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Transplantation of the Liver

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My [T.E.S.] assigned task today is to discuss homotransplantation of tissues other than the kidney. This would include the liver, lung, heart, the alimentary tract, pancreas and other endocrine glands, the spleen, skin, bone marrow, and even the brain, since experimental work is proceeding with each of these organs. I am not going to attempt to do all of this, and with good justification. There is no sound reason to believe that the essential problems of immunology will differ in other than a quantitative way with any of these tissues.

Thus, it may be that any organ will prove to have an antigenic specificity, which will make it easier or harder to control rejection, but the general therapeutic concept of immuno-suppression will be required in all. It is our belief that those drugs which prove best for potentiating renal homograft survival will provide the keystone therapy for transplantation of other tissues, and that the concept of organ-specific immuno-suppression, which recently has been propounded, will be found to be unsound.

If we are willing to accede to these generalizations—and there will be many in this group who will not be—why cannot we then undertake clinical homotransplantation of other organs with the same success as has already been attained with kidneys? The reason is that a variety of specific details need to be clarified for the transplantation of every tissue concerning problems of surgical technique, preservation, tolerance to ischemic injury, the effects of denervation upon function, metabolic requirements of the transferred tissue, and the effects of that tissue upon the host.

Unlike the kidney, the homografted lung sustains a severe functional impairment by virtue of its denervation. The heart has shorter

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FIGURE 21. This dog with orthotopic liver transplantation is now 4½ months post-operative. Immuno-suppression was with azathioprine. The animal also received 100 microcuries S^{32} every 5 days in 1.8 mg methionine. All immuno-suppression was discontinued after 120 days with no subsequent delayed rejection. Note the abdominal midline incision through which the dog's own liver was removed and the homograft inserted.

ischemic tolerance than any other tissue, excepting only the brain. In addition, it must function effectively without a time lapse even for a few seconds, so that the implications of a "rejection crisis" are unacceptable even though the process is potentially reversible. The spleen participates in a graft-versus-host reaction, which is manifested by a hemolytic process. The transplanted pancreas is peculiarly sensitive to pancreatitis.

Such problems, many of which are nonimmunologic, probably have

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been responsible for failure to obtain chronic survival with life-sustaining function of vital organs other than the kidney, excepting only the liver. Because of this fact and because we believe that the liver will probably be the next major organ to be transplanted successfully and since its study demonstrates the variety of unique requirements which must be met with each new organ system, I would like to tell you what we have learned about hepatic transplantation (1).

Figure 21 shows a dog, about 4½ months postoperative. Through

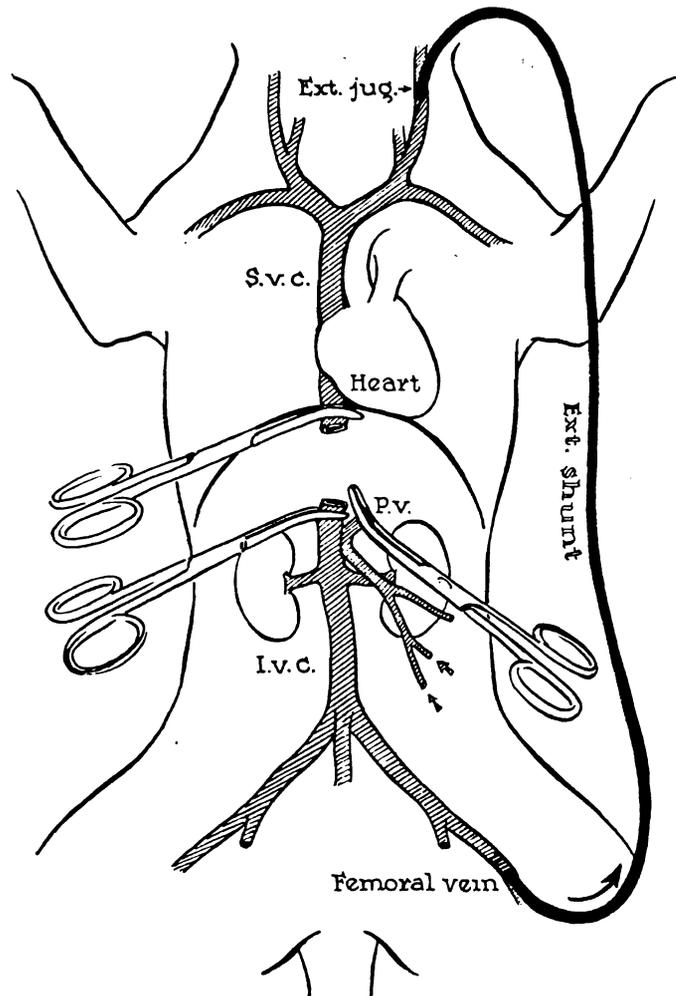


FIGURE 22. Use of temporary portacaval shunt during hepatic homotransplantation. By connecting the splanchnic and vena caval systems a single external bypass can be used for venous decompression during the actual insertion of the homograft (by permission of *Surg., Gynec. & Obst.* (3)).

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an upper midline incision, an orthotopic homotransplantation was performed with removal of her own liver and replacement with a homograft. The chronic survival of this and other dogs carries implications of the future clinical usefulness of such operations.

Orthotopic Transplantation in the Untreated Dog

The technical requirements for hepatic homotransplantation are stringent. In dogs, we first perform a temporary portacaval shunt con-

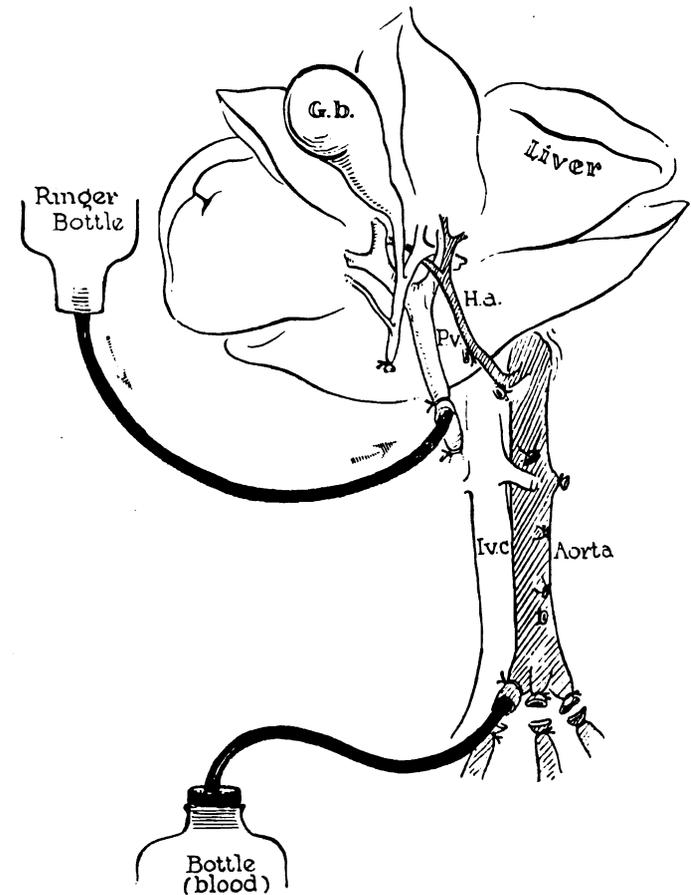


FIGURE 23. Method of liver preservation used for the dog. Total body hypothermia is initially carried out to 29 to 31°C. Just before removal of the liver, chilled lactated Ringer's solution is perfused in the portal vein at the same time the animal is exsanguinated through the aorta. The temperature of the homograft falls to 10-15°C. Livers cooled in this way can tolerate an ischemic interval of as long as 2 hours (by permission of *Surg., Gynec. & Obst.* (3)).

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necting the splanchnic and systemic venous systems, so that a single bypass may be used to decompress these blocked venous areas during the actual transplantation (Fig. 22).

The method of organ preservation used in the laboratory is shown in Fig. 23 (2). The donor animal is initially cooled. Just before organ removal, the liver temperature is lowered further by perfusion of chilled lactated Ringer's solution into the portal vein. For reasons I will discuss later, this simple method of organ preservation may be inadequate for clinical transplantation.

Transplantation is then carried out after removal of the recipient animal's own organ, connecting the upper vena cava just below the diaphragm, the lower vena cava below the liver, the portal vein, and the hepatic arterial supply (Fig. 24). The temporary portacaval shunt

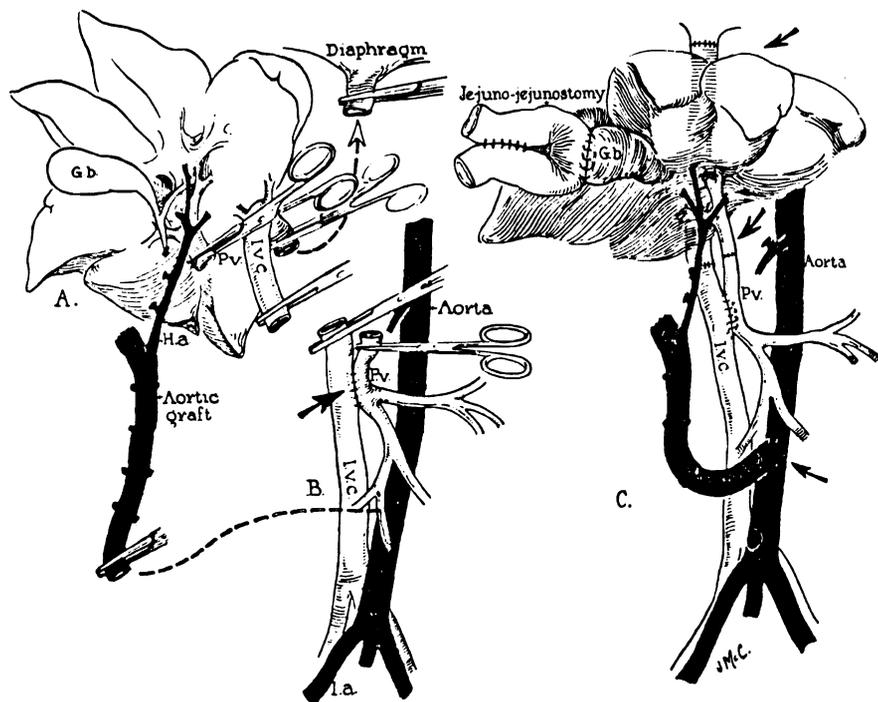


FIGURE 24. Technique of orthotopic liver transplantation in the dog. Note the temporary portacaval shunt which is removed after insertion of the homograft is complete. The aorta is removed in continuity with the hepatic artery and attached to the recipient aorta in order to have a larger vessel for anastomosis. Internal biliary drainage can be provided as shown or a cholecystoduodenostomy may be constructed (by permission of *Surg., Gynec. & Obst.* (3)).

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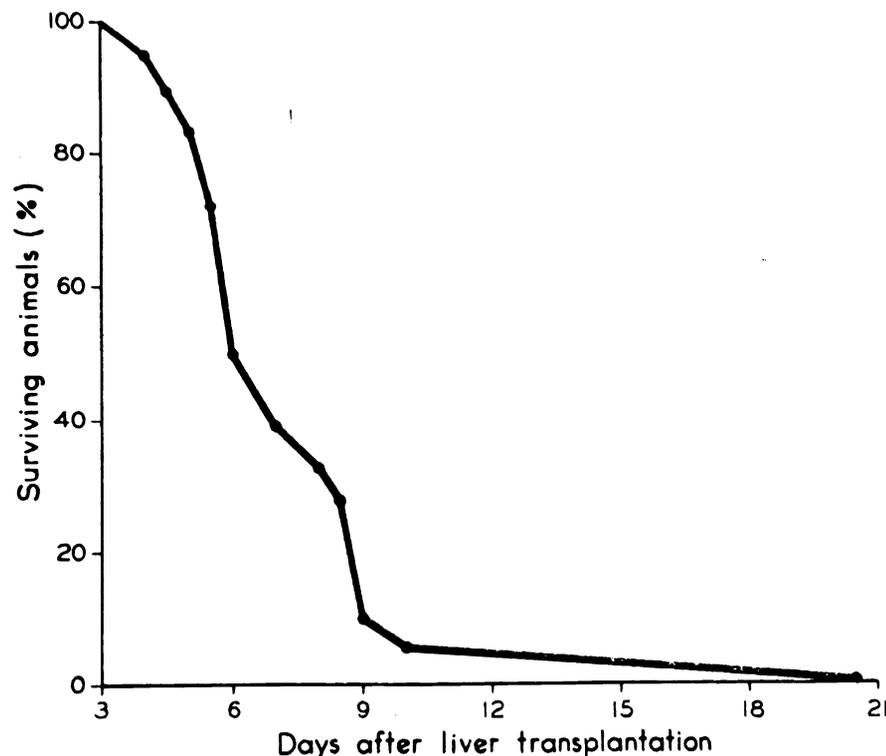


FIGURE 25. Survival of a group of untreated animals after orthotopic liver transplantation. Note that the heaviest loss rate is between days 5 and 9. One animal had an unusually protracted survival of 21 days (by permission of *Surg., Gynec. & Obst.* (4)).

is then removed. Internal biliary drainage is provided with a cholecystenterostomy (3).

Figure 25 shows the survival curve of a group of nontreated dogs. The life expectancy is not dissimilar to that reported after renal homotransplantation to untreated animals. The dogs began to die off within 3 or 4 days, the peak mortality being after 6 or 7 days. In this group there was 1 exceptional survival, a dog who lived for 21 days, probably because a good antigenic match was accidentally obtained between donor and recipient.

The biochemical changes after hepatic homotransplantation in the untreated dog are characteristic. Most of the animals develop unremitting jaundice after 4 or 5 days (Fig. 26). Alkaline phosphatase begins to rise a day or so earlier than the bilirubinemia (Fig. 27). Many of the animals in the untreated series become hypoglycemic terminally.

