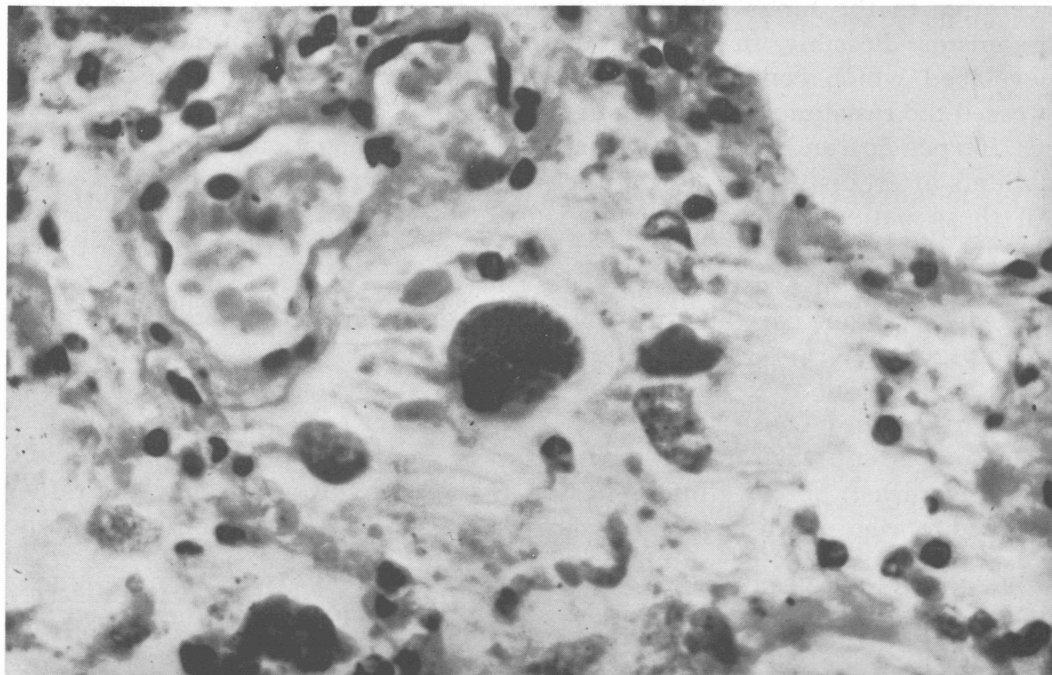


Figure 114. Microscopic section of lung of Patient LD 8. Large intranuclear inclusion bodies and an occasional intracytoplasmic inclusion are seen. H and E (X 660). (Courtesy of Rolla B. Hill, Jr., M.D.)

Table 24. Herpes Zoster following Renal Transplantation

Series and Number	Onset (Days) Post-Transplant	Immunosuppression		Preceding X-Ray	
		Azathioprine	Prednisone	Diagnostic	Therapeutic
LD 25	*	-	-		
SD 6	22	+	+		+
LD 49	26	+	-		+
LD 30	187	+	+	+	+
LD 39	15	+	+		+
LD 22	191	+	-	+	
LD 1	509	+	-		

*Onset 30 days prior to transplant.

these agents characteristically produce disease in the debilitated patient and usually invade in the presence of depressed host resistance. It may be that in these cases other factors aside from immunosuppressive drug therapy were operative in the lungs which predisposed them to invasion by these micro-organisms. Possibly, in reacting to the graft kidney, an immune response developed which concomitantly attacked the lung and thereby further depressed the resistance of this organ to infection.

Herpes Zoster. Herpes zoster has appeared in one patient before, and in six patients after, institution of drug therapy for renal transplantation (Table 24). All these patients had had a previous history of varicella. In four cases, therapeutic irradiation to the implant had been given several days prior to the appearance of the zoster lesions. In two cases extensive diagnostic x-ray procedures, in one case an intravenous pyelogram, and in the other studies of the hips and pelvis preceded the development of zoster. These lesions have run an essentially normal and benign course despite the immunosuppressive therapy, and in no case has varicella pneumonia or encephalitis developed. In only one patient was therapy, in the form of gamma globulin, administered.

In contrast, herpes simplex has been surprisingly rare despite the rather marked febrile responses which occurred in many of these patients during the rejection crisis and in conjunction with infectious diseases. A severe "cold sore" has been observed in only one patient. This virus, like the varicella-zoster agent, is frequently carried in the latent state, and it could have been anticipated that activation of the virus in renal transplantation patients would have been common.

Hepatitis. Hepatitis has occurred in two patients following transplantation, in one (LD 36) three months and in the other (LD 15) six months post-

operatively. On the basis of clinical symptoms it would appear that the first case may represent homologous serum jaundice, and the second, infectious hepatitis. The possibility that the liver disease in either case is related to immunosuppressive drug therapy, however, cannot be excluded. The course in both these patients has been prolonged, and progression into a subacute or chronic form of the disease may occur. Therapy in these cases has consisted primarily of bed rest, diet, and adjustment of steroid dosage.

The rather severe course of hepatitis in the two patients receiving immunosuppressive therapy is of interest in light of the observation by Good that patients with agammaglobulinemia do poorly following hepatitis, while they handle the usual viral infections in an apparently normal fashion. It would appear that an intact immune system may be necessary for recovery from hepatitis while in other viral diseases ancillary defense mechanisms, such as interferon, may facilitate recovery. In addition, it has been noted that viral upper respiratory disease in transplant patients is no more severe or protracted than in other persons.

Infections after Heterotransplantation. The problems of infectious disease associated with heterotransplantation (Table 20) were similar to those encountered in homotransplant recipients. It is interesting to note that no evidence for the transmission of infectious agents from the baboon to the recipients occurred. In one instance, *Hepaticystis kochii* was identified in the liver of a donor baboon.* Only the gametocyte stage of this malaria parasite appears in the blood of the *Papio doguera* and for this reason transmission to man by means of inoculation would be very unlikely. In addition, viral cultures of segments of ureters of four baboon donors failed to reveal any latent agents.

PROSPECTS

In general the disease pattern in renal transplant patients is that of severe and fatal infections caused by a wide variety of agents which are infrequently associated with disease in the otherwise normal host. The problems of infectious disease are similar to those which occur in patients with a variety of hematologic, neoplastic, and metabolic disorders who are receiving anti-inflammatory and cytotoxic agents. The methods which are evolving for the management of infections in patients treated by transplantation could be applicable, in part at least, to a wider range of medical problems and could have direct applicability in future clinical trials of transplantation of other organ systems. Detailed studies of the cellular and molecular alterations produced by immunosuppressive drugs which result in heightened susceptibility to infection could yield important information regarding the multiple defense mechanisms of the normal host against invading microorganisms.

*Identified by George L. Graham, Associate Professor of Parasitology, University of Pennsylvania.

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Chapter Twenty-two

IMMUNOLOGIC ASPECTS OF RENAL HOMOTRANSPLANTATION

*by W. E. C. Wilson, M.D., and
Charles H. Kirkpatrick, M.D.*

The developments in surgery which are described in the preceding portion of this book have resulted in technically successful transplantation of human kidneys prior to the solution of many fundamental problems in transplantation immunology. Nevertheless, a remarkable, and to some extent unexpected, degree of success has been achieved with human kidney homografts. Analysis of the reasons for this are important in order to determine if, and to what extent, the growing body of knowledge about renal homotransplantation can be applied generally to the transfer of other tissues and organs.

This chapter summarizes an inquiry into the immunological capability of the chronically uremic patient and the changes in immunologic responsiveness which follow operative correction of the kidney failure. In addition, current laboratory approaches to a number of practical problems in human tissue transplantation will be reviewed, including selection of the most appropriate donor, early detection of rejection, and recognition of a tolerant state.

EVALUATION OF IMMUNOLOGIC RESPONSE IN UREMIA

In 1955, Hume and his associates reported the transplantation of kidneys from unrelated donors to nine patients with terminal renal failure. Four of the nine kidneys functioned for five to 25 weeks even though immunosuppressive therapy was not given. Dammin, Couch, and Murray subsequently observed histologic evidence of skin homograft survival in six uremic subjects for as long as 115 days. These observations all indicated that the graft rejection mechanism was impaired in the presence of uremia. A detailed study of the im-

munologic capability of patients with advanced renal disease was therefore undertaken.

Delayed Cutaneous Hypersensitivity. Of the various expressions of immunologic potential, delayed cutaneous hypersensitivity is believed to be the type most similar to homograft rejection. In both instances, the reaction has been thought to be mediated by an "antibody" which is intimately associated with lymphoid cells rather than by demonstrable humoral antibody.

A group of 33 uremic patients who were being evaluated for renal transplantation were studied with respect to their responses to a panel of seven antigens which were expected to elicit delayed cutaneous reactions. All patients had irreversible renal failure and, in the majority of instances, this was the result of chronic glomerulonephritis. Intracutaneous testing was performed prior to the institution of any therapy known to alter cutaneous reactivity. The antigens used were commercial preparations and were diluted according to the manufacturers' directions. Intermediate strength purified protein derivative, histoplasmin, blastomycin, coccidioidin, and mumps skin test antigen were administered in 0.1 ml intradermal inocula. Purified extracts of *Candida albicans* and *Trichophyton inguinale* (1:100 dilutions) were given intradermally in 0.03 ml inocula. Reactions were measured at 24 to 48 hours, and induration of 5 mm diameter or greater was considered to be a positive response. For each uremic patient, a normal subject was tested with the same antigens. The normal subjects were being evaluated as kidney donors, and in 26 of the 33 a genetic relationship to the uremic patient existed. Three potential donors were related only by marriage, and the remaining four were unrelated volunteers.

Of 212 tests applied to the donor and to the recipient groups, 89 positive responses were seen in the normal subjects. There were only 28 positive reactions in the uremic patients. Table 25 reviews the reactions observed with each antigen and demonstrates that delayed cutaneous responsiveness to all the antigens was impaired in the presence of chronic uremia.

To characterize the nature of this impaired responsiveness, the skin tests were repeated in 23 patients who were selected for renal transplantation, and who were available for retesting in the immediate postoperative period prior to the institution of adrenal steroid therapy. Because the kidney transplantation operation involved the transfer of leukocytes within the renal vasculature and the transfer of hilar lymphoid tissue, a potential model for the passive transfer of delayed hypersensitivity existed. The majority of the patients were retested during the 48 hours following surgery, and all were studied by the twelfth day. Some patients were retested a second time in association with the rejection crisis, and the results of this study will be discussed later in this chapter.

In the postoperative period, each patient was found to have reactions in addition to those present preoperatively. In each instance of acquired reaction, sensitivity to the antigen had been previously demonstrated in the donor. Table 26 illustrates that 61 specific reactivities were possessed by the donors, but not by their recipients. Forty-seven (77 per cent) of these reactivities were demonstrable in the kidney recipients after the operation. Immediate function of

Table 25. Preoperative Tests for Delayed Cutaneous Hypersensitivity in Uremic Patients and Their Donor Candidates

Antigen	No. of Donor-Recipient Pairs Studied	Positive Reactions in Recipients	Positive Reactions in Donor Candidates
Intermediate PPD	33	0	5
Histoplasmin	33	1	9
Blastomycin	33	0	0
Coccidioidin	33	0	7
Mumps	33	11	26
Candida	30	11	29
Trichophyton	17	5	13
Total	212	28	89

Table 26. Passive Transfer of Delayed Cutaneous Hypersensitivity following Kidney Transplantation

Antigen	Reactivities Possessed by Donors but Not Recipients Preoperatively	Reactivities Demonstrated in Recipients Postoperatively	
	No.	No.	Per Cent
Intermediate PPD	7	3	43
Histoplasmin	6	1	17
Blastomycin	0	0	--
Coccidioidin	9	7	78
Mumps	16	15	94
Candida	14	13	94
Trichophyton	9	8	89
Total	61	47	77

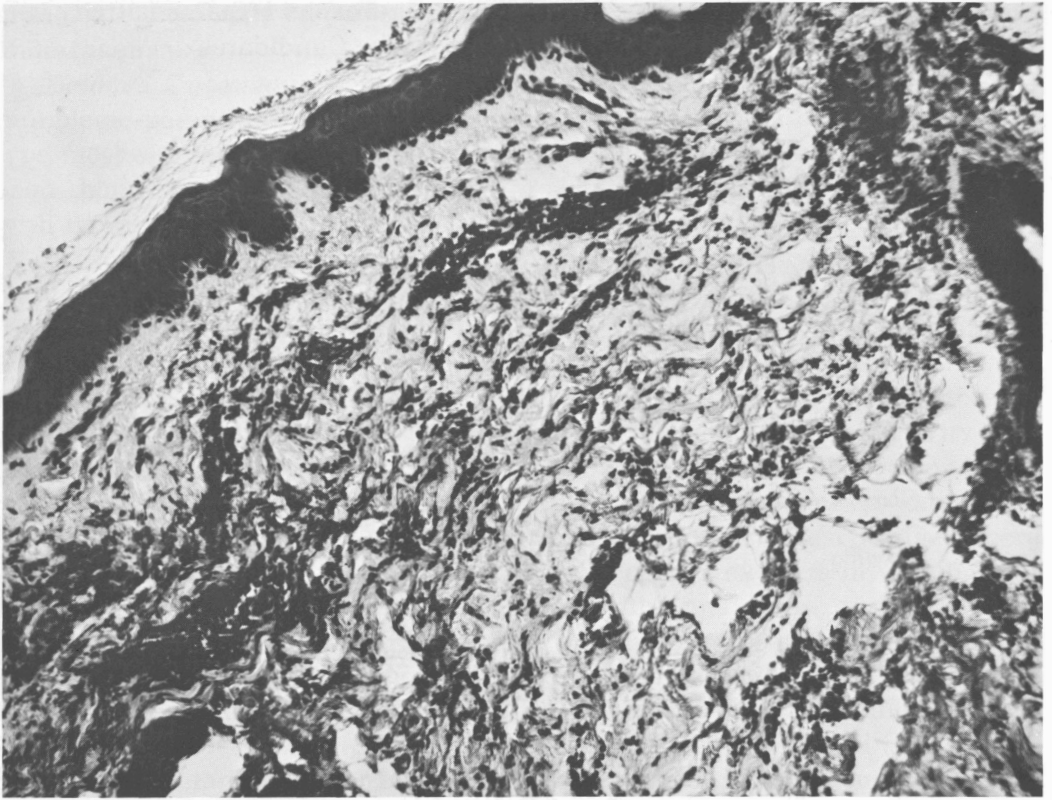


Figure 115. Photomicrograph of a skin biopsy of a passively acquired reaction to *Candida albicans* in a 40-year-old male kidney recipient (LD 30). Note the perivascular aggregation of mononuclear cells, which are predominantly lymphocytes. The biopsy was obtained 24 hours after antigen injection.

the kidney did not occur in all patients, indicating that correction of the uremia was not a prerequisite for the postoperative change in skin reactivity. The results indicate that passive transfer of immunologic competence accompanied the kidney transplantation.

Furthermore, it is apparent from this investigation that the basis for the impaired cutaneous reactivity in uremia is not an inability of the skin to respond, and the defect must therefore lie in the preparative phase of reaction. Similar observations have been made by Sones and Israel in patients with sarcoidosis. The decreased incidence of delayed cutaneous reactivity demonstrated by Kelly in Hodgkin's disease appears to have a different pathogenesis, since passive transfer studies in patients with this disease were unsuccessful.

The histologic features of the delayed cutaneous hypersensitivity, passively acquired by the kidney recipients, were studied by Rowlands with serial skin biopsies. The microscopic reaction appeared to reach its peak at 24 to 27 hours, and by 36 hours the infiltrate had decreased. Figure 115, a photomicrograph of a biopsy taken 24 hours after antigen injection, demonstrates the

perivascular accumulation of lymphocytes which is a characteristic of the delayed cutaneous response.

A number of features which characterize delayed cutaneous hypersensitivity have been observed in homograft rejection. Because of these similarities, many investigators consider these two immunologic reactions to have a common pathogenesis. It was therefore of interest to study the relationship of the suppressed delayed cutaneous reactivity demonstrated in these uremic patients to their ability to respond to the transplanted kidneys.

Assessment of delayed cutaneous reactivity was carried out in 25 patients who later received renal homografts and who survived the immediate post-operative period. These patients were divided into two groups on the basis of their reactions to the panel of antigens. Fourteen patients had no reactions to the antigens, and the 11 members of the other group reacted to at least one antigen. These groups were compared with respect to the time of onset of the rejection reaction. Criteria for the diagnosis of rejection have been discussed in Chapter 15, and include BUN elevation, decrease in urinary volume, fall in creatinine clearance, and fever. A favorable response to adrenal steroid therapy supported the diagnosis.

One patient who showed no response to the antigens has not manifested a rejection crisis (LD 14) and will not be considered further. The mean time of onset of the rejection reaction in the remaining 13 unresponsive patients was 14.8 days. By contrast, the onset of the reaction occurred on an average of 4.3 days after transplantation in the group of 11 patients in whom delayed cutaneous reactivity had been demonstrable.

The two groups compared were similar with respect to age and to the duration of operative renal ischemia. When these 24 patients who experienced a rejection crisis were divided on the basis of donor relationship, no difference in the time of onset of rejection was noted between those who were related to their donors and those who were not.

These observations support the concept that impaired immunologic responsiveness in uremia is an important factor in successful human kidney transplantation. Furthermore, the difference in rejection times between the responsive and unresponsive groups suggests that the reactive group might benefit from additional immunosuppressive therapy prior to the rejection crisis (see Chapter 18).

Immediate Cutaneous Hypersensitivity. To further evaluate immunologic responsiveness in uremia, a group of patients were studied with respect to their ability to manifest cutaneous wheal and erythema. Such a response, following 15 minutes after intracutaneous injection of antigen, indicates the presence of a specific circulating antibody, which has skin-fixing properties. In this chapter, antibodies possessing this characteristic are referred to as reagins.

The reactions obtained in 30 uremic patients were compared to the reactions of their donors to a panel of antigens expected to elicit the wheal and erythema response. Twenty-three donor-recipient pairs were genetically related.

Table 27. Preoperative Tests for Immediate Cutaneous Reactivity in Uremic Patients and Their Donor Candidates

Antigen	No. of Donor-Recipient Pairs	Positive Reactions in Recipients	Positive Reactions in Donor Candidates
Molds	30	6	19
Trees	30	2	5
Chenopods	30	1	4
Ragweeds	30	3	7
Grasses	30	1	7
Candida	30	8	20
Trichophyton	17	5	12
Total	197	26	74

The antigens were commercial preparations of mixed molds, mixed tree pollens, mixed chenopod pollens, mixed pollens of ragweed and sage, mixed grass pollens, *Candida*, and *Trichophyton*. All were used in 0.03 ml intracutaneous inocula. Table 27 summarizes the results of this comparison in which 74 positive reactions (greater than 1 cm wheal or erythema) were observed in the normal group, compared to only 26 in the uremic patients. When comparison was made between only the genetically related pairs, 18 positive responses were seen in the uremic group and 61 in their normal relatives.

To define the pathogenesis of this impaired response, the skin reactivity to a standard intradermal injection of 0.03 ml of histamine (1:100,000) was measured in 14 uremic patients. The mean wheal and erythema response was 2.5 cm with a range from 1.4 cm to 4.0 cm. These reactions were not significantly different from those seen in the normal subjects. Passive sensitization to ragweed was successfully demonstrated after intracutaneous injection of 0.1 ml of serum, containing reaginic antibody to ragweed, in all 15 patients studied in this manner. In seven of these patients, the response to passively administered reagin was determined quantitatively using tenfold dilutions of the antibody-containing serum. All seven showed reactivity following injection of the 1:10 dilution, and three had reactivity with the 1:100 dilution. Injection of a 1:1,000 dilution did not produce passive sensitization. These responses were similar to those of the donor population.

These results indicate that the decreased response in uremia is the result of an absence of a sufficient quantity of a specific reagin since the cutaneous

Table 28. Humoral Antibody Response to Typhoid Vaccine in 10 Uremic Patients and 22 Normal Subjects

Antigen	Group	*Prestimulation Titer	*Poststimulation Titers		
			1 wk	2 wk	3 wk
O	Normal	20	50	80	50
O	Uremic	**10	10	30	20
H	Normal	30	70	80	60
H	Uremic	10	10	40	20

*Titers expressed as the mean of the reciprocals of the titer observed in each member of the group.

**Patients and normal subjects with an observed titer of less than 1/20 were arbitrarily assigned a reciprocal value of 10 for determination of the mean titer of each group.

receptor sites for reagin, the histamine content of cutaneous mast cells, and the vascular response to histamine are normal. Recent studies by Heremans and Vaerman indicate that reagin is a constituent of the γ_{1A} globulin fraction of serum proteins. The presence of grossly normal γ_{1A} globulin bands following immunoelectrophoresis of the sera of 18 patients in this group does not suggest a gross disturbance of γ protein metabolism. Quantitative determinations of serum γ globulin levels showed values in the normal range.

Induction of Humoral Antibody Production. Because the previously outlined results were indicative of suppression of pre-existing immunologic capability in individuals whose effector pathways appeared to be intact, a study was undertaken to measure the ability of the uremic patient to produce humoral antibody in response to a standard antigenic stimulus. Typhoid vaccine was selected as the test antigen because the normal response has been shown by LoSpalluto to involve both γ_{1} and γ_{2} globulin production. Typhoid vaccine in a dosage of 0.5 ml was administered subcutaneously to 10 uremic subjects, most of whom had by history been previously immunized. The responses seen at intervals after the booster injection are summarized in Table 28. The patients' responses were significantly suppressed when compared to those of a normal population.

Conclusion. The impaired ability of the chronically uremic patient to reject homografted tissue appears to be only a part of a more generalized suppression of immunologic capability which includes the delayed cutaneous hypersensitivity reaction and the production of all three classes of humoral antibody (i.e., γ_{1A} , γ_{1M} , and γ_{2} globulin).

DONOR SELECTION

The problem of selecting the optimal tissue donor remains a major obstacle in successful organ transplantation. Since most uremic patients do not have an identical twin, a donor must be selected who is of necessity genetically dissimilar to the recipient. It has already been pointed out (see Chapters 5 and 18) that use of the patient's mother or a sibling greatly increases the likelihood of success of a renal homograft, presumably because of reduction in the number of differences in histocompatibility antigens.

Matching of Tissue Antigens. Although little is known about tissue antigens in man, the existing data suggest that a great many are present and that a few are organ specific. Until more refined methods of tissue typing become available, the immunologist must devise experimental models which will permit some degree of comparison of tissue antigens between donor and recipient. In this section we propose to review some immunologic studies which have been undertaken in an attempt to identify antigenic relationships between the recipient and a prospective donor. Although several tests have been proposed by various groups, no one method has received sufficient clinical trial to warrant its general application. The problem is further complicated by the fact that the long-term success of a transplanted organ will require months or years to determine.

Because transplantation of any test tissue from a donor to the recipient may result in sufficient sensitization to jeopardize the success of the final graft, Wilson, Henry, and Merrill have described a method for detection of common antigens which is based upon the observed behavior of skin grafts transplanted from both the prospective donor and recipient to a third person. The first graft is transplanted from the recipient to the third person, and 15 days later skin from the donor is similarly placed. The rate of rejection of this second graft gives an indication of the degree of antigen-sharing between the two grafts. A second graft which is rejected rapidly (accelerated rejection) or without becoming vascularized (white graft rejection) implies a high degree of antigenic similarity between the donor and recipient. Conversely, a second graft which is slowly rejected indicates that few antigens are shared. Although this system does permit assessment of antigens which are common to skin from different subjects, the direct application of the method to organ transplantation is limited because it does not provide for assessment of antigenic differences. Experimental verification of the importance of this limitation has been reported by Rapaport and his associates, who observed a poor correlation between the indirect demonstration of shared antigens with this method and the survival of test skin homografts.

A different approach to donor selection has been proposed by Brent and Medawar. In their experiments, lymphocytes from a potential recipient guinea pig were injected intradermally into several other guinea pigs which were later used as skin graft donors. The intensity of the cutaneous reaction was found to correlate directly with the order of breakdown of skin grafts after

subsequent transplantation. The animal that showed the weakest cutaneous reaction was the donor of choice. This inflammatory response was believed to be a localized "graft versus host" phenomenon in which the injected leukocytes reacted against antigens in the donor skin. This scheme permitted some classification of donors based on the severity of the reaction of the immune mechanism of the recipient. It also has the advantage of not sensitizing the recipient.

When the method was investigated in guinea pigs, in which it could be evaluated by test skin grafts from all members of the panel, it was successful in predicting relative rejection times. A similar direct evaluation of its applicability in human kidney transplantation is impossible. A partial appraisal of the method may be derived from the study of a large group of patients who are to receive kidneys from genetically related donors. These patients are known to have a more favorable prognosis than those receiving kidneys from unrelated volunteers. Indirect support for the utility of the method would be obtained by the demonstration that lymphocytes from the patients of this preferred group cause a less intense reaction in the skin of their related donors than they cause in the skin of nonrelated individuals.

We have applied this approach in two patients who were being evaluated for renal transplantation. In the first case (LD 23), the panel of donors included the patient's brother and two unrelated volunteers. All members of the group were given 3.52×10^6 lymphocytes from the recipient, and the cutaneous induration was measured at 24 and 48 hours. These data are summarized in Table 29. The patient's brother was selected to be the donor because of his genetic relationship. Unfortunately the kidney failed immediately, probably as the consequence of a blood group incompatibility (see Chapter 6) that will be discussed in more detail. A second transplantation was subsequently done using an unrelated donor. This organ was still functioning nine months later.

Another patient (LD 28) was also studied in a similar manner. His lymphocytes were injected into his sister and an unrelated volunteer prior to transplantation (Table 29). Minimal reactions occurred in both, possibly because the cellular inoculum was small. The kidney transplanted from his sister functioned well for several weeks and then deteriorated. The patient died as the result of drug toxicity, rejection, sepsis, pulmonary embolization, and intestinal infarction (Chap. 19).

While our experience with this method of donor selection is limited, and definitive conclusions are not possible, in the two patients studied the system did not reveal differences between related and unrelated potential donors. We have also attempted to apply the principle of this method to the early detection of graft rejection. These experiments will be described further on.

The relationship of leukocyte antigens to survival of renal homografts in six cases has been reported by Hamburger and his associates. Leukocytes from both donor and recipient were "typed" according to their reaction in leucoagglutinating sera obtained from a large number of hyperimmune subjects. All

Table 29. Donor Selection Using Intradermal Injection of Lymphocytes from Recipients

Group	Relation	Inoculum Total Lymphocytes	Induration (cm)
Recipient (LD 23)		3.52 X 10 ⁶	0.6
First donor	Brother	3.52 X 10 ⁶	0.5
Second donor	Unrelated	3.52 X 10 ⁶	0.5
Control	Unrelated	3.52 X 10 ⁶	0.7
Recipient (LD 28)		0.89 X 10 ⁶	0
Donor	Sister	0.89 X 10 ⁶	0.1
Control	Unrelated	0.89 X 10 ⁶	0

these patients were related to the donors, and in three of the six the mothers donated kidneys to their children. In the three cases in which the donors possessed leukocyte antigens different from those of the recipient, function of the transplanted kidney ceased within hours after surgery, and all three recipients were dead by the twentieth postoperative day. However, in three cases all donor antigens were possessed by the recipients, and graft survival was prolonged. Two patients were alive, one and two and a half years after transplantation, at the time of reporting. While the number of patients in this series was small, the results suggest that leukocyte typing may have more general application in donor selection. The number of antigens which are common to leukocytes and kidney tissue is not known.

An additional approach to the study of antigenic relationships, which is now being evaluated in several centers, is investigation of the behavior of the recipient's lymphocytes in the presence of histocompatibility antigens from the proposed donor. Bain, Vas, and Lowenstein observed that, when peripheral blood lymphocytes from two individuals are mixed in tissue culture for five days, the lymphocytes enlarge and synthesize DNA. It was suggested that histocompatibility differences between the two lymphocyte populations may have been the stimulus for the changes which occurred. That cells from a single individual or mixtures of cells from identical twins did not undergo this transition supported this postulate.

The application of the technique to the selection of donors for organ transplantation was complicated by inability to quantitatively separate the responses of each population in the lymphocyte mixture. Bach and Hirschhorn circumvented this difficulty by demonstrating that transformation required viability of only one population of lymphocytes, since the antigenic stimulus could

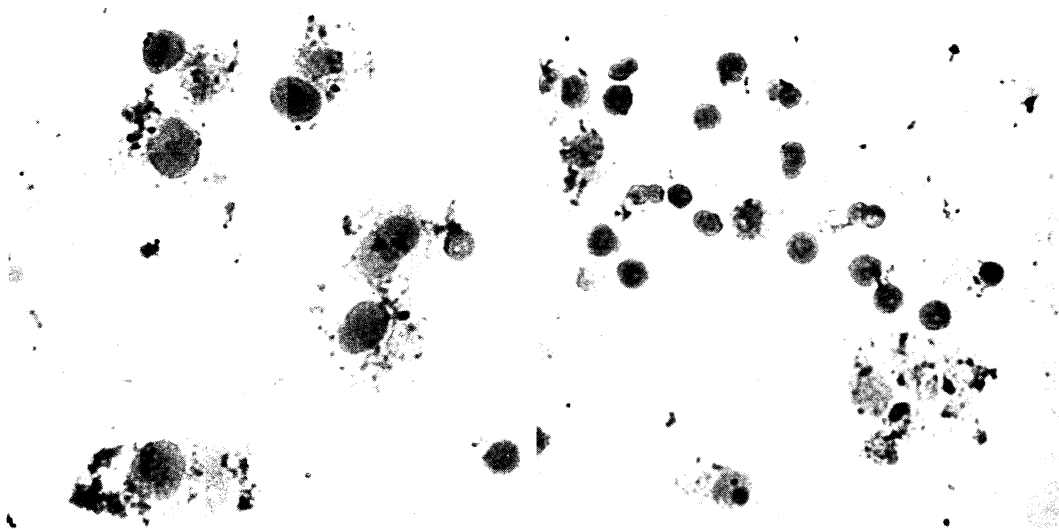


Figure 116. Left—The microscopic appearance of peripheral blood lymphocytes which were cultured for 10 days in the presence of frozen-thawed leukocytes from an unrelated individual. Right—The response of the same lymphocytes to frozen-thawed autologous leukocytes.

be provided in the form of frozen-thawed cells of the donor candidate. Figure 116 (left) illustrates the changes which are induced in peripheral blood lymphocytes following culture for 10 days in the presence of frozen-thawed leukocytes from an unrelated individual. The companion photomicrograph (Fig. 116, right) shows the lymphocytes of the same test subject which were exposed to his own frozen-thawed leukocytes in the control experiment.

Preliminary studies of the applicability of this concept to donor selection in human transplantation have been reported by Rubin and his associates. They studied the reactivity of the lymphocytes of two individuals to the antigens of their donors prior to transplantation and observed a correlation between the cellular response and the rapidity of onset of graft rejection. Because studies of this type are performed *in vitro*, they do not involve the possibility of sensitization of the recipient during repeated testing. Substantiation of the validity of this technique for selection of donors in human organ transplantation must await demonstration of a correlation between the *in vitro* lymphocyte response and the subsequent fate of the homograft in a large number of patients.

Donor—Recipient Blood Group Matching. Matching of erythrocyte antigens of the donor and recipient does not ensure success after a homografting procedure because many tissue antigens are not represented in the red cell. Nevertheless, ABO blood group antigens can pose formidable barriers to transplantation. In Chapter 6, the practical guidelines for donor selection are outlined in detail, as they have developed from our experience with the use of donors whose blood groups differed from those of their recipients. In five cases, mismatches were used which would no longer be considered acceptable.

Table 30. Isoagglutinin Titers in Patients following Transplantation of a Kidney from a Donor with an Incompatible ABO Blood Group

Case	Blood Group	Postoperative Day	Recipient Antibody	Titer
LD 2	Sister Donor B Recipient A	56	Anti-B	1:2
		85	Anti-B	1:2
		195	Anti-B	1:2
LD 19	Mother Donor A Recipient O	-1	Anti-A	1:32
		3	Anti-A	1:512
		7	Anti-A	1:256
		11	Anti-A	1:128
		32	Anti-A	1:128
LD 20	Mother Donor A Recipient O	-1	Anti-A	1:128
		1	Anti-A	1:4
		12	Anti-A	1:16
		24	Anti-A	1:16
LD 23	Brother Donor B Recipient O	-1	Anti-B	1:64
		3	Anti-B	1:64
		5	Anti-B	1:64
		13	Anti-B	1:128
		68	Anti-B	1:64

In one instance (LD 2), a patient of blood type A received a kidney from his sister who was type B. The kidney functioned well postoperatively. A severe rejection crisis which began on the twenty-fifth day was reversed, and the patient remains well 16 months after operation (see Figure 98, Chapter 20). Another patient of blood type O received his kidney from a type A cadaver (CD 1). The kidney never functioned well, but the contribution of the blood group incompatibility to the unsuccessful outcome is difficult to assess because of a long period of ischemia. In a third case, a type O recipient (LD 20) received a homograft from his mother (type A). There were signs of rejection within 24 hours which were reversed (see Figure 60, Chapter 14). Adequate renal function was maintained during the ensuing 202 days until death from a "stroke" (Chap. 19). The fourth patient (LD 23), also type O, received one of his brother's kidneys (type B) which was the target of an acute immunologic reaction upon completion of the vascular anastomosis. The organ was immediately removed (see Figures 18 and 19, Chapter 6). A similar series of events was observed following transplantation of a kidney from a type A mother to her son (LD 19), whose blood type was O. This kidney was also removed at the time of the original operation (see Figures 17, 18, and 19, Chapter 6).

The isohemagglutinin responses of four of these five patients are summarized in Table 30. Studies on patient LD 2 were not begun until 56 days after the transplantation, and consistently low titers were observed. In patient

LD 20, an abrupt fall in anti-A agglutinin followed insertion of the kidney, suggesting adsorption of the antibody. In one of the remaining patients whose mismatched kidneys were immediately removed a significant rise in isoagglutinin titer occurred. In this case (LD 19) donor erythrocytes which were not removed by perfusion prior to the transplantation probably provided the stimulus for the rapid rise in isoagglutinin titer, a finding which was not present in the other comparable patient (LD 23).

From these observations, it appears certain that blood group mismatched kidneys bind specific preformed host isohemagglutinins promptly after reconstitution of blood flow. In two cases (LD 19 and 23), this apparently occurred with such rapidity that there was immediate homograft failure. In two others (LD 2 and 20), in which renal function was obtained, the low antibody titers postoperatively indicated that binding persisted for long periods. Similar findings in other mismatches have been reported by Porter (see Chapter 25), Shackman, and Hume. This is not surprising in view of the previous demonstration by Glynn and Holbrow and by Szulman that A and B antigens are present in renal parenchymal and vascular cells.

The precise mechanism by which specific isoagglutinins inflict injury on the homograft is not known. Exposure of the antigens in the renal blood vessels to circulating isoantibody is one explanation for the immediate immunologic reaction observed in two patients (LD 19 and 23). There is little direct evidence to support this concept, but the observations made at the time of the operation and shortly thereafter clearly indicated a major disturbance of renal parenchymal blood flow (see Chapter 6). It is also possible that the intravascular erythrocyte clumping illustrated in Chapters 6 (Fig. 19) and 25 (Fig. 148) may have contributed an obstructive component blood flow reduction in these cases, but the evidence of isohemagglutinin binding for prolonged periods in the patients with good renal function (LD 2 and 20) cannot be explained on this basis, and it is unlikely that such a simple mechanical etiology is the sole explanation in the two examples of instant organ failure. The role of agglutinating humoral antibodies in the etiology of rejection is considered further in Chapter 24, based upon observations on six patients who received heterografts from baboons. All were found to have circulating heteroagglutinins to the donor erythrocytes prior to transplantation. None of these patients had immediate organ failure. The titers of these heterospecific antibodies fell following transplantation, again suggesting absorption of the humoral antibody on the foreign tissue, although the declines occurred more slowly than with the anti-A titer in LD 20. One interpretation of this difference is that the concentration of antigenic determinants with A specificity within the human kidney was higher than the concentration of heterospecific determinants within the baboon kidneys.

To our knowledge, kidneys from Rh positive donors have not been transplanted into recipients presensitized to the Rh antigens. No difficulty has been observed with several transplants from Rh positive donors into Rh negative patients who were not sensitized. In a study by Lawler and Shatwell, the Rh antigens could not be demonstrated on platelets, leukocytes, epithelial cells, and secretions of Rh positive subjects.

Table 31. The Evaluation of Passively Transferred Delayed Hypersensitivity before and during Attempted Kidney Rejection

Patient	*Positive Tests Immediately after Transplantation	Positive Tests during Rejection	Steroid Therapy
LD 22	1/3	3/3	Yes
LD 25	2/3	3/3	Yes
LD 33	2/3	3/3	Yes
LD 38	1/1	0/1	No
LD 37	2/2	1/2	Yes
LD 30	2/2	2/2	Yes

*The fraction designates the number of successful passive transfers to the recipient over the number of possible transfers.

DETECTION OF REJECTION

In discussing the use of immunosuppressive measures (see Chapters 14, 15, and 18) it is disturbingly clear that the methods used to diagnose the onset of rejection are crude, that they become diagnostic only after the immunologic response is well advanced, and that it is accordingly necessary to employ an inherently dangerous form of therapy without precise criteria. Consequently, a number of investigations have been undertaken in an attempt to characterize immunologically the degree to which current cytotoxic therapy is immunosuppressive and to specifically identify the rejection crisis at its onset.

The first approach to the problem of identification of the rejection crisis again involved the delayed cutaneous hypersensitivity reaction. As outlined earlier in this chapter, skin reactivities of donor specificity were passively acquired postoperatively by the kidney recipients. Assuming that some degree of antigenic identity existed between the transplanted renal tissue and the accompanying leukocytes, the rejection process would be expected to be directed against both types of cells. Rejection would, therefore, be manifested by a reduction or loss of passively transferred delayed hypersensitivity as well as by deterioration of kidney function. This hypothesis was studied in six patients who had passively acquired reactions. They were tested immediately following transplantation and again during the rejection crisis. The results of these studies are summarized in Table 31. It is apparent that no regular qualitative loss of transferred reactivity occurred in association with rejection, although in several instances in which adrenal steroids were being given the intensity of the reaction was reduced.

Table 32. Serum Complement Activity following Renal Transplantation

Patient	Preoperative Activity	Postoperative Prerejection Activity	Activity during Rejection	Peak Postoperative Activity
LD 37	44	78	80	85
LD 44	58	58	58	58
LD 16	--	35	33	33
LD 38	54	--	62	62
LD 30	37	--	63	75
LD 24	31	--	32	42
LD 43	43	--	50	52
LD 39	38	--	36	58
LD 23	38	--	26	82

It is thus apparent that this method will not be successful for identification of the rejection crisis. One possible explanation for this is that rejection of the kidney and destruction of the immunologically competent cells occur at the same rate, and the cutaneous test may be insufficiently sensitive to discriminate a fractional decrease in the number of cells. In addition, the persistence of the acquired skin reactivities may be related to the "transfer factor" reported by Lawrence. This is a constituent of human peripheral blood leukocytes which will passively transfer delayed cutaneous hypersensitivity.

A number of diseases which are believed to have an immunologic pathogenesis are associated with low serum complement activity. In 1955, Hume reported that serum complement levels did not change significantly in six homografted patients who were not receiving immunosuppressive therapy. The results of serial estimates of total complement activity in nine of the patients from the treated Colorado series are recorded in Table 32. The range of normal values for complement activity in this laboratory is 35 to 70 (50 per cent hemolytic) units. The preoperative levels were in the low normal range, and in general the activity increased progressively during the early postoperative period. In the later postoperative period, complement values decreased to near preoperative levels. In several patients, evidence of graft rejection appeared within a few days after transplantation, and in these cases complement activity was not measured until after graft rejection had begun. The complement level of patient LD 23 fell following transplantation of a kidney which failed immediately because of a B to O mismatch. It is difficult to be certain that this transitory fall was significant, but it is of interest that it did occur

in association with the kind of antigen-antibody combination which will fix complement in vitro. These results indicate that changes in total serum complement levels were of no value in either prediction or detection of graft rejection. However, preliminary studies by Guiney, Austen, and Russell showed that the activity of a complement fraction (C'2) was depressed during rejection, suggesting that more detailed analysis of the role of the complement system will be necessary.

Hume (1963) reported the temporary appearance of an unusual protein band on microelectrophoresis of the serum of one of six patients undergoing kidney rejection while on immunosuppressive therapy. We have performed serial immunoelectrophoresis on the sera of nine patients. No definite changes in the patterns were observed at the time of rejection.

Hume (1963) reported the temporary appearance of an unusual protein first six patients in his later series. Lymphocytes were noted in the urine of one patient after the BUN had risen significantly. A second patient appeared to have an increase in the number of lymphocytes in the urine as a prelude to three rejection attempts beginning 72, 107, and 137 days after transplantation. The third patient had a fall in urine output on the eleventh postoperative day, and, in association with this, microscopic hematuria and a few lymphocytes were noted in her urine. Assessment of the value of this technique as a method for the early detection of kidney rejection requires further investigation. The mechanism by which the cells are excreted has been illustrated by Porter (see Chapter 25).

The principle of Brent and Medawar's method for donor selection has been applied to predict graft rejection. The concept tested was that at the time of rejection an increased number of immunologically competent cells, specifically sensitized to the kidney donor, should appear in the recipient's circulation. The studies of Merrill provided some support for this approach. These investigators reported that normal subjects, at the time of skin graft rejection, had a sufficient number of immunologically competent cells in their peripheral circulation to produce a delayed hypersensitivity response when a suspension of these cells was injected intradermally into the donor.

Lymphocytes obtained from the peripheral blood were injected intradermally into the kidney donor and, as controls, back into the recipient and into a third unrelated volunteer. Figure 117 demonstrates the clinical course of one patient in whom this study was carried out. The arrow indicates the postoperative day on which the lymphocyte transfer was made. It can be seen that at this time she had an unexplained fever, and a fall in creatinine clearance was beginning. The lymphocytes injected into her mother's skin were responsible for a reaction with an induration of 14 mm and erythema of 35 mm compared to 11 and 8 mm in the patient and the nonrelated control. Similar studies were carried out on eight patients, and the results are summarized in Table 33. The

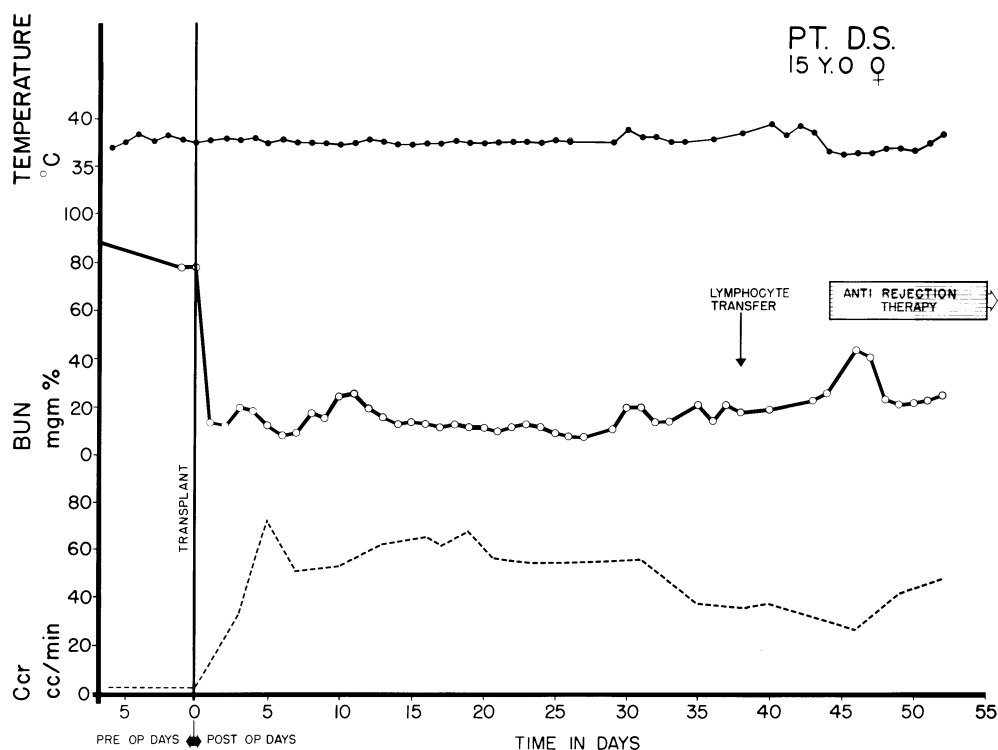


Figure 117. Clinical course of patient (LD 17) with unexplained fever following transplantation. The arrow indicates time when lymphocytes from the patient were injected intradermally into the patient's donor as described in the text. The onset of vigorous drug therapy corresponds to the time of recognition of rejection from clinical evidence.

Table 33. Detection of Rejection Using Intradermal Injection of Lymphocytes from Recipients

Lymphocyte Donor (Kidney Recipient)	Lymphocyte Recipient	Relation	Lymphocytes Transferred	Postoperative Day of Test	Induration (cm)
LD 40	LD 40	Self	2.1×10^6	6	0.5
	Kidney donor	Mother	2.1×10^6	6	0.7
	Control	None	2.1×10^6	6	0.4
LD 39	LD 39	Self	1.5×10^6	9	0.2
	Kidney donor	Mother	1.5×10^6	9	0.5
	Control	None	1.5×10^6	9	0.4
LD 17	LD 17	Self	23.2×10^6	38	1.1
	Kidney donor	Mother	23.2×10^6	38	1.4
	Control	None	23.2×10^6	38	0.8
LD 28	LD 28	Self	6.7×10^6	6	0.4
	Kidney donor	Sister	6.7×10^6	6	0.5
	Control	None	6.7×10^6	6	0
LD 19	LD 19	Not done	--	--	--
	First kidney donor	Mother	2.0×10^6	8	0.6
	Second kidney donor	None	2.0×10^6	8	0.7
	Control	None	2.0×10^6	8	0.4
LD 27	LD 27	Self	3.5×10^6	9	0.4
	Kidney donor	None	3.5×10^6	9	0.4
	Control	None	3.5×10^6	9	0
LD 21	LD 21	Self	3.9×10^6	26	0.3
	Kidney donor	None	3.9×10^6	26	0.7
	Control	None	3.9×10^6	26	0.5
LD 23	LD 23	Self	8.1×10^6	18	0.4
	Kidney donor	None	8.1×10^6	18	0.5
	Control	None	8.1×10^6	18	0.4

reactions in the donor skin were more intense than those in the control skin, but in general the differences were small.

A serious drawback to in vivo investigations of this type is the possibility of transmission of the serum hepatitis virus. All the kidney recipients receive multiple blood transfusions shortly before the transplantations which makes them dangerous cell sources for such a study. The in vitro method of Bach and Hirschhorn is under study currently in an attempt to obtain quantitative information on the number of specifically sensitized cells in the recipient circulation.

The addition of phytohemagglutinin, an extract of *Phaseolus vulgaris*, to cultures of peripheral blood lymphocytes stimulates morphologic transformation and cell division. An in vitro method for detection of kidney rejection employing lymphocyte responsiveness to this extract has been described by Rubin and his associates. They reported that the administration of azathioprine to recipients of renal homografts abolished the responsiveness of the patients' lymphocytes to phytohemagglutinin. In one patient lymphocyte responsiveness reappeared coincident with the onset of kidney rejection. The stimulatory effect of phytohemagglutinin on the lymphocytes of three renal homograft recipients in the Colorado series who had manifested no evidence of rejection for several months has been studied. In each instance a typical mitogenic effect was observed. Figure 118A demonstrates the response of the lymphocytes of an 18-year-old male four months after kidney transplantation (LD 37). Figure 118B shows the appearance of his lymphocytes in the absence of phytohemagglutinin in the control experiment. For comparison, the response of lymphocytes from a normal subject is shown in Figures 118C and 118D. These observations indicate that azathioprine does not produce permanent suppression of the response to phytohemagglutinin. In view of these results, the relationship of the onset of the rejection reaction in the early postoperative period to the suppression of the mitogenic effect of the extract requires further evaluation.

