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Rejection of the Homotransplanted Dog Liver in the Absence of Hepatic Insufficiency.* (28163)

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Transplantation of a donor liver has repeatedly been attempted(1) particularly in dogs, such as abdominal implantation with one 6-day survivor of 40 animals(2), orthotopic transplantation with one 2-week survivor, and several 1-week survivors in 35 attempts(3) or with preliminary porta-caval shunting and cool saline perfusion(4) with one 3-week survivor and several 6-day survivors. These attempts to study the process of rejection were complicated by hepatic insufficiency and jaundice resulting from the destruction of the donor liver. Therefore, a method has been developed to transplant a whole liver into the pelvis of a recipient animal with its own liver undisturbed *in situ*. This report presents preliminary observations dealing with: a) surgical procedure; b) function of the homotransplanted liver during rejection as indicated by flow and constitution of its bile; c) microscopic changes in the rejected liver; and d) functional and structural response of the host liver as a reflection of the effect of the graft on the host.

Materials and methods. Using donor dogs about 10 kg in weight under intermittent positive pressure endotracheal Nembutal anesthesia, an aortic pedicle is prepared extending from above the celiac axis to the iliac bifurcation. A corresponding vena caval pedicle is constructed extending from the liver to the iliac veins. The portal vein is skeletonized to the superior mesenteric vein, dividing the coronary, splenic and pancreatic branches. In about 20 kg recipient dogs, a second team exposes the infrarenal vena cava, the entire left external iliac artery and the entire left common iliac vein. The hepatic artery connections of the donor dog are then traced to the celiac axis, ligating and excluding superior mesenteric anastomoses as well as left gastric and splenic arteries. The common duct is cannulated with a polyethylene tube and the gall-bladder resected. Hypothermic perfusion of the isolated arterial circuit of the donor liver is commenced with proximal and distal cross clamping, utilizing an external iliac artery as inflow tract. This perfusion with 500 ml of an aqueous solution containing 15% dextran, 5% dextrose and 1,000,000 units penicillin cools the donor liver and avoids intravascular coagulation.

The incision in the donor dog is then extended into the chest with division of the inferior vena cava at the atrium. The liver is separated from its diaphragmatic attachments and the superior mesenteric artery is divided at the aorta. The isolated sterile liver is then transferred to the recipient (Fig. 1). An end-to-side venous anastomosis between the suprahepatic donor vena cava and the infrarenal recipient cava is followed by an end-to-end anastomosis between donor aortic segment and recipient external iliac artery. An iliac-portal venous anastomosis is then performed and the donor liver is stabilized by envelopment beneath the Fallopian tube mesentery. This permits immediate liver perfu-

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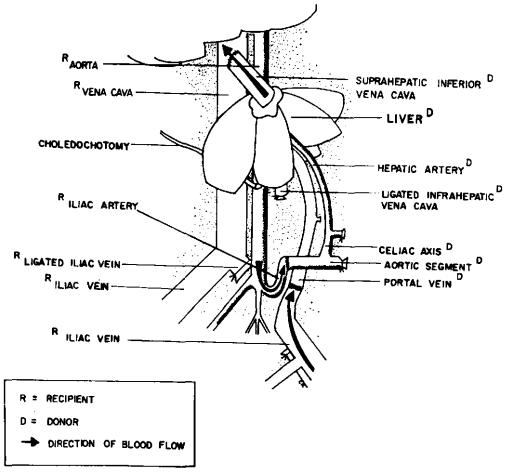

FIG. 1. Schematic drawing of reconstruction following homotransplantation of liver into pelvis of recipient dog.

sion. After closing of the abdomen, the choledochotomy tube is brought out through a posterior stab wound. After the operation the recipient dog receives chloromycetin and penicillin and parenteral fluids for 3 days.

Serum of some dogs was examined for bilirubin level and activities of transaminase and alkaline phosphatase. The bile of the donor livers was studied for flow and for concentration of bile salts, bicarbonate and electrolytes. Liver biopsy specimens of both host and donor liver were obtained at intervals by Menghini biopsy needle and examined histologically as were 2 donor livers removed on the 21st and 24th day after transplantation as well as the livers obtained at autopsy of dogs surviving more than 3 days.

