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Immunosuppression after Experimental and Clinical Homotransplantation of the Liver *

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THE FATE of whole organ liver homografts after transplantation to untreated canine recipients is well known.[3, 9, 23, 25, 26, 34, 39, 41] For several days after operation, the transferred tissue is life-sustaining, but after this there is rapid functional failure. The histologic abnormalities in the rejected liver consist of infiltrates of mononuclear cells which tend to be concentrated in the periportal areas, a necrotizing arteriolitis, and dissolution of hepatic parenchymal cells with retention of a relatively normal reticulum.

In the present report, an attempt will be made to add an additional dimension by describing the behavior of canine and human hepatic homografts in recipients treated with immunosuppressive agents. Under these circumstances, a modified rejection was observed in many instances despite the absence of significant cellular invasion of the homograft. In addition, it was noted that severe hepatocyte injury often occurred in livers in which the duct system was selectively preserved or even hyperplastic. Finally, several previously unrecorded observations will be documented regarding more esoteric biochemical alterations in patients after hepatic homotransplantation. These include serial determinations of plasma or serum immunoglobulins, haptoglobins, amino acids, pyruvates, and lactates.

Methods

Types of homotransplantation. Orthotopic homotransplantation was carried out in 25 dogs, after removal of the animal's own liver.[40] The reconstructed blood supply to the revascularized homograft was essentially normal.[40] The time for transfer and complete revascularization of the cooled organ averaged 70 minutes. The effects of ischemia were minimized by perfusion of the liver with cold (10–15° C.) lactated Ringer's solution prior to its removal.[40] Internal biliary drainage was provided with a cholecystjejunostomy or cholecystoduodenostomy. Splenectomy was performed. Eleven animals died during or within three days following operation. These failures were considered to be technical,[42] and are not considered in the pathologic analysis (Table 1).

In 15 dogs, an auxiliary liver was placed in the right paravertebral gutter (Fig. 1) using a modification[42] of the method of Welch and his associates,[9, 47] revascularizing the portal vein from the terminal inferior vena cava and the hepatic artery

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TABLE 1. *Pathologic Findings in Auxiliary Canine Homografts*

No.	Time of Biopsy or Autopsy (days)	General Architecture	Intrahepatic Hepatic Artery	Intrahepatic Bile Ducts	Perivenular Necrosis Central V.	Immunocytes	Cause of Death
			Only Hepatic Artery Open				
AHH 1	30	Good, central necrosis, moderate	Medial and intimal thickening	Well preserved decreased number	Moderate	Periportal, severe	Hepatic inficiency after removal of own liver (28 days)
AHH 2	28	Fair, central necrosis, moderate; generally vacuolated hepatocytes	Medial and intimal thickening	Well preserved	Moderate	Periportal, severe	
	72	Poor, only few hepatocytes remain	Severe medial and intimal thickening	Well preserved	Severe	Periportal, severe; general, moderate	Sacrificed
*AHH 7	28	Good, central necrosis, slight	Medial thickening, Focal intimal necrosis	Columnarization, hyperplasia, and bile stasis	Slight	Periportal, moderate	
	65	Fair, central and scattered necrosis, moderate	Intimal and medial thickening and focal necrosis	Hyperplasia and bile stasis	Moderate	Periportal, severe; general, slight	Sacrificed
*AHH 9	27½	Good, some vacuolated hepatocytes	Focal intimal and medial necrosis	Columnarization	None	None	Hepatic insufficiency after removal of own liver (27 days)
*AHH 10	19	Poor, diffuse loss of hepatocytes	Medial thickening, focal medial and intimal necrosis	Columnarization, bile stasis	Severe	Periportal, moderate	Pneumonia
AHH 14	33	Poor, central necrosis, severe	Medial thicking and focal necrosis	Columnarization	Severe	Rare	Not known
			Portal Vein and Hepatic Artery Open				
AHH 3	26	Excellent, central necrosis, slight	Medial thickening	Columnarization, bile stasis	Moderate	Periportal, slight	After angiogram
AHH 4	29	Fair, central necrosis, moderate; general hepatocyte vacuolization	Medial and intimal thickening	Normal, decreased number	Moderate	Periportal, moderate	Hepatic insufficiency after removal of own liver (27 days)

TABLE 1. *Continued*

No.	Time of Biopsy or Autopsy (days)	General Architecture	Intrahepatic Hepatic Artery	Intrahepatic Bile Ducts	Perivenular Necrosis Central V.	Immunocytes	Cause of Death
AHH 5	29	Good, central necrosis, slight, focal periportal necrosis	Medial thickening	Columnarization	Slight	Periportal, moderate	Hepatic insufficiency after removal of own liver (28 days)
AHH 6	27	Good, central necrosis, moderate; focal hepatocytes vacuolization	Focal medial necrosis, intimal thickening	Columnarization	Moderate	Periportal, slight	After angiogram
*AHH 8	13	Poor, central necrosis, severe; periportal necrosis, severe	Intimal thickening, focal medial necrosis	Columnarization, bile stasis	Severe	None	Hepatitis own liver
*AHH 11	24	No sections					After angiogram
AHH 12	20	Excellent, central necrosis, slight	Intimal and medial thickening; focal medial necrosis	Normal	Slight	Periportal, slight	After angiogram
*AHH 13	21	Poor, central necrosis, severe	Medial thickening	Well preserved, bile stasis	Severe	Periportal, slight	After open liver biopsy
AHH 15	45	Good, central necrosis, slight; moderate periportal necrosis	Medial thickening	Hyperplasia	Slight	Periportal, moderate	Sacrificed

* Received eight day course of prednisolone, starting on day of operation. See text.

from the common iliac artery (Fig. 1) or aorta. The vascular supply to the graft was thus comparable to that of a portacaval transposition[4] in that systemic venous blood passed to the portal vein. Cold perfusion of the homografts was done with the same technic as with the orthotopic livers.[40] The period of ischemia averaged 32 minutes. Cholecystoduodenostomy was performed (Fig. 1). The spleen was removed. Postoperatively, patency of the vascular anastomoses was checked with transfemoral venous and arterial angiograms (Fig. 3). In four animals, the recipient dog's own liver was removed at a second-stage operation after 27 to 28 days, leaving the homograft as the only residual liver tissue.

Five clinical hepatic homotransplantations were performed with previously described technics.[42, 44, 45] The homograft was placed in normal anatomic position (Fig. 3) after removal of the recipient's diseased liver. Four of the patients (ages 29–67) had primary malignancies of the liver, and the fifth was a three-year-old child with congenital biliary atresia. The livers were

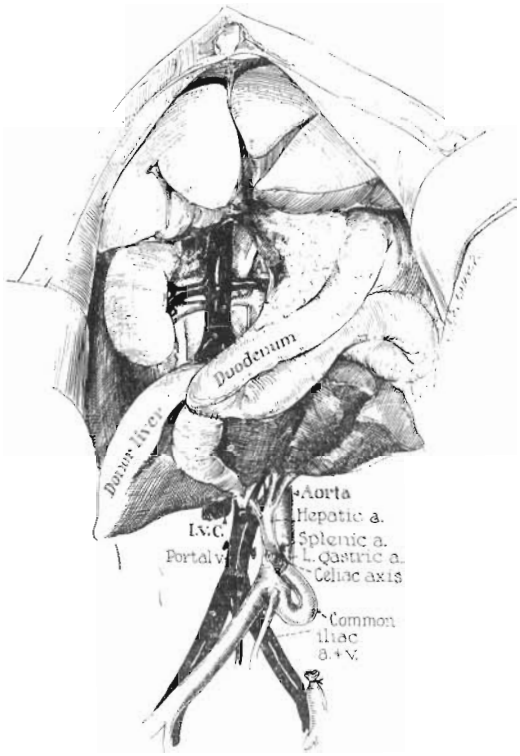

FIG. 1. Auxiliary liver homotransplantation in dogs. Note that the reconstituted portal blood supply is from the inferior vena cava. Cholecystoduodenostomy is performed.

obtained from cadaveric sources,[42, 44] using an extracorporeal perfusion system to provide interim circulation and cooling as the organ was removed.[22] The times from donor death to revascularization in the recipient ranged from 164 to 420 minutes.[42] Splenectomy was performed in only one case (Patient 2).

Immunosuppression. Azathioprine was used for the canine orthotopic experiments (Fig. 9), in quantities of 8–15 mg./Kg. per day for two to four days after operation and 2–10 mg./Kg. per day thereafter. The highest dose possible was selected which did not cause leukopenia. In three cases, this was supplemented after four to 12 days with a short course of subcutaneous prednisolone, administering 20–100 mg./day for four to ten days.

Azathioprine was used in a comparable manner for canine auxiliary homotransplantation studies (Fig. 4). In six of the 15 dogs, 100 mg./day subcutaneous prednisolone were also started on the day of operation, with subsequent reductions in the dose every two days to 50, 25, 10 and 0 mg.

The use of azathioprine was similar in the clinical cases (Fig. 11, 12). Prednisone was used (100–200 mg./day) within one-half to three and one-half days after operation in Patients 2, 4 and 5, and started before operation in Patient 3. This drug was continued until death. Intravenous actinomycin C (200–400 micrograms/day) was administered every two to five days. In one case (Patient 2), 10 mg./day azaserine was given intravenously for two days after operation.

Studies of function. Numerous liver function studies were obtained. Analytic methods will be documented only for those determinations considered in detail in the results. Bilirubin content of serum, urine, and T-tube drainage was measured by the method of Malloy and Evelyn,[19] the one-

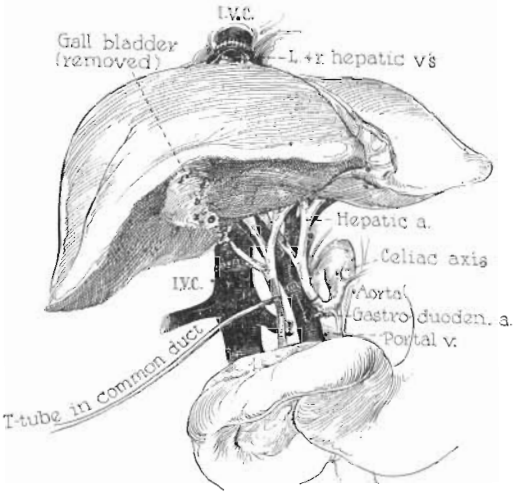

FIG. 2. Completed clinical homotransplantation. The reconstruction is anatomically normal. The T-tube is placed through a stab wound in the recipient common duct, rather than through the anastomosis as shown.

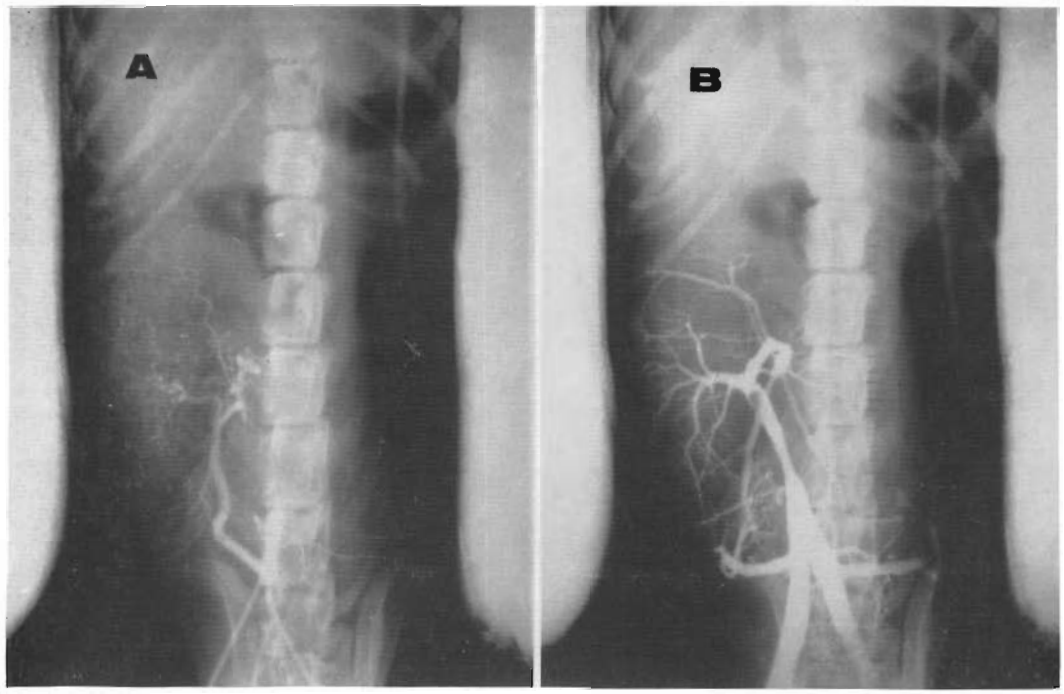

FIG. 3. Angiographic studies of auxiliary liver homograft (AHH 6) 27 days after transplantation. A. Hepatic arterial supply B. Venous supply. Note excellent filling of small ramifications of both systems.

minute reading being taken as the *direct* (conjugated glucuronide) fraction, and the 30-minute reading as the total bilirubin. Serial serum alkaline phosphatases were measured by the Bodansky method. Serum glutamic-oxalacetic acid transaminase (SGOT), serum glutamic-pyruvic acid transaminase (SGPT), lactic acid dehydrogenase (LDH), and isocitric acid dehydrogenase (ICD) followed similar postoperative patterns and, therefore, only the results of the SGOT determinations will be described.

