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22 Experimental and Clinical Preservation of Orthotopic Liver Homografts

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There are several reasons why effective preservation techniques play a crucial role in orthotopic transplantation of the human liver. The liver is especially sensitive to the effects of anoxia although the rate of injury can be markedly reduced by prompt cooling.[1-3] The technical difficulties of recipient total hepatectomy may be time consuming, requiring many hours. In addition, it may be necessary to invest postmortem time for the performance of histocompatibility tests on prospective recipients and the potential donor. Finally, the penalty for failure to provide a well-functioning organ is death of the recipient, as survival in the functionally anhepatic state is possible for only a brief interval.

For a number of years, research in whole-organ liver preservation provided little reason for optimism. In dogs it was soon learned that prompt core cooling with chilled perfusates provided a safe ischemic time of about 2 hr.[1,2] When perfusion was added to hypothermia, Mikaeloff and Kestens *et al.*[4] and Marchioro *et al.*[5] were able to extend organ viability after donor death; however, both of these systems involved in-situ techniques. Attempts to excise the liver and preserve it outside the donor body were uniformly unsuccessful.

During the last year and a half, it has been established that livers can be maintained in good condition for many hours after their extirpation. It is the purpose of this report to describe the techniques which have made possible these experimental results and the subsequent successful clinical application of the same methods.

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EXPERIMENTAL STUDIES

Hypothermia, hyperbaric oxygenation, and low-flow perfusion through the portal vein and hepatic artery were used together to preserve livers of dogs which were first cooled to 30-32°C and sacrificed by performance of total hepatectomy. In all experiments, the organs were further cooled by infusion with a chilled solution, then preserved, and finally evaluated after placement as orthotopic homografts in nonrelated mongrel recipients (Fig. 1). The test system is a technically difficult one, but it has the overriding advantage that the life of the recipient animal is dependent upon the new organ at all times subsequent to operation. Thus, decisive information can be obtained about both the early and chronic function of the preserved organs and the influence of variations in methodology on the results

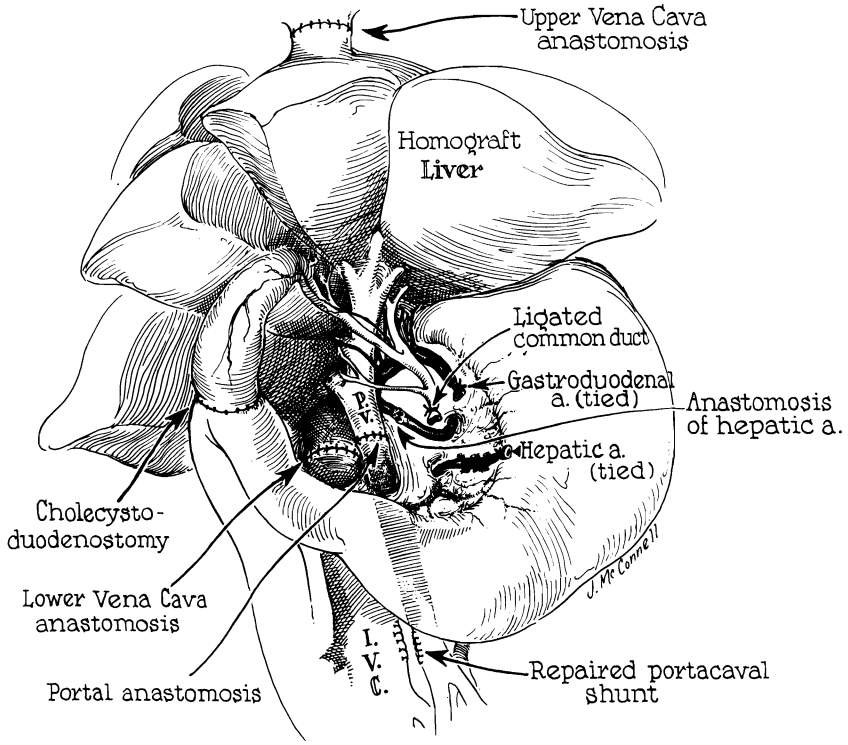

Fig. 1. Technique of orthotopic liver transplantation in dogs. Arterial supply is derived from proximal part of common hepatic artery by end-to-end anastomosis to proximal part of recipient hepatic artery. Distal portion of recipient artery and its inferiorly directed branches are left undisturbed; failure to do so may result in duodenal necrosis. (By permission of *Surgery, Gynecology & Obstetrics* [*126*:263, 1968].)

during both periods of convalescence. The effects of several modifications in technique were analyzed as previously reported elsewhere,[6] and these findings will be summarized below.

