Homotransplantation of the Canine Liver: *

A New Technic

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WITH refinement in technics for surgery of small blood vessels, considerable investigation has been carried out in the field of whole organ transplantation. Liver transplantation in the dog has been handicapped by difficulties primarily related to outflow block, dual blood supply, and marked sensitivity to anoxia.

During the past two years, reports of functioning hepatic homotransplants in totally hepatectomized recipients have appeared in the literature.[13, 14, 20, 21] This has resulted in increased interest in liver transplantation.

The purpose of this investigation was to develop a simplified technic for transplantation of the canine liver in which the graft and the recipient are subjected to minimal surgical trauma allowing for a high percentage of successful transplants. In addition, this method was designed to provide the opportunity to observe, accurately, the pattern of rejection as well as to create a method for testing the various means of delaying rejection of the hepatic homograft.

Material and Method

Mongrel dogs, previously immunized against canine distemper and hepatitis, were used. Pairs of dogs were selected on the basis of weight; the donor animal weighed 15 to 20 pounds, the recipient 40 to 50 pounds.

Anesthesia: Both animals received 0.4 mg. of atropine sulfate, intramuscularly, one half prior to operation. The donor was anesthetized with ether. The recipient received thiopental sodium intravenously prior to ether. Both animals were intubated with cuffed endotracheal tubes and maintained on ether and oxygen in a semi-closed system throughout surgery. Controlled ventilation was provided by a positive pressure automatic respirator during the period that the chest was opened. Blood transfusions to the donor were usually not necessary, but 500 cc. of blood was always administered to the recipient prior to the release of the occluding clamps and the re-establishment of circulation in the transplant. Blood samples of both animals were subjected to direct cross matching prior to surgery.

Differential intraperitoneal cooling [8] with selective intrahepatic hypothermia was used during dissection and removal of the transplant (Fig. 1). This was accomplished by the initial administration of cold saline (4° C.) intravenously followed by intraperitoneal irrigation with two, or three liters of cold saline (4° C.) to reduce the mid-esophageal temperature from 34 to 29° C. The esophageal temperature was maintained between 28 and 29° C. by additional irrigations. In addition, cold saline (4° C.)

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was infused into the portal venous system through a polyethylene catheter introduced into the splenic vein after splenectomy. This resulted in a drop in intrahepatic temperature to about 18 to 20° C. with minimal effect on the systemic temperature of the animal which rarely dropped below 28° C. Vasopressor agents were not used.

Operative Technic: Donor and recipient animals were operated upon, simultaneously, by two teams. Initially, an incision was made in the neck to expose the jugular vein and carotid artery and polyethylene catheters were introduced into these vessels for the administration of fluids and for the registration of blood pressure, respectively.

Donor Animal: A mid-line thoraco-abdominal incision was made through the skin and subcutaneous fat. The abdomen was entered initially. A biopsy of the liver was obtained and immediately fixed in formalin (Specimen 1). Cold saline irrigation of the peritoneal cavity was then carried out to bring the mid-esophageal temperature down to 29° C. Dissection and exposure of the portal vein, superior mesenteric vein and inferior vena cava followed. The coronary vein was divided at its junction with the portal vein. A long segment of superior mesenteric vein was isolated and later excised in continuity with the portal vein to create a longer venous channel for anastomosis to the recipient vessel. The subhepatic inferior vena cava was exposed from the level of the renal veins to the diaphragm and the two suparenal veins were carefully exposed and ligated. The spleen was removed and a polyethylene catheter was introduced into the portal vein via the splenic vein. Cold saline (4° C.) was infused into the portal venous system to cool the liver to approximately 18 to 20° C. The left gastric vessels were ligated, the upper abdominal aorta was exposed and ligated above the renal artery to restrict arterial flow to the hepatic and superior mesenteric circulations. The

