

Recent Developments in Liver Transplantation

THOMAS E. STARZL, LAWRENCE BRETTSCHEIDER
AND CARL G. GROTH

Denver Veterans Administration Hospital, Denver, Colorado U.S.A. 80220

On several occasions during the past few years, the reasons have been analyzed for the failure to successfully transplant livers in humans. There is little doubt of the feasibility of the procedure since chronic survival in mongrel dogs was first achieved after orthotopic hepatic homotransplantation in early 1964, an experience that has been since repeated in several laboratories. Four dogs which received orthotopic liver homografts in Denver in that year are still alive. None has received any immunosuppressive therapy from the fifth month onward.

In spite of this progress, all human recipients of either orthotopic or auxiliary liver homografts have died within 5 weeks after operation. The number of trials is no longer small. In Denver, there have been 7 clinical attempts at orthotopic homotransplantation and 3 at auxiliary transplantation. In the former group, one patient bled to death during operation; the other 6 died after 6.5, 7, 7.5, 10, 22, and 23 days. After auxiliary transplantation, one patient died immediately of a technical complication and the other 2 succumbed 22 and 34 days later. Similar efforts are known to have been made elsewhere, between 10 and 20 in all.

The care of these patients was made difficult by gastrointestinal ulcerations and hemorrhage, sepsis, serious electrolyte disorders and swift and life threatening intraoperative and postoperative changes in blood coagulation. To some extent each of these complications was ultimately founded upon the inability to either obtain immediate good liver function or, alternatively, to maintain it for more than a few days or weeks without causing the patient's death with excessive immunosuppression. In the first instance, the failure was in not providing adequately preserved tissue. In the second, the technique of drug therapy did not have a sufficient margin of safety to permit long-term survival. The following remarks are concerned with efforts to ameliorate these deficiencies.

Tissue Procurement and Preservation

It is our present view that the greatest deterrents to successful liver transplantation in man are concerned with organ procurement and storage. The sensitivity of liver tissue to anoxia under normothermic conditions is well known. If some means of preservation is not instituted, canine livers become unsuitable for transplantation within 30 minutes after donor death. Sicular and Moore (1961) have shown that the ability to oxidize glucose is rapidly lost after 30 to 60 minutes.

In dogs, these adverse effects can be delayed and minimized during the period of homograft devascularization by inducing donor hypothermia in advance of operation, and by further cooling the graft with intraportal infusion of chilled electrolyte solution (2-4°C). Livers so prepared regularly provide good function if their blood supply is reestablished in the recipient within 2 hours. For laboratory experiments, this is ample time since donor and recipient operations can be precisely coordinated.

For clinical transplantation the situation is quite different. The donor is usually normothermic. Furthermore, there is a period of failing tissue perfusion before the death of the donor except under the most extraordinary circumstances. Finally, some minutes are required after death is pronounced before the organ can be cooled by intraportal infusion of a cold fluid or by other emergency means. A starting point thus exists at which the margin of acceptable ischemia has already been partly used up.

The technical requirements for the recipient operation compound the dilemma, whether an orthotopic or an auxiliary procedure is contemplated. Bleeding problems due to poor hepatic function are to be expected. Patients who need either kind of homograft almost invariably have portal hypertension. The technical requirements for preparation of a bed for an auxiliary

(From the University of Colorado School of Medicine and the Denver Veterans Administration Hospital, Denver, Colorado, USA. The work was supported by Grants F05-TW-1154, AM-06283, AM-06344, HE-07735, AM-07772, AI-04152, FR-00051, and FR-00069 from the United States Public Health Service).

organ may be tedious. Those necessary for removal of the patient's diseased liver for an orthotopic transplantation are even more formidable. In either case, efforts to proceed with speed are apt to lead to uncontrollable hemorrhage. A number of hours may be required before it is possible to even begin the vascular anastomoses.

