

211

A LOOK AHEAD AT TRANSPLANTATION

THOMAS E. STARZL, M.D.*

IN THE FOLLOWING REMARKS, I am going to concentrate on some specific problems which have not been solved or upon which more research effort needs to be invested. The first concerns *Organ Preservation*.

There is no way that widespread and efficient utilization of human organ homografts will ever be possible without major new developments in organ preservation which will allow banking for weeks or months. Progress in this direction has been relatively minor. Thanks to the work of Lillehei, Humphries, Marchioro, Brettschneider, Belzer, and many others it has become possible to keep vital organs in good condition for a number of hours or even for as long as 2 or 3 days. In general, the techniques have either involved cooling or perfusion. In addition, hyperbaric oxygenation or the use of metabolic inhibitors has been employed as adjuvant measures.

In Fig. 1 is shown an example of the kind of device that can be built for organ conservation, this being the preservation unit developed by Brettschneider for livers. The excised organ is protected by three means. It is perfused through the portal vein and hepatic artery, cooled at 4°C, and kept in a hyperbaric

oxygen environment. Conceivably, one could add to the perfusate a variety of metabolic inhibitors such as those reported by Webb, by Fonkalsrud, and others.

In spite of the importance of these efforts, they have not held much promise of permitting long-term storage, and it must be conceded that there has been less progress toward organ banking than in any other aspect of transplantation. This fact has been recognized by the Surgical Study sections of the NIH as well as by the Transplantation Subcommittee of the National Research Council. In a sense, the NIH and National Research Council committees are involved in long-term scientific planning. This fact is worth emphasizing to a group of young academic surgeons for at least one very practical reason. Presently, research grants are generally hard to obtain but at the same time significant sums of money are still available to support truly innovative proposals in the field of preservation. It will, of course, be necessary to have original ideas about how either solid state or perfusion techniques can be applied rather than submitting protocols that have already been thoroughly tested. The thought that I would like to leave is that new and old workers with fresh ideas about organ preservation can reasonably expect to receive tangible encouragement in the form of money.

The next topic I would like to touch on is *Histocompatibility Typing*. The last 5 years has been a time of intense activity of a small but devoted group of tissue typers in the

*Talk delivered at The Association for Academic Surgery Meeting in Boston, Massachusetts on Nov. 21, 1969.

From the Department of Surgery, University of Colorado School of Medicine, and the Veterans Administration Hospital, Denver, Colorado.

Supported by United States Public Health Service Grants AM-06344, AM-07772, FR-00051, AI-04152, FR-04152, FR-00069, AM-12148, and AI-AM-08898.