OPTIMAL TECHNIQUE

The best results were obtained when hypothermia (4°C) and hyperbaric oxygenation (40 psig) were combined with perfusion of the portal vein and hepatic artery with diluted blood at a total flow rate of 6 milliliters per gram liver tissue per hour; the portal vein to hepatic arterial flow ratio was 4:1. The way in which cannulas were used to connect to the pump system is shown in Fig. 2. A simple baffle runoff system placed within the hyperbaric chamber provided adequate oxygenation. At the end of the preservation period, the chamber was decompressed slowly over a period of 180 to 240 min.

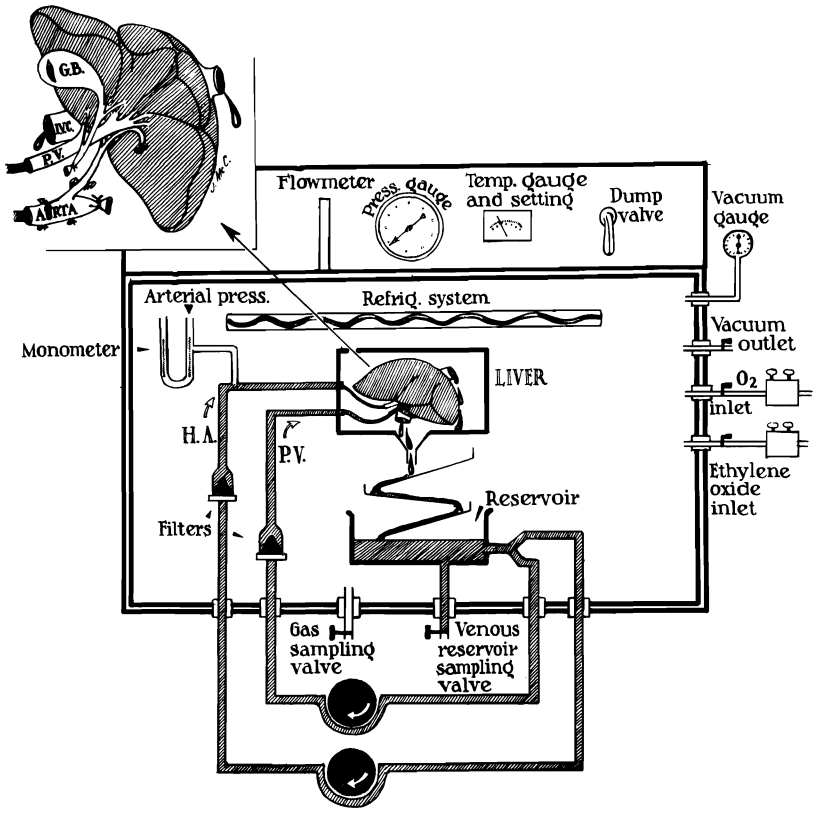

Fig. 2. Preservation unit. Perfusion pumps are located outside hyperbaric chamber. Organ receptacle, oxygenator, and venous reservoir are inside. Various chamber inlets permit sampling of perfusate, gas sterilization, and oxygen delivery and removal. Temperature is electronically controlled. (By permission of *Surgery, Gynecology & Obstetrics* [*126*:263, 1968].)

The composition of the perfusate was one part homologous blood per one part balanced electrolyte solution* containing 5.0 g% low-molecular-weight dextran,† 150 mg% glucose, 2 mEq/L magnesium sulfate, 50 mg/L procaine, 100 mg/L heparin, and sufficient sodium bicarbonate to adjust the final pH to 7.4-7.5. A glass wool filter was inserted at the runoff of the baffle and standard intravenous blood filters were placed in both the inflow lines.