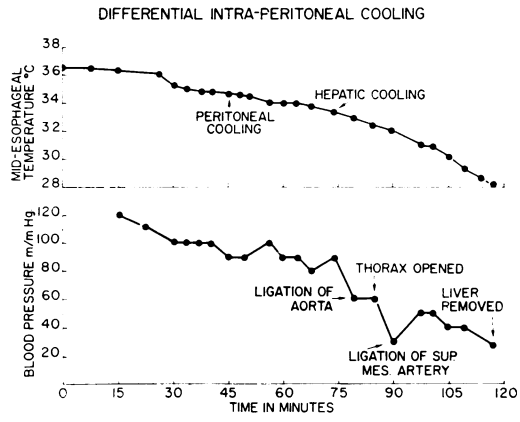

FIG. 1. Temperature and blood pressure record of a donor animal during removal of the liver.

common bile duct was ligated close to its entrance into the duodenum. The pancreatic and duodenal arteries and veins were isolated and ligated.

With the abdominal phase of the dissection completed, the chest was opened through a mid-line sternotomy. Ligation of the superior mesenteric artery was per-

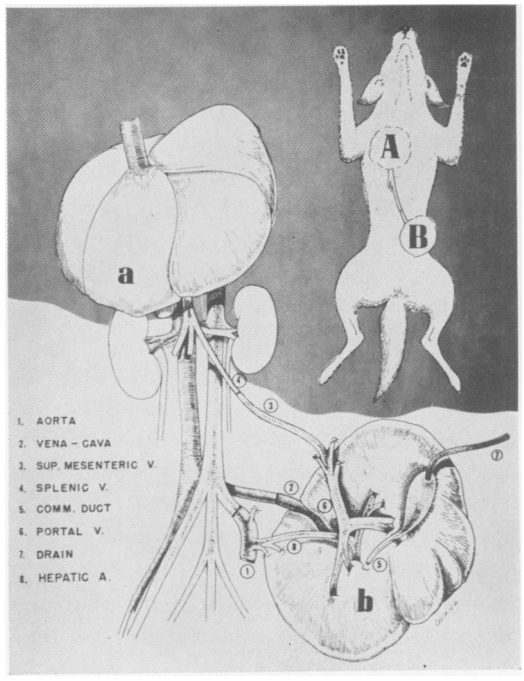

FIG. 2. Diagram showing hepatic transplant in position. (A) Autologous Liver; (B) Homologous Liver.

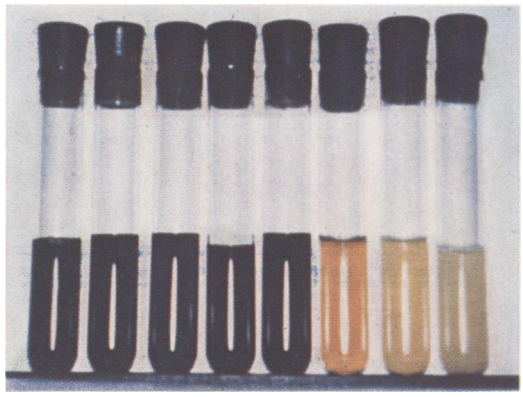

FIG. 3. Character of bile obtained from transplant on day of operation and first seven postoperative days (from left to right). Note striking change in color on the fifth day.

formed prior to sternotomy. In this manner, the abdominal aortic blood flow was routed entirely into the liver via the hepatic and phrenic arteries.[9, 10,15, 16]

The diaphragm was severed between clamps, the lower three intercostal arteries were tied and a segment of aorta including the celiac axis was resected in continuity with the hepatic artery. The segment of inferior vena cava cephalic to the liver was clamped and severed close to its entrance into the right auricle. The inferior vena cava was divided immediately above the entrance of the renal veins. The liver was removed and transferred to the recipient. One end of the inferior vena cava was unclamped to allow free outflow drainage from the liver and the cold saline infusion was discontinued.

Recipient Animal: A second team simultaneously exposed, and transected the left iliac artery and vein at the level of the inguinal ligament. A splenectomy was then performed preserving the splenic vein for future anastomosis with the portal system of the hepatic transplant which was then positioned into the left iliac fossa of the host. An end-to-end anastomosis was performed between the donor's subhepatic vena cava and the recipient's iliac vein. Similarly, an anastomosis was performed between the donor's aortic segment (containing the hepatic artery) and the iliac artery (Fig. 2). A unit of blood was administered prior to the release of the occluding clamps. Re-establishment of arterial blood flow was almost always associated with a moderate degree of temporary hepatic engorgement which gradually improved except in instances where the transplant was subjected to prolonged ischemia. When severe outflow obstruction developed, the liver assumed a dark blue congested appearance with marked stretching of its capsule and the development of multiple spontaneous tears leading to profuse, generalized, and uncontrollable hemorrhage.