One of the most promising recently reported tests to diagnose rejection in its premonitory phase is that of Kountz, Laub, and Cohn. Rather than using immunologic methods, the examination is based upon serial measurements of renal blood flow using a single injection radioactive iodohippurate technique. They have demonstrated that a sharp fall in flow occurs before overt clinical or biochemical evidence of homograft rejection, a disclosure which is in accordance with the growing body of evidence that tissue ischemia is an important component of rejection (see Chapters 15 and 25). By delivering maximum immunosuppressive treatment before rather than after physiologic evidence of tissue injury, they have greatly improved their results after canine renal homotransplantation.

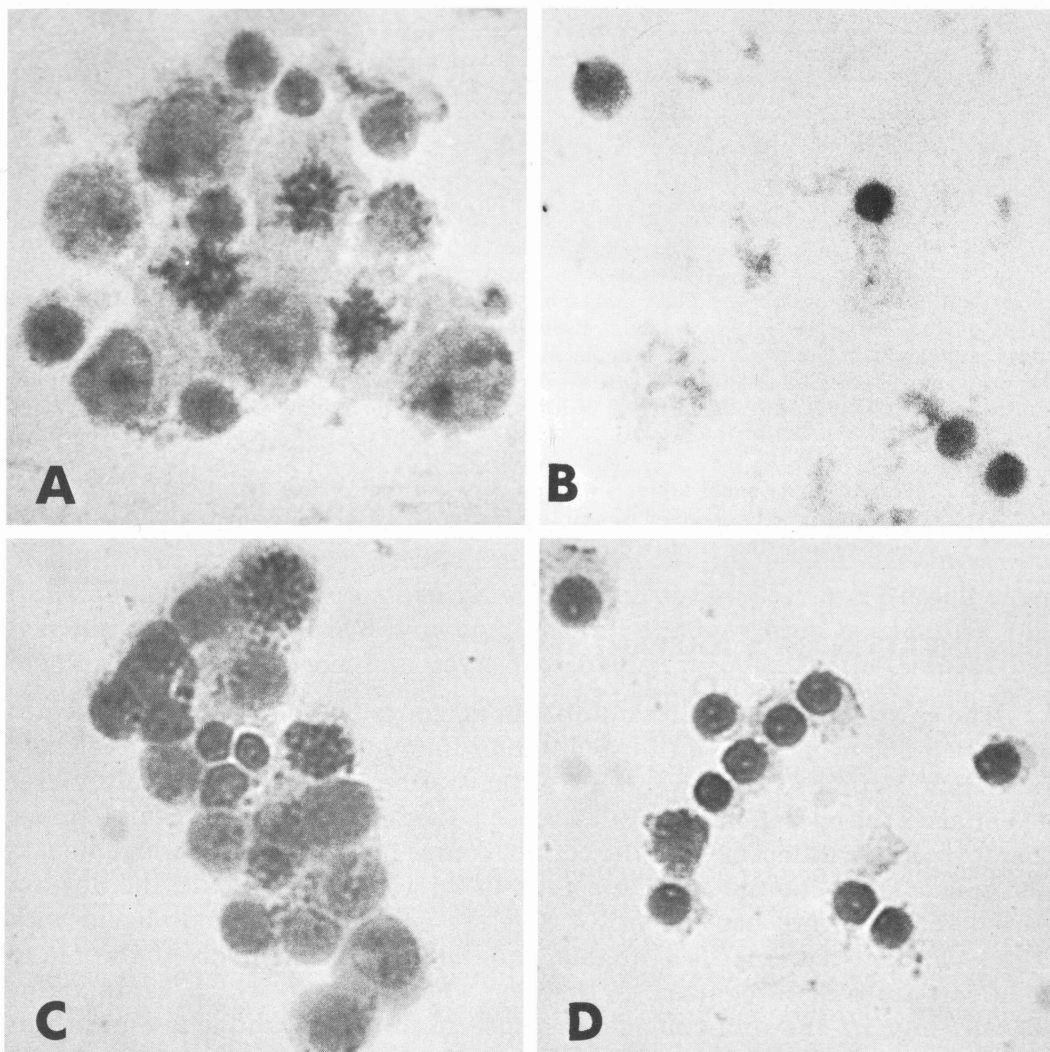


Figure 118. The effect of therapy with a purine analogue on the behavior of lymphocytes in tissue culture. The response of the cells from a patient (LD 37) who had received azathioprine and prednisone for six months to phytohemagglutinin is shown in (A). The same cells in the absence of phytohemagglutinin are shown in (B). These responses are comparable to parallel experiments using lymphocytes from an untreated patient as shown in (C) and (D) with and without phytohemagglutinin, respectively.

Table 34. Humoral Antibody Response to Typhoid Vaccine by 11 Kidney Recipients and 22 Normal Subjects

Antigen	Group	*Prestimulation Titer	*Poststimulation Titers		
			1 wk	2 wk	3 wk
O	Normal	20	50	80	50
O	Recipients	**10	40	40	40
H	Normal	30	70	80	60
H	Recipients	20	20	70	20

*Titers expressed as the mean of the reciprocals of the titer observed in each member of the group.

**Patients and normal subjects with an observed titer of less than 1/20 were arbitrarily assigned a reciprocal value of 10 for determination of the mean titer of each group.

IDENTIFICATION OF A TOLERANT STATE

The early success in maintaining function of renal homografts has created another problem in human transplantation immunology which deserves consideration. Patients from several institutions are now apparently healthy over a year after the operation. Almost all these patients are receiving maintenance therapy with azathioprine, but the correct course for long-term management is not clear. The potential risks inherent in continuous cytotoxic therapy for periods of years are not known. X-ray is the only other cytotoxic agent with which similar experience is available, and the deleterious effects of chronic irradiation are well recognized. In addition, the degree to which azathioprine therapy impairs the immunologic response to microbial antigens and in this manner compromises the patient in his environment is not known. To investigate this problem, 11 patients, all doing well three to 14 months after transplantation, were injected with typhoid vaccine and their antibody titers measured (Table 34). Although their responses appear to be less than those of the normal subjects, a larger series will have to be studied with additional antigens before a firm conclusion can be drawn.

Patients who attempt rejection unsuccessfully and then do well for long periods may achieve a stage of tolerance which does not require cytotoxic therapy for perpetuation. In dogs, several isolated examples of this have been observed. However, other dogs which were tolerant while receiving therapy rejected the graft when the drugs were withheld, as was discussed in Chapter 17. These experiences indicate that before drug therapy can be stopped with safety, methods for the detection of tolerance are required.

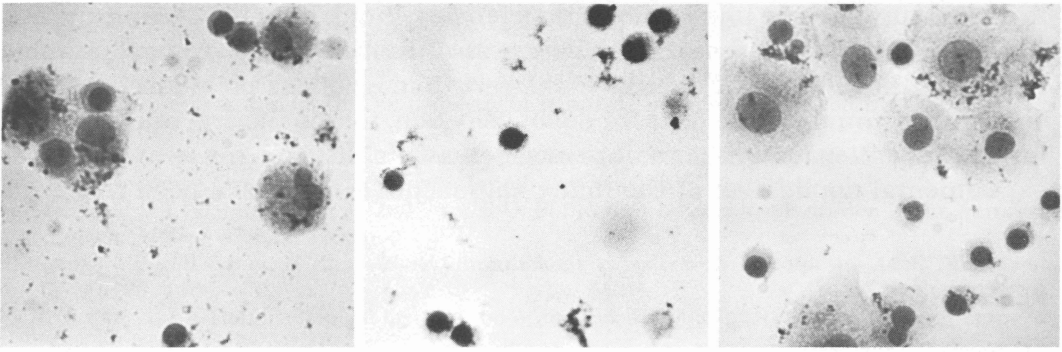


Figure 119. The response of the lymphocytes from a kidney homograft recipient (LD 36) to frozen-thawed leukocytes from his wife donor (left), himself (middle), and an unrelated subject (right), after 10 days of culture. This study was done six months following the transplantation, while the recipient was receiving azathioprine and prednisone; he showed no clinical or laboratory evidence of active graft rejection. Note that the response to tissue from the donor is similar to that with the unrelated person.

One possible approach to this problem is the model proposed by Brent and Medawar for detection of specific immunologically competent cells in the peripheral blood. A test of this nature was carried out by Gray and Russell using lymphocytes from a patient who had received a kidney from her brother four months before. The inoculum caused a smaller reaction in the skin of her brother than it did in the skin of an unrelated volunteer. Whether the obtunded response was the result of the close genetic relationship or some degree of tolerance is uncertain, but it is evident that the recipient did have cells capable of recognition of the donor's skin antigens. Because of the risk of serum hepatitis, an *in vitro* method would be preferable.

Employing the lymphocyte culture technique, the responsiveness of three homograft recipients to their donors' antigens has been evaluated. These patients manifested no evidence of rejection at the time the studies were performed, six to nine months postoperatively. Figure 119 illustrates the response of a recipient's lymphocytes to the antigens of his donor, himself, and a third person after 10 days in culture. Reactivity against his donor was present and was indistinguishable from his response to the third person. Similar results were obtained in each instance. The observation that cells capable of recognition of the donor's antigen can be identified several months after kidney transplantation in the apparent absence of rejection raises the distinct possibility that renal damage may be taking place continuously at a subclinical level.

PROSPECTS

The purpose of this chapter has been to review those areas of current research in which answers are being sought to certain problems in human transplantation immunology. Chronically uremic patients have been demonstrated

to have many specific immunologic deficiencies, indicating that chronic uremia may be an important factor in the success of human kidney transplantation. It is clear, however, that definitive answers to most of the problems discussed concerning immunologic tests for donor selection, for diagnosing rejection, and for identification of tolerance are not yet available, and must await wider experimental application of the many approaches which have been reviewed.

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Chapter Twenty-three

RENAL HETEROTRANSPLANTATION

Despite the advances that have taken place in the field of renal homotransplantation, unique social and moral issues exist which have no counterpart in other forms of medical endeavor. Much of the clinical progress of the last 10 years has resulted from the performance of procedures involving the use of healthy living donors, a practice which imposes upon these well-motivated volunteers an immediate operative risk as well as a negligible but unknown long-term liability. With the use of living donors, it has been possible to obtain an increased insight into many aspects of the problem. The importance of technical factors in determining the outcome has become appreciated, paramount being the degree of anoxic injury during transfer of tissue. Although there are recorded examples of urinary excretion after very long periods of ischemia, inconstant function results when the interval of homograft devascularization exceeds 40 minutes, as described in Chapter 7. When cadaveric sources are employed, the inequity is to the recipient patient instead (Chapter 8), since homograft injury during the agonal and postmortem period of the prospective donor may be so great that all hope of survival is unknowingly lost from the beginning.

Because these problems of tissue procurement will in the long run greatly limit the widespread application of therapeutic clinical homotransplantation, the introduction of heterotransplantation by Reemtsma in the autumn of 1963 was an event of great significance. With his first chimpanzee-to-man renal heterograft, Reemtsma dispelled many preconceived notions regarding the unfeasibility of this approach to therapy. His subsequent chimpanzee cases as well as our own later experiences with baboon-to-man heterografts have shown that survival for more than two months can only rarely be obtained with the immunosuppressive techniques available at the present time. Yet they have also demonstrated that the behavior of the heterografts is in many important respects similar to that of homografts, and they point to the important future possibility of employing living animal tissues for replacement—a concept no more repugnant than the use of bovine insulin or other biologic derivatives.

Although modern interest in heterotransplantation must be dated from the time of Reemtsma's clinical investigation, the first attempt to transplant an animal kidney to man was made by Jaboulay almost 60 years ago (Table 35). In 1906, he revascularized heterografts from a pig and a goat to the antecubital space in two patients in terminal uremia, using nonsuture anastomotic techniques for the vessels. In both cases the vascular connections thrombosed within three days. Later Unger (1906), Schönstadt (1913), and Neuhof (1923) each performed a transplant to the femoral area of a uremic patient, using a *Macacus* monkey, an "ape," and a lamb, respectively (Table 35). The first and third of these patients lived for 32 hours and nine days. The fate of the second is unknown. In each instance, provision for urinary drainage was with a skin ureterostomy.

Although measurable renal function was not obtained in any of these early cases, the experience was valuable as a background for later work. The applicability of vascular suture techniques was established. It was shown that toxemia, anaphylaxis, or sudden death did not follow revascularization of the foreign tissue, and that thrombosis of the graft's vascular system could be avoided. The principle of discontinuity removal and revascularization of both kidneys, aorta, and vena cava was clearly described, as was the technique for transplantation of a single organ. Perfusion of the heterograft to remove donor red blood cells was alluded to. In addition, future developments were predicted with prophetic insight, including (1) the advantages of the pelvic implantation site, (2) the desirability of ureteral anastomoses to the patient's own lower urinary tract, (3) the lesser degree of pathologic damage after heterotransplantation between primates than that observed with more distant genetic relationship, and (4) the potential reversibility of the histologic changes in the heterografts. Finally, the problem was discussed of providing total renal mass equivalent to that of a single human kidney.

After Neuhof's case, no human renal heterotransplantations were recorded in the ensuing 40 years. On February 16, 1963, the first heterotransplantation of modern times was carried out by Hitchcock and his associates at the Minneapolis General Hospital, under immunosuppressive coverage with azathioprine and a leucine analogue (DON). A baboon kidney (*Papio doguera*) was placed in the femoral triangle of a 65-year-old Indian woman, using Neuhof's technique. A brisk diuresis began promptly and continued for five days. Urine production then abruptly ceased. At the time of removal of the heterograft, the renal artery was found to be thrombosed. An account of the case was not prepared for publication until more than a year later, and its existence was not known when Reemtsma performed the first of his simian transplants in New Orleans.

Reemtsma's experience is outlined in Tables 35 and 36. In the first of his cases, a pair of rhesus monkey kidneys were anastomosed with an aortic and vena caval pedicle (Figs. 120, 121) to the iliac vessels of a 32-year-old uremic female, and the ureters were implanted into the bladder. There was unequivocal proof of renal function, but this was of relatively poor quality. The hetero-

Table 35. World Experience with Heterotransplantation
Exclusive of Colorado Baboon Cases* **

Author	Year	Donor	Anastomosis	Immuno- suppression	Significant Function	Survival	Pathological Findings
Jaboulay	1906	Pig	Payr prosthesis	No	No	3 days	Thrombosis, renal vessels
Jaboulay	1906	Goat	Payr prosthesis	No	No	3 days	Thrombosis, renal vessels
Unger	1910	Macacus nemestrinus (swine ape)	Suture	No	No	32 hours	Tubular necrosis; round cell infiltrate
Schönstadt	1913	"Ape"	Suture	No	No	"Several hours"	Not known
Neuhof	1923	Lamb	Suture	No	No	9 days	Marked degeneration without round cell infiltrate
Hitchcock	1963	Baboon (papio doguera)	Suture	Azathioprine "DON"	Yes	4 days	Minimal round cell infiltrate
Reemtsma	1963	Macaca mulatta	Suture	Azathioprine Actinomycin C Azaserine	Yes	12 days (removed)	Marked round cell infiltrate; fibrinoid necrosis, arterioles
Reemtsma	1963	Chimpanzee	Suture	Azathioprine Actinomycin C Local irradiation Prednisone	Yes	9 weeks	Acute tubular necrosis; no cellular infiltration
Reemtsma	1963	Chimpanzee	Suture	Azathioprine Actinomycin C Local irradiation Prednisone	Yes	7 weeks	Acute tubular necrosis; no cellular infiltration
Reemtsma	1964	Chimpanzee	Suture	Azathioprine Actinomycin C Local irradiation Prednisone	Yes	Living and well at 18 weeks with normal renal function	No biopsy
Reemtsma	1964	Chimpanzee	Suture	Azathioprine Actinomycin C Local irradiation Prednisone	No Yes	2 weeks	Tubular degeneration; interstitial edema; moderate cellular infiltrate; tubular degeneration
Reemtsma	1964	Chimpanzee	Suture	Azathioprine Actinomycin C Local irradiation Prednisone	Yes	18 days	Tubular degeneration, edema; perivascular infiltration with lymphocytes
Reemtsma	1964	Chimpanzee	Suture	Azathioprine Actinomycin C Local irradiation Prednisone	Yes	Transplant removed 19 days after implantation	Tubular necrosis; interstitial edema; mild cellular infiltration

*More complete notes on the chimpanzee heterografts are in Table 36.

**Historical data prepared by Dr. George N. Peters.

Table 36. Features of First Six Chimpanzee to Human Heterotransplants Performed by Reemtsma*

No.	Donor Weight	Donor-Recipient Blood Groups	Average of All Daily Postop BUNs	Average of All Postop Ccr (ml/min)	Rejection Crises	Average Postop WBC	Primary Cause of Death	Anuria
1	41 kgm	A—A	34.8 mgm%	60.6	4 days: ? 32 days	6,705	Sepsis	No
2	29.5 kgm	O—O	32 mgm%	41.1	10 days: 30 days: 40 days**	9,036	Sepsis; rejection	Yes
3	30 kgm	O—B	18.6 mgm%	49	24 days	4,208	Living 18 weeks 6-1-64	No
4	29.5 kgm 38.6 kgm	A—O O—O	Acute failure 97.8 mgm%	-	- Poor function difficult to determine	- 3,430	- Sepsis; unrelieved uremia	Yes No
5	43 kgm	A—A	48.4 mgm%	23.7	12 days	5,040	Sepsis	No
6	33 kgm	A—A	63.5 mgm%	24.3	8 days**	10,370	Rejection	Yes

*Analyses are from raw data provided us by Dr. Keith Reemtsma, New Orleans, and from the published accounts of his first cases.

**Irreversible.

grafts were removed on the twelfth postoperative day, and the patient died 24 hours later. Histologically, there was evidence of very aggressive rejection with moderate mononuclear cell infiltration and diffuse vascular injury (Table 35).

Using an identical surgical technique and immunosuppression with azathioprine, prednisone, actinomycin C, and local transplant irradiation, Reemtsma then performed six chimpanzee-to-human heterotransplantations (Tables 35 and 36). Five of the patients died after 14 to 63 days. The sixth was living and well as of June 5, 1964, with normal renal function 18 weeks postoperatively.

Encouraged by Reemtsma's early results and having obtained accurate information about the Minneapolis case, the group at the University of Colorado Medical Center attempted a clinical trial of renal transplantation in December, 1963, and January, 1964, using baboons as donors. The investigation was done in collaboration with Dr. Claude Hitchcock, and advice was sought from most of the American authorities in the field of clinical renal homotransplantation in order to obtain the maximum in useful information from these cases. This clinical experiment was decided upon for several reasons. Comparison of the relative merits of the baboon and chimpanzee was not otherwise possible. In

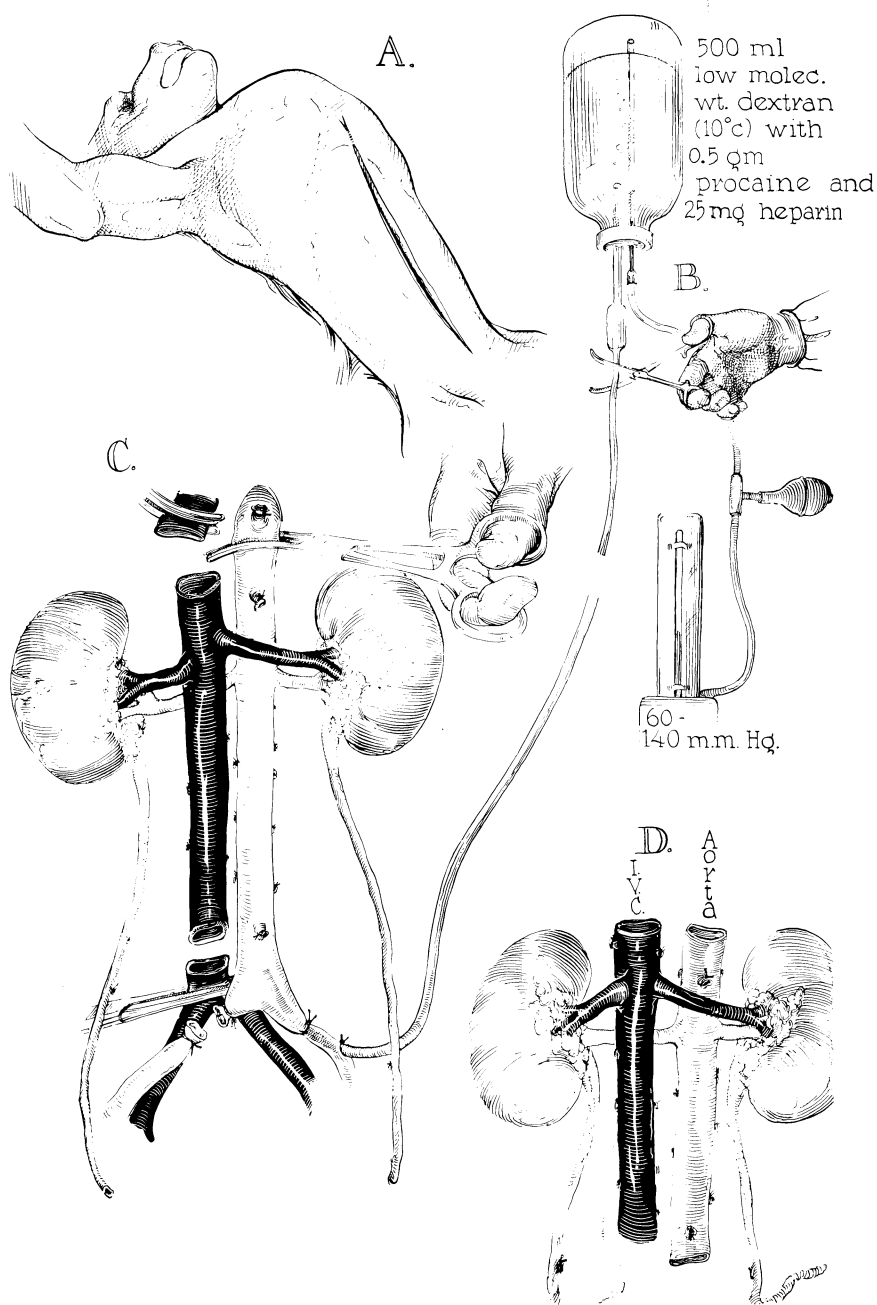


Figure 120. Preparation of heterografts. A—Complete midline incision. B—Method used to cold perfuse the heterograft complex. See text. C—In situ perfusion. D—The heterograft after removal.

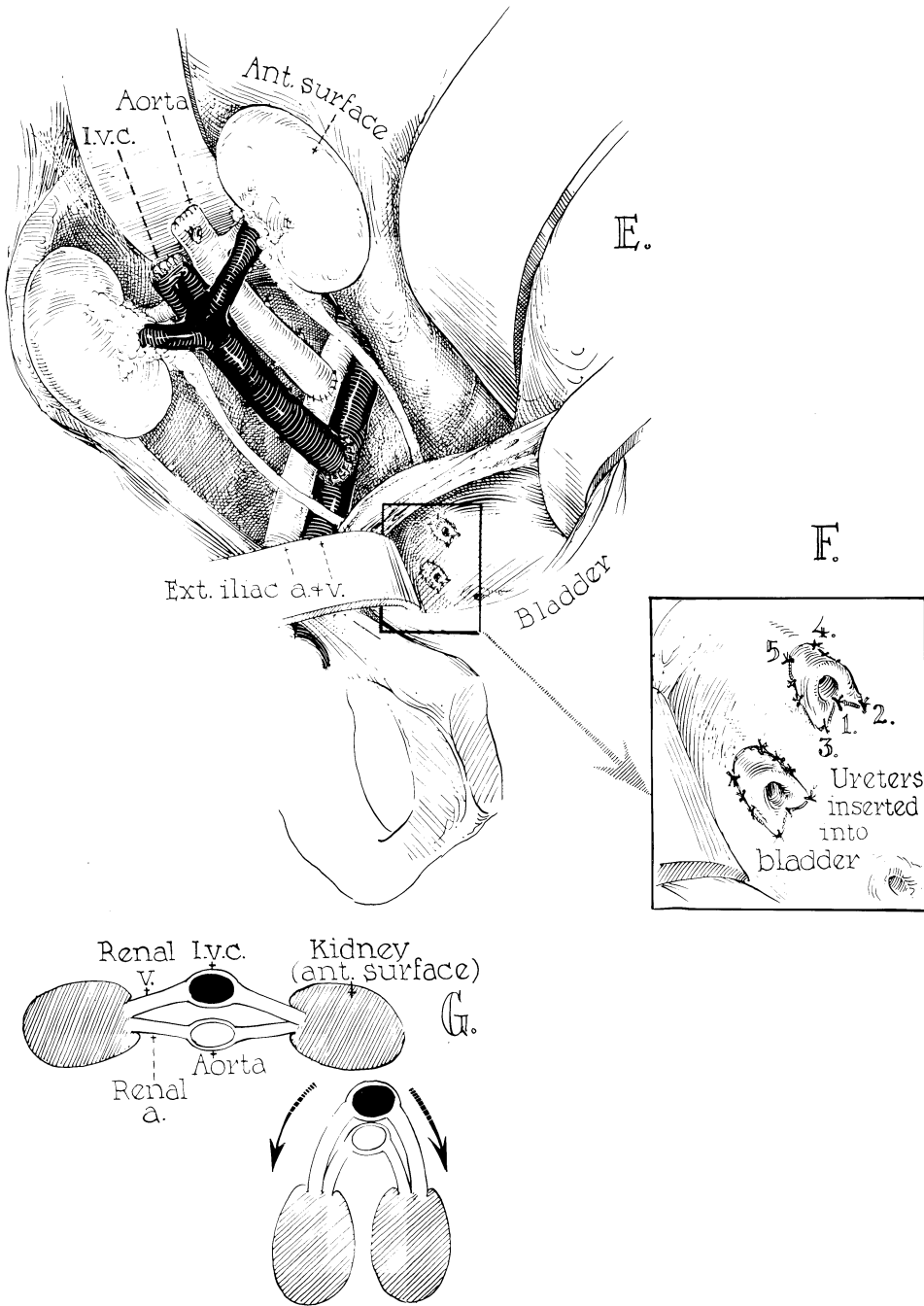


Figure 121. Insertion of heterografts after technique of Reemtsma. E—Anastomosis of distal aorta and vena cava to external iliac vessels. F—Parallel ureteroneocystostomies. G—Folding back of kidneys in order to occupy less space. (By permission of *Transplantation* 2: November, 1964.)

addition, the baboon is the cheapest and most readily available of the large subhuman primates, in contrast to the chimpanzee, which is a rapidly disappearing species. Finally, it had been previously established by Wiener and Moor-Jankowski that the chimpanzee has only two ABO blood types, the O and A, making it impossible to obtain donor-recipient blood group identity for all clinical heterotransplantations. The baboon, which has A, B, and AB blood groups, could fill this gap if it proved to be a suitable donor in other respects.

The six patients treated with baboon heterografts all had immediate fair renal function, and survival before death or removal of the heterografts was obtained for 19 to 60 days. Ultimately, the biologic penalty with the use of baboon heterografts was mobilization of a much stronger immunologic host response than that described for homografts elsewhere in this book. Eventually, this proved uncontrollable in two months or less in each case.

Despite failure of all of the baboon and most of the chimpanzee heterotransplantations, the temporarily favorable early course of a number of patients, with maintenance of life-sustaining renal function for many weeks, provides hope that animal donors will be useful when better immunosuppressive measures become available. The experiences to date should serve as the base upon which future progress can be built. For this reason, it is as worthwhile to completely record all data accruing from this work as it is to thoroughly document progress in homotransplantation.

METHODS IN HETEROTRANSPLANTATION

Donor Operations. Because of the expendability of the donors, it is possible to remove the kidneys under even more favorable circumstances than with human volunteers. Special methods are necessary for control and manipulation of the animals during the performance of preoperative studies and in order to make them susceptible to standard anesthesia techniques. A tranquilizer, Sternyl,* is useful for this purpose. This agent, in an intramuscular dose of 1 mgm/kgm, paralyzes voluntary movement in five to 10 minutes. For donor nephrectomy, the animals are then anesthetized with sodium pentobarbital, intubated endotracheally and cooled in an ice water bath to 28 to 32° C.

The heterografts are removed transabdominally through a complete midline incision (Fig. 120), reflecting the ascending and descending colon off the respective kidneys. The organs can be removed individually, using the principles of block excision described in Chapter 10 for human homograft removal. When this is done, it is advisable to remove a small ellipse of aortic wall in order to facilitate the arterial anastomosis in the recipient's operating room (Fig. 122).

Perhaps the simplest technique when both kidneys are to be transplanted is that described by Reemtsma: the skeletonized aorta and vena cava are

*Parke, Davis & Co., Detroit, Michigan.

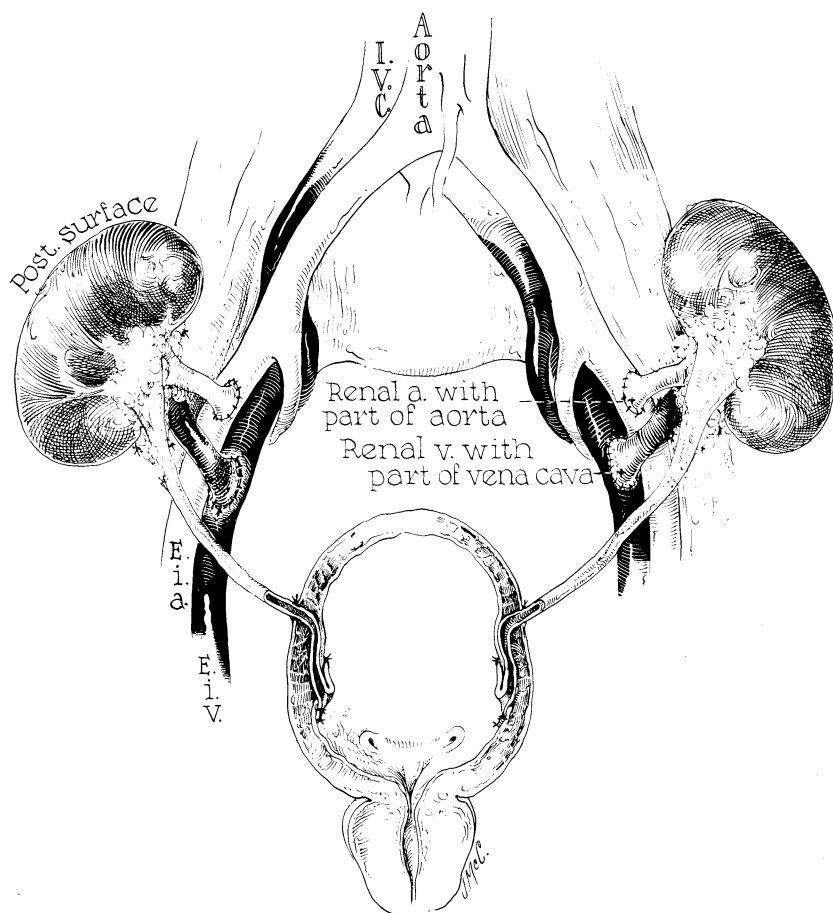


Figure 122. Double heterotransplantation of individual kidneys used for SD 1 of baboon series. Note inclusion of aortic and vena caval cuffs with renal vessels in order to provide larger vascular anastomoses.

removed in continuity with the renal vessels, the kidneys, and ureters (Fig. 120), avoiding dissection in the central hilar areas. Just before removal, a cannula can be inserted proximally into the aorta through the common iliac artery (Fig. 120) and perfusion commenced with a pressure of 70 to 120 mm Hg, using a cold electrolyte or low molecular weight dextran infusate. The heterograft complex can be cooled in this way within seconds after interruption of its circulation.

Recipient Operation. In all of Reemtsma's cases and in five of the six University of Colorado heterotransplants, the heterograft complex was placed in the right retroperitoneal space and revascularized to the distal donor aorta and inferior vena cava (Fig. 121). By folding the posterior surfaces of the organs together, less space is occupied (Fig. 121). The ureters are implanted with the ureteroneocystostomy described in Chapter 11, the ureteral implants being placed approximately 1 cm apart. In one of the baboon heterotransplants, the

Table 37. Changes in Urine Volume, BUN, and Renal Clearances for the First Three Postoperative Days after Baboon Heterotransplantation*

Case No.	Urine Volume (ml)				**BUN (mgm%)				**C _{Cr} (ml/min)				**C _{PAH} (ml/min)	
	First 24 hr	Second 24 hr	Third 24 hr	Pretransplant	Hrs post-transplant				Hrs post-transplant				Hrs post-transplant	
					6	18	42	64	6	18	42	64	6	18
1	8,710	5,790	2,770	93	102	98	74	78	15	43	--	--	80	316
2	24,290	4,390	2,575	59	48	31	18	16	50	45	--	--	150	257
3	8,915	2,555	2,895	92	83	67	68	62	25	37	22	35	74	130
4	20,250	3,540	2,780	132	105	62	39	37	39	58	38	46	80	333
5	12,345	3,700	3,800	81	73	48	54	60	39	34	--	30	139	--
6	24,510	6,000	2,060	111	80	55	--	58	59	61	93	37	216	241
Mean	16,500	4,330	2,810	95	82	60	51	52	37.9	46.3	51	37	123	255

*Analyses performed and table prepared by Dr. David A. Ogden.
**BUN—blood urea nitrogen; C_{Cr}—endogenous creatinine clearance; C_{PAH}—para-aminohippurate clearance.

individual kidneys were placed in the contralateral retroperitoneal spaces of the recipient, using end-to-side vascular anastomoses to the external iliac artery and vein (Fig. 122).

Postoperative Care. Most of the chimpanzee and baboon heterografts have had an acute postoperative diuretic phase of at least as great a magnitude as that described in Chapter 12 for homografts. Urine volumes during the first 24 hours following placement of the baboon heterografts are shown in Table 37, the first-day diuresis being as much as 25 liters in two cases. The principles of care are similar to those for patients after homotransplantation (see Chapter 12), although the large volume losses necessitate even closer management of fluid and electrolyte replacement.

Immunosuppressive Therapy. All of Reemtsma's chimpanzee heterotransplants as well as those of the baboon series were given a pretreatment regimen of both prednisone and azathioprine, similar to that described in Chapter 18. In the Colorado cases, azathioprine was given seven to 10 days preoperatively, and prednisone, 100 to 200 mgm per day, was given one to two days before operation. Postoperatively, these drugs were continued. In addition, intravenous actinomycin C, 200 to 400 µg per day, and local irradiation to the transplant site were given either prophylactically or for the treatment of rejection. When used, irradiation was given every other day for three or four times with the techniques described in Chapter 16. Individual doses of irradiation were 150 to 200 R at depth, delivered with a 250-K.V. machine. Details of therapy for the individual baboon transplants are described in Figures 123 to 128. Later in the postoperative course, an effort was made to reduce the quantity of steroids as quickly as possible in both the New Orleans and Colorado series, but in both groups, this was not done as easily as with patients who

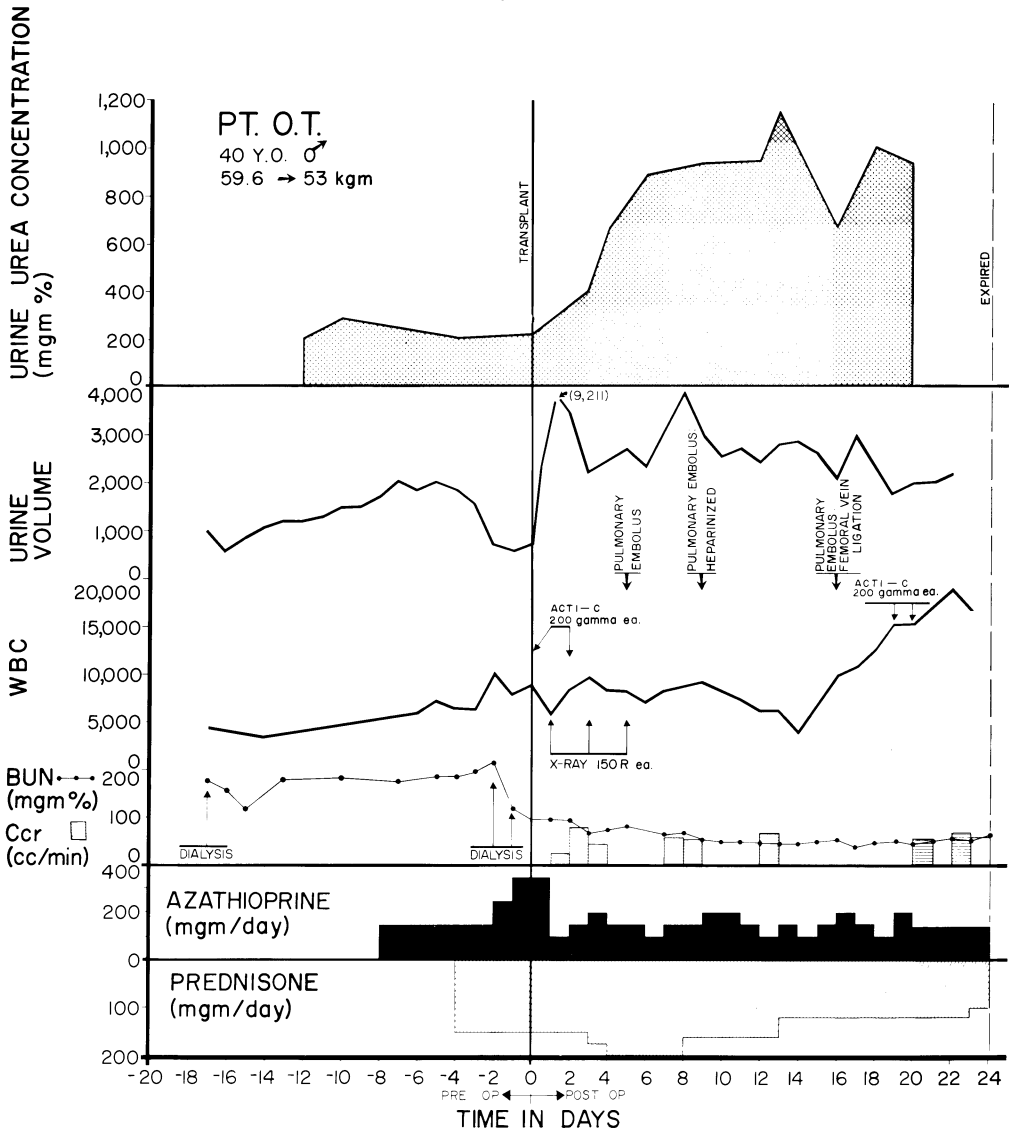


Figure 123. Course of SD 1. The baboon and recipient were both A blood type. Bilateral nephrectomy and splenectomy were performed concomitantly with heterotransplantation. Pretreatment was given with azathioprine and prednisone. Secondary therapy with actinomycin C and local irradiation is indicated. The case differed from the other five in that a definite rejection episode was not diagnosed. The patient died of pulmonary sepsis after multiple pulmonary embolization.

had received homografts. As will be mentioned subsequently, dependence on massive steroid dosages for maintenance of renal function was much less with the chimpanzee heterotransplants than with those of the baboons.

INFLUENCE OF HUMAN BLOOD TYPES UPON HETEROTRANSPLANTS

Only O and A blood groups have been found in chimpanzees. Animals of this species with A blood type have this antigen in their erythrocytes, making blood grouping analysis possible by direct hemagglutination techniques, and leading to the prediction that the directions of tissue transfer in relation to ABO groups should follow the rules outlined in Chapter 6. This expectation was confirmed by Reemtsma, who transplanted a chimpanzee heterograft of type A to a patient of type O. There was immediate failure of the transplant (Table 36), similar to that described in Chapter 6 for two comparable human cases. The heterografts were removed a few days later and a new chimpanzee donor of O blood type was used. Prompt function was obtained after the second operation.

With baboons, a different situation exists. Baboon red cell antigens do not react with specific human isoagglutinins, the blood groups being identifiable only by reverse hemagglutination techniques or by salivary testing. Because of the absence of erythrocyte antigens, and because of the possibility that renal tissue was also free of these substances, the possibility existed that baboon-to-man heterografts would not be adversely affected by blood group mismatches. The converse would not be true. Because baboon serum does contain preformed hemagglutinins to human A and B erythrocyte antigen, mismatches in human-to-animal transplants would be predicted to be dangerous.

In order to test the postulate that baboon-to-human transplants would not be adversely affected by blood group incompatibilities, three patients with O blood type were treated with kidneys of baboons of B or AB blood type. These patients fared slightly less well than three others who received matched kidneys (Table 38). Renal function was obtained for an average of 28 days. Two of these three patients developed anuria after 25 and 10 days, respectively, although one excreted substantial quantities of urine until the day of death. (Table 38). The three patients who received heterografts from baboons with compatible blood types had an average duration of urinary excretion of 44 days. Anuria was not observed in the latter three cases.

Although the survival figures in the two subdivisions of baboon heterotransplants cannot be said to have statistical significance, the serial changes in anti-A and anti-B titers to be described in the next chapter indicate that there were important specific immunologic changes consequent to transplanting mismatched kidneys to these patients. This evidence, added to the fact that mismatched combinations did not do as well clinically, makes it inadvisable to accept such donor-recipient incompatibilities in future cases.

Table 38. Function and Survival of Heterografts, and Relation to Blood Group Matching (Baboon Series)

Case	Ischemia	Donor Blood Type	Recipient Blood Type	First Urine	Duration Urine Excretion	Survival with Heterograft In
1	Rt-29 min Lt-37 min	A	A+	4.5 min	23 days	23 days
2	44 min	B	O-	10 min	25 days	35 days
3	34.5 min	B	AB+	8 min	60 days	*60 days
4	29 min	B	B+	90 min	49 days	*49 days
5	27.5 min	AB	O-	8 min	10 days	19 days
6	37 min	AB	O+	90 min	49 days	49 days

*Heterografts removed. Homografts placed.

COURSE AFTER HETEROTRANSPLANTATION

The course of patients receiving chimpanzee heterografts is summarized in Table 36. All except one died after 14 to 63 days. The notable exception was still alive and reported to have normal renal function on June 1, 1964, more than 18 weeks postoperatively.

The patients treated with baboon heterografts lived for 19 to 98 days after operation (Figs. 123-128). Four died with the baboon kidneys still in place after 19, 23, 35, and 49 days (Table 38). In two of the cases, the heterografts were removed after 60 and 49 days, respectively, and homografts from volunteer convict donors were placed on the opposite side (Figs. 125, 126). Survival after the second operation was 38 days in one of these patients, and 44 days in the

Table 39. Baboon Heterotransplants

Case	Became Anuric	Direct Cause of Death	Ultimate Heterograft Function
1	No	Pulmonary emboli with abscess formation	Failing
2	Yes	Rejection	Failed
3	No	*Regrafted	Failing
4	No	*Regrafted	Failing
5	Yes	Rejection	Failed
6	No	Pneumonia: poor renal function	Failing

*Died of sepsis 38 and 44 days, respectively, after the secondarily performed homotransplantation (see text).

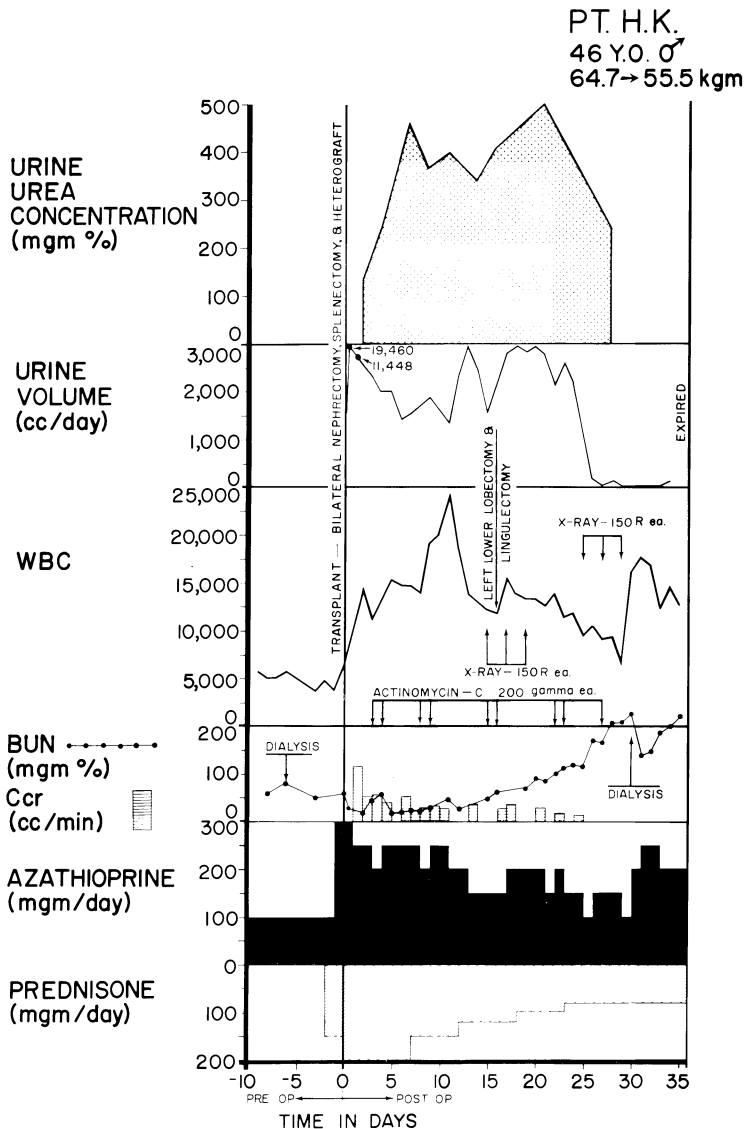


Figure 124. SD 2. Recipient was O-. The baboon was B blood type. The pulmonary resection was necessitated by a perforated lung abscess which resulted from pulmonary embolization. The patient had been anuric for two months before transplantation. Note repetitive rejections after four, nine, and 15 days. The last of these was irreversible and proceeded to anuria 10 days before death.

other. In each of the secondarily operated patients, the primary cause of death was sepsis.

It will be noted (Table 39) that complete cessation of urine excretion occurred in only two of the baboon cases (Figs. 124, 128), although renal function was failing in the remainder prior to death or before removal of the homograft (Figs. 123, 125, 127).