Plasma sugars were analyzed colorimetrically on an autoanalyzer. Plasma pyruvates and lactates were analyzed by the methods of Friedman and Haugen [7] and Barker and

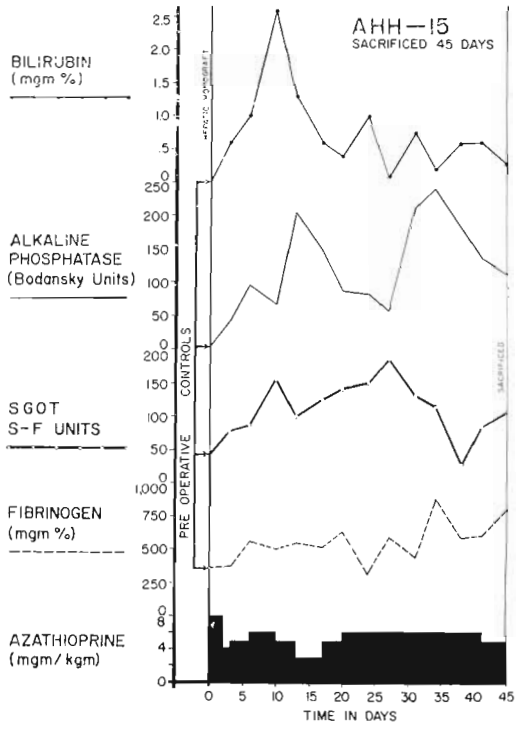

FIG. 4. Course of dog with an auxiliary hepatic homograft. Azathioprine was the only immunosuppressive agent used. Note the irregular postoperative rises in alkaline phosphatase, SGOT and fibrinogen. The slight bilirubinemia depicted was uncommon.

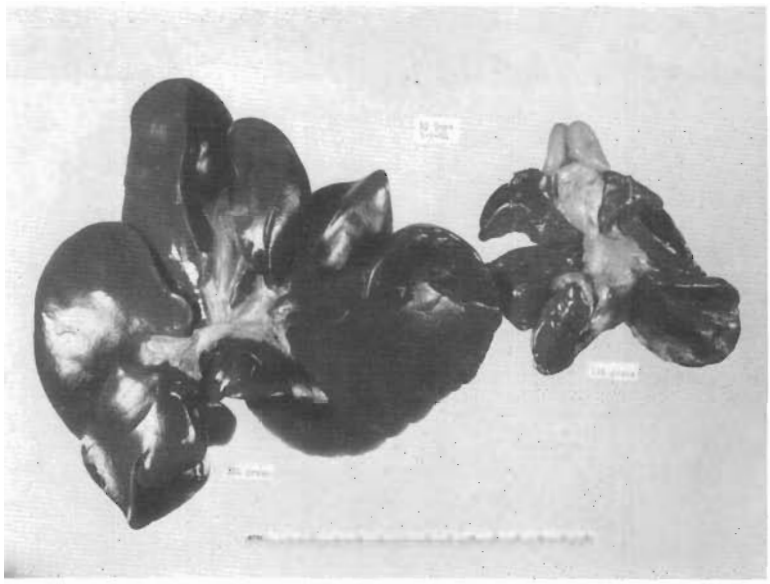

FIG. 5. The auxiliary homograft (right) and the recipient dog's own liver (left) in experiment AHH 15. Note the well preserved but dimensionally reduced general structure of the homograft. The gallbladder did not shrink proportionately. The specimens were obtained 45 days after transplantation.

Summerson,[2] respectively. Venous blood for lactate and pyruvate determination was collected without stasis in a tube containing 20 mg. potassium oxalate and 15 mg. sodium fluoride. The tube was immediately placed in an ice water slush at 0° C. and taken to the laboratory where the cellular elements were removed by centrifugation at 3,000 RPM for 10 minutes. The plasma thus obtained was then frozen until the time of definitive analysis.

Plasma protein fractionation was determined by paper electrophoresis and fibrinogen was measured by the technic of Ratnoff and Menzie.[33] Quick one-stage prothrombin times were obtained. Analysis of serum and urinary amino acids is referred to in the results section. A detailed account of changes in euglobulin lysis times has been published elsewhere.[45]

Haptoglobin types. In Patient 5 of the clinical series, the serum haptoglobin genotypes of the donor and recipient were determined by vertical starch gel electrophoresis.* [35] The wells were charged with a solution consisting of 15 parts of undiluted serum and one part of 10 per cent hemoglobin solution. After electrophoresis for 18 hours, the gel was divided into 2–3 mm. slices and stained with 0.1% o-tolidine in acetic acid. The hemoglobin binding proteins appeared as blue bands.

Serum immunoglobulins. In addition, each of the three classes of immunoglobulins (gamma globulins) was quantitatively estimated for Patient 5 with a modification [5] of the immunochemical diffusion method of Huntley.[14] Commercially prepared ** goat anti-human gamma$_2$ globulin, anti-human gamma$_{1M}$ globulin and anti-human gamma$_{1A}$ globulin were each incorporated into separate volumes of 1% agar in 0.85% saline (buffered with veronal, pH 7.4, μ 0.15) in a concentration of 2:1. The agar, containing the heterologous antibody was drawn into the lower portions of a series of silicone-coated capillary tubes, which were sealed and supported vertically. An aliquot of the patient's serum was layered over the

* Hydrolyzed starch was the product of Connaught Medical Research Laboratories, Toronto, Canada.

** Hyland Laboratories, 4501 Colorado Blvd., Los Angeles, California.

agar, and a precipitin band formed which migrated slowly through the agar. Tubes containing anti-gamma$_2$ globulin in the agar and overlaid with the patient's serum were allowed to stand for 24 hours. Tubes containing anti-gamma$_{1M}$ and anti-gamma$_{1A}$ stood for 72 hours. The distances traversed by the precipitin bands during these standard reaction times were measured. The level of each class of immunoglobulin in each serum sample was determined from standard curves. These curves were prepared simultaneously using pooled normal human serum, to demonstrate the relationship between distance traversed by the precipitin band and the $\log_{10}$ of the concentration of the respective immunoglobulin.

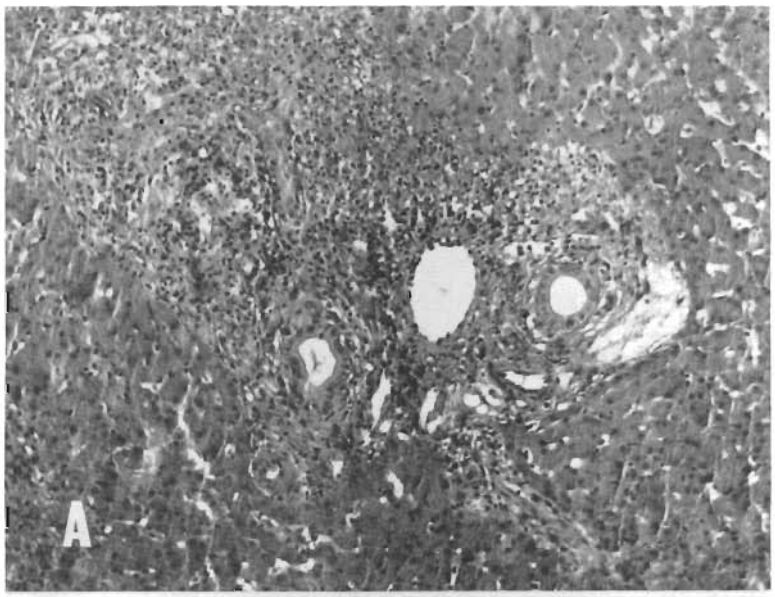

FIG. 6A. Biopsy of auxiliary hepatic transplant at 28 days from dog AHH 7. There is increased cellularity in the portal area with good preservation of the ductal system. The adjacent hepatocytes are intact (from × 80).

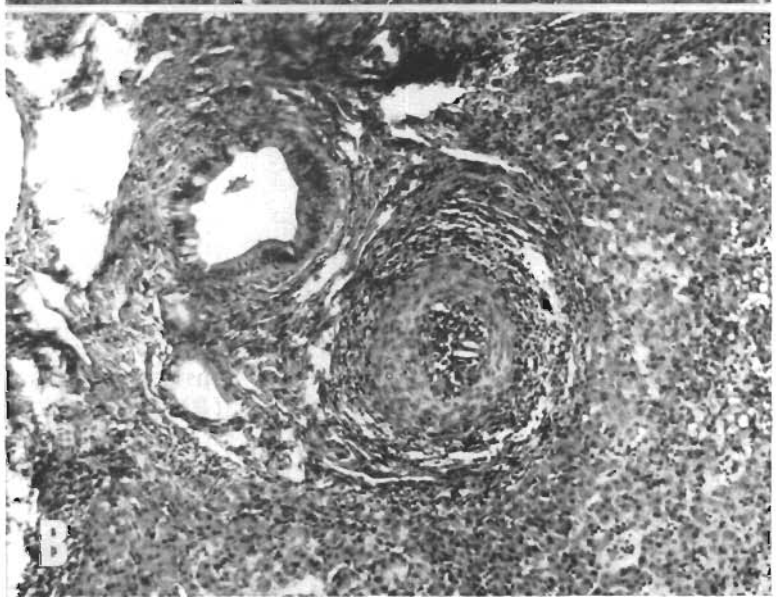

FIG. 6B. Auxiliary hepatic transplant (AHH 7) after 65 days. The ductal system remains well preserved with columnarization and hyperplasia of the mucosal cells accompanied by moderate dilatation. The artery shows marked intimal and medial hypertrophy and a perivascular inflammatory response. Diffuse loss of hepatocytes is seen in the neighboring area. H and E (from × 80).

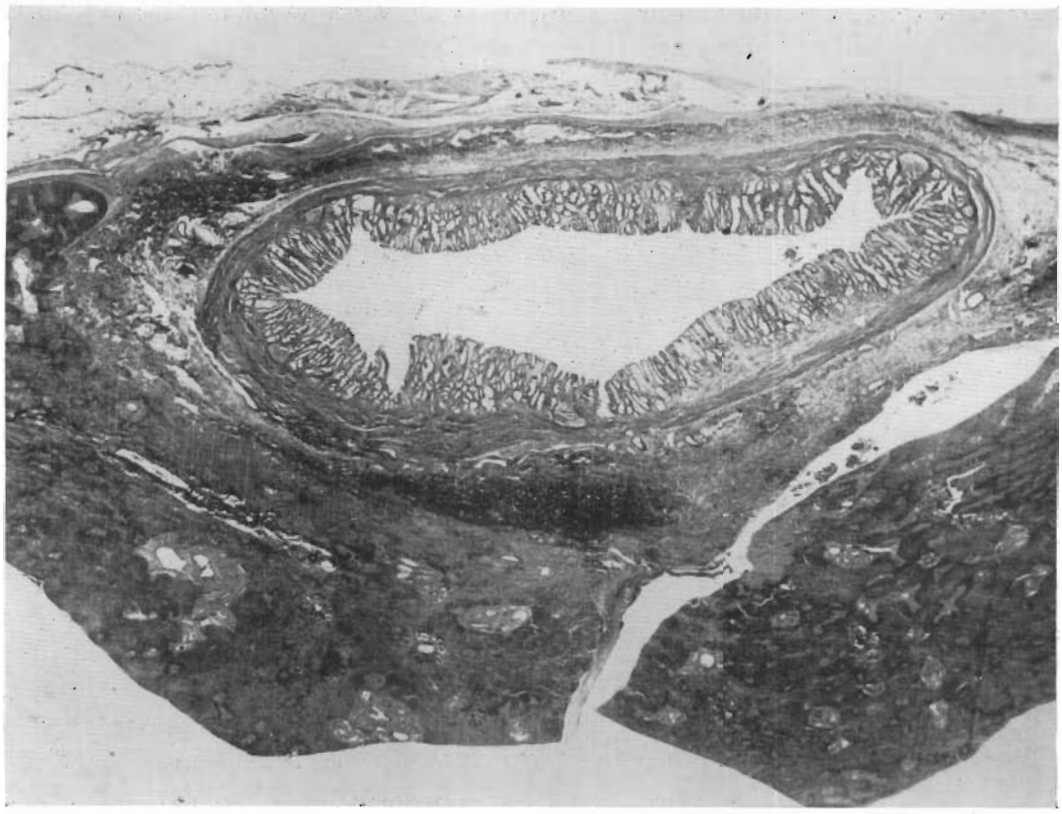

FIG. 7. Low power scan of transplanted liver from dog AHH 7. The gallbladder is seen in the lower central portion of the figure. Note the good preservation of both the gallbladder and the intrahepatic portal tracts. The portal tracts appear to be hypertrophic and compressed. Mallory Trichrome Stain.