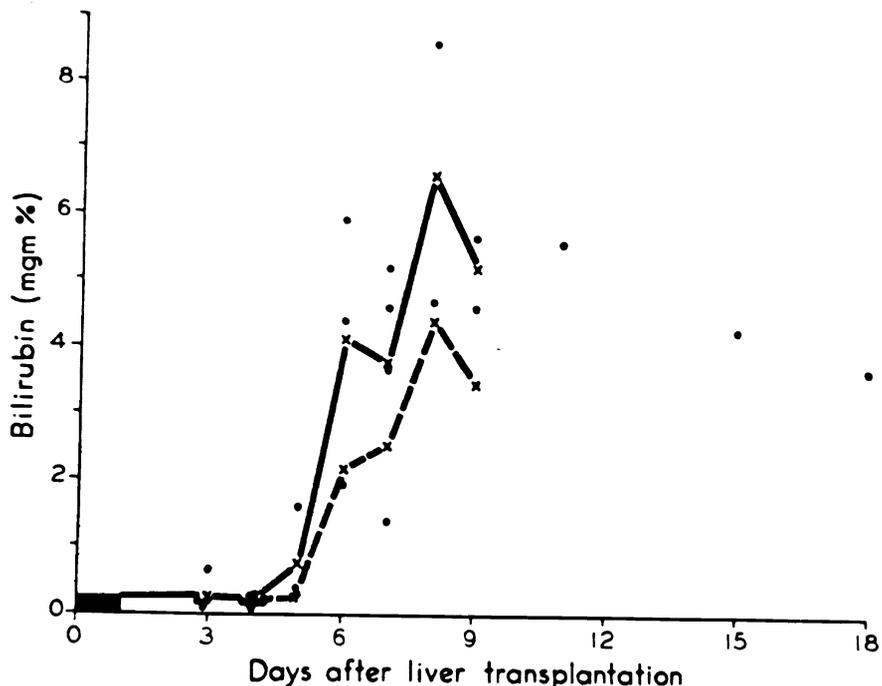


FIGURE 26. Development of jaundice in untreated dogs after orthotopic liver transplantation. Hyperbilirubinemia is invariably present after 4 days (by permission of *Surg., Gynec. & Obst.* (4)).

When this happens, death usually follows within a few hours (Fig. 28) (4).

The histologic changes in the homograft also are characteristic (4-6). After about 4 days, a few round cells begin to appear in the periportal area, but with good preservation of the general hepatic architecture (Fig. 29A). This proceeds to a heavy focal cellular accumulation, and later with generalized scattering of immunocytes throughout the microscopic field. There is at this time loss of hepatocytes (Fig. 29B) which tends to be concentrated in the periportal areas and around the central veins.

Orthotopic Transplantation to Dogs Treated with Azathioprine

When dogs are treated with azathioprine, the inexorable events of rejection are altered, and in some instances avoided altogether (13). In Fig. 30, the course of an unusually long-surviving control animal (*left*) is compared with that of a dog treated with azathioprine (*right*).

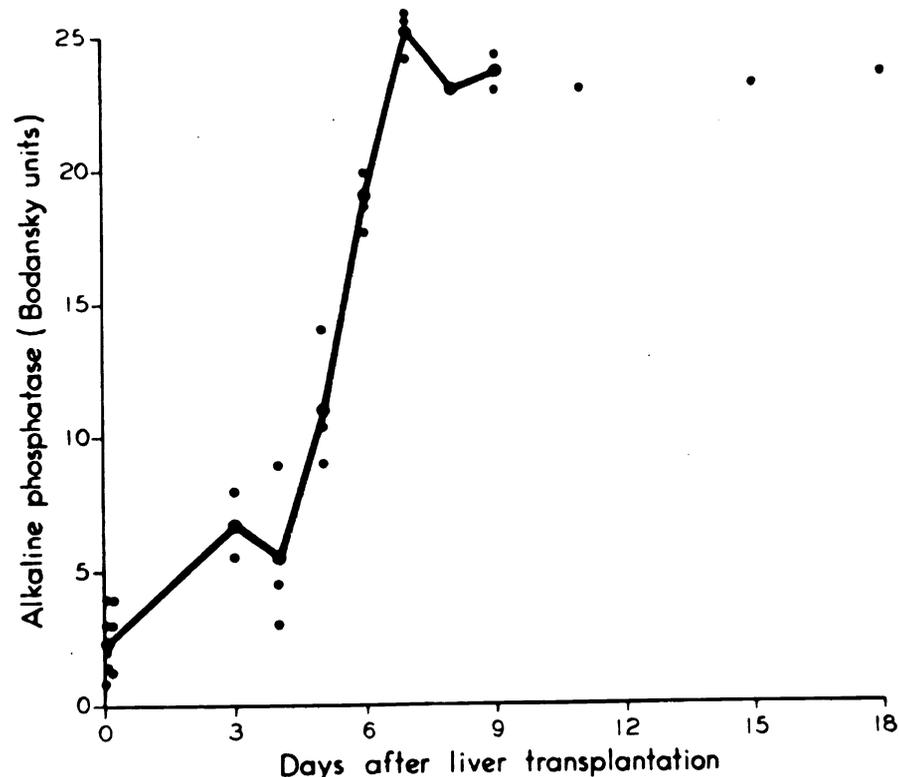


FIGURE 27. Serum alkaline phosphatase in untreated dogs after orthotopic liver transplantation. Elevations tend to occur at a slightly earlier time than those in serum bilirubin (by permission of *Surg., Gynec. & Obst.* (4)).

In the treated dog, hyperbilirubinemia did not appear for almost a month, and changes in the alkaline phosphatase and SGOT waxed and waned. The treated dog ultimately died after about 31 days—of a perforated gastric ulcer.

Histologic findings are also markedly changed. During the first month, destruction of hepatic parenchyma may be very minimal (Fig. 31). In some dogs, hepatocyte loss may occur, particularly around the central veins, but with little or no evidence of mononuclear cell invasion (Fig. 32), a variety of noncellular rejection comparable to that observed in some renal homografts.

Despite these encouraging observations, we were not able until about a year ago to obtain long-term survival after hepatic homotransplantation. More recently, we have had extended survival of animals. I will show you first some examples of the tissues obtained from these

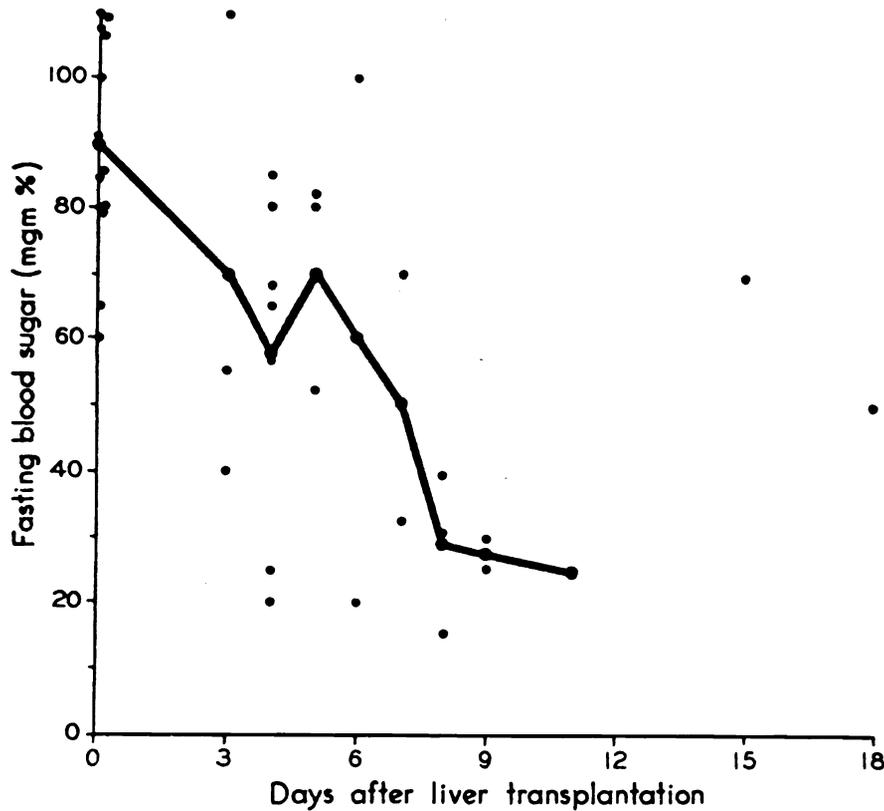


FIGURE 28. Changes in blood sugar in untreated dogs after orthotopic liver transplantation. Note the tendency to terminal hypoglycemia (by permission of *Surg., Gynec. & Obst.* (4)).

dogs, and then later mention the alterations in experimental protocol which preceded the improved results, hastening to add that we are not sure that the changes were responsible for the increased success.

Figure 33 shows a liver 34 days after transplantation into a dog which died of subacute bacterial endocarditis. He had essentially normal liver function. Histologically, there were some focal accumulations of round cells. Figure 34 represents a liver biopsy from a dog with good liver function after 60 days. Figure 35 shows a biopsy from a dog 120 days after operation. I apologize for the artifact in the portal area. There appears to be hypercellularity of the hepatic parenchyma, but those pathologists who have looked at this illustration do not believe it has any clear stigmata of rejection.

The late behavior of dogs after hepatic homotransplantation deserves special comment (7-10). In the past, several laboratories have

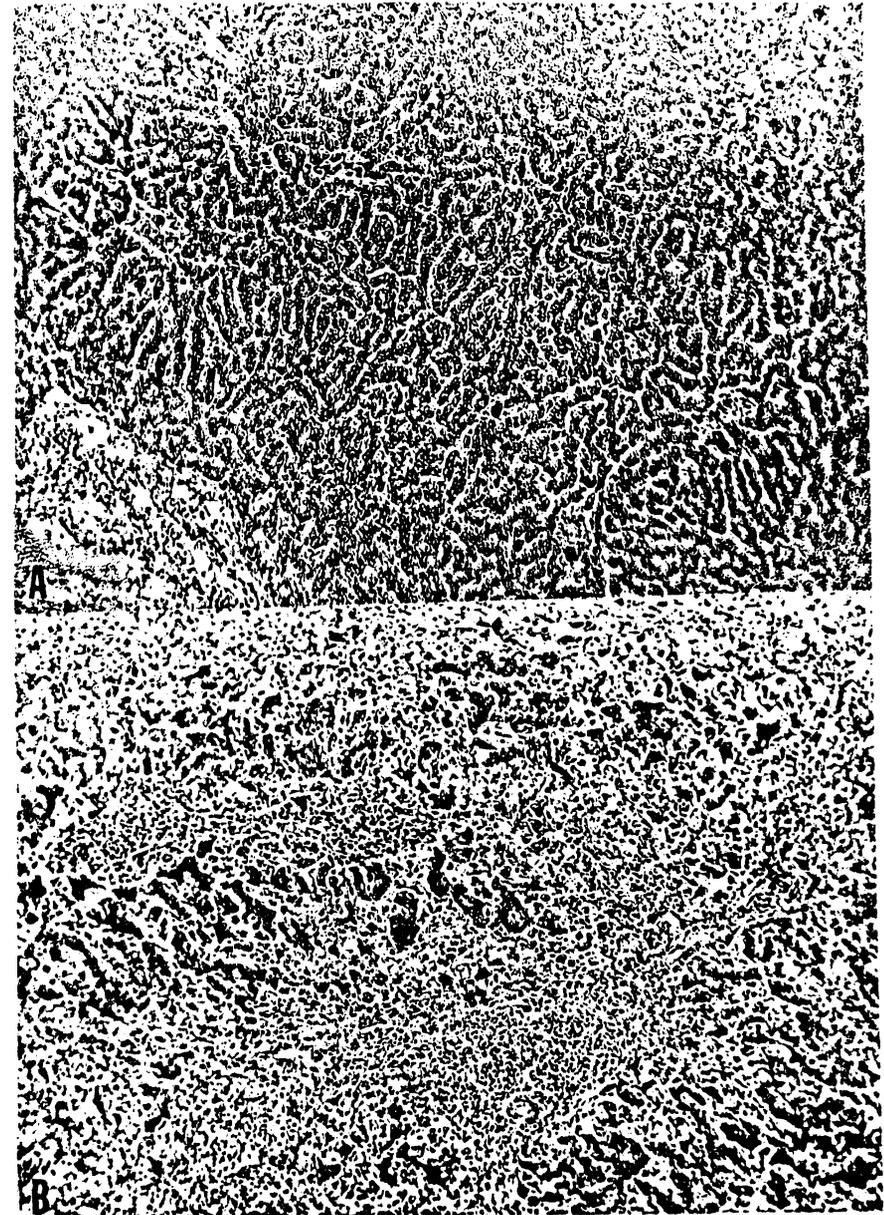


FIGURE 29. Stages in the rejection of an orthotopic canine liver homograft transplanted to an untreated recipient.

A: After 5 days. Architecture is intact but there are mononuclear cell infiltrates in the portal area (*lower left*).

B: After 13 days. Parenchymal pattern is disorganized and there is a massive cellular infiltrate. The periportal and centrilobular areas are affected most severely. (H & E $\times 60$). (By permission of *Surg. Clin. N.A.* 42:55, 1962).

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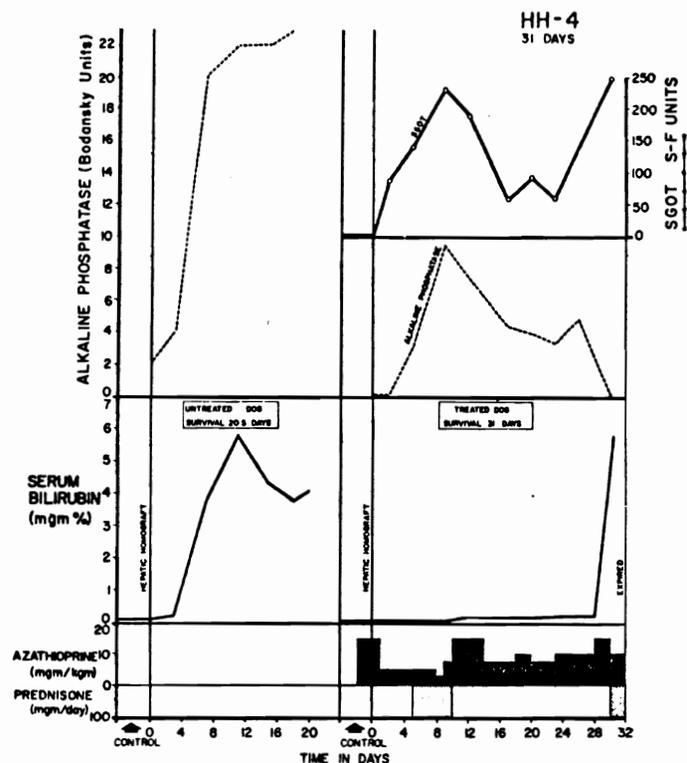


FIGURE 30. Contrasting courses of an untreated (left) and a treated (right) dog after orthotopic liver transplantation. Note the absence of early jaundice in the latter dog and the variations in alkaline phosphatase and SGOT (by permission of *Ann. Surg.* (13)).

demonstrated that some dogs which receive renal homografts develop a state of host-graft nonreactivity and that immuno-suppressive therapy can be discontinued with continuing function of the kidney. The explanation for this happy circumstance is unclear. The phenomenon is unpredictable even after a year or more, and the majority of animals challenged by withdrawal of therapy proceeded to reject their homograft.

