Analysis of Mechanism of Immunosuppressive Drugs in Renal Homotransplantation *

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ALTHOUGH survival of kidney homografts can be prolonged by immunosuppressive drugs [6, 12, 15, 17, 18, 24] their mode of action is not clear. Chemical blockades by individual drugs at specific sites may be postulated [1] but correlation of these reactions with the biological effect cannot be assumed. Theoretically, the drugs classified as antimetabolites compete with naturally occurring purines or pyrimidines in the synthesis of nucleic acids thus producing abnormal antibodies, the antibiotic Actinomycin blocks DNA control over messenger RNA synthesis, and the corticosteroids act at a later point in the immune reaction aborting the antigen-antibody union and destroying lymphocytes.[10]

The initial impression that these drugs make the host an immunological cripple has proven incorrect; nevertheless, some drug treated hosts, especially those receiving combinations of drugs, do develop adverse side effects such as wasting and hyperpyrexia. As greater numbers of patients and animals survive on prolonged drug therapy many previously undetected phenomena are appearing in both the host and the graft.

A long term laboratory study was started four years ago to find more effective drugs with less adverse side effects. In the analysis of these experiments unexpected findings appeared which raised provocative questions regarding the nature of the drug induced survival of homologous kidneys. In a group of long term survivors treated according to many different protocols the following 19 questions were posed:

1. Do the drugs completely suppress the host immune response?
2. Can the drugs be stopped and still have the kidney survive?

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3. Is the long survival of a kidney homograft due to adaptation of graft to host?

4. Does a long surviving kidney graft lose its antigenicity?

5. Is prolonged survival due to immune paralysis produced by the continual release of donor antigen?

6. Is the response to drug therapy determined by the absorption or metabolism of the drugs?

7. Do the drugs prevent sensitization of the host?

8. Does drug induced immunological tolerance apply to all tissues of the donor?

9. Does drug induced immunological tolerance apply to all *renal* tissue of the donor?

10. Can drug therapy inhibit a second-set response?

11. Can a second homograft ever be made to survive?

12. Is the tolerant dog off drugs still sensitized against the kidney donor?

13. Is a dog successfully withdrawn from drugs still immunologically competent?

14. Can skin grafts be protected by drug therapy as successfully as kidney grafts?

15. Does simultaneous placement of skin and kidney grafts affect survival of either?

16. Does the rejection of a skin homograft affect an established kidney graft?

17. Can a kidney homograft regain good renal function after successful treatment of a rejection crisis?

18. Are drug treated dogs fertile?

19. Can a rejection process initiate a generalized immunological disease pattern in the host?

This report describes experiments which attempt to answer these questions.

Material and Methods

The bilaterally nephrectomized dog with an iliac renal homograft is the basic experimental test model. The operative technic and the care of the animals have been described.[1, 7, 14] The experimental design was altered when indicated in the following aspects: in some instances the second, third or fourth kidney transplant in the same recipient was placed in the neck for ease of observation and biopsy; in some animals two kidneys were transplanted from one or from two donors; and in some others one host kidney was left in situ for a short test period. In one series the donor animals were kept alive for as long as the recipients survived.

Drug therapy was started on the day of the transplant in all instances. Over 1,000 individual kidney transplant experiences have been completed with 24 basic drug regimens analyzed.[1, 7, 14] All skin homografts were full thickness, measured 5×4 cm and were placed on the thoracic wall. Initiation of rejection was indicated by ulceration of a previously well healed hair-bearing graft; rejection was termed complete when all skin remnants had disappeared.

Results

Two experiments described in detail (Fig. 1, 2) serve as examples of the methods used to follow each animal experiment. Note that the drugs are started on the day of the bilateral nephrectomy and renal homotransplant. In Figure 1 a rejection crisis on day 20, indicated by a rise in the blood urea nitrogen level, followed diminution and cessation of the Imuran therapy. This crisis was reversed with cortisone. A second rejection crisis on day 330 indicated by an increase in the BUN and proteinuria was reversed with Actinomycin C. Note that the total white cell count rose to a high level after an initial depression. The hematocrit remained normal throughout. The gradual rise in BUN and fall in urine specific gravity after day 365 probably indicate a chronic rejection process. Note also that this animal rejected a donor skin

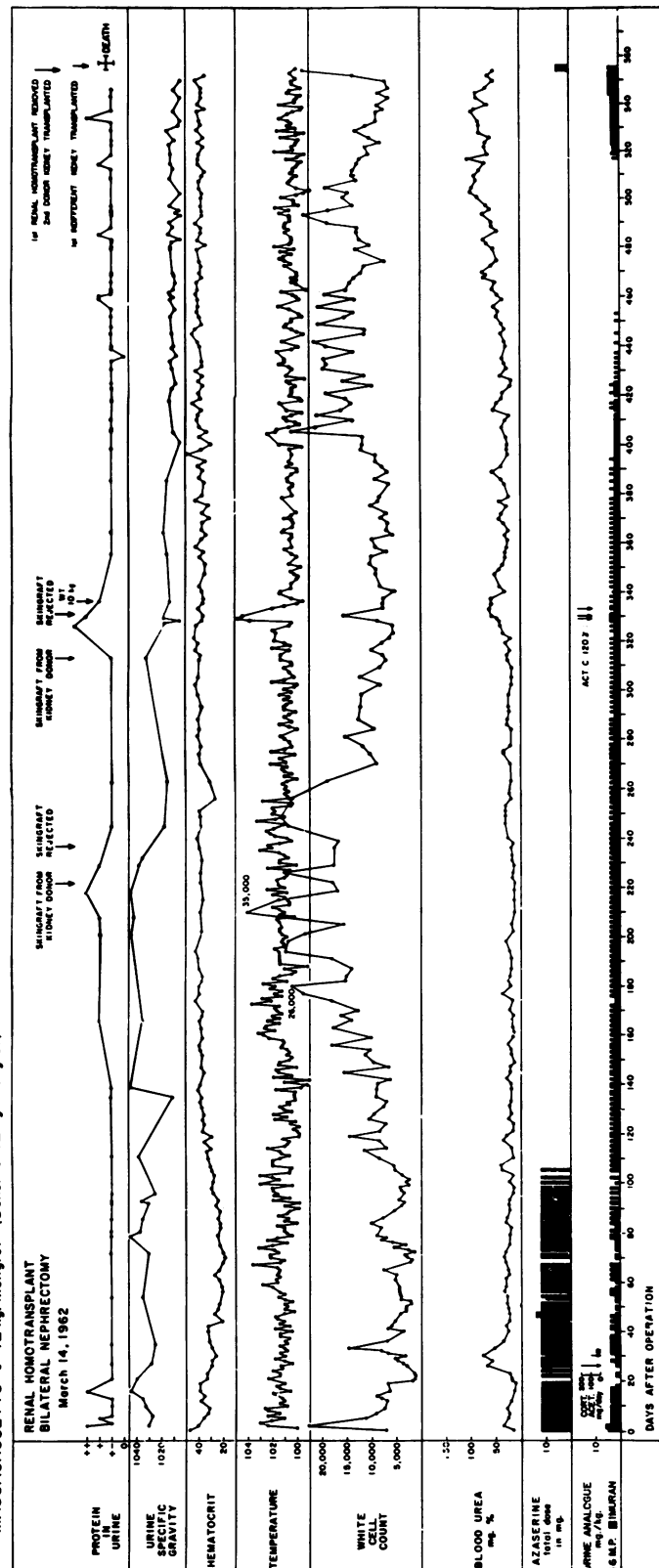


FIG. 1. A detailed clinical course of one long surviving bilaterally nephrectomized dog with a homotransplanted kidney. The clinical parameters are plotted against time and are discussed in the text. This serves as an example of the detailed study involved in each animal.

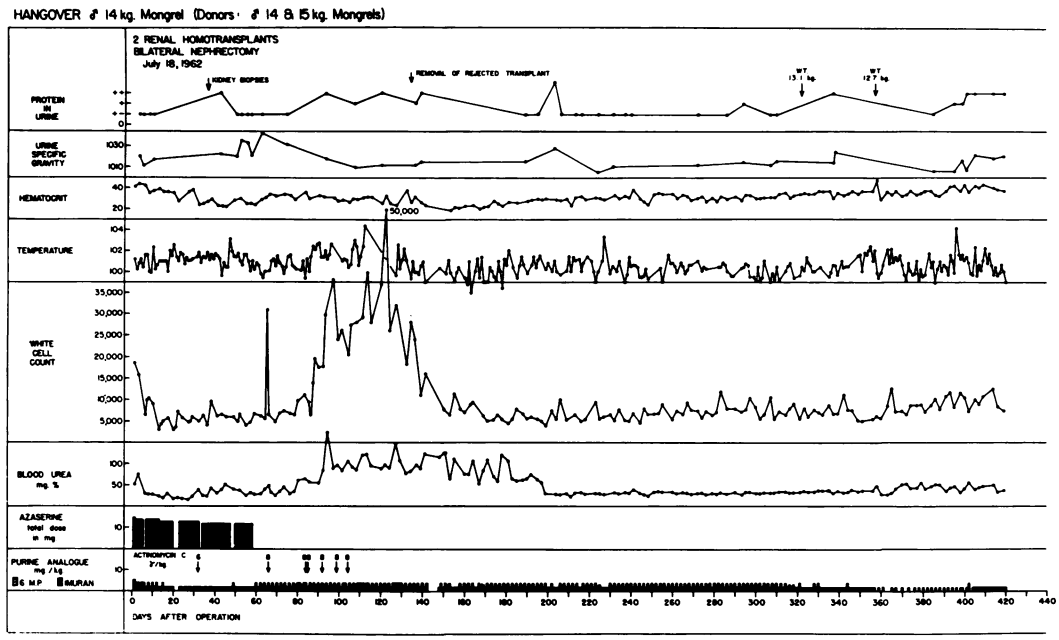


FIG. 2. Another detailed chart of a long surviving kidney homograft in a drug treated host. The transplantation of two kidneys, each from separate donors, constitutes a variation in the basic protocol. Note that one rejected homograft was removed on day 134 while the animal survives on the other. The rejection is reflected in the fever and leucocytosis and BUN rise at days 90 to 120.

homograft at day 222 in 16 days without any apparent deleterious effect on renal function; yet the second skin graft from the same donor placed at day 315 was rejected in 15 days with an increase in BUN and proteinuria. Note also that this animal was never successfully withdrawn from drugs. An attempt at drug withdrawal from days 455 to 515 led to a rising BUN which was not reversed by resumption of therapy. This animal rejected the second kidney in an accelerated fashion, as will be described below in more detail.

