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Transplantation Immunology[1]

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I. Introduction

In considering transplantation immunology there is obviously a major overlap with each of the other chapters in this volume. As long as this fact is acknowledged, there should be a real advantage in summarizing the immunology of transplantation from the clinician's point of view. In particular, it is worth focusing upon certain phenomena that have been seen after whole-organ transplantation, which form the basis for the clinical discipline of transplantation but which have not yet been precisely and satisfactorily explained by those interested in the definition of mechanisms.

Major progress toward organ transplantation has been achieved only in the past few years. Before then an almost total ignorance of the biological problems that would be encountered after transplantation precluded the development of appropriate methods of therapy. As recently as 1940, there was still a widespread belief that application of tissue transplantation needed only the refinement of better surgical techniques. Despite the slow accumulation of evidence that this was not the case, the situation remained obscure until the first of the studies by Sir Peter Medawar and his colleagues in England.

A. Medawar and the Turning Point

Medawar's investigations were conceived and executed under trying circumstances. Questions concerning skin replacement had become more urgent than ever because of the need to treat mass wartime casualties from the Battle of Britain. The answers were provided on the basis of investigation with rabbits in which the genetic homogeneity of the donor and recipient animals was sufficient to permit a reasonable reproducibility of results. The conclusions were precise and can readily be summarized as they apply to humans.

First, skin grafts placed with perfect surgical technique were rejected after a rather predictable interval which in the rabbit system was about 10 days. In humans the rejection time is highly variable, but within a few days to several weeks, repudiation of skin grafts is ordinarily complete in that initially viable skin becomes a blackened and necrotic eschar.

In Medawar's report there was evidence that the repudiation was due to an immunological reaction of the host to the foreign tissue. The key observation in support of this concept was the fact that a second skin graft from the original rabbit donor strain was destroyed in an

accelerated fashion, suggesting the acquisition of immunity by the host. The immunity conferred by contact with the first graft was permanent or of long duration and pertained to all tissues subsequently transplanted from the donor strain. The sensitization was specific inasmuch as grafts from *other* donor strains were *not* usually rejected in an accelerated manner. The initial delay between the actual transplantation and the subsequent development of active immunity prompted comparison between these events and the delayed hypersensitivity that permits immunity to develop to diseases such as tuberculosis.

B. Nomenclature

The terminology used in transplantation is based upon genetic and phylogenetic relationships which, in turn, roughly determine the hostility with which a graft is viewed by its recipient. When tissue is transplanted from one location to another place on the same person (an *autograft*), it is identified as "self" (Fig. 1) and therefore does not evoke a defensive host reaction. The success or failure of the graft is exclusively dependent upon the technical adequacy of the procedure and upon other well-accepted principles of surgical care.

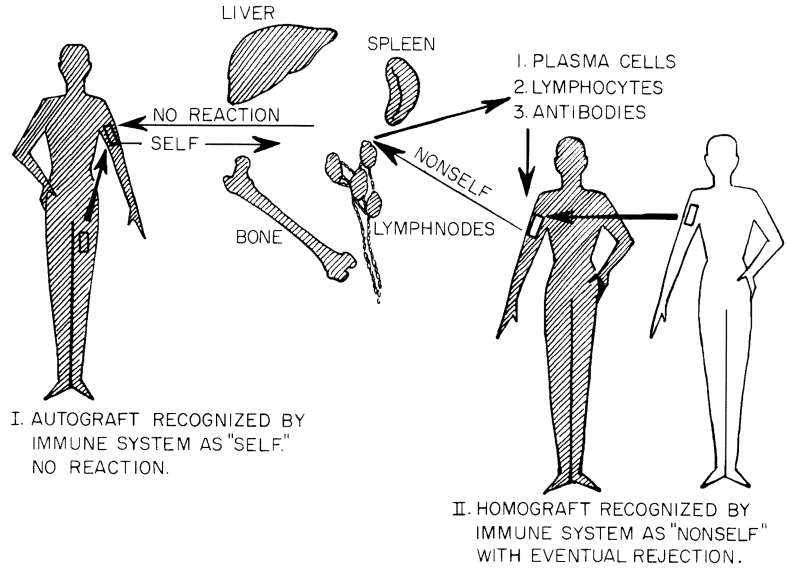

Fig. 1. Fundamental difference between autografts (left) and homografts (right). Tissues transferred between identical twins behave as autografts. They are termed isografts and are not rejected. [From *Surg. Clin. N. Amer.* **42**, 55 (1962); by permission of W. B. Saunders Co.]

The same applies when tissues or organs are exchanged between identical twins. These are called *isografts*. Because there is total genetic identity of identical twin donors and recipients, the grafts are not recognized as foreign and can be expected to have the same life expectancy as that of the host. This was first proved in man by Dr. J. B. Brown of St. Louis with skin transplantation experiments, reported in *Surgery* in 1937, and later applied to the transplantation of kidneys and, more recently, bone marrow between identical twins.

Tissue transplanted from nonidentical members of the same species (Fig. 1) are called *homografts* (or *allografts*). The host response that follows is defined above as *rejection*, the intensity of which is roughly determined by the degree of genetic dissimilarity between donor and recipient. The genetic factors of transplantation, often referred to as *Snell's laws* (after Dr. George Snell) were precisely worked out in inbred rodent experiments. Unfortunately, in the outbred canine and human populations, there is a tremendous and as yet unpredictable variability in the vigor of the attack a homograft elicits. These observations have led to an intensive search for methods that would allow identification of a favorable donor-recipient combination in advance of clinical transplantation. These techniques are referred to as *tissue typing*, a subject to which we return later (see Section VII).

If transplantation is from a donor belonging to a different but similar species, the tissue is called a *heterograft*, and on the average the rapidity and intensity of rejection are even greater than with homografts. However, studies with chimpanzee to human heterografts pioneered by Dr. Keith Reemtsma have shown that such transplants can sometimes be tolerated for long periods in patients receiving immunosuppressive therapy.

Tissues or organs transferred between widely divergent species (as, for example, between pigs and dogs) are called *xenografts*. In most instances xenografts are destroyed within a few hours by a kind of hyperacute rejection which is apparently subserved by preformed heterospecific humoral antibodies (see Section II,C,3).

II. Mechanisms of Rejection

A. Cell-Mediated Immunity

The means by which transplanted tissues and organs are rejected are poorly understood. There has been abundant evidence that lympho-

cytes participate in the process in an important way. This was illustrated by experiments of the late Dr. Glenn Algire, who used Millipore chambers in which enclosed fragments of tissue were shielded by a mesh barrier of appropriate sized interstices to exclude lymphocytes and other mononuclear cells but through which barrier nutrient fluid and even red cells could pass (Fig. 2). Survival of the transplants was longer than with tissue that was not thus protected. Corneal homografts, which have been used clinically for many years, presumably escape rejection at least partially for similar reasons; their nutrition is obtained from the cell-free aqueous humor in the anterior chamber of the eye. If a corneal graft becomes revascularized, it usually fails.

The participation (if not the precise action) of mononuclear cells in the rejection of tissues and organs can be appreciated in a more direct way by histopathological studies. As the graft is repudiated, the normal architectural organization becomes distorted as variable necrosis develops (Fig. 3). At about the same time, massive infiltration by lymphocytes and plasma cells occurs. It is not hard to understand how the liver in Fig. 3 nearly ceased to function 6 days after its transplantation to an unmodified canine recipient. Eventually, the blood supply to whole-organ grafts is diminished and later all but cut off, so that more-or-less complete ischemic necrosis is the ultimate fate of the transplant if the recipient animal lives long enough for this stage to be reached.

The hallmarks of classic unmodified cellular rejection are much the same in all acutely rejecting organs, whether they be liver, kidney, heart, skin, or others. The tissues become overrun with millions of lymphocytes

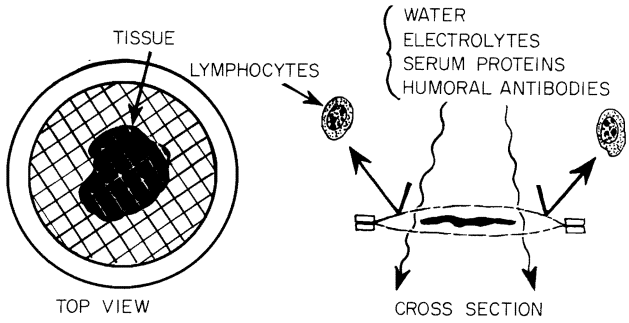

Fig. 2. Diffusion chamber experiment, after Algire. The enclosed homograft, which is protected from physical contact with lymphocytes, can survive for protracted periods. [From *Surg. Clin. N. Amer.* **42**, 55 (1962); by permission of W. B. Saunders Co.]

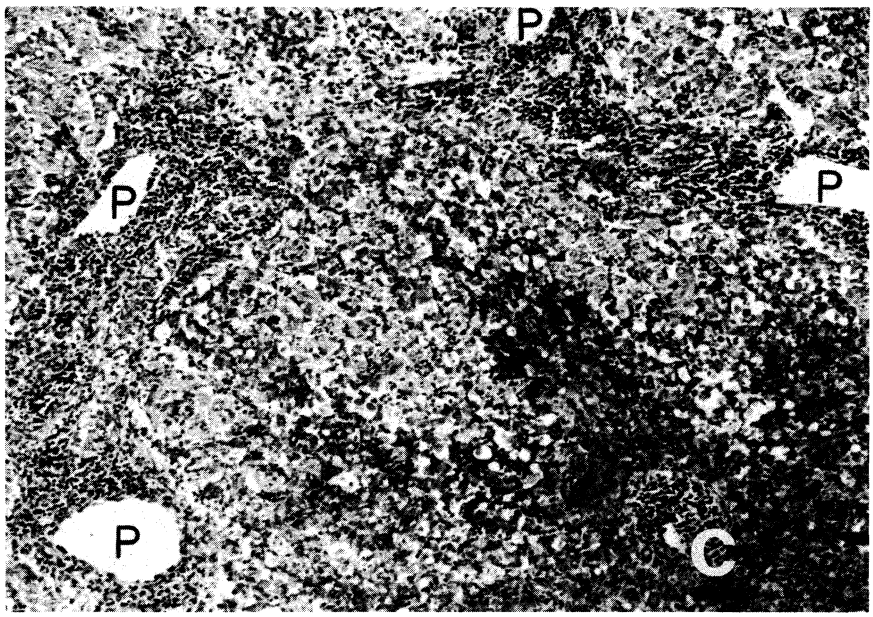

Fig. 3. Untreated canine hepatic homograft at 6 days. Portal veins (P) and central vein (C) are surrounded by a dense infiltrate of mononuclear cells. There is centrilobular necrosis with hemorrhage. The cytoplasm of the surviving hepatocytes in the middle and peripheral zones of the lobules contains abundant lipid. Hematoxylin and eosin stain. ×27. The photomicrograph was prepared by Dr. K. A. Porter, London, England. (From *Advan. Surg.*, 1966; by permission of Yearbook Publishers, Inc.)

and plasma cells. There is concomitant necrosis of the distinctive parenchymal tissues that give the grafts their structural identity.

