per cent protein hydrolysate, vitamins, sodium, potassium, magnesium, and chloride. Adult patients are gradually brought up to 24 hour volumes of 5 liters infused through meticulously cared for, central venous catheters. Once again, attention to detail and reliance on fundamental principles win the day in surgical care. This sort of approach to the preparation of high risk patients for operation and monitoring methods will prevent shock or lead to its early recognition. There have been no dramatic breakthroughs in the study of shock and metabolism during the past year, but steady progress of this kind will result in the saving of many lives.

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TRANSPLANTATION

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THERE IS a widespread interest in the experimental and clinical problems inherent in the transplantation of tissues and organs. There is a pervading spirit of optimism about the practical use of grafting procedures tempered by a realization of their imperfections.

PRESERVATION

There is no way that widespread and efficient utilization of organ homografts will ever be possible without major new developments in organ preservation which will allow banking for weeks or months. With the vital organs, the progress in this direction has been minor; almost all the investigations have been found to be concerned either with short range conservation or else with the pathophysiology of ischemic damage.

Kidney. Brodman has shown a self-perpetuating effect of ischemia in rabbit kidneys caused by an intermittent holdup of blood flow at the efferent arterioles at some time after initially satisfactory revascularization. Under similar experimental conditions, Nanninga demonstrated a protective effect of ethacrynic acid and furosemide upon rat kidneys, provided that the drugs were administered at the beginning of the interval of vascular cross clamping. The reason for the benefit is not clear.

McCullough, Jacobs, and Halasz described kidney preservation, perfusing a fluorocarbon in a cold salt solution emulsion at low flow rates. Fluorocarbon is a chemically inert liquid which allows for the exchange of carbon dioxide and oxygen but not of other metabolites. Canine kidney autografts could be kept viable for as long as 24 hours.

An argument for simplicity of short term preservation was contained in the results of another canine study by Martin. He found that kidney autografts which were protected by surface cooling alone remained in good condition for as long as eight hours after nephrectomy. In clinical practice, this should be sufficient time to find a recipient on the basis of histocompatibility matching and even to fly a renal homograft from one city to another.

Heart. Two studies with excised canine hearts are of interest because of the similarity of results, despite different experimental conditions. McCord removed the hearts and made no attempt whatever to protect the anoxic organs, whereas Lande used relatively sophisticated perfusion with oxygenated blood. Under both circumstances, the decay of quality of the hearts became pronounced after about two hours. As the organs became unacceptable, oxygen consumption fell. Perhaps the results highlight the inadequacies of presently available means of supporting the artificial circulation of single organs.

Skin. Some of the most interesting observations on preservation have been made by Abbott, who tested freeze-dried skin in mice for its ability to sensitize recipients to subsequent, similarly processed grafts or to fresh tissue from the same donor strain. There was no loss of antigenicity with freezing alone, but after freezing and lyophilization, histocompatibility antigens could no longer be identified or could second set reactions be induced. At a practical level, the clinical implication is that this kind of biologic dressing can be used without the danger of recipient sensitization.

THE DIAGNOSIS OF REJECTION

Efforts to sharpen the criteria of diagnosis of homograft rejection are still being made, even with the kidney. Andrews, Coppola, and Villegas re-examined urinary and serum concentrations of lactic dehydrogenase and one of its isoenzymes, alpha hydroxybutyric dehydrogenase, as indexes of either physical or immunologic injury to renal homografts or autografts. With kidney damage, there were elevations with both measures, but the organ specificity was greater with the isoenzyme.

In an exhaustive investigation, Graham and Lower and their associates examined the incidence, severity, and laboratory findings of cardiac rejection in dogs being treated with azathioprine to which methylprednisolone or homograft irradiation were intermittently added. There were 39 dogs which lived from nine to 422 days after heart replacement. These 39 recipients had 59 episodes of rejection, approximately a fourth of which were promptly fatal. In the others, rejection was at least partially, and often completely, reversible by intensification of immunosuppressive treatment. A number of serum enzyme determinations were evaluated as diagnostic aids. None of these tests was particularly helpful, and the best diagnostic indexes were provided by clinical observation and electrocardiography.