Figure 2 illustrates slight variations in the protocol. This animal received two kidneys from separate donors on the day of his bilateral nephrectomy. Imuran and Azaserine therapy was started on the day of the transplant. Kidney biopsies on day 34 had revealed that one kidney was more swollen and further along its rejection pattern than the other. A rising white count and blood urea level at day 80 indicated rejection of at least one of the transplanted kidneys. The peak white count reached 50,000 at the height of this rejection crisis. By day 90 this kidney had been rejected and was removed on day 134. Following this the temperature fell to normal, the BUN gradually fell to normal, and the animal has maintained a normal renal function ever since on the one transplanted kidney. On day 420 all therapy was stopped and this animal is surviving today with good renal function of his remaining homograft.

Answers to the individual questions as suggested by the following experimental observations are presented sequentially at this time and constitute the body of this report. The subsequent clinical charts are abridged to clarify the question under study. Some animals are presented more than once because their course illustrates different mechanisms of drug actions. Some of these experiments have been described in part previously but are included now as part of the completed study.

1. The animals on drug therapy are not

immunological cripples. Some withstand ordinary kennel infections of severe degree. One healed a saucerized mandibular defect secondary to an osteomyelitis. Wound abscesses healed. The 20 animals tested have rejected skin homografts regardless of the source. Fourteen well established kidney homografts which had been biopsied all showed some evidence of immunological rejection. Figure 3 illustrates the rejection of three successive skin homografts from the kidney donor. The grafts survived for 21, 30 and 24 days, respectively, indicating indubitable immunological capacity on the part of the host while still on drug therapy. In addition, biopsy of the kidney on day 176 reveals many foci of mononuclear cellular infiltrate (Fig. 4b).

2. Five animals with well functioning renal homografts have had an attempt to withdraw drug therapy after several months. One animal after six months rejected following cessation of drug.[7] Another, (Fig. 1) was kept off drugs for two months and then required re-institution of therapy because of decreasing renal function. Another animal surviving more than two years still requires drug treatment despite efforts to withdraw it. However, two animals have been successfully withdrawn from all therapy after 14 months without any evidence of rejection. One of these survives 16 months later and still has normal renal function (Fig. 4a). Biopsy 12 months following cessation of drugs reveals many areas with normal

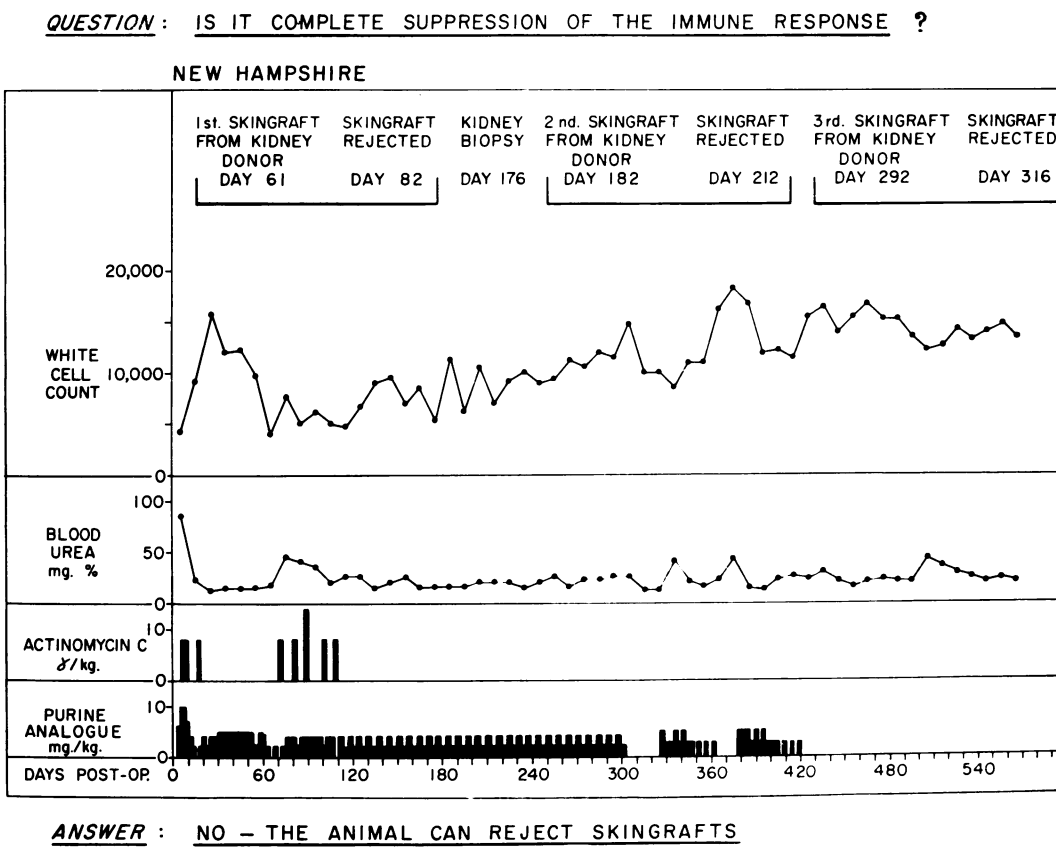


FIG. 3. The abridged clinical course of a dog on drugs over 420 days which has rejected three successive skin homografts from the kidney donor while the kidney homograft still has normal function.

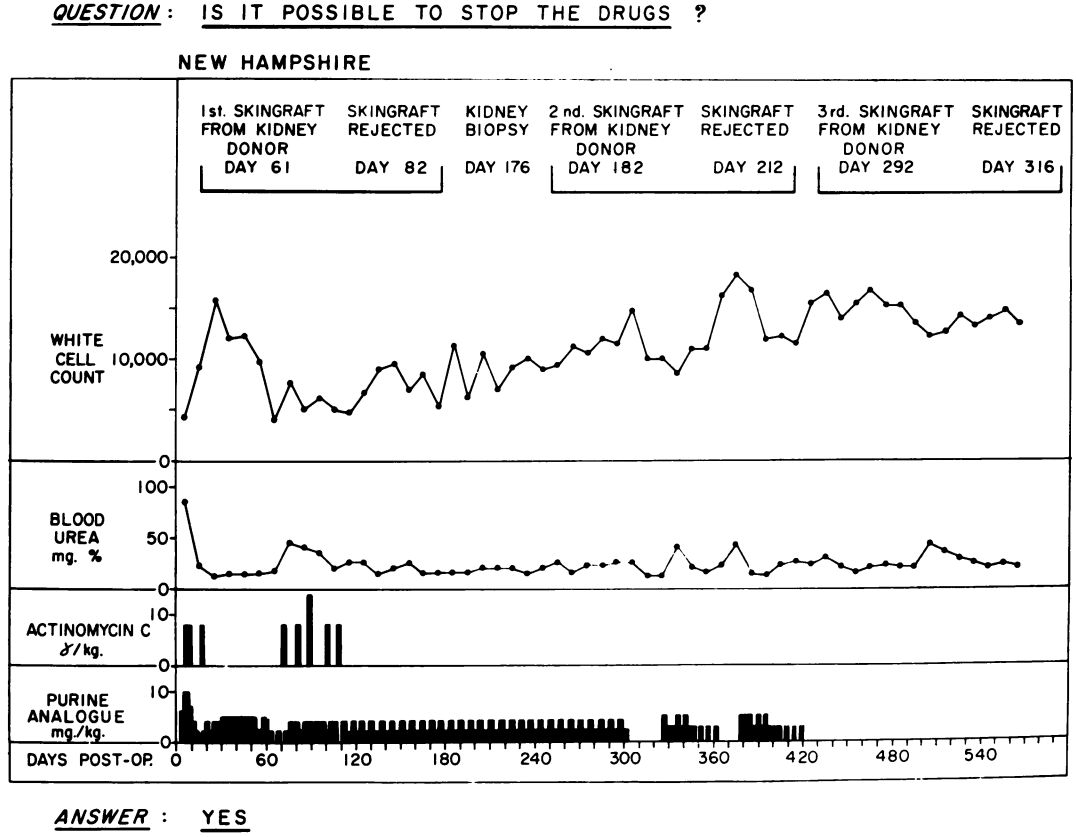


FIG. 4a. Same dog as Figure 3. This illustrates ability to stop drugs after 14 months. This animal is still alive 16 months later with normal renal function.

renal architecture and a few areas of cellular infiltrate and fibrosis (Fig. 4c). There is neither clinical nor microscopic evidence that drug withdrawal has led to any impairment of renal function in this animal.