Curiously, the development of host-graft nonreactivity appears to be a much more common event after orthotopic hepatic homotransplantation, possibly because a much greater antigenic mass is involved than with the kidney. To date, 5 dogs with orthotopic liver transplants have had all therapy stopped after 120 days (Fig. 36). There has been no delayed rejection in any of these animals in subsequent follow-up intervals of 1 to 5 months. In several, hepatic function has actually improved. If these animals continue to do well, it will be

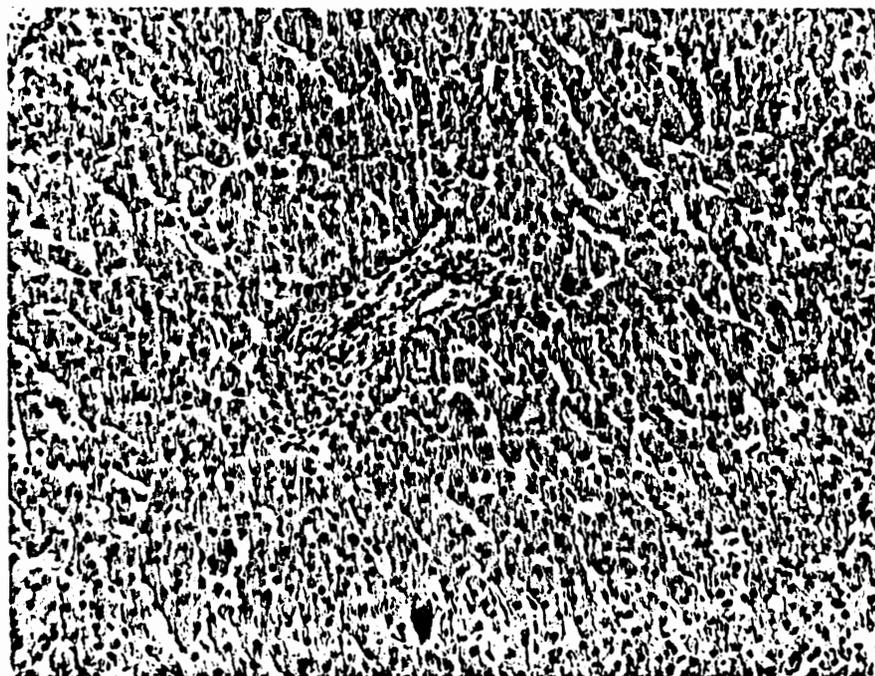


FIGURE 31. Homografted liver after 25 days' residence in host treated with azathioprine. Architecture is essentially normal (H & E $\times 80$).

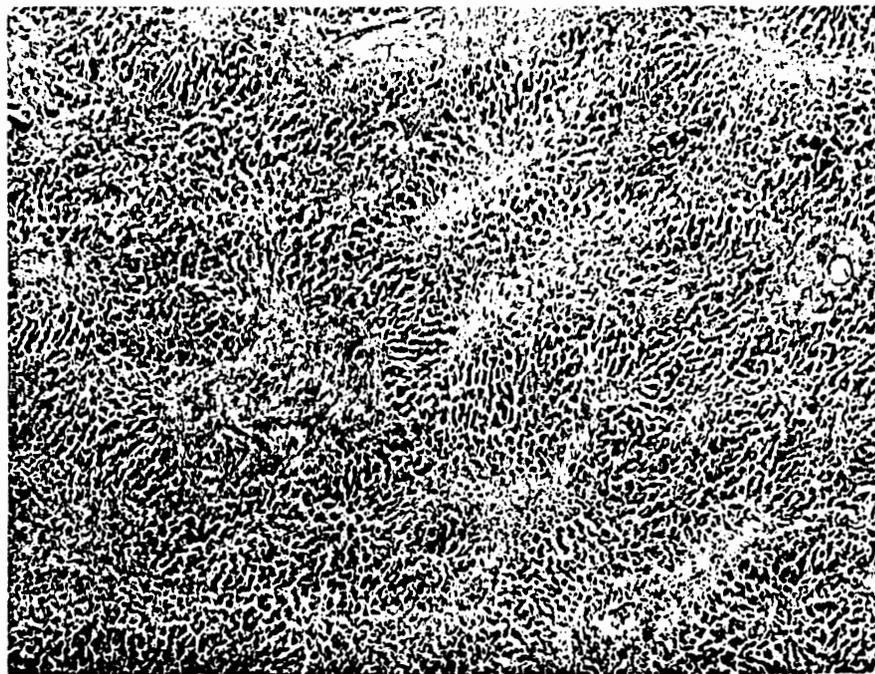


FIGURE 32. Homograft in dog treated with azathioprine after 31 days. Note hepato-

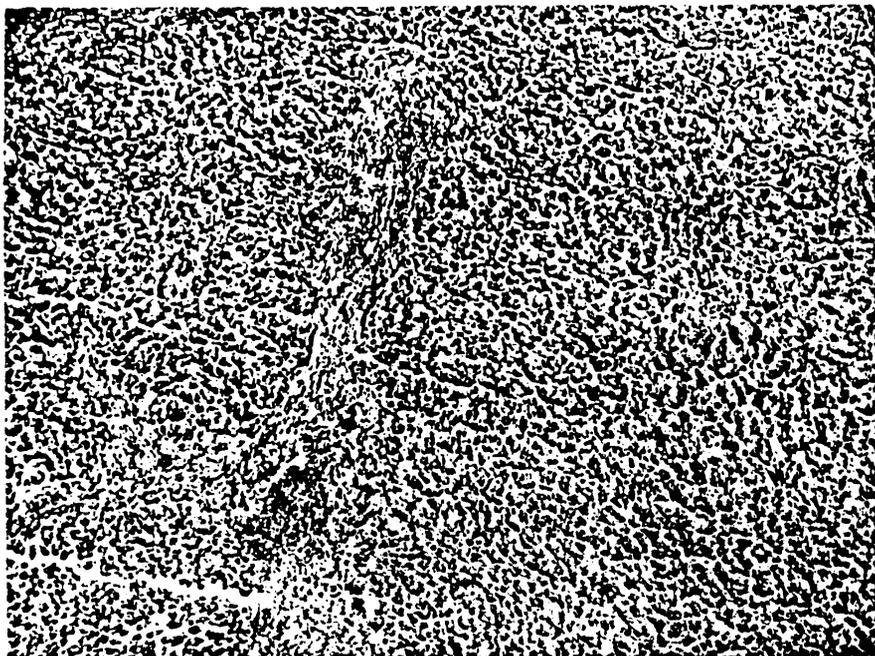


FIGURE 33. Orthotopic liver homograft after 34 days. The dog died of bacterial endocarditis. Note good preservation of general structure but with focal accumulations of mononuclear cells (H & E \times 32).

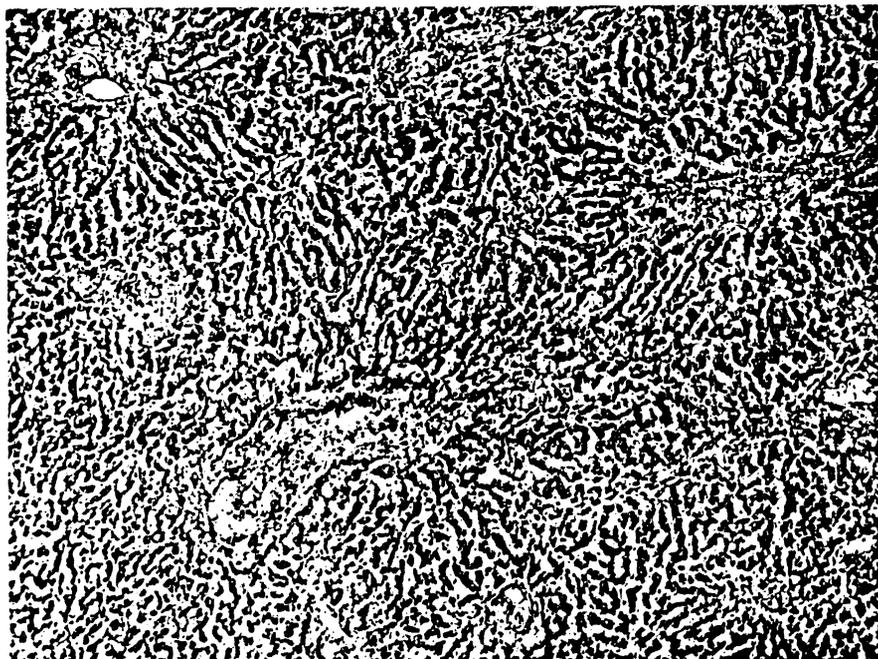


FIGURE 34. Orthotopic liver homograft after 60 days. Histologic alterations are

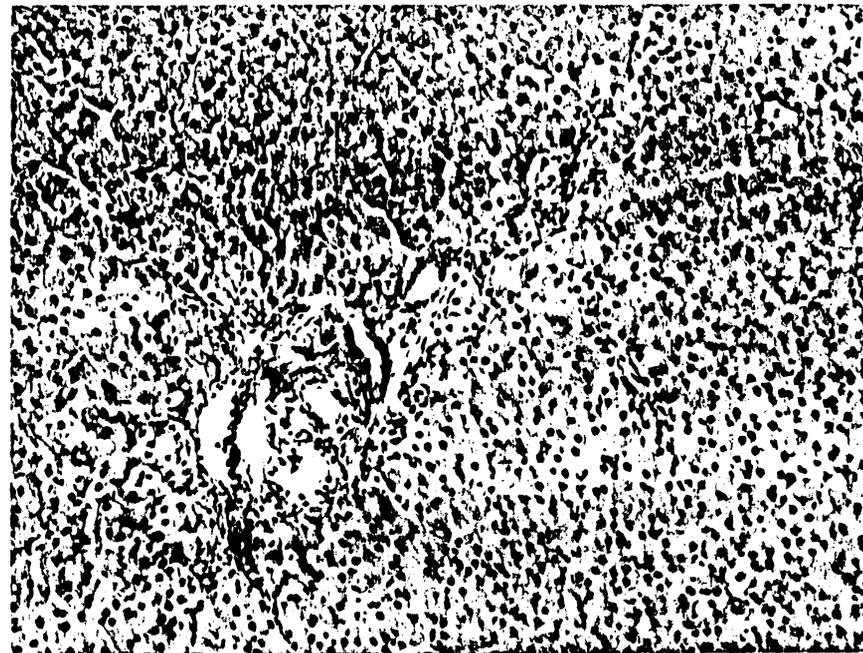


FIGURE 35. Biopsy from homograft after 120 days' residence in a recipient treated with azathioprine. The dog also received intermittent ^{35}S methionine. The cleft in the portal area is an artifact. This dog had immuno-suppression stopped at the time of biopsy without deterioration of hepatic function in the ensuing 5 months (H & E \times 80).

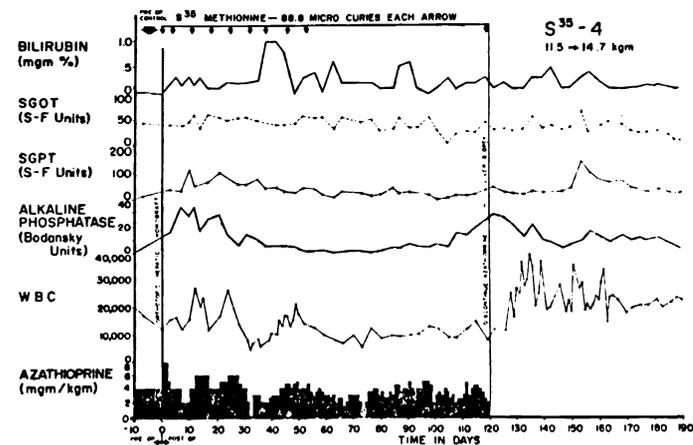


FIGURE 36. Course of a dog who received an orthotopic liver homograft in March, 1964. Note discontinuance of azathioprine therapy after 120 days. No evidence of rejection has been evident in the ensuing 5 months. During the early postoperative course the animal was given 90 microcuries ^{35}S every 5 days in 1.8 mg methionine. The 120-day biopsy from this animal is shown in Fig. 35 and his portrait in Fig. 37.



FIGURE 37. Dog S⁴. This animal is the longest survival after orthotopic liver homotransplantation. The operation was in March, 1964, and the dog is in good health 9 months later, all immuno-suppressive therapy having been stopped 120 days after operation.

another encouraging sign inasmuch as the late care of recipients after liver transplantation would seem to be simpler than with the kidney homotransplants. Figure 37 illustrates an animal more than 9 months after orthotopic liver transplantation. Except for his surgical scars it would be difficult to distinguish him from a normal, lively animal.

Earlier, I mentioned that some changes in experimental protocol were made just prior to the attainment of chronic survival after hepatic homotransplantation. These changes involve the addition of cold or radioactive methionine as an adjuvant therapeutic measure. When non-tagged methionine was used, a gram a day was given intravenously. Radioactive methionine was administered in another series

using 80 to 100 microcuries of ³⁵S in 1.8 mg of methionine every 5 days.

Whether or not the use of methionine had anything to do with the improvement of results is completely unproved and much more work will be required to demonstrate if the lipotropic substances are valuable. It is possible that general improvements in surgical technique and postoperative care are entirely responsible. Whatever the explanation, the fact is that considerably more than half of all dogs provided with orthotopic liver homografts can now be expected to live more than 25 days and approximately one-fourth will survive 100 days or longer.