B. Humoral Antibodies

There is no reason to believe that the cellular immune response is the only means by which delayed homograft rejection can occur. In the serum of patients undergoing acute rejection, cytotoxic or other kinds of antibodies have been described. Even in the serum of patients who have tolerated renal homografts for years, there are often circulating antigraft antibodies, but in these cases apparently with a low capacity to cause transplant injury. Nevertheless, homografts in such recipients commonly contain deposits of γ-globulin, as well as host complement.

Antibody deposition has been very well documented after transplantation of human kidneys, livers, hearts, and lungs. The patterns of the

immunoglobulin binding are particularly interesting in renal grafts since they resemble those of two major kinds of experimental glomerulonephritis, namely, Masugi nephritis caused by anti-GBM antibody, and the kind of nephritis caused by the filtration by the kidney of soluble antigen–antibody complexes. In many cases the glomerulonephritis in these transplants has been similar or identical to that which destroyed the native kidneys, indicating a recapitulation of the original disease. However, it may also be said that glomerulonephritis can be one manifestation of humoral homograft rejection. This position has received support from the fact that "glomerulonephritis" has been observed in homografts transplanted to recipients whose renal failure was due to polycystic kidney disease, cystinosis, pyelonephritis, or other disorders not suspected to be of autoimmune etiology.

In other organs, such as the liver, immunoglobulin deposits have been somewhat less extensive than in renal homografts, and they have tended to be unevenly distributed throughout the vasculature.

C. Presensitization States

1. Second-Set Rejection

In Medawar's original experiments, which were mentioned earlier, skin transplanted to rabbits sensitized by one exposure to donor tissue was rejected in an accelerated, or *second-set* fashion. Instead of being repudiated after an average of 10 days, this time was shortened to 6 days. The assumption was (and still is) that lymphoid tissue or other contributors to cell-mediated immunity are mobilized more quickly than normal because of their prior antidonor instruction. An additional role of circulating humoral antibodies may also be important, and in more extreme degrees of sensitization these antibodies may come to be the dominant factor in what has been called hyperacute rejection (Section II,C,2).

After whole-organ transplantation under immunosuppression, there have been numerous reports of accelerated rejection apparently comparable to that in Medawar's rabbit system. It has been speculated that the advance sensitization in these humans could have been induced to antigens also found in donor tissues, during the course of pregnancies, by the previous administration of white cells or platelets in multiple blood transfusions, or by other means including prior renal homotransplantation. In such patients rejection occurs earlier and often more vigorously than expected, but it does not necessarily proceed to immediate

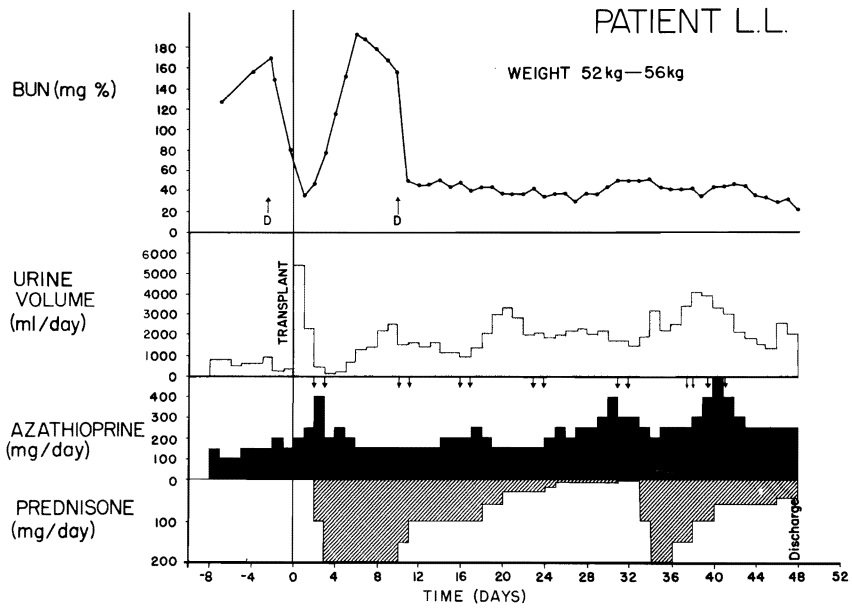

Fig. 4. Development of a rejection crisis less than 36 hours posttransplantation. Although transient anuria resulted, the process was reversed after the addition of high-dose prednisone therapy. Note that a dialysis (D) was required before adequate function returned. Each arrow represents 200 μg actinomycin C administered intravenously. This patient, whose transplant operation took place on July 8, 1963, still has excellent function of the same homograft almost $8\frac{1}{2}$ years later. It is probable that presensitization had occurred in this case and that the violent and early crisis was a second-set or accelerated rejection. [By permission of *Surg., Gynecol. Obstet.* **118**, 819 (1964).]

destruction of the graft. Reversal of this kind of uncomplicated accelerated rejection has often been observed (Fig. 4).

2. *Preformed Antibodies and Hyperacute Homograft Rejection*

a. ABO Incompatibility. The first clear examples of hyperacute rejection of renal homografts were in patients who received kidneys from ABO blood group-incompatible donors. An effective blood flow to some of these transplants was not restored when the vascular anastomoses were opened. The small vessels of the excised kidneys were demonstrated by angiography to be closed and, histopathologically, the arterioles and capillaries were plugged with formed blood elements, particularly erythrocytes. A rational although partial immunological explanation was available since the blood group substances that allow red cells to be

typed had been shown by Högman and Szulman also to be found in other tissues including the kidneys. Consequently, if the kidney of an A, B, or AB donor were placed in a patient whose serum contained naturally occurring anti-A and/or anti-B isoagglutinins (an example would be a recipient with O blood type who would have both kinds of isoagglutinins), these antibodies might be predicted to bind with the renal red cell antigens. Serological studies in some of our cases showed that falls in systemic isoagglutinin titers actually occurred. Subsequent investigators have reached similar conclusions about the role of red cell isoagglutinins in precipitating accelerated rejections. The rules of red cell compatibility as they apply to whole-organ transplantation are summarized in Table I.

b. Cytotoxins and Other Antibodies. It is unlikely that future organ transplantations will be carried out under the foregoing adverse conditions of ABO mismatching. However, hyperacute rejection in the presence of *red cell group compatibility* has been seen with increasing frequency and, in fact, this kind of rejection has become the chief cause of acute homograft loss in most major transplantation centers. The first case was described by Dr. Paul Terasaki of Los Angeles in a patient whose serum contained lymphocytotoxic antibodies that killed donor cells. Terasaki theorized that, in the course of being transfused prior to operation, the recipient had been immunized (probably on multiple occasions) to white cells that shared histocompatibility antigens with the eventual renal donor. Since then, no one has seriously challenged this general hypothesis of presensitization. The concept has been indirectly supported by the high rate of hyperacute rejection with retransplantation in patients whose first homografts were rejected and who were thereby presumably immunized to some antigens also present in the second graft.

TABLE I
DIRECTION OF ACCEPTABLE MISMATCHED TISSUE TRANSFER[a]

O to non-O	Safe
Rh^- to Rh^+	Safe
Rh^+ to Rh^-	Relatively safe
A to non-A	Dangerous
B to non-B	Dangerous
AB to non-AB	Dangerous

[a] O is universal donor; AB is universal recipient.

Subsequently, many other investigators have confirmed the adverse implications of preformed antidonor antibodies as detected with several techniques. The most commonly employed methods have measured lymphocytotoxins and leukoagglutinins but, according to G. M. Williams and Felix Milgrom, the most sensitive examination is the mixed agglutination test.

While certain tests may be more sensitive than others for the detection of the preimmunized state, it does not seem likely that a single antibody will be found to have unique predictive significance. In our laboratories deliberate sensitization of dogs by repeated skin grafts led to the formation of a variety of antiwhite cell and antired cell antibodies with antidonor reactivity. However, the titer of these antibodies is not well correlated with the rapidity of rejection of a kidney from the skin donor. Moreover, it has been emphasized in reports of clinical cases that hyperacute rejection presumably due to presensitization may occur even though antidonor antibodies cannot be found with any currently available technique including the mixed agglutination method. Under these circumstances it has been necessary to assume that an immediate, albeit undiscernible, immunological reaction is the initiating event in the destructive process that follows. With or without demonstrable antibodies in the recipient serum, the immunoglobulin deposition in the transplants may be in such small quantities that their specificity as judged by strictly morphological criteria in immunofluorescence studies could be open to question even though on other grounds it is reasonable to believe they are significant.

c. Coagulation. A simplistic view of hyperacute rejection might be that the antidonor antibodies discussed in the preceding section were destructive of renal homografts by their direct nephrotoxicity. The observations already cited in the ABO-incompatible cases were not consistent with such a conclusion since the most obvious lesion in the rapidly repudiated kidneys was occlusion of their blood supply by clot and mechanical debris including formed blood elements.

In cases with hyperacute rejection despite red cell compatibility, there was also evidence of interference with the blood supply. When Kissmeyer-Nielsen described the histopathology of two hyperacutely rejected kidneys, he noted that the glomerular capillaries and the arterioles were full of microthrombi, making the morphological features indistinguishable from those of a generalized Shwartzman reaction. Similar observations were made in our own first cases (Fig. 5).