In some of the early attempts at orthotopic transplantation in man, these problems led to use of staged procedures. At a first operation, the major structures entering and leaving the liver were skeletonized. Later, when a cadaveric organ became available, the incision was reopened, the diseased liver quickly removed, and the homograft inserted. The approach had serious disadvantages. These ill patients did not well tolerate 2 consecutive procedures of such magnitude. In addition, it could not be predicted at the time of the first intervention when a suitable cadaveric donor might arrive. In one case, the interval before the definitive operation was more than 2 weeks.

Fortunately, there is now good evidence that whole organ liver preservation is practical for much longer than the limit of 2 hours which was alluded to earlier. Mikaeloff and Kestens and their associates (1965) were the first to demonstrate this. They perfused dog livers *in situ* by means of a pump oxygenator into which a heat exchanger had been incorporated for induction of hypothermia. When the organs were removed from the donors 6 hours later and transplanted as orthotopic grafts, a number of recipients, which were being treated with azathioprine lived for several weeks or months. However, Kestens has told us that these results could not be duplicated if the liver was extirpated and then perfused extracorporally. Efforts by Marchioro *et al* (1963) to apply a similar but simpler system for whole or lower-half cadaver perfusion also resulted in unsatisfactory liver preservation.

Within the last few months, 2 groups of investigators have reported benefits of preservation with a combination of hypothermia, intravascular perfusion, and hyperbaric oxygenation. Slapak *et al* (1967) stored puppy livers for 24 hours and inserted them into the neck of adult recipients. In 7 of 19 experiments, bile production was noted. The perfusate used did not contain red blood cells.

Brettschneider and his associates (1967) have evaluated the same method in dogs employing diluted blood for the perfusate. The adequacy of organs stored for 8 to 25 $\frac{3}{4}$ hours was tested by placing them as orthotopic homografts in mongrel recipients which were under immunosuppressive therapy. During preservation, the

temperature and oxygen compression within the chamber were 2-4°C. and 40 pounds per square inch respectively. The best perfusion rate studied was 6 ml/gm tissue/hour, divided in a 4:1 ratio between the portal vein and hepatic artery.

Livers preserved in this way for 8 to 10 hours performed almost as well as those cooled and transplanted without delay. Even after 24 hours storage, such homografts were good enough to allow chronic survival in 2 of 5 experiments. Deviations from the above protocol were notably less successful. Elimination of high oxygen compression, reduction in flow rate, omission of blood from the perfusate, and elimination of perfusion all caused a deterioration in results.

When the suboptimal preservation methods were used, the recipients sometimes were able to survive operation but they developed a syndrome which has already been seen after several human liver transplantations. A bleeding diathesis during operation was the first sign of trouble. In many dogs, this led to immediate fatal hemorrhage. In others, it was eventually controlled with high doses of epsilon amino caproic acid and protamine. The latter animals were slow to arouse from barbiturate anesthesia. Massive therapy with electrolyte solutions and plasma was required for the first day, apparently because of the formation of a third fluid space. There was a high incidence of acute gastrointestinal ulcerations and hemorrhage. These dogs could be kept alive for several days but none was ever able to eat; all died within 10 days.

With immunoelectrophoresis, Kashiwagi *et al* (1967) examined the serum protein components in these animals and compared the results with those reported earlier by Kukral (1962). In untreated dogs which had received optimally preserved homografts, the latter author had observed heightened protein synthesis for at least the first 4 postoperative days as well as maintenance of essentially normal serum proteins. In contrast, Kashiwagi found early reduction or even complete disappearance of a number of discrete protein moieties in the recipients of the damaged livers. These tended to return, but never completely to normal. In such experiments, the combination of ischemic injury plus an unanalyzable further insult due to subsequent rejection, apparently precluded chronic survival.

The demonstration that livers can be kept in good condition for at least 8 and often for 24 hours is encouraging, but it would be unrealistic for the reasons cited at the beginning of this section to accept these figures as applicable

in a clinical setting. No presently known technique of preservation can resuscitate liver tissue which has already been seriously injured. Thus the necessity for quickly obtaining and rapidly cooling cadaveric livers looms as the most important impediment to success.