After liver transplantation in human beings, sepsis of the homografts has been reported. Alican and Hardy showed in their study of autografts that this complication should not arbitrarily be ascribed to rejection, since hepatic abscesses and cholangitis were seen in their experiments in the absence of an immunologic barrier. However, their studies did not disprove that rejection could not contribute to this kind of infectious problem.

A decline in blood flow is apparently a characteristic feature of all rejecting homografts. This principle was confirmed by Rosen and his associates who transplanted canine larynges to unmodified recipients. With the onset of rejection, or sometimes preceding it, flow declines were described with a krypton washout technique.

HUMORAL ANTIBODIES AND REJECTION

The classical view of rejection has been that the destructive agents are mononuclear cells and that there is little participation of humoral antibodies. In recent years, there has been a growing appreciation that circulating immunoglobulins may play an important ancillary role in rejection or, under certain circumstances, that they may be the most important element in the induction of injury to the transplant.

Cochrum and Kountz examined the sera of 24 human recipients of renal homografts at various times after transplantation. The sera from patients who did not have overt rejection did not develop easily detectable antibodies. However, in 11 of the patients whose kidneys underwent clinically diagnosed rejection, cytotoxic antibodies were regularly found. Two of these homografts were ultimately removed and shown by immunofluorescent studies to contain immunoglobin G, immunoglobin M, and beta IC complement. Antibodies eluted from the kidney specimens contained the same cytotoxins as had previously been present in the sera. It is conceivable that the humoral antibodies originated from the same sensitized lymphoid cells that were responsible for classical rejection. The work of Irvin supported the concept that the division of immunity into cell-mediated and humoral varieties may be artificial.

When cytotoxic antibodies are present in a recipient prior to transplantation, there is an increased risk that the homograft may undergo immediate destruction. Robertshaw and Hume and their associates studied this problem in dogs by immunizing animals with the skin and other tissue of an organ donor. The donor kidney was then removed, exposed briefly to serum from the hyperimmunized dog, and then returned back to the original host in which it promptly underwent cortical necrosis. The observations provided further evidence of the nonspecificity of the destructive process of hyperacute rejection, at least in the sense that the end stages of this process were served by elements within the autologous circulation after the stage had been set by initial exposure to homologous antibodies.

ANTILYMPHOCYTE SERUM

Of all the immunosuppressive agents, the one that received the greatest attention was heterologous antilymphocyte serum.

Preparation. Antilymphocyte serum is, in no sense, a standardized product like azathioprine or prednisone. Moreover, its mechanism of action is not known, although there has been increasing acceptance that these antisera selec-

tively destroy long-lived circulating lymphocytes of thymic origin. The results of a tissue culture study by Lundgren with the lymphocytes of renal homograft recipients were interpreted as evidence against this particular hypothesis.

If antilymphocyte sera act by destroying special populations of lymphocytes, it might be anticipated that the use of different lymphoid antigens for immunization could influence the activity of the end-product. Miller and Cohn examined this possibility in mice, using the rabbit as a heterologous serum donor. As a first step, they compared the ability of antithymocyte and antilymph node lymphocyte serum to slow the first set rejection of mouse skin grafts. The degree of protection was identical. However, under special recipient conditions which included near total body irradiation or prior induction of sensitivity to donor strain tissues, they obtained data supporting the idea that antisera could be raised against specific populations of lymphocytes.

Means to study this question further may have been provided by investigations on noncellular thymic extracts. Quint, Hardy, and Monaco administered thymosin to mice which were also given rabbit antimouse-lymphocyte serum. Provided that the thymosin was given in advance, it potentiated the immunosuppressive effect of the antiserum. It was speculated that the thymosin acted by mobilizing thymus-dependent circulating lymphocytes which were thereby rendered more susceptible to the antilymphocyte serum.

Davis, Cooperband, and Mannick worked with a soluble material obtained from human thymic tissue that had almost the opposite properties of thymosin in that this substance was an inhibitor rather than an augmentor of lymphocyte proliferation in tissue culture. In view of this action, it might be predicted that the humoral factor of Davis would not be synergistic with, and might even cancel, the antirejection potency of antilymphocyte serum.