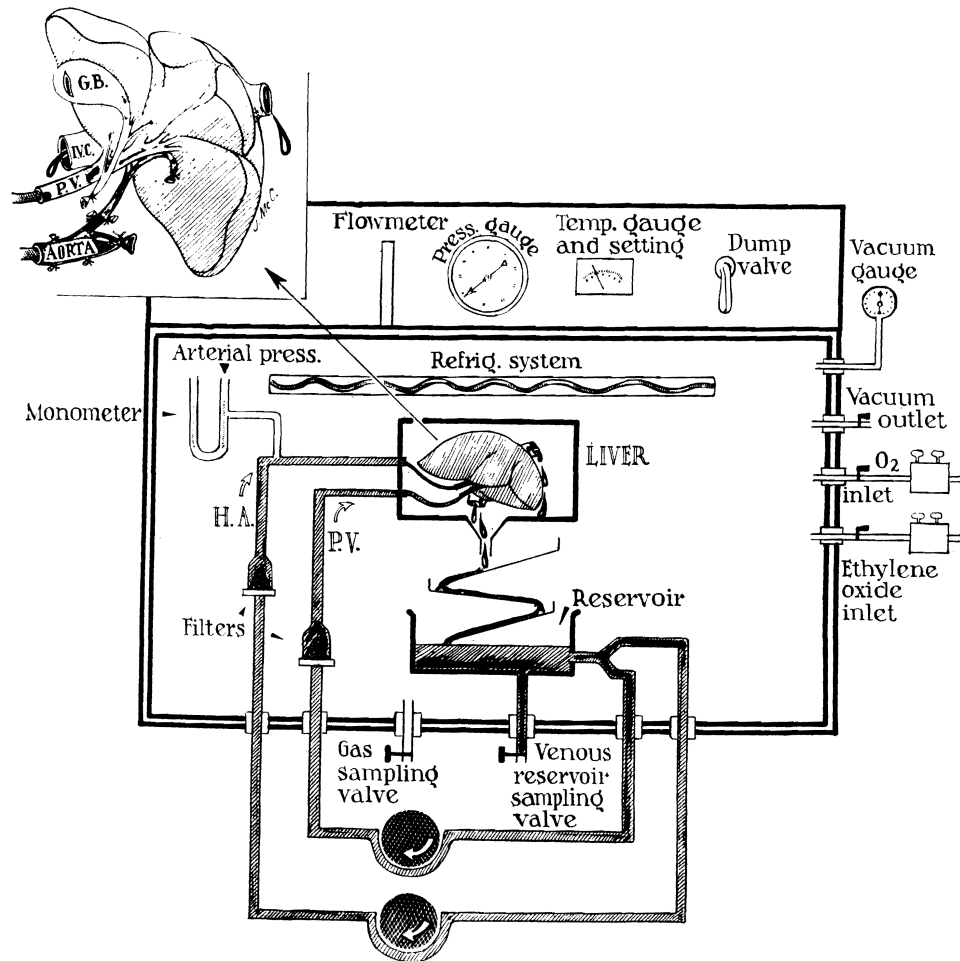


Fig. 1. Preservation unit. The perfusion pumps are located outside the hyperbaric chamber; the organ receptacle, the oxygenator, and the venous reservoir are inside. The various chamber inlets permit sampling of the perfusate, gas sterilization, and oxygen delivery and removal. The temperature is electronically controlled. (By permission of *Surg. Gynec. Obstet.* 126:263, 1968).

United States and Europe. These men and women have developed methods to identify antigens in lymphocytes and other tissues. In related cases, particularly involving siblings, it has been repeatedly demonstrated that a good tissue match is a highly favorable prognostic condition and that the converse is true if the match is a poor one. With more distant relationships, the correlation between tissue matching and the clinical outcome may be tenuous at best and in the present state of the art, such correlations may not exist at all with cadaveric transplantation. It is particularly important in 1969 to realize this since donors for most extrarenal organs must come from

nonrelated cadaveric sources. Our own confidence in tissue matching with serologic techniques is so limited in nonrelated cases that we do not anticipate a good result simply because of a fine match, nor do we refrain from using cadaveric organs on the basis of a bad match. This point of view can be more easily illustrated than described.

Shown in Fig. 2 is a human orthotopic liver homograft at 68 days. This organ was A matched despite which it was inexorably rejected in a little more than 2 months. The rejected liver was removed and replaced with a homograft which had frank mismatches in groups HLA 5 and 6. The second, or D-



Fig. 2. A rejecting orthotopic liver homograft in a patient who was said to have an A histocompatibility match with the donor. The protruding homograft as it was seen when the transverse abdominal incision was reopened. Note the enormous swelling, as well as the heterogeneous appearance of its surface. This liver was removed at the same operation and replaced with a second homograft which came from a donor with a D match. Although the second organ also underwent a severe rejection, it supported life for an additional year.

matched organ then supported life for more than a year. The best result we have obtained in our liver series was in the recipient of a hepatic homograft that was mismatched in four groups including HLA 2, 3, and 7. The recipient of this D-matched kidney had two rather minor rejection episodes early in his convalescence (Fig. 3) but he has an excellent result with completely normal liver function now $1\frac{1}{2}$ years after operation.

For reasons pointed out by the tissue typers themselves, the measurement of histocompatibility between unrelated individuals is very imprecise and incomplete using the serologic methods. It is probable that a direct cross-match technique, such as the mixed lymphocyte culture method of Hirschhorn and Bach, would be much more discriminating. However, the Hirschhorn-Bach approach requires the better part of a week to be completed, a time that is much too long to permit practical

application in most cadaveric cases at the present time. However, it is also obvious that a major advance in organ banking would permit the application of such direct measures of histocompatibility. Thus, the research on organ preservation that I have already mentioned would not only cut the waste of cadaveric organs that is inevitable today, but it would also allow the use of more predictive techniques of tissue typing.