Although these histopathological findings suggested that coagulation changes had occurred, clotting studies were not available to determine

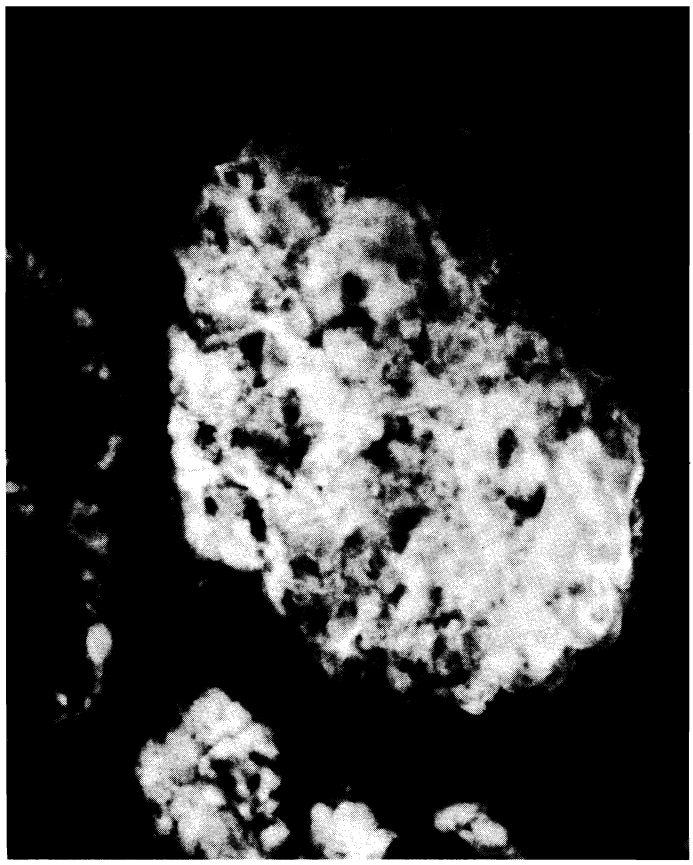

Fig. 5. A glomerulus stained for fibrin from a homograft that had undergone hyperacute rejection. There is complete obliteration of the glomerular architecture with fibrin. ×300. The photomicrograph was prepared by Drs. Richard A. Lerner and Frank J. Dixon. [By permission of *N. Engl. J. Med.* **278**, 642 (1968).]

if the alterations were systemic or if they were confined to the actual homograft. The first efforts to obtain such information were completely negative.

More recently, evidence had been found that coagulation changes are an integral feature of hyperacute rejection in the presensitized canine model as well as in man. In the dogs that were exposed to multiple skin grafts from the eventual organ donor, the subsequently transplanted kidney, spleen, or liver always consumed clotting factors and platelets locally. One of the objectives of these animal investigations was to see if transplantation of consecutive organs from the same donor would

mitigate the rejection of the second graft. It was found that the second transplant was briefly protected, possibly by the prior depletion of either humoral antibodies, clotting factors, or formed blood elements. In time, however, the final organ suffered the same fate as the first one.

All of the sensitized canine recipients in the above study developed evidence of local consumption. In addition, a minority of animals also had profound systemic coagulation changes similar to those of disseminated intravascular coagulation (DIC). The same kinds of observations have been made in patients after renal homotransplantation with a consequent severe or even fatal bleeding diathesis. Thus although the clotting abberations of hyperacute rejection are usually confined to the graft insofar as can be measured, there is now little reason to doubt that profound systemic changes may follow.

d. Formed Blood Elements. White cells, platelets, and red cells form a morphologically prominent component of the vascular plugs of hyperacutely rejecting renal homografts. Williams and Hume of the Medical College of Virginia were the first to draw attention to the dramatic appearance of polymorphonuclear leukocytes (PMNs) in such kidneys. Their observations, since amply confirmed, were made possible by systematically biopsying homografts about 1 hour after revascularization. In some instances the PMNs appeared before any other histopathological findings were evident. That the participation of these cells in the ultimate destruction was not immunologically specific has been illustrated by experiments showing that autologous PMNs were effective intermediaries of hyperacute rejection.

e. Interlocking Relationships. In sensitized recipients it is clear that a transplanted kidney almost immediately becomes a trap for antidonor antibodies, formed blood elements, and clotting factors. The removal of these various substances occurs essentially simultaneously. Nevertheless, it must be assumed that an antigen-antibody reaction induces the clotting process, presumably with the collaboration of PMNS.

f. Therapeutic Possibilities. Although clotting is prominent in the pathogenesis of hyperacute rejection, the use of potent anticoagulants, including heparin and cobra snake venom, have not provided effective prophylaxis. In contrast, the intraarterial infusion of either citrate or ethylenediamine tetracetic acid (EDTA) is of great benefit, apparently secondary to calcium binding. Calcium has an essential role in the clotting process, but it is also vital to the activation of complement. Since citrate and EDTA therapy impose predictably high risks under

the laboratory conditions tested so far, these drugs have not yet been used clinically.

Within the last few months, Kobayashi of Boston has reported some potentially practical experiments in which organ pretreatment was carried out prior to transplantation. Monkey kidneys were perfused with pepsin-digested $F(ab)_2$ fragments made from the serum of an animal specifically sensitized against the organ donor. The noncomplement binding immunoglobulin fragments prevented the subsequent hyperacute rejection of these organs after transplantation to the sensitized original serum donors.

3. *Hyperacute Xenograft Rejection*

In recent years it has been thought, on the basis of indirect evidence, that the violent rejection occurring after xenotransplantation between divergent species was initiated by the action of preformed heterospecific antibodies. Support for the hypothesis included the fact that antidonor antibodies of several kinds were often demonstrable by preoperative *in vitro* testing of the recipient animals' sera, that such antibodies were cleared by organs transplanted from that donor, that the vascularization of successive kidneys from the same donor (or donors of the same species) usually prolonged the function of the last organ, presumably by antibody depletion, and that physiochemical removal of immunoglobulins or the inactivation of complement in the recipient sometimes increased heterograft survival.

It has been of considerable interest to compare the events of hyperacute xenograft rejection to those that abruptly lead by unquestionably immunological mechanisms to the destruction of homografts placed in recipients deliberately sensitized to donor tissue (see Section II,C,2). The observations have been so similar in each circumstance that progress in ameliorating hyperacute rejection would be expected to be applicable to both situations. This prediction has been strikingly fulfilled in that both the citrate and EDTA therapy described in the preceding section can prevent the rejection of porcine-to-canine renal grafts for as long as $\frac{1}{2}$ day. Such kidneys fail within 2–10 minutes if untreated.

III. Physiological Consequences of Classic Rejection

The result of whole-organ rejection in untreated recipients is a rather sudden and progressive decline in the function of a homograft after

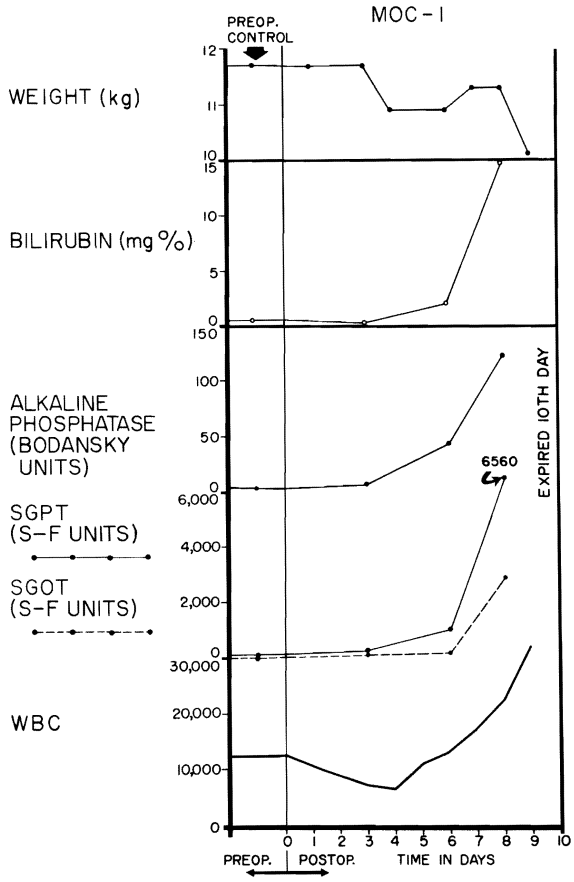

Fig. 6. The biochemical changes usually seen after orthotopic liver transplantation to an untreated recipient. A brief period of good function follows operation, but deterioration of the biochemical pattern is unrelenting once it has begun. (From *Advan. Surg.*, 1966; by permission of Yearbook Publishers, Inc.)

an initial interval of satisfactory performance. These typical events can be seen in the canine experiment summarized in Fig. 6. The recipient dog's own liver was removed and replaced with a homograft from a nonrelated donor. After several days of quite normal postoperative function, progressive jaundice developed. Not long after, astronomical increases in serum transaminase indicated massive necrosis of hepatocytes just before the death of the animal.

Of course, the same sequence of events occurs after transplantation of the kidney, heart, lung, and all other organs, with easily imagined variations according to the function of the organ in question. After ap-

proximately the same delay, host immune defenses are brought to bear upon the homografts. Then the anatomic features of the tissues become distorted, the blood supply is choked off, and rejection usually goes on to completion in which case necrosis is the eventual consequence.

The full evolution of classic whole-organ homograft rejection is essentially always seen in untreated recipients if there is a strong natural histocompatibility barrier as there is, for example, between most (although not all) nonrelated dogs or between nonrelated people. In contrast, rejection may be minor and/or atypical if there is a weak barrier. In outbred animals a special example has been provided in pigs. Professor Henri Garnier of Paris first showed that livers transplanted in pigs did not evoke a very strong reaction despite the fact that no immunosuppression was given. It was promptly confirmed by Peacock and Terblanche in Bristol, Calne of Cambridge, and in our laboratories that clinically evident hepatic rejection sometimes never occurred even when different breeds of pigs were used, or that on other occasions rejection could develop and recede spontaneously. It has been proposed by Calne that these results are due to some special tolerance-inducing substance released by the liver. However, Perper and his associates have shown that the pig is also a very "easy" animal in which to transplant the kidney, since long-term function of renal homografts can be obtained with only a day or two of immunosuppression or even with such minor manipulations as providing a coincident transfusion with donor blood. The significance of the pig model is discussed further in Section V.