It was found that livers preserved in this way for 8 to 9½ hr invariably functioned well immediately after transplantation to mongrel dogs. In five such experiments the recipients survived operation for more than 5 postoperative days. Furthermore, chronic survival in these animals, which were treated with azathioprine, prednisone, and heterologous antilymphocyte globulin, was not significantly different (Table 1, Group 1D) than in a series of control experiments in which transplantation was carried out as rapidly as possible without the intervening period of preservation (Table 1, Controls). The biochemical evidence of ischemic injury after transplantation was mild-to-moderate and highly reversible—at least in terms of changes in SGOT and SGPT and other measures of liver function.

With use of the same technique, 5 more livers were preserved for 24 hr. Two recipients of these organs died during or just after operation from an uncontrollable hemorrhagic diathesis. The other 3 (Table 1, Group 1E) lived for 8, 46, and 128 days (Fig. 3).

REDUCED PERFUSION RATES

The same experiment was performed in 10 animals but with half the rates of total perfusion. Survival at operation was obtained in 90 percent of the animals (Table 1, Groups 1B and 1C) but only 4 of the 10 lived beyond 5 days. Even in the occasional long-term survivor there was evidence of moderate-to-severe ischemic injury with high rises in the serum transaminases and the early appearance of jaundice (Fig. 4). In less fortunate animals a characteristic syndrome was observed. First, the operative bleeding was often extremely difficult to control—a fact fully explained by multiple and rapidly developing abnormalities of coagulation detected in the same animals by Pechet *et al.*[7] Second, many of the recipients in which hemostasis was finally obtained had subsequent evidence of acute hepatic insufficiency. Postoperative hypoproteinemia was common. Massive fluid therapy was usually necessary, apparently because of "third-space" sequestration. Serial electrophoretic studies in such dogs showed the disappearance of multiple protein constituents which are ordinarily present in small quantities. This resulted in a "stripped tree" appearance (Fig. 5), indicating the presence of reduced protein synthesis and/or an increased overturn of the various protein fractions. With the passage of time, the absent protein lines tended to return (Fig. 5), but usually incompletely by the time of death. Gastrointestinal hemorrhage was frequently a de-

*Travenol Laboratories, Inc., Morton Grove, Ill.
†Pharmacia Laboratories, Inc., Piscataway, New Market, N.J.

TABLE 1. EXPERIMENTAL GROUPS AND SUMMARY OF RESULTS

Group	No. of Experiments	Avascular Period (hr)	Perfusate	Hyperbaria	Flow Rate (ml/g liver/hr)	Hyperbaric Decompression (min)	Average Postperfusion Weight Gain (%)	Operative Survival (24 hr)	Survival Greater than 5 days
Controls	12	½–1½	–	None	–	–	–	12/12	11/12
1A	7	10–14½	Diluted blood	Yes	3	25–35	+16.6	6/7	4/7
B	5	8–9½	"	Yes	3	180–240	+ 0.6	4/5	2/5
C	5	11½–15½	"	Yes	3	180–240	– 1.4	5/5	2/5
D*	5	8–9½	"	Yes	6	180–240	– 4.2	5/5	5/5
E*	5	24¾–25⅛	"	Yes	6	180–240	+ 4.3	3/5	3/5
2	3	20¼–25¼	"	None	–	–	+27.1	1/3	0/3
3	3	22½–25	None	None	6	–	– 4.0	1/3	0/3
4	5	21¼–24½	5% LMDX in balanced salt solution	Yes	6	180–240	– 1.9	1/5	1/5
5	5	8–10½	Diluted hemoglobin solution	Yes	6	180–240	– 0.7	1/5	0/5
6	5	9½–10¾	Diluted plasma	Yes	6	180–240	– 0.9	3/5	2/5

*Optimal preservation technique.

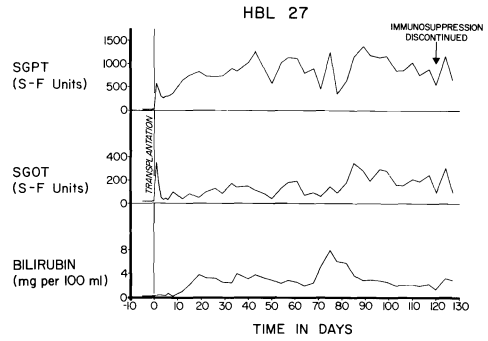

Fig. 3. Course of dog which received orthotopic liver homograft preserved for 24¾ hr. Perfusion rate was twice that used for experiment shown in Fig. 4. There is relatively minor biochemical evidence of early ischemic injury. Dog died after 128 days; immunosuppression was stopped after 120 days.