Standardization. To date, there is no totally reliable in vitro test for assessing the immunosuppressive potency of antilymphocyte sera. The technique described by Clayman determines the ability of antihuman serum to prevent rejection of monkey-to-monkey homografts. The method reported by Saleh, Gordon, and MacLean grades the quality of sera according to the suppression of graft versus host reactions induced by injecting lymphocytes beneath the capsule of the rat kidney.

Refinement. Two methods have been described for removing purified globulin from raw antisera. Alexander used a standard diethylaminoethanol sephadex technique, while Moberg used an electrophoretic separator of such efficiency that it could process a liter of raw serum per hour.

Toxicity. The most specific risk of chronic therapy with heterologous serum or globulin is sensitization to the injected foreign protein. Gewurz and his associates showed that tolerance to horse globulin could often be accidentally produced in patients during a course of intravenous treatment with antilymphocyte globulin. However, Anderson and Wood warn about the repeated intravascular infusion of high potency antilymphocyte serum, since thrombosis and vasculitis could be produced locally. In addition, intravascular coagulation was also produced in distant organs including the lung.

Reports by Wanebo, Zipp, and Kountz and by Mandel and DeCosse show that both transplanted and spontaneously occurring lymphomas in mice had an accelerated growth rate under the influence of antilymphoid sera. This effect is thought by Mandel and DeCosse to be the consequence of potent immunosuppression, rather than to be a specific risk from the antisera. This point of view was upheld by Penn who reported that only about one out of every seven patients in whom new malignant growths developed after renal homotransplantations, had received prior treatment with antilymphocyte globulin.

OTHER IMMUNOSUPPRESSIVE MEASURES

Mechanical lymphocyte depletion. By means of thoracic duct drainage, Fish and his associates removed the lymphocytes of six calves and 11 patients. Profound lymphopenia was produced but not hypogammaglobulinemia. In the calves, renal homograft survival was prolonged, providing the lymphocyte depletion was carried out in advance. The thoracic duct fistulas in the patients were introduced before kidney transplantation from cadaveric donors and remained open for ten to 123 days. In the human recipients, early rejection tended to be both late and mild. Pharmacologic means of immunosuppression were withheld for 19 to 50 days. After this

time, maintenance doses of azathioprine and prednisone were begun.

Local homograft irradiation. With as much as 1,500 rads, McCredie, Inch, and Sutherland irradiated skin transplants on the backs of mice with prolongation of graft survival. When this total dose was given to the four extremities, the same effect was obtained, suggesting that the irradiation effect was systemic rather than a local one.

Drugs. Thiocymetin® (thiamphenicol), an analogue of Chloromycetin® (chloramphenicol), was evaluated by Linehan and his associates for its ability to slow the rejection of canine renal homografts. The dogs lived for an average of 21 days compared with nine days for the untreated controls. Phytohemagglutinin, an extract of the Phaseolus vulgaris bean which causes lymphocyte blast transformation, was found by Gertner to have almost no protective effect upon canine skin homografts. However, it seemed to add to the immunosuppression of azathioprine and also of prednisone but not to that of antilymphocyte serum.

Macdonald has studied the value of medroxyprogesterone as a substitution for prednisone in a treatment protocol for canine kidney recipients that also included azathioprine. Survival using medroxyprogesterone was superior to that with prednisone. The addition of antilymphocyte serum as a third agent did not confer additional benefit.

Any clinical trials with medroxyprogesterone as a substitute for prednisone will be awaited with great interest because of the excessive morbidity rate that has followed the use of high dose prednisone therapy in kidney transplant recipients. Crutchlow and Bright emphasized the profound changes in bone metabolism or structure that can be induced with chronic prednisone administration. In a badly needed study, Fisher and Bickel showed how steroid treatment in rabbits could cause increases in serum lipids with the formation of fat emboli that passed to terminal arterioles of bone and caused both osteoporosis and osteonecrosis.

In a different approach, Murphy attempted to increase the safety with which large doses of azathioprine could be given by concomitantly administering a yeast extract that has been said to prevent the bone marrow toxicity of nitrogen mustard. The azathioprine seemed to be better tolerated and to cause less marrow depression.