3. To test the possibility that these long surviving grafts have changed antigenically, retransplantation of the graft from the homologous host back to the original donor was performed in two animals after 296 and 554 days, respectively. Figure 5 is an abridged chart of one of these animals. Note the same level of renal function as indicated by the BUN following return to the original host on day 554. In this instance, and in the 296 day transplant (Fig. 6a, b) the kidney survived in the original donor at the same level of renal function; neither kidney was functioning normally before transplantation, nor did improvement in function occur later in what must presumably have been a more favorable environment. These retransplantation observations indicate that host antigenicity was not incorporated in the graft during this long period of residence and make it unlikely that any antigenic alteration of the donor tissue can be achieved by perfusion or by contact with a homologous host. Explanation why these kidneys are tolerated

FIG. 4c. Same kidney as in 4b at 26 months, 12 months after all immunosuppressive therapy had been discontinued. Large areas consist of apparently normal parenchyma but in the area of dense cellular infiltration parenchymal elements are lacking and there is evidence of focal, slowly progressing, rejection.

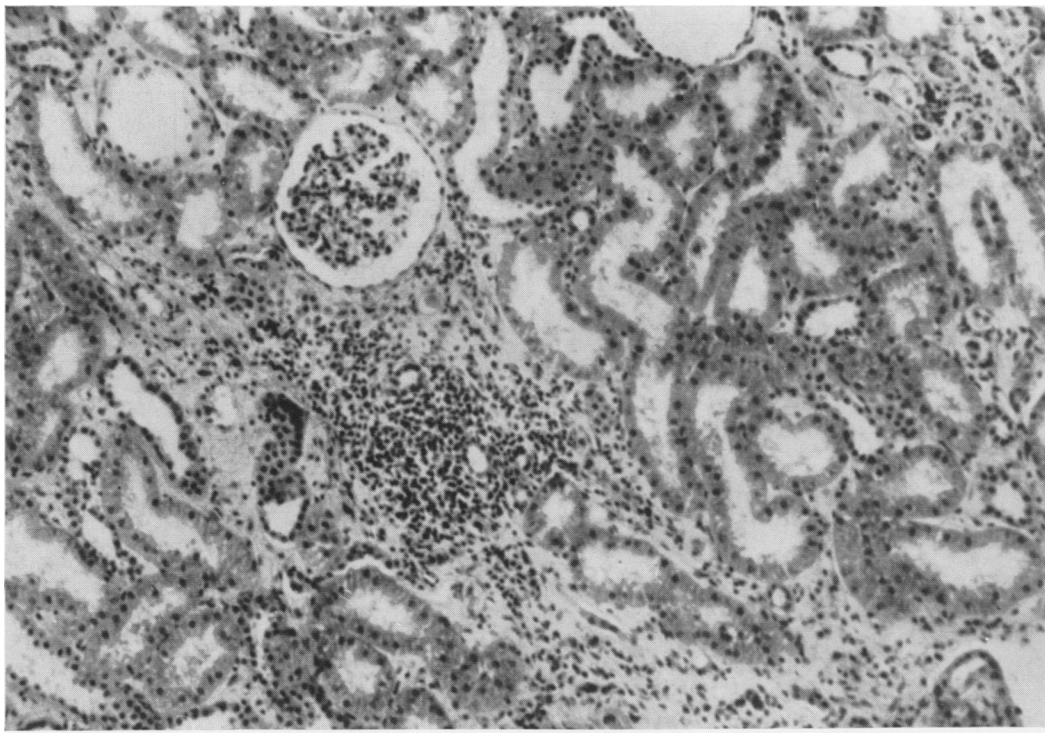
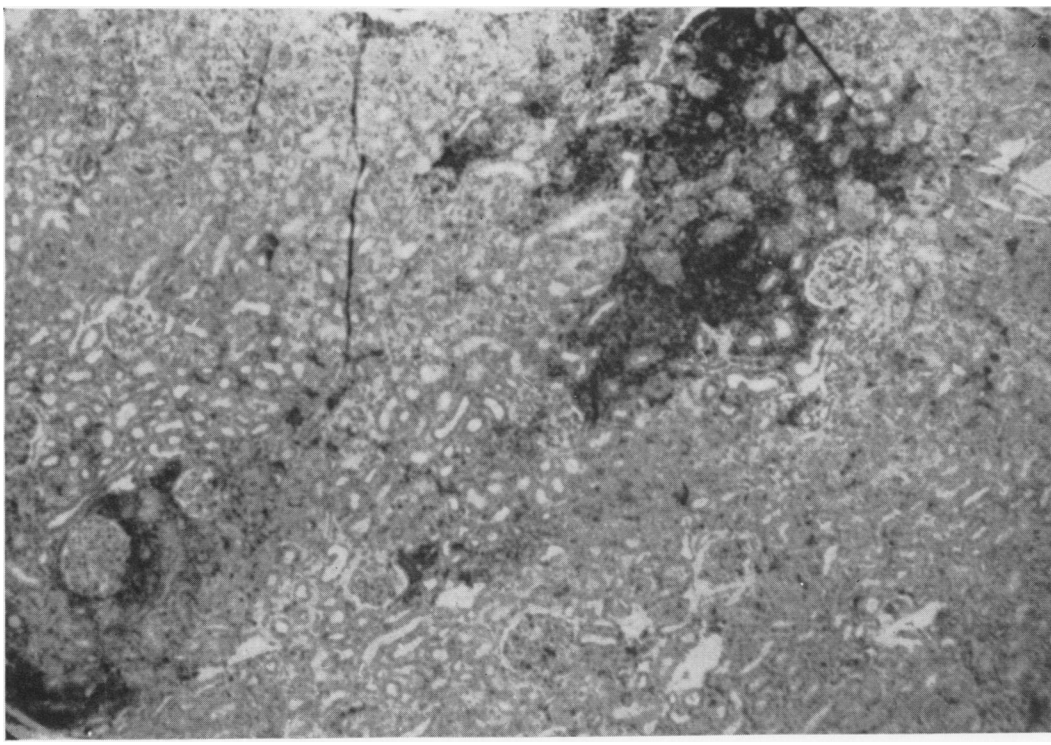


FIG. 4b. Microscopic pattern in the renal homograft on the 176th post-transplant day. Parenchymal elements present appear normal except in the small area where there is prominence of the interstitial tissue with a collection of mononuclear cells, predominantly lymphocytes.



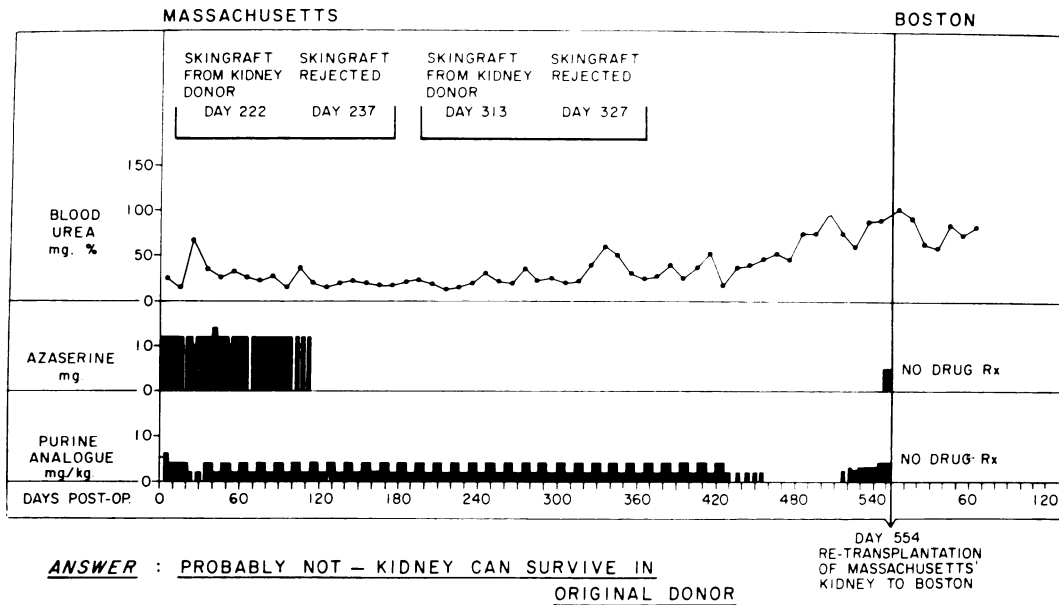
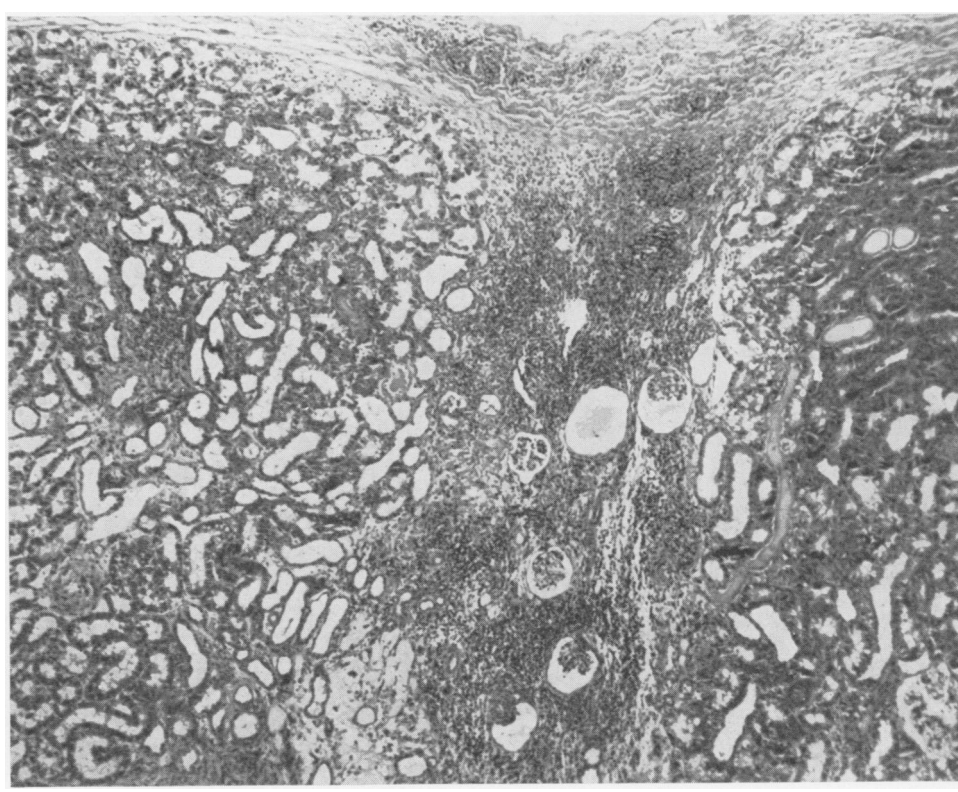