Auxiliary Liver Transplantation

At this juncture I would like to go into some other problems of hepatic homotransplantation which are not so directly concerned with

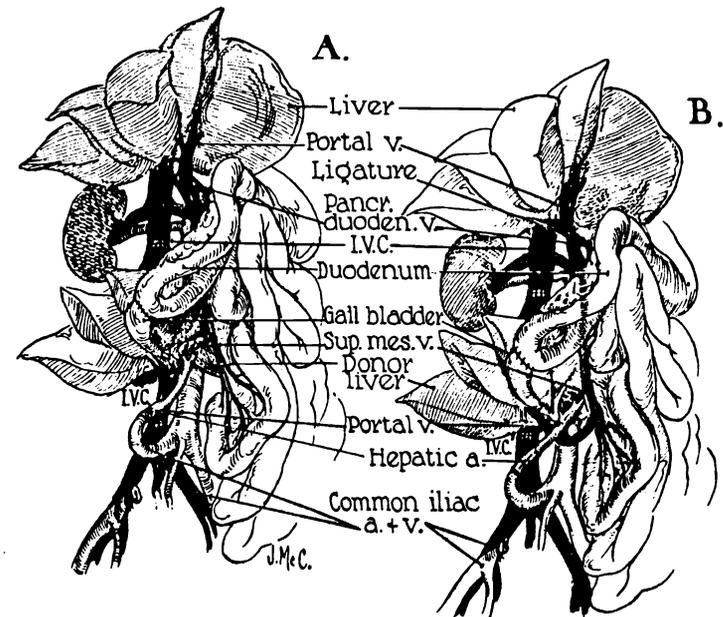


FIGURE 38. Auxiliary liver homotransplantation in the dog.

A: Transplantation by modification of Welch's technique. Note that the reconstituted portal blood supply is from the terminal inferior vena cava.

B: Modification of auxiliary liver transplantation in which portal blood supply is derived from the superior mesenteric vein. In order to obtain retrograde flow through the splanchnic system the recipient portal vein is ligated at the hilum of the liver. In this preparation most of the splanchnic flow passes through the auxiliary homograft (by permission of *Surg., Gynec. & Obst.* 120: June, 1965).

immunology but which have to do with the metabolic and nutritional requirements for successful transplantation of a second or "auxiliary" liver. These experiments were designed to test the potential clinical value of the preparation originally described by Welch, Goodrich and their associates (11,12). With this operation, the homograft is placed in an ectopic site in the right paravertebral gutter, leaving the recipient liver *in situ* (Fig. 38A).

With the Welch procedure, the homograft is fully vascularized. The hepatic artery is anastomosed to the aorta or iliac artery. Portal venous inflow is from the inferior vena cava (Fig. 38A,B), a situation comparable to that of portacaval transposition.

These animals were treated with azathioprine. Postoperatively, retrograde angiograms were obtained through the contralateral femoral artery and vein. There was good vascularization of the homograft (Fig. 39). In spite of this, the behavior of the auxiliary homograft was quite different from that observed in the orthotopic livers.

Figure 40 shows the changes in the gross structure of the liver. The homograft undergoes a remarkable symmetrical shrinkage, beginning within 2 weeks. The dog's own liver is not altered (13). Under



FIGURE 39. Angiographic study of auxiliary liver homograft 27 days postoperative.
 A: Hepatic arterial supply.
 B: Venous supply. Note excellent filling of small ramifications of both systems (by permission of *Ann. Surg.* (13)).

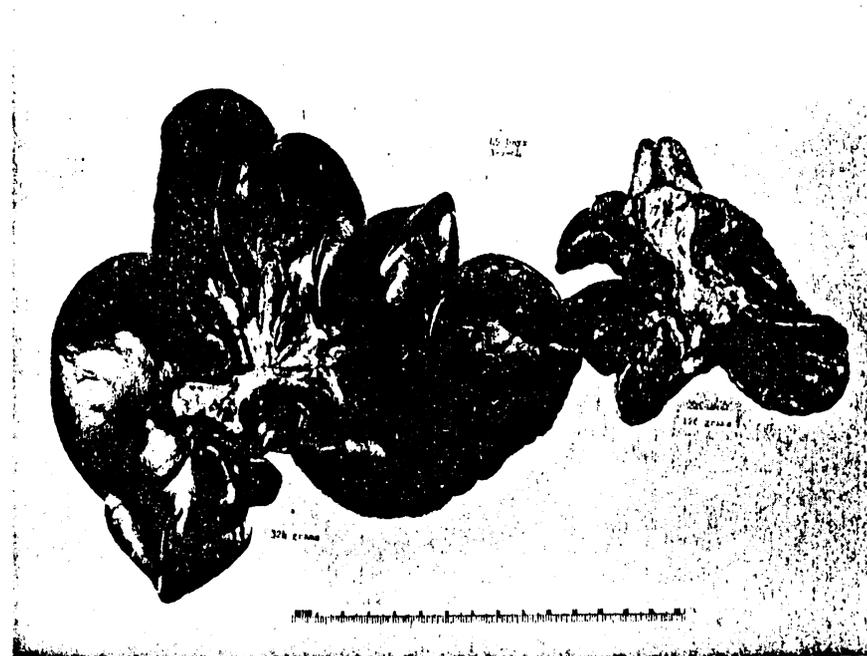


FIGURE 40. An auxiliary liver homograft (right) and the recipient dog's own liver (left) after revascularization by the method depicted in Fig. 38A. The specimens were obtained 45 days after transplantation. Note the extraordinary atrophy of the auxiliary homograft (by permission of *Ann. Surg.* (13)).

low-power microscopic study, it can be seen that the portal areas of the homotransplanted liver are compressed (Fig. 41). Hepatocytes have disappeared, but there is good preservation of the duct system. Reticulin collapse is widespread. Under higher power, one can see aggregates of round cells. There are large areas of hepatocyte loss which tend to be centrilobular in location (Fig. 42).

These atrophic auxiliary livers cannot provide life-sustaining function. In several experiments the recipient animal's own liver was removed from 25 to 35 days after transplantation. All of these animals died within 48 hours.

The remarkable shrinkage of the auxiliary livers in the foregoing experiments was discouraging in regard to any possible clinical application of the method. Since this acute atrophy had not been seen in orthotopic homografts, the possibility remained that this change was in some way caused by the abnormal blood supply of the extra liver.

Inasmuch as the auxiliary liver is revascularized by the same prin-

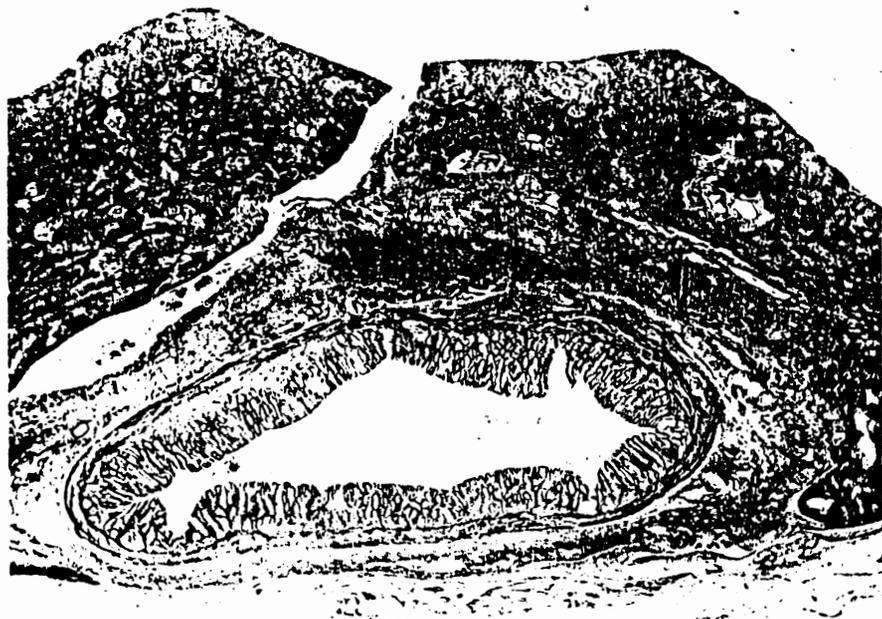


FIGURE 41. Low-power scan of an auxiliary dog liver vascularized as shown in Fig. 38A. Note the good preservation of the gallbladder and the apparent compression of the intrahepatic portal tracts. Mallory trichrome stain (by permission of *Ann. Surg.* (13)).

As with portacaval transposition, the metabolism was re-investigated of animals receiving transposition alone (14). In these dogs, it was observed that a remarkable decrease in hepatic glycogen content followed within 1 to 2 months after operation (Fig. 43). The total glycogen concentration was halved, and the TCA soluble (labile) glycogen was reduced by an average of 70 per cent. Despite a number of previous reports to the contrary, these findings indicated that the liver in dogs with transposition is not normal. The conclusion seemed justified that this method of reconstructing the blood supply placed the auxiliary liver at a physiologic disadvantage.

In order to test this concept, a new experiment with auxiliary transplantation was designed. The extra liver was placed in exactly the same position and arterialized in the same way as before. Instead of providing a venous inflow from the inferior vena cava, the portal vein was anastomosed to the superior mesenteric vein of the recipient animal (Fig. 38B). Proximal ligation of the recipient portal vein was then carried out so that most of the splanchnic flow was directed through

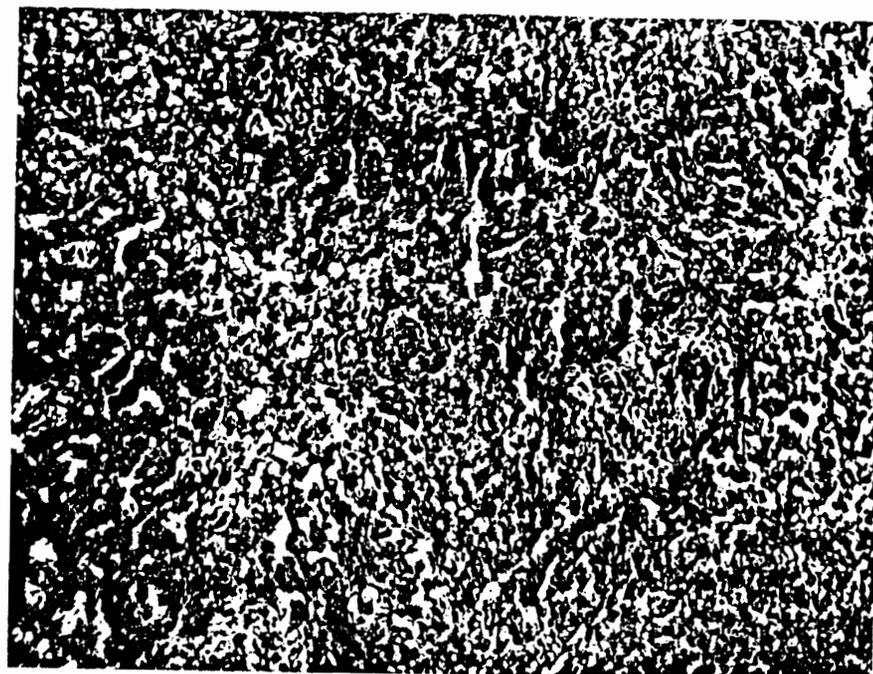


FIGURE 42. Auxiliary liver homograft after 30 days using vascularization shown in Fig. 38A. Note the diffuse loss of hepatocytes and the focal accumulation of mononuclear cells (H & E $\times 80$).

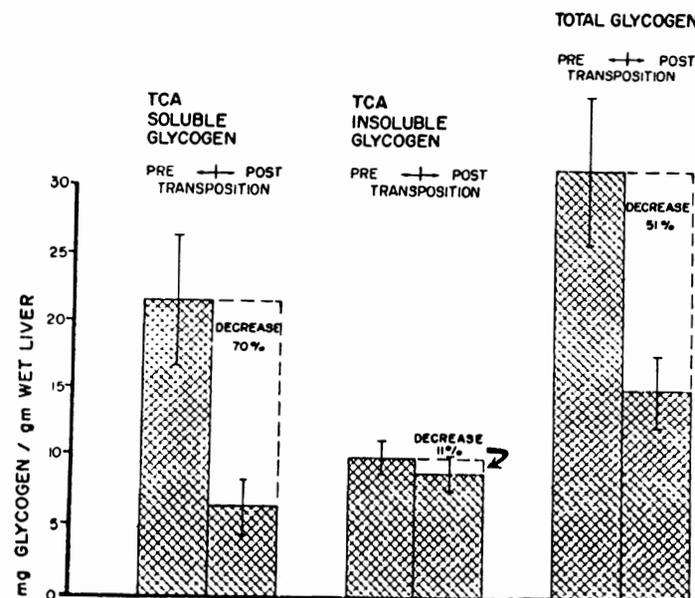


FIGURE 43. Mean changes in liver glycogen fractions of 17 dogs following transposition. Vertical lines represent ± 1 SE.



FIGURE 44. Operative venous angiogram of a dog vascularized as shown in Fig. 38B. The ligature around the portal vein is evident (arrow). The dog's own portal system has also filled with dye from collaterals. Note the large size of the homograft compared to the small dimensions of the dog's own liver (by permission of *Surg., Gynec. & Obst.* (14)).

the homograft (Fig. 44). It will be noted (Fig. 44) that the homograft now retains its large size and that the animal's own liver becomes atrophic. Thus, by altering the blood flow so that the homograft receives first exposure to venous return from the intestinal tract, the process of atrophy is reversed, the deleterious effect being manifest in the host liver. The weights of the autologous and homologous livers in this group of experiments are indicated in Table 14.

In 3 of these animals the ultimate test of homograft function was imposed by performance of host hepatectomy. All 3 animals woke promptly from anesthesia and lived for varying times thereafter. The longest survival was an animal who lived for 2 months after removal of his own liver, with a total survival of 126 days after the original homotransplantation (Fig. 45).

The later studies with auxiliary hepatic homotransplantation have done much to clarify the physiologic requirements for the employment

TABLE 14

Weights of Autologous and Homologous Livers after Transplantation

Dog #	Weight of Donor (kg)	Weight of Recipient (kg)	Total Survival (days)	Removal Host Liver	Survival after Hepatectomy (days)	Weight of Homograft at Autopsy (gm)	Weight of Host Liver at Autopsy or Hepatectomy (gm)
2	14.1	15.2	28	No	Not done	325	322
4	14.5	19.5	25	No	Not done	380	308
5	15.4	18.2	126	Yes	49	650	225
8	18.6	20.5	69	Yes	8	478	211
9	18.2	18.6	52	No	Not done	440	300
10	20.0	22.0	101	Yes	28	492	310

of an extra liver. When 2 livers are present, there is evidently competition for some metabolic substrate or other hepatotrophic factor. The liver which has first access to splanchnic flow operates at a physiologic advantage, whether this be the homograft or the host's own liver.