GRAFT ACCEPTANCE

As reillustrated by the canine study of Shanfield, a homograft may come to be more or less well tolerated in its new host and, in some instances, may continue to function chronically, even though all immunosuppression is stopped. There may be more than one explanation for the privileged status.

Tolerance. The term tolerance has been used to indicate a selective loss of host responsiveness to specific antigens with retention of otherwise normal immunologic reactivity. Within the limits of this definition, experiments of Graff and Newton were interpreted as showing a relation between sensitization and tolerance that depended upon the dose of sensitizing antigen and the strength of the histocompatibility barrier.

Enhancement. Evidence was presented by Raju and Grogan, Yussman and Hines, Holl-Allen and his associates, and Stuart and his associates that specific immunologic tolerance was not the full explanation for graft acceptance. In the experiments of Stuart, renal homotransplantation was performed in rats across a strong histocompatibility barrier without the aid of immunosuppressive agents. The recipients were conditioned before operation by the intravenous administration of donor genotype spleen cells as well as by the injection of specific antidonor serum. The kidney homografts survived for as long as 18 months, in spite of the fact that the recipients sometimes did not have a loss of immunologic memory for the donor strain tissues. Stuart suggests that both tolerance and enhancement were factors in the graft protection.

Other factors. The possibility exists that portions of grafted tissues may eventually assume the genetic characteristics of the recipient. Williams and Alvarez report a technique by which the sex of vascular endothelium of transplanted kidneys could be studied. Their preliminary results suggest that a sex change of arterial endothelial cells might occur, a finding that was not confirmed by Kashiwagi in the hepatic arteries of human liver homografts. However, Kashiwagi did note that the Kupffer cells of hepatic transplants became of host origin at all times after three months.

HISTOCOMPATIBILITY MEASUREMENT

The advantages and especially the limitations of histocompatibility typing with serologic meth-

ods have been described by Terasaki in relation to cardiac transplantation. A more cumbersome and time-consuming but potentially more discriminating technique for matching donors and recipients is the mixed lymphocyte culture test. The latter method was evaluated in untreated dogs by Sullivan who found consistently longer survival with good compatibility.

GRAFT FUNCTION

A number of problems have been raised concerning the function of various transplanted organs and the effects upon these grafts of denervation, blood supply, and other factors.

Limbs. In puppies, Furnas showed that reimplanted right forelimbs almost never provided normal subsequent function but that most puppies were eventually able to use the leg for auxiliary support.

Spleen. Kelly, Pechet, and Eiseman demonstrated that perfused porcine spleens in an extracorporeal system synthesized antihemophiliac globulin and at a level that supported the concept of splenic transplantation for the treatment of hemophilia.

Liver. Investigations by Lee and by Sawada appear to reaffirm the desirability of perfusing the portal system of liver homografts with venous blood from the splanchnic bed. Even with this advantage, Sawada usually observed atrophy if the hepatic transplants were used as auxiliary organs in dogs. The shrinkage was apparently due to the combined adverse effects of rejection plus competition of the transplanted liver with the host's own organ. Bell also pointed out that not all the injury of liver homografts can be attributed to rejection. He studied the cholestasis that can be caused by the immunosuppressive agent, azathioprine. This finding was minimized in the dogs of his study by the administration of choleretic agents.

As confirmed by the studies of Giles and Slapak, one of the most interesting features of liver transplantation is that the homograft can synthesize protein of a new type. This is not surprising with those proteins that are normally produced by hepatocytes. Since the immune globulins are thought to be produced extrahepatically, Kashiwagi added a further dimension by showing that new gamma G globulin phenotypes were conferred upon human recipients of orthotopic livers. Some of these livers were eventually studied at autopsy and were shown to contain lymphoid tissue. Presumably, these lymphoid deposits had been accidentally transplanted along with the liver, had remained viable, and had synthesized and released the new gamma G globulin for long periods.

Lungs. Graf, Nadel, and Edmunds found that by two to six months, pulmonary autografts had regeneration of the efferent autonomic nerves. The afferent autonomic nerve conduction had not returned and, consequently, the animals did not have Hering-Breuer or cough reflexes.