Results. Of 13 completed experimental procedures, 5 succeeded with survival of the recipient dogs beyond 3 days. Two survived for more than 3 weeks. One dog survived 10 days with late vena cava thrombosis. Two died suddenly on the 4th day with intraabdominal bleeding. The time of anoxia of the donor liver never exceeded 45 min and blood transfusions were not required. The serum level of alkaline phosphatase never rose above 29 KA units and that of bilirubin never above 1.0 mg % (Table I). The highest transaminase level recorded was 35 units. Bile drainage was continuous from the time of recipient perfusion. In those dogs surviving beyond 24 hours, sanguinous bile changed to dark green bile initially. Subsequent bile drainage was greenish until the 4th day and thereafter colorless and mucoid in character. In the 10-day survivor, bile was yellow and thinner on the 4th day, changing on the 6th day to whitish yellow, and on the 7th day to mucoid white; on the 8th day it became sanguinopurulent. In the 24-day survivor, bile was clear and green for 4 days, light green on the 5th, and white on the 6th, at which time the dog bit out its catheter. In this dog the donor liver produced initially a bile rich in bile acids and bilirubin and then poor in them, while the electrolytes changed relatively little (Table II).

In biopsy specimen from the host liver, foci of centrolobular ischemic necrosis were noted 2 days after transplantation, which were not seen after the 4th day. Otherwise the Kupffer cells were more numerous and rich in PAS positive material while the portal tracts showed excess lymphoid cells with basophilic cytoplasm. After 5 days, focal necroses appeared associated with accumulation of mononuclear basophilic cells, some macrophages and isolated segmented leucocytes. At 8 days, although the lobular architecture still was entirely preserved, the focal necroses were increased and the portal tracts were conspicuously enlarged and contained proliferated bile ductules surrounded by dense inflammatory infiltration (Fig. 2) consisting of basophilic cells with large nuclei, macro-

TABLE I. Survival Time and Available Laboratory Findings Following Homotransplantation.

Recipient dog	Days survival	Days of bile output	Serum bilirubin (mg %)	Serum alkaline phosphatase (King Armstrong units)
3556	24+	6+	1	
3590	4	3		
3530	3+	3		
3699	22	–	.3/.1	14, 18, 29
3698	10	7	.7/.3	29.2

TABLE II. Biochemical Analysis of Bile Secreted by Donor Liver Removed Eventually 24 Days After Transplantation (#3556).

Days after transplantation	Icterus index	Bile electrolytes (in milliequivalents)				Bile acid (mg %)	
		Na^+	K^+	Cl^-	HCO_3^-	Desoxycholate	Cholate
0	56	160	6	112.6	18.7	6315	6280
½	59	162	6	95.5	13.1	3335	3030
1	52	168	5	88.1	18.5	1800	555
2	39	172	6	87.1	27.2	320	565
4	3	196	8	81.9	35.7	240	265
6	1	180	12	80.1	39.7	10	155