IV. Immunosuppression

A. A Theoretical Impasse?

For several years after the features of rejection were defined, the not unreasonable assumption was made that this process was one of nature's most powerful and perservering reactions which could be prevented only by relatively complete crippling of the host's immune defenses. In view of the evident connection between the capacity to mount an effective rejection and to react forcibly against a variety of other inimical environmental antigens, including those of pathogenic microorganisms, the possibility of achieving chronic graft survival without killing the host was seriously questioned.

The first clue that host death was not the requisite penalty for homograft protection came from the observations that permanent acceptance

of adult donor tissue could be induced in fetuses or newborn animals. The initial disclosure was made by Dr. Roy Owen, who noted that dizygotic calf siblings, whose circulation *in utero* communicated freely, could have each others white cells and red cells persist indefinitely after birth. These are known as the freemartin experiments of nature (Fig. 7).

On the basis of Owen's observations, Burnet of Australia suggested that exposure of the fetus to donor tissue might similarly confer protection persisting after birth to subsequent grafts from the same donor but not to those from other donors. The hypothesis was confirmed by Billingham, Brent, and Medawar by injecting adult lymphoid cells into mouse fetuses. The experiments were later extended to other species.

While of no practical clinical value, these remarkable observations were of great theoretical interest. They appeared to be the result of exposing the host to donor antigens at a time when its immune mechanism was too rudimentary to recognize the graft tissue as foreign. After maturation of the immune mechanism, neither the graft nor other tissues from the same donor were identified any longer as alien. The observations indicated the feasibility of inducing acquired tolerance, and thereby

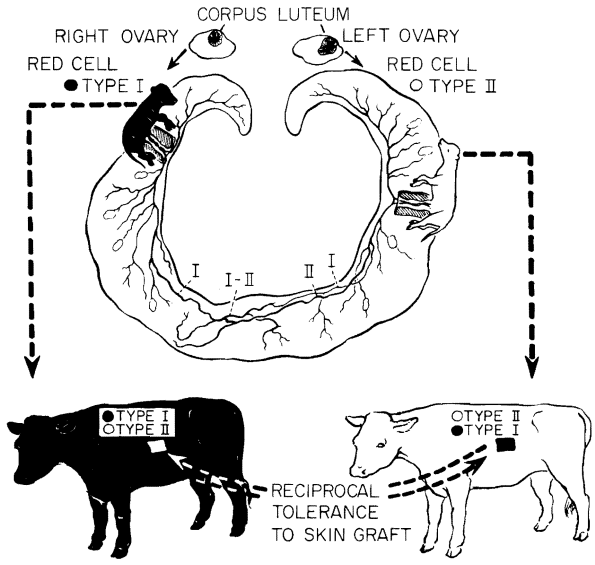

***Fig.* 7.** Chimerism in cattle siblings. There is cross-tolerance to formed blood elements and to subsequently placed homografts as the result of intrauterine circulatory communication. [From *Surg. Clin. N. Amer.* **42**, 55 (1962); by permission of W. B. Saunders Co.]

stimulated the search for immunosuppressive agents with which it was hoped that a similar sequence of events could be duplicated in adult recipients.

Total body irradiation was the first kind of therapy demonstrated to prolong the life of homografts in adult animals. However, the treatment was dangerous, requiring doses sufficient to cause bone marrow depression. There was a consequent acute mortality which was so excessive that clinical organ transplantation proved to have little chance of success from the years 1957 to 1962 during which total body irradiation was given several clinical trials. Nevertheless, there were two patients treated before 1962 who survived more than a decade after renal transplantation under irradiation, one from Boston and the other from Paris. Both received kidneys from fraternal twins.

B. Clinically Important Drugs

1. Azathioprine

A highly significant subsequent advance was the development of azathioprine, a potentially radiomimetic drug with the predominant effect of inhibiting DNA synthesis. With this drug chronic homograft function could often be obtained without the need for doses large enough to cause leukopenia. For the first time, whole-organ grafts could successfully be performed in dogs in a standard laboratory environment in which no extraordinary precautions against infection had been taken. In Fig. 8 is the course of recovery of a dog which had its own liver removed and replaced with that of a nonrelated mongrel donor. Azathioprine was given for only 4 postoperative months. During this time bone marrow depression did not develop, as shown by the normal white blood counts. Liver functions were essentially normal. After 4 months all therapy was stopped. There was no deterioration in hepatic function. The dog is still alive almost 8 years later, not having received any treatment at all for more than $7\frac{1}{2}$ years of this time.

2. Cyclophosphamide

On the basis of laboratory investigations in mice, rats or other rodents, and rabbits, cyclophosphamide has been thought for more than a decade to possess strong immunosuppressive properties. Unfortunately, when cyclophosphamide was tested in the dog kidney or intestinal transplantation models as an intermediate step to clinical application, no prolongation of graft survival was obtained, or else the effect was a minor one.

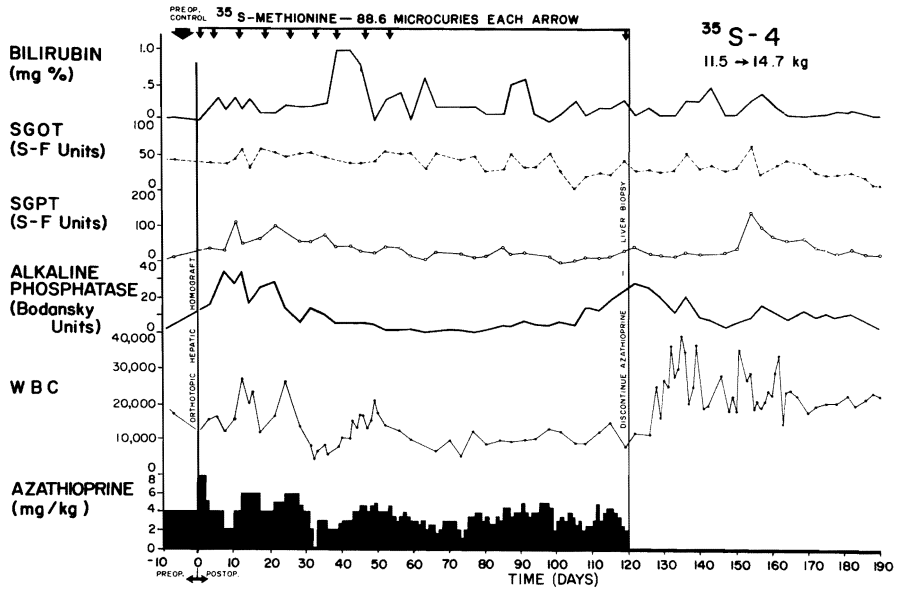

Fig. 8. Course of recovery of a dog after orthotopic homotransplantation of the liver. Overt clinical rejection has never been observed. Liver biopsies after 3, 6½, and 10 months were normal. The operation was performed on March 23, 1964. The animal is in good health more than 8 years later. Note that immunosuppression was discontinued after 4 postoperative months without subsequent deterioration of liver function. Resumption of treatment has never been required. [By permission of *Surgery* 58, 131 (1965).]

It may now be suggested that the dampening influence of the discouraging canine experiments was based upon a species difference that made the dog an inappropriate animal to evaluate cyclophosphamide for human immunosuppression.

Despite the experience in dogs, there has been evidence in man supporting the propriety of testing cyclophosphamide for clinical whole-organ transplantation. Some of this information came from efforts to promote tolerance to bone marrow grafts, as proposed by Dr. George Santos of Baltimore and subsequently carried out by several other workers. Prodigious doses of cyclophosphamide (45–100 mg/kg per day) were given, but only for a few days in close temporal approximation to infusion of the homologous bone marrow. Although such efforts represent an essentially different therapeutic approach than that used with whole-organ transplantation, it is worth emphasizing that Santos' data on several immunosuppressive drugs have indicated that, in man, cyclophosphamide should be a rival to other agents, including azathioprine.

Almost a decade ago cyclophosphamide was given a very brief clinical

trial for renal homotransplantation but was promptly abandoned because of its toxicity. Within the past year cyclophosphamide was reintroduced at our institution as a substitute for azathioprine in a triple-drug combination that also included heterologous antilymphocyte globulin (ALG) and prednisone. More than 100 human recipients of livers, kidneys, and hearts have been treated with this regimen. The conclusion from these studies has been that cyclophosphamide is equivalent to azathioprine as a component of this kind of drug combination.

3. *Adrenal Corticosteroids*

Cortisone, the first major immunosuppressant to be discovered, was described by Billingham, Krohn, and Medawar in 1951 to delay the rejection of first-set skin grafts in rodents. Krohn demonstrated in 1954 that cortisone could partially abolish a preexisting state of delayed hypersensitivity in rabbits. The crucial role of prednisone in the control and reversal of the rejection process has been unequivocally established in cases of clinical whole-organ transplantation under conditions to be described in Section IV,C.

4. *Heterologous Antilymphocyte Serum*

Since 1965, heterologous antilymphocyte serum (ALS) and its globulin derivative (ALG) have received an enormous amount of attention, and since 1966 ALG has been used clinically with increasing frequency. ALS is obtained from animals (such as the horse) previously immunized against the lymphoid tissue of the species that is eventually to be treated (Fig. 9). For example, horses can be inoculated with human lymphocytes obtained from spleens, lymph nodes, thymuses, thoracic duct lymph, or tissue culture. The resulting antibody response of the horse can be measured by determining the ability of the serum to agglutinate or to lyse human white cells *in vitro*. After intensive immunization the equine titers may rise to spectacular heights; antiwhite cell titers of 1:16,000 are not at all unusual.