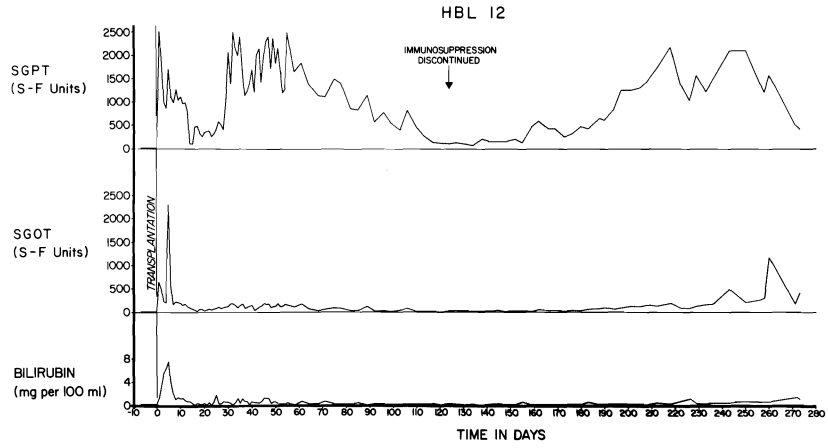

Fig. 4. Course of dog which received orthotopic liver homograft after preservation for 14½ hr. Total liver perfusion was only 3 ml/g/hr, a rate thought to be inadequate. Note early evidence of reversible ischemic injury. Later rise in SGPT was probably caused by low-grade rejection. This dog, as well as all others in the study, was treated with azathioprine, short postoperative course of prednisone, and antilymphocyte globulin; immunosuppression was stopped after 4 months. Dog died after 274 postoperative days.

layed cause of death. The overall picture was one which could be explained by a general impairment of liver function. These latter findings emphasize the importance of not judging the adequacy of preservation techniques simply by the ability to achieve operative survival.

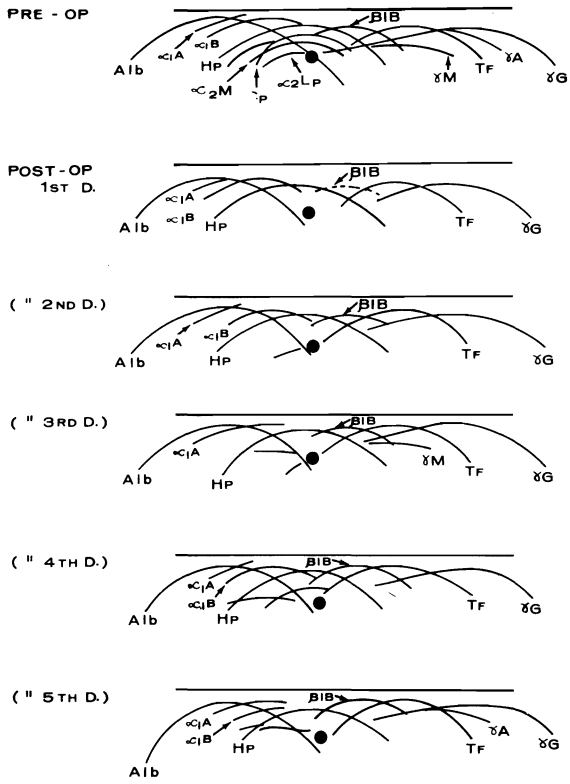

Fig. 5. Changes seen by immunoelectrophoresis in serum of dog which received damaged liver homograft. Note early postoperative disappearance of several precipitation bands including ceruloplasmin (Cp), α_{2M}, α_{2L}, β_{1A}, γ_M, and γ_A. These tended to return, but never completely, to control status. (By permission of *Surgery* [*63*:247, 1968].)