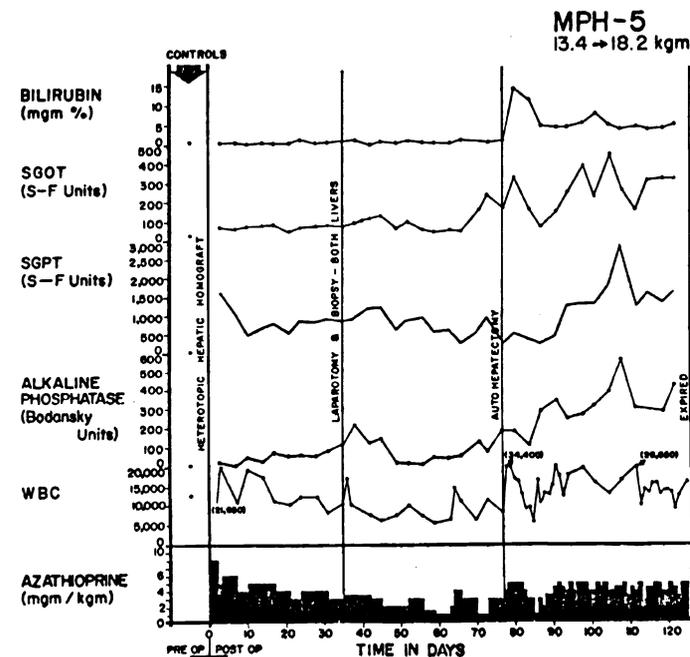


FIGURE 45. Clinical course of a dog vascularized as shown in Fig. 38B. Note the abrupt bilirubinemia which followed removal of the dog's own liver (autohepatectomy). After autologous hepatectomy, the dog lived for 49 days with sole dependence on the homograft, ultimately dying as the result of a wound dehiscence and evisceration which followed repeat biopsy (by permission of *Surg., Gynec. & Obst.* (14)).

Clinical Homotransplantation of the Liver

With the remaining time I would like to tell you something about the experience gained from human liver homotransplantation with emphasis on those unique lessons which could only be learned by clinical trial. We have done 5 cases (15) and single attempts have been carried out in Boston (9), Paris (14), and Minneapolis (17), respectively (Table 15); all but the Minneapolis case had orthotopic replacement and all had an ultimately unfavorable outcome.

One of the great problems in extrapolating the lesson of the laboratory to the clinical scene involves that of organ procurement. With the human case, it is, of course, necessary to use a cadaver homograft, the only alternative being a heterograft which we would not consider at this time. In order to get quality homografts from cadavers and to allow sufficient time for meticulous removal of the liver, we have used a method of postmortem extracorporeal perfusion (Fig. 46) which has several advantages (6).

One is that catheters can be placed in the femoral artery and vein of the recently deceased cadaver within a few minutes after death. By incorporation of a heat exchanger into the extracorporeal circuit, the corpse can be cooled as well as perfused and oxygenated. Dissection and removal of the liver can be carried out with this method without the tremendous urgency which would be necessary without some form of artificial circulation. By placing a clamp on the terminal thoracic aorta, selective perfusion of the lower half of the body is obtained, but venous return can be retrieved from the entire cadaver (Fig. 46). Using this method, functioning livers were obtained in 4 of the 5 Colorado cases. Although substantial ischemic injury occurred in each instance, the degree of acceptable damage seemed greater than in the dog, in which species a self-perpetuating ischemic injury termed "outflow block" is a much more serious problem than in the human.

Another species difference concerns the need for portal decompression. The dog can tolerate portal occlusion for only a few minutes and failure to use an effective external bypass during placement of the liver (Fig. 22) inevitably leads to failure. In the first case of human clinical transplantation it was attempted to use an external bypass from both the splanchnic and the inferior vena caval pools (Fig. 47). The splanchnic bypass failed almost immediately without harmful effects. In the subsequent cases only a single bypass from the inferior vena cava was used. It is conceivable that even the vena caval bypass is unnecessary but we have not dared to test this possibility in practical application.

TABLE 15
World Experience with Clinical Homotransplantation of the Liver

Case	Reference	Date of Transplant	Donor-Recipient Age-Sex	Donor-Recipient Blood Types	Recipient Disease	Cause of Donor Death	Donor Death to Revascularization	Survival	Cause of Death
1 (Denver)	1, 13, 15	3-1-63	3M-3M	A+—A+	Biliary atresia	Cardiac arrest during craniotomy	420 min	4 hr	Operative hemorrhage
2 (Denver)	1, 13, 15	5-5-63	55M-48M	A+—A+	Cirrhosis & hepatoma	Terminal brain tumor	152 min	22 days	Pulmonary emboli
3 (Denver)	1, 13, 15	6-24-63	69M-67M	O—O+	Cholangio carcinoma	Stroke	192 min	7½ days	Pulmonary emboli; GI bleeding
4 (Denver)	1, 13, 15	7-16-63	73M-52M	O+—A-	Cirrhosis & hepatoma	Coronary	174 min	6½ days	Pulmonary emboli; congestive heart failure
5 (Denver)	1, 13, 15	10-4-63	64M-20F	O+—O+	Cirrhosis & hepatoma	GSW head (suicide)	164 min	23 days	Common duct necrosis; bile peritonitis; ? rejection; hemorrhagic diathesis
6 (Moore)	9	9-14-63	Not known	Not known	Metastatic colon carcinoma	GSW head (homicide)	Not known	12 days	Liver failure
7 (Demirleau)	16	Jan. 1954	71M-75M	O—A	Metastatic colon carcinoma	Bilateral gangrene of legs	180 min	3 hr	Operative hemorrhage
8* (Abrolon)	17	11-3-64	2F-1M	A+—O-	Biliary atresia	Cardiac failure post-pump	About 4 hr	13 days	Common duct necrosis; bile peritonitis; septicemia

* Heterotopic transplant to left iliac system. Donor died while on pump oxygenator support, and perfusion was continued until liver was removed. Period of completely absent circulation during insertion of liver was 37 min.

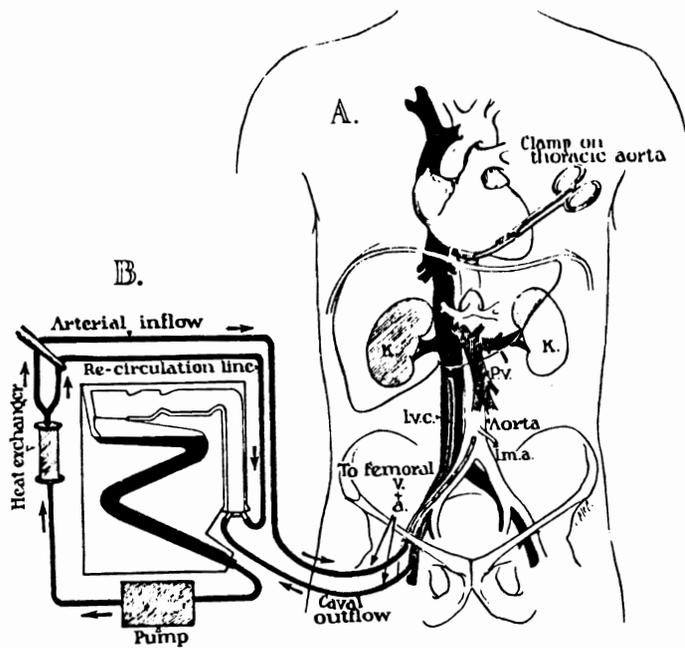


FIGURE 46. Method of extracorporeal cadaveric perfusion used for procurement of liver homografts. The cannulas are inserted into the abdominal aorta and vena cava shortly after death. A heat exchanger is incorporated into the pump oxygenator circuit. Note the clamp on the lower thoracic aorta which is used to increase perfusion to the lower half of the body. The pump is primed with electrolyte glucose solution to which heparin and procaine are added (by permission of W. B. Saunders Company, Experience in Renal Transplantation, 1964).

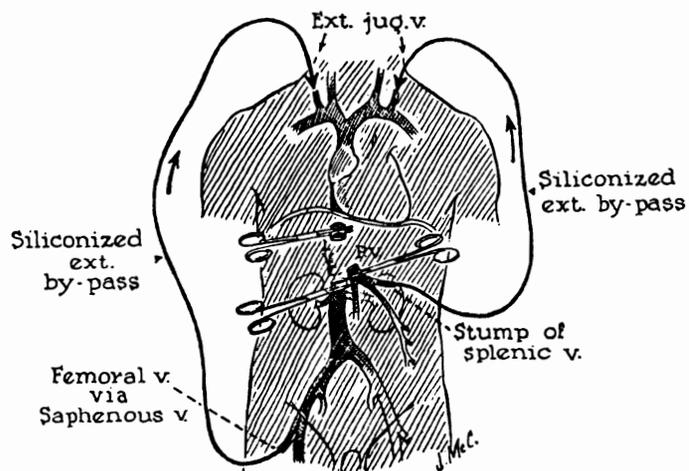


FIGURE 47. External bypass system used for clinical orthotopic transplantation. It was found that the shunt connecting the splanchnic to the jugular systems was unnecessary since portal occlusion was well tolerated in the human (by permission of W. B. Saunders Company, Experience in Renal Transplantation, 1964).

TRANSPLANTATION OF THE LIVER

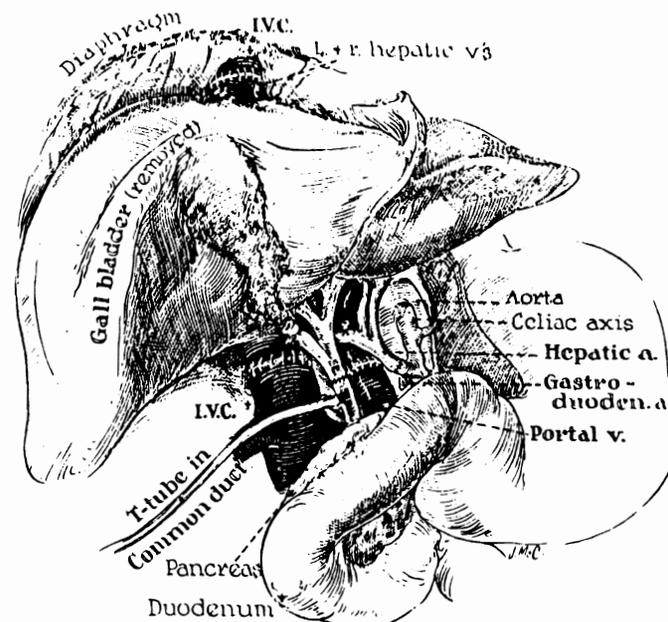


FIGURE 48. Completed orthotopic clinical homotransplantation. The T-tube is placed through a stab wound in the recipient common duct (by permission of *Ann. Surg.* (13)).

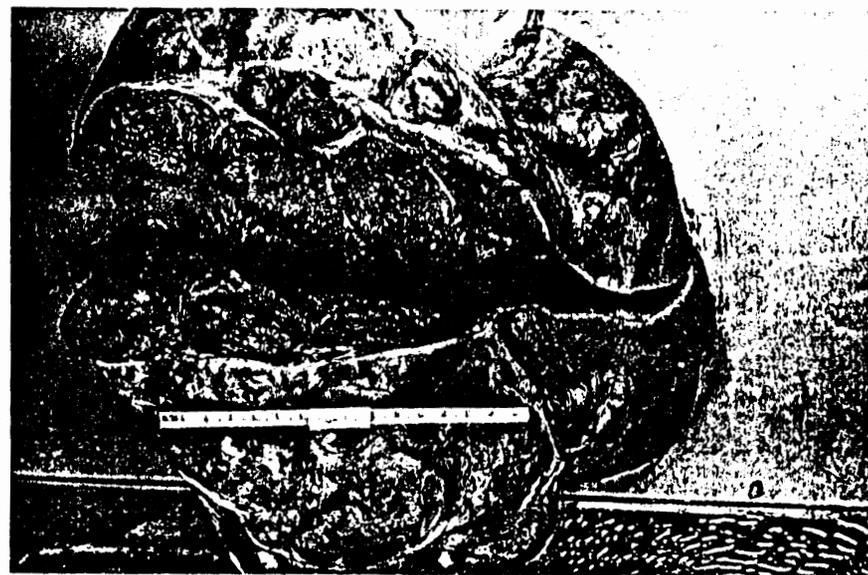


FIGURE 49. Enormously enlarged liver of Patient 5 in the University of Colorado series of liver transplants. The tumor was a hepatoma which developed in a liver with postnecrotic cirrhosis.

HETEROGRAFTING PROBLEMS AND LIVER TRANSPLANTATION

Figure 48 shows the liver homograft in place. The anatomic reconstruction of structures passing to and from the liver is normal.

The indications for homotransplantation of the liver are listed in Table 15. In 4 of the 5 Denver cases there was a primary intrahepatic malignancy, either a hepatoma or a cholangio-carcinoma. The massiveness of some of these tumors (Fig. 49) has posed a serious technical problem, in some cases the livers being as much as 4 or 5 times normal size. In addition, all of the cases treated in Denver had portal hypertension, making removal of the diseased organ exceedingly difficult.

After operation there was early evidence of severe ischemic damage to the homograft. Sharp rises in SGOT, SGPT and LDH occurred within the first 24 hours. There was deepening of jaundice which progressed for several days (Fig. 50). The differentiation of this technical injury from that of rejection presented a diagnostic dilemma. Fortunately, there was reversal of the early malfunction in 4 of the 5 Colorado cases (Fig. 50). The 4 patients who survived the operative procedure lived from 6½ to 23 days. In 3 of these patients the direct or an important contributory cause of death was pulmonary embolization.