FIG. 5. This abridged chart illustrates that a long surviving kidney homograft can be successfully re-transplanted back to its original donor, indicating no accretion of antigen from the homologous host. This kidney apparently was not altered by its long contact with and perfusion by the new host, suggesting that alteration of antigenicity of the donor organ as an aid to transplantation will be difficult to achieve.



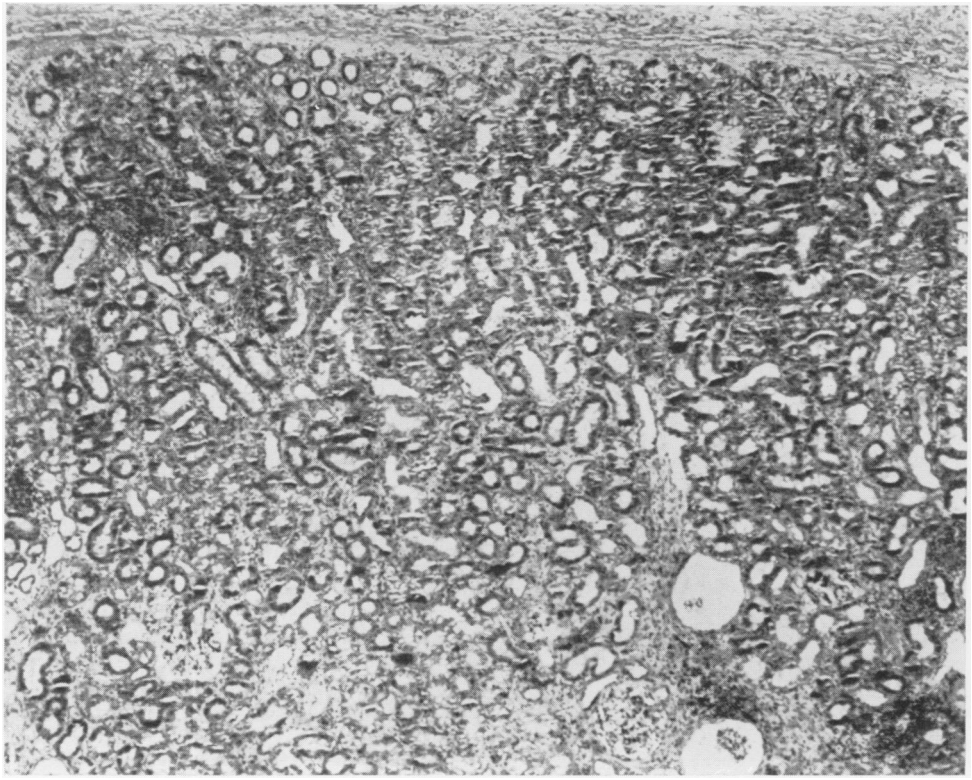


FIG. 6b. This is the same kidney three months after its return to the original donor. Foci of atrophy are present and there is moderate prominence of the interstitial tissue with slight cellular infiltration but the tubular epithelial cells appear more normal.

in the two diverse environments is most likely related to the development of some protective mechanism, possibly antibody coating, rather than by any genetic alteration of donor tissue.

4. To test the possibility that the retransplanted kidney had lost all antigenicity and is incapable of initiating any rejection process, three long surviving kidneys were retransplanted to non-drug treated homologous hosts. In all instances prompt rejection of these previously well tolerated grafts occurred, indicating definite immunogenic potential. Although it is theoretically unlikely that an organ could lose its antigenicity this experiment was performed to supplement question 3 and documents some degree of antigenicity in long surviving homografts.

5. The immunologic principle of immune paralysis may be invoked to explain the survival of these kidney grafts on the grounds that the vascularized kidney protested initially by the drug may overload and paralyze the host's immune response by continual release of antigen. The insertion of a second kidney from the same donor at a time when the first kidney is still

FIG. 6a. Microscopic appearance of the renal homograft on the 296th post-transplant day. This graft had sustained and recovered from a rejection crisis. The central portion is dominated by an area of atrophy and cellular infiltration. On the right, parenchyma appears normal and the major abnormality on the left consists of slight tubular epithelial atrophy with interstitial edema and cellular infiltration.

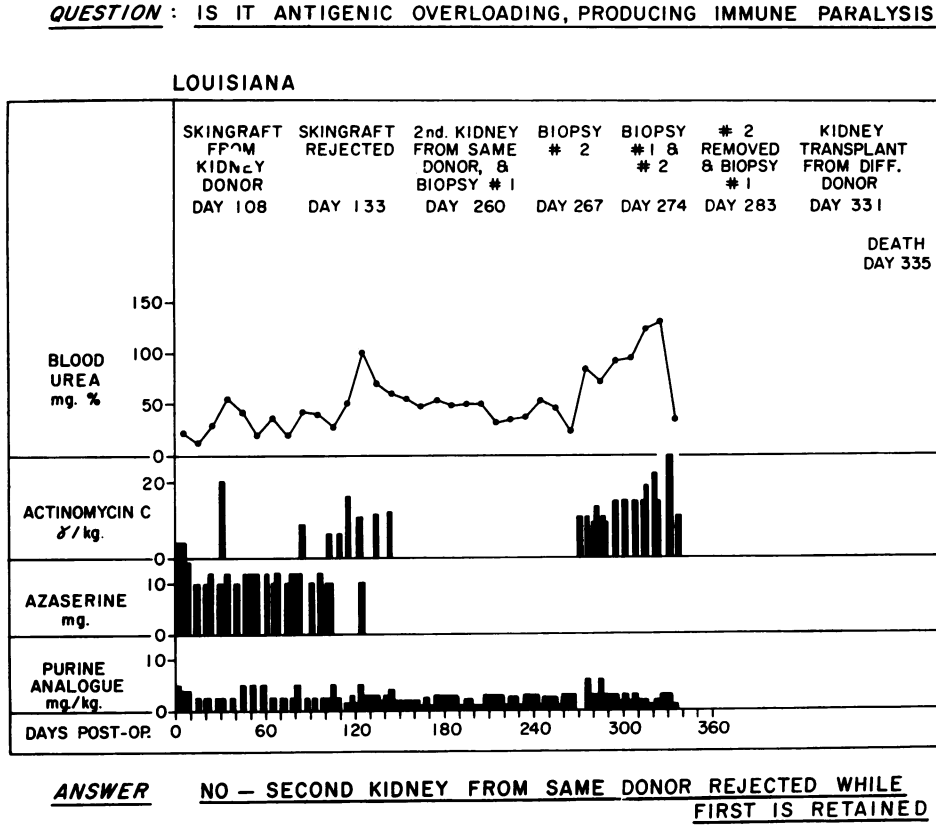


FIG. 7. This abridged chart illustrates that a double dose of donor antigen does not insure survival of the homografted kidney. Therefore, it is unlikely that the principle of immunoparalysis activated by steady release of donor antigen from a long established kidney graft accounts for drug induced immunological tolerance.

functioning would test this possibility because the amount of donor antigen would be doubled and therefore the second kidney should survive if immune paralysis had been produced. Figure 7 illustrates one of two such experiments. The second donor kidney inserted at day 260 was rejected within 23 days, while the first kidney continues to function without change. During the 23 days when both kidneys were in situ gross inspection and microscopic examination after seven and 14 days revealed steady progression of rejection of the second kidney while the first remained unchanged. Clearly, a steady release of excess antigen by the first kidney was not the sole factor in its long survival.

