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*Impediments to successful liver transplantation in man, and possible solutions**

by

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BY NOW, considerable information has become available on hepatic transplantation. Two things seem clear. First, the feasibility of such procedures has been proved beyond doubt,^{7,10} inasmuch as a number of dogs are still alive from 2½ to more than 3 years after orthotopic homotransplantation of livers obtained from non-related mongrel donors (Figure 1). Second, the possibility of success with application of such techniques for the treatment of human disease has been sharply reduced or perhaps even precluded altogether by two bottlenecks. The following remarks will focus upon these key deficiencies and upon the efforts being made to correct them.

IMMUNOSUPPRESSION

The immunosuppressive agents used in renal homotransplantation are also effective for the prevention of liver homograft rejection. In the laboratory, prolonged survival can be obtained after orthotopic hepatic homotransplantation to dogs treated solely with azathioprine⁷ with approximately the same consistency as that which is possible after canine kidney homografting under the same conditions. When randomly selected non-related mongrel donors are used as the source for either organ, there is a great variability in the subsequent events. Some (15–20%) of the recipients never undergo a clinically overt rejection. Another third have repudiation of their grafts which is relentless, and mitigated only slightly if at all by azathioprine therapy. The rest have detectable rejection which may be severe but which may be compatible with long survival in spite of substantial consequent deficiencies in the function of the homografts.

After homotransplantation of either the canine kidney or liver, it is sometimes possible in favourable cases to stop all immunosuppressive therapy after a postoperative interval

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FIGURE 1. A portrait taken on 23 March, 1967, of a dog which received an orthotopic liver homograft on 23 March, 1964, from a non-related mongrel donor. Azathioprine was used as the sole immunosuppressive therapy, and discontinued after 4 months. The dog has received no medications for 2½ years. Biopsies were normal after 4, 8, and 12 months. Liver function is normal.

as short as four months or even less, with subsequent survival for years. In our laboratories, there are four healthy recipients of orthotopic liver homografts who have not received any medication for 2 to 2½ years.

At present, there is no single immunosuppressive drug which permits truly chronic survival in more than a minority of animals. In man, the same is true. For clinical homotransplantation it has, therefore, been necessary to use a variety of agents in addition to azathioprine, of which prednisone has been by far the most important. This steroid, if given as a second drug in large quantities from the day of operation, can greatly reduce the incidence of rejection after human renal homotransplantation. Alternatively, when administered at a later time, prednisone can reverse an established rejection which has developed in spite of daily prior therapy with azathioprine. For the latter purpose, other measures can be taken such as the intravenous administration of actinomycin C or local homograft irradiation, but their effect is relatively feeble.

The combination of azathioprine and prednisone has been responsible for much of the recent progress in clinical renal homotransplantation, although the cost has been high. In many cases, control of homograft rejection has been incomplete, and in others

this objective has been achieved only by such immunologic crippling that the host died of sepsis caused by common pathogens, or in many cases by opportunistic bacteria, viruses, or fungi. Nevertheless, the margin between the desired therapeutic effect and toxicity has been sufficient to permit a moderate number of successes.

In contrast, all efforts at either orthotopic or auxiliary liver transplantation in man have failed in 5 weeks or less. In each patient who lived for more than a few days, drug toxicity and consequent sepsis have played important adverse roles. The magnitude of the requisite surgical procedures, the hepatotoxic effects which both steroids and azathioprine may cause, the necessity for intraperitoneal placement of the transplants, and the imperfect homograft function which was achieved may all have contributed to an eventually unmanageable therapeutic situation as discussed elsewhere.⁶ Whatever the reasons, it has become evident that the 'standard' program of immunosuppression is unacceptable for clinical liver transplantation.