Following establishment of systemic arterial and venous anastomoses, the portal circulation of the donor liver was reestablished by end-to-end anastomosis between the recipient's splenic vein and the superior mesenteric vein of the homograft over a temporary internal shunt of polyethylene tubing, which was removed at the completion of the anastomosis.

The liver was fixed to the left iliac region by suturing a part of the diaphragm retained on the posterior surface of the transplant to the psoas fascia of the host.

The portal vein of the recipient was then partially constricted with a fine cotton ligature to reroute part of the host's splanchnic circulation into the transplant. Bile was collected by means of a catheter which was introduced into the gallbladder and exteriorized through a stab wound in the left flank. A biopsy of the transplant was obtained prior to closure of the abdominal wall.

The fate of the hepatic transplant was assessed by the daily output of bile and the determination of some of the physical and chemical properties of the bile. Daily percutaneous needle biopsy specimens were obtained from the homograft.

Results: Of 17 animals receiving hepatic transplants, eight survived for 24 hours or

longer and six survived from 5 to 45 days (Table 1). The surviving animals regained consciousness at the end of operation although they remained drowsy for several hours. After the second day, the animals tolerated regular diet in increasing amounts. One hundred milligrams of tetracycline was administered intramuscularly twice daily for four days and then reduced to once a day for three additional days.

Production of Bile. The production of bile was variable, but no bile was produced by the hepatic transplant during the first six hours. In the first 24 hours, one to 30 ml. of dark green bile was collected. This gradually increased in quantity and reached a maximum of 67 cc./day. On the fourth or fifth postoperative day, the color of the bile changed to brown, golden yellow, and ultimately to turbid white (Fig. 3). Following the sixth or seventh day, copious amounts of foul smelling hemorrhagic fluid flowed for several days, after which secretion stopped entirely.

Composition of Bile. Quantitative determinations of cholesterol, bilirubin, bile salts, and bile pigments in the daily bile specimens revealed steady decreases during the first eight days following transplantation (Table 2).

TABLE 1. *Survival Time and Cause of Death of Recipient Animals*

Survival Time	No. Dogs	Cause of Death
1 to 24 hr.	9	Shock with or without hepatic outflow block
1 to 24 hr.	2	Undetermined
5 da.	1	Undetermined
9 da.	1	Intussusception of entire small intestine
16 da.	1	Peritonitis with possible septicemia
22 da.	1	Peritonitis
22 da.	1	Exsanguination from femoral arteriotomy site post arteriogram
45 da.	1	Sacrificed

Hematological Determinations. A. *Hematocrit:* serial hematocrit determinations showed a progressive drop throughout the postoperative period. In survivors of 14 days and over, the hematocrit values dropped to less than 50 per cent of the preoperative levels.

B. *White Cell Count:* there was a considerable increase in the white cell count.

C. The non-protein nitrogen, total protein, and bilirubin values showed no remarkable change.

TABLE 2. *Composition of Bile Produced by Animal 46*

Day	Vol. (cc.)	Color	Consistency	Cholesterol (mg./100 ml.)	Bilirubin (mg./100 ml.)	Bile Pigment (units)*	Bile Acids (units)**
Oper.		Dark green	Viscid	824	40	2250	19.10
1	15	Dark green	Viscid	952	46	6320	2.15
2	25	Dark green	Viscid	437	13	505	1.55
3	42	Dark green	Viscid	398	3.8	453	1.33
4	40	Dark green	Viscid	257	1.7	216	0.69
5	46	Golden yellow	Watery	225	1.4	135	0.46
6	42	Lighter	Watery	31.1	1.95	11.4	0.32
7	35	Lighter	Watery	28.5	0.1	3.62	0.29
8	12	Turbid	Watery	32.6	0.1	13.7	1.14

* Bile pigments: Units are icteric index units (described by Hawk, P. B., B. L. Oser and W. H. Summerson, Practical Physiological Chemistry The Blakiston Co., New York, 12th ed. p. 542, 1951.