All patients in both the New Orleans and Colorado series were critically ill at the time of heterotransplantation. All exhibited early clinical improvement

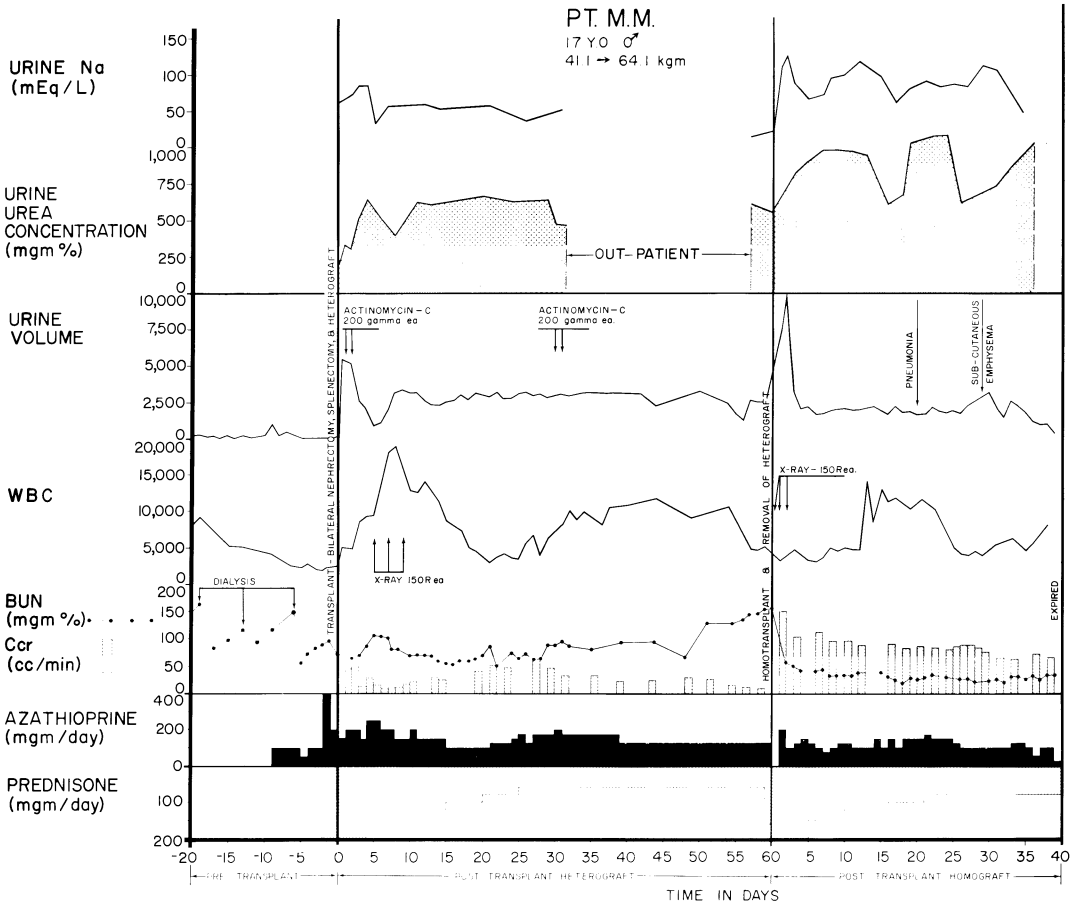


Figure 125. SD 3. Recipient was AB+ blood group, and donor was B. The patient was anuric pre-operatively. The difference in quality of function of the heterografts, compared to the secondarily placed homograft, is evident. Heterotransplant rejection crises occurred after five and 50 days. Urine function continued until heterografts were removed. (By permission of *Transplantation* 2: November, 1964.)

at the time of initial diuresis and for varying periods thereafter. However, recovery was interrupted in each instance (Table 40, baboons; Table 36, chimpanzees) by early rejection crises which were characterized by transplant site tenderness, and by the other stigmata of rejection (see Chapter 15). The influence of these episodes upon renal function of the baboon heterografts is graphically portrayed in Figures 123 to 128.

The management of rejection in both the chimpanzee and baboon cases was more difficult than after homotransplantation. The rejection crises tended to be repetitive and closely spaced, a feature more prominent in the baboon than in the chimpanzee series. The individual crises in both types of case could be at least partially controlled in most instances with local transplant irradiation, actinomycin C, or increases in steroid dosage. With the baboons, the adverse consequences of rejection could not be completely reversed before the onset of the next assault (Figs. 124-128), and the cumulative effect was

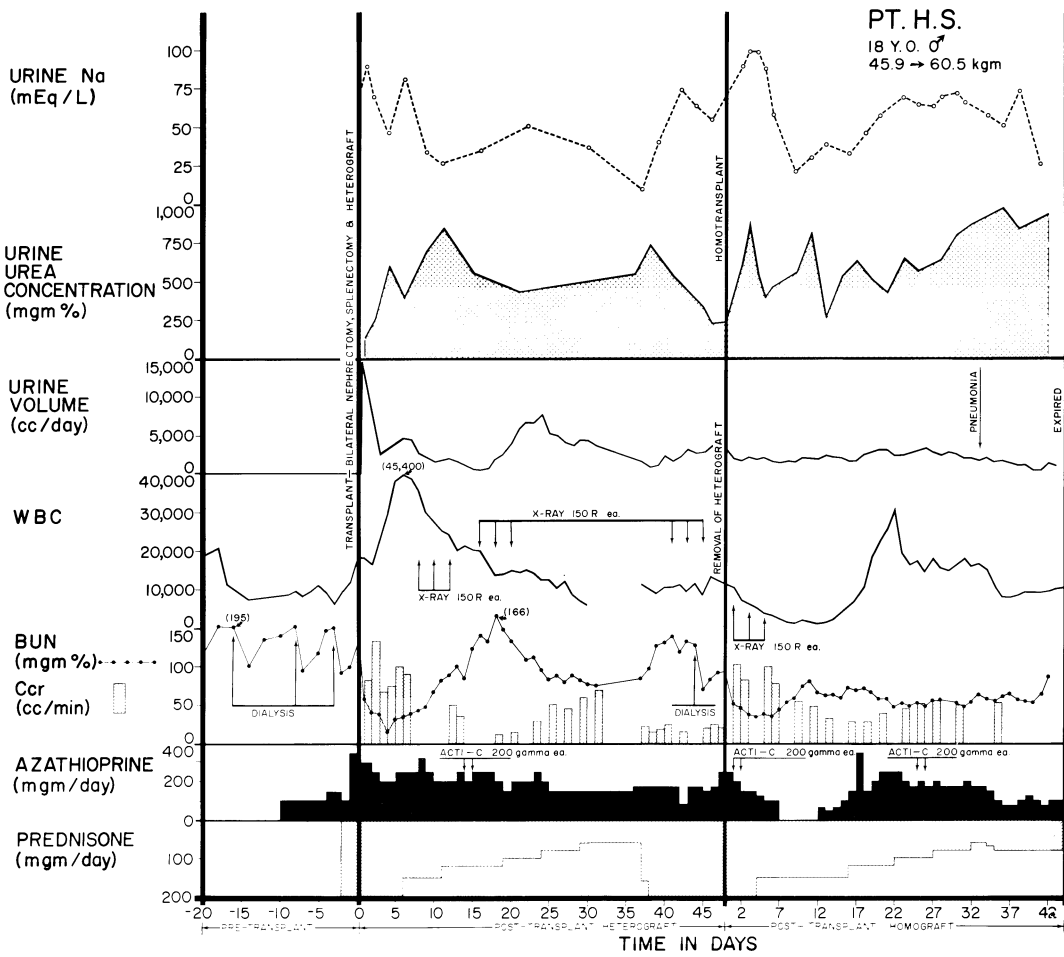


Figure 126. SD 4. Course after heterotransplantation (left) and subsequent homotransplantation (right). The patient was B+ blood type, and received donations from a B baboon and an O human volunteer. The cause of death was pneumonia. Note sharp falls in urine sodium concentration with heterograft rejection episodes. (By permission of *Transplantation* 2: November, 1964.)

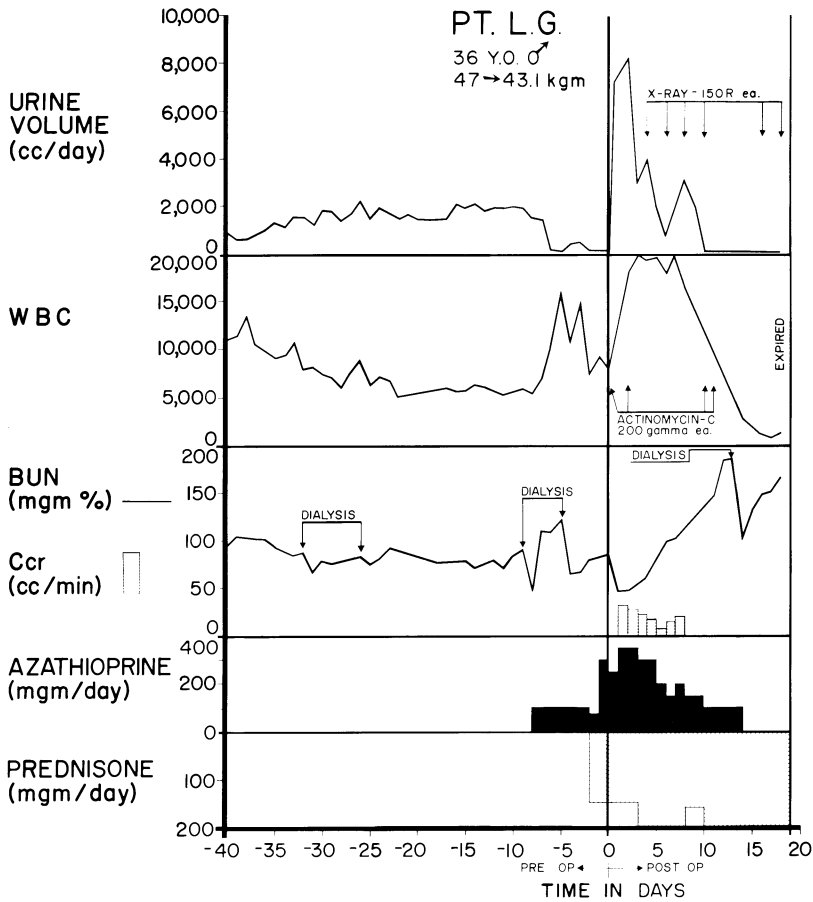


Figure 127. SD 5. Fulminant rejection with development of anuria in 10 days. Patient was O— blood type. Baboon was AB.

progressive deterioration interrupted by incomplete remissions. In the two longest surviving patients of the baboon series, removal of the heterografts was necessitated by the sudden formation of masses in the transplant areas, of such size in one as to produce massive edema of the right leg which was apparently due to local compression of the venous and lymphatic systems. At the time of reoperation, the swollen and boggy heterografts were surrounded with 500 to 1,000 ml of serosanguineous fluid under considerable pressure.

Because of the intensity of rejection, it was difficult to successfully relax the stringency of immunosuppressive measures in the chimpanzee heterotransplants, and it was impossible in the baboon series. In the latter group, every

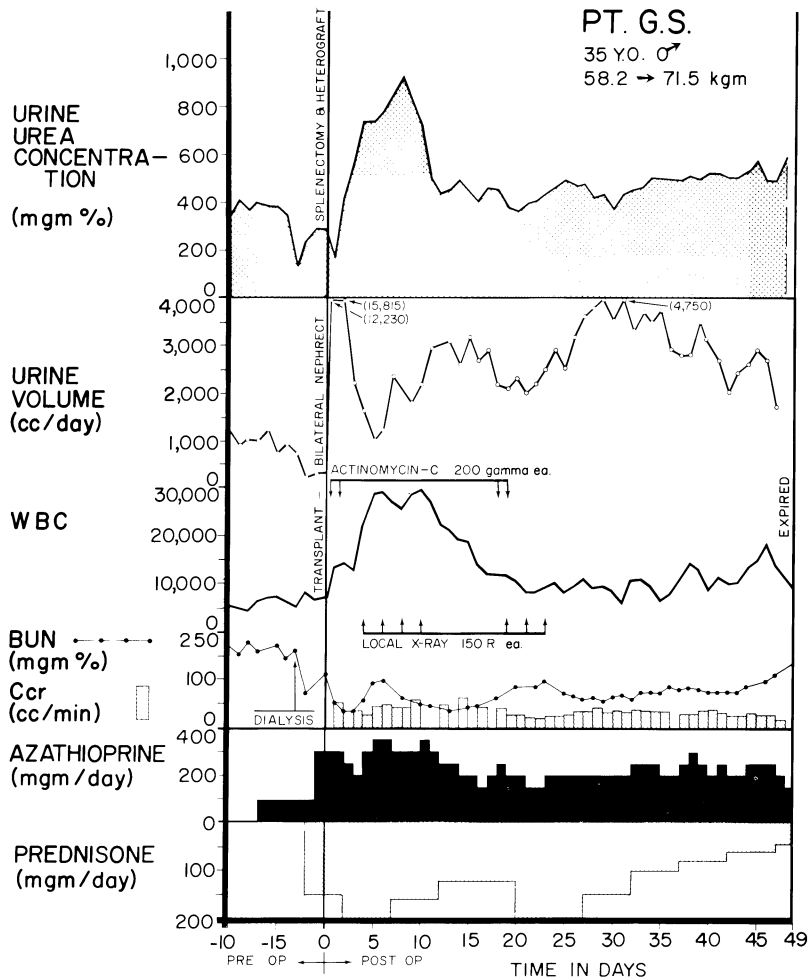


Figure 128. SD 6. Patient (O+) received AB baboon heterografts. Rejections occurred at four, 18, and 48 days. Direct cause of death was pneumonia. Note gradual falls of creatinine clearance and slow progression of azotemia between partially reversible rejection crises. (By permission of *Transplantation* 2: November, 1964.)

attempt to reduce the dosage of prednisone was followed by serious rejection. The prolonged use of dosages in excess of 100 mgm per day resulted in profound hypercorticism. The extraordinary degree of immunosuppression required to maintain even mediocre function after baboon heterotransplantation (Figs. 123-128) undoubtedly contributed to the septic complications which played a role in the unfavorable outcome of the majority of cases (see Table 20, Chapter 21). Similar difficulties were encountered by Reemtsma in maintaining chimpanzee heterografts, although his longest living patient had had steroids reduced to 40 mgm per day after 18 weeks with continuation of essentially normal renal function.

BABOON HETEROGRAFT FUNCTION

Early Function. The most detailed functional studies of the baboon heterografts were obtained for the first few days after operation. Table 37 indicates 24-hour urine volume, changes in BUN, and clearance of PAH and endogenous creatinine for the first three postoperative days.*

A large diuresis was observed in each case. Urine volume for the first 24 hours ranged from 8,710 ml to 24,510 ml with a mean of 16,500 ml. Volume fell sharply in the second 24 hours to an average of 4,330 ml with a range of 2,555 ml to 6,000 ml.

Blood urea nitrogen fell progressively for the first three days in the majority of baboon heterotransplants, but reached normal only in Patient 2 (Table 37). Creatinine clearances six hours post-transplant averaged 37.9 ml per minute with a range of 15 to 59. Twelve hours later, Ccr had increased to 34 to 61.1 ml per minute with a mean of 46.3. Simultaneous PAH clearance ranged from 74 to 216 with a mean of 123 ml per minute at six hours and 130 to 316 with an average of 255 ml per minute 18 hours post-transplant (Table 37).

Later Heterograft Function. Figures 123 to 128 portray 24-hour urinary output, BUN, Ccr, and urinary sodium (in two cases) before operation and for the duration of the patient's survival. In two of the cases, comparison with the subsequent behavior of secondarily placed homografts was possible (Figs. 125, 126). It should be emphasized that the recipients' diseased kidneys were removed in each case so that the function studied was entirely that of the transplanted tissue.

The early renal function described above was not normal, but it was sufficient to produce immediate clinical benefit, and if sustained, it seemed to be compatible with long survival. Unfortunately, acute deterioration was noted in all but the first baboon heterotransplant within eight days, with the onset of the first of multiple rejection crises (Figs. 124-128). Even with partial reversal of these crises, there was a progressive deterioration of function, and renal failure contributed to or was the direct cause of death in each instance (Table 39).

COMPARISON OF HETEROGRAFT REJECTION TO THAT OF HOMOGRAFTS

The characteristics of heterograft rejection have been found in both the New Orleans chimpanzee series and in the baboons to have many features in common with those of homografts. After almost all the recently performed heterotransplants, function was prompt in the early postoperative period, and in several it was only slightly less adequate in many respects than that

*Examinations were performed by Doctor David A. Ogden.

described by Homer Smith for the normal animal. In the majority of both chimpanzee and baboon heterograft transplants, the urine composition during diuresis was similar to that described in Chapter 12. Nor were the subsequent events qualitatively unique in comparison to the behavior of homografts. Rejection crises occurred which were partially reversible, which had the same general characteristics seen after homotransplantation, and which responded to the same therapeutic measures. The differences were quantitative in that rejection of the alien tissue was more vigorous and insistent and that evidence of host-graft adaptation was not seen in any but Reemtsma's longest surviving patient.

The alterations in renal function seen during heterograft rejection are shown in Figures 124 to 128. There were diminutions of urine volume, sudden rises in BUN and serum creatinine, and declines in creatinine clearance. The changing patterns of urine composition described in Chapter 15 were also observed in both the baboon (Figs. 125, 126) and chimpanzee transplants. This sequence consisted of a sharp diminution of urine sodium concentration at the time of oliguria, usually with an increase in urea and creatinine concentration.

Despite the similarities to homografts and the ease with which partial rejection reversal could be achieved with actinomycin C, local x-ray therapy, or adjustment of steroid and azathioprine dosage, the improvement in most cases was transient (Tables 36, 40). Especially with the baboon heterografts, restora-

Table 40. Features of Rejection and Its Reversibility after Baboon Heterotransplantation

Case	Onset (days)	Duration before Reversal	Agent Used for Reversal Therapy	*Degree of Reversal
1	None definite	---	---	---
2	5	2 days	Actinomycin C	Excellent
	9	3 days	Actinomycin C: local x-ray	---
	15	Irreversible	Actinomycin C: local x-ray	None
3	5	4 days	Local x-ray	Good
	50	---	Not attempted	---
4	8	21 days	Actinomycin C: local x-ray	Fair
	37	6 days	Actinomycin C: local x-ray	Fair
5	4	2 days	Local x-ray	Fair
	7	Irreversible	Actinomycin C: local x-ray; increased steroid	None
6	4	3 days	Local x-ray	Good
	18	5 days	Actinomycin C: local x-ray	Fair
	48	---	Not attempted	---

*Complete reversal was not achieved in any instance since improvement ceased before pre-existing renal function had been reached. The consequence was a progressive deterioration of function, interrupted by partial remissions.

tion of renal function was either short-lived or incomplete. With the baboon donor, the shortest interval between the first and subsequent rejection crises was three days, and the longest was 45 days. Between these dramatic occurrences, there was frequently a subtle diminution in function (Figs. 124-128).

COMPARISON OF BABOON AND CHIMPANZEE HETEROGRAFTS

In the pathological section (Chap. 25), it will be pointed out that the degree of structural damage was greater in the baboon heterografts than in Reemtsma's chimpanzee transplants. In Tables 36, 38, 40, and 41, other measures can be compared following the use of the two types of donors. On superficial examination, it appears that the survival of patients receiving baboon donations was at least as good as in those receiving chimpanzee kidneys if one excludes from consideration Reemtsma's longest living patient (Table 36). Nevertheless, there seemed to be a definite functional superiority in the latter type of heterograft. The average of daily creatinine clearances during the entire postoperative period was much higher in three of these patients than that attained in any of the baboon series (Table 36). Relief of azotemia was also more complete (Table 36). Although the weights of the chimpanzee

Table 41. Recipient Patients*

Case	Wt.	Age	Wt, Sex of Donor	Renal Disease	Contraindication for Volunteer Homograft
1	61.6 kgm	40	20.9 kgm male	**CGN	None available; age; neuropathy
2	62 kgm	46	17.3 kgm male	CGN	Age: advanced neuropathy
3	47.2 kgm	17	16.4 kgm male	CGN	Multiple recent pulmonary emboli and abscesses; ? tuberculosis
4	53.6 kgm	18	24.8 kgm male	CGN	None available; neuropathy
5	46.3 kgm	35	15.7 kgm female	Pyeloneph.	None available; pyelonephritis
6	69.8 kgm	35	16.4 kgm female	CGN	None available; neuropathy

*All were male.

**Chronic glomerulonephritis.

donors (Table 36) were approximately twice those of the baboons (Table 41), the functional disparities between the two groups exceeded those of the weight disproportions.

Furthermore, the rejections in the chimpanzee heterotransplantations of the New Orleans series did not occur at such brief intervals (Table 36), and were more completely reversible. Finally, the results in Reemtsma's chimpanzee cases were obtained with far less dependence upon steroid therapy, the maximum doses of prednisone ranging from 40 to 100 mgm per day. Thus, better control seemed to have been possible without such a high degree of immunoparalysis, despite which septic complications still contributed heavily to his mortality figures. On the basis of these findings, it may be tentatively suggested that the chimpanzee is a biologically more satisfactory source of donated kidney tissue for human use than the baboon.

THE PRESENT UTILITY OF HETEROGRAFTS

Results of recent clinical heterotransplantations provide little hope that heterografting procedures will be of immediate consistent value for the treatment of human renal disease. Although there appears to be a slight advantage in the use of the chimpanzee, only one survival of significantly more than two months was obtained with either species of donor. Nevertheless, the amount of potentially useful information that can be obtained from a limited number of well monitored clinical heterotransplantations is great from the physiologic, immunologic (Chap. 24), and pathologic (Chap. 25) points of view. As will be discussed in the ensuing chapters, many new observations have been made after those procedures which may contribute to a more fundamental understanding of the rejection process, and may lead to the development of better immunosuppressive techniques.

These benefits of heterotransplant research have been of little comfort to the unsuccessfully treated patient or his bereaved family. Consequently, a misleading prognosis must not be given before such an undertaking. The use of any form of heterotransplantation must be considered as the purest form of investigative effort, and it is necessary to make this clear to all involved. There is, at present, no place for unplanned or casual procedures of this type. What must emerge from a minimum number of cases is a clean body of unassailable factual data upon which to build future progress. There is no other justification for such a surgical experiment.

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Chapter Twenty-four

IMMUNOLOGIC STUDIES OF BABOON-TO-MAN RENAL HETEROTRANSPLANTATION

*by Charles H. Kirkpatrick, M.D.,
and W. E. C. Wilson, M.D.*

The homograft rejection phenomenon is an immunologic process which is determined by genetic factors. The survival time of skin transplanted between mice of different inbred strains varies directly with the antigenic similarity between the donor and recipient strains. Although transplantation between members of different species has been the subject of relatively little systematic study, the fate of heterospecific organ transplants appears to be an extension of this concept.

It was documented in the early transplantation literature that kidneys, grafted between members of widely disparate species, failed rapidly following revascularization. However, the more recent demonstration by Makinodan that mice may be protected from otherwise lethal irradiation by infusions of rat bone marrow suggested that immediate destruction of the heterografted tissue might be prevented if there was intensive suppression of the immune response.

The temporary function of kidneys transplanted from baboon donors to human recipients (Chap. 23) supports this postulate, but the vigor of the immunologic response which was evoked emphasizes the importance of the genetic disparity that existed. Inasmuch as each of the six patients was demonstrated preoperatively to possess a circulating antibody which agglutinated the erythrocytes of his donor baboon, a unique opportunity existed to study the relationship of a humoral antibody to the rejection reactions.

Table 42. Clinical Data from Recipients of Baboon Kidneys before Transplantation

Patient	Age	*Diagnosis	**Ccr (cc/min)	***BUN (mgm %)
1	40	CGN	2.6	184
2	46	CGN	0	155
3	17	CGN	0	69
4	18	CGN	1.6	147
5	35	CPN	0	90
6	35	CGN	1.8	111

*CGN—chronic glomerulonephritis.

CPN—chronic pyelonephritis.

**Creatinine clearance.

***Blood urea nitrogen. All patients had received multiple hemodialyses, so the figures noted do not reflect the severity of the uremic process.

Table 43. Comparison of ABO Types of Recipients and Baboon Donors

Patient	Recipient Blood Group	Baboon Blood Group
1	A	A
2	O	B
3	AB	B
4	B	B
5	O	AB
6	O	AB

BLOOD GROUPING

The six patients who received baboon heterografts had irreversible renal failure with severe uremia. In five cases the underlying disease was chronic glomerulonephritis, and in one, chronic pyelonephritis. All the patients were males, and their ages ranged from 17 to 46 years. The clinical features of these six patients are summarized in Table 42. Typing of the human erythrocytes was done according to standard procedures using commercially prepared anti-sera. The results of the blood grouping are shown in Table 43. Patients 1, 3, and 4 were group A, AB, and B, respectively, and the remaining patients were group O.

The AB groups of the baboons (Table 43) were determined by Doctor J. Moor-Jankowski. One baboon was type A, three were type B, and two were AB. Human erythrocytes are classified as A, B, AB, or O according to their reactions with type-specific antisera. Determinants cross-reacting with human anti-A and anti-B typing sera have been found on the red cells of chimpanzees, orangutans, and gibbons, but not on the red cells of Old World monkeys (Cercopithecidae) such as baboons and rhesus monkeys. Therefore, the "blood group substances" of the donor baboons were determined by two indirect methods. The saliva and other secretions of these animals contain the blood group substances A, B, or AB which react with type-specific human antisera and specifically inhibit the agglutination of the human erythrocytes. For example, the saliva of a type A baboon will abolish the agglutinating property of anti-A serum for group A human erythrocytes. In addition, the serum of the baboon contains agglutinins for human red cells of the reciprocal type with the result that the serum of a type A or B baboon will specifically agglutinate human type B or A erythrocytes respectively. Using these methods, Wiener and Moor-Jankowski have found baboons with A, B, and AB types, but none with O.

SEROLOGICAL STUDIES

Blood samples were collected from the six recipients preoperatively and serially after transplantation of the baboon kidneys. The serum was removed after centrifugation, divided into aliquots, and stored at -20°C . Thus erythrocyte agglutinin activity in a series of samples from an individual patient could be assessed using a single preparation of test red cells. When all the samples from one recipient were not evaluated together, sera from the first group were restudied with the second to provide control for nonspecific variation. To quantitate the agglutinin activity, serial doubling dilutions of each serum sample were prepared with saline. An aliquot of a 2 per cent saline suspension of washed test red cells was added to each dilution. The titers were read after incubation at 25°C for one hour.

Anti-A and Anti-B Agglutinins. Four patients (Cases 2, 4, 5, and 6) had agglutinins for type A human erythrocytes preoperatively. Patient 4, type B, received the kidneys of a type B baboon, and a very gradual decrease in anti-A activity was noted during the postoperative period (Fig. 129). Patients 5 and 6 were group O and received kidneys from type AB baboons, and in both patients a rapid fall in titer occurred (Figs. 130, 131). Patient 6 subsequently showed a brief rise in titer and then a slow fall similar to that seen with patient 4. Patient 5 became anuric on the ninth postoperative day, and his anti-A activity increased rapidly (Fig. 130). Patient 2 was also group O and received a kidney from a type B baboon. The level of anti-A activity in this patient also decreased; however, the fall was somewhat slower than that noted with patients 5 and 6 (Fig. 132).

PT. 4

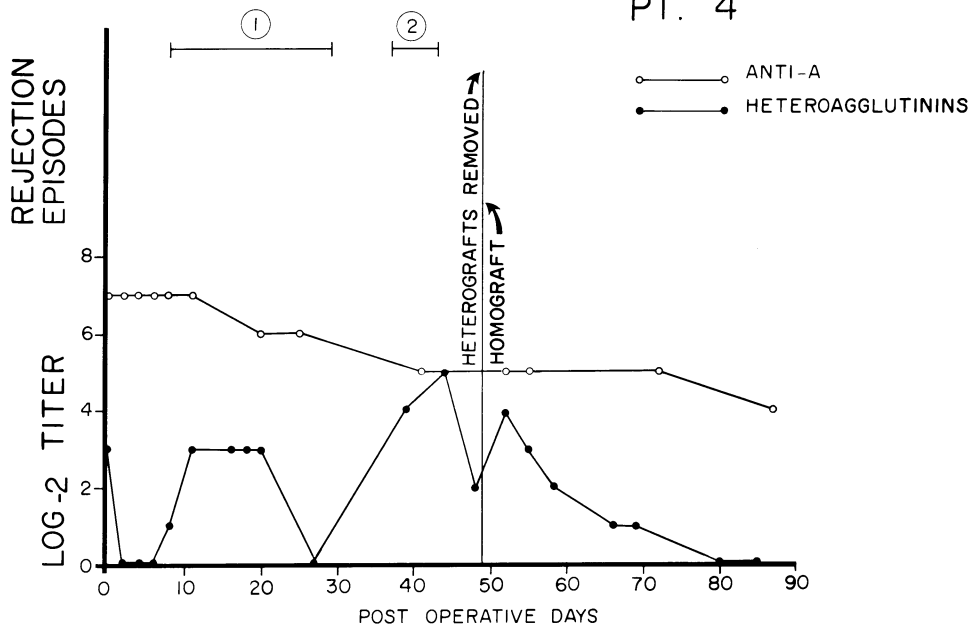


Figure 129. The anti-A hemagglutinin and heteroagglutinin activities in Patient SD 4, type B, following transplantation of kidneys from a type B baboon. The rejection episodes occurred from day 8 to day 29 and from day 37 to day 43. The heterografts were removed and replaced by a homograft on day 49 (arrow). (By permission of *Transplantation* 2: November, 1964.)

PT. 5

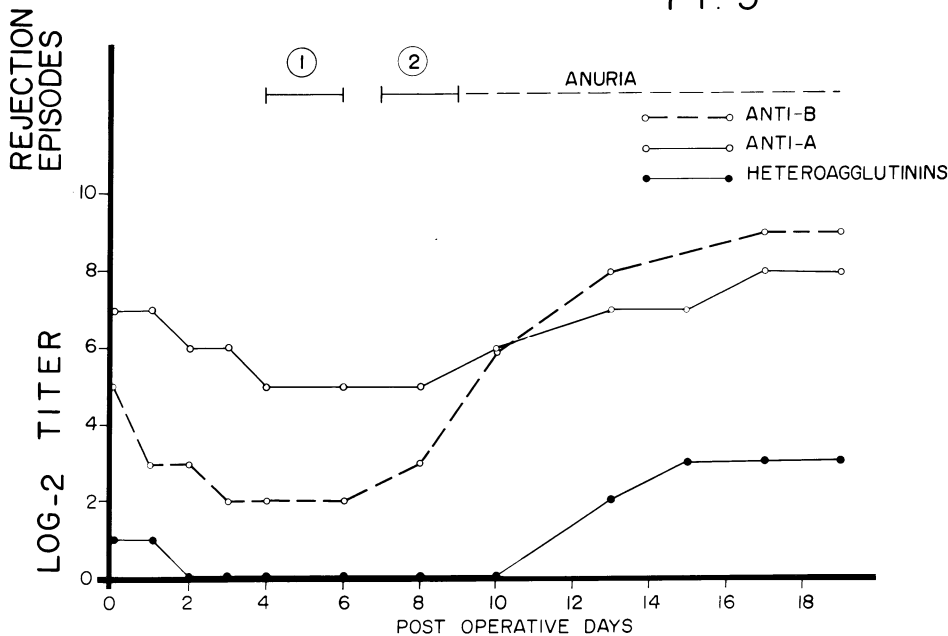


Figure 130. Serial measurements of the activities of anti-A and anti-B hemagglutinins and heteroagglutinins of Patient SD 5, type O, following transplantation of the kidneys from a type AB baboon. Note that the antibody titers rose after the patient became anuric during the second rejection episode. (By permission of *Transplantation* 2: November, 1964.)

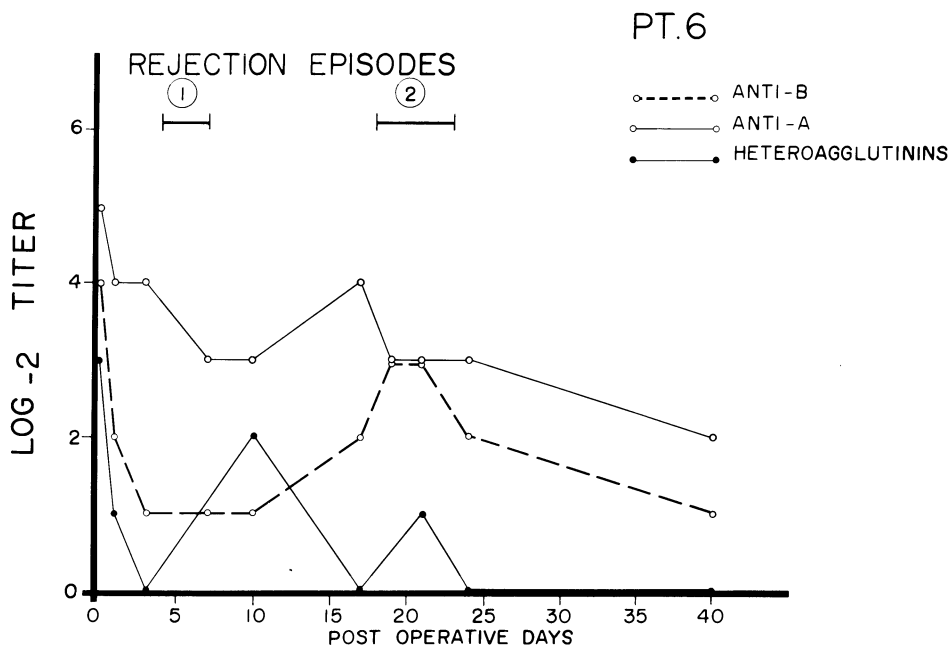


Figure 131. The activities of the anti-A and anti-B agglutinins and heteroagglutinins of Patient SD 6, type O, following the transplantation of kidneys from a type AB baboon. (By permission of *Transplantation* 2: November, 1964.)

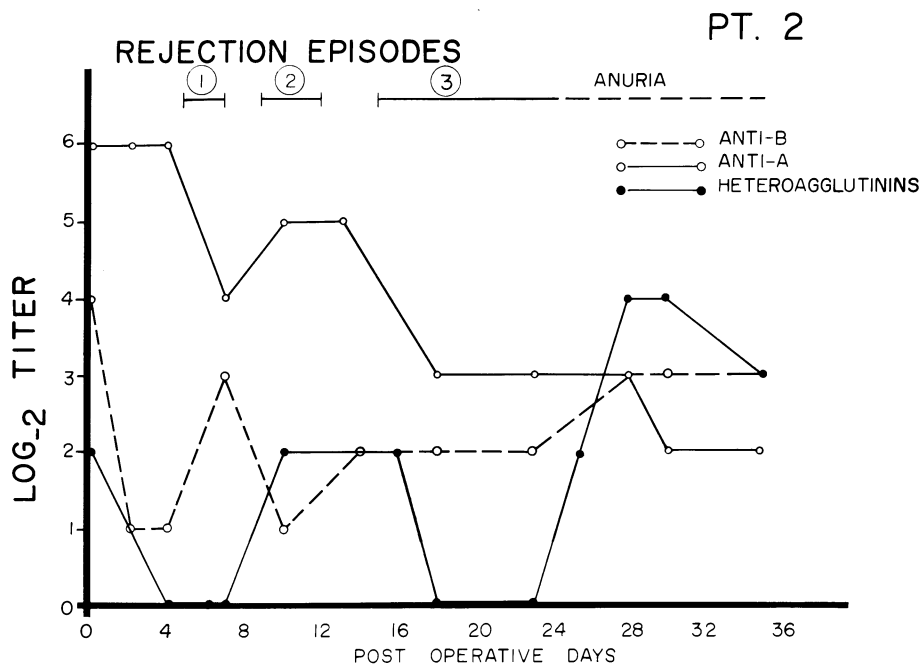


Figure 132. The postoperative changes in titers of anti-A and anti-B hemagglutinins and heteroagglutinins of Patient SD 2, group O, following transplantation of the kidneys of a type B baboon. The clinically apparent rejection episodes are also shown. (By permission of *Transplantation* 2: November, 1964.)

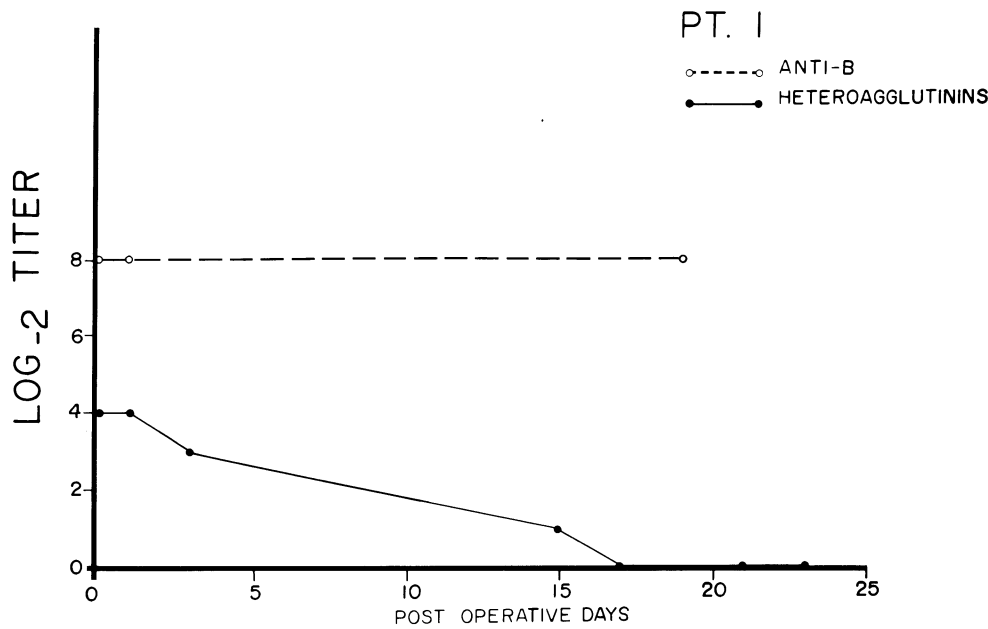


Figure 133. Serial measurements of the anti-B hemagglutinin and heteroagglutinin activities in Patient SD 1, blood group A, following transplantation of kidneys from a type A baboon. (By permission of *Transplantation* 2: November, 1964.)

Patients 1, 2, 5, and 6 had anti-B agglutinins before the transplantation operation. In Case 1, both the donor and recipient were group A, and no change in anti-B occurred (Fig. 132). The remaining three patients were group O and received kidneys from type B or AB baboons. In all three patients a rapid decrease in activity occurred following the transplantation (Figs. 130-132). However, late increases in titer were seen in each case. Two patients, Cases 2 and 5, became anuric (Chap. 23) and in one (Case 5) a marked increase in agglutinin activity was seen. The relationships of the antibody titers and the active rejection reactions for each patient are shown in Figures 129 to 133.

In summary, postoperative decreases in the activities of preformed antibodies to human A and B erythrocytes were observed in all three patients who received kidneys from baboons mismatched with respect to the AB system. These three patients were group O. One received the kidneys of a type B baboon, and two received the kidneys from type AB baboons. The fall in anti-A and anti-B titers in Patients 5 and 6 and anti-A titer in Patient 2 was presumably the result of absorption of the antibodies on the antigenic sites within the transplanted organs. The A and B agglutinogens have been demonstrated on the capillary endothelium of human kidneys by Szulman, and also in aqueous extracts of organs (including kidneys) from rhesus monkeys by Wiener, Candela, and Gross. Although the rate of disappearance of anti-B appears to exceed that of anti-A, the differences are not significant when the data are considered

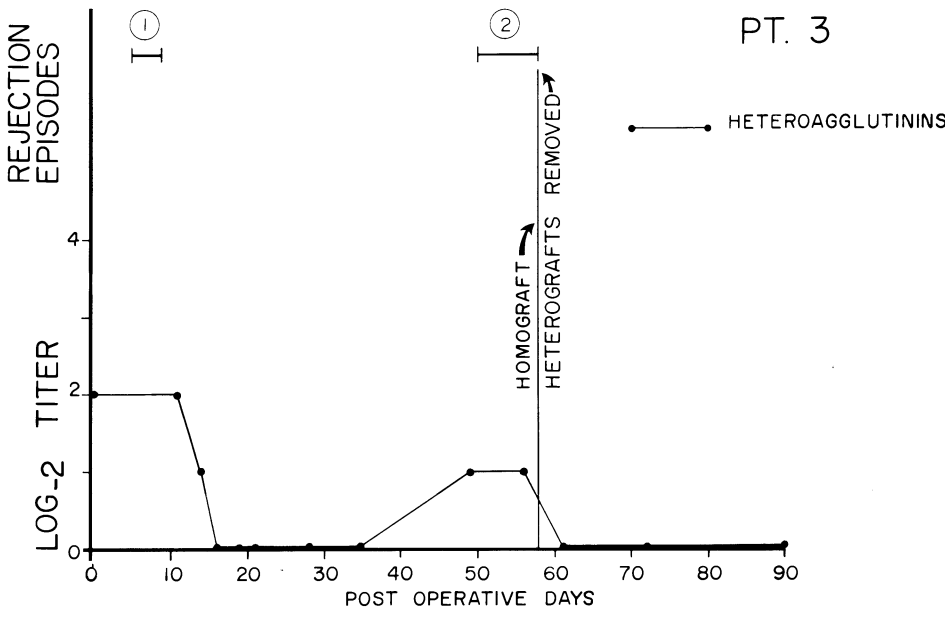


Figure 134. The changes in heteroagglutinin activity and the rejection crises in Patient SD 3, group AB, following transplantation of the kidneys from a type B baboon. The heterografts were replaced by a homograft on day 60. (By permission of *Transplantation* 2: November, 1964.)

in terms of absolute amounts of antibody removed. That the isoagglutinin titers never fell to zero suggests that saturation of the combining sites within the kidney occurred.

The decrease in anti-A as well as anti-B activity observed in Patient 2 may be a result of a cross-reacting antibody present in group O serum. Wiener, Samwich, Morrison, and Cohen identified this antibody by the observation that absorption of human group O serum with A cells also reduced the titer of anti-B agglutinin and, conversely, that absorption with B cells reduced the titer of anti-A. Although the mechanism of this cross-reactivity is not clear, an antigenic determinant, C, has been proposed. The C substance is present on both group A and B cells; therefore, the antibody occurs only in group O serum. Absorption with A presumably removes both anti-A and anti-C and consequently reduces the activity for B cells.

The fluctuation in hemagglutinin titers in the three patients who received AB mismatched heterografts did not have a clearly defined correlation with the rejection episodes. Nevertheless, specific exceptions to this general statement are of interest. Patient 2 showed an elevation of anti-B titer during the course of each of three rejection crises. The highest titers were seen shortly after he became anuric on the twenty-fifth postoperative day (Fig. 132). Patient 5 underwent a mild and reversible rejection reaction on the fourth postoperative day, but an intractable rejection crisis occurred on day 7. The patient was anuric from day 9 until his demise on the nineteenth day after transplantation (Fig. 130). During this anuric phase his anti-A and anti-B activity rose markedly,

and hemolytic antibody activity was demonstrable. Patient 6 also showed a rise in anti-A and anti-B activity during his second rejection episode. Serum samples were not available during the final rejection reaction which began 48 days after transplantation.

Although a precise explanation for these changes in antibody activity is not apparent, it is of interest that each patient showed an increase in antibody activity during his second, and in one case, first and third rejection reactions. These changes are consistent with the hypothesis that one component of the tissue rejection is reduction in blood flow (see Chapters 15 and 25), a change which would result in functional exclusion of the circulating antibody from the combining sites within the kidney. The increases in agglutinin activities also suggest that new antibodies were being synthesized while the patients were under intensive immunosuppressive therapy.

Heteroagglutinins. Although baboon erythrocytes do not possess A or B antigenic determinants, they frequently agglutinate in human sera. The responsible human heteroagglutinin is usually present in low titer, and its activity may be abolished by slight dilution. Preoperative serum samples were analyzed for heteroagglutinin activity in all six recipients. With the exception of Patient 5, each recipient was tested for this antibody employing the red cells of his donor baboon. The preoperative sera of all six patients contained heteroagglutinins for baboon erythrocytes (Figs. 129-134).

The baboons were exsanguinated following nephrectomy, and the donor erythrocytes were stored in ACD solution in small aliquots at 4°C. Under these conditions the cells retained their immunologic properties for approximately two months. In five of the six cases the early postoperative serum samples were tested using the red cells of the donor baboon. Sera obtained more than two months after the transplantations and all sera from Patient 5 were studied using erythrocytes that were freshly obtained from another baboon of the same blood type as the actual donor.

Following transplantation the heteroagglutinin activity disappeared in all six instances. In four patients (Cases 2, 4, 5, and 6) the antibody was not detectable by the fourth postoperative day (Figs. 129-132). In the remaining two patients activity was demonstrated on the fourteenth postoperative day but had disappeared by the seventeenth (Figs. 133, 134). The decrease in titer of this antibody following transplantation suggests that the antigen was represented on the baboon kidneys as well as on the erythrocytes. It is unlikely that the number of donor erythrocytes remaining in the kidney following perfusion was sufficient to cause the fall in antibody titer. The slow removal of the antibody from the circulation implies that the combining sites in the kidney were relatively inaccessible, or that the avidity of the antibody for the combining sites was low. Finally, the complete disappearance of the heteroagglutinin indicates that initially there was insufficient antibody to saturate all the available antigenic determinants. A rapid fall in heteroagglutinin activity has not been observed following transplantation of chimpanzee kidneys into man by the New Orleans group. DeWitt has observed that chimpanzee spleen and kidney tissues will not absorb the heteroagglutinin from human serum.

The changes in antibody activity which were observed in the later post-operative period are of interest when considered relative to the patient's clinical course. In all recipients, except Case 1, antibody activity reappeared at some time during the postoperative period. While in Patient 4 the reappearance of heteroagglutinin was associated with his first rejection reaction, there was no apparent relationship between heteroagglutinin activity and the initial rejection episodes in the other patients. However, the titers were observed to rise in association with the subsequent crises in the five patients in whom rejection crises were identified (Cases 2-6). A rapid rise in titer was seen in the two patients who became anuric during a rejection reaction (Figs. 130, 132).

The reappearance of heteroagglutinin in five of the six recipients indicates that the kidney transplantation stimulated synthesis of the antibody. It is also apparent that the amount of antibody produced was sufficient to saturate all the available combining sites within the transplanted kidneys.

IMMUNOLOGICAL CONSIDERATIONS REGARDING GRAFT REJECTION

The Role of Humoral and Cell-Bound Antibodies. The pathogenesis of destruction of organ grafts has not been completely characterized, but there exists strong evidence that more than one immunologic pathway may be involved, at least under certain experimental conditions.

Human kidneys, transplanted from donors mismatched with respect to the ABO antigen system, may be rejected immediately upon revascularization (see Chapters 6, 22, 25). The rapidity of onset of the graft failure suggests that preformed humoral antibody was involved in the process, a postulate supported by the observation of an abrupt fall in the isohemagglutinin titer. A second model which implicates humoral antibody in graft rejection has been termed the white graft reaction. One example of this, reported by McKhann and Berrian, is the fate of a second skin homograft transplanted to a mouse that is rejecting a first graft from the same donor. The second graft does not become vascularized and consequently is rejected even more quickly than a typical second-set skin graft. Since erythrocyte agglutinins and cytotoxic antibodies are produced in association with graft rejection, it has been postulated that these humoral antibodies mediate rejection of second grafts placed at this critical time. Employing canine kidney transplantation as the model system, Calne has reported a similar phenomenon. He observed that a renal homograft sensitized the recipient dog to a second kidney graft from the same donor with the result that the second transplant was rejected immediately. Because of the rapidity of rejection of the second graft and the absence of cellular infiltration, he concluded that humoral antibody, produced in response to the first graft, mediated the rejection of the second.

Preformed antibody has also been strongly incriminated in the rejection of heterospecific organ transplants. The rapid failure of organs grafted between members of widely disparate species was documented in the early transplanta-

tion literature by Neuhof and summarized recently by Calne. He observed that a goat kidney, transplanted into a dog, failed within minutes, following release of the vascular clamps. The dog's preoperative serum contained an agglutinin directed against the goat's erythrocytes.

Although these observations clearly implicate humoral antibody in graft repudiation under certain circumstances, equally impressive evidence indicates that graft rejection may proceed in the absence of humoral antibody production. Newborn infants (Fowler) and agammaglobulinemic children (Good) may reject homografts despite the absence of humoral antibody-forming capability. A second observation is that by Algire that tissue grafts placed within millipore chambers, impermeable to lymphocytes, remain viable for protracted periods. It has been demonstrated by Billingham that allogeneic skin grafts on highly tolerant mice can be rejected only by lymphocytes from nontolerant mice of the same strain. Finally, Brent and Medawar have observed that serum from sensitized mice was without effect on skin grafts in tolerant mice.