Richards determined that the function of five pulmonary autografts and one homograft was not normal eight to 12 months postoperatively. However, the high vascular resistance, low pulmonary blood flow, and low oxygen uptake could all be partly reversed when the contralateral normal lung was exposed to pure nitrogen. Thus, the impairment of the grafted lungs was partly a reflection of the functional interrelationship with the contralateral lungs rather than an indicator of a serious intrinsic deficiency.

HETEROTRANSPLANTATION

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 more severe than after many homotransplantations. On the other hand, a xenograft may be repudiated within a matter of minutes, depending upon the kind of donor-recipient animal combinations. 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.

Cerilli and Gideon studied relatively easy heterotransplantations from rats or hamsters to mice. The skin grafts were repudiated by unmodified recipients after an average of 5.8 and 4.5 days, respectively, long enough to conclude that the rejections were chiefly by cell-mediated immunity. Treatment with antilymphocyte serum significantly prolonged skin survival. The addition of procarbazine hydrochloride to antilymphocyte serum was even more effective.

Gunnarsson and his associates used a more difficult system, transplanting porcine kidneys to goats. Kidney survival in untreated recipients was about one day, an indication of destruction

by preformed heterospecific antibodies. The survival time was extended to two days by the administration of cytosine arabinoside, an agent which is said to suppress humoral antibody response. With the use of antilymphocyte globulin, the period of viability was seven days. When antilymphocyte globulin and cytosine arabinoside were used together, the organs were not rejected until 14 days. These results are the best that have yet been reported with whole organ transplantation using such a difficult species combination.

Rabbit to dog renal heterotransplantation results in violent repudiation of the kidneys within six to 12 minutes. Ellis and his associates were able to slow this process as much as ten times by pretreating the kidneys with a collagen emulsion prepared from calf skin. They concluded that the protective effect was by coating of the vessels and glomeruli, thereby preventing an antibody attack on the vascular endothelium and secondarily avoiding platelet aggregation.

Cross circulation of patients with baboons was recently reported for the treatment of hepatic insufficiency. Gayle, Williams, and Hume described the consequent lethal effects upon the animals and suggested that they had died from heterograft rejection. There was evidence that the baboons absorbed antibodies present in human beings. In some tissues, such as the lung, there were morphologic findings suggestive of a hyperacute rejection.

SURGICAL TECHNIQUE

Benzing and his associates performed orthotopic cardiac transplantation in dogs with the Shumway-Lower technique, except that a Teflon® (polytetrafluoroethylene) coupler was used to reconnect the aorta and pulmonary artery. The ischemia time was lowered, and the annoying hemorrhage encountered in suturing the canine aorta was eliminated.

Dwoskin, Soderdahl, Purtilo, and Harrison tested new methods of ureteroneocystostomy after canine renal transplantation, bringing the homograft ureter through the distal portion of the recipient's own ureter in two ways. Neither method yielded satisfactory results, and these investigators did not recommend these technical modifications for clinical trial.

CLINICAL NOTES

There are highly significant, purely clinical reports on extrarenal organs that deserve more than passing notice. For example, Kelly and Lillehei and their associates, after encountering many difficulties in their early trials, have now been able to discharge two patients from the hospital after combined pancreatic and renal transplantation. The longest follow-up period in those patients still living is now five months. The insulin requirements in these brittle diabetic patients has been minimal or absent.

Steady progress has been recorded with liver transplantation. In six instances, there has been survival of a year or more, the longest living patient has now survived for a period of 20 months. Several of the late deaths after liver transplantation have been in patients in whom the original disease was hepatoma and who ultimately died of metastases. Consequently, the prime indication for liver transplantation in the future will certainly be for benign diseases, such as biliary atresia and cirrhosis.

Between six and ten patients with cardiac disease have lived for a year or more after cardiac transplantation, the longest survival period being 17 months. The autopsy findings in a patient after 19 postoperative months have been reported. In essence, the transplanted heart, which eventually failed, was small and contained minor interstitial fibrosis and few mononuclear cells. The major finding was acquired obliterative disease of the coronary arteries, such as that reported in the past in canine cardiac transplants by Lower and Shumway.