Control of Rejection

The high incidence of infectious complications after clinical liver transplantation has made it clear that the margin is exceedingly slim between desirable and lethal immunosuppression, using azathioprine and prednisone by the same general techniques that have permitted a large number of successes after clinical renal transplantation. This does not imply that it is more difficult to prevent rejection of livers than of kidneys; indeed, experiments in our laboratory with the 2 organs have led us to the converse conclusion. It probably does mean that a patient who is a candidate for liver transplantation is more apt to be killed by the side effects of these agents. He is more ill, requires a much larger operation, and suffers from a far more complicated metabolic disorder than the patient with terminal uremia.

The features of rejection of orthotopic liver homografts have been well described in both untreated canine recipients and in dogs modified by azathioprine, heterologous antilymphocyte globulin and other agents. By and large, the findings are analogous to those in other rejecting tissues and organs. In unmodified recipients, there is early invasion by immature mononuclear cells which are similar to the same kind of cells found in increased numbers in the host lymphoid organs. These tend to be concentrated around the portal tracts and central veins. Within a few days, mature plasma cells and lymphocytes appear. Meanwhile, there is necrosis of the hepatic parenchyma, particularly around the portal tracts and in the centers of the liver lobules.

Provision of immunosuppression may not prevent these changes. However, in many treated dogs the acute assault upon the homograft loses its intensity during the second or third weeks. The number of mononuclear cells decreases, and from this point onward the predominant histologic findings are those of regeneration and repair. Patterns of late fibrosis seen in many chronically tolerated homografts are dictated by the earlier localization of most serious injury. In long term survivors, the appearance of the homografts months or years later may range from totally normal to that of moderately severe cirrhosis.

In some of the earlier studies of hepatic homotransplantation, it was suggested that an important component of liver rejection might be interference with the blood supply either at the level of very small (Starzl *et al*, 1961, McBride *et al*, 1962) or of larger vessels (Moore *et al*, 1964). The latter possibility was made less likely by the subsequent observation (Starzl *et al*, 1965) that significant histologic lesions of large intrahepatic arteries were uncommon in either treated or untreated dogs. However, electron micrographs in the latter study showed fusion of mononuclear cells to the sinusoidal endothelium, reraising the possibility of disturbances at the microcirculatory level.

Recently, support has been provided for this hypothesis (Groth *et al*, 1967). Sharp falls in total liver blood flow were observed with a xenon washout technique. In untreated animals, these changes were noted concomitantly with the diagnosis of rejection. They were progressive, and generally were not accompanied by falls in cardiac output. The hemodynamic alterations could be avoided if adequate immunosuppression was given or they could even be reversed if an existing rejection was brought under control.

Such research, designed to obtain knowledge about the events of rejection may prove to have important implications for the care of patients. The testing of alternative methods for the prevention or treatment of this process is an even more clinically oriented kind of investigation. During the past few years, many agents have been evaluated. Of these, none has shown more promise than heterologous antilymphocyte serum (ALS) and its globulin derivative (ALG), as will soon be reviewed in the proceedings from an international Ciba symposium held in London in January, 1967, (Wolstenholme and O'Connor, 1967).

The remarkable developments in this field cannot be recapitulated here, but it is worth noting that mitigation in dogs of liver homograft rejection has been unequivocally demonstrated with both ALS and ALG (Starzl *et al*, 1967a). The serum used was raised by immunizing horses against canine spleen, thymus, and lymphnode lymphocytes.

The immunosuppressive effectiveness was comparable to that of azathioprine in that rejection was prevented altogether in a small number of dogs, substantially delayed in the majority, and little effected in a minority. The investigations also showed that these substances are potentially dangerous since histopathologic evidence of serum sickness nephritis was not uncommonly found in the kidneys of animals

after a course of either ALS or ALG (Iwasaki *et al*, 1967).