Although graft rejection in different experimental models can frequently be ascribed primarily to humoral antibody or alternatively to "cell-bound" antibody, there is little understanding of either mechanism at a molecular level. Most of the fragments of evidence implicating humoral antibody imply that acute vascular injury is a major factor, and Stetson has postulated that an Arthus-like reaction is the lesion involved. As noted earlier, the experience with transplantation across species barriers suggests that humoral antibody was important in the rejection of heterospecific grafts.

Hemagglutinating Antibody and the Rejection of Baboon Kidney Transplants. The six patients who received baboon heterografts all had early renal function in spite of the presence of heteroagglutinins against baboon erythrocytes in the serum of each recipient. *In vivo* activity of the heteroagglutinin was demonstrated by the rate of clearance of Cr^{51} donor erythrocytes from the recipients' circulations. In Cases 1 and 3 the survival of donor baboon erythrocytes was measured within a few hours following the kidney transplantation. Baboon red blood cells were incubated with 150 microcuries of Cr^{51} for 30 minutes at 25°C , and the reaction was stopped by the addition of ascorbic acid. A small volume of the tagged cells was injected into the patient. Blood samples were collected at frequent intervals, and the radioactivity was determined with a well-type scintillation counter. The heterologous erythrocytes were removed from the circulation with a 50 per cent clearance time of 12 minutes (Fig. 135), a rate comparable to that observed by Cutbush and Molli-son when labeled human erythrocytes, mismatched with respect to the ABO antigens, are infused.

The reasons for the failure of the humoral antibody to evoke an immediate immunologic reaction are not clear. One possibility is that the erythrocyte-agglutinating antibody did not have affinity for the kidney, but this seems unlikely in view of the consistent fall in heteroagglutinin titers following transplantation. A second possibility is that the steric configuration of the

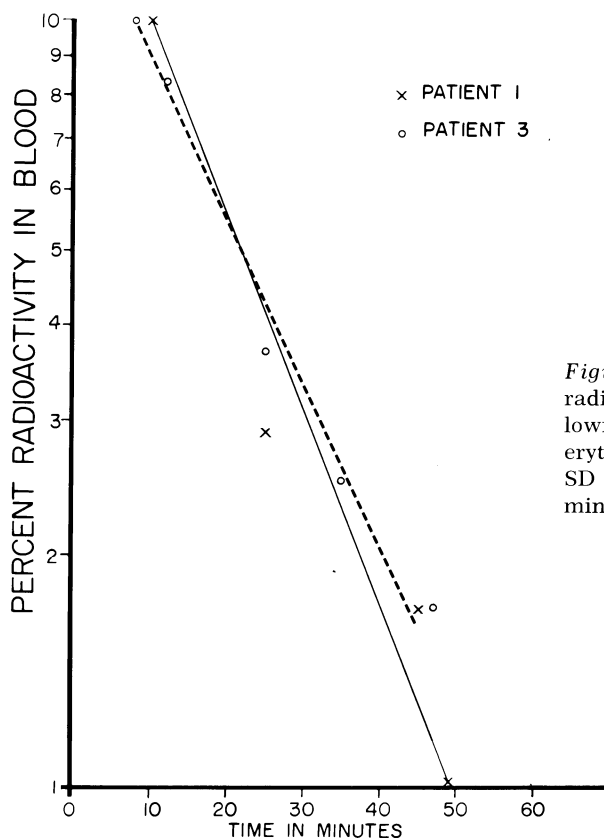


Figure 135. The disappearance of radioactivity from the circulation following injection of Cr^{51} -labeled baboon erythrocytes into patients SD 1 and SD 3. The $T_{1/2}$ in each instance is 12 minutes.

antigen-antibody complex was not correct for initiation of an immediate vascular response such as that observed by Calne. The association constant of the antibody for its combining site is probably not very high. Since the agglutinin apparently cross-reacts with the erythrocytes from a number of distinct species of infrahuman primates, it is unlikely that the reaction with the cells of any particular animal is highly specific. The possibility of a low association constant is strengthened by the observation of the comparatively slow decline in heteroagglutinin titer following baboon kidney transplantation in contrast to the rapid fall in specific isohemagglutinin level following transplantation of a human ABO mismatched kidney (Chap. 22). Finally, the general reduction in immunologic capacity which is present in uremic patients (Chap. 22) probably contributed to an attenuation of the host response. This could be an important factor since the intensity of the Arthus reaction is a function of the amount of precipitating antibody in the circulation.

In three patients there was an even greater possibility of immediate rejection of the heterografts because of the additional presence of another group of preformed humoral antibodies, the anti-A or anti-B hemagglutinins, or both. The evidence for cross-reaction between these hemagglutinins and the hetero-specific transplants was just as clear as with the heteroagglutinins. It is of

interest, in contrast, that abrupt rejection of a chimpanzee kidney by a human recipient has been described using a similar ABO mismatch (Chap. 23). In the latter case, there was an abrupt fall in the host isohemagglutinin titer. The difference in results with the chimpanzee as opposed to the baboon donor is presumably the result of dissimilarities in the number of combining sites in the kidneys of these two species and in the comparative avidity of the isohemagglutinins for the combining sites. The slow decline in isohemagglutinin titer after baboon heterotransplantation is in accord with this concept.

The response of the patients to the baboon kidney transplantation was generally similar. Following an initial period of renal function, abrupt deterioration occurred. This was reversed temporarily to a greater or lesser extent by additional immunosuppressive therapy. Nevertheless, rejection was not successfully controlled in any case, since severe pathologic alterations were present (Chap. 25) in all the heterografts, and sustained adequate renal function could not be maintained in any case (Chap. 23).

The findings in these patients suggest that both humoral and cell-bound antibodies may have been operative in the pathogenesis of the rejection reactions. Two observations may indicate a role of the heteroagglutinins. In Case 1, no acute rejection episode was observed, and heteroagglutinins did not reappear during the postoperative course. In the other patients, there was a temporal relationship between some rejection crises and the reappearance of circulating antibody. Whatever the role of the humoral antibodies, the heterografts also evoked a vigorous cellular response. The rejected organs all had an extensive mononuclear cell infiltration (see Chapter 25).

Possible Pathologic Effects of Hemagglutinating Antibody. While the heteroagglutinins and isohemagglutinins may not have mediated the rejection in a classic sense, these antibodies were bound to antigenic determinants within the kidney and undoubtedly contributed to tissue injury. The distribution of these combining sites within the kidney is not known. Using another heterologous antibody system, Feldman has demonstrated that antibody, produced in rabbits following immunization with rat kidney, was directed against the basement membranes of rat kidneys. Within 30 minutes following rejection of this antiserum into a normal rat, structural abnormalities were found in the basement membrane, and proteinuria developed. Proteinaceous deposits appeared in the basement membrane, followed later by similar accumulations in the subendothelial region of the glomerular capillaries. The material in the basement membrane was composed of the rabbit antibody, basement membrane, and complement. Later, larger deposits composed of host antibody, rabbit antibody, and complement were found. Enlargement of the fenestrations and proliferation of the endothelial cells were noted, and the foot processes of the epithelial cells were broadened.

Proteinuria was observed during the early postoperative period in all six recipients of baboon heterografts. This proteinuria, coupled with the absorption of the humoral antibody, prompted a study of the ultrastructure of the glom-



Figure 136. Electronmicrograph of a glomerular capillary of the heterograft removed from Patient SD 4. The basement membranes (b) show areas of focal subendothelial thickening (arrows) and the foot processes (f) of the epithelial cells are smeared. Endothelial cells (e) show some hyperplasia. 1—Capillary lumen. (Approx. X 5,500.)

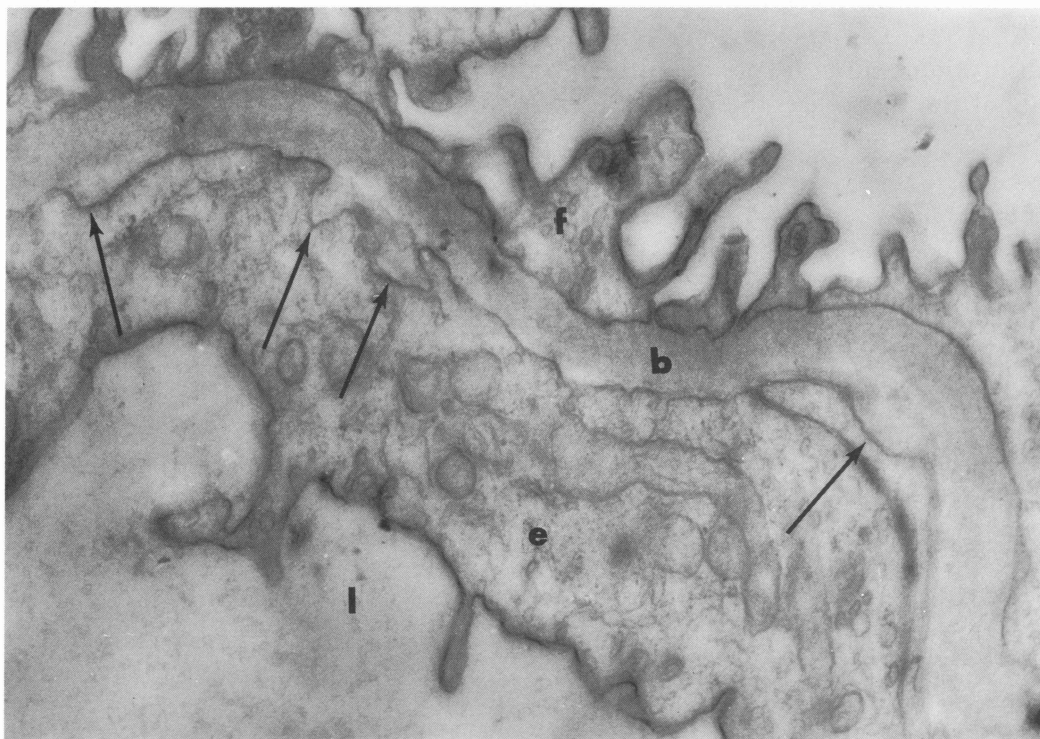


Figure 137. A small part of a glomerulus of the heterograft removed from Patient SD 4. The arrows indicate areas of subendothelial thickening of the basement membrane (b). 1—Capillary lumen; e—Endothelial cells; f—Foot process of epithelial cell. (Approx. X 21,000.)

eruli of the heterografts which were removed from Patient 4. Immediately following extirpation, sections of the kidneys were fixed in osmic acid for examination in the electron microscope. The glomeruli of the rejected kidneys were abnormal. There was hyperplasia of the endothelial cells with considerable thickening of the cytoplasmic lining of the capillaries. The endothelial cells were swollen and vacuolated. There was smearing of the foot processes of the epithelial cells. However, these changes are not specific, as they may occur with a variety of renal injuries that result in proteinuria. While the basement membranes showed some fusiform thickenings without consistent subendothelial deposits, occasional subendothelial thickenings were observed (Figs. 136, 137). The relationship of these abnormalities to the heteroagglutinin absorption cannot be proved although the capillaries might be expected to be a site of the heterospecific antigenic determinants.

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Chapter Twenty-five

PATHOLOGICAL CHANGES IN TRANSPLANTED KIDNEYS

by K. A. Porter, M.D., D.Sc.

Although this chapter will deal primarily with the histopathological changes encountered in the human renal transplants performed at the University of Colorado Medical Center, an attempt will be made to relate these findings to those of other investigators doing similar work elsewhere and to those obtained from animal experiments.

Of the 64 patients treated in the living donor (LD) series at the University of Colorado between November, 1962, and March 31, 1964, 24 died by June 1, 1964. During the same period, three cadaveric homotransplantations (CD series) were performed, all unsuccessfully. Six baboon heterotransplantations were also attempted, with death of the patients from 19 to 98 days later. An autopsy was granted for 30 of the 33 patients who died. In addition, seven homo- or heterografts became available for study after their surgical removal. Finally, one ureter was resected eight months after the initial homotransplantation because of a stricture. In considering the findings in the 37 retrieved kidneys and in the single ureter, the homotransplants will be separated from the heterotransplants.

RENAL HOMOTRANSPLANTS

Before discussing these cases, all of which were treated with azathioprine, prednisone, and, in most instances, actinomycin C, it is necessary to consider at some length the changes that occur in untreated renal homotransplants. In this way a background will be provided against which modifications in the rejection pattern brought about by treatment can be assessed. Much of this information must inevitably be drawn from the results of renal homotransplantation in dogs, because not only has almost all the fundamental work been done in these experimental animals, but also because on no occasion has a kidney been homotransplanted into a healthy nonuremic human.

At this point, a distinction has to be made between first and subsequent renal homotransplants. In their classic descriptions, both Dempster and Simonson drew attention to the fact that a second transplant from the same donor not only functioned for a shorter time than the original transplant, but showed different and rather characteristic histological changes. However, a second transplant from a donor other than the original one looked and behaved like a primary transplant. Except where specifically stated, the following account refers only to primary homotransplants.

UNTREATED CANINE RENAL HOMOTRANSPLANTS

Homotransplants in dogs rapidly become swollen so that they are two or three times as heavy by five days as they had been originally, and the capsule and perirenal tissues become thickened. When the kidney is examined on the day urine flow has ceased, the cut surface can show several patterns. The cortex is usually wider than normal, pale, and swollen. There may be hemorrhagic foci scattered throughout the kidney; sometimes the hemorrhage is predominantly medullary in distribution.

Microscopically there is an infiltration with lymphocytes and a variety of cells with pyronine-positive cytoplasm (Fig. 138). This invasion starts in the cortex, can easily be seen in conventional sections at about the third day, and progresses until the kidney becomes anuric. At first, the cellular infiltration is focal (Fig. 139, top), but later the cells are diffusely distributed in the interstitium (Fig. 139, bottom). Electronmicroscopic studies have shown that at 24 hours small lymphocytes can be found adhering to the endothelial cells lining the peritubular capillaries and venules in the cortex (Fig. 140, top). From 48 hours onward two groups of larger cells appear.

Many of these cells resemble either large lymphocytes or monocytes (histiocytes). Two such cells are seen in Figure 140, bottom. The nucleus is often indented, and there is usually a large nucleolus. The cytoplasm is abundant and contains many ribosomes, a few granules, and some vacuoles. Small projections from these cells indicate micropinocytosis. Endoplasmic reticulum is frequently present but rarely abundant. Mitochondria are large and numerous.

Some of the infiltrating cells, however, seem to be of the plasma cell series (Fig. 141). They have a central or slightly eccentric large nucleus in which the chromatin is loosely arranged and nucleoli are sometimes seen. The cytoplasm is usually abundant with a prominent well-developed endoplasmic reticulum with many ribosomes attached to the surface of the membranes. In some the reticulum is lamellar, but in a few the cavities are dilated and contain amorphous material (Fig. 142). A Golgi zone is often apparent close to the nucleus. Mitochondria are fairly numerous and prominent.

The heterogeneity of the cell population entering homotransplanted kidneys contrast with the homogeneity of the infiltrate in skin homografts in which Wiener, Spiro, and Russell have demonstrated that all cells are of the "lymphocytic" type until the final stages of rejection.

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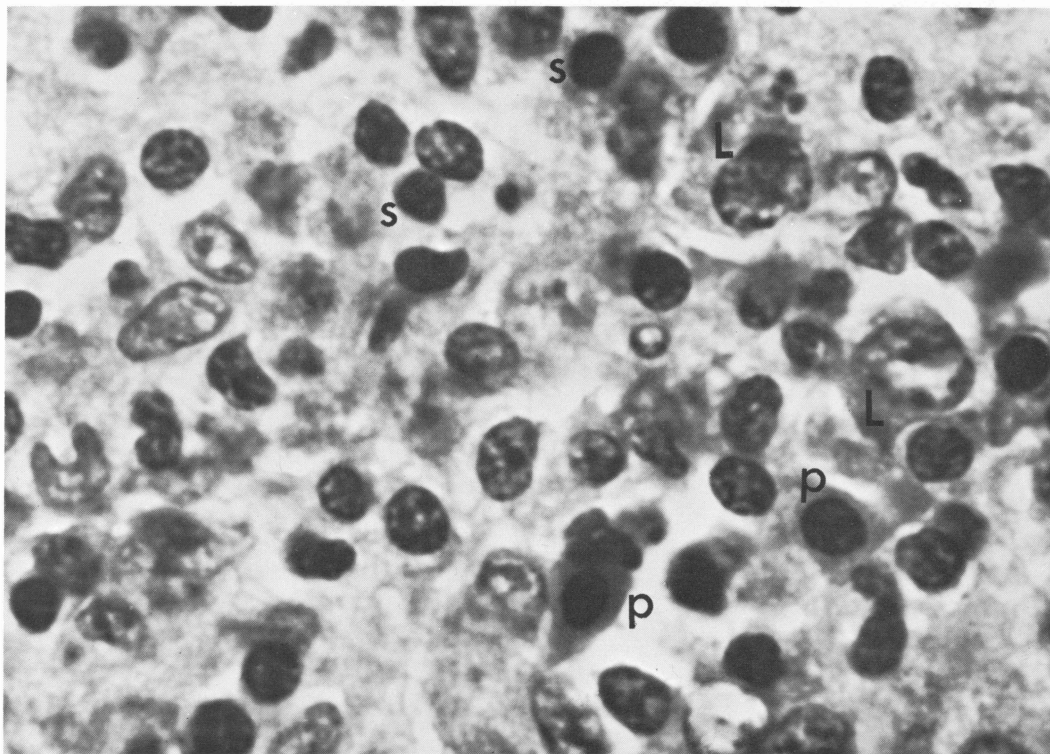


Figure 138. Dog kidney five days after homotransplantation into an untreated recipient. High power view showing heterogeneity of cellular infiltration. Many of the cells have basophilic cytoplasm. A few are obvious plasma cells (p); some are small lymphocytes (s); a few are large "lymphoid" cells (L); many are intermediate in their size and cytoplasmic characteristics between these main groups. H and E (X 870).

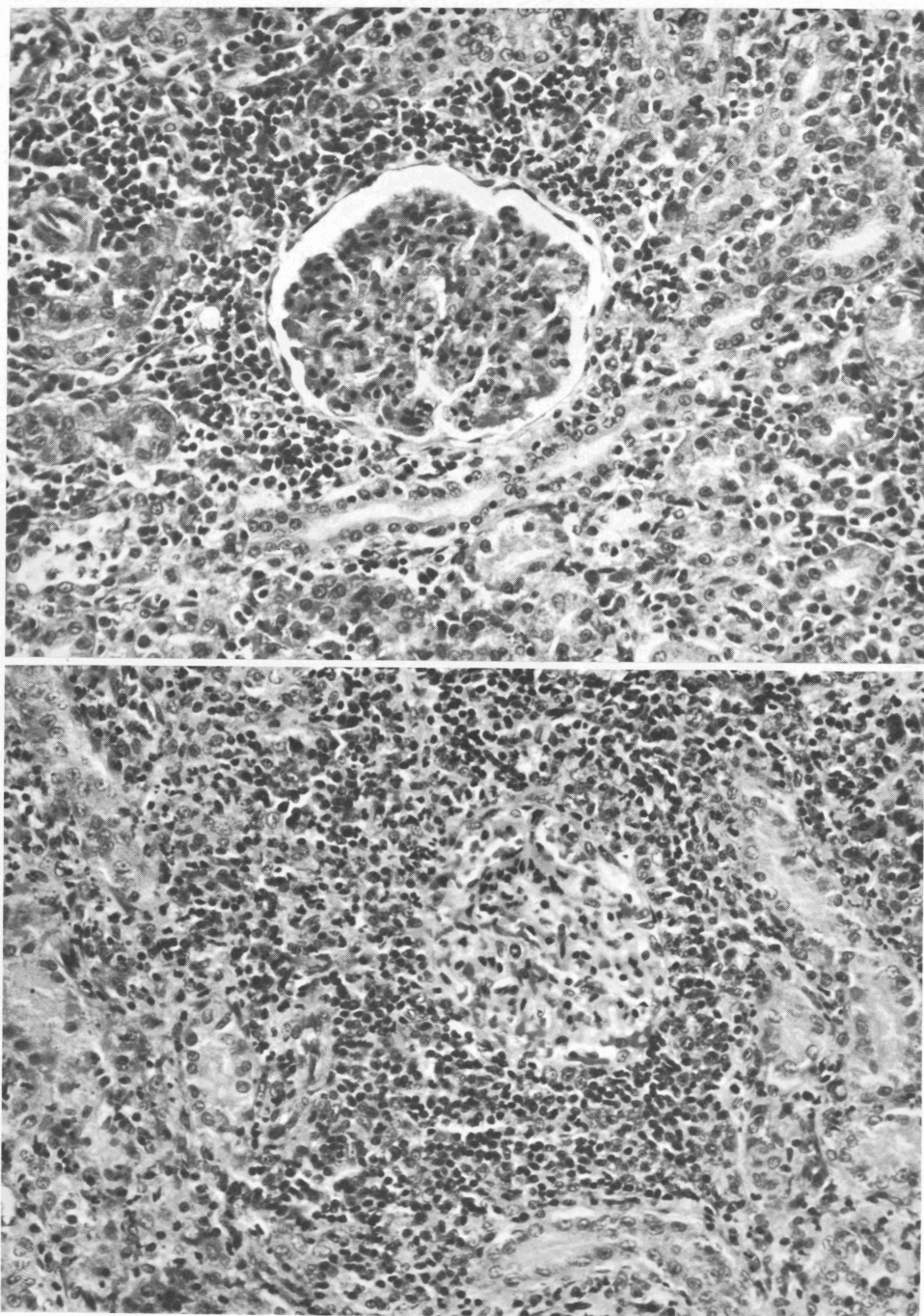


Figure 139. Top—Dog kidney five days after homotransplantation into an untreated recipient. There is a heavy, but focal, cellular infiltration. Some of the proximal convoluted tubules are undergoing necrosis, and epithelial debris is seen in the lumina.

Bottom—Untreated canine renal homotransplant at eight days. The heavy cellular infiltration is diffuse, and includes some neutrophils. There is much tubular necrosis, and a glomerulus is undergoing destruction. H and E (X 250).

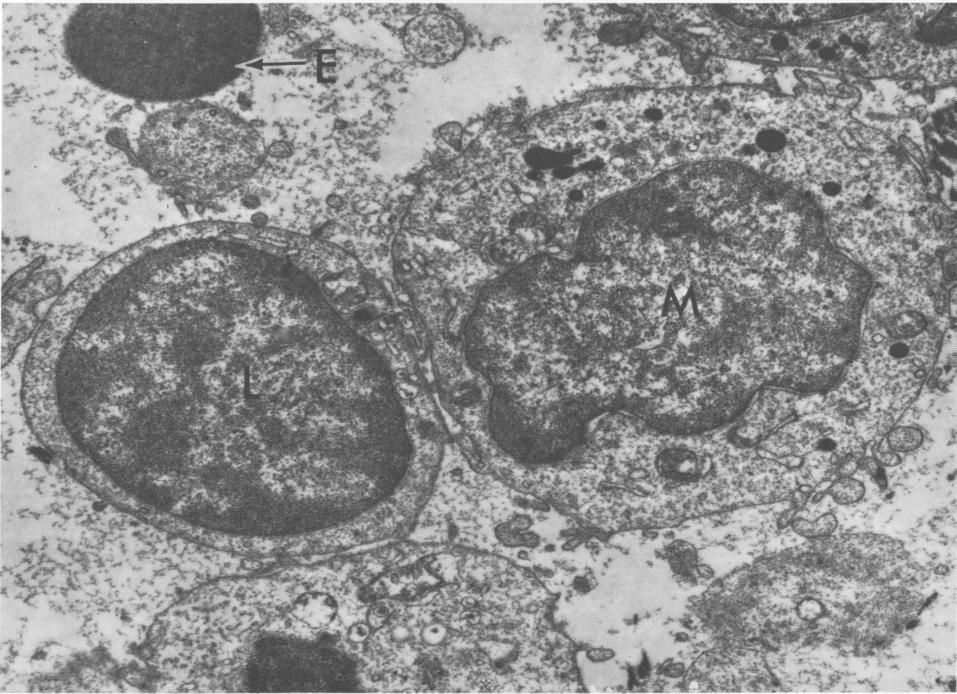
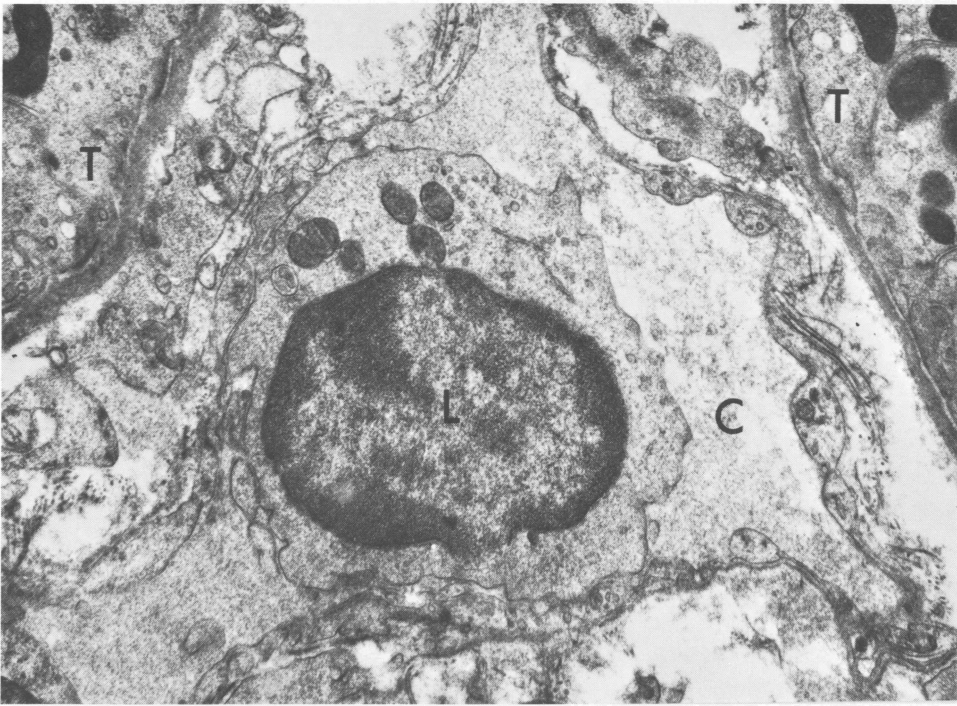


Figure 140. Top—Electronmicrograph of a dog kidney 24 hours after homotransplantation into an untreated recipient. A lymphocyte (L) is seen lying in close contact with the endothelium of a peritubular capillary (C) near the corticomedullary junction. On either side of the capillary are the basement membranes of adjacent tubules (T). The lymphocyte has several large mitochondria at its upper pole and a few scanty ergastoplasmic profiles (X 10,000).

Bottom—Electronmicrograph of a dog kidney 72 hours after homotransplantation into an untreated recipient. Two infiltrating host cells, one resembling a lymphocyte (L) and the other a monocyte or histiocyte (M) are seen lying in the edematous interstitium. Both cells contain some profiles of endoplasmic reticulum and mitochondria. There are also granules in the larger cell and many projections from the cell periphery. An erythrocyte (E) is present in the upper part of the photograph (X 8300). (By permission of *Lab. Invest.* 13: September, 1964.)

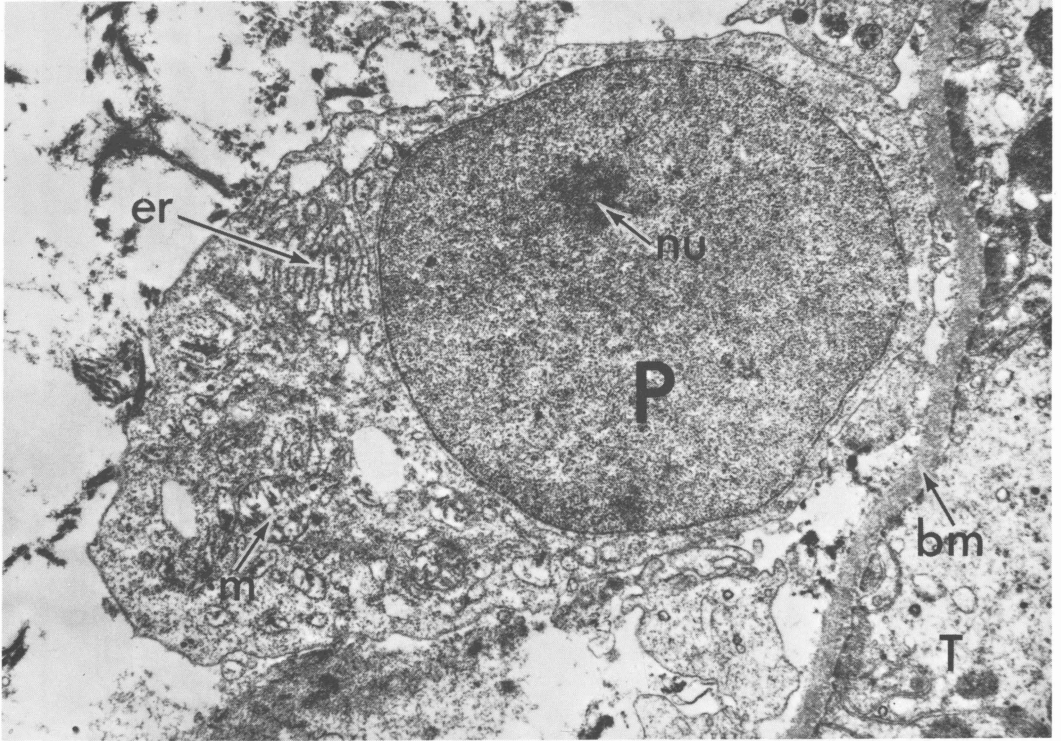


Figure 141. Electronmicrograph of a dog kidney 72 hours after homotransplantation into an untreated recipient. An infiltrating host cell resembling a plasma cell (P) is lying in the interstitium adjacent to the basement membrane (bm) of a tubule (T). Endoplasmic reticulum (er) is abundant and rough. Mitochondria (m) are frequent, and there is a conspicuous nucleolus (nu). (X 8,250.) (By permission of *Lab. Invest.* 13: September, 1964.)

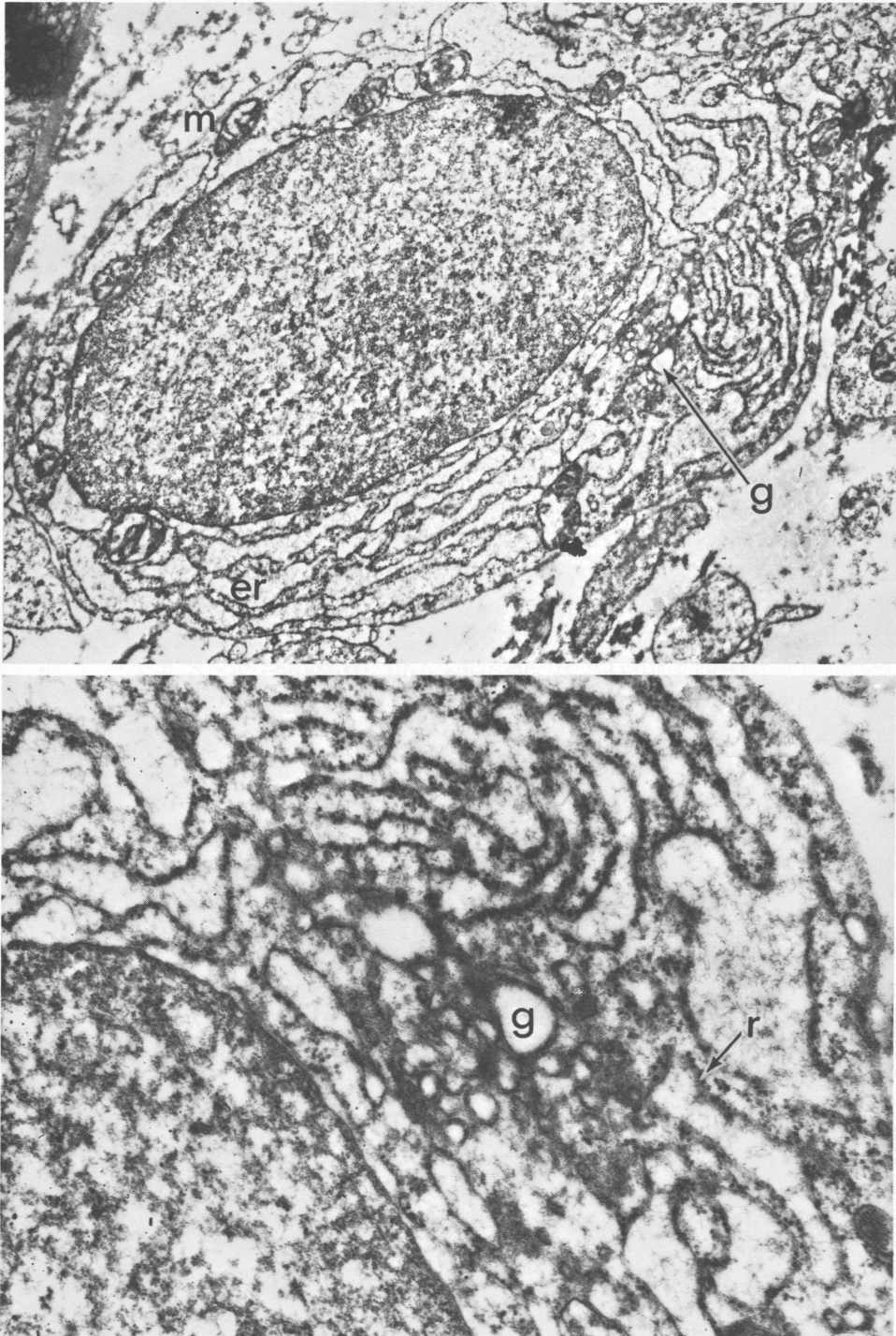


Figure 142. Top—Electronmicrograph of a dog kidney 72 hours after homotransplantation into an untreated recipient. A plasma cell is lying in the interstitium. It shows plentiful endoplasmic reticulum (er) in the form of cisternae, numerous mitochondria (m), and a Golgi zone (g). (X 10,000.)

Bottom—Higher power view of part of the plasma cell shown above illustrating the arrangement of ribosomes (r) on the membranes of the endoplasmic reticulum. The Golgi zone is at g (X 58,000). (By permission of *Lab. Invest.* 13: September, 1964.)

The cytoplasm in both groups of large cells contains much ribonucleic acid (RNA) and is consequently pyronine positive. In thin paraffin-embedded sections these pyroninophilic cells can be seen marginating in the peritubular capillaries and venules (Fig. 143, top). Intimate association of these infiltrating cells with the endothelium is followed by disruption of the capillary walls (Fig. 143, bottom). Under the electronmicroscope apparent temporary establishment of cytoplasmic continuity between the infiltrating cells and the endothelial cells has been described by Kountz and Dempster and their associates (Fig. 144, top). After this, there is escape of fluid and cells into the interstitium of the transplant (Fig. 144, bottom). Within the graft some of the cells undergo division, the total cell cycle being about 12 hours. As time passes, more of the cells entering the kidney have the appearance of mature plasma cells.

There is swelling of the endothelial cells lining the arterioles and small arteries (Fig. 145, top). The glomeruli undergo few changes, but the tubules become necrotic. One of the earliest tubular changes is a shedding of the superficial part of the proximal tubular epithelium into the lumina. This damage affects most commonly the outer cortical tubules and particularly the very first part of these, according to Darmady, Dempster, and Stranack. In the terminal stage there is widespread interstitial hemorrhage and edema. Polymorphonuclear leukocytes and macrophages are prominent in the cellular infiltrate. Sometimes the small arteries and arterioles show fibrinoid necrosis of their walls with plugging of their lumina with fibrin, platelets, and cells.

Such primary renal homotransplants in dogs usually cease to function after four to eight days, although Zukoski and Jeejeebhoy have observed function for 60 and 30 days, respectively. In the latter experiment, a biopsy at 18 days showed massive cellular infiltration, destruction of peritubular capillaries, severe tubular degeneration, and obstruction of damaged interlobular arteries by cells, fibrin, and platelets (Fig. 145, bottom; Fig. 146, top). When the dog died at 30 days, there was widespread tubular atrophy, interstitial fibrosis, far less cellular infiltration, and fibrous obliteration of many peritubular capillaries, arterioles, and small arteries (Fig. 146, bottom). There was commencing fibrous intimal thickening of some larger arteries.

These occasional prolonged survivals are probably due to chance genetic compatibility of the host and donor, the transplant not possessing any strong histocompatibility genes which are lacking in the host.

UNTREATED HUMAN RENAL HOMOTRANSPLANTS

When homotransplants have been performed for acute renal failure in humans, the pattern of rejection has been similar to that seen in dogs, although rather more protracted, anuria occurring at about 22 days. One of the best documented cases is that reported by Michon and his associates in which a boy of 16 years tragically had his only kidney removed because of hemorrhage

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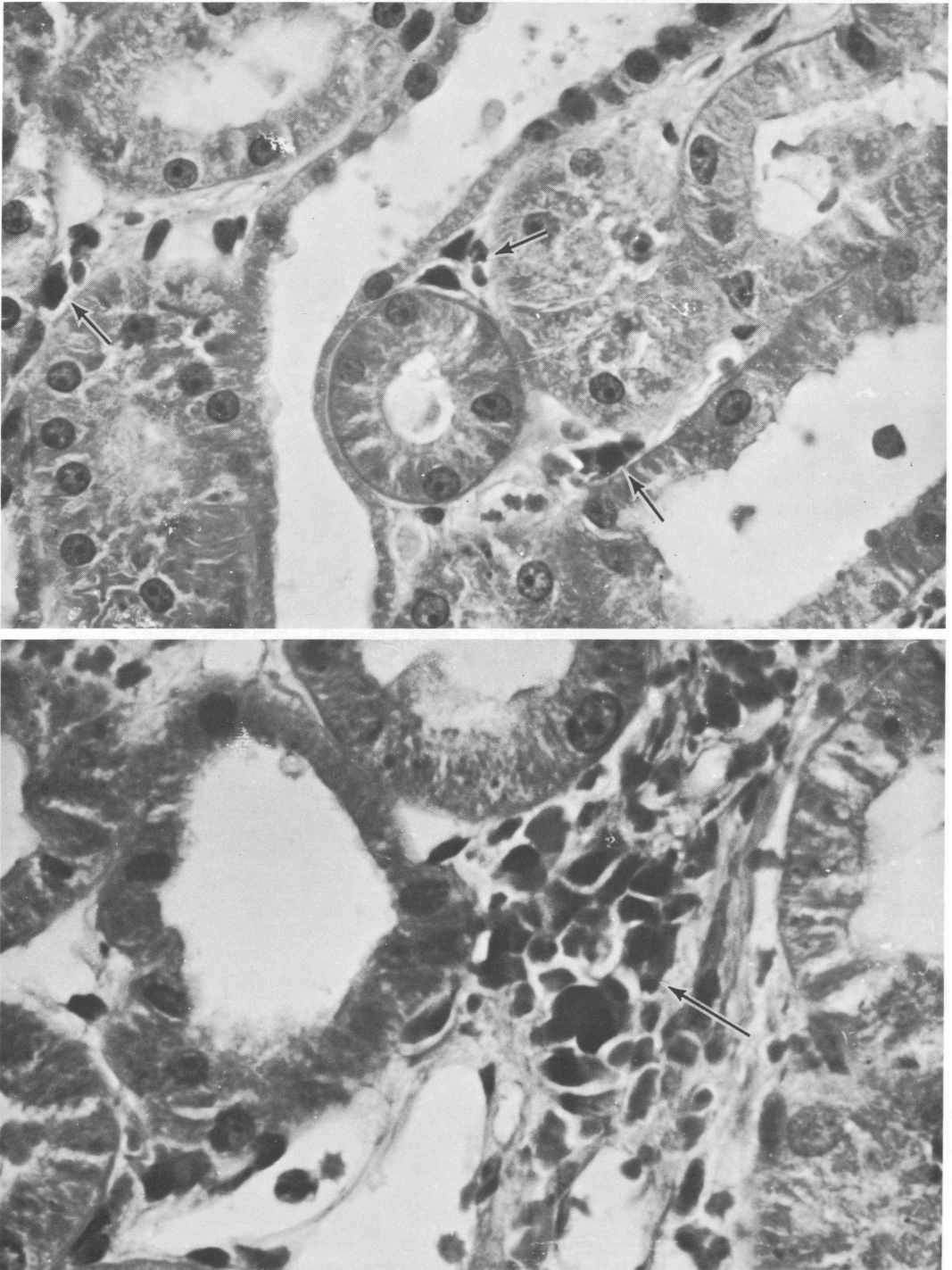


Figure 143. Top—Dog kidney 48 hours after homotransplantation into an untreated recipient. Host cells with basophilic cytoplasm (arrows) can be seen marginating in three peritubular capillaries.

Bottom—Dog kidney 72 hours after homotransplantation into an untreated recipient. A peritubular capillary (arrow) has partially disintegrated, releasing erythrocytes and host cells with basophilic cytoplasm into the interstitium. H and E (X 550).

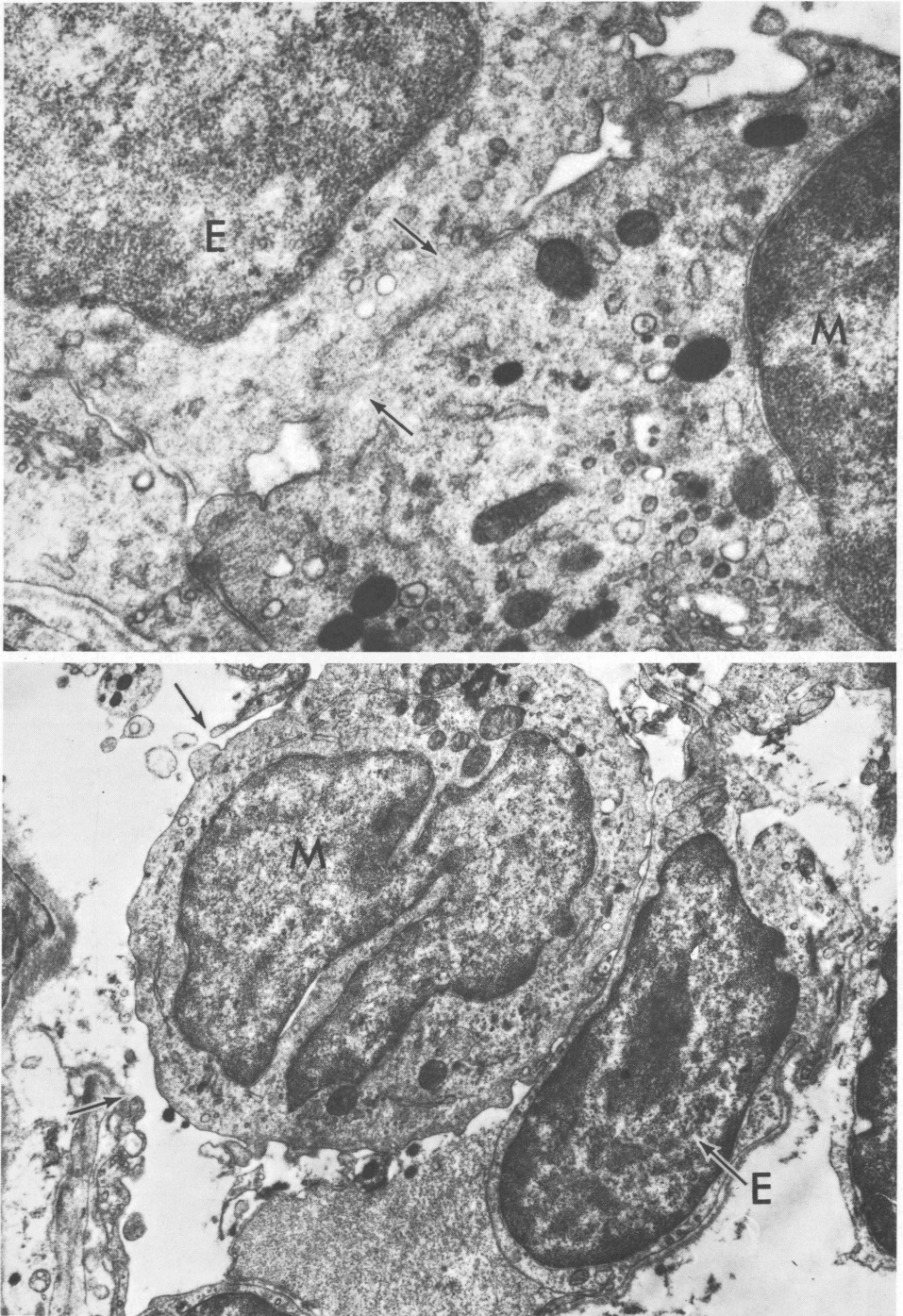


Figure 144. Top—Electronmicrograph of a dog kidney 48 hours after homotransplantation into an untreated recipient. There is intimate association between an infiltrating “monocyte-like” host cell (M) and an endothelial cell (E) lining one of the peritubular capillaries. At the points marked with arrows there is apparently breakdown of the membranes and fusion of the cytoplasm of the two cells (X 33,000).

Bottom—Electronmicrograph of an untreated canine renal homotransplant at 48 hours. A monocyte type of infiltrating cell (M) is lying in a ruptured peritubular capillary in close association with an endothelial cell (E). The gap in the capillary wall is marked by arrows (X 8,300). By permission of *Lab. Invest.* 13: September, 1964.)

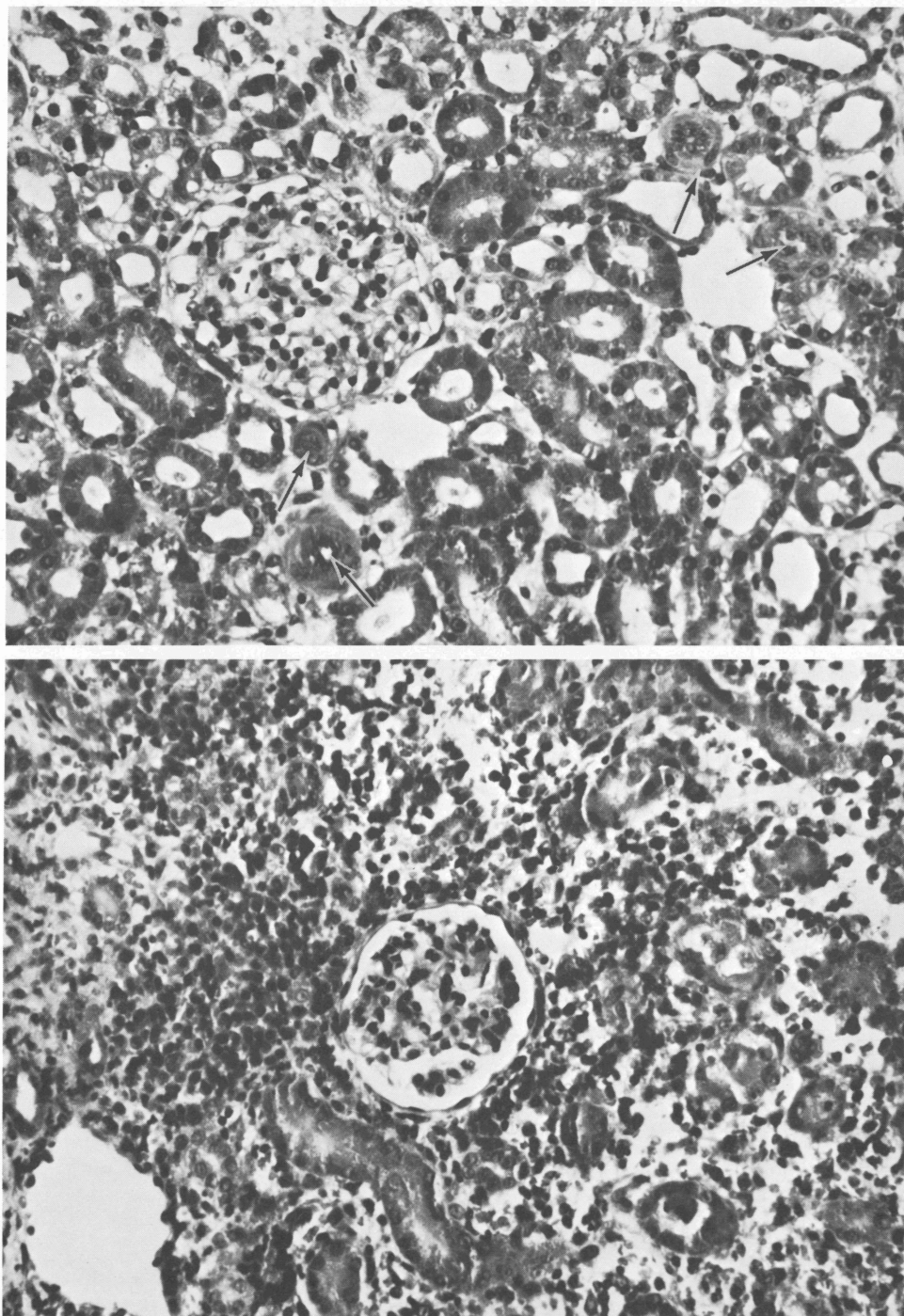


Figure 145. Top—Biopsy of untreated canine renal homotransplant at 72 hours. The tissue is from Jeejeebhoy's experiment (see text) in which unusually prolonged homograft function was obtained, probably due to chance genetic similarity of the donor and recipient. There is swelling of the endothelial cells lining the arterioles and small arteries (arrows).