Fortunately, there are reasonable prospects of improving immunosuppressive protocols by the addition of heterologous antilymphocyte globulin (ALG) to reduced doses of azathioprine and prednisone. We have prepared ALG by ammonium sulphate precipitation of the serum (ALS) of immunized horses.^{3,8}

When used as the sole immunosuppressive agent, either the ALS or its ALG derivative result in a striking prolongation of life after orthotopic liver transplantation (Figure 2) which is almost of the same magnitude as that provided by azathioprine. In eighteen

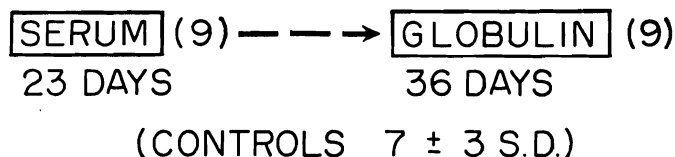


FIGURE 2. Effect of antilymphoid serum (ALS) or globulin (ALG) upon survival after orthotopic liver transplantation. For both of these mean values, survival credit for any individual animal was limited to 70 days.

dogs, nine treated with ALS and nine with ALG, mean survival was more than a month as computed with a maximum credit for individual dogs of 70 days. In actuality, four of these animals lived for more than 4 months. One which is still alive after 1 year, received therapy only for the first three postoperative weeks (Figure 3). Another, which died of a midgut volvulus after 6 months, had received only six preoperative injections. In the majority of experiments, serum or globulin therapy was stopped after a few postoperative weeks, and in none was it continued beyond 60 days. The desired effect was not dependent upon the production of lymphopenia (Figure 3).

While encouraging, the results in these animals and in companion experiments with renal homotransplantation were not good enough to warrant a clinical trial with ALG alone. In addition, the fact that ALS or ALG eventually caused a significant incidence of serum sickness nephritis in dogs³ further restricted the way in which they could be employed. The demonstration that ALS or ALG have a synergistic action when used with cytotoxic drugs^{2,8} or with steroids⁴ supported the feasibility of a compromise program of adjuvant therapy with ALG in which its use would be limited to the early postoperative period.

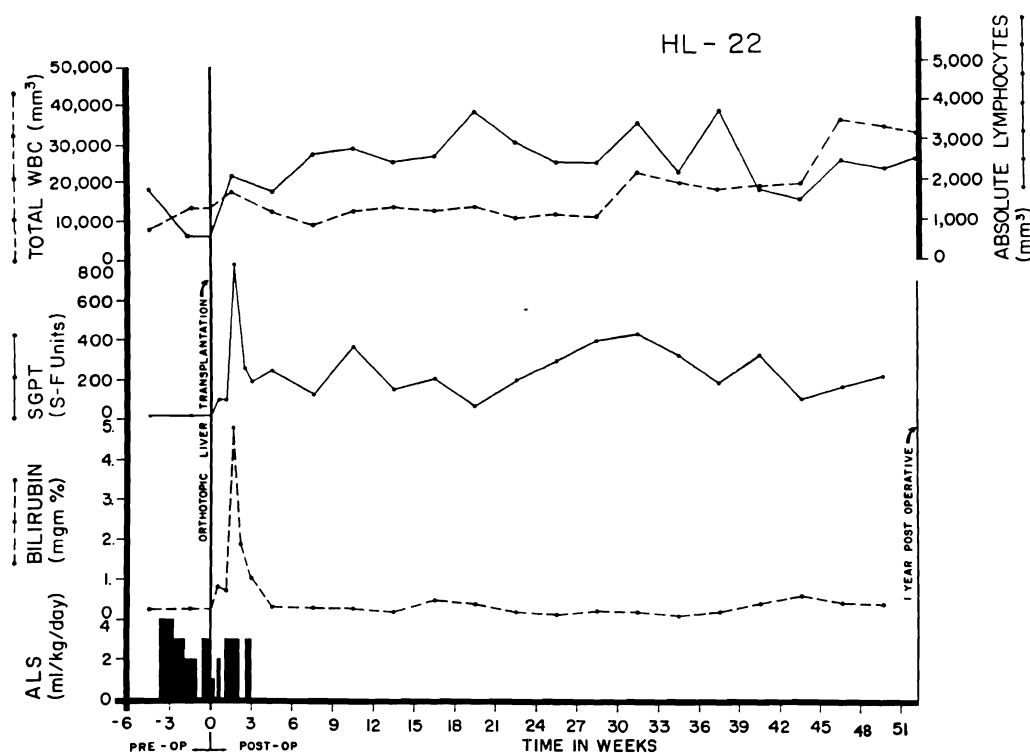


FIGURE 3. A chronically surviving dog which was treated before and for 20 days after orthotopic liver transplantation with intraperitoneal antilymphoid serum (ALS). Note the pronounced lymphocytosis late in the postoperative period. The animal is still alive after more than one year.