RAPID CHAMBER DECOMPRESSION

In all the foregoing experiments in which either the higher or the reduced flow rates were used, the hyperbaric chamber was gradually decompressed during 3 to 4 hours at the end of the preservation period. The organs did not tend to gain weight even after as long as a day (Table 1, Groups 1B-E).

In another series of experiments (Table 1, Group 1A), similar low flow perfusion was carried out but the chamber was decompressed in 30 min. Weight gain of the homografts was a prominent finding, averaging 16.6 percent. An additional complication was the development of gas bubbles within the organ. These issued forth in the residual fluid in the major vessels and could be identified within the parenchyma by x-ray examination. After the livers were revascularized, some of the dogs did not awaken and it was suspected that they had suffered air embolization. Although 6 of the 7 recipients survived operation, and 4 lived longer than 5 days, all were dead by the ninth postoperative day.

OMISSION OF HYPERBARIC OXYGENATION

Three homografts were preserved for approximately a day by using the higher flow perfusion described earlier and with 100 percent oxygen at ambient pressure. The oxygen tension of the perfusate was maintained at about 250 mm Hg. The homograft weight gain was greater (27.1 percent average) than in any other experimental group in this study (Table 1, Group 2). Two of the recipients of these organs bled to death after revascularization, and the third died after 28 hours.

OMISSION OF PERFUSION

Three homografts were placed in the cold hyperbaric chamber at a pressure of 40 psig, and not perfused. Two of the recipients of these organs failed to survive operation (Table 1, Group 3) and the third died after 3½ days. The last animal never awakened from anesthesia.

EVALUATION OF ACELLULAR PERFUSATES

Unsatisfactory homografts were obtained when the balanced and oncotically controlled salt solutions used to dilute the homologous blood were used for 24-hr perfusions (Table 1, Group 4). Two of the organs did not immediately support life and the recipient of the third had poor liver function during the survival period of 9 days.

In five more experiments, hemoglobin was prepared with Folkman's modification[8] of Pennell's technique[9] and added to the above solution so that the final hemoglobin concentration was 5 to 7 g%. The electrolyte composition and pH were adjusted to that of normal whole blood. Five livers perfused at 6 milliliters per gram liver tissue per hour for 8 to 10½ hr were apparently badly damaged. Only one of the recipients of these organs survived operation (Table 1, Group 5), and that animal died after 5 days.

In a final group of experiments, homologous plasma was added to an equal volume of balanced and oncotically controlled solution; the technique was similar to that described by Slapak *et al.*[10] Five organs were perfused at 6 milliliters per gram liver tissue per hour for 9½ to 10¾ hr. Three of the 5 recipients survived operation and 2 of these animals lived for 19 days. The mediocre results in these trials may be explained by our failure to remove the lipoprotein flocculate which Belzer has demonstrated to be an extremely adverse factor in plasma perfusion systems used for kidney preservation.[11]

CLINICAL APPLICATION

The most effective of the above techniques (Groups 1D and 1E) has been used for preservation of 7 cadaveric human livers. Pains were taken to reduce