The serum collected from an immunized animal is a powerful immunosuppressive agent when given by a variety of routes to members of the lymphoid donor species. In dogs it has been possible to give as few as six doses of ALS or ALG to recipients of transplanted kidneys or livers and to have them live for as long as a year without any other kind of treatment.

In patients ALG is usually given intramuscularly in combination with azathioprine (or cyclophosphamide) and prednisone, and its use is limited to the first few postoperative months. By administering ALG

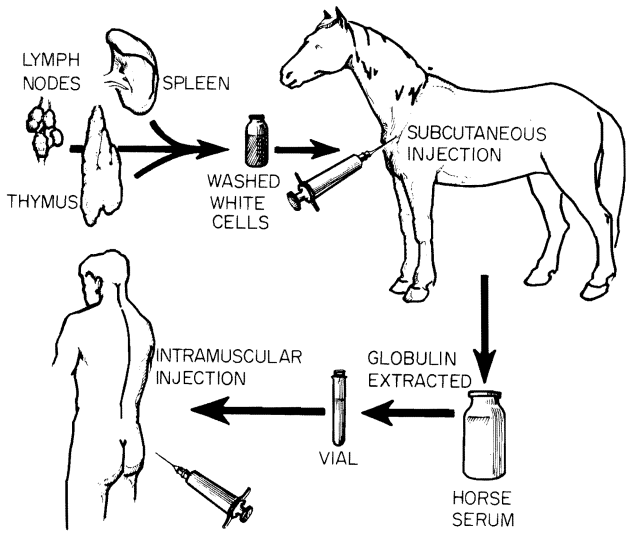

Fig. 9. The preparation in the horse of heterologous antilymphocyte globulin for use in patients. (From "Christopher's Textbook of Surgery," 1968; by permission of W. B. Saunders Co.)

within these guidelines, the risks of foreign protein sensitization and anaphylaxis are minimized.

C. Drug Synergism

In dogs, and probably in humans as well, consistent survival after renal homotransplantation is not obtainable by treating solely with any one of the four immunosuppressive agents described above. With transplantation between nonrelated mongrel dogs, the best results have been with azathioprine or alternatively with ALG. However, even by using one or the other of these agents, it is possible to obtain survival exceeding 100 days in only 15–30% of animals.

Consequently, the clinical application of organ transplantation has been based upon the combined use of immunosuppressive measures. The first combination that was widely exploited was azathioprine plus prednisone, hereafter referred to as the "double-drug" regimen (Fig. 10). In 1966, heterologous ALG was added to make the "triple-drug" regimen (Fig. 11) that has become increasingly widely used. Finally, a triple-drug program in which cyclophosphamide is used in place of azathioprine (Fig. 12) has received an extensive clinical trial during the last year.

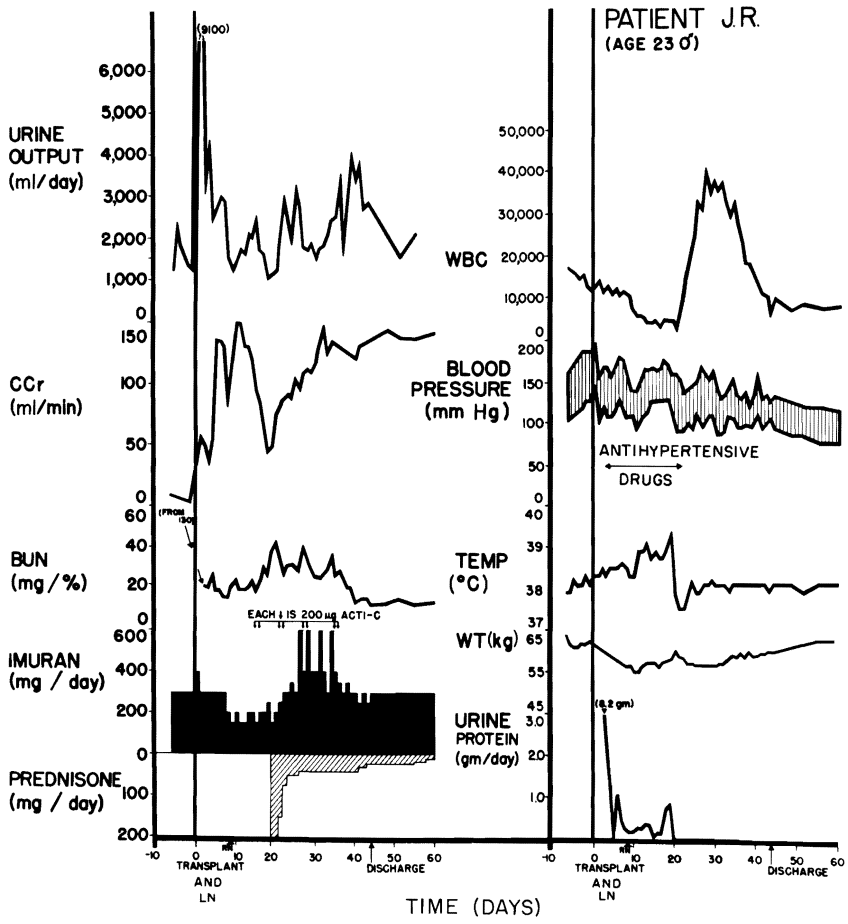

Fig. 10. Classic rejection crisis in a patient treated with azathioprine (Imuran) to which prednisone was added. Deterioration of renal function began 19 days after transplantation. All stigmata of rejection were present except for acute hypertension and weight gain, which were successfully prevented by medical treatment. Acti C, actinomycin C; LN, left nephrectomy at time of transplantation; RN, right nephrectomy. [By permission of *Surg. Gynecol. Obstet.* **117**, 385 (1963).]

V. Changing Host–Graft Relationships

Since each of the major systemic immunosuppressive agents mentioned above can cause general immunological crippling, it has been customary to categorize as nonspecific all the treatment protocols (Figs. 10–12) in which they have been employed. The implied criticism of using a

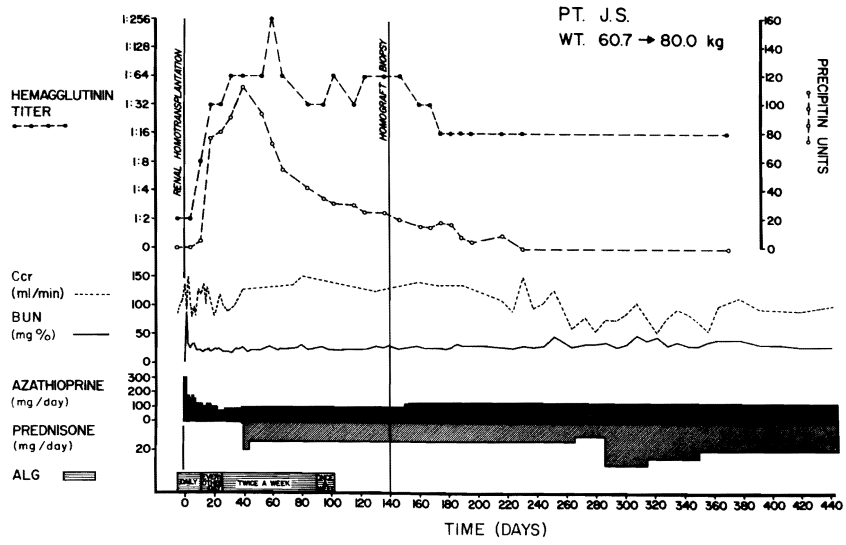

Fig. 11. The postoperative course of a patient who received ALG before and for the first 4 months after renal homotransplantation. The donor was an older brother. There was no early rejection. Prednisone therapy was started 40 days postoperatively because of high rises in the serological titers which indicated a host response against the injected foreign protein and which warned against a possible anaphylactic reaction. Note the insidious onset of late rejection after cessation of globulin therapy. This was treated by increasing the maintenance dose of steroids. [By permission of *Surg., Gynecol. Obstet.* **126,** 1023 (1968).]

sledge hammer where a therapeutic scalpel would be preferable is not without justification.

Nevertheless, there has been for nearly a decade an impressive body of information indicating that whole-organ homotransplantation with such therapy can eventually lead to selective abrogation of the host rejection response, that the success with which this can be done is related among other things to histocompatibility factors, and that the degree to which it is achieved is the most important determinant of prognosis in any given case. Appreciation that the immunological relation of the graft to the host is a fluid rather than a fixed one adds an important dimension to the consideration of any kind of immunosuppression.

A. Rejection and Its Remission

There are two clinically identifiable phases in the chain of events under discussion. The first consists of an attack by the host's immune

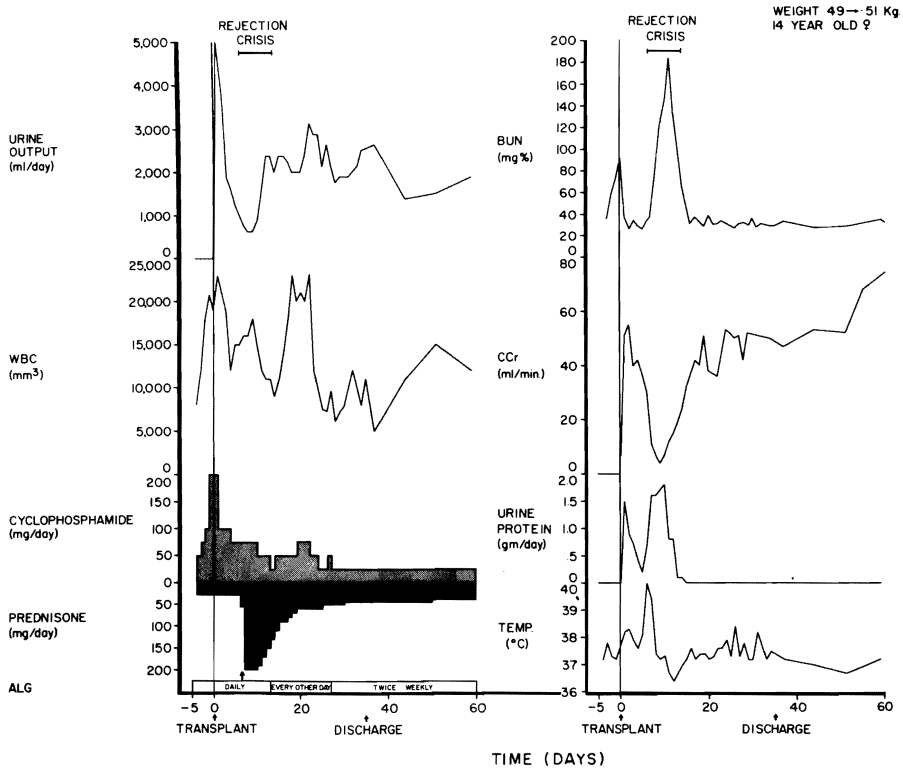

Fig. 12. The first 60 days after the transplantation of a kidney from a mother to her daughter. Although the rejection crisis after 1 week was a severe one, it was easily and completely reversed. Note that leukopenia was never produced by the daily doses of cyclophosphamide which were usually between 0.5 to 1.0 mg/kg per day. ALG, Horse antilymphocyte globulin; BUN, blood urea nitrogen; CCr, creatinine clearance; WBC, white blood cell count; arrow, 625 mg methyl prednisolone intravenously. [By permission of *Surg., Gynecol. Obstet.* 133, 981 (1971).]

defenses upon the new organ, usually within a few days or weeks after its transplantation. The vigor of the process is highly variable, as judged by the magnitude of the changes caused in the morphology and function of the homograft.