ALG has been given a clinical trial under these conditions with distinctly encouraging results. It was used during the first four postoperative months for the treatment of twenty recipients of renal homografts between June and early December of last year.⁹ Nineteen of the twenty patients are alive with good renal function from 4 to 10 months postoperative. The necessary amounts of both azathioprine and prednisone have been less than in any group of patients previously treated in Denver. Furthermore, homograft biopsies obtained in the first 8 of these cases from 108 to 145 days after operation revealed no evidence of serum sickness nephritis.

In the laboratory, similar combination therapy has been evaluated in animals receiving orthotopic liver homografts. ALG was given before and for several weeks after transplantation. Azathioprine was administered indefinitely following surgery, as well as a one-week postoperative course of prednisone in progressively diminishing daily doses which started at 3 mg/kg on the day of operation. With this regimen, a previously uncommon complication killed six of seven dogs within the first 10 days. There was thrombosis of the homograft aorta which was removed in continuity with the hepatic artery and anastomosed to the side of the recipient aorta (Figure 4A). It was suspected that this was a reflection of better early postoperative liver function.

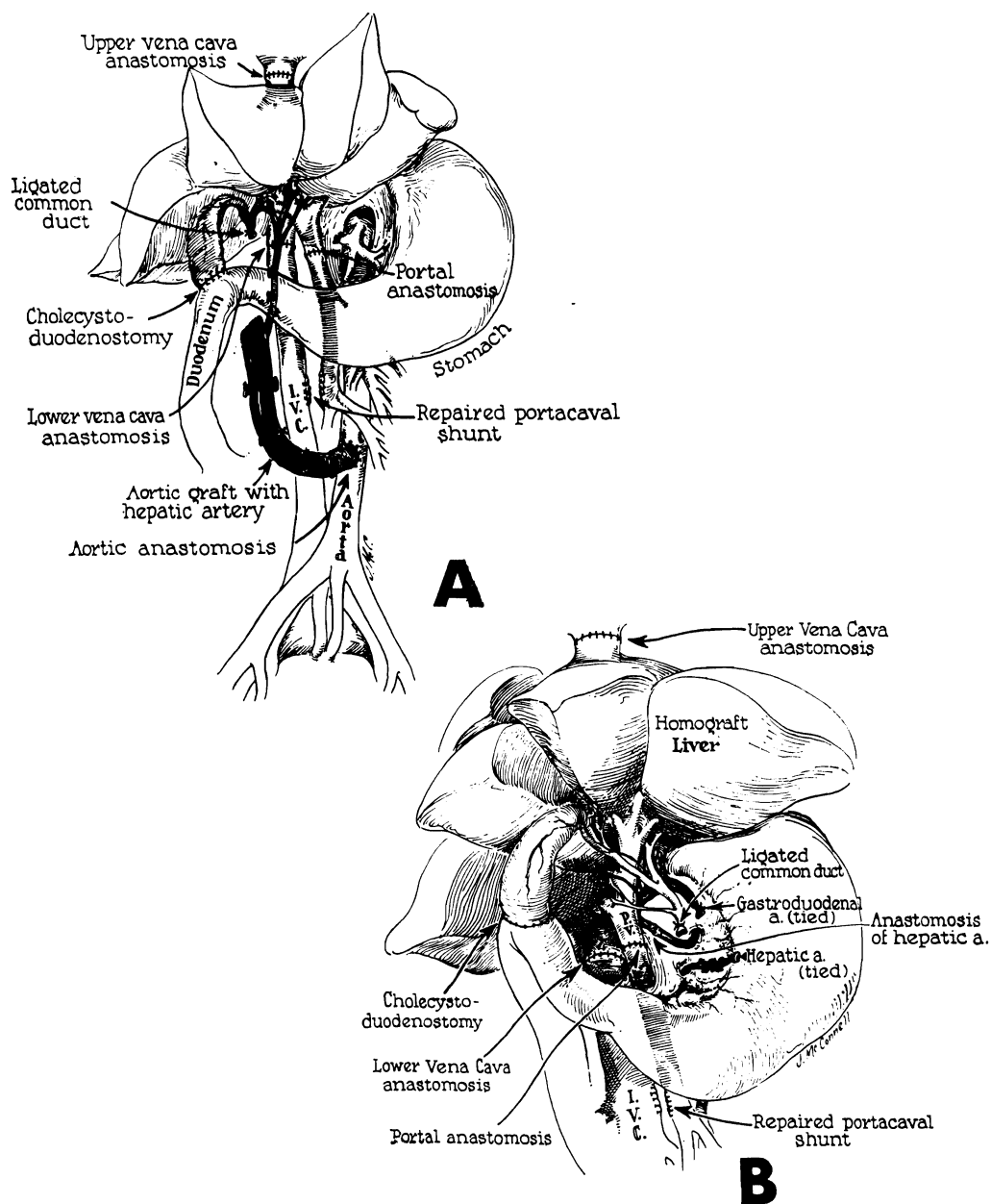


FIGURE 4. Different techniques of rearterializing orthotopic liver homografts. A third possibility which is not shown is to remove the right kidney and anastomose the renal artery to the homograft hepatic artery. (A) Removal of the donor aorta in continuity with the hepatic artery and celiac axis. The graft aorta is then anastomosed to the mid-abdominal aorta of the host. With recent improvements in immunosuppression, this technique has resulted in an extremely high incidence of aortic thrombosis. (By permission of *Surgery* 58: 131, 1965.) (B) Presently preferred method of rearterialization. The homograft hepatic artery is anastomosed to the transected hepatic artery of the recipient. Duodenal necrosis can be prevented if dissection in the area of the distal hepatic and gastroduodenal arteries is scrupulously avoided.