** Bile acids: Units are percentage of sodium taurocholate (giving the same intensity of color reactions as the unknown sample, when tested in Gregory and Pascoe's test (*loc. cit.*, p. 377) as modified by Reif, A. E.).

Arteriography. The arterial supply of the transplanted liver was evaluated by retrograde aortography and was found patent in all long-term animals. In one of the transplants, a patent arterial tree was visualized on the twenty-second post-transplantation day.

Pathological Findings. Tissue specimens obtained immediately before and after transplantation of the liver showed no appreciable change from the normal, except in the dogs in which outflow block occurred. In these animals, severe congestion and dilatation of the intrahepatic sinusoids with compression and atrophy of the neighboring hepatic cells were found.

Sections from the functioning homografts, taken after transplantation, showed the following changes:

Seven Hours: The hepatic tissue was essentially normal except for variable degrees of sinusoidal dilatation.

Ten Hours: The lobular architecture of the liver was essentially intact. The sinusoids showed a slight infiltration with inflammatory cells and the portal areas revealed a mild accumulation of round cells in focal areas.

Thirty-six Hours: The lobular architecture was still intact. There were, however, marked sinusoidal dilatation and congestion with some atrophy of the liver cells. A marked polymorphonuclear cell infiltration was seen throughout the lobule. The portal veins were markedly dilated and the bile ducts appeared intact. Round cell infiltration of the portal areas was more evident. Liver cells showed fine vascularization.

Four Days: The lobular pattern was still present, but only a few strands of liver cells radiating from the central vein areas could be identified. The sinusoids were extremely distended and infiltration of some portal and central vein areas with lymphoreticular and plasma cells was evident. A number of apparent macrophage cells resembling Kupffer cells containing pigmented material were scattered in the more central parts of the lobules. The bile ducts and portal areas were intact and in some of them a number of partially necrotic polymorphonuclear cells were present.

Nine Days: The architecture of the liver was completely destroyed. The sinusoids were markedly dilated and congested. A few bands of viable liver cells were present around the portal areas which were moderately infiltrated with round cells and fibrous tissue. Pigmented macrophages were present throughout the lobule in the portal areas.

Sixteen Days: The entire liver architecture had been destroyed. The sections consisted of a coagulated mass of liver parenchyma unrecognizable as liver tissue. The hepatic cells, portal areas, and bile ducts were completely necrotic.

Twenty-two and 45 Days: Gross examination at autopsy revealed almost complete disappearance of the previously transplanted liver. Sections failed to show recognizable hepatic tissue and resembled a fibrous wall with chronic inflammatory infiltrate and an inner layer of fibrinous material. The inflammatory cells consisted of reticulum cells, lymphocytes and some polymorphonuclear cells with a moderate number of macrophages containing brown pigmented material. Focal areas of calcification and multiple focia of giant cells were seen.

Other Organs in the Host. The recipient's own liver exhibited focal areas of centrilobular infiltration with round cells, plasma cells, reticulum cells, and lymphocytes. Some sections showed focal areas of central necrosis with numerous clostridia organisms which were also present in the portal blood vessels and sinusoids.

In the lungs, pulmonary edema was a constant finding. There was no evidence of infiltration of the pulmonary tissue with round cells or plasma cells. The kidneys showed a variable degree of tubular necrosis with focal areas of round cell and

plasma cell infiltration. The lymph nodes demonstrated active lymphopoiesis with an increased number of plasma cells and severe congestion of the medulla.

Discussion

In 1956, Welch, and Goodrich *et al.*[7, 24] reported a series of functioning hepatic homotransplants in canine recipients with intact livers. Vascular anastomoses were performed with Blakemore-Lord metallic tubes, connecting the donor's hepatic arterial and portal venous channels to the host's aorta and inferior vena cava. The venous return from the lower extremities and pelvis was routed into the portal system of the transplant to simulate the splanchnic circulation, thus re-establishing the dual blood supply of the liver. The results of these investigations showed that the hepatic transplants produced bile for four or five days after which a turbid, then hemorrhagic fluid was collected.