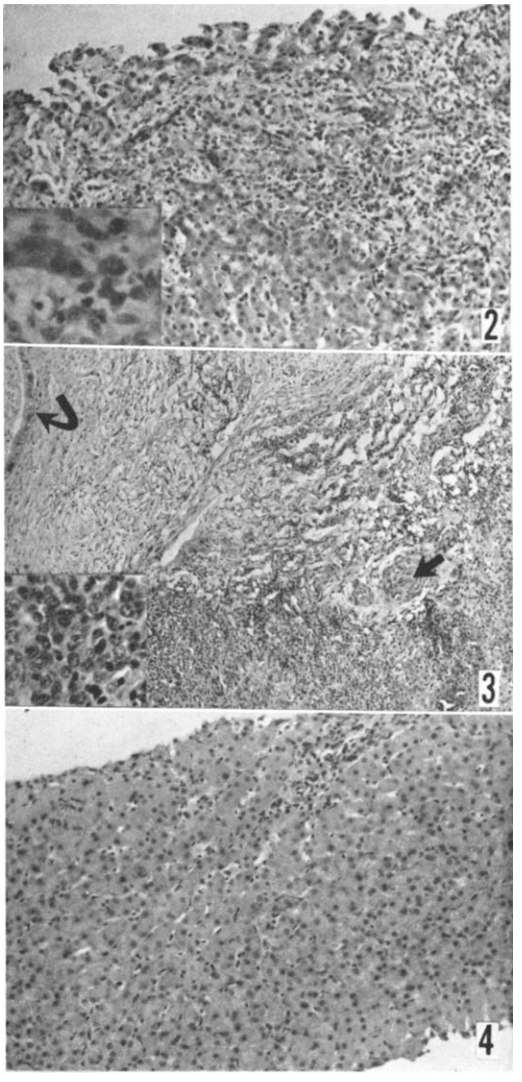

FIG. 2. Host liver on 8th day of transplantation. Note unsharp border between enlarged portal tracts and parenchyma. Proliferation of bile ductules, infiltration with mesenchymal cells and loss of single liver cells (''piecemeal necrosis''). Hematoxylin and eosin, × 120. Insert: Approximation of basophilic and lymphocytic mesenchymal cells to atrophic liver cells. Hematoxylin and eosin, × 240.

FIG. 3. Host liver removed 22 days after transplantation. Collapsed parenchyma containing blood pools and accumulation of basophilic mesenchymal cells. Hepatic vein (straight arrow) occluded by loose cellular connective tissue. Bile duct epithelium (curved arrow) is intact. Hematoxylin and eosin, × 38. Insert: Accumulation of plasma cells. Hematoxylin and eosin, × 240.

FIG. 4. Recipient liver 10 days after transplantation. Some proliferation of Kupffer cells and slight inflammatory infiltration of portal tracts (''nonspecific reactive hepatitis''). Hematoxylin and eosin, × 72.

phages, whose cytoplasm contained PAS positive diastase resistant granules, and few segmented leucocytes. Typical plasma cells were sparse. Liver cells on the lobular periphery had disappeared and those remaining on the unsharp border were surrounded by closely approximated basophilic mesenchymal cells whose cytoplasm was of increased density and contained PAS positive granules. Bile ducts and vessels were not altered. In subsequent days the number of hepatocytes gradually reduced while the number of mesenchymal cells increased. Among the latter were a rising predominance of macrophages, segmented leucocytes and typical plasma cells with cartwheel structure of the nucleus.

The two livers removed at 20 days had a wrinkled surface. Their cut surface had a spleen-like appearance. There were a few foci up to 3 mm in diameter containing purulent material. Histologically, the basic architecture of the liver was in principle preserved but portal tracts and central canals were approximated as a result of collapse. The liver cells were entirely replaced by either pools of blood or accumulated mesenchymal cells, most of which were plasma cells (Fig. 3)

frequently arranged in homogeneously appearing islands. Many phagocytic cells with PAS positive cytoplasm were noted. In some areas segmented leucocytes accumulated. The lumens and walls of the hepatic arteries were intact. The lumens of portal and hepatic veins were greatly narrowed and frequently obstructed by loose cellular connective tissue. The large bile duct, in contrast, appeared little altered.

Biopsy specimens of the host livers, taken on the 10th day after transplantation, showed some proliferation of Kupffer cells (some of them PAS positive) and slight increase of mainly phagocytic and partially lymphoid cells in the portal triads associated with slight proliferation of bile ductules (Fig. 4).

Discussion. Judging from the biochemical parameters investigated, transplantation of the donor liver to a recipient dog interferes minimally with the general well-being of the surviving recipient dog. The recipient liver reveals histologic changes best described as nonspecific reactive hepatitis(5). In view of the absence of "immunologically competent" cells, such changes are more readily explained as a result of the operative insult or of nonspecific tissue breakdown in the host liver than as an expression of a graft effect of the donor liver on the recipient liver. This concept is in agreement with previous assumptions(6).