Pathologic studies. Autopsies were performed as promptly as possible after death. In most animal experiments, tissues were obtained immediately or within a few hours. Delay between death and necropsy in the human cases was one-half, six, 12, 12, and 16 hours. Specimens were fixed in formalin and Carnoy's solution.

A variety of tissue stains were employed for study of the livers using the technics summarized by the Armed Forces Institute of Pathology.[20] The following histologic sections were prepared from formalin fixed and paraffin imbedded blocks: hematoxylin and eosin for general architecture; silver impregnation for reticulum; Verhoeff-van Gieson method for elastic and connective tissue; periodic acid-Schiff (with and without previous diastase digestion of the sections) for identification of glycogen; phosphotungstic acid hematoxylin for study of connective tissue and definition of fibrinoid necrosis; trichrome stain for connective tissue, muscle and endothelium; Prussian blue iron stain; and bile stain. Sudan IV fat stains were applied to frozen sections from formalin-fixed tissues. A methyl-green-pyronin stain was used for Carnoy-fixed paraffin-imbedded sections in order to demonstrate cells with high ribonucleic acid (RNA) content.

Results

Auxiliary Liver Homografts

Clinical Course. Homotransplantation of an extra liver was well tolerated. There

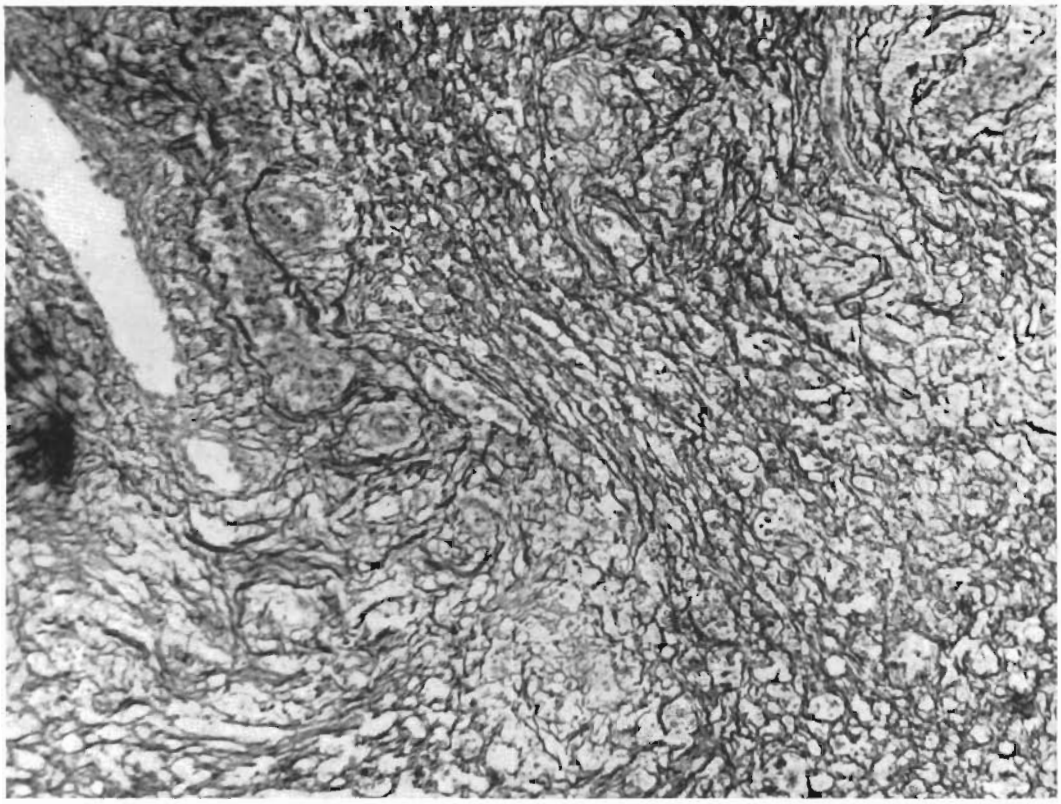

FIG. 8. Reticulum stain of auxiliary homograft obtained after 29 days from AHH 4. The reticular scaffold is collapsed, coarsened, and diffusely fragmented (from × 80).

were only three spontaneous deaths, these occurring after 13, 19, and 33 days (Table 1). The other 12 animals were sacrificed, died as a direct consequence of diagnostic instrumentation, or succumbed one-half to two days after removal of their own livers (four animals) of hepatic insufficiency. The dogs resumed alimentation quickly after operation and generally appeared to be quite normal. The period of postoperative study in the series ranged from 13 to 72 days. In six instances, the portal anastomosis

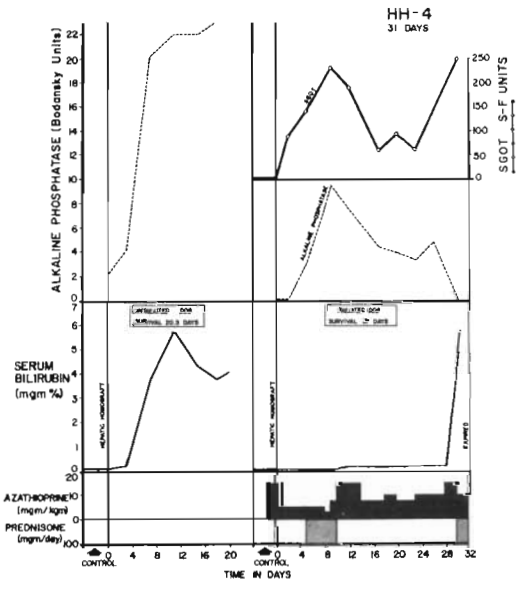

FIG. 9. Comparison of course of untreated animal (left) with that of dog treated with azathioprine and prednisolone (right). The treated dog died after 31 days with a perforated gastric ulcer. Note difference of bilirubin and alkaline phosphatase in the treated compared to the untreated animal.

was found to be occluded, an occurrence which did not influence the results in a distinctive way. Similarly, the use of steroid therapy did not demonstrably influence the biochemical or pathologic findings.

When the anastomoses were patent, angiograms showed good filling of distal arborizations of both the portal and arterial systems (Fig. 3) without evidence of small vessel block. The reduced size of the homografts, to be described below, was evident from these dye studies.

Biochemical Studies. Although slight changes in serum bilirubin concentration regularly occurred (Fig. 4), clinically detectable jaundice developed in only two animals (maximum bilirubin 4.4 mg% and 5.8 mg%, respectively).

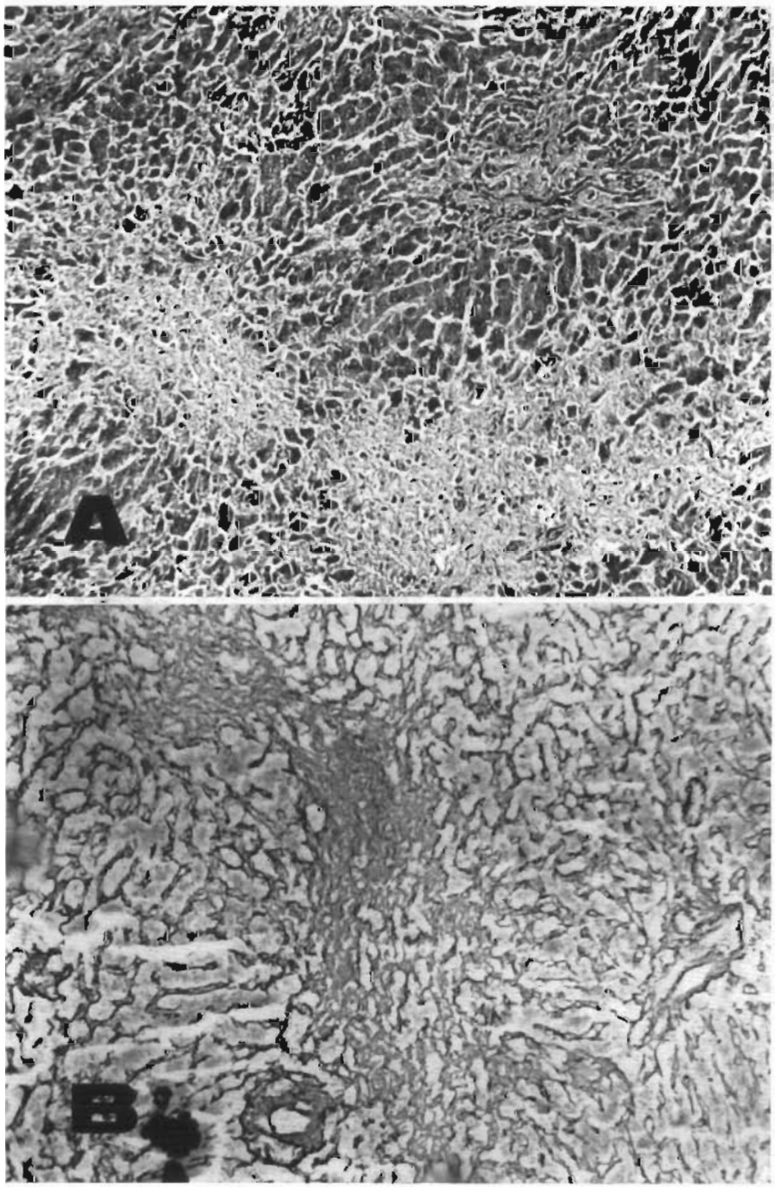

FIG. 10A. Orthotopic canine homograft (HH 4) after 31 days. Note central loss of hepatocytes with good preservation around the portal tract. Immunocytes are absent. H and E stain (from × 80).

FIG. 10B. Same dog. Note general preservation of reticulum, but with focal areas of collapse and fragmentation. Reticulum stain (from × 80).

Marked elevations in SGOT occurred in every animal. The mean preoperative values were 27.8 ± 2.3 (S.E.) S-F units. Postoperative, the peak values were 293 ± 56.9 (S.E.) S-F units after 12.7 ± 2.1 (S.E.) days. In some instances, the SGOT rises occurred remittently (Fig. 4).

The most striking and sustained changes were in serum alkaline phosphatase. From control levels of 2.2 ± .1 (S.E.) Bodansky units, the postoperative alkaline phosphatases rose to 203 ± 36 (S.E.) after 18.3 ± 2.4 (S.E.) days.

Preoperative plasma fibrinogens were 301 ± 34 (S.E.) mg.%. Postoperatively, these rose to 564 ± 34 (S.E.) mg% after 7.6 ± 2.6 (S.E.) days. The increases in fibrinogen occurred at an even earlier time than is apparent from the mean value, since the highest fibrinogen level in one dog occurred on the 34th day. Excluding this unusual animal (Fig. 4), the maximum rise occurred after an average of 5.6 days.