Bottom—Biopsy of same homograft after 18 days. There is marked cellular infiltration, edema, and tubular damage. H and E (X 210).

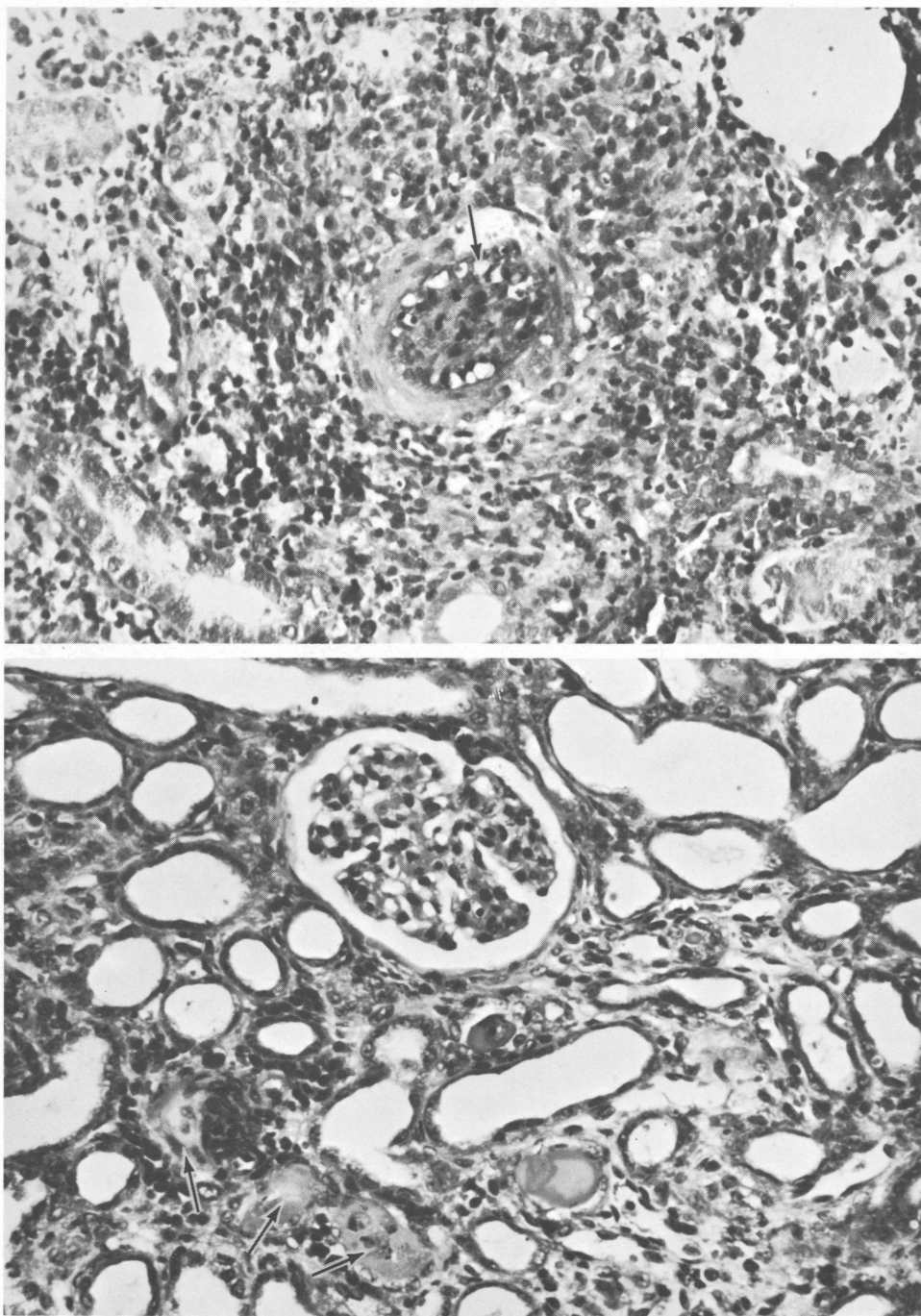


Figure 146. Top—Same 18-day biopsy of canine renal homograft as was shown in Figure 145. There is obstruction of a damaged interlobular artery by cells, fibrin, and platelets. Fat spaces (arrow) are present just inside the internal elastic lamina due to breakdown of platelets.

Bottom—Autopsy appearance at 30 days of the same renal homotransplant. There is widespread tubular atrophy, interstitial fibrosis, only a moderate cellular infiltration, and fibrous obliteration of a small artery (arrows). The tissue was kindly provided by Mr. H. F. Jeejeebhoy. H and E (X 210).

following trauma. Seven days later a kidney was transplanted from his mother who was of the same blood group. The transplant excreted 3 liters of urine in the first 24 hours and then 1.5 liters per day until the twenty-second day when it suddenly stopped functioning. When explored, the transplant was swollen, purple, and speckled with petechial hemorrhages, but the main vessels and ureter were patent. A biopsy showed accumulations of lymphocytes and plasma cells, necrosis of tubules, and intimal thickening with thrombosis in some small arteries. The patient became uremic and died 10 days later. At autopsy many of the intrarenal vessels were thrombosed, and there were many small areas of infarction. During the time the transplant was in the recipient, the serum gamma globulin level slowly rose.

When kidneys have been homotransplanted into recipients with chronic uremia, marked prolongation of survival has been recorded, and the histological features have been modified in some instances. Nine such cases were published by Hume in 1955. Four of the transplants functioned adequately and continued to excrete urine for 37, 47, 99, and 176 days. The kidneys were ischemic for 55 to 200 minutes before transplantation and because of acute tubular necrosis went through an initial period of anuria. After 8.5 to 19 days, repair of the damage had progressed far enough for urine excretion to begin. Microscopically, the kidney which functioned longest showed striking vascular changes in addition to tubular atrophy, interstitial fibrosis, and foci of infiltrating plasma cells. In many of the arteries there was a severe degree of intimal fibrous thickening. In many ways this kidney histologically resembles Jeebhoy's 30-day canine renal homotransplant which was mentioned earlier.

Since publication of Hume's study, it has been proven by Mannick that uremia does influence the survival of homotransplanted kidneys. Uremic dogs will retain a renal homotransplant for 15 to 23 days. The pathological changes in the uremic transplants are the same as those observed in kidneys homotransplanted to normal dogs, but the cellular infiltration commences later. The mode of action of uremia in prolonging homograft survival is considered in detail in Chapter 22.

MECHANISM OF REJECTION OF UNTREATED RENAL HOMOTRANSPLANTS

Although the principal morphological changes in kidney homotransplants have been recognized for many years, most fundamental aspects of the rejection process are still far from clear.

Host Recognition of the Transplant. It is known, for example, that the host's lymphoid tissues are made aware of the transplant. This is based upon three pieces of evidence. First, a second kidney homotransplanted from the same donor is rejected more quickly than the primary graft. Second, sensitization with a skin graft from the donor or cross-circulation with the donor prior

to renal transplantation will also produce rapid rejection of the kidney. Third, renal homotransplantation causes large and medium-sized cells with pyronine-positive cytoplasm to accumulate in the host around the postcapillary venules of the lymph nodes and along the arterioles of the spleen. These cells are similar to those demonstrated by Scothorne in the lymph nodes draining a skin graft. How this information about the foreign antigen reaches the host's spleen and lymph nodes is not known.

One possibility is that antigen enters the plasma that circulates through the vessels of the transplant and is conveyed to the lymphoid tissues, perhaps by passage across the endothelial cells of the arterioles and postcapillary venules.

Another possibility is that circulating host lymphocytes interact with the transplant endothelium and become altered in some way. It has already been shown that some lymphocytes are seen in the first 24 hours clinging to the lining of the peritubular capillaries (Fig. 140). These cells could then transform within the transplant into pyroninophilic cells, some with endoplasmic reticulum and some without, and enter a proliferative phase. Such transformation of sensitized lymphocytes is known to occur in vivo and in vitro (see Cowling, Gowans, and Porter). Later some of the progeny of these cells could enter the blood stream, travel to the lymphoid tissues of the host, and there form further clones of pyroninophilic cells.

A third explanation is that the lymphocytes, after contact with the kidney antigen, leave by way of the blood stream, enter the host's lymphoid tissues, and there transform into large proliferating cells. Some of the cells produced then return to the kidney via the lymph and blood stream, attach themselves to the peritubular capillary endothelial cells, and there cause local damage. The last explanation fits the known facts best. Experiments in our laboratory in which lymphocytes were labeled with tritiated thymidine and adenosine showed, first, that cells present in the thoracic duct lymph of the host four days after renal transplantation enter the transplant, and, second, that some of the cells infiltrating the graft had been manufactured in the spleen after renal transplantation.

Production of Cell-Bound Antibody. While damage to the walls of the peritubular capillaries following contact with the pyroninophilic cells is presumably caused by a cell-bound antibody, there is no proof of this. The paucity of rough endoplasmic reticulum and profusion of free ribosomes within many of the cells that infiltrate the graft in the first few days may be related to the fact that these cells are producing antibodies which are not released, but adhere to the cell surface.

Localization of the infiltrating cells in the peritubular vessels may be determined simply by the slowness of the blood flow in this capillary meshwork leading to margination of lymphocytes, just as margination of polymorphs in the venules in acute inflammation follows slowing of the blood flow. This process might be encouraged by unusual stickiness of the endothelium resulting from the slight ischemic damage which is inevitable in all renal transplantations.

Destruction of the peritubular capillaries will inevitably lead to interstitial

edema, some hemorrhage, ischemic necrosis of the proximal tubules, and functional arrest of the transplant. This sequence of events probably accounts for the abrupt termination of function of most unmodified kidney homotransplants, as suggested by Kountz and his colleagues.

Production of Circulating Antibody. In the terminal phases of renal homograft rejection in both humans and dogs, areas of fibrinoid necrosis may occur in the walls of arterioles and arteries. Recently Horowitz and his associates demonstrated by a fluorescent-antibody technique that gamma globulin appears in the walls of the arteries and arterioles of the transplant during rejection. They found that this gamma globulin was soluble in acid buffer and that it bound complement, features which suggest that it is probably part of a recently formed antigen-antibody complex. Reaction of antibody with antigen in vessel walls causes damage, and could account for the endothelial swelling and marked fibrinoid necrosis noted in some transplants.

Although this antibody might come from host cells infiltrating the vessel walls, there is some evidence that circulating antibody is involved. Following kidney homotransplantation, the morphological changes that occur in the lymphoid tissues of the host resemble those seen when antibodies are being produced against known antigen. This naturally leads to speculation that antibody against the transplant antigens is made by cells in the host's lymphoid tissues and, after a delay of some days, is released into the circulation. Such a thesis could explain the localization of fibrinoid necrotic lesions to the arteries, arterioles, and glomerular capillaries because circulating antibody would tend to be deposited most heavily in that part of the foreign vasculature first encountered.

The difficulty in demonstrating circulating antibody against a primary renal homotransplant which has been experienced by many investigators in the past may simply be due to sampling before there has been time for the antibody titer to reach detectable levels and to the use of relatively crude analytic techniques. A rise in the serum gamma globulin of four of five recipient dogs 10 to 12 days after removal of a renal homotransplant which was mentioned briefly by West, the rise in gamma globulin in the human renal homotransplant recipient reported by Michon, and the recent demonstration by Simonsen and Altman of cytotoxic antibodies appearing between seven and 14 days after transplantation would all tend to support this view.

TREATED HUMAN RENAL HOMOTRANSPLANTS FROM THE UNIVERSITY OF COLORADO MEDICAL CENTER

For convenience the findings in these homotransplanted human kidneys will be described under seven headings:

1. Three transplants which did not function at all because of prolonged ischemia.

2. Two transplants which failed immediately, probably because the major blood groups of donor and recipient were not compatible.
3. One transplant which bled, necessitating its removal after four days.
4. A transplant which was examined 12 hours after operation because of the death of the patient from electrolyte imbalance.
5. Fourteen transplants which were examined during or just after a rejection episode.
6. Eight transplants from patients who died some time after at least one rejection episode had been recognized and apparently treated successfully.
7. Three transplants from patients who died without having had at any time a clearly recognizable clinical episode of rejection.

TRANSPLANTS WHICH DID NOT FUNCTION BECAUSE OF ISCHEMIA

The three patients who received these kidneys have been referred to briefly in Chapters 8 and 19. In one case (LD 29) the renal homotransplant, which came from a living donor, had been ischemic for 85 minutes, never functioned, and was removed at 48 hours. In a second case (CD 3), both kidneys from a man who had died from a myocardial infarct were transplanted into a woman. The left kidney was ischemic for 137 minutes and the right for 215 minutes. Both failed to excrete urine and the patient died at four days. The third patient (CD 2) received a cadaveric homograft which was revascularized in the recipient 124 minutes after death of the donor. After 12 days of anuria, the kidney, which ruptured after minor trauma, was removed.

Grossly, the kidneys in these cases were found to be slightly swollen, soft, pale, and a little heavier than before transplantation. The cortex was widened and the corticomedullary junction blurred. The vessels were patent and the ureter was unobstructed.

Microscopically, there was massive recent tubular necrosis, affecting particularly the proximal part of the nephron (Fig. 147). The lumina of the tubules were filled with casts of protein and cell debris. There was evidence of active repair in the form of mitoses among surviving proximal tubular cells and the lining of some tubules by new flattened epithelium. The interstitium was edematous and contained scattered foci of small lymphocytes, plasma cells, and occasional polymorphs, chiefly in relation to severely damaged tubules. Cellular infiltrate was most prominent in CD 2 who was actively rejecting (see Figure 157). The glomeruli were normal in the transplant that had come from the living donor, but in one of the other cases there was periglomerular fibrosis and a few fibrotic glomeruli. The cadaveric kidneys also showed some fibrous intimal thickening of the interlobular and arcuate arteries.

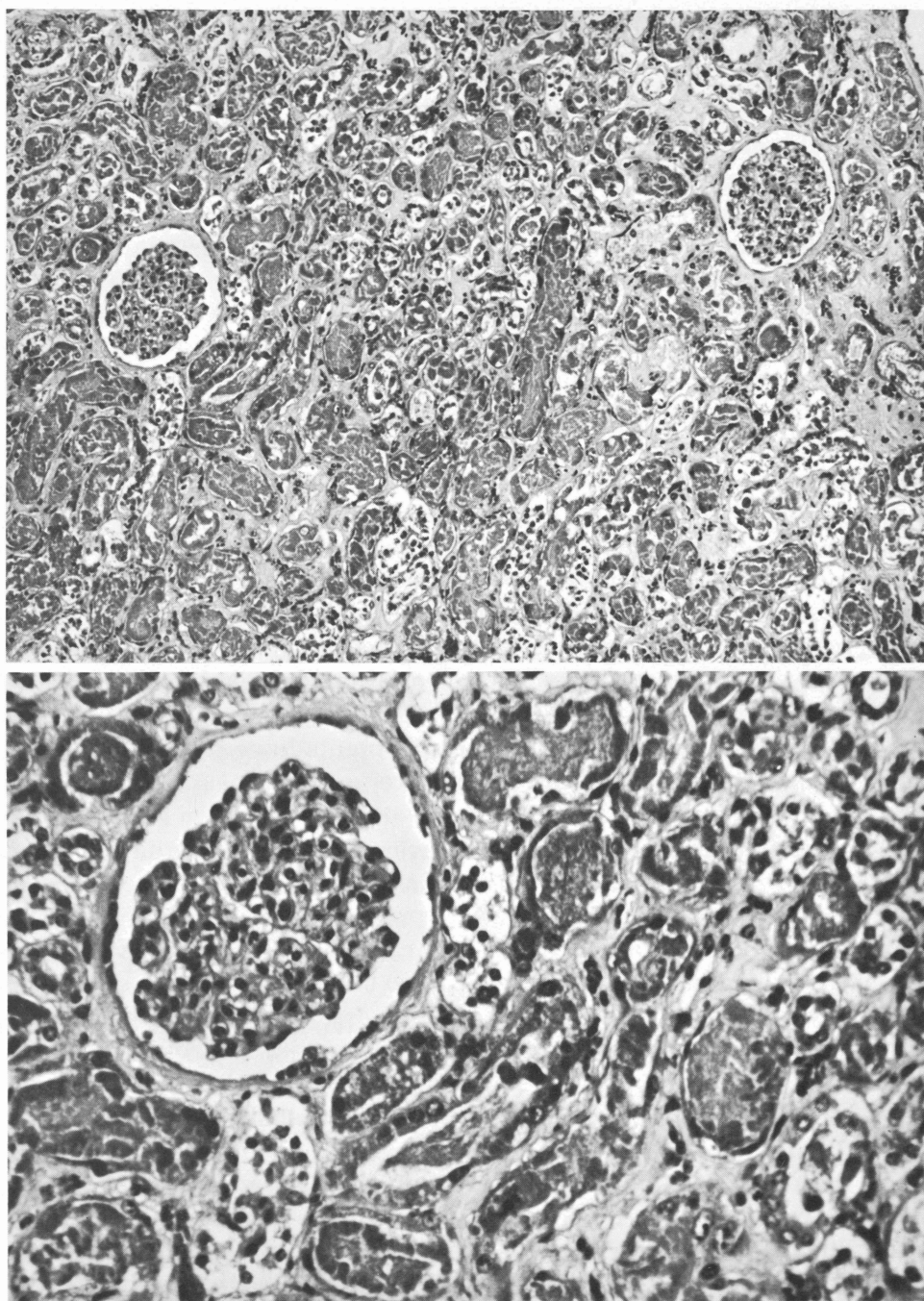


Figure 147. Top—Cadaveric human renal homotransplant from Patient CD 3 examined at four days. The transplant was ischemic for 137 minutes. There is severe tubular necrosis involving particularly the proximal parts of the nephrons which are filled with casts of cell debris. The interstitium is edematous. H and E (X 125).

Bottom—Higher power view of transplant from Patient CD 3. Some of the proximal tubules are lined by flat regenerating epithelium. The glomerulus shows slight periglomerular fibrosis, but is otherwise normal. H and E (X 300).

Such tubular changes are characteristic of temporary complete ischemia of a kidney and are often seen in transplants. Nephron dissection in one such human renal homotransplant described by Joekes in 1957 showed that in addition to severe necrosis of the proximal convoluted tubules with rupture of the basement membranes, there were occasional focal areas of necrosis in the loops and distal convoluted tubules. A mild degree of this change occurs even in most renal autotransplants and accounts for the brief period of disturbed tubular function and transient proteinuria that is often noted. That the damage is due to the period of ischemia to which the kidney has been subjected was demonstrated clearly in 1947 by Hamburger who showed that clamping the renal artery of a dog for as short a period as 15 minutes resulted in proteinuria and defective urea concentration. In over half the animals in this experiment renal function did not return to normal for over seven days. Later Mitchell and Woodruff showed that the damage was lessened if the kidney was cooled during the time its blood supply was occluded, an adjunct routinely employed in one form or another for all the Colorado homotransplants.

Most cadaveric human homotransplants that have been described have shown proximal tubular necrosis, but this damage need not be severe if the donor has not been hypotensive before death and if the kidney is cooled while awaiting transplantation. At St. Mary's Hospital, London, seven of the last eight consecutive cadaveric renal homotransplants have functioned well from the time of operation with a daily urine excretion of at least 1.5 liters. In these seven cases the mean ischemic period was 144 minutes, with a range of 69 to 187 minutes. In the one kidney that was oliguric for four days after transplantation the ischemic period was 210 minutes. None of these kidneys had double renal arteries, although they were present in Case LD 29.

Although proximal tubular necrosis is rapidly healed by regeneration of tubular epithelium, and there is every reason to believe that even a severely damaged kidney will begin to excrete urine after a period ranging from five to 20 days, the difficulties of diagnosing and treating a rejection episode under these circumstances are very great. The importance of avoiding ischemic tubular damage to homotransplants for this and other reasons has been emphasized in Chapter 7.

The glomerular and arterial changes in the cadaveric transplants (CD 2 and CD 3) were almost certainly present at the time of transplantation, and merely reflect the diseased state of the donor. This illustrates another of the difficulties in using cadaveric kidneys. There is rarely time to investigate the donor adequately, and occasionally severely diseased kidneys are inadvertently used. In the St. Mary's series a case occurred in which it was discovered, some days after a successful renal transplantation, that the donor had suffered from malignant phase hypertension. A biopsy showed fibrinoid necrotic changes in the vasculature of the transplanted kidney.

TRANSPLANTS WHICH FAILED BECAUSE OF BLOOD GROUP INCOMPATIBILITY

Two kidneys, which were incompatible on the basis of ABO blood groups, when transplanted into group O rhesus positive recipients became cyanotic within a few minutes and failed to excrete urine. The course of these transplants and the pathologic findings have been described fully in Chapter 6 (see Figures 17, 19). The first kidney (LD 19) came from a group A+ donor and the second (LD 23) was from a group B+ donor. Both transplants were removed within three hours of their insertion.

A similar example of immediate anuria following transplantation of an A+ kidney into an O+ recipient has been recorded by Shackman. In his case, as in LD 19, there was a sharp rise in the anti-A hemagglutinins within a few days after operation. Six transplants from A+ donors to O+ recipients and one from a B+ donor to an O+ recipient have been performed at St. Mary's Hospital. Three of these never functioned, and one became anuric at two days. The other three failed at various times up to 39 days. The transplants which developed immediate anuria showed sludging of red cells in the glomerular capillaries (Fig. 148, top) similar to that described in Chapter 6 (see Figure 19), and the transplant which ceased functioning at 48 hours also contained erythrocyte clumps in the tuft loops (Fig. 148, bottom). It seems clear that violations of the rules propounded in Chapter 6 must be avoided if donors and recipients of different blood types are to be paired.

Cases of anuria in which there has been MN incompatibility between donor and recipient have also been reported by Hamburger in 1962 and by Shackman, but in these cases there was no concomitant rise in anti-N antibodies.

REPORTED HOMOTRANSPLANT FAILURE BECAUSE OF OTHER FACTORS

Examples of immediate postoperative anuria or oliguria which did not seem to be due either to ischemia or to blood group incompatibility have been reported from time to time. Three of these cases proved at autopsy to have developed acute glomerular lesions in the transplant. Two of the patients were suffering from either the microscopic form of polyarteritis nodosa or from Ellis's rapidly progressive Type I glomerulonephritis. The Boston case, reported by Hume in 1955, was described as "polyarteritis nodosa." This was also the clinical diagnosis in a case studied at St. Mary's Hospital, but it was decided after autopsy that this was in reality an example of glomerulonephritis. The homotransplants were untreated and were oliguric from the time of operation. The first patient died after 38 days; the second at six days. In Hume's case, there was marked crescent formation in Bowman's capsule and obliteration of

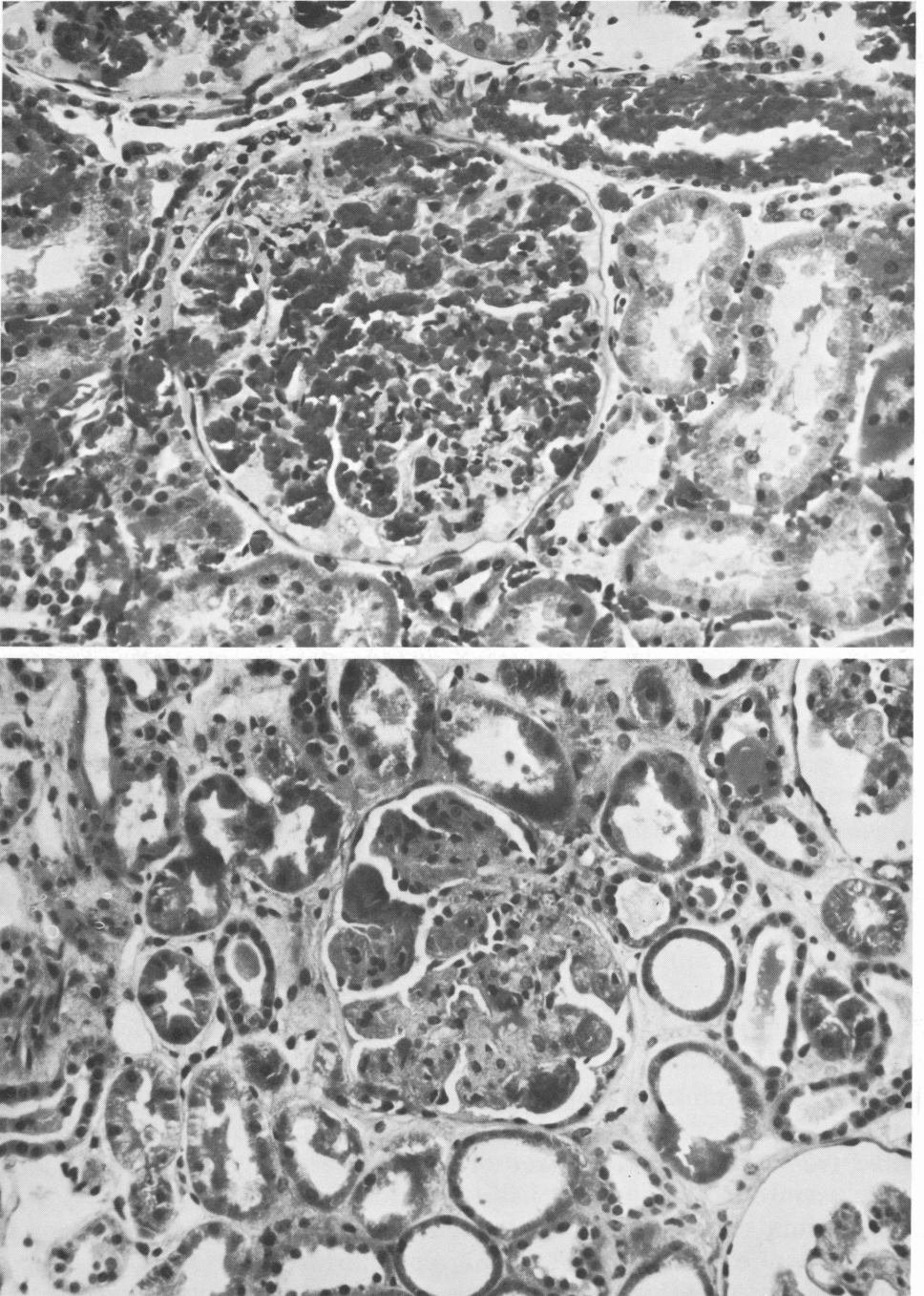


Figure 148. Top—Cadaveric human renal homotransplant from St. Mary's series, from a donor who was of blood group B+, two hours after transplantation into an O+ recipient. The kidney became cyanotic within a few minutes, and failed to excrete urine. The glomerular tuft capillaries are distended with sludged erythrocytes, and there is protein-containing fluid in Bowman's space. There is some hemorrhage into the interstitium, and the proximal tubules show early signs of ischemic necrosis. H and E (X 340).

Bottom—Biopsy at 48 hours of an untreated cadaveric human renal homotransplant (St. Mary's series) which became anuric at that time. Some of the glomerular tuft capillaries contain collections of sludged red cells presumably resulting from the major blood group incompatibility between the donor who was group A+ and the recipient who was O+. There is tubular damage due to 105 minutes of ischemia imposed on the cooled kidney during transplantation. H and E (X 300).

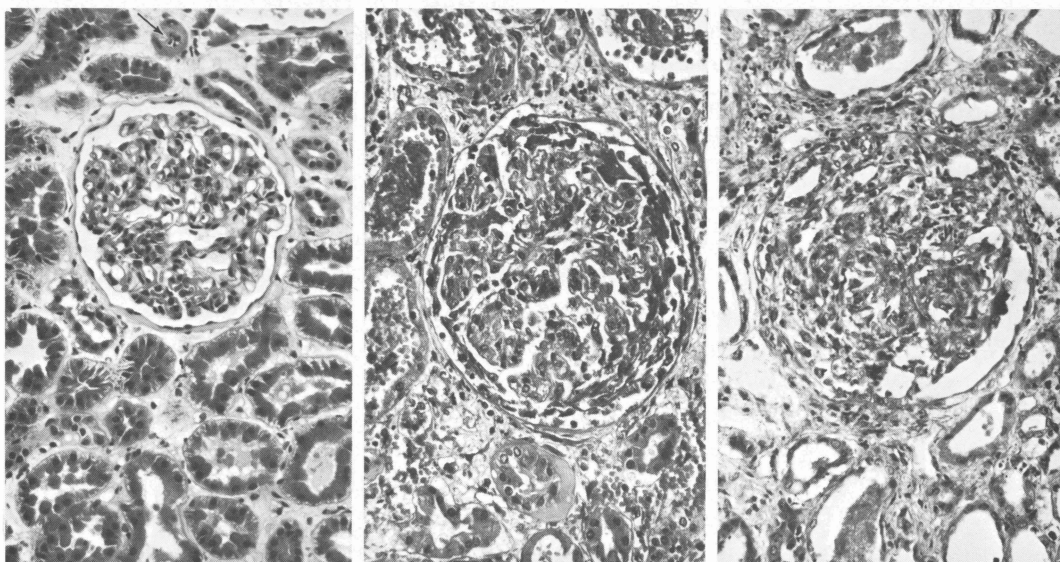


Figure 149. Left—Biopsy at 24 hours of an untreated cadaveric human renal homotransplant in St. Mary's series. The donor was a man of 36 years who died as the result of a head injury. The kidney, although cooled, was ischemic for 106 minutes. There is interstitial edema, tubular damage, and hyaline thickening of the wall of an arteriole (arrow).

Middle—The same kidney at autopsy six days after transplantation. The homograft had remained oliguric during that period. There is patchy fibrinoid necrosis of the glomerular tuft capillaries and early crescent formation in Bowman's capsule. The tubules are severely damaged, and the distal parts contain red cells.

Right—One of the patient's own kidneys from the same case. The glomerular tuft shows some fibrinoid necrosis and a large, partly fibrosed, crescent. These changes were part of a rapidly progressive Ellis Type I glomerulonephritis. H and E (X 175).

glomerular capillaries. The St. Mary's homograft showed fibrinoid necrosis of many tuft capillaries and some early crescents (Fig. 149). In these two cases it seems probable that the factors responsible for the original disease also produced the lesions in the transplants. It will be recalled that neither recipient received immunosuppressive treatment.

The third case, described by Krieg, was rather different in that the recipient, an 11-year old boy, was suffering not from glomerulonephritis but from chronic pyelonephritis. The cadaveric transplant he received was ischemic for almost three hours and never really functioned. The patient died after 10 days. At autopsy the glomeruli within the transplant were enlarged, and the capillary basement membranes were thickened. However, there was also focal fibrinoid necrosis of many of the arterioles, and infiltration of the interstitium with plasma cells, lymphocytes, and edema fluid. Although there were no changes in the patient's gamma globulin, in this case it is possible that glomerular, vascular, and interstitial changes were all part of an acute homograft rejection.

In other homotransplants sudden cessation of function after a period of

satisfactory diuresis has been reported. Some of these kidneys, all from patients who had received between 250 R and 450 R whole body irradiation, showed a predominantly hemorrhagic histological picture. A typical case was described by Murray in 1962. The patient was exposed to 210 R and 190 R of total body x-irradiation on successive days, and then received a kidney homotransplant from his brother. Function of the transplanted kidney was immediate, rapidly reducing the BUN from 225 mgm per cent to normal levels. At the fifth day a few red cells appeared in the urine. A biopsy of the transplant at the sixth day showed patchy interstitial hemorrhage, thrombosis of occasional afferent arterioles, but no infiltrating mononuclear cells. This progressed steadily to extensive tubular destruction and massive interstitial hemorrhage, and the patient died at the thirteenth day. No information was given about the presence or absence of plasma cells and other pyroninophilic cells in the lymphoid tissue at autopsy. These cases have been regarded as examples of "humoral" rejection by Murray. Hamburger has also postulated that pre-existing antibodies are the cause of failure in this type of case. There is no direct evidence for this, however, and this clinical and histological picture does not seem to have been seen except in association with treatment by large doses of ionizing radiation to the whole body. Before one hastens to incriminate immunological factors, the possibility of other causes should be very carefully considered in all such cases.

TRANSPLANT REMOVED BECAUSE OF HEMORRHAGE

When the kidney, which was donated by the recipient's sister (LD 49), was transplanted there were technical difficulties resulting in an ischemic period of 58 minutes. Flow of urine from the homotransplant did not begin for two hours, but after this there was a daily excretion of 2 to 3 liters. However, the urine was heavily contaminated with blood. Hematuria persisted, and even after three explorations no cause could be found. After four days the kidney had to be removed.

Examination of the gross specimen showed blood in the pelvis, but no bleeding point could be identified. Microscopically, there was some proximal tubular damage and many crystals of calcium oxalate in the lumina, but no evidence of blood or pigment in any of the nephrons. There was no interstitial edema, only minimal cellular infiltration, and no evidence of inflammatory changes in the pelvis of the transplant. In the peripelvic fat there was a band of hemorrhage with necrosis of several small arteries. At many tiny points the pelvic transitional epithelium was destroyed, and hemorrhagic oozing into the pelvis has occurred over a wide area. The cause of the disastrous bleeding in this case remains a mystery. A second transplant was not similarly afflicted.

TRANSPLANT EXAMINED AT HEIGHT OF POSTOPERATIVE DIURESIS

One patient (LD 26) died 12 hours after operation from hyponatremia and hyperkalemia during a massive postoperative diuresis in which urine output from the transplant averaged 1,320 ml per hour. The immediate cause of death was cardiac arrest (see Chapter 12). The kidney was a little swollen and pale, but showed a normal pattern on the cut surface.

Microscopically, the tubules and interstitium appeared to be normal, but some of the glomeruli were fibrosed and a few showed fibrous thickening of the capillary basement membranes associated with periglomerular fibrosis. There was hyalinization of the walls of occasional afferent arterioles. The glomerular changes in this kidney must have been present before transplantation. The alterations in the afferent arteriolar walls presumably were also present when the kidney was transplanted. It is interesting that no significant morphological changes were detected in the tubules.

Copious excretion of urine in the first 24 hours has been seen in many renal transplants, and at least two other patients have died because of electrolyte imbalance at this time. One was a chimpanzee heterotransplant in Hume's experience and the other a homotransplant in Murray's series.

TRANSPLANTS EXAMINED DURING OR JUST AFTER A REJECTION EPISODE

Fourteen of the Colorado homografts came from patients who were either at the peak of a rejection episode or were just beginning to recover from such an event.

Most of the homotransplanted kidneys were enlarged. The mean weight was 225 gm, with a range of 150 to 350 gm. Similar enlargement was encountered in a series of 22 kidney homotransplants examined at St. Mary's Hospital, London, where the mean was 242 gm, with a range of 180 to 350 gm. In all the cases this enlargement was almost entirely due to interstitial edema following damage to the peritubular capillaries in the acute rejection process. In one of the transplants (LD 10) that had functioned for 295 days in a bilaterally nephrectomized recipient, compensatory hypertrophy was responsible for part of the increase in weight (Fig. 150). The capsule was considerably thickened, but stripped easily leaving a smooth cortical surface (Fig. 150, top). In only one transplant (LD 24) was the capsule fairly adherent. In others the subcapsular surface was speckled with petechiae (Fig. 150, top), and similar hemorrhages were sometimes present in the swollen cortex.

In the St. Mary's experience more striking gross pathologic changes have been seen, including profound congestion of the medulla (Fig. 151). Another pattern, commonly seen in the St. Mary's patients who died during rejection but less frequently seen in the Denver series, was a band of hemorrhage and congestion at the corticomedullary junction of the swollen kidney. Some homografts in both groups showed tiny areas of infarction.

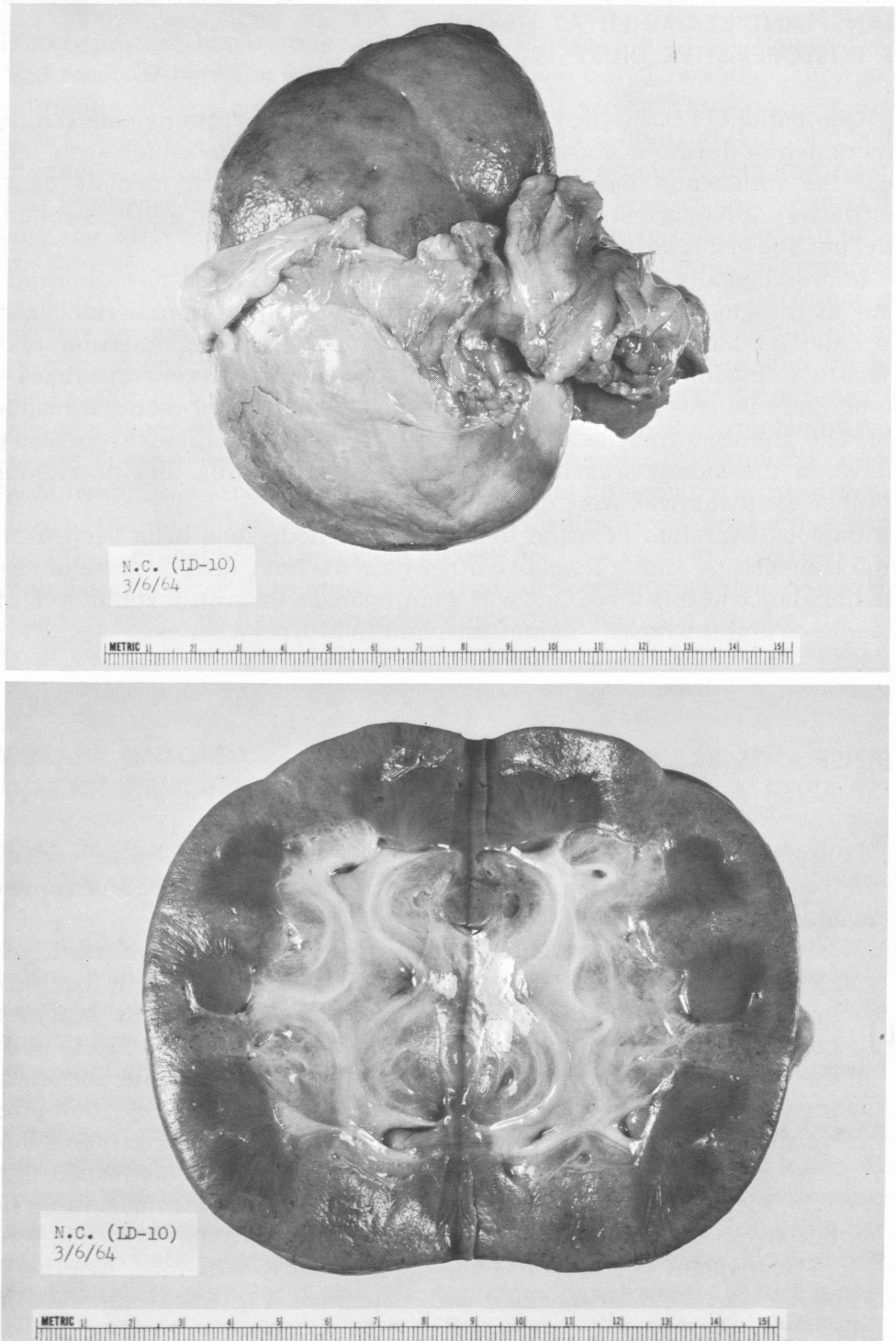


Figure 150. Homograft 295 days after operation in LD 10. The patient died of late rejection, after months of stable renal function.

Top—Partially stripped capsule. A few subcapsular petechiae are seen.

Bottom—Bisected kidney. The homograft weighed 260 gm, and is slightly swollen. (By permission of *Ann. Int. Med.* 61: September, 1964.)

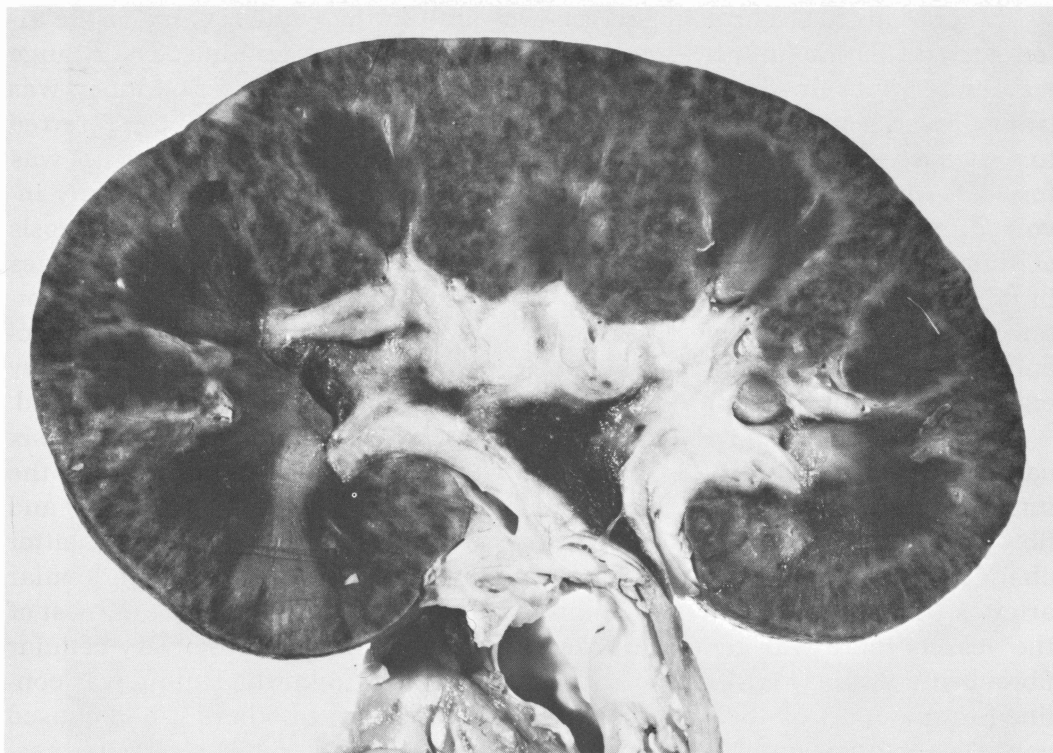


Figure 151. Cadaveric renal homograft (St. Mary's series) from a patient receiving 6-mercaptopurine, steroids, and actinomycin C. Two rejection episodes at the fifteenth and thirty-eighth post-operative days were successfully reversed, but the third, starting at the forty-fifth day, had not been reversed when the patient died at 62 days. The kidney was swollen, weighed 300 gm, and showed a characteristic speckling of the cortex with hemorrhages and congestion of the medulla.

Generally, these gross changes are the same as those commonly seen in both canine and human untreated renal homotransplants that are undergoing rejection.

In all the Colorado cases the main renal vessels were patent, and in all but two the ureters were unobstructed. The exceptions were the kidneys from LD 35 and LD 61. In LD 35 blockage of the ureter by debris during a rejection episode led to rupture of a necrotic area in the pelvis of the transplant, necessitating removal of the kidney at 20 days (see Chapter 12). The other patient (LD 61) was found after a very severe rejection episode to have a necrotic but not perforated area on the anterior surface of the pelvis and evidence of obstruction at the ureterovesical junction. The pelvic wall was reinforced with a piece of ascending colon and the ureter implanted into the bladder at a different site. When the patient died at 36 days (six days after ureteral reimplantation), there was no longer any obstruction, but the ureter and pelvis were dilated with thin walls. A similar, but more chronic, example of urine extravasation following rupture at the pelviureteric junction has been met in the St. Mary's series. In this patient a sac filled with urine was formed around the kidney by 116 days.

Microscopically, fibrinoid necrosis was seen in the walls of some of the arterioles and interlobular arteries in 12 of the 14 homotransplants. The change was most widespread and severe in CD 1, in whom a cadaveric kidney was transplanted from an A+ donor to an O+ recipient. This case has been referred to previously in Chapter 8. In the affected transplants the necrotic process was found in the afferent arterioles but not in the efferent ones, and usually involved the whole thickness of the vessel wall (Fig. 152, top). Fibrinoid necrosis of some of the glomerular tuft capillaries accompanied the arteriolar changes in the four most severe cases; CD 1, LD 10, LD 32, and LD 46 (Fig. 152, bottom; Fig. 153, top). Fibrinoid necrosis was not found in the vessels of the second transplant of LD 29, the homograft having been in the recipient for only eight days, nor in LD 28, in whom the exact period of rejection was ill-defined.

Swelling of the endothelial cells lining the arterioles was present in six cases, including case LD 28. Fibrin and platelet deposits on the intima of the interlobular arteries were seen in seven of the kidneys (Fig. 153, bottom) and fibrous intimal thickening of these vessels in seven (Fig. 154). This latter change was most severe in LD 46 in whom many of the arcuate and interlobular arteries within the graft showed obliterative changes (Fig. 155, top). In most of the vessels there was general thickening of the intima by loose, very cellular fibroblastic tissue (Fig. 155, bottom). In some, the intimal thickening was confined to only part of the circumference of the artery; in others it had caused complete obliteration of the lumen. Deposits of fat of variable size were present in the deeper layers of the thickened intima immediately adjacent to the media. Reduplication of the internal elastic lamina was present in four cases, and rupture of the same layer in two. The vascular lesions were more frequent and severe where arteries divided and gave rise to small side branches. Darmady recently obtained confirmation of this distribution of the lesions in human renal homotransplants by microdissection of the vessels. Thrombosis of occasional small veins was found in six of the transplants, with associated patchy infarction of the superficial cortex in three of these cases.

Cellular infiltration was present in all the cases except LD 28 and LD 61. It was never so severe as in the untreated dog kidneys, was usually very light and focal, and consisted of small lymphocytes, plasma cells, and larger cells with pyroninophilic cytoplasm as in canine renal homotransplants (Fig. 156). Often the cells were found only within peritubular capillaries; mitoses were not seen. The heaviest concentrations of infiltrating cells were found in Cases LD 35 (first transplant), LD 43, and CD 2.