Of course, the need for tissue banks would be reduced if animal organs could be employed. When tissues and organs are transplanted across a species barrier this is designated *Heterotransplantation* or *Xenotransplantation*. The terms are descriptive but imprecise since the kind of rejection that may follow may be no different but only more severe than after many homotransplantations. On the other hand, a xenograft may be repudiated within a matter of minutes depending

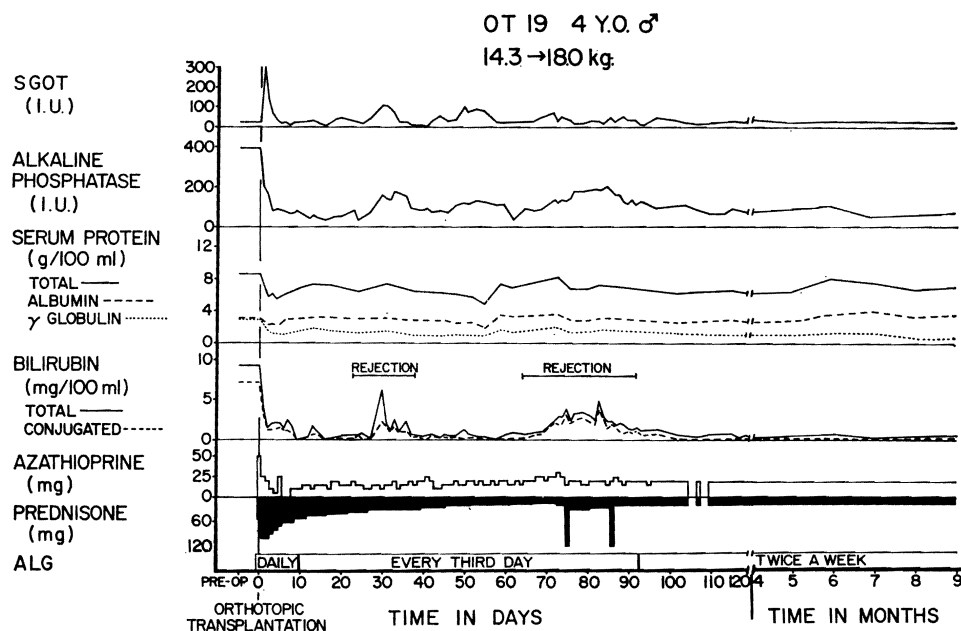


Fig. 3. A 4-year-old child with intrahepatic biliary atresia who was treated with orthotopic liver transplantation. The histocompatibility match with the donor was classified as D- (See text). A very transient rejection occurred after 1 month. This underwent almost immediate and complete remission. A late rejection which began on postoperative Day 72 was also easily controlled. Note the change in time scale after 4 months. The patient still has perfect liver function after 18 months. He is still receiving ALG. The normal enzyme values in international units at this age are: alkaline phosphatase, 57–151; SGOT, 3–27; and SGPT, 2–30.

upon the kind of donor-recipient animal combination. If hyperacute rejection occurs, it can usually be shown that preformed heterospecific antibodies are present in the recipient and that these have a high avidity for cells of the donor species.

The thought that animal organs might be clinically useful was generally conceded to be totally unfounded until the trials of chimpanzee-to-man renal heterografts performed by Reemtsma in 1963. That experience and a similar though less satisfactory one with baboon kidneys, indicated that even then heterotransplantation was almost, but not quite, good enough to have some real utility. Subsequently, many laboratories have contributed important details to an understanding of heterograft rejection and how this process might be controlled. There will be time to refer to only a few of these studies.

The first serious attempt to define the hyperacute rejection seen in unfavorable donor-recipient combinations was by Clark and

Gewurz and their associates at the University of Minnesota. They showed that rabbit kidneys transplanted to dogs cleared formed blood elements as well as hemolysins, hemagglutinins, and complement. They stressed the role of the preformed host antibodies in causing sudden devascularization of the transplants. With less divergent species, the role of immunoglobulins in the rejection process is less important, although still probably significant. In a variety of species it has been found that techniques to deplete these antibodies or complement can prolong life of subsequently placed heterografts.