6. There is great variability in the individual experiments within any one protocol whether the drugs are used singly or in combination. With some series there were never any long survivors; with others several long survivors were produced. Our best current series, for example, had four of eight animals surviving over 100 days and seven of eight survived beyond the 50 days. To test whether or not the variations in results on a uniform drug protocol was related to altered drug absorption or metabolism, one kidney from each of two individual donors was transplanted simultaneously into the drug treated host. Obviously the absorption and metabolism of the drug would be no factor in any dif-

ferential rejection of these two kidneys. In three of four animals thus tested differential rejection did occur. In one experiment (Fig. 8) differential rejection was noted grossly and microscopically by day 36 and by day 90 was complete. The other kidney still survived over a year and a half later. Clearly these observations eliminate variability in the absorption or metabolism of the drugs as the major cause of unpredictability of the therapeutic effect of these drugs.

7. The accelerated rejection of a second kidney from the same donor in one instance indicates that the drug treated host has been sensitized against the donor, (Fig. 9). The sensitization in this animal may have been heightened by the rejection of two skin grafts from the kidney donor several months previously. However, skin graft rejection while the dog was still on drugs was not accelerated in a second-set fashion. The data demonstrate that the drug treated host is capable of becoming sensitized to a donor and at the same time maintain a kidney in a *tolerant* state.

8. Several of the experiments mentioned above indicate that the drug induced immunological tolerance does not apply to all tissues of the donor. Eight of nine tolerant animals which were tested by subsequent skin grafting from the kidney donor rejected the skin while maintaining the kidney. The only exception was an animal skin grafted on day 71 who died 15 days later of kidney rejection triggered by the donor skin graft. This animal did not survive long enough to reject his skin. These animals indicate the ability of the drug treated host to distinguish between antigens of skin and kidney. This indicates that either skin possesses antigens not shared

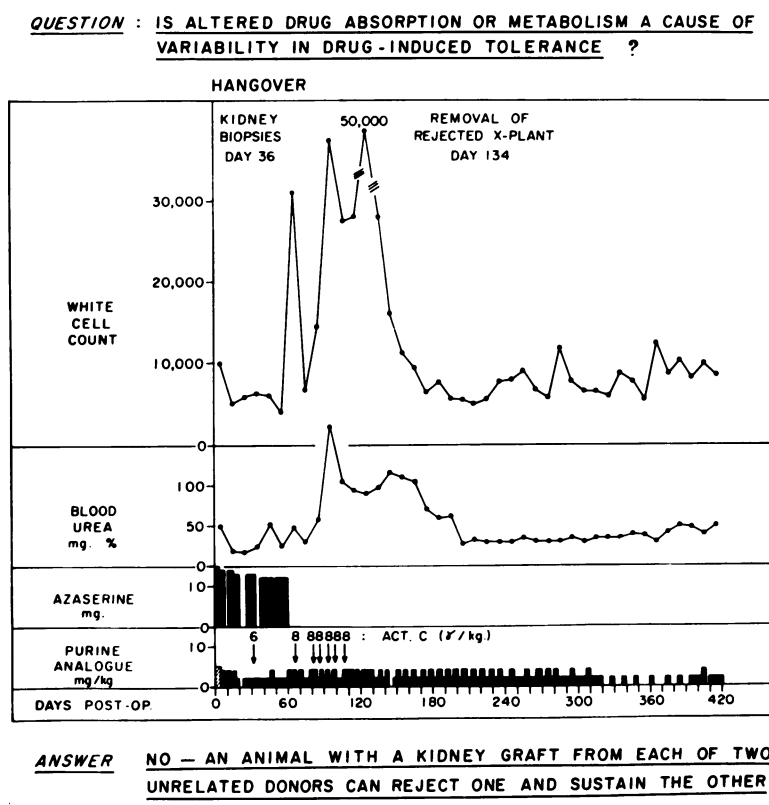


FIG. 8. This abridged chart of animal detailed in Figure 2 indicates that variability in absorption or metabolism of drugs does not account for the variability in the therapeutic effectiveness of the drugs. One kidney is rejected while another from a different donor still survives.

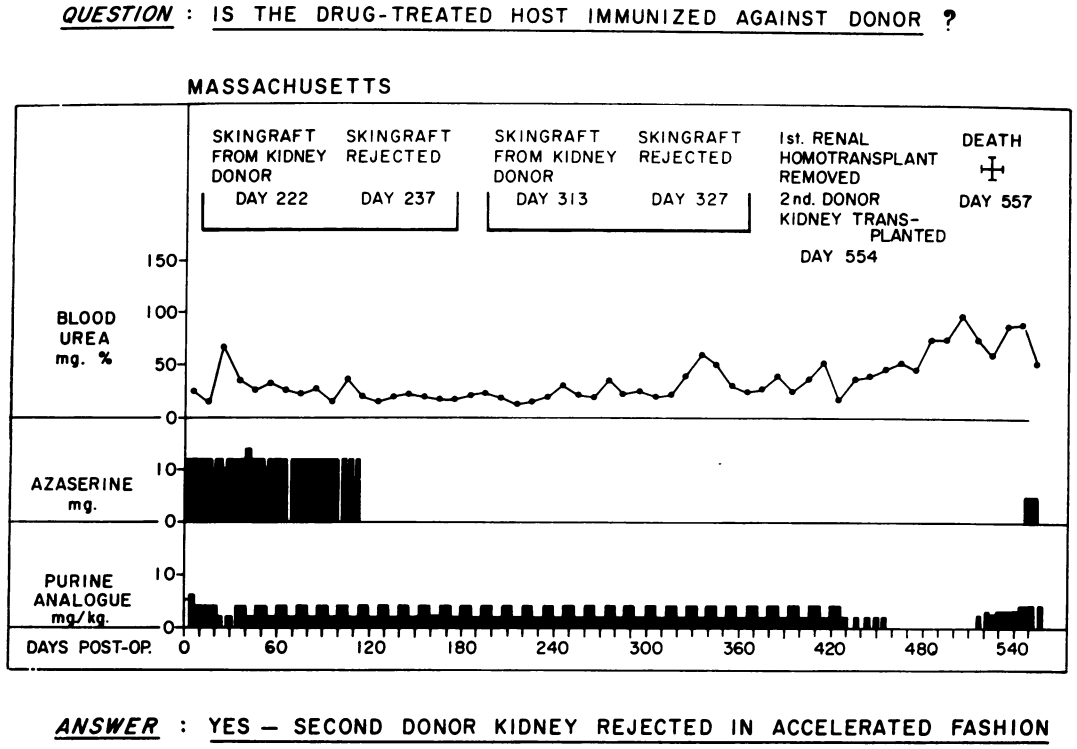


FIG. 9. An abridged chart to show accelerated rejection of a second kidney from the same donor in a drug treated host which had tolerated the first kidney for 554 days. This clearly demonstrates that the *tolerant* host has been immunized against the donor.

by the kidney or the kidney somehow has been protected or placed in a sequestered position during its long period of residence. Possibly both factors are involved to a varying degree.

9. The drug induced immunological tolerance does not apply even to all renal tissue from the donor because the animals mentioned to answer questions five and seven (Fig. 7, 9) demonstrate rejection of the second kidney from the same donor. The different types of rejection in these two instances is not clearly explainable. Perhaps the accelerated rejection in Figure 9 was stimulated by the skin grafts placed several months previously. However, the animal shown in Figure 7 had also rejected skin grafts from the kidney donor. In this animal at the time the second kidney was transplanted, the first was left in situ and the remaining kidney may have been a site of antibody absorption partially protecting the second kidney for the 23 days. In any event, the observations indicate that drug induced immunological tolerance does not apply to all *renal* tissue from the same donor.

10. Several observations indicate that drug therapy can inhibit the accelerated or second-set rejection of a homologous tissue graft. Figure 3 shows serial skin grafts from the kidney donor, none of which were rejected in an accelerated fashion. One animal of a previously published series (Fig. 10) demonstrates this point more clearly. In this animal a first kidney was transplanted and drug therapy started without removing the host's kidneys. The survival of this first kidney was prolonged for 20 days by drug therapy at which time drugs

were deliberately withdrawn to allow rejection which occurred promptly within four days. Biopsy on day 15 confirmed graft viability and biopsy on day 25 indicated florid rejection. A second kidney from this donor, implanted on day 30 when drugs were restarted and bilateral nephrectomy performed was not rejected in an accelerated fashion. Instead, a prolonged survival for over 90 days occurred. Here was an animal deliberately sensitized by allowing the first kidney to reject, yet five days later accelerated rejection of the second kidney from the same donor did not occur.