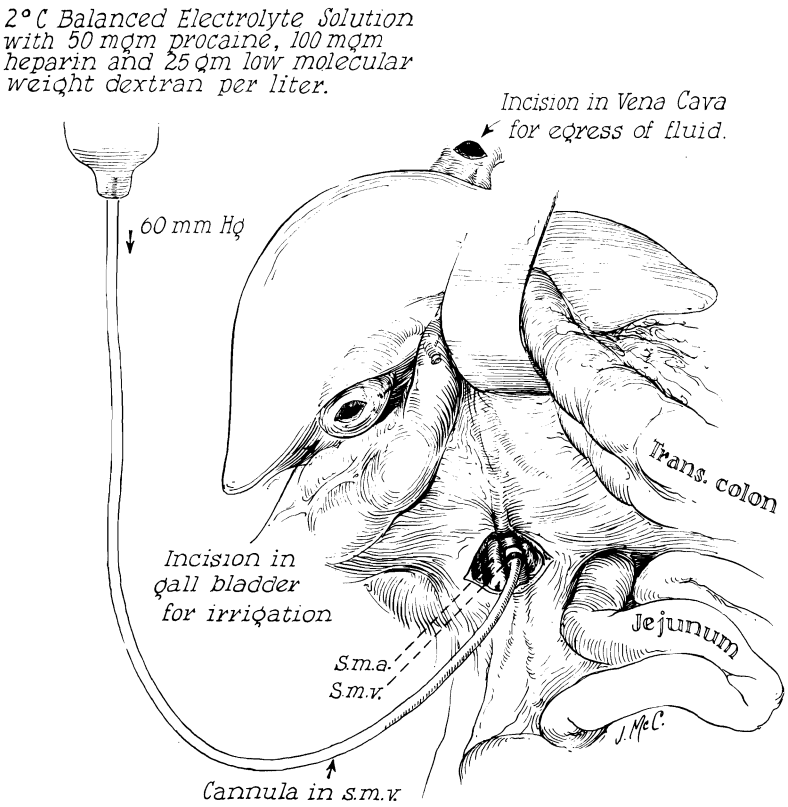

Fig. 6. Core cooling of cadaveric liver used for infant donors. Immediately after entering abdomen, cannula is placed into readily accessible superior measenteric vein. Vessel is far enough away from portal triad so portal vein, ultimately used for anastomosis, is not in danger of injury. Egress of perfusion fluid is provided by venotomy in suprahepatic inferior vena cava. Bile is washed from gallbladder through cholecystotomy. Adult donors livers were cooled by total body extracorporeal perfusion and flushing carried out after completion of hepatectomy. (By permission of *Annals of Surgery* [*168*:Sept., 1968].)

postmortem damage before delivery of the organs to the chamber. The most important step was rapid cooling.

This was accomplished in the infant donors by immediate insertion of an infusion catheter into the easily accessible superior mesenteric vein, which was isolated and cannulated within a few minutes after death (Fig. 6). By keeping the dissection away from the portal tract, the possibility of injury to the major hilar structures was circumvented. During portal perfusion egress for the fluid was provided by venotomies in the vena cava (Fig. 6). A possibly important precaution was lavage of the gallbladder for the purpose of preventing postmortem autolysis of the extrahepatic biliary system.

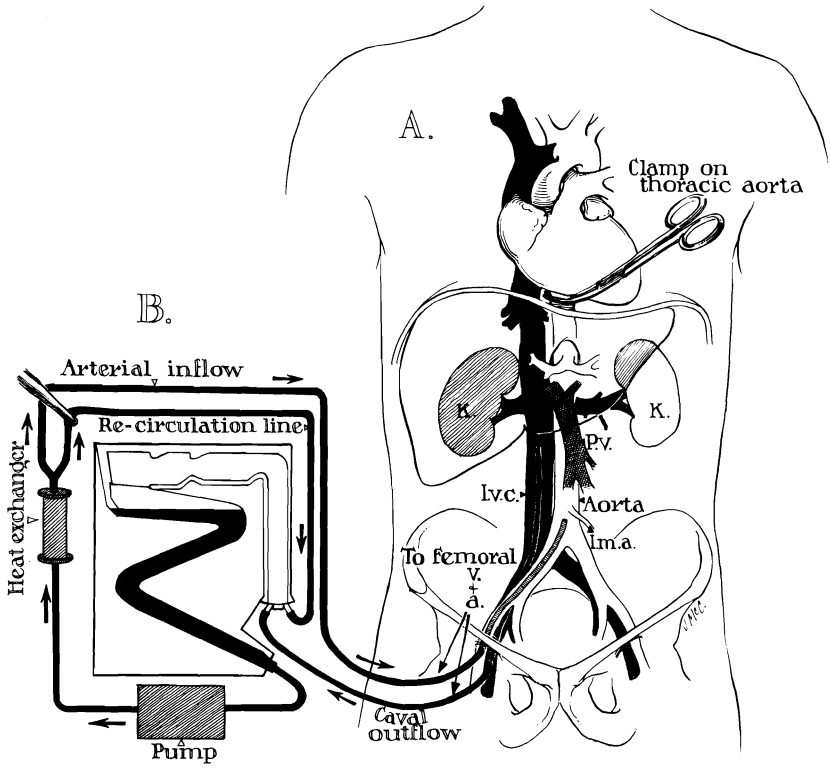

Fig. 7. Technique of extracorporeal cadaver perfusion. Catheters are inserted via fermoral vessels into aorta and vena cava as soon as possible after death. Extracorporeal circuit is primed with heparinized glucose or electrolyte solution with procaine added. Cadaver is anticoagulated with first surge of pump. Temperature control is provided by heat exchanger. (By permission of the W. B. Saunders Co., Philadelphia, 1964.)