Widespread recent tubular necrosis with active repair and casts of protein and cell debris was seen in four of the transplants (LD 29, LD 43, LD 32, and CD 1). Patchy tubular damage was present in most. Lymphocytes were found in the lumina of some tubules, but only in those cases with tubular damage and interstitial infiltration (Fig. 157). Presumably these are the cells that appear in the urine at the height of rejection (Fig. 158) and which were discussed in Chapter 15. A few oxalate crystals were present in six of these kidneys (Fig. 159).

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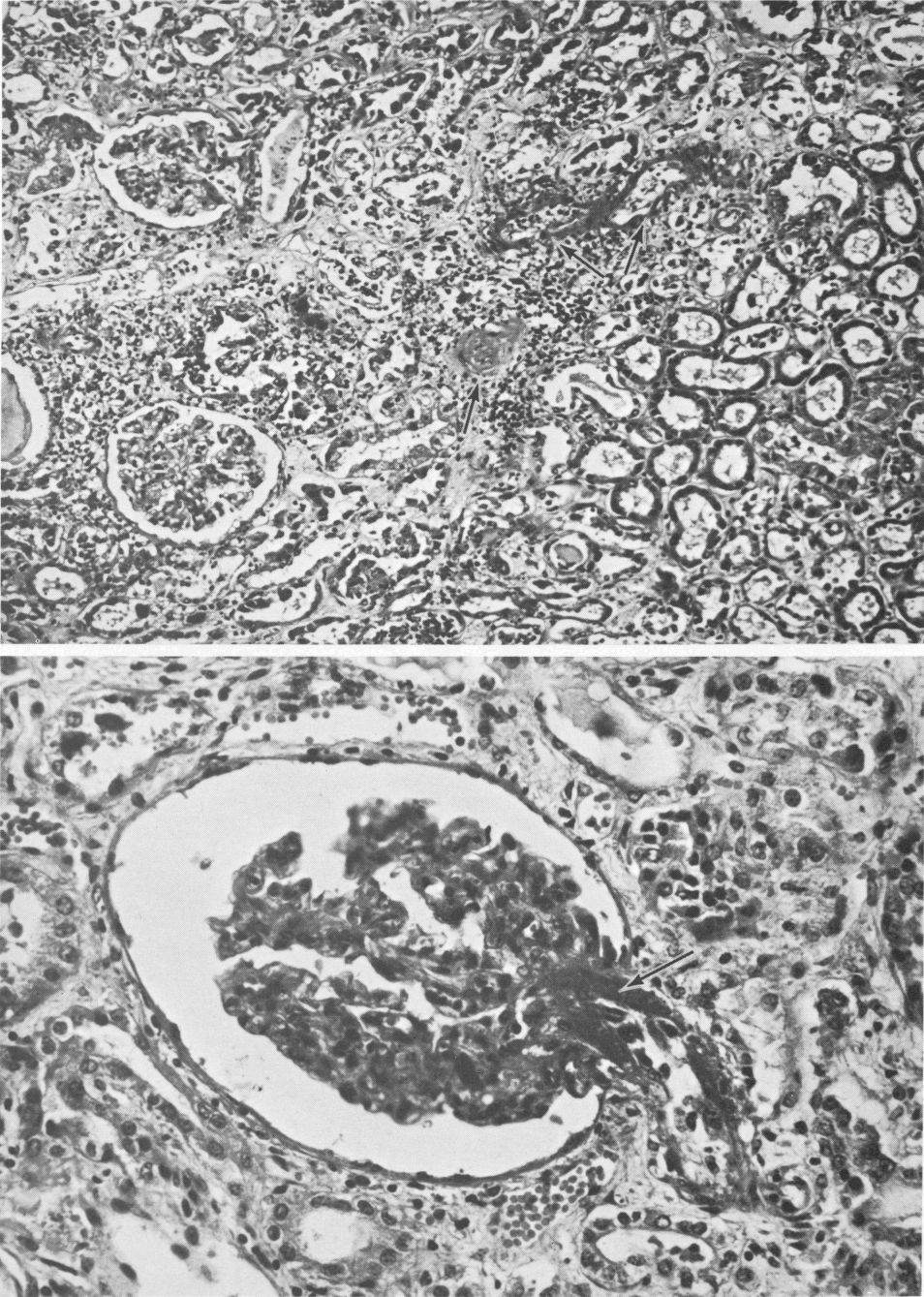


Figure 152. Top—Treated human renal homotransplant from Patient LD 43 who died at 38 days while in a rejection phase. There is severe fibrinoid necrosis of the wall of an interlobular artery (two arrows) and obstruction by fibrous intimal thickening of another interlobular artery (single arrow). There is also tubular atrophy, interstitial edema, and foci of cellular infiltration. H and E (X 125).

Bottom—Treated human cadaveric renal homotransplant from Patient CD 1 who died at 25 days while in a rejection phase. There is severe fibrinoid necrosis of the wall of an afferent arteriole (arrow) with extension of the same process into the tuft. H and E (X 340).

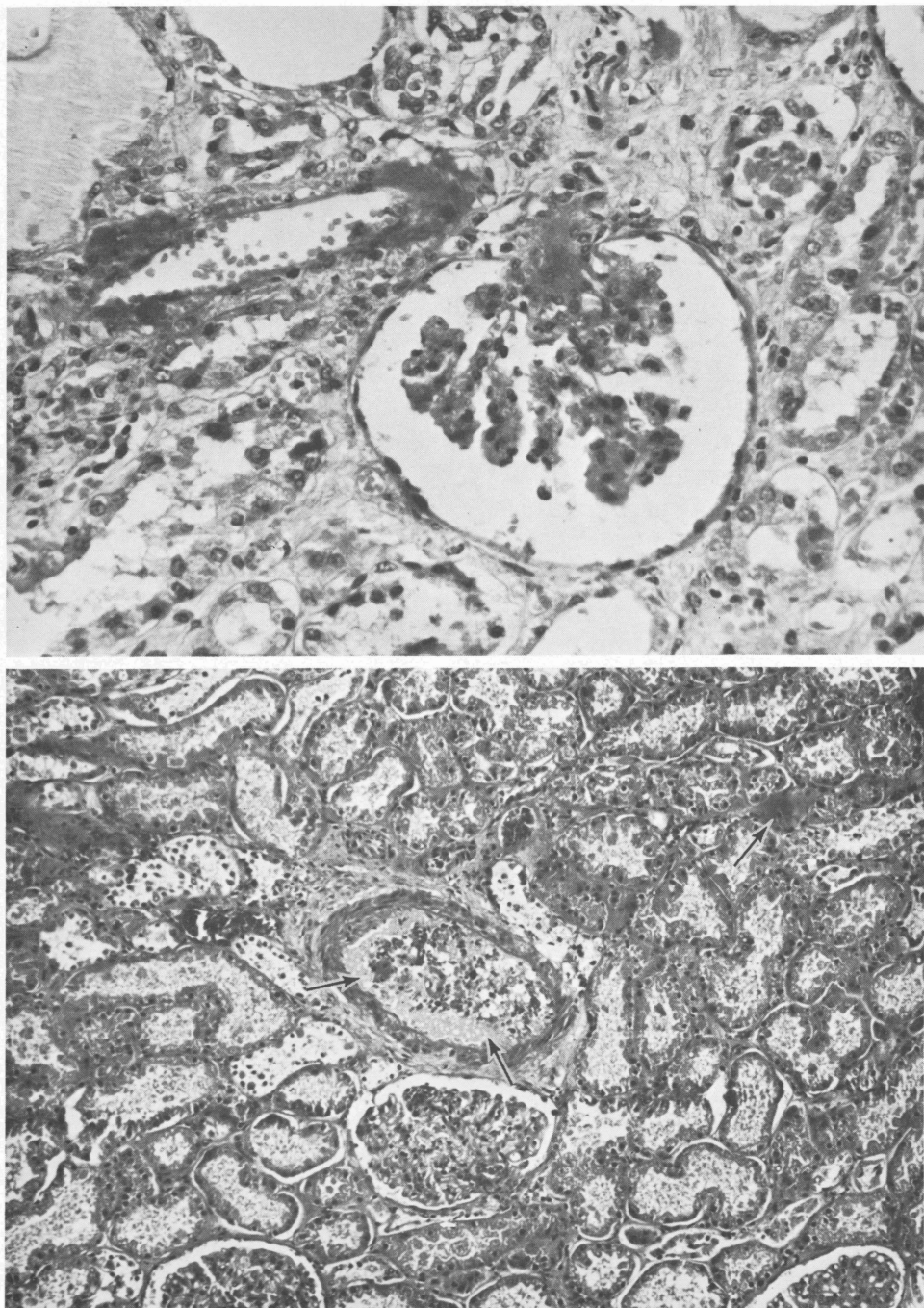


Figure 153. Top—Treated human renal homotransplant from Patient LD 10 who died at 295 days of late rejection. There is fibrinoid necrosis of part of the wall of an afferent arteriole. There is much tubular atrophy, interstitial fibrosis, and edema. H and E (X 300).

Bottom—Treated human renal homotransplant from Patient LD 11 who died at 25 days from sepsis while recovering from a prolonged rejection episode, during which anuria was present for 10 days (see Figure 5, Chapter 3). In the center of the photomicrograph there is an arcuate artery with platelets and fibrin deposited on the intima lining a damaged part of the vessel wall (two arrows). An arteriole with fibrinoid necrosis of its wall is indicated by one arrow. H and E (X 170).

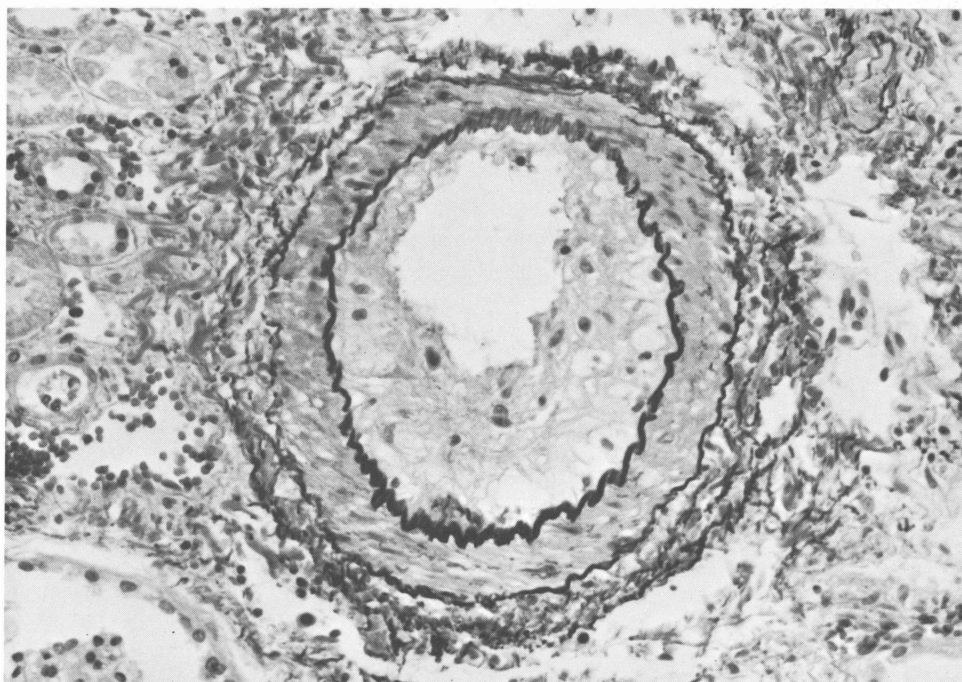


Figure 154. Treated human renal homotransplant from Patient LD 10 who died at 295 days of late rejection. There is marked fibrous intimal thickening in an arcuate artery. Elastic counterstained with hematoxylin and van Gieson (X 170). (By permission of *Ann. Int. Med.* 61: September, 1964.)

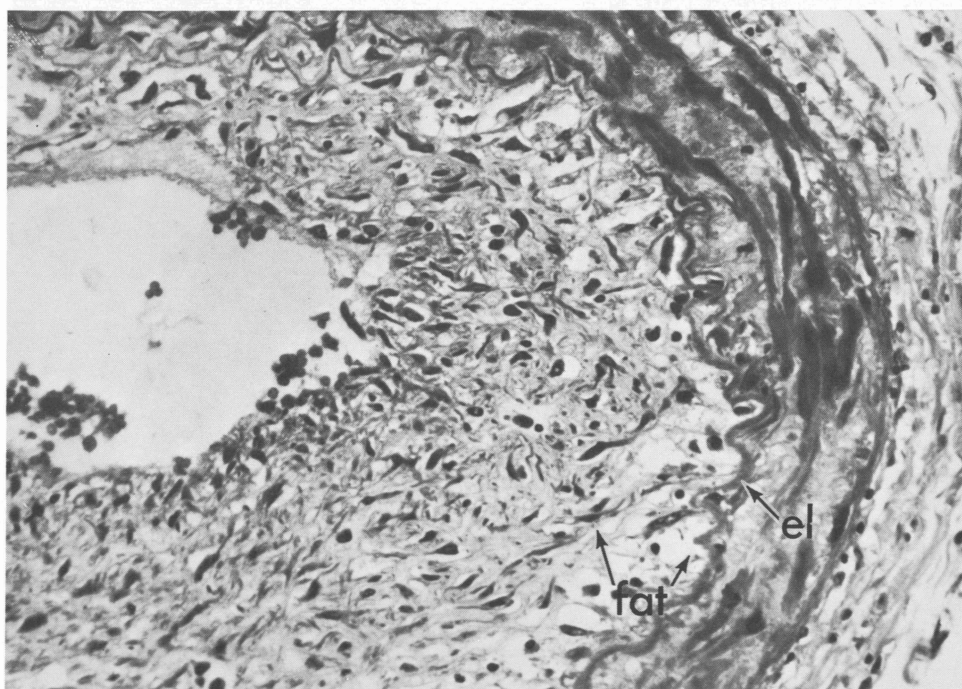
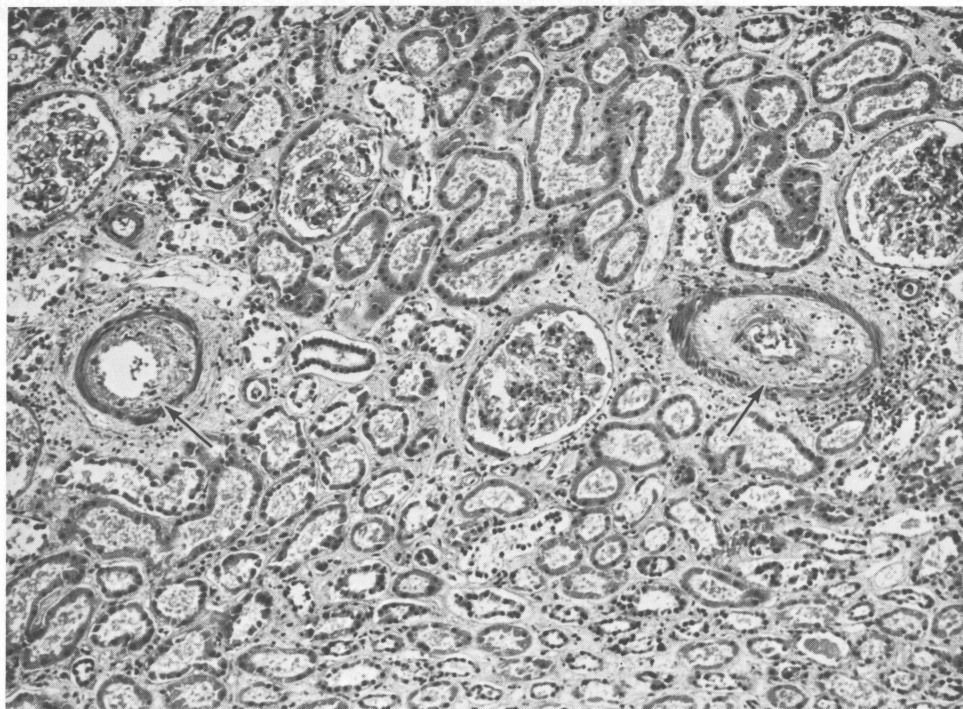


Figure 155. Top—Treated human renal homotransplant from Patient LD 46 who died at 43 days during a rejection crisis with anuria (see Figure 80, Chapter 18). Two interlobular arteries (arrows) are greatly narrowed by marked thickening of the intima by fibrous tissue. The interstitium shows edema, some fibrosis, and a scanty cellular infiltration. There is some tubular atrophy. H and E (X 125).

Bottom—Greatly narrowed arcuate artery in renal transplant from Patient LD 46. The intimal thickening is largely composed of fibroblasts with some infiltrating plasma cells. Adjacent to the internal elastic lamina (el) there are spaces (arrows) in which fat has been dissolved in the preparation of the section. These fat deposits are derived from the breakdown of platelets. H and E (X 300).

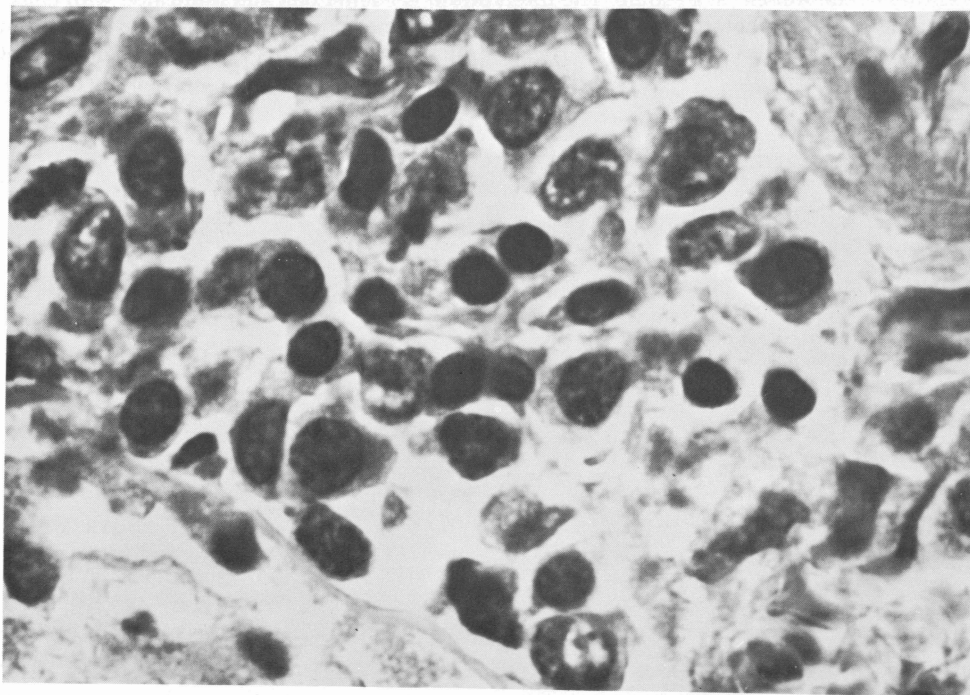
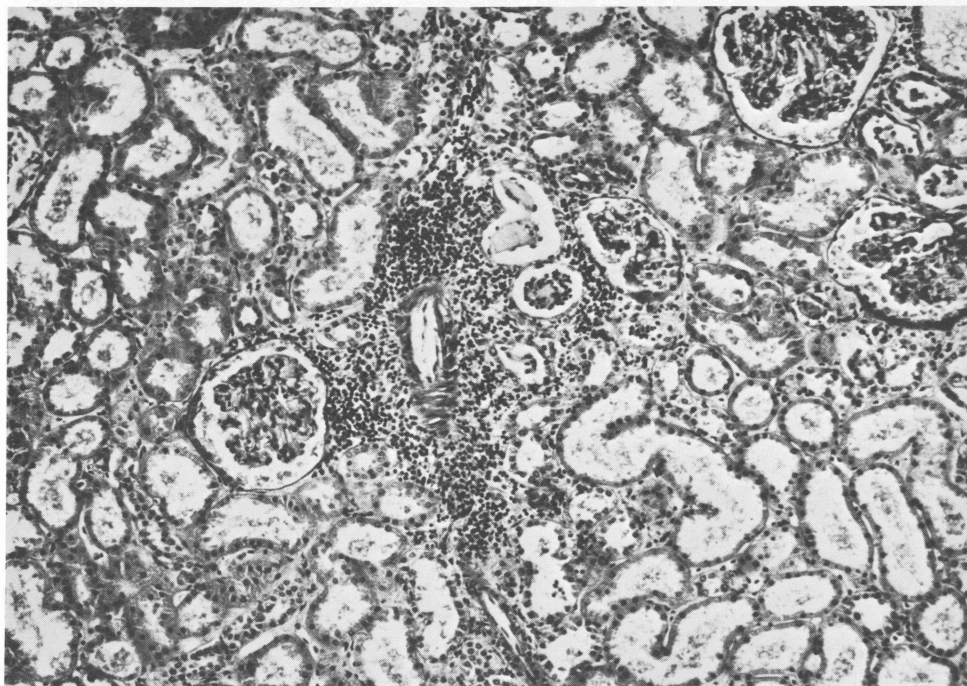


Figure 156. Top—Treated human renal homotransplant from Patient LD 35. The kidney was removed at 20 days because of rupture of the pelvis of the kidney following obstruction of the ureter by debris during a rejection phase. There is a heavy but focal infiltration of cells around an interlobular artery. H and E (X 125).

Bottom—A higher power view of the infiltrating cells (LD 35) in which many appear to be of the plasma cell series. H and E (X 800).

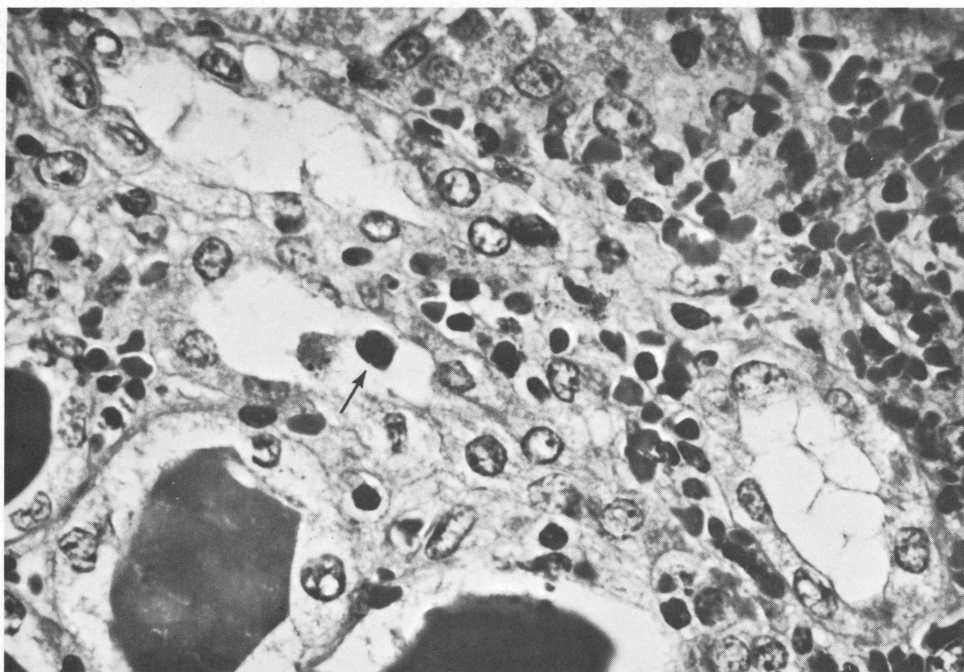


Figure 157. Treated human cadaveric renal homotransplant from Patient CD 2 showing a lymphocyte (arrow) in the lumen of a tubule. The interstitium is edematous and infiltrated with cells. At the upper right of the photomicrograph there are also some red cells. The tubular epithelium is damaged, and two tubules at the lower left contain protein casts. This kidney was actively being rejected. H and E (X 340).

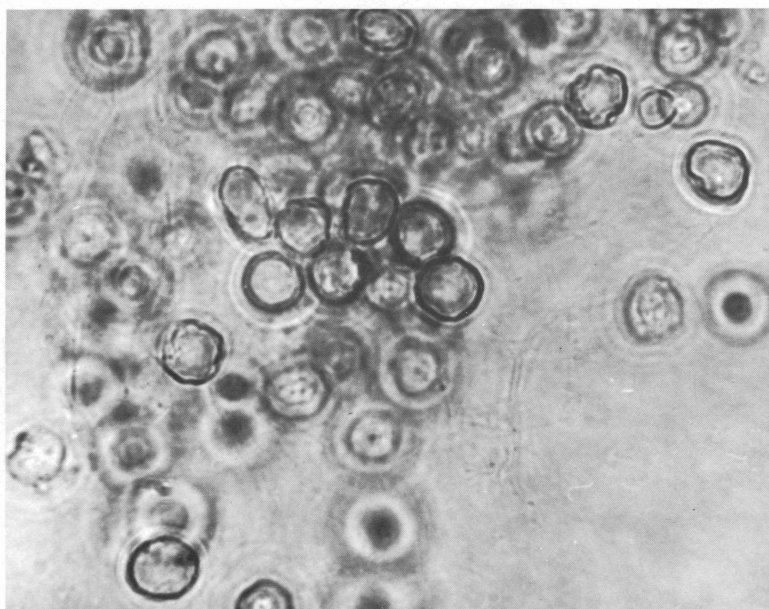


Figure 158. Cells seen in the fresh deposit from the urine of a patient in the St. Mary's series at the onset of a rejection episode. Almost every cell is a lymphocyte. Most were alive as shown by their ability to take up tritiated adenosine, and some exhibited characteristic lymphocytic movement. Phase contrast (X 450).

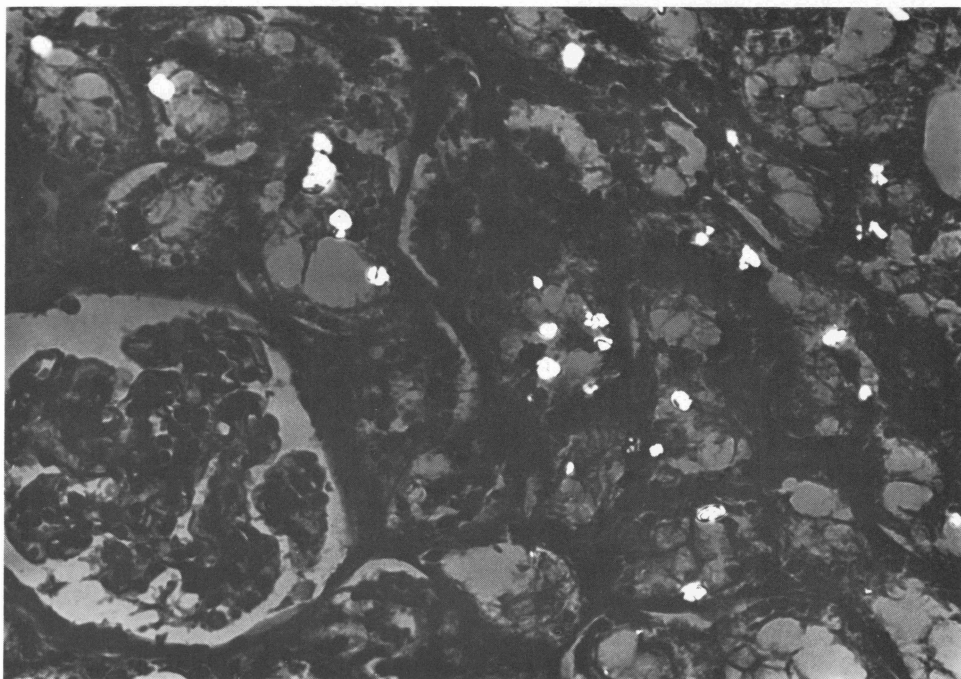


Figure 159. Birefringent oxalate crystals in the tubules of a human renal homotransplant. This patient (CD 1) was the most severely affected in the Denver series. Polaroid (X 350).

In addition to edema which was present in eight of the kidneys, there was some interstitial fibrosis in four of the five transplants which survived 36 days or longer. Small hemorrhages and collections of fibrin in the interstitium also accompanied arteriolar fibrinoid necrosis in these five cases.

Glomerular changes were not significant in these cases, but Merrill has noticed endothelial cell hyperplasia and proliferation in the tuft capillaries of one case during a rejection phase.

Monilial lesions were seen in two of the kidneys. In Patient LD 32, who had systemic fungus infection (see Chapters 19 and 21), the lesions consisted of necrotic areas filled with budding forms centrally and hyphae peripherally with no cellular response. In Case LD 43 fragments of hyphae were surrounded and partly engulfed by giant cells (Fig. 160).

Ureteric changes are common during a rejection episode. In the St. Mary's series edema and cellular infiltration of the wall were common, with endothelial swelling and fibrinoid necrosis of the small arteries and arterioles. Rupture of the internal elastic lamina in the affected vessels was frequent. Later in the process areas of focal infarction and hemorrhages into the ureteric interstitial tissues and the peripelvic fat were seen. Loss of the transitional-cell epithelium lining the damaged ureteric segments was usual. Fibrin and platelet deposition on the intima of affected vessels and organization of this material to fibrous tissue also occurred. These changes were present in LD 61. Similar find-

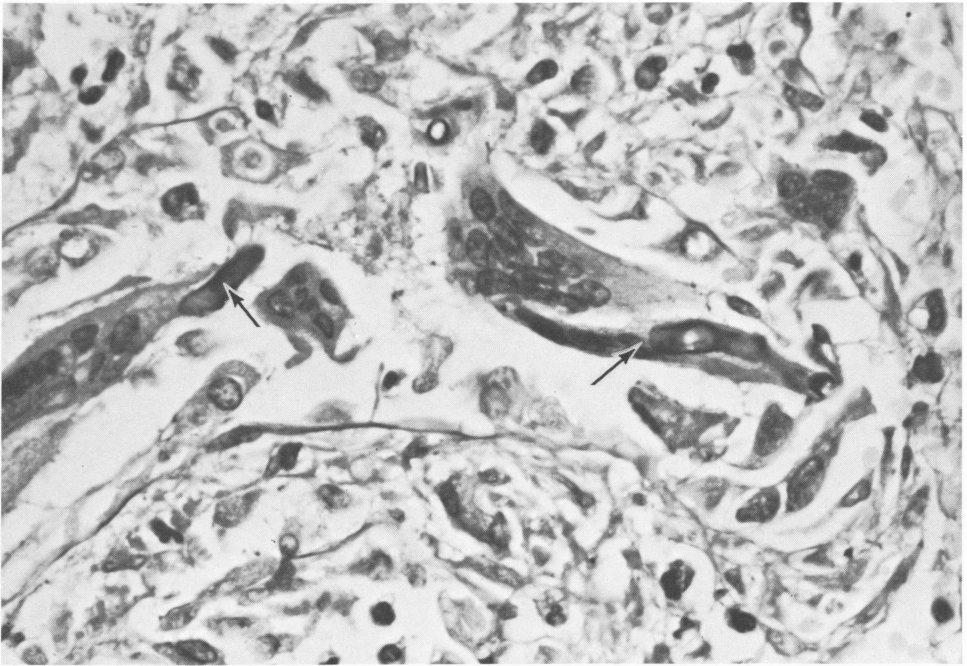


Figure 160. Treated human renal homotransplant from Patient LD 43 who died at 38 days. Two candida hyphae (arrows) are seen surrounded by foreign body giant cells. Periodic acid-Schiff (X 300).

ings were clearly illustrated by Küss in the renal homotransplant from their patient, who died at 490 days.

Early swelling of the afferent arteriolar endothelial cells and fibrinoid necrosis of the walls of both these vessels and the interlobular arteries are now recognized as important features of the acute rejection process in renal homotransplants modified by treatment. The longer the rejection episode lasts without being reversed by therapy, the more common it becomes to find fibrin and platelet deposits on the damaged endothelial lining of these vessels, and, later, fibroblastic intimal thickening with progressive narrowing of their lumina.

Vascular changes in a homotransplanted kidney were first described by Hume in 1955, and have since been mentioned in 18 other human renal homotransplants (Table 44). Similar lesions are seen in dogs. Analysis of the pathological changes in 200 canine renal homotransplants recently performed in collaboration with Calne and Zukoski showed that when the life of a kidney homotransplant was prolonged by immunosuppressive drug treatment, arterial and arteriolar lesions, which are relatively minor and late features of the unmodified graft, often came to dominate the histology. In the transplants which ceased to function before 28 days, disintegration of the peritubular capillaries, swelling of arteriolar endothelial cells, and fibrinoid necrosis of arterial and arteriolar walls were common. Fibrinoid necrosis was seen in the vessels of as many as 35 per cent of the renal transplants which functioned for 15 to 21 days

Text continues on page 336.

Table 44. Nineteen Cases of Human Renal Homotransplantation in Which Vascular Lesions Developed in Transplants

Reference	Case	Donor (sex, age, blood group)	Recipient (sex, disease, blood group, age)	Ischemia (min)	Treatment	Rejection Episodes (day of onset postop)	Function (days)	Vascular Lesions
Hume et al. 1955	G.W.	Cadaver 37	F M A+ A+	180	None	162	176	At autopsy fibrous intimal thickening of arteries.
Küss et al. 1962	Gen.	Unrelated 32	M O+ F O+	42	400r total body radiation; 200r to spleen; *6- MP, steroids	44	490	At autopsy fibrous intimal thickening of arteries; fibrinoid necrosis of vessels and glomeruli. First changes seen in biopsy at 57 days.
Hamburger et al. 1962	G.S. P.Y.	Dizygotic twin 37 Mother 50	M O+ F M B+ B+	40 38	460r total body irradiation 450r total body irradiation	15 None	960 (alive) 660	Biopsy at 8/12: "an intralobular artery with moderately marked fibrous endarteritis." Biopsy at 70 days: "the vessels showed a moderate fibrous endarteritis of non-specific type." No autopsy report.
Goodwin et al. 1963	D.M.	Mother 43	F B+ F B+	70	Nitrogen mustard; cyclophosphamide; prednisone	40, 88, and others	144	At autopsy: "marked arteriosclerosis and glomerulitis."
Parsons et al. 1963	I.	Cadaver 44	F A- M A+	133	Cyclophosphamide; local irradiation to spleen and graft site	25	33	At autopsy lesions affected whole arterial tree from renal artery to arterioles. Extreme intimal thickening of interlobular arteries and fibrinoid necrosis of arterioles and glomeruli.
Murray et al. 1963	T.G.	"Matson" kidney 11	M O+ M O+	45	Azathioprine; azaserine	22, 60, and 123	160	Arteritis seen in biopsy at 135 days. "Healed arteritis" at autopsy.

*6-mercaptopurine.

Table 44. Nineteen Cases of Human Renal Homotransplantation in Which Vascular Lesions Developed in Transplants (*continued*)

Reference	Case	Donor (sex, age, blood group)	Recipient (sex, disease, blood group, age)	Ischemia (min)	Treatment	Rejection Episodes (day of onset postop)	Function (days)	Vascular Lesions
Porter et al. 1963	484708	Cadaver 16	F AB+ Chr. GN 46	140	6-MP; acti.C; corticosteroids	15 and 38	59	Lesions first seen in biopsy at 41 days. Fibrous intimal thickening of arteries at autopsy.
	557629	Cadaver 52	M A+ Chr. PN 11	210	6-MP; acti.C; corticosteroids	15 and 38	62	At autopsy fibrous intimal thickening in whole renal arterial tree including main renal artery.
	580739	Cadaver	M Chr. PN	69	6-MP; acti.C; chlorambucil; azathioprine; corticosteroids	19 and 45	143	Lesions first seen in biopsy at 52 days. Fibrous intimal thickening of arteries at autopsy.
Németh et al. 1963	580594	Cadaver 48	M O+ Chr. PN 44	85	6-MP; acti.C; chlorambucil; azathioprine; corticosteroids	38	86	Lesions first seen in biopsy at 52 days. Fibrous intimal thickening of arteries at autopsy.
	I.	Brother 21	M A+ Chr. PN 26	64	150r total body irradiation; 200r to spleen; local irradiation to kidney	26	79	At autopsy severe fibrous intimal thickening of arteries; fibrinoid necrosis of vessels and glomeruli.
Merrill et al. 1963	M.D.	Cadaver 30	M O- Chr. GN 24	125	Azathioprine; acti.C; prednisone	29, 64, 99, and 222	450 (alive)	Biopsy at 141 days: "small artery shows narrowed lumen with subendothelial deposition of lipid."

Table 44. Nineteen Cases of Human Renal Homotransplantation in Which Vascular Lesions Developed in Transplants (*continued*)

Reference	Case	Donor (sex, age, blood group)	Recipient (sex, disease, blood group, age)	Ischemia (min)	Treatment	Rejection Episodes (day of onset postop)	Function (days)	Vascular Lesions
Porter et al. 1964	588813	Cadaver 40	F M O- A-	187	Azathioprine; acti.C; corticosteroids; local irradiation	24	51	Biopsy at 34 days showed fibrinoid necrosis of arteries and arterioles. At autopsy patchy infarction.
	584487	Cadaver 34	M F O+ O+	180	Azathioprine; acti.C; corticosteroids; local x- irradiation	36	54	Biopsy at 36 days showed fibrinoid necrosis of arterioles and occasional tufts. Biopsy at 44 days fibrin and platelet intimal thickening. At autopsy intimal fibrous thickening of arteries with elastic rupture.
Kincaid- Smith 1964	1.	Cadaver			Azathioprine; acti.C; prednisone			Arteriotar fibrinoid necrosis at 7 days.
	2.	Cadaver			Azathioprine; acti.C; prednisone	28		Fibrinoid necrosis of vessels at 28 days. By 40 days widespread vascular and glomerular lesions.
Dempster et al. 1964	1.	Cadaver 55	F	350	"Antimetabolic drugs"	28		"Strange fibrinoid necrosis" of vessels.
Hopewell et al. 1964	3.	Father 54	M M O+ O+		6-MP; prednisolone	10 and 35	49	Fibrinoid necrosis of some glomerular capillary loops. Intimal thickening of medium sized and small arteries.

(Fig. 161, top). From the twenty-eighth day arteriolar narrowing and arterial intimal thickening became increasingly frequent. Deposits of fibrin and platelets on the endothelium of the interlobular and larger arteries were often seen in the transplants surviving up to 70 days; after this fibrous intimal thickening was commoner, affecting 64 per cent of the transplants surviving beyond 70 days (Fig. 161, middle). There was apparent transitions between the fibrinous and fibrous lesions, and rupture of the internal elastic lamina was also common (Fig. 161, bottom).

In seeking an explanation for these arterial changes a number of possibilities can be excluded. Neither x-irradiation, corticosteroids, nor purine analogues can be responsible, because they have not always been used in the treatment of patients who have shown such lesions. Indeed, no *one* drug or agent is common to all the cases that have been reported.

Total renal vascular occlusion for periods exceeding two hours can cause severe damage to arteries and arterioles, and in the repair process intimal hyperplasia is prominent. Although some of the homotransplants that showed vascular lesions were ischemic for long periods, this was not a factor in all cases. For example, in the Denver series, the kidney transplanted into patient LD 46 was ischemic for only 31 minutes, and yet during an uncontrolled rejection episode developed the most severe arterial lesions seen in this group of patients (Fig. 155).

Hypertension seemed the most likely explanation to Hume. Admittedly many of the recipients of renal transplants have been hypertensive, and the view has to be considered that all these changes occur because the vessels of the transplant, accustomed to a normal blood pressure, have suddenly been exposed to a considerably higher pressure. However, vascular lesions have occurred on many occasions when the recipient's blood pressure has been carefully controlled with hypotensive drugs and in several cases when the recipient was not hypertensive at all at the time of transplantation. While it thus seems clear that vascular lesions are not initiated by a raised blood pressure, any existing hypertension will probably further damage the weakened vascular walls. When hypertension emerges late in the course of a renal homotransplant, this often appears to have been due to ischemia caused by the obstructive arterial lesions.

If the arterial changes are not initiated by hypertension, an immunological cause becomes a strong possibility. Certainly vascular damage of this type has not been reported in kidneys transplanted between identical twins. It could be that in patients receiving renal homotransplants chronic uremia and treatment depress but do not halt the immune reaction by the host against the transplant. If this is so one could expect to find histological changes similar to those seen in acute canine renal rejection, but modified and spread over a longer period, the duration and severity being dependent upon the efficacy of the treatment and the genetic relationship between donor and recipient. In general this prediction appears to be correct. Practically all human renal homotransplants surviving more than a few days have shown some degree of infiltration by cells resembling

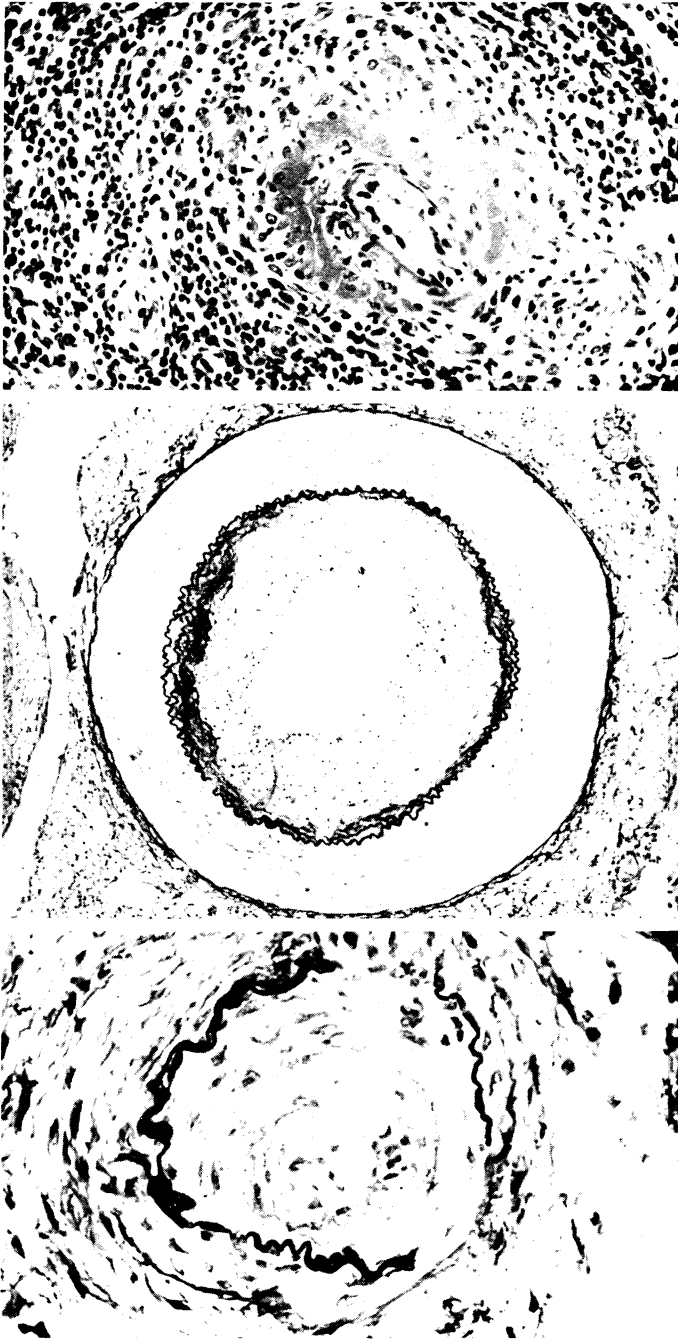


Figure 161. Top—Vascular changes in canine renal homografts. Renal homotransplant at 19 days from a dog treated with azathioprine and actinomycin C. There is fibrinoid necrosis of the whole wall of an interlobular artery, and the surrounding tissues are heavily infiltrated by cells. H and E (X 180).

Middle—Renal homotransplant at 739 days from a dog treated with 6-methyl mercaptopurine. An interlobular artery shows diffuse intimal thickening by fibrous tissue. This material was kindly provided by Doctor C. Zukoski.) Elastic counterstained with hematoxylin and van Gieson (X 30).

Bottom—Renal homotransplant at 330 days from a dog treated with azathioprine and actinomycin C. An interlobular artery shows marked fibrous intimal thickening which has almost obliterated the lumen of the vessel. The internal elastic lamina has been ruptured. Elastic counterstained with hematoxylin and van Gieson (X 160).

those seen in the early stages of dog homografts. The infiltration has been **maximal** in those cases in which chronic uremia and treatment were lacking or genetic disparity was combined with ineffective treatment; and **minimal** in those cases in which close genetic similarity was combined with vigorous treatment.

It will be remembered that in the later stages of renal homograft rejection in a normal untreated dog the capillaries, arterioles, and arteries may show fibrinoid necrosis of the whole or part of the circumference of their walls and that some evidence was produced to support the thesis that these lesions may be due to circulating antibody.

An extension of this hypothesis would be that with continued persistence of the graft in the host the humoral aspect of the host's response assumes greater importance in that during a rejection episode circulating antibody is rapidly produced. The resulting antibody-antigen reaction occurring in or on the arteriolar walls would damage the intima and cause swelling of the endothelial cells, and might induce spasm of the smooth muscle in the vessel wall. Such lesions, widespread in the transplant, could explain the alterations in water, electrolyte, and creatinine excretion that occur at this time. The similarity between some of the functional changes observed during acute rejection of a renal homograft and those which can be induced by a constricting clip on the renal artery has been commented upon in Chapter 15. That it is possible to abolish the arteriolar endothelial swelling and to reduce vascular spasm in canine renal transplants by giving cortisone, as Demptster has shown, might explain why prompt treatment with prednisone has been so successful in inducing a diuresis from human renal transplants that have shortly before suddenly ceased to function.

Halting the rejection process at the stage of endothelial swelling and probably even at that of fibrinoid necrosis of arterioles should lead to complete restoration to normal. Once the damage has involved the interlobular arteries and intimal change has induced deposition of platelets and fibrin, ending the rejection episode will not prevent healing with replacement of the intimal deposits by fibroblasts. This progression has been clearly demonstrated in the St. Mary's experience by successive biopsies taken from a human renal homotransplant during and after treatment of a severe rejection episode. The deposition of platelets on the damaged arterial segments is probably due to release from the vessel wall of substances with properties similar to adenosine di- and triphosphate which, in high concentrations, have been shown by Honour and Mitchell to induce clumping of the platelets and their adherence to the intima.

When the vessel wall has been appreciably weakened by the necrotic process, compensatory intimal thickening will contribute substantially to narrowing of the lumen. This aspect of the process is comparable to the rapid laying down of fibroblastic tissue on the intima (endarteritis fibrosa) in malignant phase

hypertension. If the necrosis is initially widespread in the larger arteries, this healing process can produce crippling obliterative fibrous lesions like those that have been seen in both humans and dogs. The breakdown of the many platelets in the early intimal deposit accounts for the fat spaces later found in the deeper parts of the thickened fibrous layer, because 1 per cent of the dry weight of platelets consists of lipid.

When there is extensive fibrinoid necrosis involving the media, rupture of the internal elastic lamina is likely to occur. During healing, this elastic damage is not repaired and, just as in healed polyarteritis nodosa, the fragmented elastic persists indefinitely as evidence of the earlier destructive phase.

The vessels of the transplanted ureter are not immune from these processes. Fibrinoid necrosis of the small arteries and arterioles is followed by focal infarction of the ureteric wall. If at the same time the ureter distally becomes blocked by debris due to tubular destruction resulting from the intrarenal vascular changes, then rupture of the necrotic ureteric or pelvic wall may occur. This was the mechanism in LD 35.

TRANSPLANTS EXAMINED LONG AFTER REVERSAL OF A REJECTION EPISODE

Eight of the Colorado renal homotransplants were from patients whose last clearly recognizable rejection episode had occurred 14 to 117 days previously. Two of these patients had survived two rejection episodes, and in one case (LD 9) three such crises had been encountered. A ureter was also available for examination from Patient LD 27.