The alterations of the recipient liver function as reflected by the secretion and composition of the bile are those of an initial preservation followed by gradual secretory failure. Bile flow is preserved for a considerable period but secretion of biliary substances ceases first as might be expected from destruction of hepatocytes. Histologically, these hepatocytes reveal initially transient contiguous ischemic changes. The delayed onset of the biliary secretory failure indicates that the anoxic period of approximately 40 minutes which is unavoidable in the surgical procedure does not interfere with successful transplantation. The subsequent focal necrosis of hepatocytes associated with the accumulation of "immunologically competent" cells gradually destroys the liver. A continuous "piecemeal" necrosis rather than contiguous ischemic necrosis is the crucial process. The persistence of the lumen of the hepatic arteries excludes disturbance of the circulation as of major importance in the elimination of the hepatocytes. This piecemeal necrosis is particularly prevalent in the lobular periphery around the portal tracts and is associated with ductular proliferation and periportal cellular infiltration. Peripheral "piecemeal" necrosis(7) has been designated previously as the structural characteristic of progression of cirrhosis. This indication of chronic active hepatitis has been related to immunologic processes by the demonstration of the local depositions of gamma globulin (8). The morphologic similarity with earlier stages of the rejection phenomenon in the liver is apparent from previous experimental models(9) and it throws light on the pathologic processes in chronic active hepatitis in man. It is interesting that the destruction of hepatocytes and obliteration of veins is not accompanied by significant alteration of bile duct epithelium. Independent of the immunologic implications, it is pertinent that continuous "piecemeal" necrosis *per se* even without acute massive necrosis may lead to massive collapse of the liver. This experimental preparation therefore may explain why human postnecrotic cirrhosis apparently resulting from a continuous, relentless piecemeal necrosis can be found without a preceding history of jaundice(10,11). The experimental model presented appears to be useful for the study of the hepatic rejection processes by immunologic and immunocytochemical technics.

Summary. A method is presented for transplantation of a donor liver into the pelvis of recipient dogs without disturbing the recipient liver. This surgical preparation has been designed for the study of the immunologic rejection of the liver in the absence of hepatic insufficiency and of jaundice. The recipient liver shows only nonspecific changes. No evidence of an immunologic attack of the graft on the host liver is recognizable. The donor liver exhibits transient anoxic changes which do not appear to interfere with hepatic function as judged by its sequential biliary secretion. Bile secretion ceases within 8 days.

Piecemeal necrosis, particularly accentuated on the lobular periphery near the portal tracts, leads to a relentless elimination of all liver cells and their replacement by immunologically active cells within 20 days. The initial stage of this rejection resembles the peripheral piecemeal necrosis of chronic active hepatitis and progressing cirrhosis in man.

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Metabolic Adaptations in Higher Animals. VII. Responses of Glutamate-Oxalacetate and Glutamate-Pyruvate Transaminases to Diet.* (28164)

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Freedland and Harper(1) found that hepatic glucose-6-phosphatase (G-6-Pase) activity of rats, fed a diet in which most of the carbohydrate (dextrin)† was replaced by protein or fat, increased within 24 to 48 hours, then gradually returned to normal. They suggested that the immediate rise in G-6-Pase activity might represent a primary adaptation to low glucose intake and that the return of G-6-Pase activity to control level might be accompanied by secondary adaptations, such as an increase in rate of utilization of amino acids or fatty acids for energy, which could reduce the need for synthesis of glucose and, hence, for elevated G-6-Pase activity.

If transaminase activity increased before G-6-Pase activity returned to normal in rats fed diets high in protein and low in glucose, as would appear to be the case from observations of Rosen *et al.*(2), such a response could be considered a secondary adaptation of the type postulated. Therefore, the activities of some transaminases in organs from rats fed high-protein diets for different periods of time were studied.

Methods. Male Holtzman rats which had been maintained on a stock diet were fed the appropriate control diet for at least 3 days before they were used in an experiment. The composition of diets used most frequently is summarized in Table I.

At the times indicated in the Tables rats were decapitated and organs removed and chilled immediately. About 1 g of liver, kidney or heart was weighed and homogenized with a Teflon pestle in 9 or 19 volumes of distilled water at 0-4°C. Muscle was homogenized in a Duall glass homogenizer (Kontes Glass Co.). Incubations were at

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† The term "dextrin" refers to the product obtained when moist cornstarch is heated for 2 hours in an autoclave at 115°C and then dried and ground.