Ten more similar experiments were then performed, differing in that the recipient right kidney was removed and the renal artery anastomosed to the homograft celiac axis. With this hemodynamically improved arterial reconstruction, there were only three failures due to thrombosis (Table 1). The other seven dogs all had prolonged survival. One animal died of rejection after 20 days. The other six lived for a minimum of 38 days; four dogs are still alive after 1½ to 4 months.

More recently, the incidence of arterial thrombosis has been reduced even further by anastomosing the homograft hepatic artery directly to the proximal hepatic artery of the recipient animal (Figure 4B). With this modification, it is important to avoid dissection of the distal hepatic and gastroduodenal arteries in order to prevent ischemic necrosis of the duodenum. In five experiments, arterial thrombosis has not been seen. Follow up is too short to allow evaluation of the efficiency of immunosuppression in this series.

TABLE 1

Results in ten consequent orthotopic liver transplantations between non-related mongrel dogs

(Note that all animals which did not develop hepatic artery thrombosis had prolongation of survival. Four of the animals are still alive.)

Number	Survival (days)	Cause of death	Last bilirubin (mg %)	Last alk. phosphatase (B.U.)*	Last SGOT (S.F.U.)†
1	117	Alive	4.4	258	520
2	115	Alive	1.0	239	138
3	47	Liver failure	11.3	458	32
4	7	Hepatic artery thrombosis	11.7	276	850
5	95	Rejection	8.3	204	276
6	20	Hepatic failure	11.6	44	140
7	8	Hepatic artery thrombosis	0.5	95	350
8	54	Alive	2.1	320	276
9	4	Hepatic artery thrombosis	5.6	56	680
10	45	Alive	12.3	389	370

* B.U.—Bodansky units.

† S.F.U.—Sigma-Frankel units.

ORGAN PRESERVATION

The results cited above provide hope that improved immunosuppressive regimens will be available for future attempts at clinical liver transplantation. Unfortunately, a second and conceivably even more serious practical problem will have to be dealt with, namely the provision of adequately preserved cadaveric organs.

In the laboratory, the timing of donor and recipient procedures can be precisely coordinated. The homograft has a blood supply until virtually the moment of its

excision. Further, it is cooled first by the use of donor total body hypothermia and later by intraportal perfusion with a cold electrolyte solution. Under these ideal circumstances good immediate liver function can usually be obtained if revascularization in the recipient is completed within 2 hours.