All the kidneys were enlarged, the mean weight being 202 gm, with a range of 180 to 230 gm. Unlike what occurred in those patients who died during or immediately after a rejection episode, edema was not an important factor in this increase in weight. All these kidneys had been for long periods (48 to 207 days) in bilaterally nephrectomized recipients, and in several compensatory hypertrophy was the main cause of the enlargement; this finding is not surprising since Couch has demonstrated that dogs with a solitary autotransplanted kidney, in which there is no question of an immune mechanism, develop increases of up to 55 per cent in the weight of the transplant in one year. In all the clinical cases the capsule was thickened and fibrous. Most of the transplants had a smooth subcapsular surface, the cortex was pale, and the medulla was reddish-brown. The only exception was Case LD 19 in whom there was an iatrogenic superior polar infarct (see Figure 47, Chapter 11). In Patient LD 4, in whom vena caval plication had been performed following pulmonary embolism (see Chapter 12), the renal and right iliac veins and the lower inferior vena cava were completely thrombosed, but in the other seven cases the renal vessels and their anastomoses were free from any obstruction. Similar relatively normal gross findings have been reported from other centers and do not differ greatly from those shown in Figure 150.

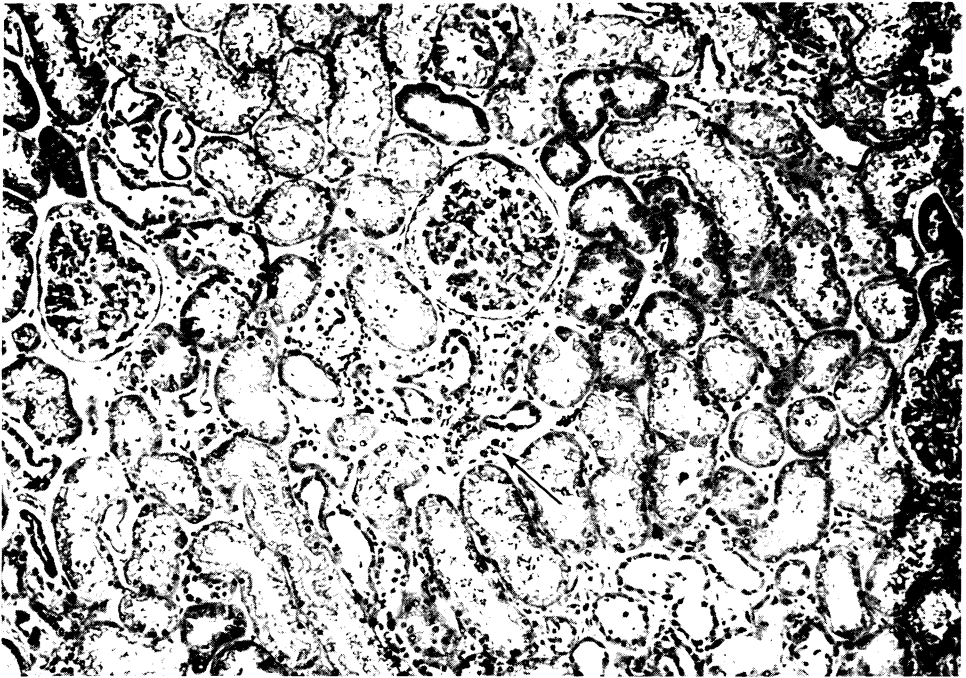


Figure 162. Treated human renal homotransplant from Patient LD 21 who died at 76 days from a fungal pneumonia and brain abscesses. A fairly severe rejection episode had lasted from the fifth to the thirty-fifth days. There is minimal cellular infiltration; one small group of cells is indicated by an arrow. The interstitium is a little more prominent than normal because of some focal tubular atrophy. H and E (X 125).

Microscopically, seven of the eight cases showed cellular infiltration. This was usually very mild and focal. Often the cells were confined to the peritubular capillaries. A typical case is illustrated in Figure 162. Most of the cells were lymphocytes and mature plasma cells, but there were a few other more primitive cells with pyroninophilic cytoplasm and nucleoli. Mitoses were never seen. Similar but usually much heavier cellular infiltrates have been described in biopsies taken from long-surviving renal homotransplants in other series. Galle and Montera made a detailed electronmicroscopic study of the cells present in one such kidney 70 days after transplantation at a time when the patient's blood urea was 25 mg/100 ml and there was only 0.1 gm of proteinuria per 24 hours. They found that about 40 per cent of the cells were not easily classified: ultrastructurally the majority of these were related to lymphocytes, while some resembled histiocytes (monocytes). These cells had in common similar size and scanty cytoplasm with sparse ergastoplasm and many free ribosomes. These are the same two categories of cells that are found infiltrating canine renal homotransplants at 48 hours (see Figures 140 and 144). A further 4 per cent were plasma cells in varying stages of maturity; 15 per cent were typical lymphocytes; 8 per cent were histiocytes; 26 per cent were fibroblasts associated with collagen fibers; 4 per cent were fixed reticular cells; and 3 per cent were eosinophils. It seems probable that if a similar analysis had been

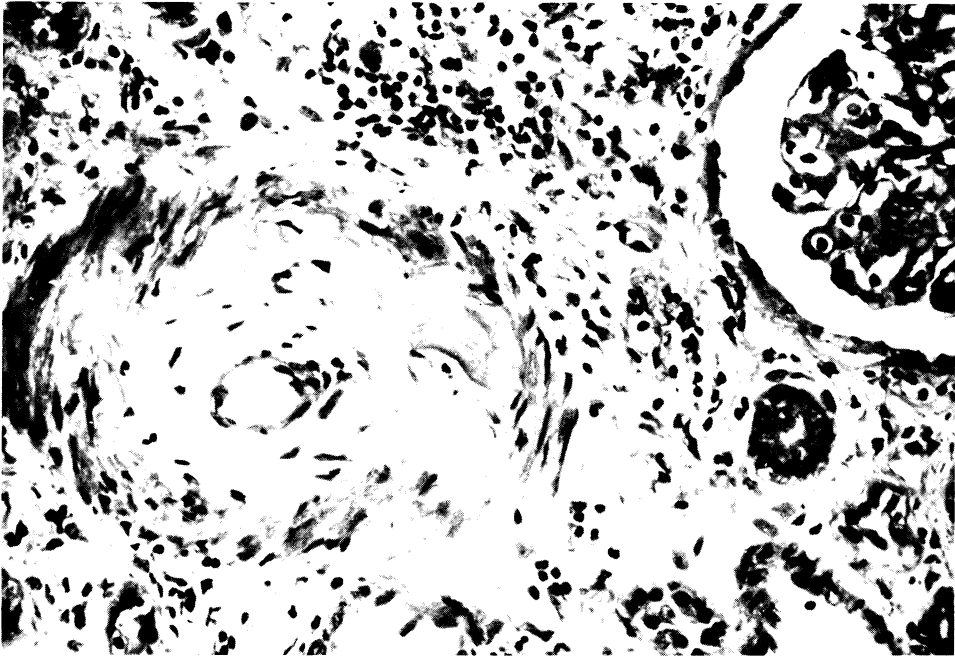


Figure 163. Treated human renal homotransplant from Patient LD 16 who died at 83 days from pancreatitis. The patient had experienced two rejection episodes, the first at the fifth day and the second at the twenty-fifth day, both apparently successfully reversed. A partly obliterated interlobular artery can be seen as well as a focus of cellular infiltration. H and E (X 300).

done on some of the Colorado cases more plasma cells would have been recorded, and far fewer of the unclassifiable "lymphoid and histiocytic" cell types.

Fibrinoid necrosis was present in the arteriolar walls of two of the eight transplants. Only in case LD 38 were an appreciable number affected, and it is perhaps significant that this patient had recovered from a rejection phase only 14 days before the transplant was examined. The characteristics and distribution of the vascular necrotic lesions were the same as in the kidneys which were examined during a rejection phase.

Four of the kidneys, however, showed intimal thickening of some of the interlobular arteries. In one the new intimal layer consisted only of fibrin, platelets, and a few lymphocytes; in the other three both this type of thickening and fibrous thickening were present (Fig. 163). Reduplication or rupture of the internal elastic lamina accompanied these changes.

Tubular damage was present in six of these kidneys. In some it was recent necrosis affecting predominantly the proximal convoluted tubules and probably related to ischemia from terminal hypotension in patients dying from such causes as pneumonia and septicemia. In others there were atrophic changes, the tubules being lined by flattened epithelium. Protein casts and crystals of calcium oxalate were present in three of these transplants. Where there was some patchy tubular atrophy the interstitium was prominent and fibrous. All

the tubular and interstitial changes were most pronounced in the transplant which functioned for 207 days (LD 9). Two of the cases showed some hyaline thickening of the basement membranes of the capillaries forming the glomerular tufts.

The very slight cellular infiltration and the paucity of severe obliterative vascular lesions in these eight patients, all of whom had suffered at least one clinically obvious rejection episode, is most encouraging. In effect, it means that if rejection is recognized promptly and treated, even by the imperfect drugs which are at present at our disposal, at least 50 per cent of the patients will suffer no permanent arterial or arteriolar damage.

The presence of some atrophic tubules in most of these transplants suggests either that tubular damage is occurring from the use of high dosages of drugs such as actinomycin C, which is suspected of nephrotoxicity, or more probably that peritubular capillaries continue to be damaged on a small scale by host cells, resulting in focal loss of tubules. Some of the tubular damage is probably corrected by regeneration, and most of these kidneys do in fact show evidence of continuing tubular repair.

Only two of the Denver transplants showed some thickening of the glomerular tuft capillary basement membranes. Much more severe lesions have been encountered by Hamburger (1963) in two human renal homotransplants seven and 15 months after operation. Similar changes developed in some of the transplants in the long-surviving dogs treated with immunosuppressive drugs (Fig. 164). The canine lesions were thought to have resulted from a combination of ischemia and deposition of antigen-antibody complex, giving a histological picture reminiscent of late Ellis Type II (membranous) glomerulonephritis. The renal lesions in Hamburger's cases were associated with splenomegaly and hypergammaglobulinemia—further circumstantial evidence of a continuing host-against-graft immunological reaction with production of circulating antibody.

These lesions must be clearly distinguished from the glomerular changes encountered by Pfeiffer and Merrill in some of the kidneys transplanted between identical twins. Of the 25 or more renal transplants that have been performed between identical twins, at least five of those who originally suffered from glomerulonephritis are said to have developed this disease in the transplants. In these cases, as in the two instances mentioned earlier, in which glomerulonephritic changes developed in anuric homotransplants, there may have been transmission of host disease to the transplant.

The ureters of homotransplanted kidneys which have successfully survived a rejection episode often show thin fibrotic walls no longer lined by transitional epithelium. There may be severe intimal fibrous thickening of the arteries and rupture of the internal elastic lamina. These changes were very prominent in the ureter removed from LD 27, a patient whose only rejection episode had been clinically reversed many months previously. Following severe diarrhea she had become anuric, and exploration revealed a thickened, swollen ureter with petechial hemorrhages and a greatly narrowed lumen 2 cm from the ureteropelvic junction. Microscopically, in addition to the vascular changes, there was much muscle necrosis, a heavy cellular infiltration, and virus inclusion bodies.

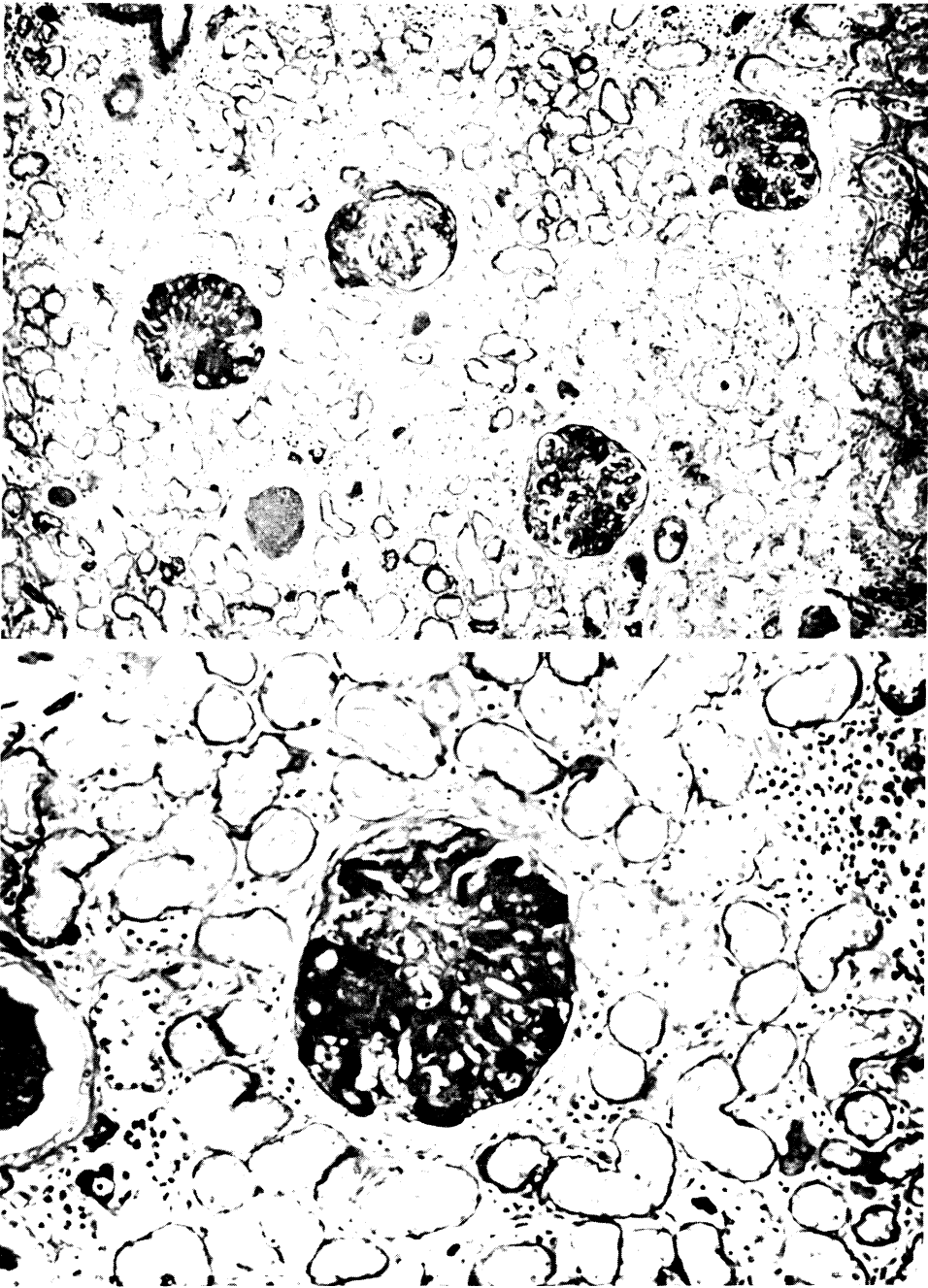


Figure 164. Top—Renal homotransplant at 739 days from a dog treated with 6-methyl mercaptopurine. Four glomeruli show thickening of their tuft capillary basement membrane by deeply PAS-positive material. There is some tubular atrophy, interstitial fibrosis, and foci of cellular infiltration. This animal also had severe obliterative vascular lesions (see Figure 161, middle). Periodic acid-Schiff (X 125).

Bottom—Higher power view. The marked thickening of the basement membranes of the capillaries in the glomerular tuft is well shown. This material may be antigen-antibody complex. Periodic acid-Schiff (X 200). (By permission of *Lab. Invest.* 13:809, 1964.)

TRANSPLANTS FROM PATIENTS WHO HAD NOT DEVELOPED CLINICAL EVIDENCE OF A REJECTION EPISODE

There were three homotransplants from patients who, up to the time of their death, had shown no clinical signs and symptoms to indicate that they were rejecting their transplants.

The first patient, a man of 50, received a homograft with two renal arteries from an unrelated donor. During the operation cardiac arrest developed just before the arterial anastomoses were completed. Resuscitation was successful, but the transplant was ischemic for 71 minutes, and urine excretion was delayed for 12 hours. After this, the kidney excreted 2 to 4 liters per day, but the patient died from mediastinitis and generalized sepsis on the tenth postoperative day (see Figure 61, Chapter 14). The transplant weighed 210 gm, and was pale and swollen. Microscopically, there was widespread tubular necrosis which was healing. Many of the tubules were lined by flattened epithelium. Mitoses in epithelial cells were frequent, and some of the tubules contained protein, pigment, and blood casts. Within the peritubular capillaries cells with pyroninophilic cytoplasm were marginating, and probably represented the beginning of a homograft reaction. The interstitium was edematous, and contained a few hemorrhages.

The second transplant came from a patient (LD 35) whose primary renal homotransplant had been removed at 20 days because of rupture of the pelvis following obstruction to the ureter during a rejection phase (see Chapter 12). Another transplant from the patient's sister was placed in his left iliac fossa and was still functioning perfectly when the recipient died from septicemia at nine days. Grossly this second kidney appeared normal, but microscopically there was some interstitial edema and a few pyroninophilic cells within occasional peritubular capillaries.

In the last case (SD 3), a pair of baboon renal heterotransplants had been removed, and at the same operation they had been replaced by a homotransplant from a living unrelated donor. The homotransplanted kidney functioned well with a diuresis of 8 liters in the first 24 hours. The BUN rapidly fell and never again exceeded 52 mgm per cent; the creatinine clearance rose, and at no time did it fall below 65 ml per minute. There was no evidence of rejection, but at the thirty-eighth day, when the transplant was still functioning perfectly, the patient died of pneumonia (see Chapter 23). The kidney was not swollen, and microscopically some of the interlobular arteries were greatly narrowed or even completely blocked by intimal fibrosis while others showed fibrin or platelet deposits on the intima; there was also fibrous obliteration of a number of the afferent arterioles. The areas of damage tended to be wedge-shaped as in chronic pyelonephritis, but there was no cellular infiltration in these areas nor periglomerular fibrosis, and the tubules were not dilated and did not contain protein casts. It seemed most probable that these scarred areas were due to healing of damage done during a clinically unrecognized rejection phase, rather than to infection.

An additional patient (LD 4), who had very equivocal evidence of rejection, was considered to be in the preceding category of patients long past rejection.

RENAL HETEROTRANSPLANTS

In the years before 1925 a number of renal heterotransplants were performed between various species, but little useful information was recorded about the histology (see Chapter 23). The two heterografts of feline kidneys to dogs, which according to Avramovici survived for 49 and 58 days, were not examined microscopically. Since that time the only heterotransplants recorded have undergone progressive vasoconstriction within a few minutes after completion of the anastomosis, and when removed after a few hours have shown very much the same histological changes as were seen in the two human renal homotransplants (LD 19 and LD 23), which failed because of blood group incompatibility.

During recent months six baboon to man renal heterotransplants (see Chapter 23) have become available for examination, and the pathological changes found in a pair of rhesus monkey kidneys and in two chimpanzee kidneys after transplantation into humans have been reported by Reemtsma and his colleagues (See chapter 23), who kindly allowed us to examine the transplanted tissues.

CHIMPANZEE-TO-MAN RENAL HETEROTRANSPLANT

A compound chimpanzee heterograft was examined. The recipient, a 43-year-old man suffering from chronic glomerulonephritis, was treated for one week prior to the transplant and for the remainder of his life with azathioprine, actinomycin C, and steroids. The donor, a 41 kgm male chimpanzee, was blood group A_{1,2}cD M; the patient was group A₁ccDee M. Following transplantation, by Reemtsma and his colleagues in New Orleans, there was an initial diuresis, but on the fourth day a rejection episode occurred which was treated with increased doses of immunosuppressive drugs and three doses of 200 R irradiation to the kidney at three-day intervals. Improvement was rapid, and function was soon restored to normal. A second rejection crisis occurred during the fourth postoperative week following a period during which azathioprine therapy had been discontinued. Restoration of drugs and further irradiation of the transplant apparently completely reversed the rejection process. Later the patient developed progressive pulmonary infection and hypokalemia. His death at 63 days was preceded by 36 hours of shock, necessitating the use of vasopressors.

At autopsy the transplanted kidneys showed tubular necrosis with repair. Many of the tubules were lined by flattened epithelium, and some contained casts of blood and protein (Fig. 165). There was interstitial edema and fibrosis, but no cellular infiltration and no glomerular or blood vessel changes.

The lack of any histological stigmata of rejection in these chimpanzee kidneys is most striking when it is remembered that in eight comparable human homotransplants, i.e., those in which the last rejection episode had been 14 to 117 days previously, seven showed cellular infiltration, and in four there was intimal thickening of interlobular arteries.

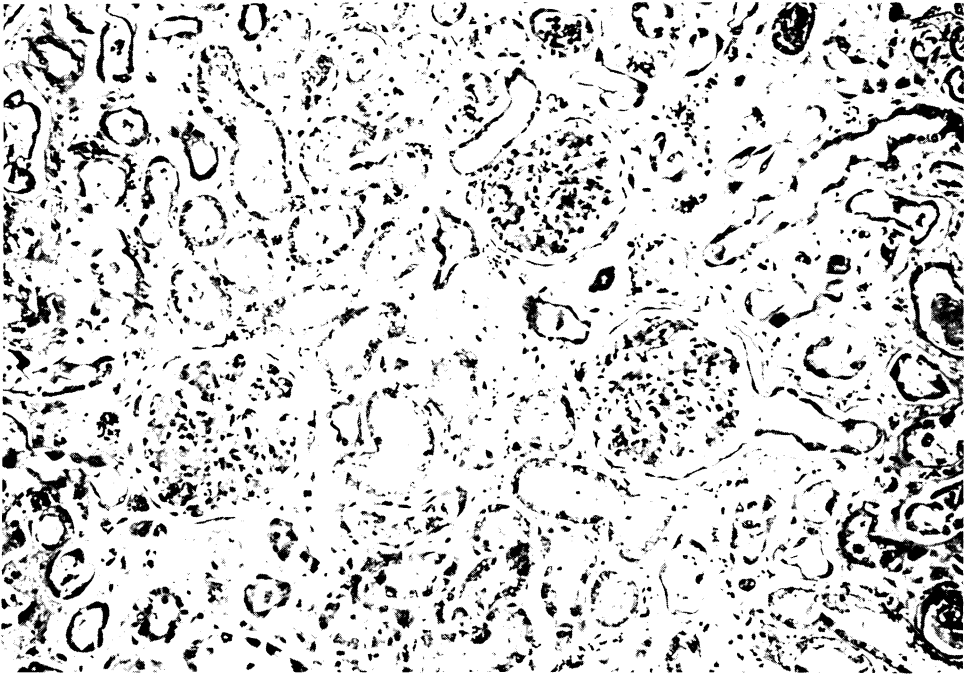


Figure 165. Heterotransplanted chimpanzee kidney from a patient who died from pneumonia at 54 days. There is extensive tubular necrosis with casts and evidence of repair. The interstitium is edematous and fibrous. The glomeruli and blood vessels are normal. There is no cellular infiltration; the few black dots that can be seen are erythrocytes lying in the peritubular capillaries. (This material was kindly provided by Doctor Keith Reemtsma.) H and E (X 125).

BABOON-TO-MAN RENAL HETEROTRANSPLANTS

One pair of baboon heterotransplants (SD 1) showed no evidence of clinical rejection before the patient died. Three other pairs of transplants (SD 3, SD 4, and SD 5) successfully survived an early rejection phase, but were in recurrent episodes when they were studied. The remaining two pairs of kidneys (SD 2 and SD 6) were in rejection for the third time when examined.

All the kidneys were swollen, each with a thickened capsule which stripped easily, leaving a smooth surface. In four of the pairs of transplants this surface was mottled with irregular hemorrhages and yellow areas each of which was surrounded by a bright red zone. The cut surface bulged, and the same hemorrhages and paler areas were seen in the cortex and extending into the deep red medulla (Fig. 166). The kidneys from SD 5 were a uniform reddish purple, and it was not possible on the cut surface to differentiate between cortex and medulla. In the sixth case (SD 1), in which there had been no clinical episodes of rejection, the kidneys were very different. They were firm, pale brown, and speckled with petechial hemorrhages. Blotchy hemorrhages and yellow areas were conspicuously absent. The vascular anastomoses were open in each case, but major branches of the renal artery and vein were thrombosed in SD 5. The



Figure 166. Gross findings (SD 6) typical of four of six baboon heterografts.

Top—Uncut paired kidneys showing mottled and greatly enlarged gross appearance. The kidneys weighed 80 gm each.

Bottom—Bisected heterografts. The dark color is due to diffuse intraparenchymal hemorrhage. Despite the advanced damage to the transplant, large quantities of urine were excreted to the time of death. (By permission of *Transplantation* 2: November, 1964.)

right kidney of SD 1 was surrounded by a large hematoma which might have partially compressed the renal artery on that side. There were many obvious large hemorrhagic infarcts in the kidneys of Patient SD 2. The ureters in SD 2 and SD 5 contained blood clots. All the other ureters had swollen walls, but were patent.

In contrast to the chimpanzee heterotransplants, the baboon kidneys showed a very heavy cellular infiltration. It was more marked than in the group of human homotransplanted kidneys which were examined while undergoing rejection, and sometimes it approached in density that seen in canine homotransplants. One of the more severe infiltrations was in SD 1, a case in which there was no blood group incompatibility and the heterotransplants were functioning reasonably well up to the time the patient died of complications of a pulmonary embolus (see Figure 123, Chapter 23). The last creatinine clearance in this case was 60 ml per minute and the BUN was 63 mgm per cent. There had been no clinical evidence of a rejection episode.

The cells were distributed in a patchy fashion in four of the cases, just as in canine renal homotransplants which are undergoing rejection, but in cases SD 1 (Fig. 167) and SD 4 the cells were scattered in a diffuse manner throughout the interstitium. Many of the infiltrating cells had pyroninophilic cytoplasm, and some of them were obviously mature plasma cells. In the kidneys from Patient SD 3 there were also eosinophils (Fig. 168). Mitoses were seen only in SD 1. Phagocytic cells containing large masses of deoxyribonucleic acid (DNA), and looking just like lupus erythematosus (LE) cells, were found in small numbers in the interstitium of two of these pairs of kidneys (Fig. 169). Such cells have not been recorded previously in any canine or human renal homotransplant.

All the kidneys were edematous, and contained many scattered hemorrhages (Fig. 170, top) and hemorrhagic infarcts (Fig. 170, bottom). These were very marked and confluent in the two pairs of heterotransplants which remained in the host for nine and 12 days after becoming anuric. Focal infarcts were least apparent in the three patients SD 1, SD 3, and SD 4 in whom function was reasonable to good and whose last creatinine clearances ranged from 19.1 to 60 ml per minute. Vascular lesions seemed to account for these changes. In all six pairs of heterotransplants there was narrowing of a variable number of interlobular arteries by fibrin and platelet deposits on the intima. In four this was associated with fibrinoid necrosis of the walls of these vessels (Fig. 171) and of the afferent arterioles; in three with swelling of the endothelial cells lining the arterioles; and in three with fibrous intimal thickening of interlobular arteries. Rupture of the internal elastic lamina of affected vessels was common, and thrombosis of occasional small arteries with damaged walls was not unusual. Destruction of peritubular capillaries was widespread in all six cases. Obvious secondary thrombosis of large arteries and veins was present in the two pairs of heterotransplants that ceased functioning several days before death of the patient.

Text continues on page 355.

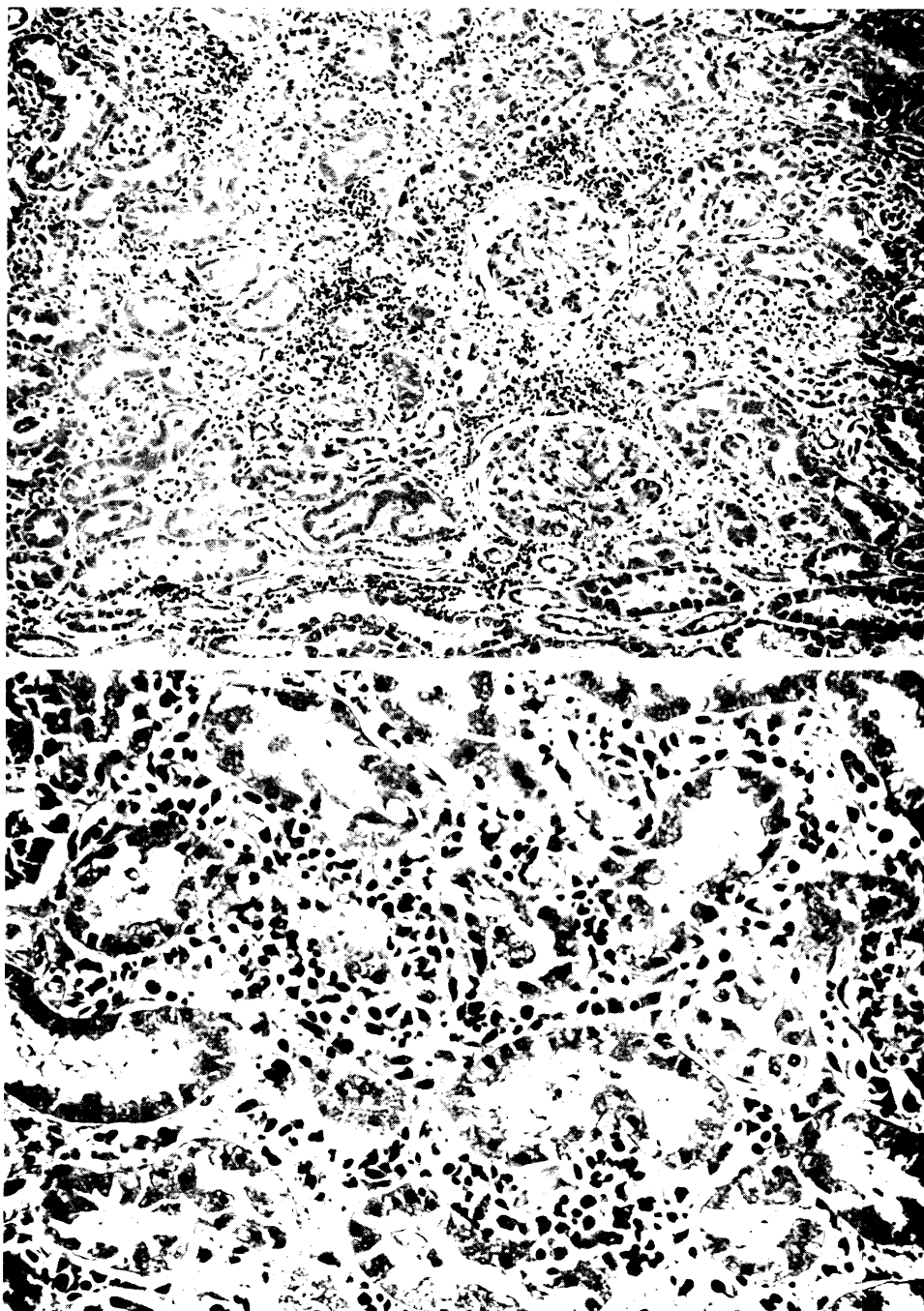


Figure 167. Top—Heterotransplanted baboon kidney from a patient (SD 1) who died from pneumonia and a pulmonary embolus at 23 days. There had been no clinical evidence of a rejection episode. There is a heavy and diffuse infiltration, with cells permeating the whole interstitium. H and E (X 125).

Bottom—A higher power view. There is interstitial edema and a heavy diffuse cellular infiltration. The tubules show recent necrosis. H and E (X 300).

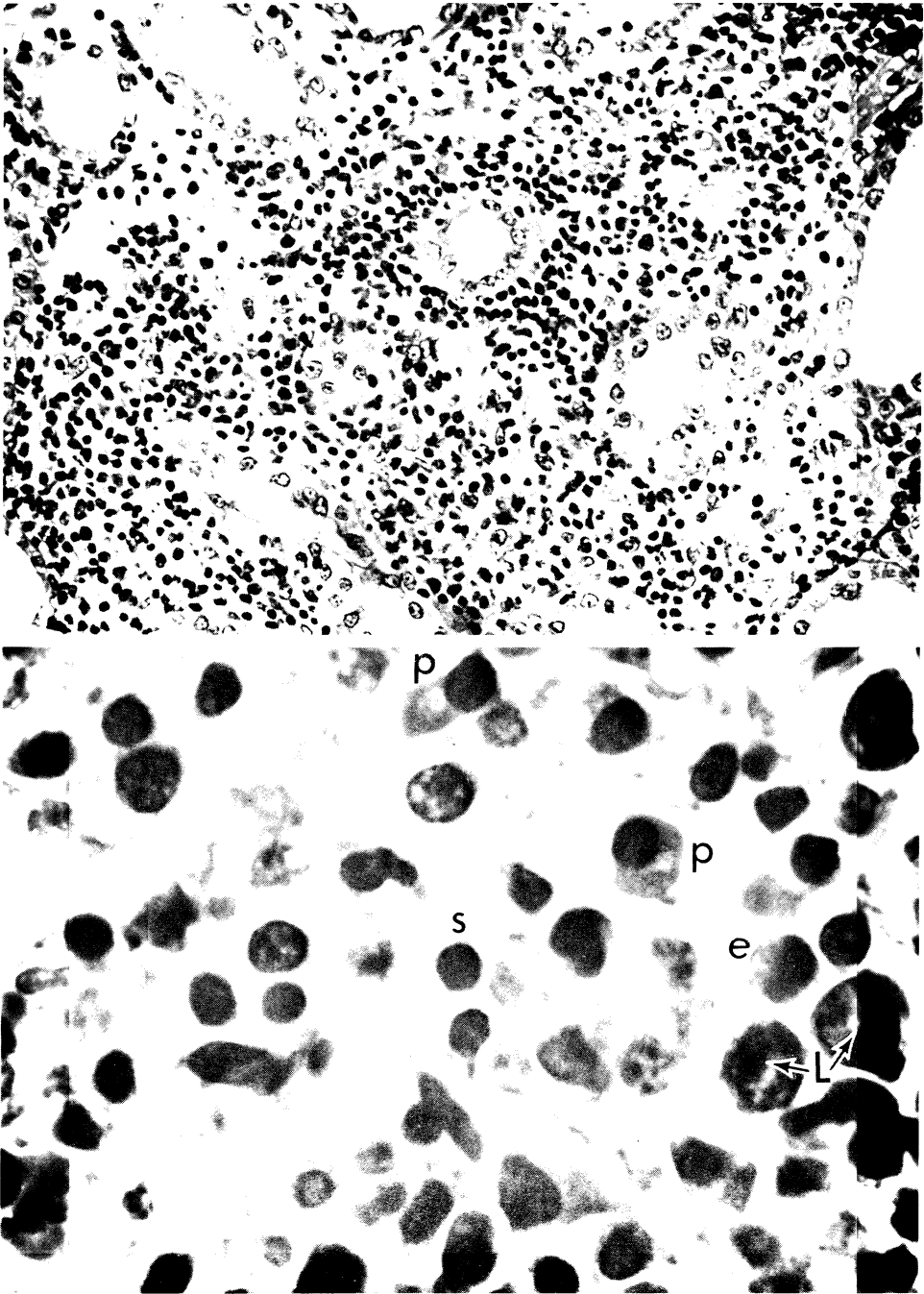


Figure 168. Top—Baboon renal heterotransplant which was removed from Patient SD 3 during a rejection episode at 60 days. A more patchy, but still very heavy cellular infiltration, is present. H and E (X 300).

Bottom—A higher power view. Many of the infiltrating cells have basophilic cytoplasm. Several are plasma cells (p); one is an eosinophile (e); some are small lymphocytes (s); and some are large lymphoid cells (L). H and E (X 800).

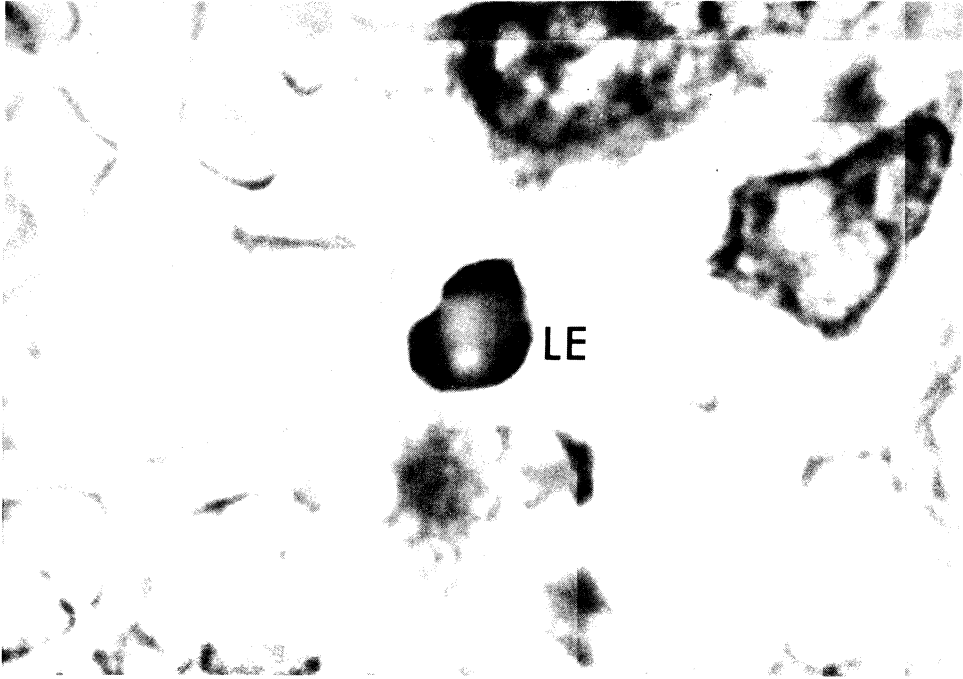


Figure 169. Baboon renal heterotransplant from Patient SD 3. A phagocytic cell (probably a neutrophil), containing a large mass of basophilic material (DNA), is seen lying in a peritubular capillary. This cell looks like a lupus erythematosus (LE) cell. H and E (X 1,000).

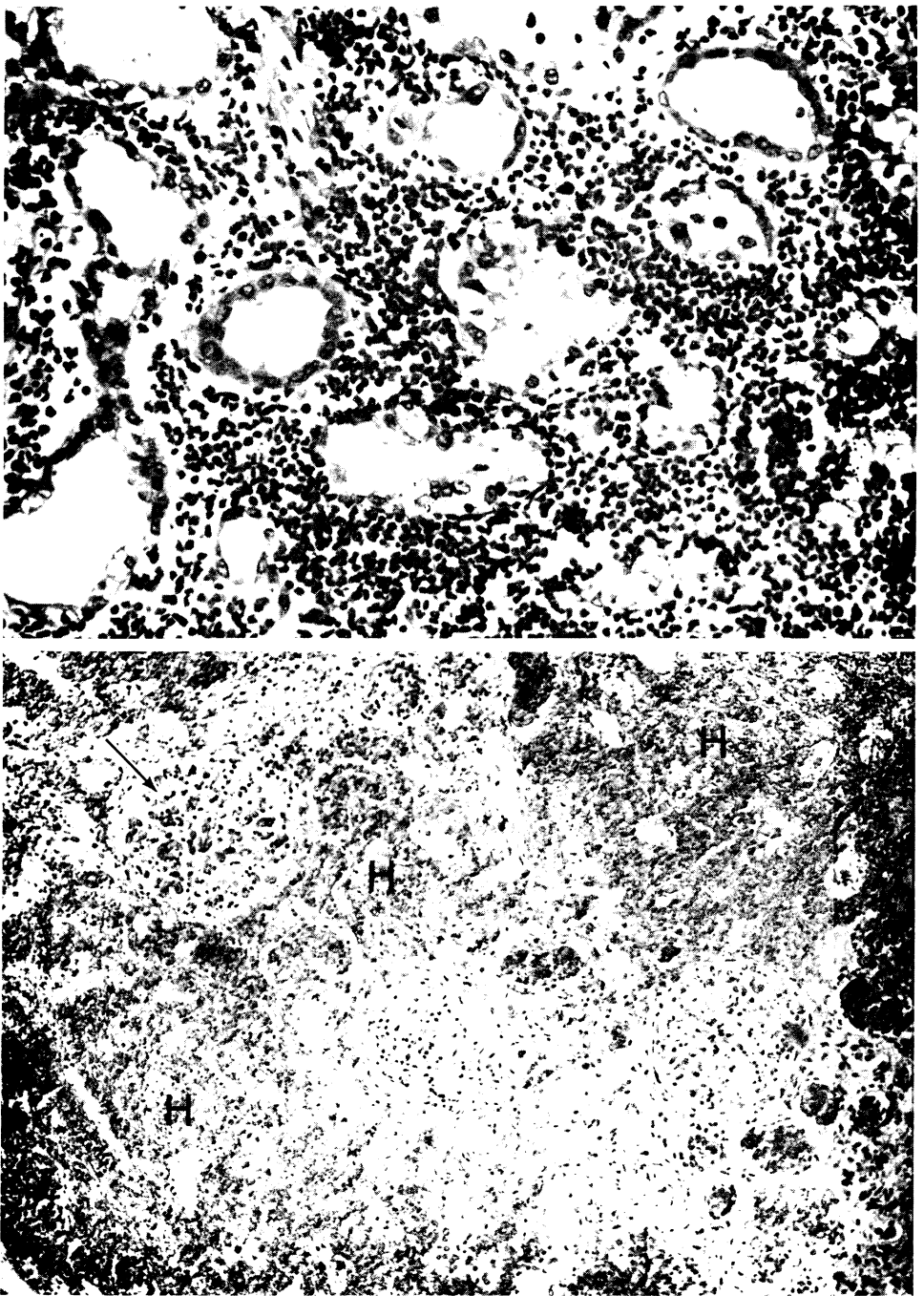


Figure 170. Top—Baboon renal heterotransplant from Patient SD 3. This photomicrograph shows an area of interstitial hemorrhage. All the dark cells are erythrocytes. H and E (X 300).

Bottom—Baboon renal heterotransplant from Patient SD 6 who was in rejection when he died at 49 days. An area of hemorrhagic infarction (H) is shown. The glomerulus indicated with an arrow is necrotic. H and E (X 125). (By permission of *Transplantation* 2: November, 1964.)

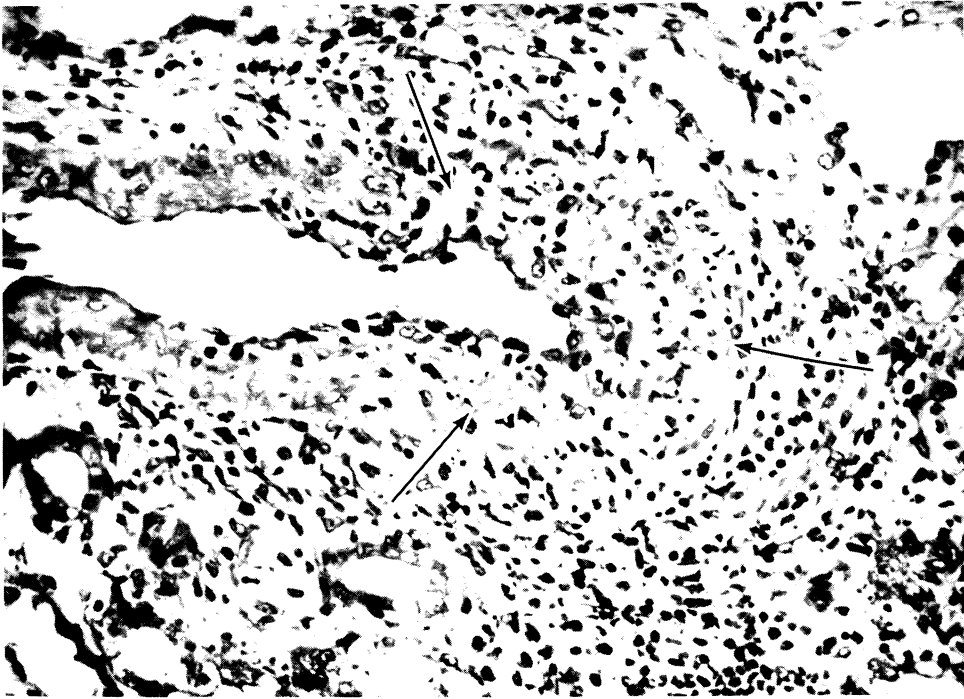


Figure 171. Baboon renal heterotransplant from Patient SD 4 who was in rejection when he died at 49 days. An interlobular artery shows not only fibrinoid necrosis of its wall, but also cellular infiltration of the damaged area indicated by arrows. This is a true arteritis. H and E (X 200).

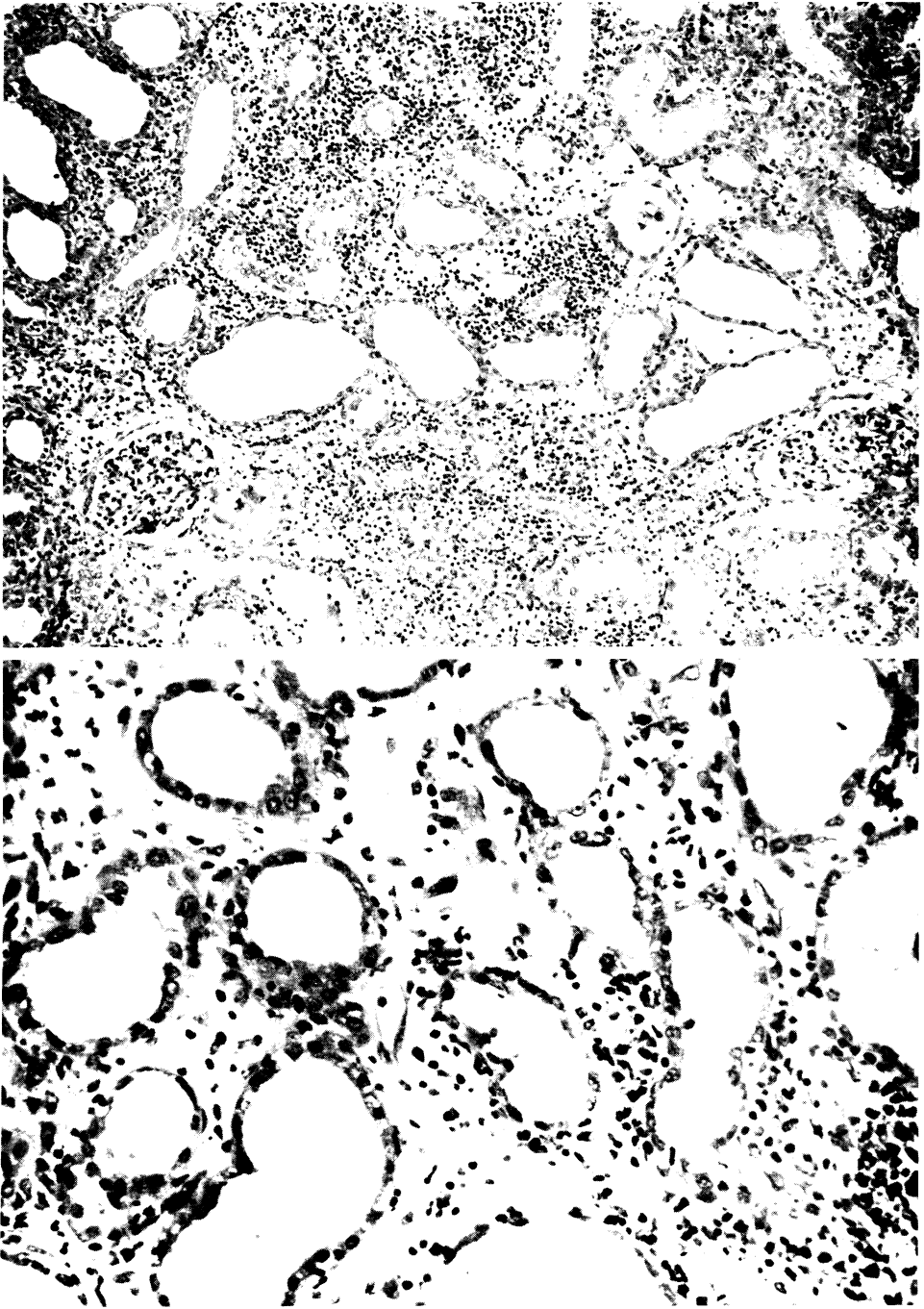


Figure 172. Top—Baboon renal heterotransplant from Patient SD 3. Many tubules have been destroyed. Those surviving are dilated and lined by flattened epithelium. The interstitium is edematous and heavily infiltrated with cells. H and E (X 125). (By permission of *Transplantation* 2: November, 1964.)