Whether severe or mild, the intensity of the acute rejection ultimately tends to abate in the second phase in many cases, particularly if short-term increases in immunosuppression are instituted. However, the forcefulness of the rejection may diminish even without making such changes in therapy, or occasionally in animals that have not received any treatment at all (see Section III).

1. Human Renal Recipients

The remission of rejection was not convincingly demonstrated in animals until it was observed following clinical renal homotransplantation. In retrospect, it is probable that the two earliest successfully treated human recipients of fraternal twin transplants alluded to earlier passed through mild and spontaneously reversible immunological crises after having been submitted to total body irradiation. However, in both the Boston and Paris twin cases, it could be speculated that the long survival may have been partially due to a high-grade although incomplete preexisting tolerance such as that seen in the freemartin cattle siblings that have had a shared placental circulation during gestation (Section IV,A).

Strong indications that rejection was a highly controllable and regularly reversible phenomenon, and that it was often followed by a state of relative "host–graft nonreactivity," came from observations of a number of nontwin recipients who were treated in late 1962 and early 1963. Many of these patients had clear-cut rejections commencing from a few days to several weeks after the operation. The process was regularly reversed by the addition of massive doses of prednisone to the preexisting therapy with azathioprine (Fig. 10). Then, within a surprisingly short time, it became possible to drastically reduce the steroids that initially had been necessary to rescue the grafts (Figs. 10, 12, and 13). In several instances the patients were soon returned to treatment only with azathioprine, the agent that at the beginning had not been capable of preventing an acute rejection crisis (Fig. 13). Many of these patients are still alive 9 or 10 years later.

There is no point in commenting further on the fully accepted fact that kidney rejection can undergo remission beyond noting that such an occurrence is uncommon in dogs and probably also in humans if immunosuppression is not increased. It is also worth mentioning that the central role of steroids in promoting this event in clinical practice was probably predictable on the basis of Krohn's report of 1954, which showed that preexisting graft sensitization could be erased in rabbits with steroids. However, the practical implications of this finding were not appreciated until many years later.

2. Animal Recipients

Although recovery of a rejecting kidney graft cannot usually be expected unless treatment is intensified, it was pointed out earlier that

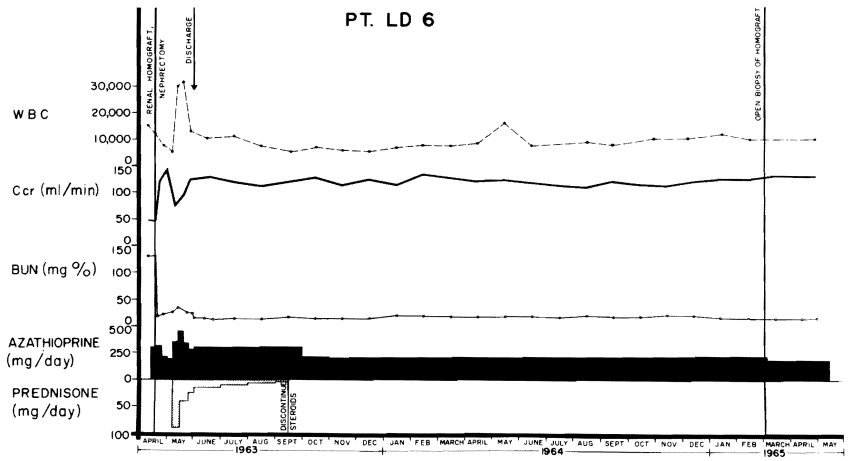

Fig. 13. The first 2 postoperative years of the same patient whose early course is displayed in Fig. 10. Note that steroid therapy was discontinued after 5 months, and that eventually the maintenance azathioprine treatment was about half the daily dosage as that which at the outset did not prevent the onset of a moderately severe rejection. [By permission of *Ann. Surg.* **162,** 749 (1965).]

spontaneous reversal sometimes can occur in both dogs and pigs and particularly with liver transplantation in the latter species. With resolution of the rejection process, abnormalities both in the graft function tests and organ blood flow tend to return toward, although often not completely to, normal.

In both treated and untreated animals, there have been histopathological studies supporting the idea that an initial forceful host attack can subsequently tend to exhaust itself or at best to become less effective. Initially, the homografts become invaded with mononuclear cells, even in some animals not undergoing biochemical and clinical signs of rejection. In surviving animals the infiltrate in various kinds of grafts may decrease in density or disappear. Subsequently, the predominant morphological changes usually become those of repair and/or regeneration.

The foregoing comments should not be construed as suggesting that the desired change in the host–graft relationship is dependent upon an overt rejection crisis. Most of the animal recipients of kidneys or livers in which a more-or-less completely "tolerant" state developed, as defined by the ability to avoid therapy, or to discontinue it, have been those in which diagnosable acute rejection had either been very minor or had not occurred at all. In these experiments the first wave of immunological reaction had apparently been insufficient to cause significant

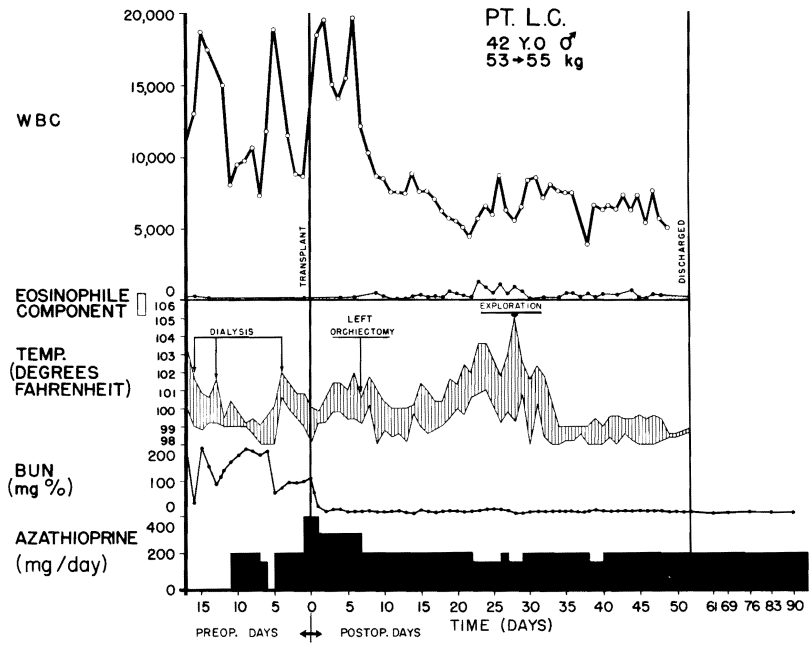

Fig. 14. A patient in whom a fully developed rejection episode did not occur after transplantation from a nontwin sibling donor. Protracted eosinophilia was observed, however, lasting for more than 1 month. In addition, the patient had a febrile crisis for which no explanation could be found. It was at first thought that the fever was due to infarction of the testis which had occurred on the side of transplantation, and later that a deep wound infection might be present. The patient was first subjected to left orchiectomy, and later to wound reexploration. Ultimately, there was defervescence without provision of specific therapy. No drug other than azathioprine was ever employed. Bilateral nephrectomy and splenectomy were performed at the time of transplantation. The operation was on July 5, 1963. Almost $8\frac{1}{2}$ years later, the homograft is still providing perfect function. [By permission of *Surgery* **56**, 296 (1964).]

deterioration of homograft function. There have been well-documented examples of this kind of "subclinical rejection" in patients (Fig. 14).

B. Mechanisms of Graft Acceptance

Although it has been well established that a homograft may come to be more or less tolerated in its new host, the explanation for the privileged status is not accepted with any more unanimity today than it was 5 years ago. One of the reasons probably is that more than one immunological pathway may be involved.

1. Specific Immunological Tolerance

It is almost certain that the continuous presence of a transplanted organ in a host being treated with immunosuppressive therapy often leads to a selective loss of responsiveness to the antigens of the homograft (tolerance). The evidence that chemotherapy can be used for the induction of narrow-range tolerance is unequivocal. The literature on this subject is not reviewed here since it has been well summarized by Dr. Robert Schwartz, who was the first to call attention to this possibility. Suffice it to say that azathioprine, 6-mercaptopurine, amethopterin, cyclophosphamide, and even total body irradiation can be used to promote specific tolerance, providing the antigen in question is administered in an appropriate dose and in close temporal approximation to the immunosuppressive treatment.

One of the theories advanced by Schwartz to explain the specific effect of chemotherapy under these circumstances is depicted in Fig. 15. The illustration suggests that a clone of lymphocytes which presumably have an active metabolism as the result of stimulation by antigen should be differentially susceptible to antimetabolites.