11. In all animals tested, regardless of the protocol used, a higher drug dosage is required early in the course of the transplant. The dose can be decreased gradually until a maintenance dose approximately one half that of the initial dose becomes adequate. After four unsuccessful attempts to get a second homograft to survive in a host already tolerating a first kidney homograft, success was obtained when drug therapy was increased to a level approximating that used at the beginning of the treatment. Figure 11 illustrates the course of a dog in whom a second kidney was grafted. Note that the first kidney homograft was functioning well on day 69,

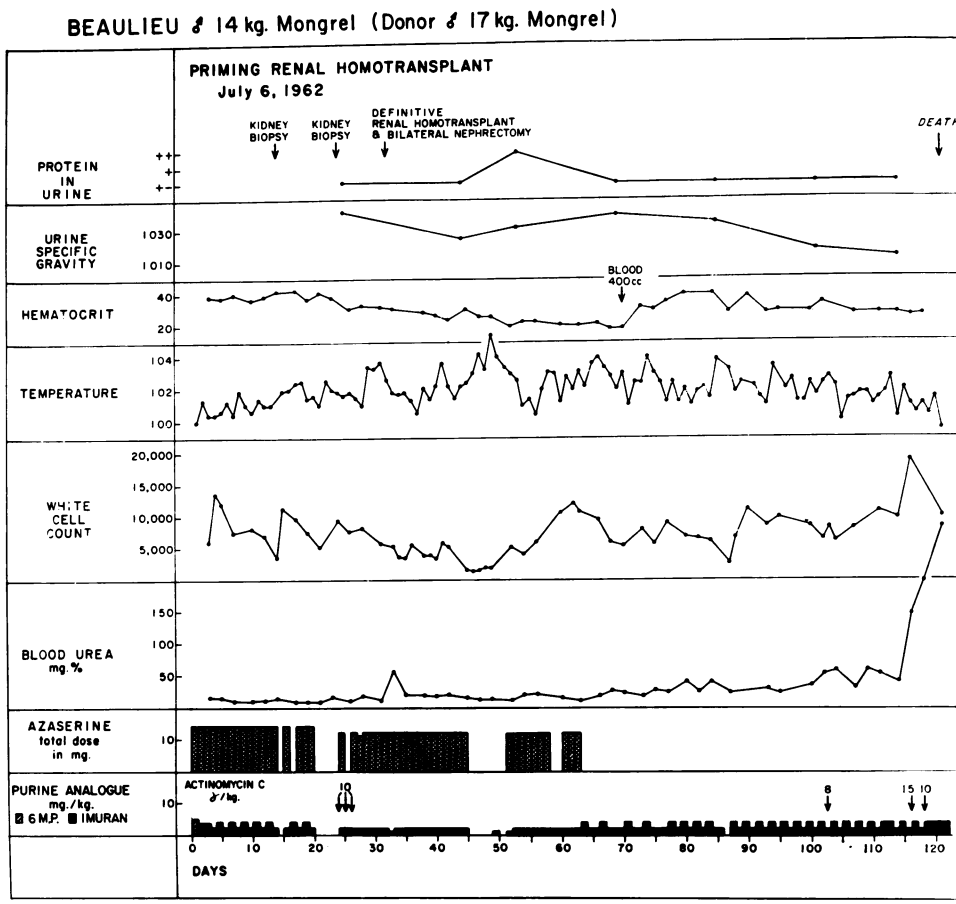


FIG. 10. (Courtesy journal *Transplantation*, published Volume 1: 457, 1963.) Previously published chart of a dog deliberately sensitized against the donor of second kidney by allowing rejection of first kidney to occur after 20 days of function. The second kidney transplant ten days later was not rejected in an accelerated fashion. Instead the drug prolonged its survival for 90 days.

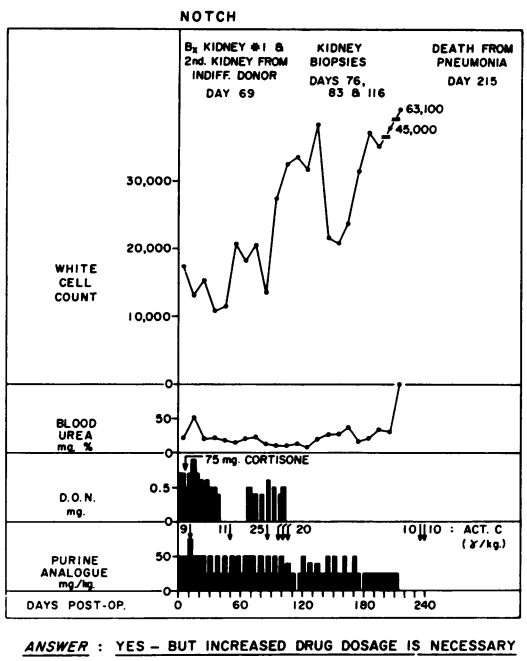


FIG. 11. Abridged chart of a dog in which a second kidney from a different donor was successfully transplanted. The first kidney continued to function normally. Note that increased drug dosage was used at the time of the second kidney graft on day 69. Viability of both kidneys was proven by biopsies on day 76, 83 and 116, and at autopsy on day 215.

at which time the second kidney from another donor was transplanted. At that time the level of drugs was increased and the animal sustained both kidneys for a total of 215 days. This animal succumbed to pneumonia, probably related to the drug therapy. At the time of the postmortem both kidneys were viable.

12. It was important to determine whether or not those animals successfully withdrawn from the drug therapy were still immunized. Figure 12 illustrates the course of a skin graft from the original donor placed approximately two years following the transplantation and one year following cessation of all drug therapy. The skin graft was acutely rejected within five days indicating that a high degree of immunity was still present. This animal, as described in Figure 3, accepted the donor skin grafts while on drug therapy in three instances beyond 20 days. Yet the dog successfully withdrawn from therapy still recognizes tissue from the donor. The drugs must have been acting to allow prolongation of skin graft survival; on drug withdrawal the sensitization effect becomes unmasked.

13. As a corollary to the above observation it was important to determine whether a dog successfully withdrawn from drug therapy would react immunologically against animals other than those to which it had been previously exposed. In other words, we asked whether or not the dog off drugs could initiate a primary response as well as a secondary response. Therefore, one animal off drugs for six months was tested with a skin graft from another animal to which no prior exposure had been made. This skin graft was rejected within ten days with a primary type rejection histologically. During this rejection there was a slight elevation in the blood urea nitrogen level but renal function returned to normal with the complete rejection of the skin graft. The animal has remained well without drugs ever since.

14. Because kidney homografts could be prolonged more consistently than skin homografts the following experiment was devised to analyze the effect of an established drug regimen on skin graft survival. Using the same drug protocol that protects 90 per cent of kidney homografts for over 50 days and 50 per cent of animals beyond 100 days, skin homografts survived an average of 20 days in 11 animals. It was obvious that the same drug program which protects kidney grafts, sometimes permanently, does not produce striking prolongation of skin graft survival.[21]

15. In order to investigate further the relationship between skin and kidney grafts the simultaneous placement of skin and kidneys from the same donor was carried out. Paradoxically, this simultaneous graft-

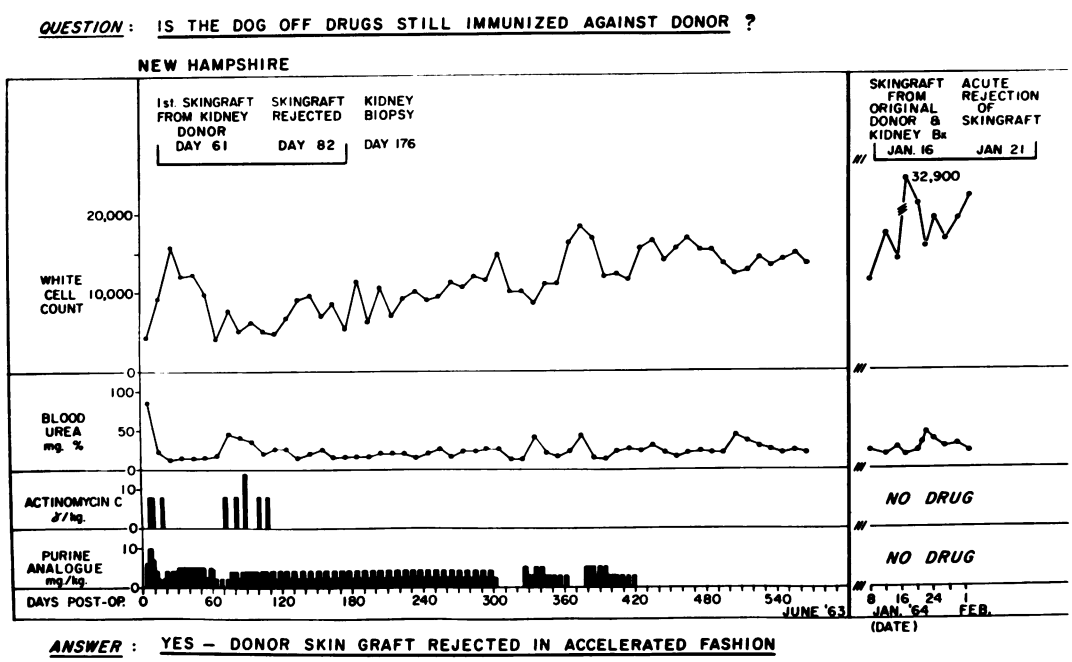


FIG. 12. Abridged chart of a dog off drugs for over one year which rejected donor skin graft in an accelerated fashion. This same animal had taken 20–30 days to reject three successive donor skin grafts while still on drugs (Fig. 3); now off drugs sensitization is unmasked.

ing prolonged skin graft survival from an average of 20 to an average of 39 days, but reduced kidney graft survival in all animals to an average of 39 days. Six of seven skin grafts remained intact until death of the animal. The stronger antigenic processes initiated by the skin graft presumably stimulated antibodies which were destructive to the kidney. Possibly the absorption of antibodies by the rejecting kidney prolonged skin graft survival. Almost certainly the skin grafts would have been destroyed a few days after the kidney had the hosts survived. This will be tested in a future protocol which leaves one normal kidney in situ at the time of the simultaneous grafting.