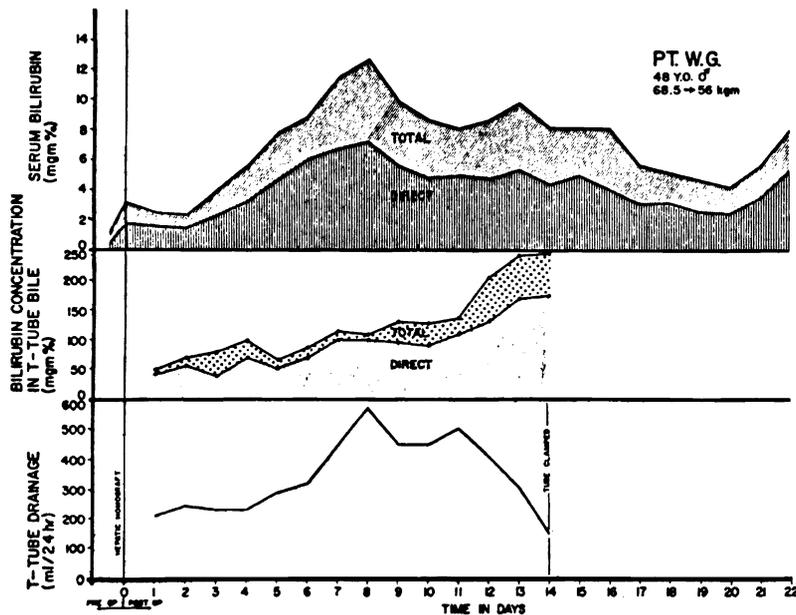


FIGURE 50. Course of Patient 2 of the Colorado series showing relationships of serum bilirubin, T-tube drainage volume and bilirubin content of T-tube bile. Note temporary worsening of jaundice after transplantation (by permission of *Ann. Surg.* (13)).

TRANSPLANTATION OF THE LIVER

Perhaps the most important information accruing from the clinical experience was an increased understanding of the changes in coagulation which occur during and after transplantation. From the animal studies, it had been appreciated that a fibrinolytic crisis commonly occurred during and just after insertion of the homograft, and for this reason the first 4 human recipients were treated intraoperatively with epsilon-aminocaproic acid (EACA) and purified fibrinogen. What had been missed in the laboratory experiments was that a phase of rebound hypercoagulability followed during the next few days. It seems probable that by our aggressive therapy of the phase of fibrinolysis we probably precipitated or potentiated the subsequent clotting phase. Our second, third and fourth patients thrombosed their iliac or terminal inferior vena caval systems and subsequently had multiple pulmonary emboli.

The homografts obtained at autopsy in these cases were quite well preserved. Figure 51 shows the liver of a patient after 6½ days. There is very little cellular infiltrate and the hepatocytes were filled with glycogen. Figure 52 is the liver from a patient after 21 days, again showing virtually no evidence of rejection. This liver had evidence

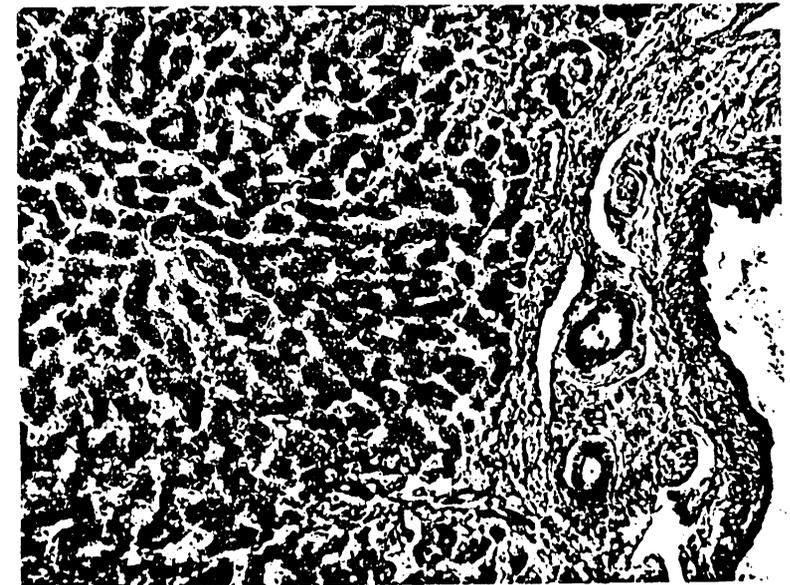


FIGURE 51. Homograft from Patient 4 of the Colorado series 6.5 days postoperatively. The liver is almost normal. Note thickening of artery in portal triad. PAS stain $\times 180$ (by permission of *Ann. Surg.* (13)).

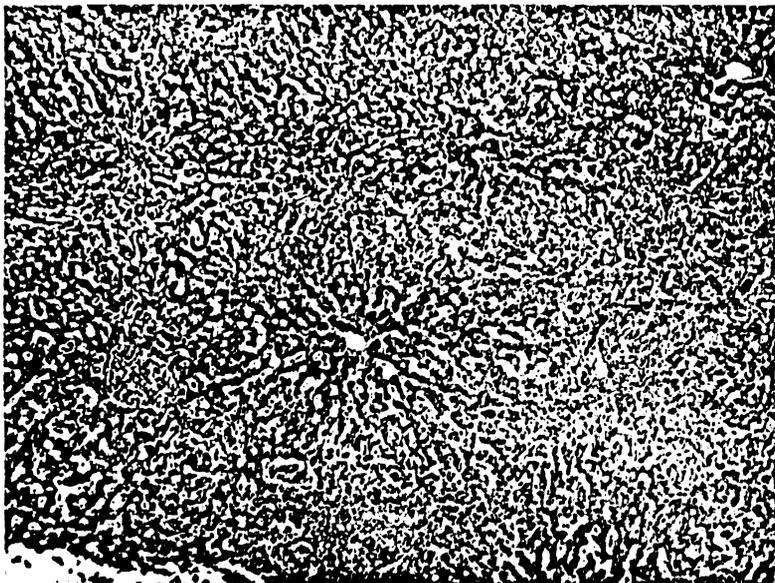


FIGURE 52. Liver homograft from Patient 2 of the Colorado series 21 days after operation. Note excellent preservation of architecture. The few cells in the portal triads are principally neutrophils. Note the dark-staining bile accumulated in the canaliculi (by permission of *Surg., Gynec. & Obst.* (15)).

of cholestasis, for which there was no clear explanation inasmuch as the extrahepatic collecting system was open.

In 7 of the 8 clinical transplants performed thus far, the diseased recipient liver was removed. Absolon (17) at the University of Minnesota has attempted the only auxiliary liver transplant to date. This case is of special interest since this type of procedure may be more applicable for the treatment of patients with benign disease. The homograft used was subjected to a minimum of anoxia since the donor patient died while on cardiopulmonary bypass. There was unequivocal evidence of homograft function postoperatively. The patient, a 13-month-old child who had biliary atresia, had clearing of the hyperbilirubinemia from 22 mg% to 5 mg% prior to death. No evidence of rejection was present at autopsy. The cause of failure was necrosis of the homograft common duct and septicemia.

Summary

Progress in transplantation of the liver has been rapid during the past few years. A number of long-term survivals after orthotopic trans-

plantation in dogs attest to this fact, the greatest postoperative longevity now being in excess of 9 months. The reasons for improved results in the laboratory have not been clarified thoroughly although the adjuvant use of lipotropic substances may have contributed. Potentiation of homograft survival in dogs after orthotopic transplantation can be achieved with approximately the same regularity as after renal homotransplantation. A state of host-graft nonreactivity appears to be established earlier after liver transplantation. Five dogs have had discontinuance of all immuno-suppressive therapy after 120 days and late rejection has not been observed in any.

Metabolic factors appear to be of critical importance in obtaining good results after auxiliary liver homotransplantation. When two livers are present, that organ which receives first exposure to splanchnic blood flow operates at a physiologic advantage, retaining its normal size and being capable of life-sustaining function. The other organ undergoes atrophy, even when the portal venous inflow is replaced from systemic sources. The livers appear to be in competition for some metabolic or hepatotropic substrate which is present in the portal blood.

Eight attempts have been made at clinical homotransplantation, all with an unfavorable outcome. The human liver seems more resistant to ischemia than that of the dog so that the problems of preservation and storage may not be so critical. Changes in the coagulation mechanism were observed with regularity in the clinical cases. There is an initial phase of intraoperative fibrinolysis which has resulted in fatal hemorrhage in 2 of the 8 cases in the world experience followed by equally dangerous stages of hypercoagulability which has caused or been a direct contributory factor in the death of 3 additional patients. A critical problem in future attempts at liver homotransplantation will be proper management of coagulation control.

DISCUSSION

CHAIRMAN MERRILL: In addition to previous participants, we will be joined by 2 other speakers: Dr. David Hume, Professor of Surgery of the Medical College of Virginia, and Dr. Crosnier from the Hôpital Necker in Paris, who has been working with the transplant group under Professor Hamburger.

I should like to begin by asking first Dr. Hume, then Dr. Crosnier, to make a few brief statements about their experiences which have been somewhat different from the one I related to you. Dr. Hume has told me that he would like to rebut almost everything I said to you! I offered him equal time, but he said he could say everything

he had to say in exactly 7 minutes, and so I will ask him to do just that.

Hume's Transplantation Experience

DR. HUME: That is not really quite the way it happened. I said I disagreed with only about half of what was said. One thing that Dr. Merrill brought up I do not quite understand: that is, about the platelets. I do not understand how platelets from a variety of donors, none of whom donated the kidney transplant, can sensitize the recipient of the transplant to his transplanted kidney. It is irrelevant that platelets share antigens with kidneys—kidneys themselves do not sensitize homograft recipients to kidneys from indifferent donors. We have given multiple platelet packs representing dozens of bottles of blood, multiple white cell packs, multiple fresh walking warm donor blood transfusions, prior to kidney transplants from other donors to multiple patients with kidney transplants without difficulty in any case, and with no evidence of sensitization.

The second thing that Dr. Merrill touched on which particularly has interested us was the early detection of threatened rejection. We have utilized everything we could think of to demonstrate rejection at an early stage, including the serum LDH, urinary LDH, LDH isozymes, changes in renal size as demonstrated roentgenographically by clips on the kidney, clinical signs, lymphocytes in the urine, antibody-forming activity in the peripheral lymphocytes, creatinine clearances, blood chemistries, renograms, scans, intravenous pyelograms, etc. Of all of these, the item that tends to give us the first sign of rejection in the majority of cases is a rise in BUN. This is not as illogical as it might seem, because, in the dog, the BUN goes up before there is massive round cell infiltration, before round cells appear in the lumina of the kidney tubules or in the urine, and before renal blood flow changes. Presumably, this occurs because tubular damage is present early in rejection and urea back-diffuses through the damaged tubule: a rise in the BUN not infrequently occurs without change in the serum creatinine. Since we have begun to treat the earliest BUN rise with an increase in immuno-suppressive agents, we find we can often prevent the appearance of the other stigmata of rejection. Dr. Merrill, if he were given a chance, would say that this is only because we are treating a number of BUN elevations that did not foretell threatened rejection. We have many control data, however, on patients whose BUN elevations were not treated promptly who then developed the full-blown picture of rejection.

Dr. Merrill touched on splenectomy. We have done splenectomies

in 30 of 52 patients. We started doing this because we felt that, by taking out the spleen, the white count and the platelet count would go up, and we could use more azathioprine and get better immuno-suppression without leukopenia or thrombocytopenia. We do not take out the spleen any more, and have not in about the last 18 cases. The reason for this is that it did not prove to be possible to give more azathioprine to splenectomized patients than to nonsplenectomized patients. Furthermore, an analysis of those patients who have had virtually no rejection at all for at least 12 months after the kidney transplant reveals that there are approximately equal numbers with the spleen out and with it in, and this likewise proved to be the case in those patients who rapidly rejected the graft. The splenectomized patients often show elevated platelet counts, and thrombosis is much more common in these patients. Of 6 pulmonary infarcts, 5 occurred in splenectomized patients. Since no benefits derived from splenectomy and since pulmonary emboli and infection occurred more often in these patients, we have stopped doing splenectomy.

Local irradiation (which has been mentioned, and which may not seem to a casual observer to be a very logical step to take) was used clinically after much experimental work indicated that local irradiation of the kidney was capable of prolonging renal homograft survival in dogs given no other treatment. It was possible to show reversal of BUN rise and to abolish the typical second-set phenomenon when the primary transplant of the dog was irradiated even when no radiation or other treatment was given to the second transplant.

I should like to say a word about hypertension in children. Here again I was unable to follow Dr. Merrill's logic. I do not see how putting an adult kidney in a child can produce hypertension by somehow producing a disproportion between cardiac output and renal mass. If the output of the heart were distributed over a larger capillary bed, one would expect a fall in blood pressure. If the blood pressure of the child is sufficient, the flow through the adult kidney should be the same as in the adult, provided that an adequate arterial anastomosis has been created. As a matter of fact, we have not seen hypertension in our children with renal homotransplants (unless they were rejecting), so it does not necessarily have to occur. We have seen hypertension developing in transplanted patients, when chronic rejection is occurring, and when the arterial lesion that has been demonstrated gets underway. The hypertension which Dr. Merrill has seen may be related to this rather than to any change in circulation, or to the sensitivity of children to prednisone and salt retention, which they seem to demonstrate to a greater degree than some adult patients.

With respect to donor risk, I was interested to hear Dr. Merrill's

figures. I hasten to emphasize that donor risk was investigated prior to the utilization of living donors and not just retrospectively. Dr. George Prout of our school contacted insurance companies relative to the risk of life with 1 kidney. He was told that the removal of 1 kidney in otherwise normal adult patients did not detract significantly from longevity, was not a reason to deny insurance, and was not accompanied by an increase in premium.

With respect to the use of the term "tolerance" in dogs or patients with kidney transplants, I should like to say that Dr. Merrill's colleagues have accumulated data which seem to me to demonstrate that tolerance has not occurred in the dog bearing a renal homotransplant and treated with azathioprine. If you transplant the second kidney from the original donor into a dog bearing a "successful" kidney transplant either on azathioprine or off azathioprine, then the second kidney promptly is rejected, while the first kidney is maintained.

This certainly would suggest that the principal reason for the acceptance of the first kidney is something that happened to the kidney, more than something that happened to the host. Probably what occurred is adaptation rather than tolerance. Furthermore, we have noted threatened rejection many months after transplantation in 2 patients who had previously had virtually no threatened rejection at all. One patient was 17 months from transplantation and the other 22 months, and in each instance the threatened rejection followed a reduction of azathioprine dosage and was reversed by an increase in azathioprine and prednisone. It did not appear, therefore, that tolerance had occurred even after this relatively prolonged period of time.