With the use of cadaveric donors, the clinical situation is quite different. Here, failing organ perfusion has often been present some time before death. In addition, the liver must be in a postmortem state for some minutes before cooling can be instituted. The acceptable margin of ischemia has thus already been partly and unpredictably used up before any means can be taken to prevent further injury.

In the past, these considerations have imposed an extreme air of urgency upon the operation which must be performed in the recipient. In order to reduce the time necessary to remove the diseased liver, staged procedures have been resorted to with preliminary dissection of all structures entering and leaving the liver. At the subsequent definitive operation, the organ could be quickly removed and replaced.

This approach has serious disadvantages. First, it can never be predicted at the time of a first stage when a suitable cadaveric donor might arrive. Secondly, the general condition of the recipient patient is invariably made worse by the preliminary procedure. It is clearly necessary to have methods available with which a hepatic homograft can be effectively preserved for a number of hours during which the patient might be prepared in a deliberate and systematic way for its receipt.

Simple though this need is, it has not until recently been achievable. In dogs, the most encouraging results with liver conservation were those described by Mikaeloff & Kestens.⁵ They were able to successfully perform orthotopic homotransplantation in dogs after *in situ* homograft preservation of from 1½ to 6½ hours using a relatively complicated technique that included organ perfusion with an oxygenated blood solution at hypothermic temperatures.

In our laboratory, a simpler method for preservation of the extirpated liver has recently been tested which combines hypothermia, hyperbaric oxygenation, and low flow perfusion with a diluted blood solution (Figure 5). The studies were undertaken on the basis of an important publication by Ackerman and Barnard¹ who used a similar technique to store renal autografts.

The liver homografts were removed from living donors, flushed with chilled balanced salt solution containing 2.5 g% low molecular weight dextran, and placed in a refrigerated (4°C.) hyperbaric chamber.

The portal vein and hepatic artery were then perfused with a solution containing 50% heparinized homologous blood and 50% balanced electrolyte solution to which low molecular weight dextran (2.5 g%), glucose, and procaine were added. The mixture was buffered to a pH of 7.45. Flow was approximately 2.5 and 0.5 ml/gm liver per hour to the portal vein and the hepatic artery respectively. The chamber was compressed with 100% oxygen to 40 PSI gauge pressure over a period of 20 minutes. Toward the end of the preservation interval, decompression was carried out over several hours; when this was done too rapidly, the organs developed air emboli within the vessels and parenchyma.

The trials which were carried out with this technique can be divided into three groups. In the first experiments, preservation was for 10 to 14½ hours, at the end of which the chamber was decompressed in approximately 25 minutes. The homografts