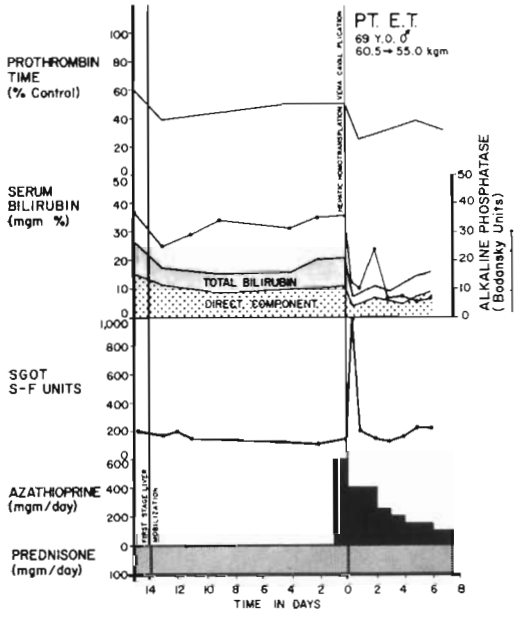

FIG. 11. Course of Patient 3, who had an intrahepatic cholangiocarcinoma. Skeletonization of all major structures entering and leaving the liver was carried out two weeks before definitive transplantation as shown. Note early rise in SGOT after transplantation. The sudden falls in serum bilirubin and alkaline phosphatase after each operation may have been a dilutional effect due to multiple transfusions with fresh blood.

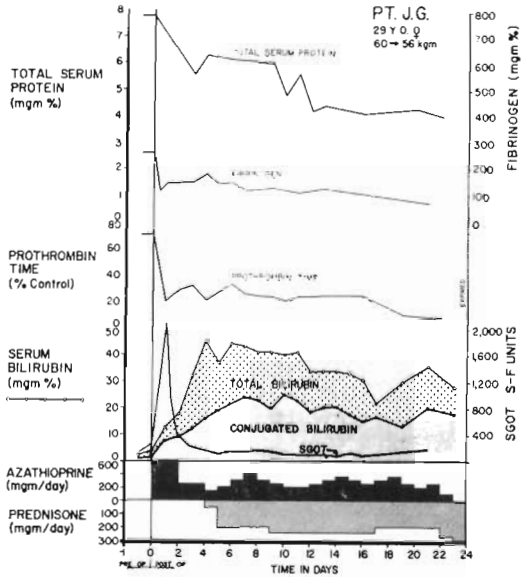

FIG. 12. Serial chemistries in Patient 5. Note serious abnormalities in various measurements. The increase in SGOT immediately after operation was the highest observed in any case. The immunosuppressive therapy is depicted at the bottom.

Gross pathology. There was marked shrinkage of the homograft (Fig. 5) in every animal which lived for more than 13 days. The homografts weighed from 120 to 265 Gm. in the animals autopsied from 19 to 72 days, as compared to 324 to 460 Gm. for the recipient's own liver. The gross architecture of the liver was extremely well preserved in each case. The lobar structure and relationships were quite identifiable (Fig. 5). The gallbladder and common duct were invariably intact, the latter structure often being even larger than normal.

Microscopic studies. Preservation of general architecture ranged from excellent to poor. There was variable hepatocyte loss in every case (Table 1), the principal areas of necrosis usually being around the central vein, although scattered focal necrosis was also sometimes present. The residual hepato-

TABLE 2. *Pathologic Findings in Orthotopic Canine Homografts**

Survival No.	(days)	General Architecture	Intrahepatic Hepatic Artery	Intrahepatic Bile Ducts	Intrahepatic Portal Vein	Perivenular Necrosis Central V	Immunocytes	Cause of Death
19	3	Good; central necrosis, slight	Slight intimal thickening	Normal	Normal	Slight	None	Pulmonary embolus
14	4	No Tissure						Intussusception
17	5	Excellent	Medial and intimal thickening	Normal	Normal	None	None	Pneumonia
24	7	No Tissure						
5	9	Good; scattered focal necrosis	Medial and intimal thickening, focal necrosis	Normal	Normal	None	Periportal, slight	Pneumonia, hepatic abscess
18	9	Good; central necrosis, moderate; periportal necrosis, slight	Medial and intimal thickening	Columnarization	Scattered small thrombi	Moderate	Periportal, slight	Pneumonia
22	9	Fair; central necrosis, moderate	Intimal and medial thickening	Normal	Normal	Moderate	Periportal and general, slight	Pneumonia
1	11	Fair; central necrosis, severe	Intimal thickening	Normal bile stasis	Normal	Severe	None	Pneumonia
9	12	No Tissue						Disrupted cholecystenterostomy
12**	12	Good; central necrosis, slight; periportal necrosis, slight	Medial thickening	Columnarization	Normal	Slight	Periportal, slight	Intussusception
16***	12	Fair; central necrosis, moderate	Intimal thickening	Normal	Large thrombus	Moderate	None	Pneumonia
20**	20	Fair; central necrosis, moderate; scattered focal necrosis	Focal intramural hemorrhages	Normal	Normal	Moderate	Periportal, slight	Pneumonia
3	25	Good; central necrosis, very slight	Medial thickening and fragmentation	Hyperplasia	Normal	Very slight	Periportal, slight	Pneumonia, pulmonary edema
4**	31	Fair; central and scattered necrosis, moderate	Medial thickening	Hyperplasia; bile stasis	Normal	Moderate	Periportal, very slight	Pneumonia, perforated gastric ulcer, pulmonary embolus

* Eleven additional unincluded animals failed to live as long as 72 hours. All dogs received azathioprine.
** Received supplementary prednisolone.
*** Segmental infarct present. Portal branch thrombosis.

cytes were relatively normal (Fig. 6A) and stained PAS positive. In most specimens, there was considerable infiltration of mononuclear cells (Fig. 6) which were localized to the periportal area in all but two experiments (Table 1). In six animals (Table 1), the degree of cellular infiltration was, however, very minimal or absent altogether, despite which other findings of rejection were present.

There was a remarkable selective preservation of the duct system. The gallbladder was invariably an easily identifiable structure, which had excellent histologic preservation of general architecture (Fig. 7). Similarly, the common duct was minimally damaged. In many animals (Table 1), the intrahepatic duct system had undergone columnarization of the lining cells, or actual hyperplasia (Fig 6B). In 40 per cent of the

specimens, there was evidence of bile stasis (Table 1) and frequently the ducts within the liver appeared to be dilated (Fig. 6B).

The intrahepatic portal venous radicals were normal. The small arteries and arterioles had widespread changes, which were most prominent in the animals followed for the longest intervals. There was thickening of the intima and media, frequently leading to marked narrowing of the lumen (Fig. 6B). These changes usually appeared to be the result of intimal or medial proliferation, but focal areas of necrosis were also present in the intima and media of many vessels (Table 1).

The hepatic reticulum was invariably preserved, but in an altered state. There was linear compression (Fig. 8) similar to that observed in one human case. In addition, there was some fragmentation and coarsening of the individual reticulum fibrils (Fig. 8).

In two animals, serial histologic studies were obtained after one and two months, respectively (Table 1). In these dogs, the features of homograft rejection were similar in both tissue specimens, although much more marked after the longer time. The progression of the changes in the intrahepatic ductal and arterial systems were particularly striking (Fig. 6). In one of these animals (AHH2), there was destruction of almost all of the parenchyma except for the ducts.

Orthotopic Canine Homografts

Clinical course. Eleven dogs died during the first three postoperative days, almost invariably as a consequence of outflow block and/or hemorrhagic gastroenteritis. These have been reported before [42] and will not be considered further. Most of the other 14 animals made an uneventful recovery from pentobarbital anesthesia. They appeared to be healthy for the first few days after operation. Most resumed alimentation. Ultimately, all 14 died, after three to 31.5 days. The gross causes of death were pneumonia, intussusception, pulmonary embolization, or gastro-intestinal perforation (Table 2). As will be described below, the extent to which homograft rejection contributed to the mortality was difficult to assess.

Biochemical studies. It was previously established that untreated dogs developed progressive jaundice starting on the fourth or fifth day (Fig. 9) after hepatic homotransplantation.[26, 41] Bilirubinemia was much less pronounced in the present treated series. The peak serum bilirubin concentrations observed were 2.8 ± 0.88 (S.E.) mg% after 9.8 ± 2.2 (S.E.) days. The onset of jaundice was frequently a terminal event (Fig. 9). In half the experiments, elevations in bilirubin were never observed. A comparison of the course of an untreated dog with that of an animal receiving immunosuppressive therapy is shown in Figure 9.

Elevations of serum alkaline phosphatase also tended to occur late in the course, the maximum rise being 22 ± 4.8 (S.E.) Bodansky units, after 8.4 ± 1.3 (S.E.) days. The very high values consistently demonstrated after auxiliary hepatic homotransplantation were not seen.

In contrast to the alterations in bilirubin and serum alkaline phosphatase, rises in serum glutamic oxalacetic acid transaminase (SGOT) tended to occur earlier, the peak values being 207 ± 21.9 (S.E.) S-F units after 5.4 ± 1.3 (S.E.) days. The early rises in SGOT, which were thought to be the consequence of ischemic liver injury during operation, were reversible to a high degree. In the preterminal period when bilirubinemia and alkaline phosphatemia were prominent, SGOT's sometimes also exhibited a secondary rise (Fig. 9), but in other animals, the enzyme levels remained relatively low despite rapid deterioration of other biochemical parameters.

Hypoglycemia, a common finding in the untreated animal [41] was never observed in

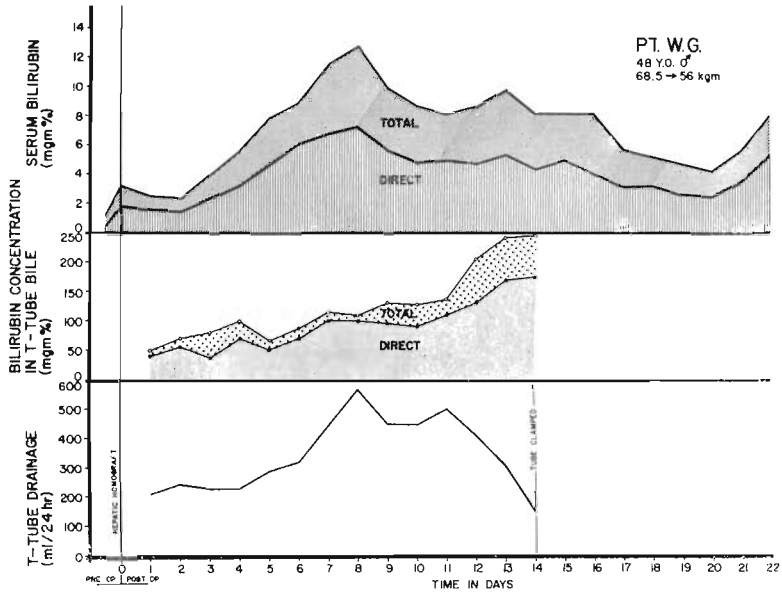

FIG. 13. Course of Patient 2, showing relationships of serum bilirubin, T-tube drainage volume and bilirubin content of T-tube bile. Note temporary worsening of jaundice after transplantation.

dogs under treatment with immunosuppressive drugs. The lowest blood sugar ever recorded was 52 mg.%.

Gross pathology. The shrinkage so characteristic of the auxiliary homografts was not observed in the orthotopic specimens. Liver weights ranged from 330 to 740 Gm. In most dogs, there was gross homogeneous preservation of the specimen, although local intrahepatic abscesses were present in two. The tissue was usually more firm than normal. The vascular supply was intact in all 14 animals under consideration for pathologic analysis. The gallbladder and common duct were intact in all but one homograft, disruption of the cholecystenterostomy having occurred in the exceptional case.

Microscopic studies. In all but one animal, there was some hepatocyte loss (Table 2), the necrosis usually being concentrated around the central vein.

There was a striking difference in the degree of round cell infiltration in the orthotopic as compared to the auxiliary hepatic homograft. Immunocytes were completely absent in four of the orthotopic livers, and cellular invasion was not prominent in any (Table 2). The characteristic hepatocyte fallout was thus present, but without consistent evidence of classical cellular rejection (Fig. 10A).

The intrahepatic duct system was well preserved in every case; in four of the homografts there was either columnarization of the bile duct epithelium, or actual hyperplasia, the latter finding being most prominent in the animals living for the longest time. Ductular bile stasis was present in two specimens. Hepatic arterial and arteriolar changes were present with medial and/or intimal thickening. In many instances, this appeared to be due to medial or intimal proliferation, although the smudge lesions of focal necrosis were also present. The intrahepatic portal veins were normal.