It is probable that the paper of Lance and Medawar published in *The Lancet* in 1968 will be regarded as a landmark in heterotransplantation. Lance and Medawar were able to transplant skin from humans, rabbits, guinea pigs, and rats to ALS-treated CBA mice and achieve survival of the skin transplants for several months. In a sense, this work was confirmatory of earlier observations of Monaco

and in turn it has been confirmed by other workers including Cerilli of Ohio. Finally, at the Surgical Forum last month we were told by Gunnarson and Najarian that pig kidneys transplanted to goats survived and functioned for as long as 2 weeks in recipients treated with ALG and cytosine arabinoside. I have mentioned only a few straws in the wind that hold more than a hope that heterotransplantation is more than a Buck Rogers concept for the academic surgeon.

Now let me turn to the question of *Graft Acceptance*. About 10 years ago it was noted by Zukoski and by Pierce and Varco that immunosuppressive therapy could sometimes be stopped after canine renal transplantation with very long subsequent functional survival of the transplanted kidneys. In these first reports, the postoperative therapy had been with 6-mercaptopurine but it has since been demonstrated that the exact nature of initial treatment is not critical to achieve this result. Thus, examples of "graft acceptance" have been reported with azathioprine, ALS, ALG, prednisone, and even total body irradiation. Shown in Fig. 4 is a dog which received an unrelated orthotopic liver homograft in March of 1964 and which was treated for 4 months with azathioprine. Therapy was then stopped and the animal is still well more than a half a canine

lifetime later. We have dogs in our laboratory which have been off the therapy for as long as 6½ years after renal transplantation under the same circumstances.

The unanswered question in experiments like these is how such transplants have come to acquire their privileged status. It is probable that more than one immunologic pathway is involved. A classical possibility is that the continuous presence of a transplanted organ in a host being treated with immunosuppressive therapy leads to a selective loss of responsiveness to the antigens of the homograft (Fig. 5). The term for this would be "tolerance." That antigen stimulation plus immunosuppression can lead to this state of narrow-range tolerance has not been seriously questioned since Schwartz first called attention to this possibility in 1959. In this concept it is suggested that a specific fraction of the lymphocyte population is stimulated to action and that these sensitized cells are thereby rendered more susceptible to the killing effect of the immunosuppressive agent than the rest of the lymphocyte population. There is evidence that I do not have time to review here that at least partial tolerance is often accidentally produced in the course of clinical transplantation. From animal work, it would be expected that thymectomy would facilitate



Fig. 4. A dog whose liver was replaced with the liver of a nonrelated mongrel donor in March 1964. Immunosuppressive therapy was with azathioprine alone. This drug was stopped after 4 months and the animal is in perfect condition more than 5 years later.