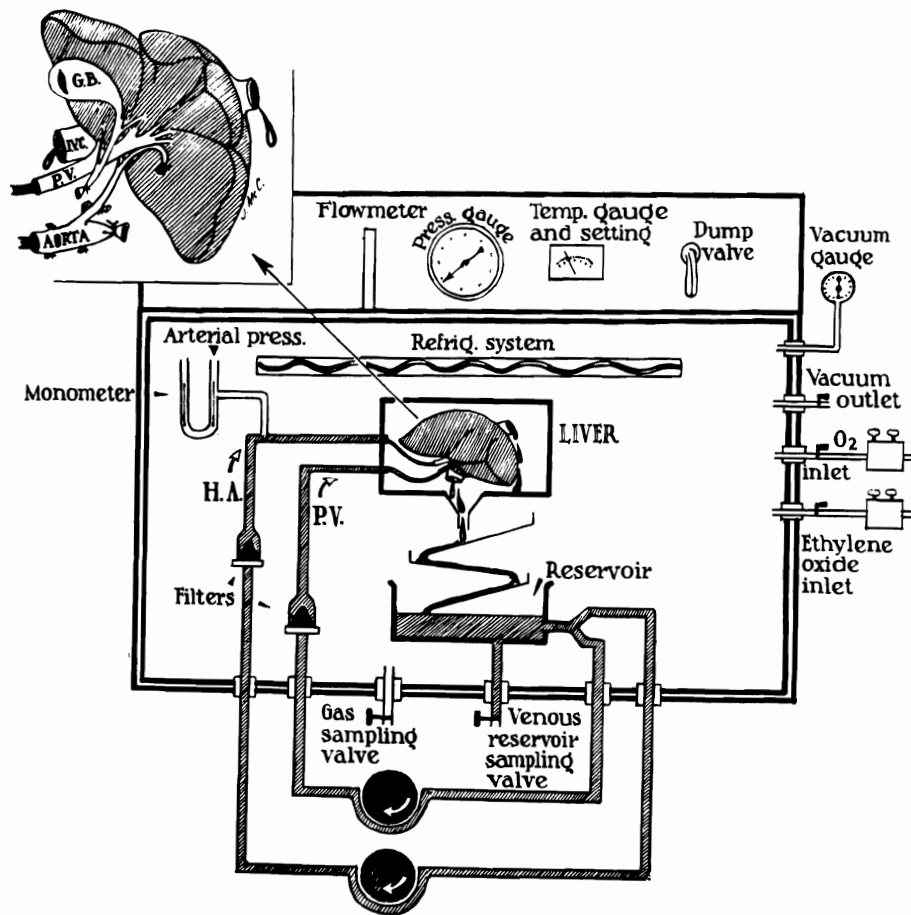


FIGURE 5. Schematic view of technique used for conservation of excised canine livers. At the time of removal cannulas are placed into the affluent vessels (insert). Note the control systems for temperature, compression and flow. The hyperbaric chamber and all its contents can be sterilized in advance with ethylene oxide.

all contained oxygen bubbles which did not prevent grossly homogeneous tissue perfusion after the organs had been revascularized as orthotopic grafts. One dog died immediately after operation. The other six, which were treated with the triple immunosuppression described in the preceding section, lived for 3, 4, 5, 5, 7 and 9 days. They were slow to awake from anesthesia. None of the animals ever were able to eat. At autopsy, the livers were badly injured. There were three dogs with perforation of the duodenum or esophagus, and two with thrombosed renal arteries.

Five additional similar homotransplantations were then performed, differing only in that decompression was during 3 hours; detectable air emboli were thereby completely avoided. Survival in this group was 5, 6, 6, 13 and 30 days, the last dog still being alive. The post-anaesthetic somnolence was somewhat less than in the earlier group.

The degree of ischemic injury in these animals, as judged by elevations in SGOT, SGPT, and alkaline phosphatase was only moderately severe (Figure 6). However, only one dog was able to resume a diet. It was thought likely that a defect in hepatic function was being created which was not accurately being measured.

Immunoelectrophoretic studies of the serum of these recipients by Dr. Nobura Kashiwagi provided strong evidence for this possibility. During the first few postoperative days, he found multiple defects of protein synthesis as manifested by diminution or complete disappearance of several small components of alpha and beta globulins, and even some of the gamma globulins. These changes were reversible but only after some days, by which time the animals were usually moribund. The studies suggested that homografts could be regularly obtained, which were immediately life-sustaining, but that they were flawed by specific defects of synthetic function.

Consequently, the experiment was repeated in five animals after preservation for 8-9 hours. One of the recipient animals bled to death during the first postoperative night. Another died after 3 days from a disrupted gallbladder anastomosis. A third dog died of liver failure after 11 days. The other two animals began to eat after several days; one died of pneumonitis in 15 days, and the other is still alive after 2 weeks. In the three