The hepatic reticulum was quite well preserved in every orthotopic homograft, although there were areas of linear compression, and some zones of fragmentation which were especially apparent around central veins in those grafts with marked hepatocyte loss (Fig. 10B).

Human Liver Homografts

Clinical course. The clinical courses of the five patients have been previously described [42, 44, 45], with particular emphasis on changes which occurred in the coagulation mechanism during and following surgery.[45] The first patient succumbed from operative hemorrhage. The next four died after 22, 7.5, 6.5, and 23 days. The immediate causes of death in these last cases were pulmonary embolization; pulmonary embolization and gastro-intestinal hemorrhage; pulmonary embolization and congestive heart failure; and bile peritonitis due to common duct necrosis. Liver failure had an obvious role in the final outcome only in the last case, in which serious deterioration of hepatic function and a bleeding diathesis developed terminally.

Biochemical studies. In all patients surviving operation, there was evidence of moderately severe ischemic injury to the homograft (Fig. 11, 12). Serum levels of SGOT, SGPT, LDH, and ICD were markedly elevated for 24 to 48 hours. The highest SGOT's were 1,150, 990, 350, and 2,060 S-F units, respectively, in the last four cases. The serum enzyme abnormalities were rapidly reversed, however, (Fig. 11, 12) and secondary rises did not subsequently occur even in Case 5.

Preoperative jaundice was present in all but Patient 5. Postoperatively, the jaundice initially deepened in all but Patient 3, the increased early bilirubinemia reaching a peak as long as eight days after operation (Fig. 12, 13). Bile which began to issue from the common duct in all the adult cases during the operation was later shown to have a bilirubin concentration as high as 250 mg.% (Fig. 13). The reversibility of the bilirubinemia was similar to that of the SGOT, except that it occurred at a later time. The early rise in bilirubin was also thought to be due to acute ischemic injury, rather than to early rejection.

Serum alkaline phosphatase was elevated preoperatively in all four of the adult cases, with a range of 12 to 35 Bodansky units. Postoperatively, these serum levels were persistently reduced to or toward normal in every instance (Fig. 11). The terminal alkaline phosphatases before death were 5.3, 6.7, 10.4, and 8 Bodansky units in Patients 2 to 5.

Prothrombin times which ranged from 40 to 100 per cent preoperatively were maintained above 25 per cent in each case except the last one. In this patient, the prothrombin time was severely depressed from the time of surgery until death (Fig. 12). In all patients, there was a slow decline in total serum protein, but severe hypoproteinemia was present only in Patient 5 (Fig. 12). Specific changes occurred in plasma fibrinogen content which have been previously described in detail.[45] These consisted of depressed fibrinogen content intraoperatively, with an almost immediate rebound which lasted for several days. In Patient 5 (Fig. 12), this sequence was seen but to a lesser degree than in any other case.

As mentioned above, progressive liver failure was present only in the last patient. In this case, restoration of liver function toward normal was interrupted on the 17th postoperative day after the patient developed acute abdominal pain. Hepatic function had been poor from the time of operation (Fig. 12), with persistently low prothrombin times, rapidly falling serum proteins, low plasma fibrinogen concentration, marked bilirubinemia, and low-volume T-tube drainage (50–150 ml./day). Although hypoglycemia did not occur, there was evidence of deranged carbohydrate metabolism. Progressive rises in serum pyruvate and lactate levels were observed (Fig. 14), beginning on the fourth postoperative day and reaching peaks of 3.4 mg.% pyruvate and 73.4 mg.% lactate. *Excess lactate,* calculated from Huckabee's formula,[11] was as high as 31 millimoles per liter.

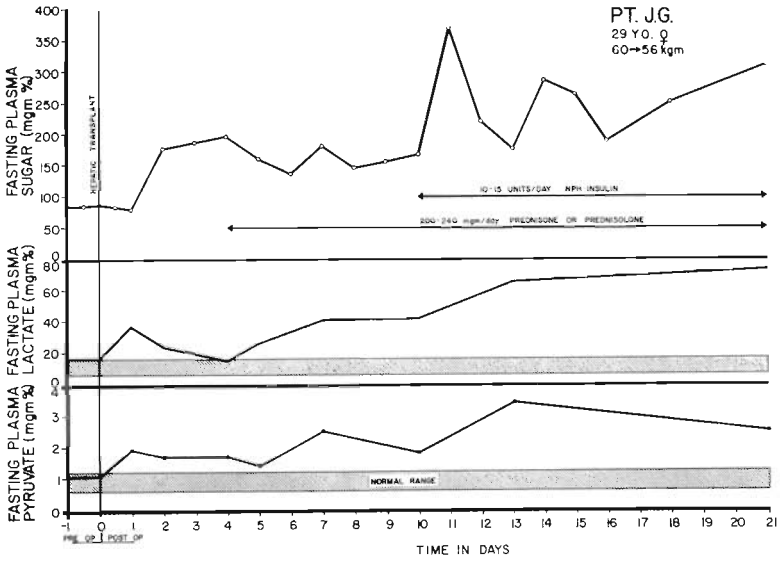

FIG. 14. Changes in serum lactate and pyruvate in Case 5. A transient elevation occurred immediately postoperatively with subsequent decline toward normal. The secondary rise beginning on the fourth day was progressive. Note the normal to increased levels of plasma sugar. Normal ranges for serum lactate and pyruvate are indicated by the shaded bars.

Several factors which are known to influence lactate levels may have been present including steroid therapy, pain, and hyperglycemia, although none of these lead to the production of calculated excess lactate.[12] It is probable, however, that the lactic acidemia is explicable by the mechanism defined by Ballinger[1] who demonstrated the inability of the hypofunctioning liver to effectively metabolize normal or increased quantities of this substrate. Less striking elevations of serum pyruvate (highest 2 mg.%) and lactate (highest 33 mg.%) were documented in Cases 2, 3, and 4, but since all three patients had hypoxemia as the result of pulmonary embolization, the significance of the findings is less clear than with Case 5 in which anoxic episodes were absent until terminally.

In Patient 5, the unconjugated plasma amino acids were studied serially by means of high voltage electrophoresis.* There was a generalized increase in the concentrations of lysine, histidine, glycine, alanine, serine, valine, glutamine, and taurine.

* Amino acid determinations performed by Thomas C. Wood, Jr., medical student University of Colorado School of Medicine. Manuscript is in preparation.

Haptoglobin Studies. The haptoglobins constitute a population of serum proteins characterized by their ability to form a stable bond with hemoglobin.[17] The liver has been postulated to be a site of haptoglobin production.[28] The molecular heterogeneity of these hemoglobin-binding proteins was demonstrated by Smithies who observed that serum protein fractions from normal subjects had different mobilities when subjected to electrophoresis in a starch gel.[36] Smithies and Walker subsequently reported that the type of haptoglobin produced by an individual was under genetic control.[37] Three major types were identified and designated 1-1, 2-1 and 2-2.[35]

In Case 5, in which the donor and recipient were of different genotypes, haptoglobin studies were performed. Electrophoresis of the donor serum, obtained on the day of his death, revealed the haptoglobin genotype to be 2-2. The serum of the recipient in Case 5 was examined prior to the surgical procedures, between the first-stage operation and the transplantation, and on several occasions after the liver transplantation. The original haptoglobin type was 2-1.

The first post transplant serum was obtained after two days. The haptoglobin

genotype had changed to an unmixed 2-2, the genotype of the liver donor. The haptoglobin patterns of the donor and the recipient, before and after transplantation, are shown in Figure 15.

Later in the postoperative period, demonstrable haptoglobins disappeared entirely. The 2-2 haptoglobin pattern was faint but probably present on the fourth postoperative day, but was not detectable thereafter (Fig. 16). The 2-1 haptoglobin did not reappear.

The biologic role of haptoglobins is not clear, although their possible functional significance and the way in which they are influenced by various disease states including

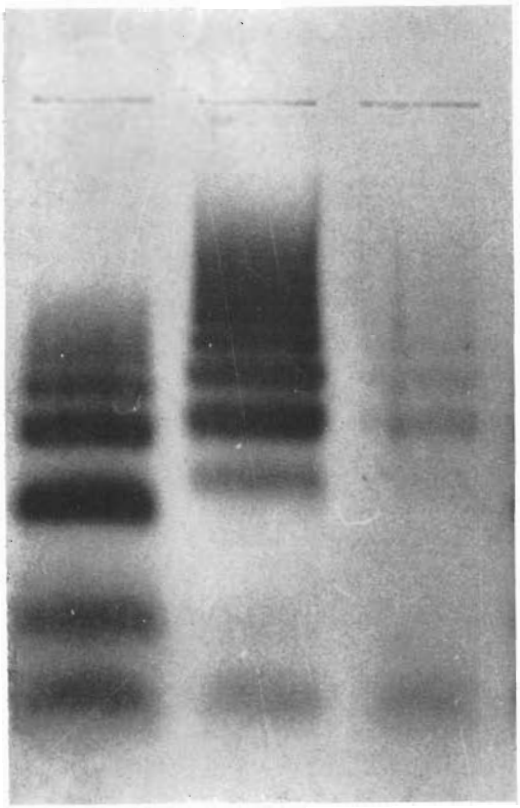

FIG. 15. Starch gel electrophoresis showing the haptoglobin genotypes of donor and recipient in Case 5. The genotype of the recipient prior to surgery was 2-1 (left) and the donor was 2-2 (center). The donor type haptoglobin was found in the serum of the recipient on the second postoperative day (right).

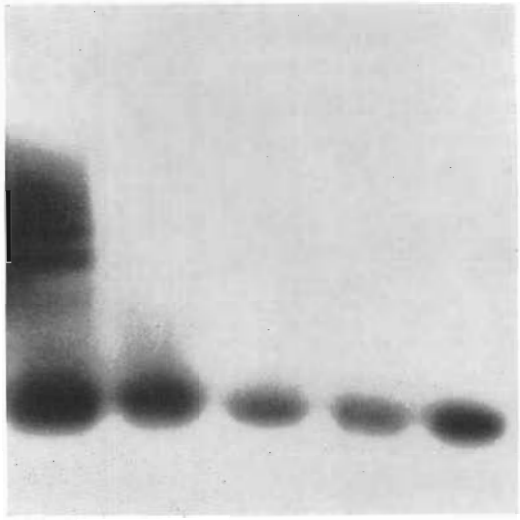

FIG. 16. Starch gel electrophoresis of the recipient's serum on the second, fourth, sixth, tenth and 17th post-transplant days (left to right). No haptoglobins could be found after the fourth day.

trauma, liver disease and steroid therapy, have been the subject of a recent publication.[8] The observance of pure donor genotype in the recipient is of interest for two reasons. It provides evidence that the source of haptoglobin is hepatic. In addition, it demonstrates a type of protein synthesizing activity of the new liver. The disappearance of the new haptoglobin after four days cannot be construed, however, as being due to complete cessation of function of the homograft in view of the subsequent survival of 19 days, and because other factors are known to mask the presence of this substance.[8]

Immunoglobulins. The serum concentrations of immunoglobulins were serially determined in Patient 5, and compared to standard values derived from analysis of pooled human sera.[5]

When the recipient patient was first studied, before both the preliminary and definitive surgical procedures, the values for $gamma_2$ globulin, $gamma_{1M}$ globulin, and $gamma_{1A}$ globulin were within the normal range (Table 4). The concentration of