Adult donors were cooled in a different way (Fig. 7). As soon as possible after the pronouncement of death, catheters were inserted via the femoral vessels into the abdominal aorta and vena cava. The cadavers then had circulation restored with an extracorporeal heart-lung machine which contained a heat exchanger.[5] The cadaver could be core-cooled to approximately 10°C within a half-hour at the same time as donor hepatectomy was being performed. The perfusion used was initially 30 to 50 milliliters per kilogram body weight per minute but after 15-30 min this was reduced to as low as 10 ml/kg/min.

After being removed, the cooled livers were first cleared of blood by infusing a balanced and oncotically controlled electrolyte solution through the portal and arterial routes. The organs were then placed in the hyperbaric chamber and per-

fused at a flow rate of 6 ml/g/hr exactly as in the most successful experimental group described in the preceding section. Special precautions were taken to insure that the blood donor for the perfusate was compatible by direct crossmatching with the liver donor and the prospective recipient. The time between donor death and revascularization of the homografts in the 7 cases was 242 to 427 min.

All 7 of these livers provided adequate immediate function, although there were always acute postoperative declines in plasma protein concentrations (Figs. 8 and 9). In each case there were rises in serum transaminases the morning after operation but these rises did not reach high levels and were quickly reversed. The

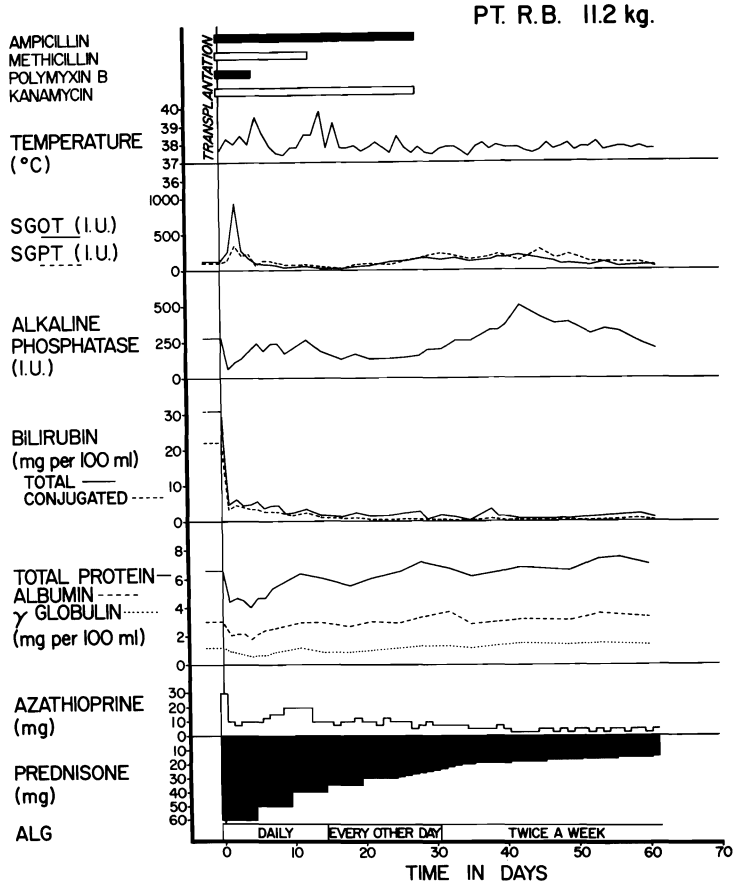

Fig. 8. Course of 24-month-old child after orthotopic liver homotransplantation for extrahepatic biliary atresia. Jaundice has not recurred, although subclinical rejection was diagnosed from third to seventh postoperative weeks because of rises in alkaline phosphatase, SGOT, and SGPT; these changes have receded. By 4 weeks, all antibiotic therapy was stopped. Child has since been afebrile. Temperatures are maximums for each day. (By permission of *Annals of Surgery* [*168*:Sept., 1968].)