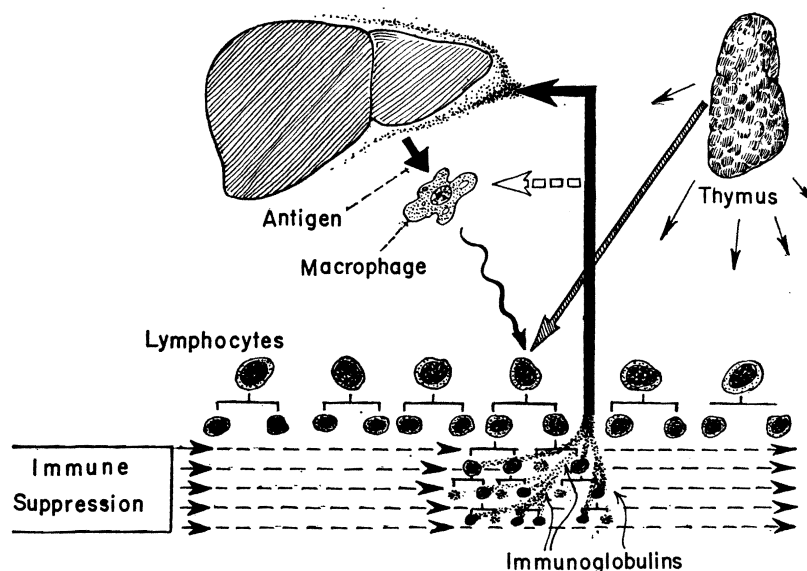


Fig. 5. Hypothetical mechanisms by which nonspecific immunosuppression may lead to selective abrogation of the host immune response. Special susceptibility to these agents of a fraction of the lymphoid population could lead to exhaustion of a clone and, hence, tolerance. Since maintenance of such cell lines even in adult life is apparently thymus dependent in experimental animals, thymectomy would be expected to aid the process; this appears to be true in rodents, but such an effect of thymus removal has not been detected in dogs or humans. A possible protective role is also shown in immunoglobulins elaborated by the replicating cells. Conceivably the antibodies could act either at the site of the antigen (enhancement) or by affecting the macrophage processing of the antigen. See text for discussion.

this process by removing the source of reinforcements to the cell line under attack (Fig. 5).

However, other lines of evidence have strongly indicated that tolerance is not the only mechanism by which graft acceptance is achieved. If tolerance were present, it should then be possible to transplant tissues and organs from the same donor to the recipient and have these new tissues accepted. This is very often not the case as was demonstrated by Woodruff 15 years ago with thyroid, and more recently by Cannon and Longmire with skin and by Murray with kidneys. Lately there has been much speculation that such primary grafts achieve their relative state of invulnerability partly by a process known as enhancement. Here the hypothesis is that antigraft antibodies are produced in response to the homograft antigens and that they return to coat or protect the transplant by some means which is not understood (Fig. 5).

The attempts to understand graft acceptance have been prompted by much more

than idle curiosity since it would be highly desirable to meet the conditions of graft acceptance before arrival of the homografts rather than hoping to achieve these conditions accidentally secondary to the actual transplantation. If this could be accomplished in advance, rejection could be prevented with far less crippling of the immune apparatus in the postoperative period.

At this year's Surgical Forum there were several papers, including those by Raju, by Yussman, and by Holl-Allen and their associates, all concerned with the questions I have just posed. More importantly, Stuart and his associates at the University of Chicago have passively immunized rat recipients with specific antigraft antibodies and have obtained prolonged function of rat renal transplants. When the use of enhancing antibodies was combined with donor-specific antigen pretreatment, homografts functioned for 18 months or longer in the presence of a strong histocompatibility barrier despite the fact that the recipients sometimes did not have a loss

of immunologic memory for the donor strain tissues. Stuart has suggested that both tolerance and enhancement may have played a role in the striking graft protection observed in his experiments without using any immunosuppression at all.

I would like to close by adding a few comments about *Clinical Transplantation*. There is no point in spending time on renal transplantation since this subject is undoubtedly well known to all of you. However, in passing, it is worth noting that recipients of vital organs other than the kidney have also had a meaningful extension of life. The longest survivor after liver transplantation is still alive after 22 months. To date the maximum follow-up of a heart recipient has been of Dr. Philip Blaiberg, who lived for 19 months. Lillehei and his associates have had recent encouraging experience with combined renal and pancreatic transplantation. I am told that two of their patients who were operated upon about 6 months ago have been discharged from the hospital and that their insulin requirements

are minimal or absent. Professor Derom of Ghent, Belgium, had a patient who lived 10 months after lung transplantation before dying of slow rejection.

In summary, I apologize to this group for having talked more about what *should be* new in transplantation than what *is* new in this field. I am going to justify having done this in the following way. Each generation contains its pessimists who regret having been born too late, at a time when the ultimate in discovery and progress has already been achieved. Surgery has been no exception. Advances in surgery have often been preceded by dire predictions of their impossibility, or worse, followed by opinions that the last frontier has been breached, leaving only small details for future cultivation. Of course, this attitude has no place in life and it certainly does not in the surgical discipline of transplantation, where almost certainly the most important advances are yet to be made, and I hope, by the members of this young and vigorous academic surgical society.