16. In practically every instance in which a skin graft was rejected renal function was impaired. This occurred whether or not the skin came from the kidney donor and indicates partial sharing of antigens by skin and kidney. The fact that a recently established kidney graft is more vulnerable than a well established graft to antibody generated by a skin graft brings up again the concept of "adaptation" mentioned in paragraph 3 above. If "adaptation" is defined as a change of the antigenicity within the organ, most likely it does not occur. If, however, *adaptation* is a protective mechanism developing on the kidney to allow it to survive in the face of an immunological attack, the term may apply and account for the more favorable position of the well established kidney.

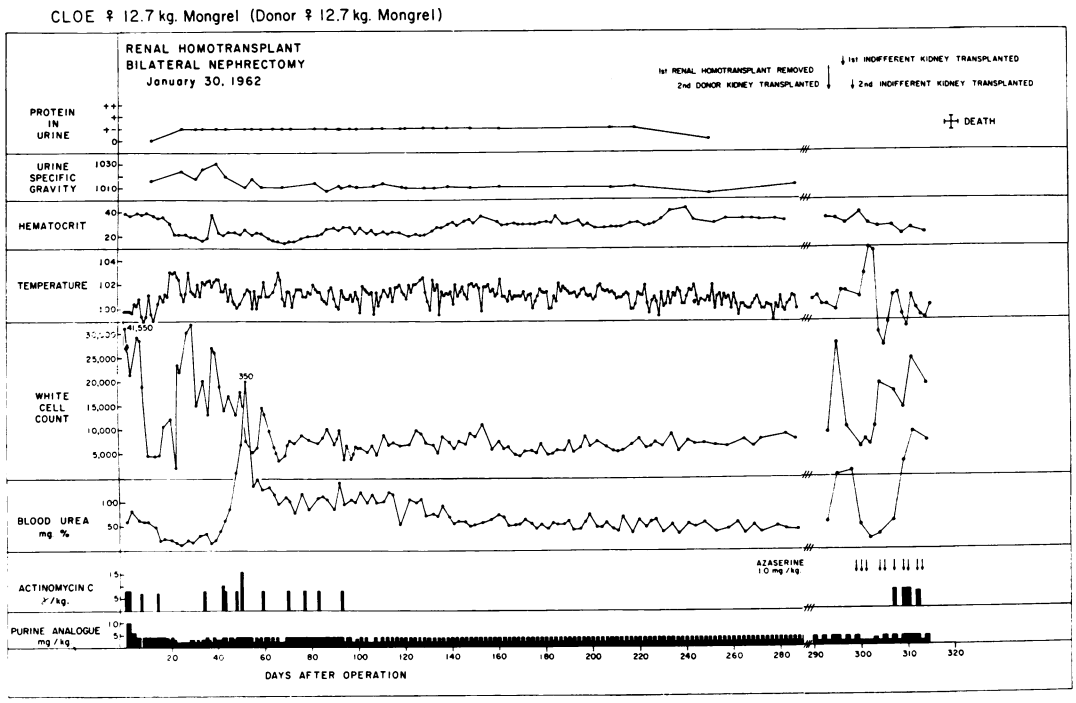
17. Five animals have been studied long enough following a serious rejection crisis to indicate that renal function can improve to a marked extent following successful reversion. One animal with a rejection crisis reversed at day 50 had continued improvement in renal function until day 296, at which time the experiment was arbitrarily terminated (Fig. 14). This indicates that the tissue damage is not neces-

FIG. 13. A photograph of a drug treated mother of a normal litter. This animal is two years following a bilateral nephrectomy and renal homotransplant. Still unable to be withdrawn from drugs she has had three pregnancies, one ending in spontaneous resorption, one with a stillborn puppy with a congenital defect, and the present pregnancy with a normal litter. Only the third pregnancy had a normal male as a father.

sarily permanent and that significant renal regeneration is possible even under continued drug therapy.

18. The drug treated female dogs were noted to undergo periods of heat at regular intervals and the male animals were active sexually. When a drug treated male impregnated a drug treated female one successful pregnancy has resulted, although one of the litter has a congenital anomaly. When a drug treated female was impregnated by a normal animal a normal pregnancy occurred with a normal litter (Fig. 13). This mother 12 months after her transplant and the initiation of drugs had a pregnancy which resorbed spontaneously. Her second pregnancy six months later produced one stillborn fetus with a congenital defect. Her third pregnancy while still on drugs produced the normal litter. These observations on sexual activity and fertility indicate the relative normalcy of the successfully treated dogs on prolonged drug therapy.

19. Figure 14 indicates the course of an



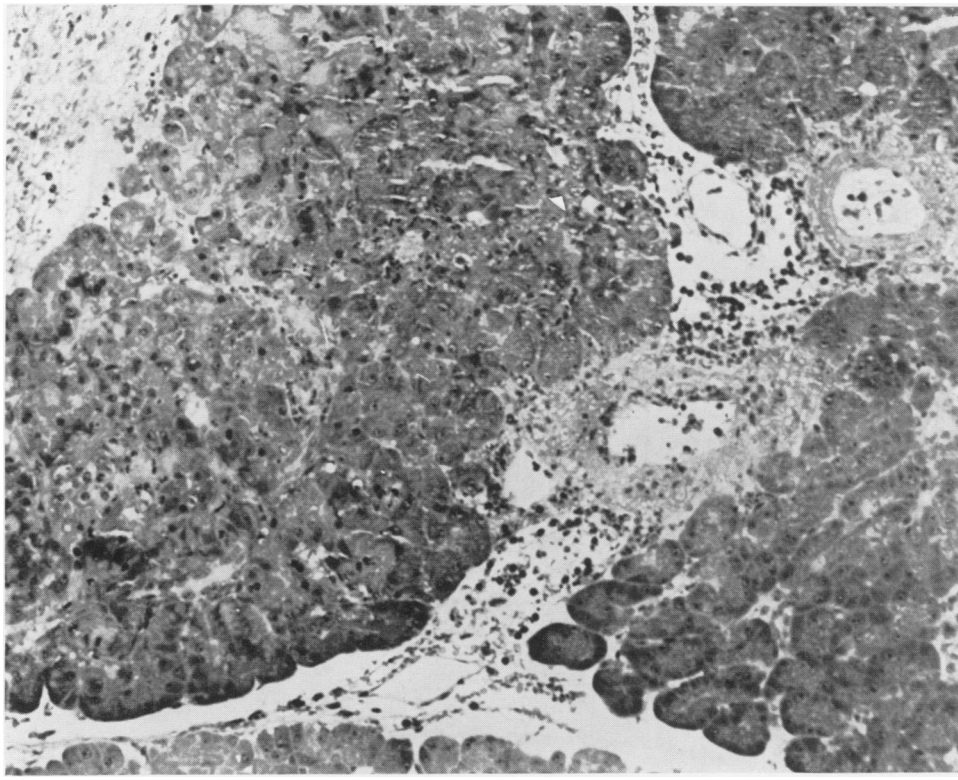


FIG. 15a. Microscopic pattern of the pancreas at autopsy of the recipient described in Figure 14. The large arteries on the left show complete necroses with fibrinoid deposition. Acute inflammatory cells including eosinophiles are contained in the arterial wall and adjacent tissue.

animal who received four kidney homografts, two from one donor and one each from two other donors. In the course of these repeated immunological attacks this animal developed a severe generalized immunological disease pattern indicating that the sharing of antigens among species is often shared by the host itself. Isoantibodies are produced which can cross-react with tissues of the host's own system producing lesions of the liver, lungs, spleen, pancreas (Fig. 15a) and gastro-intestinal tract (Fig. 15b). This indicates that a generalized autoimmune disease process can be associated with immunological rejection.

Discussion

The biological process by which a kidney homograft is rejected was considered only a few years ago to be an all-or-none process, which, when once initiated, proceeded inexorably to its final stage. Kidney destruction was presumed to be caused by lymphocytes and plasma cells which lodged in the foreign kidney producing a cytotoxic or cytostatic effect. However, with the ad-

FIG. 14. Detailed chart of a dog who had been the recipient of four renal homografts. Acute arteritis of the type seen in serum sickness was observed in the fourth homograft, the intestines and pancreas. The fourth homograft was rejected by the 21st post-transplant day although immunosuppressive therapy had been continuous. The second and third renal homografts had failed shortly after transplantation. The first homograft had been removed electively and returned to the original donor as part of another experiment (Fig. 6a, b).