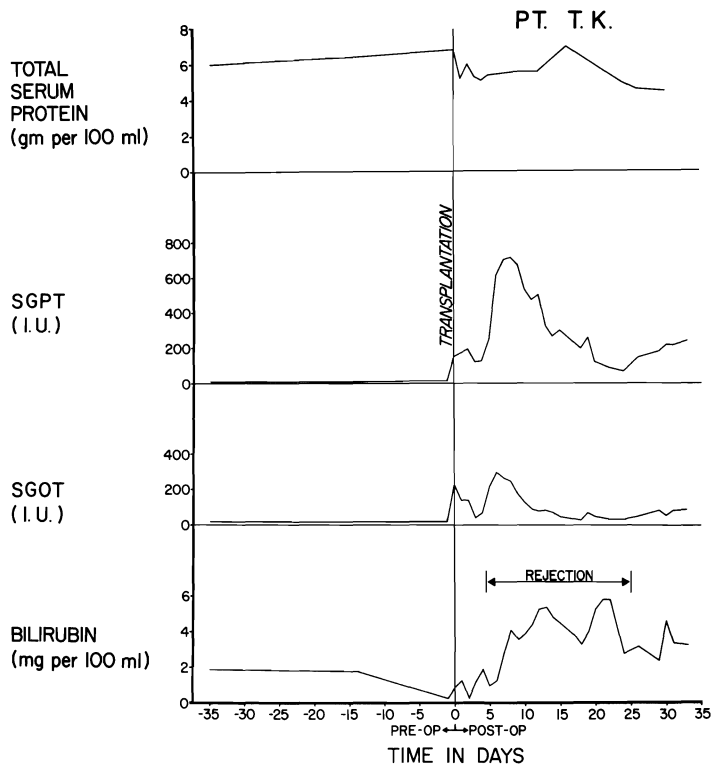

Fig. 9. Early postoperative course of 16-year-old girl who received homograft. Note absence of striking rises in early postoperative transaminases. Beginning 5 days after transplantation abnormalities of liver function became evident, but these were thought to be caused by rejection, as indicated in graph. Rejection was easily controllable and she is in good condition 6½ weeks post-transplantation.

ensuing events in these cases were determined by the severity of subsequent rejection and by other factors that have been fully described elsewhere.[12,13]

For example, the course of a child with extrahepatic biliary atresia is shown graphically in Fig. 8. His serum bilirubin level before transplantation was more than 31 mg%. The hyperbilirubinemia rapidly resolved within a few days after operation. An overt rejection crisis has not been seen although there were transient rises in SGOT and SGPT during the second postoperative month.

The minor degree of liver injury caused during the terminal course of the donor and during the times of organ removal and preservation can be even better

appreciated by observing the convalescence of a hepatoma patient who was not jaundiced (Fig. 9). This recipient was 16 years old and weighed 39 kg. Her donor was an 82-kg man, 27 years old, who died of a gunshot wound of the head. Both patients were Blood Type O. The time from donor death to revascularization in the recipient was 7 hr and 7 min. The organ functioned immediately and essentially normally until the onset of an indolent and controllable rejection on the fifth postoperative day.

The good early function in these 2 cases as well as in the 5 others is evidence of the efficiency and practicality of the preservation system. In one instance, the prospective recipient was leaving Chicago for Denver when the donor died. The organ was conserved until he arrived and was prepared for hepatectomy; the time from donor death to homograft revascularization was 6 hr.

Four of these 7 patients are still alive 2½ weeks, 1½ months, 3 months, and 9½ months post-transplantation. The other 3, as well as a fourth patient who received a nonpreserved liver, died after 2, 3½, 4½, and 6 months. Failure to provide an immediately functioning and life-sustaining homograft was not a factor in the unfavorable outcome in any of these cases.

SUMMARY

An effective method for preserving whole-organ liver homografts has been tested in the laboratory and applied clinically. The technique involves a combination of hyperbaric oxygenation, hypothermia, and perfusion with diluted blood.

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