Appreciation of the limitations of ALG therapy has influenced the way in which heterologous immune globulin has been given a clinical trial. It has been used for a period limited to the first 4 postoperative months and as an adjunct to therapy with azathioprine and prednisone. There are now indications that treatment with ALG, applied in this way, is going to significantly alter the outlook after organ transplantation in man. The principal evidence has accumulated from experience with clinical renal homotransplantation.

In Denver, horse antihuman ALG was first used as an adjuvant agent more than a year ago according to a protocol described elsewhere (Starzl *et al*, 1967b). Between 6 and 12 months ago, 21 patients were entered into the study. Twenty received renal homografts from blood relatives who were not selected on the basis of good antigen matching with their recipients; the other was provided with a cadaveric homograft. The quantities of azathioprine used in these cases were less than in any comparable previous series of patients treated in our institutions. More importantly, the requisite average doses of prednisone were halved during the first 4 postoperative months. Rejection was controlled in every instance. Twenty of the patients are still alive with good to excellent renal function. The only death was due to a technical accident. Infectious complications were virtually eliminated. It is now predicted that the 1 year survival in this group will exceed 90 per cent, an expectation which is given weight by the fact that the first 8 cases have either already passed or are within a few weeks of the first post-transplant year, and that the next 4 are not far behind.

More than 1,700 globulin injections have been given to these and subsequent recipients of renal homografts without a drug-related fatality, a record of safety to which the concomitant administration of azathioprine and prednisone undoubtedly contributed. Fever and pain at the injection site were the most common side reactions. Approximately 15 per cent of the patients had some evidence of an anaphylactic reaction during their course, but these were not difficult to treat and in several cases did not necessitate discontinuation of globulin therapy. Renal homograft biopsies after 4 months from the first 8 cases revealed no evi-

dence of either serum sickness nephritis or Masugi lesions (Starzl *et al*, 1967b).

These results suggest that a tool is at hand for general improvement of immunosuppressive management. Whether this will permit successful liver transplantation remains to be seen. Thus far, 2 patients have received orthotopic liver homografts while under treatment with the same combination of agents described above. One was a 29 year old man with a hepatoma. The other was a 13 month old child with biliary atresia. In both cases, the livers were badly damaged by ischemia and functioned poorly from the outset. Death resulted from liver failure in 7 and 10 days respectively. At autopsy, the predominant histologic abnormalities in the homografts were thought to have been caused by the tissue injury inflicted at the time of operation. Evidence of rejection was minor.

Histocompatibility Typing

The prevention of homograft rejection is, of course, not solely dependent upon the evolution of better immunosuppressive regimens. An alternative would be to characterize the histocompatibility profile of donors and recipients, and to consider transplantation only between those pairs found to be biologically suitable. The stringency of immunosuppression could presumably be thereby reduced.

The considerable progress made in identifying human histocompatibility antigens is well known. Reports on the current status of this field justly constitute a sizable fraction of the program of this meeting. All seem to indicate that the day is not far off when a rough idea of donor-recipient antigen compatibility will be obtainable very quickly and with simplified techniques.

That a good antigen match would be highly desirable is undeniable. Nevertheless, it is known that a bad match does not necessarily connote failure, at least after transplantation of the kidney. For this reason, it is by no means clear that a perfectly preserved but incompatible organ would be unsuitable. Indeed, for reasons described in the first section of this communication, such a liver would undoubtedly be preferable to a compatible but badly injured one. The ideal solution would be to combine the best techniques of liver preservation with rapid typing.