Moore and associates[13, 14] transplanted the canine liver into totally hepatectomized recipients in which preliminary temporary porto-systemic shunts were used to prevent splanchnic pooling. The homograft was placed in the space previously occupied by the animal's own liver and a segment of aorta was resected in continuity with the hepatic artery to facilitate the arterial anastomosis. Hepatic cooling was used to protect the liver cells from the effects of temporary ischemia. In these experiments, the animals were made entirely dependent for survival upon the hepatic transplant. The longest survival period reported was 12½ days.

Starzl and associates[20, 21] performed similar experiments in totally hepatectomized recipients. Hypothermia as well as temporary portacaval shunts was used. Their longest survivor lived 21 days.

The addition of hepatectomy to transplantation of the liver subjects the recipient to the additional stress of a long and difficult operation. A lesser surgical procedure should increase the chances of survival and offer better physiological conditions for recovery and function of the hepatic transplant. Our belief in these principles prompted development of the technic of liver transplantation described in this report. The sensitivity of the canine liver to short periods of total vascular occlusion is well documented.[5, 6, 17] Under normothermic conditions, the hepatic parenchyma tolerates only twenty minutes of ischemia with survival of the animal.[17, 19] When the blood supply is arrested for a longer time, the animal develops severe hypotension and dies from irreversible shock, not responding to blood transfusion or vasopressor agents. Hypothermia increases the tolerance of the liver to anoxia[1-3, 22, 23] and antibiotics provide additional protection.[4, 11, 12, 18]

Regional hypothermia, rapid resection and removal of the liver from the donor, as well as rapid anastomoses, contributed to the success of the technic described, herein. Differential intraperitoneal cooling was well tolerated by the donor and dropped the intrahepatic temperature to 18 to 20° C. while the mid-esophageal temperature remained between 28 to 29° C. Hypotension in the donor was prevented by occlusion of the abdominal aorta above the renal arteries to limit the infradiaphragmatic arterial blood flow to the hepatic and splanchnic circulations. Additional arterial flow to the liver was accomplished by ligation of the superior mesenteric artery prior to thoracotomy.

Despite these precautions, hepatic congestion was one of the most common difficulties in this investigation and in its severest form it progressed to marked intrahepatic pooling of blood, with the development of a large congested liver.

In our successful transplants where a well functioning hepatic graft was achieved, dark green viscid bile was produced for a period of four to five days. A sudden change in the physical properties as well

as the chemical composition occurred thereafter, indicating probable rejection of the homograft (Fig. 3). The liberation of a foul smelling hemorrhagic fluid at the end of the first week after transplantation indicated necrosis and autolysis of the hepatic parenchyma.

Microscopic findings showed that the lobular architecture of the transplanted liver was well preserved up to the fourth postoperative day, but was completely destroyed on the ninth day. Focal infiltration with round cells, lymphocytes, reticulum cells, and plasma cells was observed in the portal areas as early as ten hours postoperatively. Polymorphonuclear cells were also identified in variable numbers in some histological sections.

At autopsy on the sixteenth post-transplantation day, the homograft was completely necrotic and foul smelling. Thin light barium injections into the hepatic artery of the transplants demonstrated a patent intrahepatic circulation excluding vascular occlusion as a cause of autolysis. On the twenty-second and forty-fifth days after transplantation, autopsy revealed almost complete disappearance of the hepatic transplant, with heavy brownish pigmentation of the omentum overlying the transplant.

On the basis of this experiment, we believe that it may be possible to transplant an hepatic homograft to the lower abdominal, or pelvic region of the human, providing a relatively small transplant is used. Vascular anastomoses to the iliac vessels and superior or inferior mesenteric vein should be possible. Avoiding hepatectomy in a seriously ill patient would appear to be an advantage. Clinical trial obviously awaits further means of controlling the rejection phenomenon.

Summary

A new technic for transplantation of the canine liver has been described. Hypothermia by differential intraperitoneal cooling and rapid transfer of the liver proved to be an effective means of increasing the number of successful transplants.

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