TABLE 3. *Clinical Liver Homografts*

No.	Survival	General Architecture	Reticulum	Intrahepatic Hepatic Artery	Intrahepatic Bile Ducts	Perivenular Necrosis Central Vein	Immunocytes	Kupffer Cells	Hepatocytes
Case 1	4 Hr.	Diffuse autolysis	Intact						
Case 2	22 Da.	Excellent	Intact	Normal	Columnarization and hyperplasia, bile stasis, moderate	None	Periportal, minimal MGP negative	Normal; moderate iron content and small amount bile	Well preserved PAS+
Case 3	7½ Da.	Good; central and scattered focal necrosis, moderate	Intact	Intimal thickening and proliferation	Normal	Moderate	Periportal, moderate MGP negative	Normal	Well preserved PAS +Diffuse fatty metamorphosis
Case 4	6½ Da.	Good; central and scattered focal necrosis, minimal	Intact	Medial and subintimal thickening and focal necrosis	Normal	Slight	Periportal, slight MGP negative	Normal	Well preserved PAS +Intracellular bile pigment
Case 5	23 Da.	Fair; central necrosis, moderate; midzonal necrosis, moderate	Focal areas of linear compression	Medial and intimal thickening, minimal	Normal; bile stasis, moderate	Moderate	Periportal, minimal MGP negative	Normal; moderate iron content	Poorly preserved PAS−; Contain iron and bile pigment, large amounts

gamma$_2$ globulin fluctuated considerably during the first three weeks after liver transplantation. Forty-eight hours before death it fell to significantly lower levels. The concentration of gamma$_{1M}$ globulin rose significantly on the fourth postoperative day. The level subsequently fell and by the end of the third postoperative week, it had returned to the presurgery value (Table 4). Gamma$_{1A}$ globulin fell immediately after transplantation and then remained relatively constant until 48 hours prior to death when the concentration fell still further. The changes in the concentration of the specific classes of antibody proteins are not as remarkable as the changes in protein fractions, as measured by electrophoresis, reported by Hume [15] and Kukral [16] after human renal and canine hepatic transplan-

TABLE 4. *Serial Determinations of Serum Immunoglobulin in Patient 5*

Day	Gamma$_2$ mg. %	Gamma$_{1A}$ mg. %	Gamma$_{1M}$ mg. %
−4	860	684	288
−3	860	646	288
+2	640	380	224
4	780	570	512
6	860	418	512
10	740	494	352
12	700	342	416
13	540	372	416
16	600	372	240
19	700	380	416
21	420	289	240
23	420	243	192
Normal Sera			
Mean	1,194	395	191
Range	760–2000	118–1,065	64–380

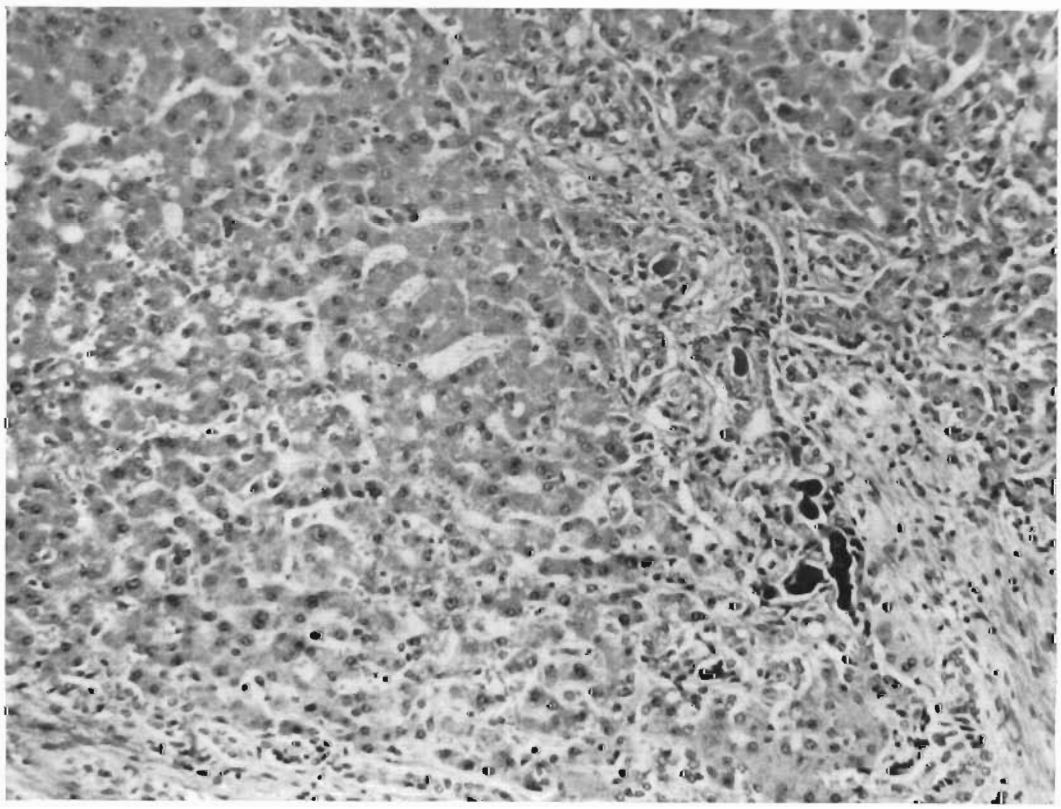

FIG. 17. Liver homograft from Patient 2 after 21 days. The hepatocytes were well preserved. Note the bile duct hyperplasia (left of field) and the bile accumulation. H and E (from × 80).

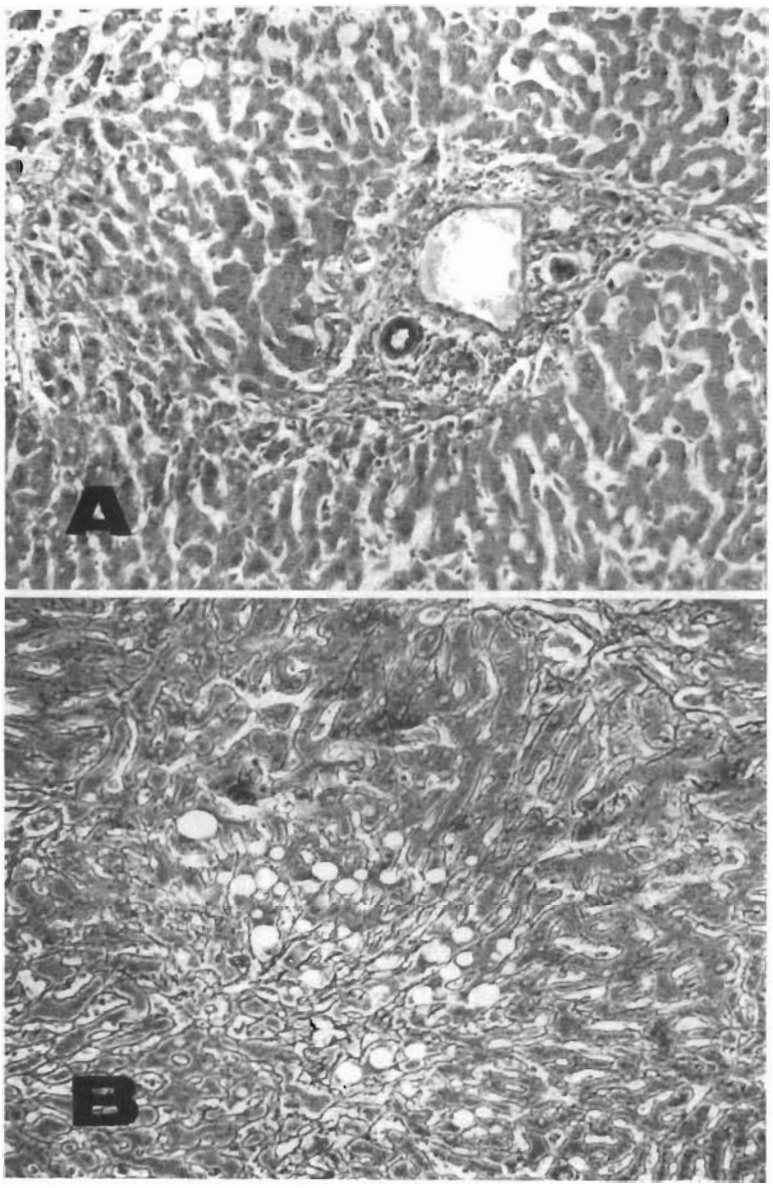

FIG. 18A. Homograft in Patient 3, after 7.5 days. A. The periportal infiltrate was greater than that seen in the other human cases, but the cells were MGP negative. Ducts are normal. Arterial medial and intimal thickening were seen. PAS stain (from × 80).

FIG. 18B. Homograft in Patient 3, after 7.5 days. Intact reticular structure. Note fat infiltration, which was proved with Sudan IV stains. Reticulum stain (from × 80).

tation respectively. The isolated increase in gamma$_{1M}$ globulins is, however, of interest.

Gross Pathology. All organs appeared to be essentially normal. The weight of the child liver was 220 Gm. The weights of the four adult livers were 1,700, 2,070, 1,720, and 1,760. Upon section, the livers seemed somewhat more pale and firm than normal. In Patient 5, there was a 2 cm. abscess in the right lobe. In the same case, there was necrosis of the distal 1 cm. of the homograft common duct.

Histologic Findings. There was extensive autolysis in the first homograft (Patient 1), which was apparently due to an excessive period of ischemia. The other homografts were generally well preserved (Fig. 17–20) with from fair to excellent over-all architecture (Table 3). The reticulum was quite normal (Fig. 18B) in all but Case 5, in

which focal areas of linear compression were present (Fig. 20B). In Cases 3–5, the intrahepatic arteries and arterioles had inconstant intimal and medical thickening similar to that described above in dogs (Fig. 18A, 19).

The intrahepatic ductal system was intact in each case. In one of the homografts (Case 2), there was columnarization and hyperplasia, but this was in a liver provided by a sporadic drinker. Trichrome stains revealed the presence of minimal periportal fibrosis in this specimen. There was evidence of moderate bile stasis (Fig. 17, 20A) in two homografts. The central veins were intact but there was slight to moderate perivenular necrosis in three of the four cases surviving operation.

Immunocytes were found in the periportal area in significant numbers only in Case 3 (Fig. 18), although a few lymphocytes and plasma cells were present in Cases 2, 4, and 5 (Fig. 17, 19, 20). MGP stains were negative in all cases.

The hepatocytes were PAS positive in Cases 2, 3, and 4, but no glycogen whatever was present in the liver cells of the homograft from Case 5. In Case 3, there was a diffuse fatty metamorphosis (Fig. 18), and in Case 5 the individual hepatocytes were poorly preserved (Fig. 20). Intracellular bile pigment was present in Cases 4 and 5.

The state of preservation of liver architecture was generally much better in the human cases than in either the canine auxiliary or orthotopic homograft series.

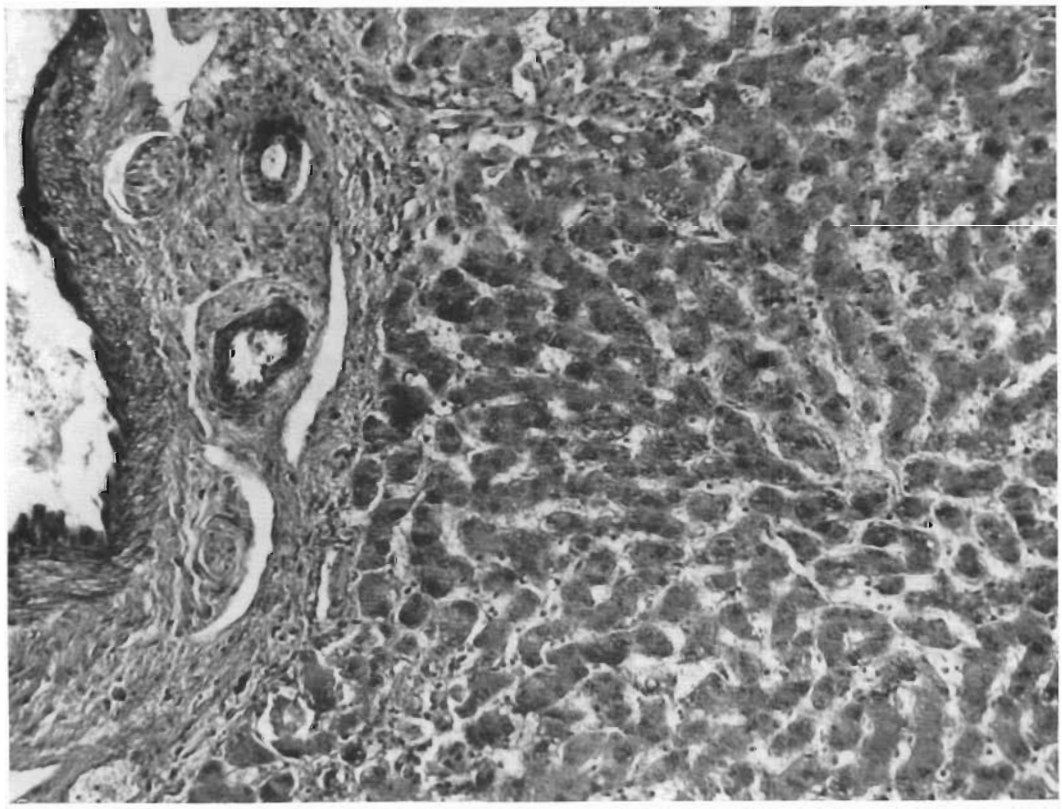

FIG. 19. Homograft from Patient 4, 6.5 days postoperatively. Liver is almost normal. Note thickening of artery in portal triad. PAS stain (from × 80).