It is interesting how compatible this hypothesis is with the events that actually take place after transplantation. Temporally, the first evidence of adaptation is often coincident with reversal of the rejection crisis. It has been noted that both the reversal of rejection and graft acceptance can occur in most cases without the necessity for even temporary suppression of the total white blood count below normal levels. Here, the peripheral white cells as well as the humoral antibodies with which the graft is in constant contact appear to have ultimately lost at least part of their capacity to injure the foreign tissue. In these cases the ultimate lymphocyte population seemed to be inactive, at least in a relative sense, against the renal antigen. It is tempting to believe that immunosuppressive therapy caused a progressive attrition of those cells that were immunologically sensitized against the homograft antigen and that the replacement cells had an absent or reduced memory of the alien tissue.

The concept of "clone stripping" in this scheme is consistent with the cyclic phenomena that characteristically occur after whole-organ transplantation both in treated animals and man. With the existence of very close biological compatibility between donor and recipient, it could also explain the apparent acceptance of weakly antigenic homografts as has apparently occurred in unmodified pigs. Under either set of circumstances, the sequence might be analogous to that demonstrated

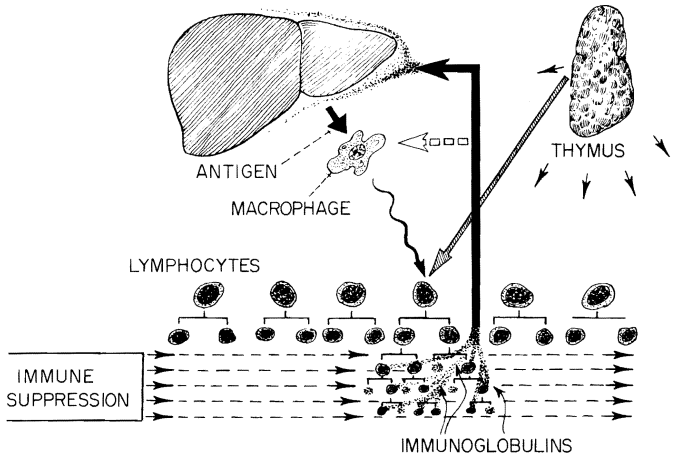

Fig. 15. Hypothetical mechanisms by which nonspecific immunosuppression may lead to selective abrogation of the host immune response. Special susceptibility to these agents of a fraction of the lymphoid population could lead to exhaustion of a clone, hence tolerance. Since maintenance of such cell lines 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 for 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. (From *Experience in Hepatic Transplantation;* by permission of W. B. Saunders Co., 1969.)

by Brent and Gowland in which tolerance in mice was preceded by a transient period of sensitization (Fig. 16).

Nevertheless, there has been a widespread reluctance to believe that specific immunological tolerance has been produced with the immunosuppressive regimens described in Section IV,C after either experimental or clinical whole-organ transplantation. The article most often quoted as contravening this possibility is that of Dr. Joseph Murray and his colleagues, which appeared in a 1964 issue of the *Annals of Surgery,* despite the fact, as the authors took pains to make clear, that the evidence in the report was inconclusive and involved only two canine experiments of a potentially crucial nature. These two dogs had been given renal homografts 9 and 18 months previously and had received long-term therapy with one of the purine analogs. Throughout the postoperative course renal function appeared from the published charts to have been unstable. Moreover, it was deteriorating at the time the other kidneys from the original donors were transplanted; in both instances the blood urea nitrogen had become significantly elevated by the time

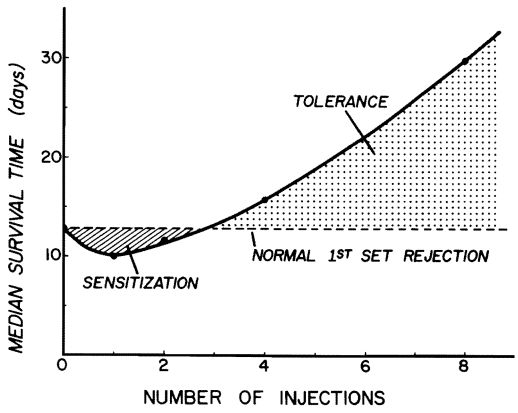

Fig. 16. The experiment of Brent and Gowland showing the induction first of sensitization and then of tolerance in mice treated with donor-specific spleen cells. The survival times of subsequently transplanted skin homografts are plotted against the number of preconditioning injections. Note that a small number of injections sensitized the animals, but that tolerance developed when the treatment was more protracted. The analogy between these findings and those of a reversible acute rejection crisis is evident. [Modified from an illustration in *Nature* (*London*) **196**, 1298 (1962).]

of the retransplantations. The second organs were rejected after 23 and 3 days, respectively. It could be persuasively argued that there was not good justification to believe that the first kidneys in these animals were well tolerated, in which case there would be little reason to anticipate that the second organs would be accepted kindly.

To date, few investigations have been carried out in human recipients of renal homografts surviving for prolonged periods to establish the presence or absence of specific immunological tolerance to their donor tissue. One of the reasons has been the potential risk that could attend some of the testing that might be done, such as skin transplantation from the original renal donor. However, it is worth mentioning that Drs. Fritz and Marilyn Bach of Madison, Wisconsin, performed mixed lymphocyte culture examinations from the peripheral blood of a group of our recipients and their donors, 2 to 4 years after transplantation, as well as upon some donor-recipient pairs of Dr. John Najarian's Minnesota series. In several cases in which clear donor-recipient histoincompatibilities were detectable by serological typing, the Bachs found that the recipient lymphocytes no longer underwent blast transformation when exposed to killed donor white cells, although they reacted vigorously to third-party lymphocytes. The findings were interpreted as indicating specific acquired immunological tolerance.

2. Enhancement

Many years ago it was shown by Kaliss that homografts of tumor tissue can be protected by the presence of certain kinds of antigraft antibodies. It is conceivable by a feedback mechanism that the same thing occurs under the conditions of whole-organ transplantation. The process could be envisioned as shown in Fig. 15, whereby immunoglobulins synthesized by the activated clone return to the target tissue and coat or protect it in some other way.

Certainly, it has been possible to demonstrate antigraft antibodies in the sera of patients carrying chronically functioning and apparently well-tolerated kidneys. Using an antiglobulin consumption test, Dr. Yoji Iwasaki of Colorado and also Dr. Felix Rapaport of New York showed that the serum of most of the tested human recipients contained 7 S immunoglobulins which could be selectively absorbed by the nucleated cells of the original donor. Moreover, there have been numerous reports of more-or-less extensive immunoglobulin deposition, as detected by immunofluorescence techniques, in long-functioning human kidney transplants. By and large, however, the latter finding does not connote a favorable prognostic sign but rather the converse.

The mechanism of enhancement may explain the kind of observation originally made by Woodruff and Woodruff and termed by them "adaptation." In their experiments with guinea pigs, bits of homograft tissue were implanted in the anterior chamber of the eye and later transferred to a subcutaneous location. There rejection occurred very slowly or not at all. In contrast, subcutaneously placed thyroid from the same donor was repudiated promptly. Some of Murray's experiments with canine renal transplantation were designed along similar lines and yielded comparable results. He showed that a secondarily placed kidney could undergo rejection while the contralateral organ that had been transplanted earlier from the same donor continued to function. The differential ability of the first kidney to resist destruction was not due to a change in its genetic character since it could also function when replaced in the original donor. The same general observations had been made in 1954 by Weber, Cannon, and Longmire, who used skin grafts in chickens.

Recently, Hellström and Hellström of Seattle have published with Pierce and Marchioro some observations that may make the term "enhancement" somewhat less mystical than it has been in the past. They showed that the sera of patients with well-accepted renal homografts contain antibodies capable of "blocking" the cytotoxic action of recipient

lymphocytes upon donor tissues. Further characterization of these blocking antibodies and their biological signficance is one of the most active areas of applied immunological research.

It is not necessary to believe that enhancement must exert its influence in an isolated way, as the recent studies of Frank Stuart of Chicago have made clear. He showed that the survival of renal homografts in rats could be more consistently obtained with the combination of tolerance induction plus the administration of enhancing antibodies than when either approach was used alone.

3. *Failure of Antigen Processing*

There is the added possibility that a defect in antigen processing by the reticuloendothelial system could be responsible for graft acceptance, a concept for which there is not yet any firm evidence. However, it is known that antisera can under certain circumstances markedly and specifically inhibit the responsiveness to the antigen recognition for long periods. The way in which an analogous sequence of events could be hypothetically injected into the picture after organ transplantation is shown in Fig. 15.

VI. Graft Pretreatment

Many of the problems of organ transplantation could be minimized if it were possible to mitigate graft rejection by modifying the transplanted tissue rather than the host immunological response. Efforts to achieve this objective have been unsuccessful with occasional possible exceptions.

A. The Removal of Passenger Lymphocytes

In 1957, Dr. George Snell suggested that donor leukocytes in grafts might play a significant role in eliciting an immune response from the recipient. Within the last 2 years, Dr. David Steinmuller and his associates at the University of Utah have proved that Snell was right. They showed that removal by one means or other of the lymphoid tissue in grafts apparently reduces the antigenicity and thereby considerably pro-

longs survival. Forms of donor or graft pretreatment that have been shown to be effective include irradiation or the administration of cyclophosphamide or ALS.

So far, there have not been efforts to exploit this therapeutic approach clinically. However, if the concept is sound, efficient removal of passenger leukocytes is certain to have important clinical implications.

B. RNA Perfusion

Some of the most intriguing experiments with organ pretreatment were described by Jolley, Hinshaw, and Peterson of Loma Linda, California. They reported that rabbit skin grafts first immersed in homologous ribonucleic acid (RNA) and then transplanted to recipient animals which were given intravenous RNA survived four times longer than controls. The role of the preliminary soaking was not analyzable in these experiments, but these investigators also reported that human skin homografts subjected to RNA soaking only had unusually protracted viability when placed upon patients with burns. Similar findings have been reported in mice.