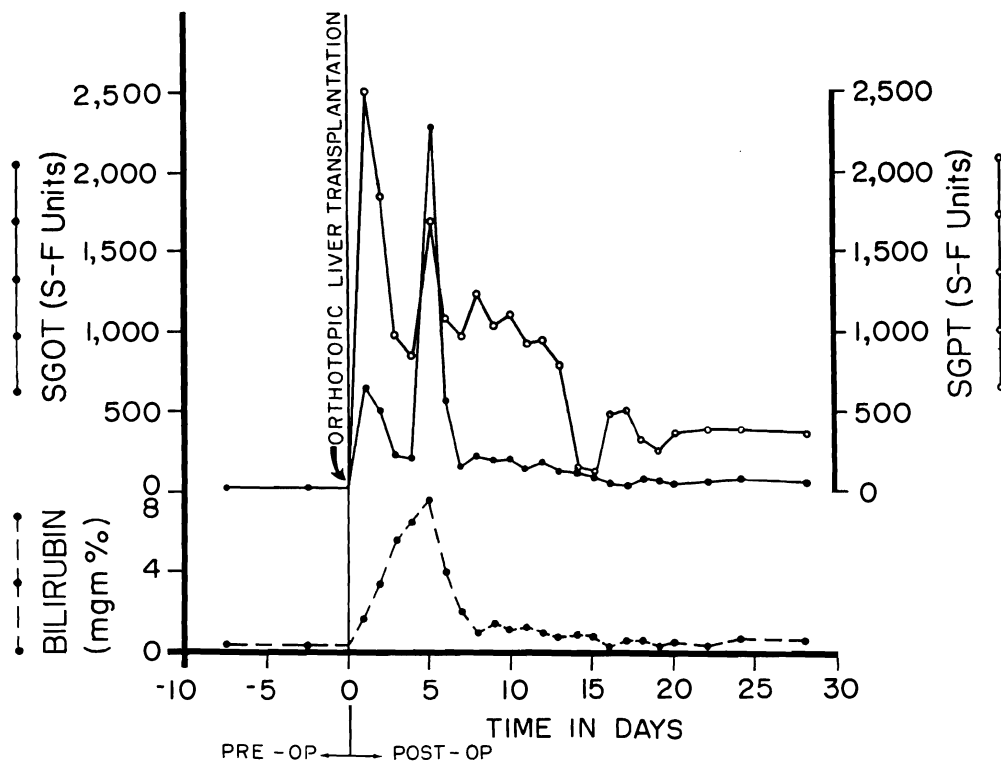


FIGURE 6. Course of a dog which received an orthotopic liver homograft which had been previously stored within the hyperbaric chamber for almost 15 hours. Note the early and reversible aberrations in liver functions.

animals with the longest survival, the defects in the serum proteins were less marked than had been observed with longer storage.

These findings highlight the deficiencies of protracted liver preservation, but they also clearly demonstrate the feasibility of the undertaking inasmuch as survival for more than a month has already been achieved with a homograft which was stored for 14½ hours. In the future, a number of variables can be tested in an effort to improve the situation including flow rate, perfusate constituency, degree of oxygen compression, temperature, amongst others.

One feature of liver transplantation with stored organs deserves special emphasis. In contrast to the normal or near normal coagulation which can usually be maintained with an acute canine orthotopic operation, virtually all recipients of stored organs failed to clot for the first several postoperative hours. High fibrinolysin levels developed almost immediately and often required aggressive therapy with epsilon amino caproic acid (EACA). In some dogs, small doses of protamine sulphate (1 mg/kl) also seemed to be helpful, possibly because of their neutralizing effect upon small doses of heparin which may have been transferred with the homografts.

SUMMARY

Research has been reviewed concerning better methods of immunosuppression which may become available for trials of clinical liver transplantation. There is evidence that heterologous antilymphocyte globulin (ALG) will be of value as an adjuvant agent, added to reduced quantities of standard immunosuppressive drugs.

Attention has also been focused upon the need for effective methods of interim liver preservation. A system has been described employing hypothermia, perfusion, and hyperbaric oxygenation which is promising but which has not yet provided consistently reliable organs after storage periods of 8 to 15½ hours.

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Discussion

FOLLOWING PAPERS BY F. D. MOORE, L. BRETTSCHEIDER AND T. E. STARZL

Moore: What do you think the hyperbaric chamber is contributing to this? You can't push oxygen through Glisson's capsule.

Starzl: The entire thing is pressurized and it is an exceedingly effective oxygenator.

Moore: At this temperature how critical is oxygen? Do you really have to go up to a PO₂ of 2200 mm of mercury. Don't you think you could do the same thing at the same temperature at one atmosphere?

Starzl: No, I don't. We have tried this, and I assume others have, and it doesn't work.

Moore: We believe at 4°C that you would need good data on hepatic oxygen removal to justify this pressure.