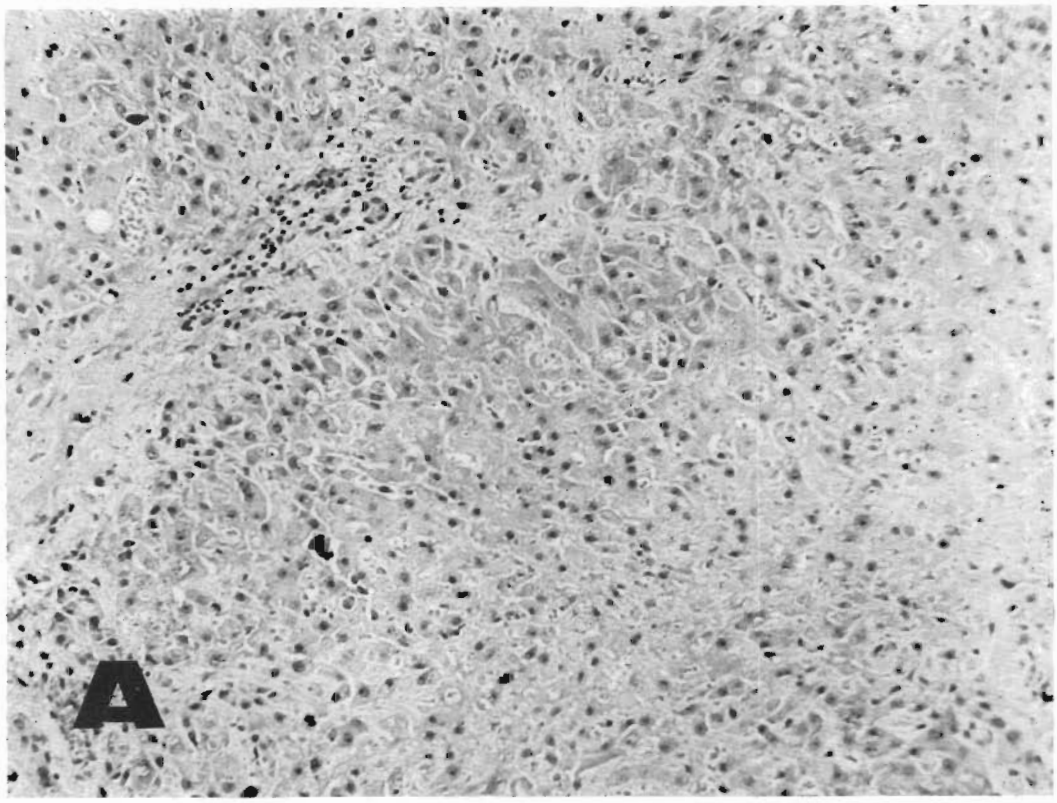

FIG. 20A. Hepatic homograft from Patient 5. Note the poor staining quality of the liver plates and individual hepatocytes. Immunocytes are rare. H and E stain (from × 80).

Discussion

From the foregoing data, there can be little doubt that the vigor of the rejection process was considerably attenuated by the immunosuppressive regimens which were employed. Prolongation of survival was obtained in the animals receiving orthotopic livers. The biochemical response was considerably different from that which invariably transpires after hepatic homotransplantation in the untreated animal.[26, 41] Finally, the state of histologic preservation of the homografts was far better in all three groups than could have been expected in untreated recipients. It is of interest that control of rejection in the human cases was more complete from a histologic point of view than in the orthotopic dogs at a comparable time after operation, suggesting that the magnitude of the problem may be less in man as has seemed to be the case with renal homografts.

Despite these encouraging findings, evaluation of refinements in drug therapy has been difficult. Past efforts to potentiate hepatic homograft survival with total body irradiation have been completely unsuccessful.[38] In the present study the only agent shown to be of unequivocal value was azathioprine, and even this compound was incompletely effective. It was not possible in dogs to demonstrate with any objectivity a consistent improvement in results with the addition of prednisone to the basic azathioprine regimen. If a beneficial steroid effect is present, as has been demonstrated after canine renal homotransplantation,[21] it is probably being obscured by the many other pitfalls which make difficult the con-

sistent attainment of a useful liver preparation.[42] Alternatively, the possibilities must be conceded that the various drugs used are not so selective in preventing hepatic destruction as they are in preventing renal homograft repudiation, that the liver is more highly antigenic and evokes a crushing immunologic response, as Greene's heterotransplantation studies would seem to indicate,[10] or that hepatic tissue is inherently more vulnerable to rejection injury.

Special attention should be directed to the influence of immunosuppression upon the pathologic features of rejection. In the untreated recipient, the most prominent findings are those of *classical cellular rejection,* with early and extensive invasion of lymphocytes and plasma cells [3, 23, 26, 41] which occurs coincidentally with or just before rapid disappearance of hepatocytes. Inflammatory changes in small hepatic arteries and arterioles have also been described.[3, 25, 38, 39, 41] It has been traditional to regard the cellular invasion as the primary event in the process with the small lymphocyte being the actual agent of destruction.[46]

With immunosuppression, the alterations are often strikingly different. The geographic distribution of hepatocyte dissolution is the same, being concentrated most heavily in areas around the central veins. The arteriolitic lesions are also found in various stages ranging from focal intramural necrosis to medial and intimal thickening and proliferation. But these changes may occur without significant invasion of immunocytes.

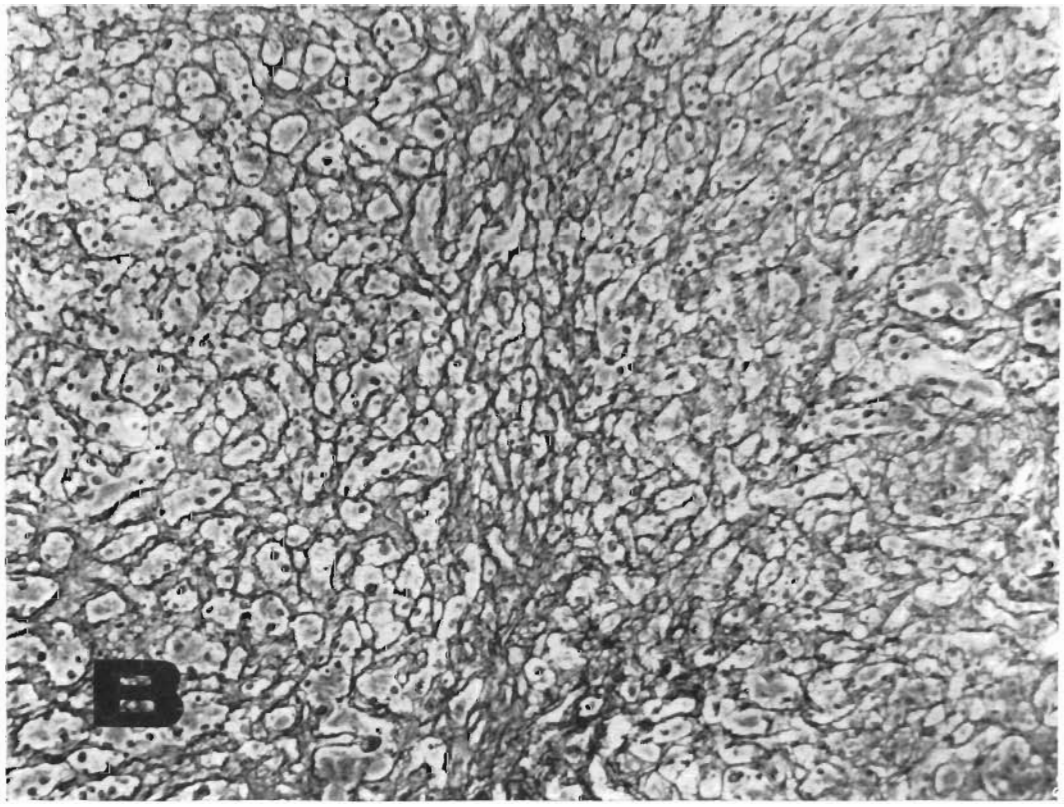

FIG. 20B. Hepatic homograft from Patient 5. Focal reticular compression, seen in the central part of the field. Reticulum stain (from × 80).

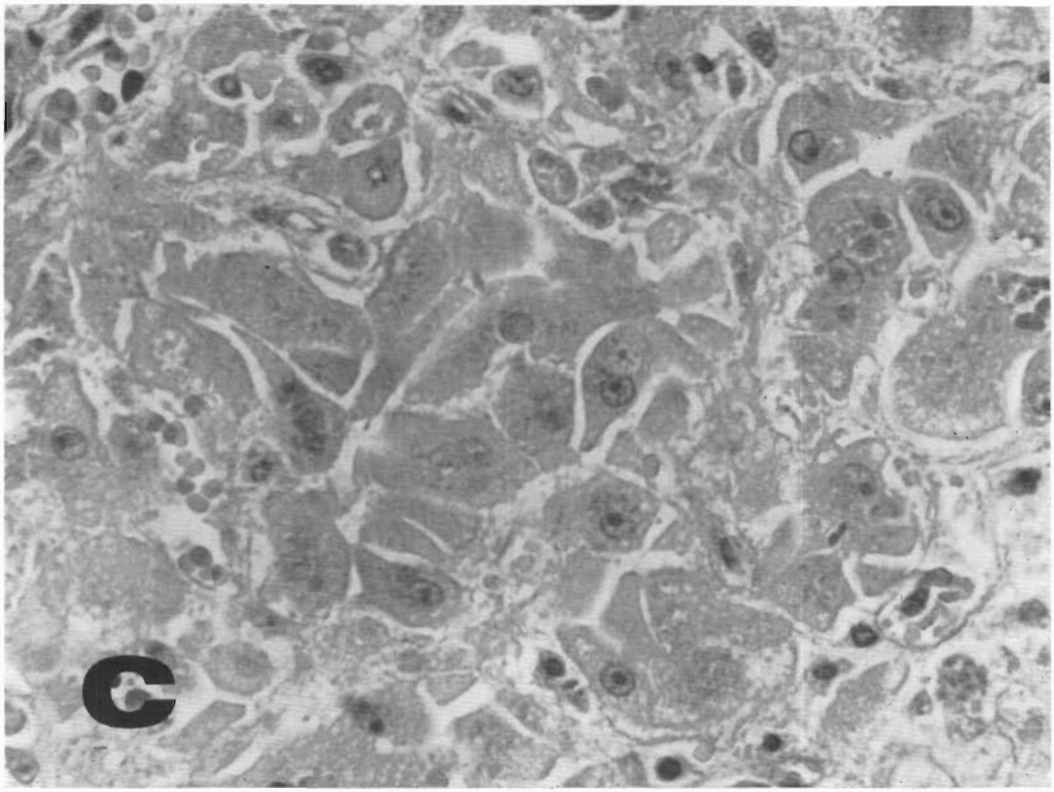

FIG. 20C. Hepatic homograft from Patient 5. High power view of hepatocytes. Note variation in size and shape of hepatocytes and their nuclei. The liver cells contained no glycogen. PAS stain (from × 320).

The high incidence of *non-cellular rejection* in the orthotopic canine series necessitates a re-evaluation of the mechanisms of hepatic homograft rejection, just as has been the case for similar reasons in the field of renal homotransplantation.[15, 31, 43] Physical contiguity of immunocytes to the parenchymal cells is apparently not a requisite.[27] Instead, there is increasing reason to suspect that humoral antibodies may play a crucial role. Sicular and his associates [34] have obtained circumstantial evidence that gamma globulin is fixed in the macrophages, bile ducts, vessels, and hepatocytes of the transplanted liver. More recently, Popper [30] reports that the greatest selectivity of antigen-antibody complexing is in the hepatocytes, a finding which could readily explain many of the pathologic findings in the presently reported series.