Bottom—Higher power view of baboon heterotransplant from Patient SD 3. Damaged tubules are lined by flattened epithelium. The interstitium is edematous and infiltrated with cells. Many peritubular capillaries have been destroyed. H and E (X 300).

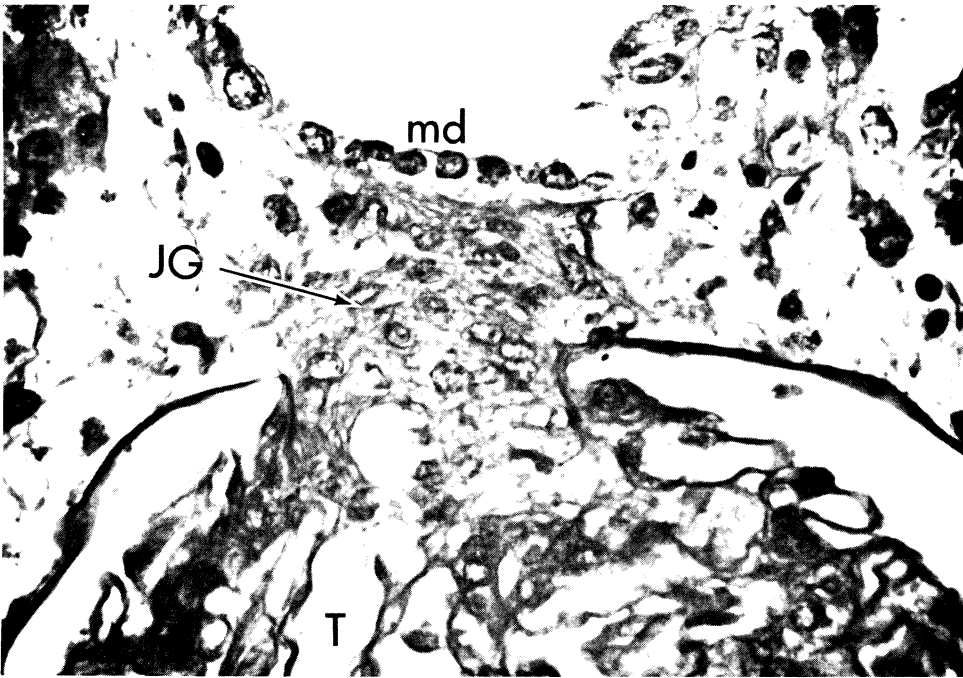


Figure 173. High power view of baboon renal heterotransplant from SD 4, a patient who was hypertensive. The heterografts were removed surgically after 49 days. The photomicrograph shows part of a glomerular tuft (T), an enlarged juxtaglomerular body (JG), and the macula densa (md). The juxtaglomerular cells contain many cytoplasmic granules. Periodic acid-Schiff (X 670).

Tubular damage with evidence of regeneration was present in all the cases. This was most severe in SD 3 and SD 6, in both of whom there was ample clinical evidence of these changes (Fig. 172). Casts of protein and cell debris were frequent.

Glomerular changes were not striking. There was some hypertrophy of the tufts in the case that functioned for 60 days (SD 3), the longest period for a baboon heterotransplant. Hyperplasia of the juxtaglomerular apparatus was present in the single hypertensive patient (SD 4) (Fig. 173).

It is apparent that there is ample evidence of a rejection process in the baboon heterotransplants and that the cellular component of this is much more pronounced than in comparable human homotransplants.

RHESUS MONKEY-TO-MAN RENAL HETEROTRANSPLANT

Both kidneys, the aorta, and the vena cava from a group A+ rhesus monkey were transplanted by Reemtsma and his colleagues into the right iliac fossa of a 32-year-old woman. The recipient was in terminal uremia from chronic pyelonephritis. Her blood group was A+. She was treated with azathioprine, steroids, actinomycin C, and azaserine. Urinary output was 3.5 liters on the first day, and the creatinine clearance rose to 24 ml per minute. At five days a rejection episode started. By seven days there was oliguria, and at 10 days the transplant was removed.

There were several punctate hemorrhagic areas on the surface of the kidneys. Microscopically, there was marked cellular infiltration. This tended to be focal, and consisted of lymphocytes, pyroninophilic cells, some mature plasma cells, and a few neutrophils. Interstitial edema was present. Many of the arterioles and occasional interlobular arteries showed severe fibrinoid necrosis of their walls (Fig. 174), and there was extension of the process into some glomerular tufts. In places the necrotic wall was surrounded by neutrophils. Hemorrhages and small pools of fibrin were seen in the interstitium adjacent to necrotic arteries. There was some tubular damage and a few red cell casts.

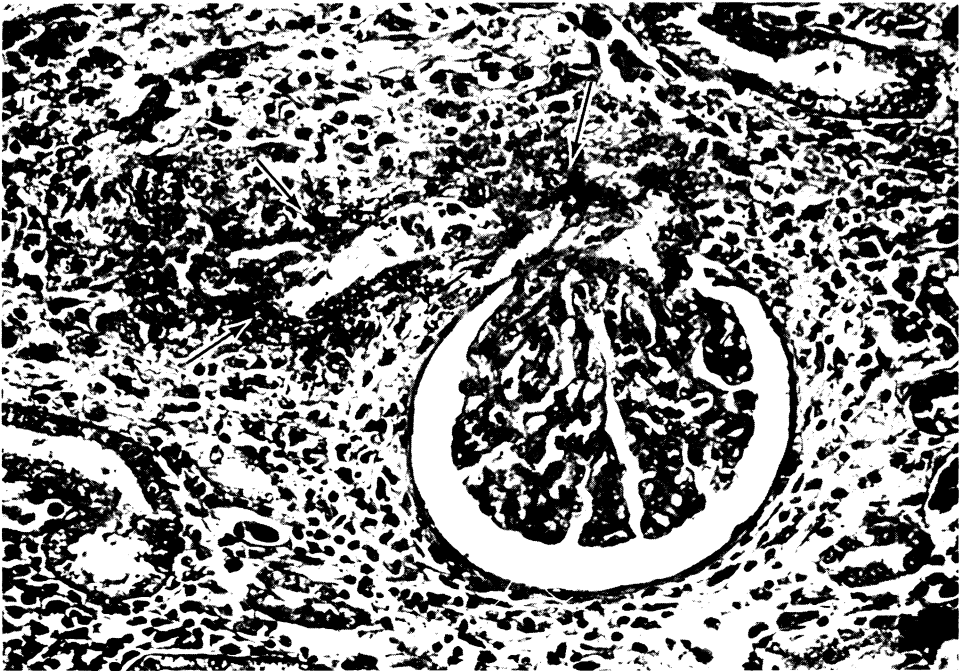


Figure 174. Heterotransplanted rhesus monkey kidney which was removed from a patient at 10 days after it had ceased to function. There is gross fibrinoid necrosis of the wall of an afferent arteriole (arrows), with spread of the necrotic process into the tuft. The interstitium is edematous and heavily infiltrated with cells. There is tubular damage and loss. (This material was kindly provided by Doctor Keith Reemtsma.) H and E (X 300).

These severe changes are very like those seen in some canine renal homo-transplants treated with immunosuppressive drugs, in which the vascular component of the rejection process is very prominent.

CONCLUSIONS

From the material discussed in this chapter it will have been seen that there are two important events which can bring about failure of renal homo- and heterotransplants: first, disruption of peritubular capillaries and venules caused by infiltrating host cells; and second, endothelial swelling and vasoconstriction of arterioles followed by fibrinoid necrosis of the walls of these and larger arteries.

When a kidney is homotransplanted into a normal, healthy, nonuremic, untreated recipient, damage to the small peritubular vessels occurs early. This causes ischemic tubular necrosis, and is the main reason why the transplant ceases to excrete urine. Although fibrinoid necrosis of vessels is sometimes seen in these cases, this only appears as a terminal event and apparently plays little part in bringing about failure of the transplant.

When rejection occurs in a renal homotransplant the life of which has been prolonged by treatment for uremia, the fibrinoid necrotic lesions assume great importance. Once they have involved the interlobular or larger arteries, halting the rejection process will result only in healing and compensatory intimal thickening with fibrous narrowing of part of the intravascular arterial tree. Successive waves of damage and repair resulting from poorly controlled rejection episodes can lead to severe impairment of renal function and death. However, it is evident from the Denver series that with prompt and vigorous treatment the earlier stage of arteriolar endothelial swelling and fibrinoid necrosis of arteriolar, as distinct from arterial walls, can be successfully repaired, leaving no appreciable residual damage.

On the basis of present information, which is admittedly rather scant, it would appear that a treated chimpanzee renal heterotransplant fares no worse in the early stages than a treated human renal homotransplant from an unrelated donor. It is clear, however, that baboon heterotransplants, and particularly rhesus monkey transplants, invoke a fierce response on the part of the host despite any treatment that is available at present. In the resulting rejection process, cellular infiltration and peritubular capillary destruction are prominent early features, but by nine days the vasculonecrotic element is marked.

There is some circumstantial evidence to suggest that whereas the peritubular capillary damage is mediated by cell-bound antibody, the fibrinoid necrotic vascular lesions are caused by circulating antibody.

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Chapter Twenty-six

PRECEPTS OF RENAL HOMOTRANSPLANTATION APPLIED TO HOMOGRAFTING OF OTHER ORGANS

In this book, the problems of tissue transplantation have been surveyed as they apply specifically to the kidney and its function. As an experimental model, this organ has unique advantages for the acquisition of knowledge which may have more general application. With renal homografts, the functional status of the kidney can be followed with precision using the specific measurements of urine volume, blood urea nitrogen, creatinine clearance, urine protein and urea, and blood pressure. The onset of rejection can be defined with accuracy. Inferential information concerning the mechanisms of tissue deterioration is available for analysis, as has been described. The effect of agents used for the prevention or reversal of rejection can be determined unequivocally since the transplanted kidney is essential for life. It is probable that at least some of these data will be useful in problems involving other organs.

Finally, the technical requirements which must be met have much in common with those to be anticipated in the employment of other tissues in clinical transplantation, inasmuch as the problems of ischemic injury, preservation, reconstitution of blood supply, and reconstruction of nonvascular visceral passages are all essential details common to most organs.

The first requirement before consideration of clinical transplantation of any organ is knowledge of the natural history of the untreated homograft. Only when this has been established will it be possible to intelligently diagnose and treat rejection. In working with liver homografts, for example, the pattern of rejection is considerably different from that which had been expected. In dogs, a clinical picture of early rejection is encountered which is indistinguishable

from that of obstructive jaundice, rather than that of diffuse parenchymal disease which might have been predicted. In pulmonary homografts, a well-integrated and distinctive pattern of rejection which could be used in day-to-day management has not yet emerged. With the use of spleen, cardiac, and endocrine homografts, specific measurements are also required to guide appropriate antirejection therapy.

In addition to cataloging those factors which permit the diagnosis and treatment of rejection, it will be necessary in the case of each organ to establish means to differentiate the early functional impairment which occurs as a result of ischemic or traumatic injury from that consequent upon immunologic attack by the host. As with cadaveric renal homografts, other organs obtained after death will be more difficult to use than those procured from living volunteers. The only available sources of most of the tissues in which there will be a future interest will be cadavers or lower animals (heterografts) making the problems both more challenging and more difficult.

The need for tissue of good quality for transplantation has been repeatedly stressed throughout this text. Successful management of the admittedly difficult problem of renal homograft rejection has undoubtedly been rendered impossible in the past by the provision of irreparably damaged kidneys. It would seem unnecessary to travel this road of tragic experience for each new organ used in clinical treatment. Instead, vigorous efforts from the beginning should be directed toward shortening the period of ischemia, toward finding methods of effective storage, and toward exploring the possibilities of heterotransplantation.

The knowledge that most attempts at rejection can be reversed provides the essential starting point in transplantation of any organ. The difficulty with which this is accomplished is subject to wide variation, necessitating such vigorous treatment in the more difficult cases as to endanger the life of the host. Nevertheless, that the usual rejection episode is both controllable and reversible makes it almost certain that homotransplantation of other organs will become a regular clinical service at some time in the future. It is possible that the intensity of the rejection process will vary with different tissues and organs. There is already evidence that pulmonary and cardiac homografts may be of low antigenicity, and that splenic and hepatic homografts may be the opposite. Whatever such differences might prove to be, the laws governing the onset, treatability, and reversal of rejection should apply, at least in a general sense.

The principle of host-graft adaptation will also have to be demonstrated for tissues other than the kidney. That adaptation appears to occur promptly and in a great many cases is perhaps the most fundamental and encouraging fact that has emerged from all previous experience with whole organ renal grafts.

Although the kidney is one of the vital organs, cessation or serious deterioration of its function can be tolerated for protracted periods because of the

availability of facilities for renal dialysis. In applying what was learned in renal homografting to the grafting of other organs, the prospect of acute temporary failure may be unacceptable. This is particularly true if one is considering the use of cardiac, pulmonary, or hepatic homografts in which complete loss of function even for short intervals would be fatal. In transplanting these organs, the prophylactic use of steroids as defined in Chapter 18 may be useful. As has been described, the full vigor of a rejection attempt can often be tempered or prevented with this approach. Even with the most intense immunosuppressive therapy, however, temporary organ failure may ensue. This fact should promote efforts to develop better mechanical devices which could be used for interim treatment. In the eventuality of temporary homograft failure, the use of artificial livers or of prolonged cardiopulmonary by-pass during the treatment of a rejection crisis may be the only suitable type of treatment.

Further demonstration will be required of the value of current immunosuppressive regimens in preventing rejection of other organs. Imperfect as it is, the therapy described elsewhere in this text (see Chapters 14 to 18) is of proven worth for long-term renal homograft survival, but its value has not been nearly so well documented with hepatic, cardiac, pulmonary, or splenic homografts. It is conceivable that azathioprine has a relatively specific protective effect for kidneys, a possibility that cannot be precluded until more is known of its mode of action and the degree of protection afforded to other alien tissues. In clinical practice, the addition of steroids to azathioprine greatly increases the likelihood of controlling renal homograft rejection, but these adjuvants have not extended survival in dogs after hepatic or pulmonary homotransplantation beyond that achieved with azathioprine alone. A continuing search will therefore be necessary, not only for agents which will improve results with renal homotransplantation, but for different immunosuppressive methods which may be more decisively directed to the protection of other kinds of homografts.

Chapter Twenty-seven

TECHNICAL TRIUMPH AND MORAL MUDDLE*

by Chauncey D. Leake, Ph.D., Sc.D., L.H.D.

The major problems in organ transplantation are almost certain to have eventual and satisfactory solutions. The technical surgical skill is already available. The immunological problem relating to the rejection of a transplanted organ is more difficult, but the way to a satisfactory solution promises to be at hand.

Experimental studies on the control of rejection factors are being successfully applied to clinical practice in organ transplantation. Mercaptopurine compounds, such as azathioprine, combined with certain steroids such as prednisone, seem to block antibody formation sufficiently in many cases to allow a transplanted organ to survive, even if obtained from an immunologically unrelated source. The problem is one of clinical judgment in achieving enough immunological block to permit the transplanted organ to survive, without interfering with the long-range immune protection of the patient against infection.

Difficult moral questions are certain to accompany further progress in this field. These ethical problems involve not only the physicians, patients, donors, and families, but also the social climate of opinion and understanding in the community.

Let it be clear that the ethical problems of organ transplantation and dialysis are those of fundamental morality, and not those aspects of "medical ethics" which are merely matters of professional etiquette. The situation can be serious, since after centuries of debate, the basic approaches to a satisfying morality are not yet generally agreed upon.

*This discussion is modified from that offered at a Symposium on Organ Transplants and Dialysis held at Rochester, Minnesota, May 18, 1964, under the auspices of the Minnesota Medical Association.

EXCURSUS ETHICUS

If we wish to make a reasonable approach to the ethical problems arising in connection with organ transplantation, it is wise to take a little excursion in order to become better acquainted with some of the complexities in the picture. It is unfortunate that philosophy is not ordinarily a part of the humanistic effort in our chaotic medical school curricula. Philosophy, however, is not popular in any of our modish educational endeavors. This may simply be a result of the declining ability of philosophers to talk the common language, or it may come from a lack of enough leisure to permit the public to reflect upon philosophical issues. The resulting moral confusion may become overwhelming as population pressures continue to increase.

There are several ethics. These are answers which have been proposed since antiquity to the basic question, "What is Good?" They are tentative and relative like the various answers to the other questions, "What is True?" and "What is Beautiful?"

Various logics have been suggested in the effort to find the truth about ourselves and our environment. Aristotelian logic, based on the validity of certain assumptions and on principles of deduction, became so powerful in medieval Europe that the only answer permitted was "The Logic." Currently, scientific logic, dependent on precision of measurement, seems to be amazingly successful in telling us what is "true," or verifiable, about ourselves and our environment.

Various esthetics try to obtain general acceptance about matters of taste and judgment. Similarly, various ethics try to win general public approval about motivations, interpersonal relations, individual and social goals and purposes, and about different sorts of moods and behavior.

Part of our difficulty over ethics results from the conditions of organization of living material: we tend to confuse, because we do not understand, the factors operating at an *individual* level of biological organization with those operating at a *social* level. Individual "good" may not always coincide with social "good." Much of the history of the development of ethical theory is concerned with attempts to harmonize these often diverging conditions of individual and social welfare.

It is not easy to try to analyze our ethical predicament in a few paragraphs. The various ethics suggest various *purposes* as being worthy of our conscious life-long endeavor. We may begin by considering biologically purposeful operants.

Biologically, as Paul McLean indicates, our purposes in living are dependent on built-in drives which direct us toward *satisfactions* in a continually recurring search for food (necessary for self-preservation), and in the postpubertal desire for sexual experience (necessary for species preservation). The biological answer to that ancient question, "What do we live for?" seems to be to obtain recurring satisfactions in our recurring drives for food and for sex. These

requirements are subject to conditioning, not only reflexly, but also at various levels of consciousness.

In a more formally conventional manner, the earliest theory of ethical behavior suggested by the ancient Greeks was that which is called *hedonism*, the proposition that our primary purpose is to obtain personal pleasure, and that our behavior is based on maximizing our individual personal happiness. This is not as simple a matter as it may sound. Practical circumstances intervene, adjustments to others become necessary, and an enlightened individualism may emerge, as Warner Fite suggested.

Hedonism, one may note, has overtones which are both epicurean and stoical. A more sophisticated ethic was proposed by Plato. This was the suggestion that the "true" good is social rather than individual, and that individual happiness is dependent on social welfare. Under *Platonic idealism*, which became the accepted Judeo-Christian ethic, an individual is expected *voluntarily* to suffer sacrifice for the benefit of the social group. This ancient tribal ethic continues to trouble us, as when conventional Christianity, which also emphasizes individual worth, confronts authoritarianism, either within itself or in the surrounding environment.

Attempts have been made to compromise hedonism and Platonic idealism. One such effort is utilitarianism, the principle of getting the "greatest good for the greatest number," as proposed by John Stuart Mill in the nineteenth century. This turns out to be a calculated hedonism, however, as Fite has explained.

Recently certain scientists, such as Julian Huxley and C. H. Waddington, have become interested in a scientific basis for an ethic. Clearly this is most readily done on the basis of evolutionary and survival factors. This trend, which started with E. G. Conklin, has been accelerated by the moral problems raised by applications of nuclear energy. Some philosophers, such as Max Otto, are particularly articulate about it.

A quarter of a century ago a deliberate attempt was made to find a naturally operating ethic, which might function on survival values. This was developed by E. G. Conklin and myself, and was critically examined by Patrick Romanell. After considering the plethora of common experience in the everyday interplay of differing personalities, we induced a principle which seems to be naturally operative in governing interpersonal relations and the motives, purposes, and behaviors associated with them. The naturally operating ethical principle may be stated as follows:

The probability of the survival of a relationship between individuals, or groups of individuals, increases with the degree to which that relationship is mutually satisfying.

Clearly, if this principle is valid, there are many significant consequences. If either party wishes a relationship to continue, it is necessary to do whatever can be done to make the relationship satisfying to the other party. This has overtones of what is called The Golden Rule. The principle also takes into

account the basic biological factor of satisfaction, an emotional feeling associated with the fulfillment of the drive for food or sex.

What does all this mean in regard to organ transplantation? At once, these ethical theories raise the immediate questions of motivation, of desires and goals, and of behavior. These questions will become of even greater importance when organ transplantation becomes an increasingly successful therapeutic method.

MORAL PROBLEMS IN ORGAN TRANSPLANTATION

Some of the problems raised in the field of organ transplantation have been surveyed by Joshua Lederberg, the distinguished Nobelate geneticist; by J. Russell Elkinton, editor of the *Annals of Internal Medicine*; and by Belding H. Scribner, who has defined five specific and practical moral questions confronting physicians and surgeons who are interested in renal transplantation and dialysis. In addition, the ethical issues have been discussed in the lay press, some of the best analyses being those of Victor Cohn and those printed in *Life* and *Newsweek*. The relation of these questions to "medical ethics" is not so much concerned with medical etiquette between members of the medical profession as it is with the more basic morality explored by Fletcher which lies at the heart of the patient-doctor relationship.

It is interesting that the current situation is historically analogous to that present in the Seventeenth Century, when blood transfusion was first undertaken. At that time, blood transfusions failed tragically, because of scientific ignorance. Under these circumstances, the moral situation became clear: one could not undertake blood transfusion without the risk of death, and the whole matter was banned by social fiat.

Two centuries were required to clarify the requirements for successful blood transfusion. It was only after Karl Landsteiner (1868-1943) classified blood-group incompatibilities that blood transfusion gradually became a successful and socially approved procedure. The resultant community blood banks paved the way for the development of corneal, cartilage, skin, bone, and endocrine depots. These banks are stocked from cadaveric sources and generally require relatively prompt utilization of the tissues.

With whole organ transplantation, it seems likely that technical skill and understanding will triumph much more rapidly than was the case with blood transfusion. On the other hand, it seems that the moral problems which are involved may multiply.

Joshua Lederberg has pointed out that the transplantation of hearts, livers, and even complete limbs may be relatively easy within the next 20 years. Vigorous exploration of the necessary techniques is under way, not only in this country but also in Russia. The humanistic problems raised by the potential

success of these efforts are quite as serious in other parts of the world as they may be with us.

Lederberg asks: Who will choose which dying cardiac patients should get new hearts and who will control the decisions concerning storage or transplantation of the homografts? He concludes that the future of organ transplantation, which may even include efforts at eugenics, may result in serious controversy. All parents would be likely to insist that their children be given the intelligence of genius, if this were possible. Who will decide how to use our genetic pool, with possible transplantation of the organs of regeneration? These problems are too important to be entrusted to biomedical scientists or to members of the health professions. They require careful and detailed public study, with solutions dependent upon moral, social, and economic factors, and most important upon enlightened public opinion.

Elkinton has critically reviewed another aspect of the problem. Is the unknown life extension of the recipient patient worth the potential, though presumably slight, reduction in the life span of the donor? Many factors must be considered in decisions regarding organ transplantation, such as relative ages of the patients, their relative productiveness, their potential social contributions and value to the community, and the motivations of the individuals involved. As Victor Cohn has indicated, it is to the lasting credit of the health-team personnel concerned in these matters that these considerations are carefully weighed and that all aspects of the case are fully explored before the team decides to let nature take its course, or, on the contrary, to intervene.

Scribner, who is President of the American Society for Artificial Internal Organs, outlines five specific practical problems facing surgeons who are proposing kidney transplantation: (1) Out of the 10,000 or so who may die yearly from acute kidney disorder, who among the 50 or 100 that can be handled by presently available kidney transplantation teams or by dialysis programs will be chosen? (2) Should homografts be obtained from the family or a friend, from anonymous volunteer donors, from cadavers, or from publicly maintained organ banks? (3) What should be done with the patient who may interfere with any therapy by means of a suicidal attempt? (4) Is there a violation of the patient's right to die when he is treated after the disease has run its natural course? and (5) In the case of dialysis, who should decide when it may have to be stopped, as in a patient who has suffered a stroke or a heart attack?

Let us consider the situation from the standpoint of recipient and donor. Here is a patient for whom a transplant may be considered: anxious, trying to repress the unpleasing contemplation of death, and perhaps appalled at the responsibility which is suddenly apparent of accepting something truly valuable beyond price from someone else. Yet, in the case of every ordinary patient, even if well self-disciplined, there is the will to live at any cost, to self or to anyone else. The moral position of the recipient is essentially the hedonistic one.

Then there are the physicians—on the surface cool, collected, self-reliant in

technical skill, troubled only by the possibility of failure through lack of knowledge and experience in controlling the immune processes, and also worried a bit over the delicacies of obtaining a suitable and willing donor. The surgeon's ethical position is essentially that of the pragmatic utilitarian.

Finally, there is the donor, often overeager to sacrifice because of some subconscious guilt complex, or simply happy to give something of great importance for a loved one. Here is the personification of Platonic idealism on which our acceptable Judeo-Christian ethic is based.

One might think that this categorization covers the interpersonal relationships involved in organ transplantation. However, the situation is much more complex, as a little reflection will reveal. Consider, for example, the somewhat related matter of blood transfusion, now a socially acceptable procedure. Despite this, there is continual difficulty in obtaining enough blood donors in spite of every inducement. Or consider the more emotionally charged problems of artificial insemination, which are still under vigorous social debate. In these examples, the scientific aspects have largely been solved, and risks to the donors are minimum. Nevertheless, difficulties remain, indicating how serious the moral problems associated with organ transplantation may prove to be.

One of the most perplexing aspects of organ homotransplantation is that there is still a good possibility that the homograft will be rejected in spite of every effort at immunosuppression. Should this happen, the recipient is clearly as poorly off as ever, and a volunteer donor is potentially worse. The recipient may have gained a few more weeks of life, but what about the potential reduction in the fullness of living for the donor, even if such subsequent limitations are purely psychological? The physicians involved can only have a sickening feeling at having operated upon a healthy, well-motivated donor, without providing any real benefit to the recipient.

Between recipient and donor, a two-way responsibility exists. If I am the sick person with a diseased organ that may be successfully removed and replaced by a transplant, am I justified in calling on even a close relative to yield a healthy organ merely to keep me alive for an as yet undetermined period of time? If I am the healthy person and wish to be generous, would I be wise to sacrifice one of my healthy organs if I had heavy family responsibilities?

The ethical issue for the donor is slight. Sacrifice is commended for generosity and for social welfare, yet sacrifice is not always justified even on the basis of social expediency, let alone enlightened self-interest. From the standpoint of the recipient, it does not seem that anyone has the right to expect that someone else would willingly give up a healthy part of his or her body for the recipient's benefit. For the recipient the problem is complicated by our sentimentalized fear of death. Few of us have disciplined ourselves to be willing to die with dignity and decency when that time comes.

The establishment of socially acceptable organ banks might reduce some of the moral difficulties inherent in organ transplantation, but these are not

yet feasible. Even donations of organs at death to an organ bank might not be as simple as would be imagined. In addition, there may be emotional factors involved for recipients who receive tissues from unknown donors.

It is apparent that a complex of psychosocial factors is in operation in the organ-transplant drama, not only with respect to the three principals (recipient, donor, and physician) but also with respect to the social environment in which each, respectively, moves, and in the larger environment of the whole community.

To maintain mutually satisfying interpersonal relationships under these conditions is difficult. It is doubtful if harmonious relations between recipient, donor, and physician and between these principals and their respective communities can be consistently maintained in the eventuality of failure. Although these interpersonal relations seem to be pleasant and promising in anticipation, maintenance of harmony is often very delicately balanced and partially dependent upon carrying the transplantation to a successful conclusion. The naturally operating ethical principle, of which I have spoken, continues to exert its force, whether we are aware of it or not, and whether we like it or not.

This ethicogenic principle, however, itself points to a way out. If those who are enmeshed in the particular interpersonal relationship under consideration and wish it to continue, then it is clearly incumbent upon each to do whatever is possible to make the relationship satisfying to the others. This does suggest the operation of The Golden Rule.

If, in the case of organ transplantation, all those who are concerned will explore together the factors that are involved and reach some clear advance understanding between themselves on the matter, there may be hope for continued harmony among them. This requires calm, honest, and open discussion between the recipient, donor, and their respective families under the leadership of the physicians and, wisely, with the guidance of religious advisers.

ETHICAL RESPONSIBILITIES OF PHYSICIANS

From all this discussion, then, one may propose the basic ethical responsibilities of surgeons in organ transplantation: (1) to undertake nothing, except for purely investigational purposes, until the technical procedures are fully under control, including the as yet incompletely solved immunological problem of transplant rejection; (2) to discuss the whole matter carefully and in detail with prospective recipient, proposed donor, their respective families, and their religious advisors; (3) to be well versed in ethical theory and in psychology, so that individual motivations can be detected, exposed, and discussed; and (4) to be prepared to do everything possible to maintain harmonious interpersonal relations between recipient, donor, families, and religious advisors, no matter what the outcome of the endeavor may be.

IN PROSPECT

The moral problems of organ transplantation are not the exclusive responsibility of physicians, nor of biomedical scientists who may be working on the technical details which must be solved for greater clinical success. Nor are they the primary responsibility of prospective recipients or donors. They are the responsibility of all intelligent people.

The chief responsibility of scientists and of members of the health professions is to place all the verifiable information available before the rest of the medical profession, before interested public agencies which must provide financial support for these efforts, and before the public. In this way, the remarkable advances that have been witnessed in the past few years can be unified, not only in a scientific sense, but for the attainment of maximum social welfare.

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Chapter Twenty-eight

**TRANSPLANTS AT THE UNIVERSITY
OF COLORADO MEDICAL CENTER**

The following pages list in tabular form the details of each patient treated at Colorado General Hospital or the Denver Veterans Administration Hospital.

Details of Patients in Living Donor (LD) Series

No.	Disease	Date Operation	Living June 1, 1964	Age	Sex	Blood Type	Donor Blood Type	Donor Relation	Ischemia (min)	Own Kidneys Retained	Thymectomy Days before Transplant	Onset Early Rejection	Onset Late Rejection	Ccr May, 1964	BUN May, 1964	Prednisone May, 1964	Antihypertensive Drugs/day May, 1964	
																	Chlorothalazine	Reserpine
*1	CGN	11-24-62	Yes (554 days)	12-M	B+	B+	B+	Mother	34	No	30 days	14 days	None	96.2	14	104/90	None	None
2	CGN	1-31-63	Yes (486 days)	38-M	A+	B+	B+	Sister	28	Right	14 days	25 days	None	94	19	100/80	None	None
3	CGN	2-9-63	Yes (477 days)	21-M	A+	A+	A+	Fraternal twin	24	No	16 days	None	None	145	14	116/70	Never used	None
*4	CGN	2-25-63	Died 6-18-63 (113 days)	35-M	AB+	A+	A+	Wife	36	No	13 days	? 17 and ? 42 days	-	-	-	-	-	-
5	Polycystic	3-26-63	Died 4-6-63 (11 days)	50-M	A+	A+	A+	Unrel. M	71 (35 min cardiac arrest: 2 arteries)	Right and left	28 days	None diagnosed	-	-	-	-	-	-
6	CGN	4-17-63	Yes (409 days)	23-M	O+	O+	O+	Brother	35	No	26 days	18 days	None	130.9	18	130/78	None	None
7	CGN	5-3-63	Died 7-21-63 (79 days)	25-M	A+	O+	O+	Wife	41 (2 arteries)	No	No	19 days	-	-	-	-	-	-
8	Accidental surg. rem. only kidney	5-8-63	Died 7-9-63 (62 days)	29-M	O+	O+	O+	Brother	61 (2 arteries)	No	No	4 days	-	-	-	-	-	-
9	CGN	5-10-63	Died 12-4-63 (207 days)	30-M	A+	A+	O+	Fraternal twin	28	No	85 days	5, 34, and 71 days	-	-	-	-	-	-
10	CGN	5-15-63	Died 3-6-64 (295 days)	47-M	O-	O-	O+	Unrel. M	38	No	No	3 days	229 days	-	-	-	-	-
11	CPN	5-17-63	Died 6-11-63 (24 days)	35-M	O+	O+	O+	Brother	30	No	49 days	1 day	-	-	-	-	-	-
12	CGN	6-7-63	Yes (358 days)	48-M	A+	A+	A+	Brother	40	No	No	7 days	300 days	45	32	140/90	1.5 gm	0.5 mgm
13	CGN	7-3-63	Yes (333 days)	15-M	A+	A+	A+	Mother	26	No	No	13 days	240 days	87.7	29	138/100	1.0 gm	0.5 mgm
14	CPN	7-5-63	Yes (331 days)	42-M	A-	A-	A-	Brother	28	No	No	None definite: eosinophilia and fever 23 day	None	91	10	154/100	None	None
15	CGN	7-8-63	Yes (328 days)	23-M	A-	A-	O+	Brother	22	No	No	7-1/2 days	None	90	16	130/80	None	None
16	Cong. hypoplasia	7-12-63	Died 10-3-63 (83 days)	44-F	A+	A+	A-	Brother	23	No	No	5 and 29 days	None	-	-	-	-	-
17	CPN	7-19-63	Yes (317 days)	15-F	O+	O+	O-	Mother	17-1/2	No	No	42 days	None	66.5	14	120/80	None	None

18	CGN	7-24-63	Yes (312 days)	39-M	O+	O+	Sister	22	No	No	6 days	None	130	23	$\frac{156}{110}$	30	1.0 gm	None	0.5 mgm
19	CPN	First 7-26-63 Second 8-9-63	Removed 7-26-63 (same day) Died 11-12-63 (95 days)	17-M	O+	O+	Mother	30	No	No	Instant	-	-	-	-	-	-	-	-
20	CGN	7-29-63	Died 2-15-64 (202 days)	6-M	O+	O+	Unrel. M	26	No	No	9 days	-	-	-	-	-	-	-	-
21	CPN	7-31-63	Died 10-15-63 (76 days)	41-M	A+	A+	Wife	41	No	No	1 day	None	-	-	-	-	-	-	-
22	Polycystic	8-12-63	Yes (293 days)	15-F	O+	O+	Mother	37	No	No	4 days	None	-	-	-	-	-	-	-
***23	Polycystic	First 8-16-63 Second 8-26-63	Removed 8-16-63 (same day) Yes (279 days)	47-M	O+	O+	Brother	58	No	No	24 days	244 days	85.7	24	$\frac{104}{60}$	25	None	None	None
24	CGN, gouty nephritis	8-19-63	Died 9-25-63 (37 days)	42-M	A+	A+	Unrel. M	43	-	-	18 days	223 days	85	32	$\frac{190}{110}$	40	None	None	None
25	CGN	8-21-63	Yes (284 days)	33-M	O+	O+	Brother	40	No	No	2 days	-	-	-	-	-	-	-	-
26	CGN	8-29-63	Died 8-30-63 2/3 day	16-F	A+	A+	Father	23	No	No	17 days	None	90.0	19	$\frac{114}{80}$	5	None	None	None
27	CGN	9-3-63	Yes (271 days)	20-F	A+	A+	Unrel. M	21	No	No	None	-	-	-	-	-	-	-	-
28	CGN	9-6-63	Died 10-1-63 (25 days)	49-M	A-	A-	Sister	34	No	No	? 19 days	None	70	28	$\frac{134}{100}$	25	1.0 gm	None	0.5 mgm
29	CPN	First 9-23-63	Removed 9-25-63 (2 days)	40-M	AB+	AB+	Sister	84 (2 arteries)	No	No	13 days	-	-	-	-	-	-	-	-
30	CGN	Second 10-3-63	Died 10-11-63 (8 days)		AB+	AB+	Unrel. M	30	No	No	None recognized	-	-	-	-	-	-	-	-
31	CPN	9-30-63	Yes (244 days)	40-M	O+	O+	Unrel. M	30	No	No	2 days	112 days	57.5	54	$\frac{160}{98}$	40	2.0 gm	2000 mgm	1.0 mgm
32	CGN	10-1-63	Died 10-16-63 (15 days)	55-M	A+	A+	Unrel. M	42	No	No	7 days	-	-	-	-	-	-	-	-
33	CGN	10-4-63	Died 11-15-63 (41 days)	20-M	A+	A+	Brother	33	No	No	5 days	-	-	-	-	-	-	-	-
34	CGN	10-7-63	Yes (237 days)	18-F	A+	A+	Mother	29	No	No	11 days	-	-	-	-	-	-	-	-
35	CGN	First 10-12-63 Second 11-20-63	Yes (233 days) Removed 11-2-63 (21 days) Died 11-29-63 (9 days)	8-F	O+	O+	Mother	42	No	No	6 days	None	75.0	18	$\frac{130}{98}$	10	1.0 gm	None	None
	CGN			38-M	A+	A+	Unrel. M	27	No	No	13 days	None	50.5	16	$\frac{114}{80}$	5	0.75 gm	30 mgm	None
					A+	A+	Sister	28	No	No	18 days	-	-	-	-	-	-	-	-
					A+	A+					None	-	-	-	-	-	-	-	-

* Steroid pretreatment ** Total body irradiation *** Receiving 25 mgm per day mecamlamine **** Receiving 250 mgm per day alpha-methyl DOPA ***** Splenectomy not performed
CGN Chronic glomerulonephritis CPN Chronic pyelonephritis M Male F Female ATN Acute tubular necrosis

Details of Patients in Living Donor (LD) Series (continued)

No.	Disease	Date Operation	Living June 1, 1964	Age, Sex	Blood Type	Donor Blood Type	Donor Relation	Ischemia (min)	Own Kidneys Retained	Thymectomy Days before Transplant	Onset Early Rejection	Onset Late Rejection	Cr May, 1964	BUN May, 1964	Antihypertensive Drugs/day				
															Chlorthalazide	Hydralazine	Prednisone May, 1964	Reserpine	
36	CGN	10-14-63	Yes (230 days)	43-M	B+	O+	Wife	38	No	No	1 day	None	121	21	140 100	20	1.5 gm	150 mgm	0.75 mgm
***37	CGN	10-18-63	Yes (226 days)	21-M	O+	O+	Mother	24	No	No	? 10 days	None	109.5	29	136 106	25	2.0 gm	None	1.0 mgm
38	CPN	11-9-63	Died 12-13-63 (38 days)	21-M	A+	A+	Mother	30	No	No	3 days	-	-	-	-	-	-	-	-
39	CGN	11-13-63	Yes (200 days)	17-M	O-	O-	Mother	18	No	No	12 days	None	104.3	21	124 88	15	None	None	None
40	CGN	11-16-63	Yes (197 days)	21-F	O+	O-	Mother	27	No	No	8 days	None	70.7	21	138 108	30	1 gm	150 mgm	0.5 mgm
41	CGN	11-23-63	Yes (190 days)	3-M	A+	A+	Mother	32	No	No	? 6 days	None	27.0	20	114 70	5	1 gm	40 mgm	0.3 mgm
42	CGN	11-27-63	Yes (186 days)	19-F	O+	O+	Father	26	No	No	4 days	None	108.0	21	130 80	20	1 gm	None	None
43	CGN	12-3-63	Died 1-10-64 (38 days)	25-M	O+	O+	Mother	46	Left	No	? Immediate ? ATN	-	-	-	-	-	-	-	-
44	CGN	12-7-63	Yes (176 days)	48-M	A+	O+	Sister	38	No	No	2 days	None	67	30	156 100	30	1 gm	100 mgm	None
45	CGN	12-10-63	Yes (173 days)	35-M	A+	A+	Sister	28	No	No	43 days	None	93.0	16	118 100	25	1.5 gm	None	None
*46	CGN	1-3-64	Died 2-16-64 (43 days)	25-F	O+	O-	Husband	31	No	No	31 days	-	-	-	-	-	-	-	-
*47	CGN	1-4-64	Yes (148 days)	37-M	A+	A+	Brother	28	No	No	3 days 14 days ? 34 days	None	113	27	126 102	25	1.5 gm	None	0.75 mgm
*48	CGN	1-10-64	Yes (142 days)	34-M	B+	B+	Sister	31	No	No	? 2 days	None	80	19	120 80	25	1 gm	None	None
*49	CGN	First 1-15-64	Removed 1-22-64 (7 days)	32-M	A+	A+	Sister	57	No	No	Not clear	-	-	-	-	-	-	-	-
		Second 3-3-64	Yes (89 days)			A+	Sister	38			No	None	65	30	120 80	20	1 gm	None	None
*50	CGN	1-25-64	Yes (127 days)	16-F	A+	A+	Father	27	No	No	1-1/2 days	None	107.7	41	130 104	30	1.5 gm	200 mgm	0.75 mgm
*51	CGN	2-10-64	Yes (111 days)	18-M	O+	O+	Maternal aunt	25	No	No	? 32 days	None	86.4	36	120 84	35	1.0 gm	200 mgm	None
*52	CGN	2-17-64	Yes (104 days)	15-F	O+	O+	Father	30	No	No	None	None	45.3	24	126 102	25	1.5 gm	150 mgm	0.75 mgm
*53	CGN	2-22-64	Yes (99 days)	15-M	O+	O+	Maternal uncle	19	No	No	? 8 days	None	91.0	21	124 90	35	2.0 gm	200 mgm	0.75 mgm
*54	CGN	2-24-64	Yes (97 days)	21-M	O-	O-	Unrel. M	25	No	No	? 13 days ? 33 days	None	95.0	40	120 70	45	0.5 gm	100 mgm	0.75 mgm
*55	CGN	2-26-64	Yes (95 days)	21-M	O+	O+	Father	27	No	No	? 6 days ? 52 days	None	75	30	110 60	25	1.5 gm	None	None

*56	CGN	2-28-64	Yes (93 days)	26-F	A+	A+	Mother	30	No	No	No	7 days	None	97.6	20	110 82	40	None	None	None
*57	CGN	First 3-12-64 Second 3-18-64	Not removed Died 5-27-64 (71 days)	36-F	O+	O+	Mother Unrel. M	35 34.5	No	No	No	No function 2 days 32 days 53 days	None	-	-	-	-	-	-	-
*58	CGN and CPN	3-13-64	Yes (79 days)	27-M	A+	A+	Sister	26	No	No	No	None	None	85	21	150 90	25	1.5 gm	100 mgm	0.75 mgm
*59	CGN	3-16-64	Died 4-30-64 (45 days)	48-M	B-	B+	Wife	43	No	No	No	37 days	-	-	-	-	-	-	-	-
*60	CGN	3-17-64	Yes (75 days)	21-M	O+	O+	Second cousin M	25	No	No	No	None	None	90	15	140 100	35	1.5 gm	None	0.75 mgm
*61	CGN	3-23-64	Died 4-28-64 (36 days)	5-F	O+	O+	Mother	30	No	No	No	3 days 14 days 25 days	-	-	-	-	-	-	-	-
*62	CGN	3-24-64	Yes (68 days)	38-M	A+	O+	Sister	21	No	No	No	17 days ? 35 days	None	70	26	114 80	45	0.5 gm	None	None
*63	CGN	3-27-64	Yes (65 days)	35-M	A+	A+	Unrel. M	28	No	No	No	None	None	60	22	150 100	45	1.5 gm	200 mgm	0.75 mgm
*64	CPN	3-30-64	Yes (61 days)	30-M	O+	O+	Brother	19	No	No	No	44 days	None	25	87	140 100	120	2 gm	200 mgm	0.75 mgm

IDENTICAL TWIN (ITD) SERIES

*****1	CGN	3-27-62	Yes (795 days)	27-M	O+	O+	Identical twin	36	No	No	No	None	None	120	14	120 80	None	None	None	None
*****2	CGN	6-29-63	Yes (336 days)	18-M	O+	O+	Identical twin	30	No	No	No	None	None	112	12	110 70	None	None	None	None

CADAVERIC DONOR (CD) SERIES

*****1	CGN	3-19-63	Died 4-13-63 (24 days)	21-M	O+	O+	Cadaver M	106 min from death	No	No	No	? 10 days	-	-	-	-	-	-	-	-
2	CGN	4-19-63	Died 5-28-63 (40 days)	29-M	A+	A+	Cadaver M	124 min from death	No	No	No	No function	-	-	-	-	-	-	-	-
*****3	CPN	12-8-63	Died 12-12-63 (4 days)	42-F	A+	A+	Cadaver M	Rt 217 min from death Lt 137 min from death	No	No	No	No function	-	-	-	-	-	-	-	-

SIMIAN (BABOON) DONOR (SD) SERIES

*1	CGN	12-20-63	Died 1-12-64 (23 days)	40-M	A+	A	Baboon	Rt 29 Lt 37	No	No	No	None	-	-	-	-	-	-	-	-
*2	CGN	1-6-64	Died 2-10-64 (35 days)	46-M	O-	B	Baboon	44	No	-	-	5 days 9 days 15 days	-	-	-	-	-	-	-	-

* Steroid pretreatment ** Total body irradiation *** Receiving 25 mgm per day mecamlamine **** Receiving 250 mgm per day alpha-methyl DOPA ***** Splenectomy not performed
CGN Chronic glomerulonephritis CPN Chronic pyelonephritis M Male F Female ATN Acute tubular necrosis

Simian (Baboon) Donor (SD) Series (continued)

No.	Disease	Date Operation	Living June 1, 1964	Age, Sex	Blood Type	Donor Blood Type	Donor Relation	Ischemia (min)	Own kidneys Retained	Thymectomy Days before Transplant	Onset Early Rejection	Onset Late Rejection	Ccr May, 1964	BUN May, 1964	Prednisone May, 1964	Antihypertensive Drugs/day May, 1964	
																Chlorthalazide	Reserpine
*3	CGN	1-8-64	Removed 3-7-64 (60 days)	17-M	AB+	B	Baboon	34.5	No		5 days 50 days	-	-	-	-	-	-
		Secondary homograft 3-7-64	Died 4-15-64 (38 days)			O+	Unrel. M	22			None	-	-	-	-	-	-
*4	CGN	1-11-64	Removed 2-29-64 (49 days)	18-M	B+	B	Baboon	29	No		8 days 37 days	-	-	-	-	-	-
		Secondary homograft 2-29-64	Died 4-14-64 (44 days)			O+	Unrel. M	31-1/2			8 days	-	-	-	-	-	-
*5	CPN	1-14-64	Died 2-2-64 (19 days)	35-M	O-	AB	Baboon	27.5	No	-	4 days 7 days	-	-	-	-	-	-
*6	CGN	1-17-64	Died 3-6-64 (49 days)	35-M	O+	AB	Baboon	37	No	-	4 days 18 days 48 days						

*Steroid pretreatment.

EPILOGUE

September 1, 1964

In preparing this book, all statistics were brought up to date to June 1, 1964. As of September 1, 1964, there have been no additional deaths in the first 55 patients. Thus the statistics in Chapters 1 to 17 and Chapter 20 are valid as they stand, all 34 surviving patients within this first group of 55 now being six months or longer past operation. Within this group there are now 13 patients who are alive more than one year after transplantation.

In the later patients, three additional deaths occurred (LD 56, LD 62, and LD 64). These deaths influence the statistics only in Chapter 18, the chapter concerned with an evaluation of steroid pretreatment. These additional deaths have eliminated the advantage that the steroid pretreated patients seemed to have enjoyed during the early postoperative course: the number of deaths was just as large, but did tend to occur at a later time. The three additional deaths occurred 151 (LD 56), 111 (LD 62), and 65 (LD 64) days after operation.

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