We have had some interesting experiences with second transplants. In 5 instances second transplants have been carried out in patients who rejected their first, and all of the second transplants have worked. There has been no rejection in any second transplant.

Our oldest second transplant is 15 months. This patient seems to be completely satisfactory. He is working as a plumber. His first transplant was rejected in 2 months, in spite of all attempts to keep it from being rejected. A second rather interesting patient is one who had a violent rejection of a cadaver transplant at 4 days, and has a second cadaver transplant which is 6 weeks now and doing nicely.

I should like to make 2 more comments, neither of which specifically relates to Dr. Merrill's presentation. The first of these has to do with a group of patients in whom we have done double-transplants; that is to say, the 2 kidneys of the cadaver are removed and one is transplanted into each of 2 patients. We have done 3 such pairs. All 6 kidneys are working well though the recipients have been vastly different. In some instances the recipient was a young child, in another

50 years old. In some instances the recipient had rejected a transplant previously. So far, in all of these 3 pairs, the transplant is performing in a similar manner, though of course one cannot draw definitive conclusions from such a small experience. If one patient starts to get a rejection at 30 days, the other one of the pair tends to do the same thing, a day or two later. This suggests that perhaps the antigen is equally as important as the immunologic capabilities of the host. We plan to do more experiments on dogs to prove or disprove this.

Finally, I would like to summarize our results at the present time. They are as follows: counting all non-twin homotransplants we have done since the inception of our program August 7, 1962, including the patients who got total body radiation, cadaver transplants, non-related volunteers (of whom we did 4 but which we do not do any more), 62 per cent of all cases are surviving at the present time. It is important to qualify survival figures by the period of time covered, because if one were to take the last 7 months in our series, for example, the survival is 93 per cent, which obviously is not what it will be some months hence. Of all the patients we transplanted in the first year of our experience, 44 per cent are living now, 1 to 2 years after transplantation. At the present time, 70 per cent of our patients are more than 6 months; 50 per cent are more than 9 months, and 2 are more than 2 years.

In the cadaver donor group, 65 per cent of all patients are surviving. Why the cadaver results are as good as they are, I do not know, but I think that it may be in part that many of the cadaver transplants were second transplants, and these seem somehow to enjoy privileges not accorded the first transplant.

CHAIRMAN MERRILL: Thank you very much. I think I have a reason for Dr. Hume's good survival in cadaver series: I think his patients are afraid to reject.

DR. STARZL: Dr. Hume has taken a very positive position about this state of post-graft nonreactivity, which he has said he believes is due entirely to something that is happening in the graft.

CHAIRMAN MERRILL: That is a liberal translation.

DR. STARZL: I believe that is his position.

DR. HUME: I thought it was probably more closely related to something happening in the graft than to alterations which could be called tolerance in the host.

DR. STARZL: I agree with you, but I would like to ask you this: You have this group of patients that have a second homograft, and you have implied perhaps that something has happened as the consequence of the first graft. Obviously, whatever happened, happened to the recipient, not to the graft, because you have taken the graft out. Is this not at variance with your belief that the alteration is in the graft, but not in the host?

DR. HUME: I would not say that that was necessarily incompatible with the theory of adaptation. After all, one could speculate that some antigen-antibody complex may have been formed with the first graft, which somehow partially coated and protected the graft from rejection.

This same complex may be floating around at the time of the second graft and be able to coat and protect it better than it did in the case of the first graft. In any event, tolerance certainly does not enter the picture with the second grafts because tolerance should be specific for the tissues of the first kidney donor only. I further base the thought about tolerance on the idea that there has not been any demonstration of tolerance that I know of with any kidney transplant, although attempts have been made to demonstrate it.

CHAIRMAN MERRILL: Perhaps we are mincing words about tolerance. What I mean by tolerance—and I am not entirely sure what anybody else means by it—is the fact that the graft is in place in the host for a considerable period of time. In this sense the host has tolerated it. Toleration perhaps is a better word than tolerance. What the mechanisms are of this in the patients is still a question.

I would like to ask Dr. Simonsen and Dr. Lawrence what they would predict has happened to either the host or the graft in a dog which has tolerated a kidney but rejected a skin graft or another kidney from that same donor. Dr. Simonsen, would you like to comment on that?

DR. SIMONSEN: What you have in mind are experiments in which a dog gets a first kidney graft, retains it; gets the second kidney from the same donor, rejects the second but retains the first one (1,2). Is that correct?

CHAIRMAN MERRILL: Yes.

DR. SIMONSEN: The question, then, is what this can be, because obviously it is not tolerance alone. Dr. Hume mentioned adaptation of the graft. I am not quite sure, in fact, what he means by adaptation.

I would have understood better had he said enhancement. We do know that specific antibodies sometimes can prolong the life of a homograft. This mechanism might be involved in these particular dogs.

But as an adaptation hypothesis, I presume that it would imply that the first kidney had lost some of its antigens. This does not seem very likely, and could at any rate be put to a critical test only by re-grafting the first kidney to another dog isogenetic with the first host. This would require identical twins. Or inbred dogs, which are not yet available.

DR. HUME: What I meant was adaptation as defined by Woodruff; namely, that some change has occurred in the graft during residence in the host, which permits the specific graft to survive, while other grafts from the same donor are rejected. Isn't that the way he defined it?

DR. SIMONSEN: As you say he defines it, it would also comprise an efferent enhancement mechanism.

I would still presume that the first kidney survived initially because immuno-suppressive treatment facilitated development of tolerance, as I described it in my talk. However, that state of tolerance cannot have been complete in these dogs, and must in the main have vanished by the time the second kidney was grafted.

When tolerance broke down, antibodies presumably were formed; and I could well believe that these would be predominantly 7S antibodies which may have attached themselves to the first kidney and provided a protective coat against cellular immunity and against 19S antibodies.

Grafting of the second kidney, which has been done without renewed immuno-suppressive treatment, may have boosted production of both 19S and cellular immunity. The second kidney which, contrary to the first one, has not been coated first with 7S antibodies, therefore succumbs more readily.

If such a mechanism operates, it would be a self-enhancement of efferent type. But it is all rather free speculation, and the phenomenon is very difficult, if at all possible, to analyze adequately without access to inbred material.

CHAIRMAN MERRILL: There is one experiment which demonstrates that at least no major antigenic change has occurred. This is the experiment in which the homograft which has survived well in dogs is removed and put back into the original donor, where it continues to

survive well. In this sense, at least, the original donor recognizes it as his own antigen and apparently therefore does not reject it.

Might we now ask Dr. Lawrence how he would explain this phenomenon.

DR. LAWRENCE: Your last comment, that perhaps some of the antigenic constituents of the recipient were percolating through that kidney and had coated it, perhaps reduced its foreignness to this extent. It had some similarity to the skin graft on brain experiment, where a piece of untraumatized tissue *in situ* is not being recognized as foreign. You do not get into any difficulty until it is injured in some way.

I should think in terms of the lymphatics from the transplanted kidney in reference to coating with host antigen, and also in terms of Simonsen's enhancement experiments, which I think is another way of coating over the antigen and making it inaccessible. The situation reminds me somewhat of Patterson's observation with allergic encephalitis. That is, if an animal is to develop allergic encephalitis, say a rat with guinea pig brain constituents, and the rat develops a high titer of complement-fixing anti-brain antibody, it does not get the disease.

To prove this experimentally, if such animals are about to get the disease and are given a high titer complement serum antibody, you can prevent the disease from occurring. All of this leads one to believe that serum antibodies may protect against autoimmune disease, and perhaps, in this less natural situation, may protect a piece of foreign tissue.

CHAIRMAN MERRILL: Dr. Crosnier, could you tell us about your experience in Paris at the Hôpital Necker?

Crosnier's Transplantation Experience (Hôpital Necker)

DR. CROSNIER: As far as our experience is concerned, I shall make but 4 remarks concerning: (1) the preparation of the recipient, (2) the selection of the donor, (3) the technique of remodeling the excretory pathway, and (4) the accidents in the evolution of the transplanted kidney.

1. Of the 34 patients, 24 were prepared by total body irradiation, either with cobalt therapy alone or together with drugs, and 10 received only immuno-suppressive drugs (and no irradiation). These last few months in particular, we have decided to divide our patients into 2 groups: one group of patients receiving irradiation and immuno-sup-

pressive drugs, the other receiving no irradiation but only immuno-suppressive drugs, wondering which of these 2 methods of preparation will prove, immediately and in the long run, to be the most effective.

2. As far as the choice of the donor is concerned, we have tried to use a certain number of tests in order to determine the most favorable donor. Our criteria are based on:

(a) Closely related donor: father, mother, brother, sister.

(b) Identical blood groups in the A-B-O-Rh system and identical subgroups.

(c) Study of the leukocytic antigens by the Dausset method, the most suitable donor having the smallest number of leukocytic antigens pertaining to the recipient.

(d) Study of the main sera groups.

(e) Study of the skin reactions 24 and 48 hours after the intradermal injection into the donors of a lymphocyte suspension taken from the recipient.

(f) Study of the duration of survival of skin grafts, taken from the patient and applied to each of the donors.

(g) Study of the recipient's lymphocytes cultivated with each of the donor's lymphocytes, the most suitable donor being the one whose lymphocytes, cultivated with those of the patient, show the least number of abnormal cells.

Up to now, it seemed to us that, in general, the results given by these different tests proved to be in satisfactory agreement.

3. Of the 34 patients, 18 have had a bladder-ureter anastomosis and 16, a uretero-ureteral anastomosis. In the first group, we had to reoperate several times because of a ureteral reflux, so now we always elect to anastomose the ureter of the transplant with the distal end of the patient's right ureter.

4. In the course of these 34 transplantations, a number of complications have arisen, impairing either momentarily or definitively the function of the transplanted kidney.

(a) In 4 cases, a rejection response suddenly occurred, ascertained by cessation of diuresis. For each of them, anatomic examination of the kidney showed a diffuse hemorrhagic infarction with multiple arterial and venous thromboses but without any noticeable obstruction of either renal vein or artery.

(b) Most of our patients have once or several times experienced what we call the "crises of the transplant." These crises are characterized by the functional insufficiency of the transplanted kidney which proved reversible either spontaneously or as a result of massive corticosteroid therapy. These crises generally appear early, between the

third and the thirtieth day following the transplantation, but they have also happened much later, toward the twenty-fourth month after transplantation.

Physical examination reveals high temperature, swelling of the kidney, oliguria, proteinuria, deterioration of renal function, precipitous decrease of sodium concentration in the urine frequently contrasting with maintenance of a high concentration of urinary urea. Much more seldom, the patient has hematuria and hypertension.

Histologically, in most of the cases there exists a more or less important diffuse edema of the kidney associated with cellular infiltration, more especially in the belated rejection crises. These histologic lesions seem reversible after the patient's recovery.

(c) Of our patients, 3 secondarily showed glomerular alterations of the transplanted kidney. In 1 case, the patient's own kidney had already been affected by glomerulonephritis and the evolution was fatal: the patient died 21 months after the transplantation. In the 2 other cases, the patient's kidney had no glomerulonephritis. Both now have satisfactory renal functions 1 year and 2½ years after the transplantation and, only a small amount of protein is to be found in their urine; nevertheless, recent biopsies have confirmed the persistence of a slight glomerular alteration.

(d) Finally, in 2 cases, we have noticed a progressive impairment of the function of the transplanted kidney and the appearance of arterial hypertension without proteinuria. The biopsy revealed a significant, mainly cellular, fibrosis of the renal interstitial tissue.

CHAIRMAN MERRILL: Thank you, Dr. Crosnier. I think that your cases are of acute vascular rejection, and resemble the one that I showed and some of the others that we have seen.

I do not know why this occurs in Boston and Paris and not in Richmond. However, I would point out that both Dr. Lawrence's group and ours have demonstrated a number of times that it is possible to immunize individuals to skin grafts and presumably to kidney grafts by the prior injection of circulating leukocytes. Therefore, there is a cross-reaction. It is obviously not entirely individual-specific.

It is well known, of course, that uremic patients are individuals who receive numerous transfusions and who on occasion do develop platelet antibodies.

While I do not need to emphasize it as a major consideration, it is one that worries us considerably and certainly does occur both in our patients and in those of the French group. I think that it has considerable interest in terms of transplantation immunology.

I should like to say a word now about the hypertension we have seen. How many clinical transplanters have seen hypertension when putting adult kidneys in children? That is, early.

DR. STARZL: We have seen it, whether we put adults' kidneys into children, or adults' into adults.

CHAIRMAN MERRILL: In the first couple of days?

DR. STARZL: Yes, we have seen that commonly in adult-to-adult transplants.

CHAIRMAN MERRILL: It really is not surprising to me. What I was getting at is this: were one to put a big kidney into a child with a small vessel, you would have a classic set-up for producing angiotensin-induced renal hypertension. I refer to any one of the 14 books on the subject published in the last 2 years.

DR. STARZL: Where one gets an unfavorable discrepancy between renal blood flow and renal mass? I cannot see the hemodynamic physiology which you have alluded to, which would regulate flow in such a way as to reduce it.

The size of the anastomosis is just as big as when you put the homograft into the adult. The source of arterial flow is from a vessel (the aorta or iliac artery) that is larger than the one into which it is flowing. I do not see how that would work.

CHAIRMAN MERRILL: It is not purely hemodynamic. We had better not belabor this.

DR. REEMTSMA: There is a related phenomenon, the return to normal of blood pressure following transplantation in individuals whose abnormal kidneys have been left in place. We have seen this after both homologous and heterologous transplantation in patients who were previously hypertensive.

CHAIRMAN MERRILL: I would emphasize that this is in the homograft situation, and that it occurs acutely and disappears in the identical-twin situation. One very striking and very happy thing has been the reduction of severe hypertension by transplanting a normally functioning kidney.

I should now like to ask Dr. Dixon a question: You have stated that your studies indicate that the rat anti-rabbit globulin, which forms

during the course of the production of nephritis, fixes only to the rabbit anti-rat kidney complex. How do you explain the production of nephritis in the rat which is parabiosed to its mate with acute pseudonephritis?

DR. DIXON: I do not believe that this occurs. The experiments on which this claim has been based were done at a time when isologous rats were not available and the transferred disease was never severe. We have had occasion to repeat these studies with isologous animals and got absolutely no transfer of disease.