Whether or not the non-cellular rejection seen during immunosuppression is fundamentally different from the better known variety of tissue repudiation in untreated animals is open to question. It is possible that the primary mechanism in either case is served by humoral antibodies, and that the immunosuppressive treatment merely slows the process sufficiently to allow observational dissection of the serial events. In this concept, the monocytic invasion of the *classical cellular rejection* could be viewed as a secondary phenomenon. It is also equally possible that humoral and cellular rejection are different and independent processes.

The high incidence of arterial abnormalities in the livers is of special interest in view of the growing evidence that an ischemic vascular factor is of importance

in the rejection of skin,[6] cardiac [32] and kidney [15, 31, 43] homografts. The significance of these changes in hepatic transplants is not known. Moore [24] in postmortem studies has demonstrated areas of poor filling in the hepatic arterial tree, and has suggested that similar devascularization may occur during life. Such changes could not be demonstrated in the present study with *in vivo* angiography, but the degree of small vessel definition was not so great as with his technic. Although acute vascular disease may contribute to the homograft failure, Popper's [30] observations which were cited above as well as the frequent observation in the present study of selective liver cell injury carry the important implication that the hepatocyte rather than the vascular or duct systems may be the primary target.

The auxiliary homograft preparation of Welch and Goodrich and their associates [9, 47] proved to be a useful tool in studying many aspects of hepatic homotransplantation. Delineation of the homograft vascularity could be conveniently accomplished. Consistent chronic survivals could be kept for late histologic examination. The selective preservation of the duct system was most evident in this group of animals. The hypertrophy and hyperplasia of the intrahepatic ducts, changes described by Mackay [18] and Paronetto [29] in liver diseases suggested to have an autoimmune basis, were most evident in this preparation.

Nevertheless, the relative ineffectiveness of immunosuppression in the dogs with auxiliary livers is both noteworthy and discouraging in regard to clinical application of this technic. The degree of cellular invasion and hepatocyte loss was much greater than with the orthotopic livers. Furthermore, a very rapid diminution in size occurred after two weeks.

Several possible explanations may account for these findings. Immunosuppressive therapy, which is partly dependent upon monitoring of function, was not delivered so incisively. The abnormal revascularization may have contributed although this is unlikely since dogs with portacaval transposition do not have loss of hepatic mass.[4] Competition with the dog's own liver for nutritional substrate may have been an unfavorable condition. Finally, the dog's own liver with its large reticuloendothelial mass may have contributed substantially to the immunologic reaction in the auxiliary homograft series, a factor which would be eliminated in the orthotopic preparation.

Summary

The influence of immunosuppressive drugs upon rejection was evaluated in 40 dogs which received orthotopic or auxiliary hepatic homografts, and in five clinical cases of orthotopic liver transplantation. The effectiveness of azathioprine and steroid therapy was judged upon serial measurements of function, upon duration of survival, and upon findings at pathologic examination.

Definite mitigation of rejection was demonstrated in all three groups. From a histologic point of view, immunosuppression was most successful in the human cases despite the employment of badly ischematized cadaveric organs. Orthotopic canine homografts were less completely protected, presumably because of a species difference in the vigor of rejection. The most severely damaged specimens were the canine auxiliary livers which were placed in the right paravertebral gutter without removal of the recipient's own liver.

In dogs treated with immunosuppressive agents, rejection was often observed in the absence of mononuclear cell invasion. The homografts in such dogs commonly had central necrosis and diffuse vascular lesions, with selective preservation of the duct system, the general location of principal hepatocyte loss being the same as previously reported in non-treated animals. The pathophysiologic mechanisms in such noncellular rejection are considered. It seems

possible that the hepatocyte is the primary target of attack by recipient antibodies. Alternatively, the vascular lesions may cause secondary ischemic injury to the parenchyma, in spite of the fact that angiograms in this study failed to support this possibility.

In dogs, the use of optimally preserved homografts makes possible the accurate identification of rejection with biochemical measurements. This diagnosis was made difficult in the clinical cases because of the employment of cadaveric organs, which do not function normally as a consequence of agonal and postmortem ischemic injury. Nevertheless, the results of serial liver tests suggested that rejection was not functionally present in any but Case 5 of the human series during survival periods of 6.5 to 23 days.

Several previously unrecorded observations are described concerning more esoteric biochemical changes after hepatic homotransplantation including alterations in serum or plasma immunoglobulins, amino acids, pyruvates and lactates. In addition, a case is documented in which the haptoglobin genotype of the recipient converted to that of the donor, a finding which supports the concept that the liver is the only source of this substance.

Addendum

A greatly increased survival after orthotopic canine homotransplantation has been achieved with the combination of daily azathioprine and intermittent intravenous doses of S^{35} methionine which was alluded to in the discussion. Methionine was given every 5 days in a dose of 1.8 mg. carrying approximately 90 microcuries of S^{35}. Eleven of 20 dogs so treated have had survival of more than a month. The longest survival obtained thus far has been 4¼ months, the animal still being alive with normal hepatic function on July 28, 1964. Whether or not the improved results are actually due to the addition of radioactive methionine to the regimen or to some other unsuspected factor has yet to be determined.

Since the submission of this manuscript, Dr. Thomas Marchioro has produced strong evidence that competition for alimentary nutritional substrate occurs with the use of the auxiliary liver and that this is the cause for the remarkable diminution of homograft size described in the body of the paper. When the homograft rather than the dog's own liver is vascularized with splanchnic venous flow, the auxiliary homograft retains its size and selective shrinkage of the autologous liver occurs. Thus, the situation reported in the present study is reversed. The latter observation is of immediate practical significance since it has done much to clarify the physiologic requirements which must be met for the successful use of auxiliary hepatic homografts.

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Discussion

Dr. Francis D. Moore (Boston): I would like to congratulate Dr. Reemtsma and Dr. Starzl for their interesting and very impressive efforts to discern the immunogentic sequences in heterotransplantation and hepatic rejection.

(Slide) This slide is from a paper that we presented here in 1961, and it shows the healthy liver cells and the immunocytes in the portal area of an untreated hepatic homograft with good liver cell function for many, and a later rejection sequence characteristic of cholangiolitic hepatitis with small bile duct obstruction.

Under immunosuppressive chemotherapy the whole picture is changed. This shows a similar animal at about ten to twelve days. There is practically no immunocytic rejection whatsoever.

(Slide) However, the vascular lesion is most impressive. This is a postmortem hepatic arteriogram of a dog with a hepatic homotransplant under immunosuppressive chemotherapy, and it shows large areas of ischemia with closing off of terminal arterioles which, on serial section, do not show thrombosis.

(Slide) This is an *in vivo* hepatic homotransplant arteriogram under immunosuppressive chemotherapy. This shows the arterial anastomosis. We can see the dye running into the liver well, but not filling it as well as we would like.

(Slide) And here is the matching portal venogram. Here is the anastomosis here (indicating), filling the liver fairly well but evidently not enough to keep it alive. This is definitely a phase of rejection; it is not seen in the autografts.

While the immunogenetic rejection response is easily abated in liver homotransplantation by the use of immunosuppressive chemotherapy, this has not resulted in the long-term maintenance of canine liver transplants after total hepatectomy. Dr. Starzl has had better results than we have had. We have never had a dog go longer than 14 days. He has had one at 30 days.

In this rapidly growing field of homotransplantation it is important to recognize things that we have *not* achieved. The long-term laboratory survivor is needed, and we do not have any!

In the case of liver we believe that the cause of this failure is vascular, probably a vasospastic or nonthrombotic vaso-occlusive feature of rejection. Our single human experience corroborated this interpretation.

Such long-term maintenance has likewise not been attained for heart, lung, adrenal, or pancreas, to name but a few organs with which members of this society are laboring.

This point is an important one, because the first successful long-term kidney transplantation in man between individuals related more remotely than fraternal twins was preceded by long-term survival in the laboratory using a protocol very similar, if not identical, to that later used in man.

Intra-order heterografts, such as chimp (or baboon) to man are likewise susceptible to careful laboratory preparation and study. Now that this area has been invaded by assault, it would seem wise to slow down and entrench our position by careful laboratory study of the rejection immunology in intra-order primate heterografts involving the several available primate

species *other than man,* in the hope of obtaining laboratory verification before pressing the human patient, *who has something to look forward to from a homograft.*

The ethical problem, in short, is that of science as a whole. Good science is ethical science, and in relation to Dr. Firor's talk, good biological science views the whole man and the whole problem with care and caution.

DR. C. STUART WELCH (Albany, New York): I rise to add my congratulations to Dr. Starzl and his group for the experimental work which they have been performing, and particularly that which has related to their studies on rejection reversal.

My own interest in transplantation of the liver and in discussing this work is on a technical aspect rather than on the immunosuppression side of it. As Dr. Starzl said, we did our first transplants of the dog's whole liver in the lower abdomen. It is not necessary to place the liver transplants in the usual anatomic site in dogs, even though hepatectomy be done, and it is my belief that when the rejection phenomenon can be shown to be controlled, liver transplants in the human being will then be justified. At the present time we have not attempted to do any.

I also believe that liver transplants may have their greatest application in treating patients with cirrhosis of the liver, which is a particular interest of mine. In most instances of this disease hepatectomy need never be done.

Dr. Starzl also suggests here today that from the immunosuppressive aspect it may be important to do it, but if the latter is controlled, hepatectomy should not need to be done in cirrhosis of the liver. At most, perhaps, a portacaval shunt would be necessary, leaving the old liver in place, plus a homotransplant of the liver, which could be done in the lower abdomen. This field of transplantation of the liver should be more rewarding than hepatectomy and transplantation for cancer of the liver.

The size of the whole liver—and this is one of the technical aspects I wish to discuss—offers some obstacle to lower abdominal transplantation, and we have been working on this subject. In recent experiments we have shown that only half of a liver (homotransplant) will sustain life in hepatectomized dogs and not only do some of these livers live but dogs will live hepatectomized when only the arterial blood supply is reconstituted.

Of 22 experiments of hepatectomy plus homotransplantation of half the liver, six were successful, in that they survived over 24 hours, but four had rather longer survivals. Four homotransplants of the middle and left lobe in hepatectomized dogs survived from four to 12 days. These are only preliminary experiments, but the suggestion is present that portions of the whole liver may ultimately survive, which means that a source of homotransplants from live sources may be possible.

I believe the transplantation into the lower abdomen is easier to do, and may well be the site of choice for transplantations in many diseases, and particularly in cirrhosis, when it becomes feasible to do these on a large scale.

DR. JOSEPH E. MURRAY (Boston): I'd just like to ask one question of Dr. Starzl, and possibly Dr. Moore.

Is it a valid assumption that immune suppression is adequate because cellular infiltrate is lacking? In kidney transplants under immune suppression we see as many as five or six different microscopic patterns of rejection only one of which is characterized by cellular infiltrate. Dr. Moore has intimated that the vascular lesion which he described is on an immunological basis and I wonder if the liver program might better be directed toward testing some other drugs unrelated to azathioprine. The vascular lesion which Dr. Starzl and Dr. Moore describes almost certainly has an immunologic component to it.

DR. T. E. STARZL (closing): In answer to Dr. Murray's comments, it has not been our assumption that the absence of immunocytes means that the rejection process has been controlled. On the contrary, it is our view that noncellular rejection is typical of the reaction which we are going to see with increasing frequency in livers, just as has been observed with kidneys.

I think his other comments are very appropriate also. At the present time we are working with S-35-methionine in the laboratory, using this radioactive amino acid as an adjuvant to Imuran. The study is not complete, but the results are encouraging.

Concerning Dr. Welch's comments, I think the reasoning behind his pioneer studies was the same as ours. We had hoped that the patient receiving an organ as functionally complex as the liver would have a better chance to survive if during the rejection crisis there were a functional backstop from the animal's or the patient's own organ which could be left in place. However, the inability to control the rejection under these circumstances has been a discouraging one, and I think it is an ill omen as far as any use of this technic employing auxiliary organs.

The explanation for the less favorable behavior of the auxiliary homograft is not clear. It may be that the injured liver is competing for substrate with the animal's own liver; that we are not tracking liver function so well, and therefore not treating rejection as effectively; that diversion of the portal flow and venous revascularization from the systemic system might be a factor, although Dr. Child's studies of ten years or more ago with transposition are against this; or that by removal of the animal's own liver there is a loss of the reticuloendothelial mass in one versus the other situation.

Dr. Moore's philosophic comments are certainly appropriate and, as he knows, they express our opinions also, at least as far as the liver work is concerned.