References

- Brettschneider, L., Daloze, P. M., Huguet, C., Groth, C. G., Kashiwagi, N., Hutchison, D. E. & Starzl, T. E. (1967). Successful orthotopic transplantation of liver homografts after 8–25 hours preservation. *Surg. Forum*. In press.
- Groth, C. G., Porter, K. A., Otte, J. B., Daloze, P. M., Marchioro, T. L., Brettschneider, L. & Starzl, T. E. (1967). Studies on blood flow and ultrastructural changes in rejecting and non-rejecting canine orthotopic liver homografts. *Surgery*. In press.
- Iwasaki, Y., Porter, K. A., Amend, J., Marchioro, T. L., Zühlke, V. & Starzl, T. E. (1967). The preparation and testing of horse antidog and antihuman antilymphoid plasma or serum and its protein fractions. *Surg. Gyn. Obst.* **124**, 1.
- Kashiwagi, N., Groth, C. G., Brettschneider, L., Guptill, J. & Starzl, T. E. (1967). Effect of homograft ischemia upon serumproteins after orthotopic liver transplantation. *Surgery*. In press.
- Kukral, J. C., Littlejohn, M. H., Williams, R. K., Pancner, R. J. Butz, G. W. & Starzl, T. E. (1962). Hepatic function after canine liver transplantation. *AMA Arch. Surg.* **85**, 157.
- Marchiori, T. L., Huntley, R. T., Waddell, W. R. & Starzl, T. E. (1963). The use of extracorporeal perfusion for obtaining postmortem grafts. *Surgery* **54**, 900.
- McBride, R. A., Wheeler, H. B., Smith, L. L., Moore, F. D. & Dammin, G. J. (1962). Homotransplantation of the canine liver as an orthotopic vascularized graft. *Am. J. Path.* **41**, 501.
- Mikaeloff, P., Kestens, P. J., Dureau, G., Rassat, J. P., Haxhe, J. J., Alexandre, G. P. J., Dubernard, M., Cuilleret, J., Hassoun, A., Maldague, P. & Moreau, M. (1965). Conservation du foie en vue de la transplantation. *Rev. Int. Hepat.* **15**, 1401.
- Moore, F. D., Birtch, A. G., Dagher, F., Veith, F., Krisher, J. A., Order, S. E., Shucart, W. A., Dammin, G. J. & Couch, N. D. (1964). Immunosuppression and vascular insufficiency in liver transplantation. *Ann. N.Y. Acad. Sci.* **120**, 729.
- Sicular, A. & Moore, F. D. (1961). The postmortem survival of tissues. II. The effect of time and temperature on the survival of liver as measured by glucose oxidation rate. *J. Surg. Res.* **1**, 16.
- Slapak, M., Wigmore, R. A., McLean, L. D. (1967). Twenty-four liver preservation by the use of continuous pulsatile perfusion and hyperbaric oxygen. *Transplantation*. In press.
- Starzl, T. E., Kaupp, H. A., Brock, D. R., Linman, J. & Moss, W. T. (1961). Studies on the rejection of the transplanted homologous dog liver. *Surg., Gynec. & Obst.* **112**, 135.
- Starzl, T. E., Marchioro, T. L., Porter, K. A., Taylor, P. D., Faris, T. D., Herrmann, T. J., Hlad, C. J. & Waddell, W. R. (1965). Factors determining short- and long-term survival after orthotopic liver homotransplantation in the dog. *Surgery* **58**, 131.
- Starzl, T. E., Marchioro, T. L., Porter, K. A., Iwasaki, Y. & Cerilli, G. J. (1967a). The use of heterologous antilymphoid agents in canine renal and liver homotransplantation, and in human renal homotransplantation. *Surg. Gyn. Obst.* **124**, 301.
- Starzl, T. E., Porter, K. A., Iwasaki, Y., Marchioro, T. L. & Kashiwagi, N. (1967b). The use of heterologous antilymphocyte globulin in human renal homotransplantation. In *Antilymphocytic Serum*, ed. Wolstenholme, G. E. W. and O'Connor, M. J. & A. Churchill, Ltd., London.
- Wolstenholme, G. E. W. & O'Connor, M. (eds.) (1967). *Antilymphocytic Serum*. J. & A. Churchill, Ltd., London.