Attempts to "pretreat" whole-organ homografts have been made in dogs by Dr. Carl Groth and his associates. They perfused kidneys for about 30 minutes with RNA prepared by phenol extraction from the spleens of the prospective recipients ("autologous" RNA) or other dogs ("homologous" RNA). After transplantation to unmodified recipients, about one-fourth of these life-sustaining organs had prolonged homograft viability. Maximum survival of recipients subjected to simultaneous removal of their own kidneys was more than 4 months. The mean survival in a group of 40 recipients was more than 20 days, as opposed to approximately 10 days in 30 control animals. Furthermore, there were 7 homografts of the 40 that had no histological evidence of rejection, whereas all the control homografts had the typical findings of unmodified rejection.

It is not yet certain that the foregoing findings represented more than an experimental artifact, since a logical explanation for the surprising results was not available and because the degree of homograft protection was so relatively limited that its statistical significance in terms of survival was marginal. It will be of interest in laboratory experiments first to confirm these observations and then to determine whether or not this kind of graft conditioning can be advantageously combined with effective host immunosuppression.

C. Exposure to Enhancing Antibody

As described earlier (Section II,C,2), the serum of an animal or patient sensitized to a given donor can precipitate the hyperacute rejection of a kidney from that donor. If the responsible antidonor antibodies are digested with pepsin, they may still attach to the antigen, but such altered immunoglobulins do not bind complement and are no longer injurious. Richard Wilson of Boston and several other investigators have been interested in the perfusion of homografts with digested cytotoxic antibody in the hope that the digested $F(ab)_2$ fragments may lead to "local" enhancement. In view of the potential harm that could result in the event of an immunological accident, very complete experimental evaluation will be required before this approach can be tried clinically.

VII. Histocompatibility Typing in Patients

In Chapter 7 is found a description of what has been thought to be the major human histocompatibility system (HL-A). The antigens of this system have been measured in laboratories from one end of the earth to the other. The reagents used for typing have been lymphocytotoxin-rich human isoimmune antisera obtained from persons who have been sensitized accidentally or deliberately to white cell antigens.

The cytolysis of test lymphocytes by such antisera indicates the presence of the same or a similar antigen as that which originally sensitized the serum donor. Failure of such a reaction implies the absence of the antigen. When the lymphocytes of both the donor and recipient react in the same way to a given antiserum, *identity* of that antigen is said to be present. The absence of an antigen in a donor that is present in a recipient is defined as *compatibility.* When an antigen is found in the donor lymphocytes but not in those of the recipient, a *mismatch* exists. Identity of antigens is preferable, compatibility is the next most satisfactory condition, and the least desirable is an overt mismatch.

The present view of the HL-A system is that there are two major loci on the same chromosome and that each locus has two antigens (alternating allele hypothesis). The possibility of a perfect match of all four antigens between randomized members of the nonrelated population is statistically rather remote, substantially less in most cases than

the chance of having all these antigens mismatched. It can be appreciated from these considerations that major logistical problems would have to be dealt with if a well-matched cadaveric organ were to be sought for a given recipient, since the chance of obtaining a four-antigen or "full house" match would ordinarily be less than 1%.

The foregoing statistical factor would be a deterrent enough to the hope of regional or national tissue-matching and organ-sharing schemes. Even more serious is the fact that correlations between the quality of HL-A tissue matching and the outcome after renal, hepatic, and cardiac transplantation have so far not been significant in large and well-studied series such as our own and those of Murray (Boston), Hamburger (Paris), Hume (Richmond), Najarian (Minneapolis), and Kountz (San Francisco).

In our clinics HL-A typing has been carried out in all cases since 1964. The clinical and histopathological observations were then compared with the widely used A–E, F-antigen match classification popularized by Dr. Paul Terasaki of Los Angeles. With this system the letters are a statement in code about the compatibility, incompatibility, or identity of the HL-A antigens.

Following intersibling transplantation it is encouraging that histocompatibility matching by this method correlates at least partially with the outcome. Specifically, the designation of an A (good) match endows a slight advantage in terms of survival and quality of homograft function, as well as a highly significant advantage in terms of the histopathological appearance of the kidneys at varying times postoperatively. In practical fact, the designation of an A match in sibling cases usually is an indication that the donor and the recipient both have the same two histocompatibility haplotypes, one from each parent, and therefore have achieved identity of the HL-A antigens; for the HL-A chromosome it could be said that there is *genotype* as well as *phenotype* identity (see Chapter 7).

In contrast, significant clinical or histopathological correlations could not be demonstrated between the phenotype grades and the results with the parent-to-offspring transplantations. With this familial combination half of the HL-A antigens of the donor (one haplotype) are by definition identical to those in the recipient. The remaining 50% of HL-A antigens are in essence unrelated. It is the unrelated half of the donor-recipient genetic mass that finds expression in the A–E score.

In the parental cases there could be several explanations for the failure to find significant relationships between the alphabetical grades and survival, function, or histopathology. The possibility that HL-A antigens are irrelevant to histocompatibility cannot be seriously entertained in

view of the evidence to the contrary cited above in connection with sibling cases and in view of supporting evidence from other kinds of studies. A second possibility is that "immunological artifact" caused by the transmission of preexisting host glomerulonephritis to the transplants is responsible (see Section II,B). However, in our case material the latter factor alone could not explain all or even the majority of the poor correlations. It could also reasonably be speculated that: (1) the completeness and/or accuracy with which HL-A phenotypes currently can be measured is substantially less than is generally realized; (2) significant and presently undetected histocompatibility loci exist on other than HL-A chromosomes; (3) variable host immunological reactivity in different patients is comparable in importance to the antigen match in determining the outcome; or, (4) host presensitization to antigens present in the homografts jeopardizes the outcome in some instances but is not always recognized as a factor.

Whatever the explanation, the experience with the parental cases reaffirms a finding first noted several years ago. In many of these same patients, as well as in mismatched siblings, it was learned that immunosuppressive treatment could very often override significant or even apparently ominous incompatibilities, with subsequent renal grafts surviving for many years. The durability of related but mismatched kidneys has become even more evident with the longer follow-ups, now as long as 9 years. As a consequence, our policy continues to be to accept parental donors despite the fact that they may be badly matched. Moreover, siblings and other relatives are not arbitrarily excluded from giving kidneys simply because of antigen incompatibilities, although an effort is usually made in these circumstances to determine if the donor and recipient share one HL-A haplotype and are therefore genetically similar to a parent-offspring combination. If only one donor is available, the only absolute immunological contraindication to intrafamilial transplantation in our center is the demonstration of preformed antidonor humoral antibodies.

Since the A–E grades fail to conform to any identifiable spectrum of outcome after the parent-to-offspring transplantations, it is hardly surprising to find the same lack of correlation within the much less fortunate group of patients who receive nonrelated kidneys and who tend to die or lose their transplants at a higher rate both early and late after operation. Reducing the risk in this kind of case has become one of the great challenges of applied immunology. It remains to be seen whether or not more assiduous application of serological tissue-typing techniques will be instrumental in improving the outlook for the nonrelated recipient. If so, it now seems certain, in view of the negative

results thus far obtained, that improved methods of interpretation must be evolved or else that nearly perfect matching of a truly complete set of antigens will be required.

Before concluding these necessarily brief comments about tissue typing, it should be emphasized that methods of matching are available other than the serological ones just discussed. It is probable that a direct cross-match technique, such as the mixed lymphocyte culture method of Bach and Hirschhorn, would be much more discriminating. However, the Bach–Hirschhorn 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 progress in long-range organ preservation 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.

VIII. Is Clinical Organ Transplantation Practical?

It is not the purpose of this article to present clinical data. Nevertheless, a brief statement is in order about what has been achieved so far. The modern era of whole-organ transplantation began in late 1962 and early 1963, from which era there are still about two dozen patients living after having had continuous subsequent function of their transplants. Since this time, thousands of patients have benefitted from renal homotransplantation and have thereafter undergone relatively complete social and vocational rehabilitation. This has been particularly true in recipients of consanguineous grafts, who now can expect to survive the first post-transplantation year at the rate of approximately 90%. It is less true of recipients of unrelated (cadaveric) transplants in whom only 60 or 70% of grafts function for as long as a year.

In successfully treated patients there has not been the theoretical impasse referred to in Section IV,A, namely, having an unacceptable susceptibility to infection. These patients have not required a controlled bacteriological environment after discharge from the hospital. All that is necessary is that they seek prompt medical attention if they develop any kind of infectious disease. Then they can usually be treated successfully with antibiotics or other standard measures. The reason why this kind of happy outcome is so often possible is that complete host crippling is not required to achieve "graft acceptance," as was explained in Section

V. Thus although there is always an increased rise of infection, this is not so grave as to vitiate the value of the procedures.

The other highly identifiable risk of chronic immunosuppression is an increased incidence of *de novo* malignancy. From the premises of Burnet and Thomas regarding the immunological control of malignancy (*surveillance hypothesis*) it could have been predicted, and was, that an increased incidence of *de novo* tumors would develop in people with naturally occurring immunological deficiency diseases or in patients whose immune reactivity was deliberately depressed in order to permit their acceptance of organ homografts. The hazard of malignancy consequent to spontaneous immunological deficiency is so well known from Dr. Robert Good's surveys that it is not reviewed here.

Analogous data in iatrogenically immunosuppressed transplant recipients was not publicized until the spring of 1968. Since then, more than 60 examples of new malignancy in transplant recipients have been recorded in an informal registry maintained at the University of Colorado. More than one-third of these neoplasms have been of the lymphoreticular system. The main practical consequence of this information is that chronically immunosuppressed patients should be watched closely for any evidence of new growth. Early diagnosis is especially important since many recipients with this complication have now been cured with standard means of treatment including excision, irradiation, and even chemotherapy—especially if this therapy is combined with a lightening of immunosuppression.

Transplantation of organs other than the kidney has not yet become comparably practical in spite of the fact that the feasibility stage of liver, cardiac, and lung transplantation has already been passed. There are several reasons for the high failure rate after transplantation of the extrarenal organs, but for the most part these reasons are nonimmunological. They include greater technical difficulties; the lack of artificial organs comparable to renal dialysis which could tide the hepatic, cardiac, or pulmonary patient over transient periods of poor function; and even the lack of discriminating techniques to diagnose rejection in its early and most reversible phases.

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