But we did find something that Beatrice Segal described earlier. If you take a rat with Masugi nephritis, produced by injection of ¹²⁵I-labeled nephrotoxic antibody a month earlier and parabiose it to a normal, isologous partner, some of the labeled antibody finds its way to the kidneys of the normal parabiont. In our experience, the normal parabiont not further treated did not get nephritis. However, if after a month or two of union the partners were separated and the normal parabiont immunized with rabbit gamma-globulin in adjuvant, it then developed nephritis. Apparently the amount of rabbit nephrotoxic antibody-fixing in the normal partner was insufficient to induce an antibody response, but was sufficient to serve as a target for host antibody induced by adjuvant immunization.

CHAIRMAN MERRILL: The second question along these lines, I think, is based on some speculations of mine. We wondered whether, let us say, if there were similarities between streptococcal protein and kidney antigen—because of nephritis—that there might be cross-reacting antibodies which would affect the normal kidney in the absence of its contamination with streptococcal protein.

I wondered, therefore, whether bilateral nephrectomy for a period before transfer of the kidney to the recipient might remove the source of antigen and allow the immunologic memory responsible for the nephritis to “die out” before the transplant was put in. Dr. J. C. Cerny (University of Michigan) wonders if there is any definite experimental or clinical evidence to suggest that the removal of the glomerulonephritic kidneys will lessen the possibility of the nephritis developing in the transplanted kidney.

I think the clinical evidence is available, but is there any experimental evidence?

DR. DIXON: There is no direct experimental evidence to support this contention. In the studies with parabiosis or kidney transplants between

normal and nephritic isologous rats, the normal kidneys remained normal even in the presence of diseased kidneys. Theoretically, a diseased kidney might shed renal antigens which could stimulate an anti-kidney response injuring a normal transplant. It is also possible that, as in nephrotoxic nephritis, an anti-kidney antibody might dissociate from a sick host kidney and fix to and injure a normal transplant. However, demonstration of these 2 possibilities is still lacking.

DR. HUME: Dr. Merrill and I had a case some years ago, in which a patient with polyarteritis nodosa and glomerulonephritis had destroyed her own kidneys and was given a renal homotransplant which was biopsied and found to be free of disease. In 30 days the transplant had developed severe glomerulonephritis. The recipient kidneys had been left in.

DR. DIXON: May I interrupt? What makes you think that the kidney that was left in had anything to do with it?

DR. HUME: Nothing. The second patient I was going to refer to had acute and subacute glomerulonephritis with a recent acute exacerbation. We performed a renal transplant from his identical twin, leaving his own kidneys in place. Three weeks after transplantation he developed some proteinuria and red cells in the urine, together with a slight rise in BUN, which alarmed us. We then carried out the first simultaneous bilateral nephrectomy. It is now 7 years and he has not developed the disease in the transplant.

We cannot be sure, of course, that he was getting glomerulonephritis in the transplant, but it is suggestive.

The final point is that, although quite a few of the identical twins have developed glomerulonephritis, this certainly has been less common in homotransplants.

Dr. Merrill has the 2 cases he referred to, although they are not absolutely clear-cut. I do not think that any of the Denver cases or any of ours, which total more than 50 patients, more than 6 months after transplant, show any evidence of glomerulonephritis, in spite of the fact that quite a few of our cases had acute nephritis at the time of transplant. All patients had nephrectomies.

CHAIRMAN MERRILL: I think it is important to emphasize, because there have been so few cases of recurrent glomerulonephritis in the true homografts, that one wonders if they may not be due to the use of immuno-suppressive therapy, which is not used in isologous twins.

We have been treating glomerulonephritis as one would treat a

transplant on this basis, and so has Dr. Vernier, with very interesting results.

DR. STARZL: There have been 2 very interesting cases of post-homograft transplantation glomerulonephritis. One is a case report described by Kreig from Western Reserve, in which the patient had pyelonephritis, received a transplant, and developed histologic evidence of transplant glomerulonephritis, within about 10 days.

Dr. Hamburger has had 2 cases who developed this. He wrote me recently that one of them did not have glomerulonephritis in the recipient's original kidneys, but had something he called interstitial nephropathy.

CHAIRMAN MERRILL: I think here we have to remember Dr. Dixon's admonition, to be careful of what you call glomerulonephritis in long-standing homografts.

Glomerular changes do occur, which are not glomerulonephritis. Nevertheless, the one case I am talking about is where it occurred rather acutely in a homograft, and certainly in Dr. Dammin's opinion, was glomerulonephritis.

One of the problems is ureteral. Certainly, I am not technically capable of saying anything about this, except to say it has been a major problem, as I indicated, in our own experience. I would like to hear Dr. Reemtsma, Dr. Starzl and Dr. Hume comment on this.

DR. REEMTSMA: We have not had many lower urinary tract problems, with the exception of patients who have had preexisting disease of the bladder. In general, I have the impression that most of these complications can be related to technical factors involved in the operation, or to the high doses of steroids that are often required. We continue to use the method of ureterocystostomy.

It may be important that, although we have studied extensively the rejection phenomenon in the kidney itself, we know very little about the ureter in this respect. I believe that Dr. Starzl has been particularly interested in this matter recently.

DR. STARZL: We were quite interested in this problem, and shipped a number of ureters to Ken Porter for examination. We were especially interested because we had some late ureteric problems with those patients who lived for 6 months or longer. In this group of 40 there were 4 cases of ureteric stricture. One of those was right at the ureteral-pelvic junction, and obviously was not technical. In this case,

the stricture was thought to be due to scarring secondary to previous ureteric rejection. In the other 3 cases, the complication was probably technical. The ureters had lost their musculature. They had the same lesions as you have described previously. Frequently mucosal sloughing was present. There was evidence of healing of a slough, which involved almost a full length of the ureter. This occurs in 10 per cent of the late cases. In the early cases we had two in which chunks of wall fell out, one in the pelvis in an area with a good blood supply, and the other one near the ureteral-pelvic junction.

These cases also had arterial lesions and have slough of the mucosa and necrotic muscle in the wall.

It is interesting that Dr. Küss, in his case that died at 17 or 18 months, published a photomicrograph which showed the same ureteric lesions. The ureter in his case was not occluded, but I think that we will see occluded ureters in the future by this mechanism. We do ureteral-cystostomy because, if it fails, one can take the patient's ureter at a second operation and hook this to the homograft. In the 4 cases where we have done this, the patients are still alive. One has a good mechanical hookup, although a fungus ball has developed in the pelvis. We are trying to get it out with irrigations.

DR. HUME: We put the ureter in the bladder in all cases, and one of the main reasons we think it is a good idea is because many of our patients—about 50 per cent—have diseased ureters.

We wouldn't want to use an infected refluxing ureter to hook up to a transplant. We take the host ureter out in most cases and put the ureter on the trigone. We have had 50 cases. One leak developed into an abscess which proved rapidly fatal. In the other 4 cases, all bladder leaks, the leak stopped and the patients are all doing well.

CHAIRMAN MERRILL: There is another question which any member of the panel may answer. It is from L. W. Bluemle. Has complement deficiency been demonstrated in animals or man in whom renal-homografts have survived for extended periods after immuno-suppressive drug therapy has been stopped?"

Not to my knowledge. However, I think it should be pointed out that a drop in serum complement requires fixation of a large amount of complement, and when it occurs probably indicates rejection of the kind with which we are concerned. However, quantitatively such rejection might occur without any gross change in the measurable levels of serum complement whatever.

Dr. Moorhead, of Georgetown University Hospital, Washington, D.C..

asks, "How much importance does the panel attach to the careful matching of blood groups in the selection of homo-transplants?"

DR. CROSNIER: In all our cases, we have required an identity or a compatibility between the groups and subgroups of donor and recipient.

DR. STARZL: We have done about 20 patients in whom the donors and recipients had different blood groups. There were in this group 4 that had a major mismatch in which the kidney was placed in the direction of the preformed hemagglutinins. The direction was A to O, B to O and B to A. Of these 4, in which we challenged the kidney in this way, 2 were destroyed immediately, and 2 others worked for a long period of time. One of them is our best case, now almost 2 years postoperative. This mismatch is exactly the same as one tried unsuccessfully by Dr. Hume. One of the A to O transplants went 7 months, with good renal function. The B to A homograft has perfect function and this patient has been off steroids for about a year. The transplants that failed acutely were A to O and B to O.

The subgroups might deserve a comment. We sent our blood subgroups, as well as donor and recipient bloods, to Dr. Paul Terasaki (U.C.L.A.) for examination. He was anxious to compare the results with his leukocyte antigen typing with the data that we already had on the subgroups. There appears to be no correlation whatsoever with the blood subgroups, although there was good correlation of results with his white cell antigen typing.

I think that the only significance that the blood group has, according to the data which we have, is in the avoidance of an acute immunologic reaction, which I do not think is rejection, but which is killing the kidney in another way, with pre-formed antibody.

CHAIRMAN MERRILL: You would not do an incompatible blood type transplant?

DR. STARZL: Not in the direction that places the kidney in contact with preformed hemagglutinins. As for the other direction, we would have no hesitation about doing that.

CHAIRMAN MERRILL: One could say, for the ABO blood groups and probably for the minor ones, too, you cannot quantitate histocompatibility on that basis. This was very nicely shown by Dr. Woodruff some time ago.

On the other hand, there is good reason for not perfusing a kidney with obviously mis-matched blood. The perfusion of kidneys with in-

compatible blood has certainly caused acute tubular necrosis. Furthermore, we know that at least one of the major red cell antigens is present on tubular cells and perfusion of such a kidney with anti-A containing blood might well be harmful. I think it is possible that you might get away with it, but I doubt whether it should be done if you have a choice.

Dr. Onesti of Philadelphia asks, "Would you give some more details on the 'wasting disease' of the recipient and its relationship to immuno-suppressive therapy? How much of this is due to steroids?"

The question is for either Dr. Lawrence or Dr. Simonsen. But I might ask either one of them to comment on the disease which we see with general immuno-suppressive therapy, which is similar to the secondary disease one sees in the experimental animal. Is this a good analogy?

DR. SIMONSEN: I do not believe that the wasting disease you see in your patients during that treatment has an early phase of splenic and lymphoid hypertrophy as has immunologic runt disease before lymphoid atrophy sets in.

CHAIRMAN MERRILL: But do you conceive of this wasting disease as a block in the normal immunologic mechanism, and, if so, would you visualize it as destruction of the recipient's spleen by the graft-versus-host reaction or possibly by repopulation by donor cells, or alternatively might the whole syndrome be due to nonspecific drug suppression of general immunologic potential?

DR. SIMONSEN: It is obvious that the nonspecific drug suppression may promote wasting, but I think that there might be more to it than that. As I tried to say this morning, if you do manage to exhaust the reactivity to a strong antigen, with or without the help of immuno-suppressive drugs, I would predict that reactivity to many other antigens would be diminished at the same time. Therefore, the ability of the patient to react to other foreign antigens, including his bacterial flora, also would be impaired. This has nothing to do with graft-versus-host reaction, but with exhaustion of the host's own apparatus. The same may not happen during exhaustion to weak transplantation antigens.

May I say that I am surprised at the statement of Dr. Lawrence that the graft-versus-host reaction should be impaired in germ-free animals. My information does not support that. Miller says that germ-free animals do not waste after neonatal thymectomy, whereas the

same germ-free animals, the same strain, if given homologous spleen cells, go through the whole process of runting, just like conventional mice.

DR. LAWRENCE: Could I add to that? In reference to recent findings in producing runting with using unrelated tissue antigens, if one treats the newborn mouse with an antibiotic, you do not get runting in as high incidence as you get otherwise.

In germ-free animals, where it seems that you do not get the runting the way you do in animals that have bacteria, I wonder if the answer to your question may be that with immuno-suppression you are not only diminishing response of the tissue to the foreign antigens, but also in the myriads of bacterial cells that are floating throughout the R.A. system in the ordinary course of events.

CHAIRMAN MERRILL: This is exactly my interpretation of what happens.

DR. LAWRENCE: What I was referring to arose from a recent article in *Nature*—I cannot remember the name of the author—where thymectomized animals runted; but in another group of thymectomized animals that were given cells and treated with antibiotics, runting did not occur. The suggestion is that, in addition to the foreign cells, you need to grow microbial cells somewhere in the host to have a runt response.

DR. STARZL: I might ask Dr. Hume about this specific point. At the meeting last year much was said about runting and about metabolic disturbances up to and including the syndrome of kwashiorkor that had been seen some place. We became quite interested in this syndrome. In all of those cases which we thought were runts, we ultimately found that they had brain tumors, chronic pancreatitis, and lung abscesses, or perivertebral abscesses. Each of them had something specifically wrong.

In looking back over the situation, we have treated a lot of patients and I cannot remember seeing anyone die of runting who did not have some other specific explanation.

I think this is fairly important, as it might relate to the liver homografts, because, in this circumstance, you can demonstrate a graft-versus-host disease, which I did not go into. It consists of a hemolytic process, in which the red cell half-life is affected. In some dogs, the red cell half-life was reduced to 2 or 3 days for quite a while, and

then it became 6 days, and, ultimately, it became normal for a dog, which is about 13 days.

We were pleased to see at the time of withdrawal of the drugs that this did not unmask, or make worse, a graft-versus-host reaction, and red cell half-life continued to improve.

CHAIRMAN MERRILL: We have time for 2 more questions. The first one I would like to ask the entire panel. Is there anyone who believes that, at the present time, thymectomy is indicated as an adjunct in renal transplants in man? If not, under what circumstances do you think it might be performed in the future?

DR. STARZL: We do not know the answer to the question, but we are doing it, and, because we do not know the efficacy of thymectomy we are doing a blind study, in which half of the patients receive thymectomy and the other half do not.

The reason we are intrigued by this question is that 4 of our original 6 cases are alive, now all 18 to 24 months post-transplant. They had thymectomy. They have all been off steroids for more than a year, all within 6 or 7 months after operation. There have been no late rejections in that group, whereas, with the rest of the patients, over 30, that are alive, only 1 is off steroids. We have had a high incidence of late rejection in the latter group. The value of thymectomy is not proved, but the urgency of establishing this one way or another is evident.

CHAIRMAN MERRILL: This is the final question. I think it is a good note on which to close the meeting. We have had a considerable amount of theory and speculation. Here is, perhaps, the most practical question we can be asked. Does the donor's or the recipient's Blue Cross cover the services to the donor? The answer is, at least in our experience, that it does for the recipient, but only in exceptional instances for the donor.

I should like to close now, and thank the panel very much for their participation.

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