McLean (A.): There have been studies carried out on the isolated perfused liver showing that oxygen does nothing as far as the liver is concerned but support oxidative phosphorylation which at this temperature has stopped. It is difficult to know what function oxygen performs, certainly active transport processes have stopped. You may be merely ensuring adequate oxygenation at the critical rewarming phase.

Moore: I don't think we should spend too much time on this. I am sure the hyperbaric need will be sorted out in Dr. Starzl's laboratory. The same argument goes on with kidneys—is hyperbaric oxygen necessary?

Kestens: In 1965 I showed our results on hepatic preservation to Dr. Starzl who thought the technique probably unnecessary. He has shown that in fact our technique does work. The technique is roughly the same as ours, the liver lies in a hyperbaric chamber lying on a plate punched with holes and venous blood drips out and is oxygenated prior to being returned via the pump into the artery. The flow we use is much higher (120 ml/min) and we perfuse for 24 h before returning in the orthotopic position. The results were disappointing, maximal survival being 10 h. There was necrosis on histological examination. Perhaps perfusion of the portal vein might give better results.

Peacock: Can I ask Dr. Starzl whether in the animals with an anastomosis of hepatic to renal artery he knows the cause of death in the fatal cases?

Starzl: The three technical failures were due to arterial thrombosis, and death occurred in the first week. After six days death, as far as we are concerned, is due to rejection or drugs being used to prevent it. Three dogs died between 20 and 100 days of rejection. There are four dogs alive.

Moore: I would like to say that I think the most exciting event in the transplantation field is the introduction of antilymphocyte serum.

McDermott: I would like to comment on the support of an anhepatic animal by hepatic perfusion as there are considerable difficulties concerned with the clotting of blood. Running heparinized blood into the perfused liver which is then returned after treatment with protamine means that one is supplying blood to the animal defective in platelets. A recent patient being perfused developed 'consumptive coagulopathy'—with fibrinogen and platelet deficiency. There was a fibrinolysin present and her fibrinogen was very low. What appeared to be happening was that she was forming microthrombi massively, lysing them and using all the fibrinogen and platelets we were giving her. To treat this we anticoagulated her and gave epsilon aminocaproic acid. She was then able to retain the platelets and fibrinogen we gave her. Maintenance may be very difficult in view of these clotting problems. My present view is that support of the anhepatic animal will be by a combination of exchange transfusion, perfusion and dialysis in a titrated way.

Moore: Maybe patients with acute hepatic necrosis being treated by exchange transfusion need haemodialysis in order to get rid of low molecular weight substances which are not managed by exchange.

Moore: Dr. Kestens, do you think that long term perfusion of the liver might be associated with a lesser risk of rejection?

Kestens: If you start with a good liver there is less tendency for rejection to be complete simply because of the high percentage of normally functioning liver cells. Others have shown that skin allograft rejection can be lessened by incubation of the graft in RNA or DNA. This may be a useful tool and perhaps we could perfuse the liver with such a mixture to prevent rejection.

Wright: Does the deposition of horse globulin in the kidneys of animals receiving antilymphocyte serum occur after single or after repeated injections?

Moore: We can't answer this—all ours had multiple injections.

Wright: This might be important. Repeated injections might lessen efficiency by promoting renal excretion.

Starzl: I can't answer that one. I think that there is a possibility of nephrotoxicity and in lower animals 50 mg/Kg/week of the globulin is probably a nephrotoxic dose. Two lesions result—there is a direct nephrotoxic effect by antigen-antibody reaction on the basement membrane. We also see a serum sickness reaction with a generalized antigen-antibody reaction with precipitation of this complex in glomeruli. This is the dangerous reaction but there follows an inflammatory reaction.

Moore: In your longer term dogs and in patients have you seen a functional disorder of the kidneys due to antilymphocyte serum?

Starzl: We have seen uremia after massive injections intravenously, but not otherwise.

Sircus: What about the dogs who died from peptic ulcers?

Moore: These are late complications at about 60 days post operation. This is an acid peptic ulcer and it also occurs in a Roux γ loop. There is often massive haemorrhage.

Sircus: Was there any pancreatic defect in these animals?

Moore: In the earlier dogs we produced marked post operative pancreatitis but not in the later ones.