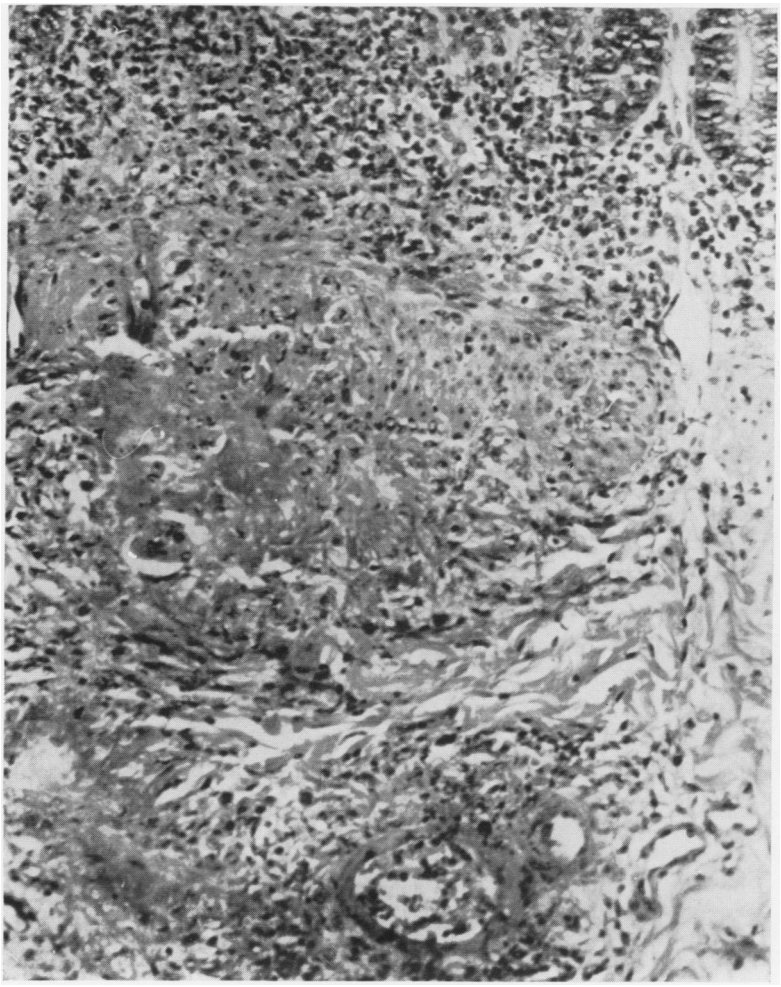


FIG. 15b. Acute arteritis with abundant fibrinoid is present in the submucosa of the intestine of the same animal. Necrosis of mucosa with exudation may be seen in the upper portion of the field.

vent of immunosuppressive drugs two pertinent observations have become evident. First the rejection process can be reversed, even if far advanced as first demonstrated by retransplantation of a rejecting kidney back to its original host [2] and later by drug therapy alone.[16] The second observation relates to the variety of rejection patterns being observed. Rejection following the use of drugs rarely reveals the *classic* picture of mononuclear cellular infiltration with tubular destruction. The drugs singly or in combination may inhibit either the cellular infiltrate, the arteritis, the macroscopic swelling or the capillary disruption of the rejecting kidney. These varieties of gross and microscopic rejection patterns probably are a resultant of the drug effect, the genetic relationship between host and graft and the fundamental reactivity of the host. The multiplicity of rejection patterns is not dissimilar from the varieties of autoimmune diseases in which antigen-antibody complexes are capable of causing a wide assortment of disease patterns whose specific features are determined not by specific antibodies but by such determinants as site of interaction, antigen-antibody ratios, solubility of the complexes and accessibility of the complexes to phagocytes.

In this framework of reversibility and

diversity no single immunological theory accounts for drug induced immunological tolerance as applied to the kidney homograft. Final cessation of renal function is a combination of many events and its pathogenesis is not always clear from the clinical and microscopic data. Many drug treated dogs with kidney transplants die with renal failure, but on microscopic examination there seems to be a sufficient amount of normal renal tissue to sustain life. Many such *rejected* kidneys have more normal renal mass than many other kidneys which are, in fact, life sustaining.

The original observation of Schwartz and Dameshek [20] on the specificity of drug induced immunological tolerance applies to our experiments. Our dogs seem to have a specific tolerance for that antigen introduced with the initiation of or an increase in drug therapy. The chief limitation in the use of drugs is the lack of consistent success; drug toxicity likewise is unpredicable. However, with more experience increasing numbers of long survivors are being obtained.

Undoubtedly the unknown genetic differences between host and graft contribute in part to the variations in results. Our *successes* may be due in part to chance close genetic compatibility and our *failures* a result of strong genetic differences which drug therapy is unable to overcome. Although admitting the existence of some degree of genetic self-selection in our experiments, we must immediately recognize that never in our laboratory has a kidney homograft in an untreated host survived longer than 12 days. However, all of the tolerated kidneys in drug treated hosts demonstrate signs of rejection indicating the existence of at least some genetic disparity.

Within the limit of the lack of information of canine genetics, we believe our data has validity in the analysis of the mechanism of immunosuppressive drugs. The hosts are capable of being immunized, the kidney cell is not altered antigenically by long perfusion in the host, the drug treated host can resist bacterial infection and can even distinguish between skin and kidney from the same donor, the second kidney from the same donor can be differentially rejected, drug treatment can be stopped in some but not all animals, drug treatment can prevent second-set rejection of skin and kidney, yet after drug withdrawal accelerated rejection can occur, the animals are fertile and can produce normal litters, and a generalized autoimmune process can occur in these immunized hosts. All these observations are valid and applicable to clinical problems regardless of the genetic backgrounds.

These hosts are simultaneously tolerant and immunized. The tolerated or *enhanced* kidney most likely is protected or sequestered in its new environment by some mechanism, possibly antibody coating, which nullifies the efferent action of the immunological reflex arc. Woodruff's original observations [23] that partially tolerant hosts can sustain primary skin grafts and rapidly destroy a second skin graft from the same donor are pertinent. It is doubtful that any fundamental change in the antigenic structure of the graft itself has resulted to account for this *adaptation;* most likely a protective mechanism has developed, as described above in the answers to Questions 3 and 16.

Fisher and Schewe [9] have obtained similar results in homografted skin in pyridoxine deficient rats. Well nourished, long surviving, hair-bearing skin homografts retransplanted back to the original donors survived normally with continued growth. However, all were rejected if placed on third party recipients. They likewise demonstrated rejection of second skin grafts from the same donor while the first continued to flourish.

Observations that intraperitoneal injections of antiserum in rats will destroy only those skin homografts less than four days

in situ and not effect any graft *in situ* longer than six days, even with large doses of anti-serum,[22] suggests another possible explanation for the differential handling of grafts from the same donor. We may postulate that during the critical period in which the graft is establishing its new blood supply, it is more vulnerable to the antibody either circulating, cell-bound, or in the form of an antigen-antibody complex. Of course the blood supply to a kidney homograft is re-established immediately but possibly the kidney may be more vulnerable for the first few days because of the ischemia and surgical trauma involved in the performance of the transplant.

Pierce and Varco [19] first demonstrated that some drug treated dogs with kidney homografts could eventually be withdrawn from drug therapy. They also demonstrated rejection of the second donor kidney while the first continued to function in one dog for seven days and in another for 27 days. These animals were off drug therapy at the time of the second kidney graft. Although differential rejection of these kidneys was produced and documented microscopically, the death of these animals relatively soon after the second transplant prevented complete analysis of the process.

The production of a generalized autoimmune pattern in the host (see Question 19 above) was observed and incisively analyzed by Holman in his remarkable clinical report which preceded the modern era of transplantation biology by 20 years.[11] Holman not only described the accelerated rejection of the second group of grafts from the same donor, but he also details "a rather widespread exfoliative dermatitis . . . over the entire body." This generalized process included fever, high pulse rate and bloody stools, indicating possible desquamation of intestinal mucosa. Ten days following the removal of the still surviving first-set skin graft the patient's general condition improved tremendously. He postulated an intoxication due to a foreign protein in a sensitized host. In our animals described above and in several others the pattern of autoimmune disease undoubtedly has been altered by the drug therapy. Many animals developed exfoliative dermatitis, occasional unexplained skin ulcerations and one developed a full-blown picture of polyarteritis nodosum.

It is tempting to identify the state of unresponsiveness induced in immunologically mature animals by means of drugs in conjunction with the introduction of antigens with the production of classic acquired tolerance produced by inoculation of young animals with much lower dosages of cells. Billingham [3] has noted several outstanding similarities: both types of tolerance may be complete or partial, both types of unresponsive animals are cellular chimeras, and, moreover, persistence of unresponsiveness almost certainly requires persistence of homologous isoantigens. In some small animals a low dose of homologous cells can produce sensitization, whereas higher doses produce tolerance. The intravenous route in the production of tolerance is the most effective method of antigen administration, at least where major histocompatibility differences are involved. In this context the kidney may be considered an intravenously administered antigen as contrasted to the orthotopic skin graft.

The differential handling of skin and kidney from the same donor may be analagous to the *split tolerance* phenomenon described by Billingham and Brent who produced tolerance to only one component of the cells injected from a hybrid into a newborn.[4] The injection of (C57BL × CBA) F_1 cells into a newborn A strain resulted in tolerance of CBA, but not of (C57BL × CBA) F_1 skin. Similarly, injection of *homologous* male cells into female newborn results in tolerance of *isologous* male grafts but not of homologous grafts of either sex.[8,13] These recipients had become tolerant of only a portion of the total antigenic moieties of the injected cells. *Split toler-*

ance is difficult to explain in terms of survival of injected cells, although it may be considered a restricted tolerance in which the host may reject skin homografts yet retain homologous lymphoid cells. These observations do not apply directly to our experiments, but they do hint at analagous situations in other experimental animals.

A *stem cell* theory may account for most of our observations. If we postulate that throughout life immunologically competent cells are being produced constantly from newly differentiated stem-cells and that at some stage of their maturation these cells can respond to antigens by becoming tolerant rather than immune, then exposure of adult animals to an antigen could produce a specific state of unresponsiveness of these immature cells and concomitantly should sensitize those already immunologically competent. This immune response will normally conceal any tolerance produced. However, if the sensitized cells undergo some form of elimination by a prolonged contact with antigens, the lymphoid cell population will gradually change from one which is predominantly sensitized to one that is tolerant.[5]

Regardless of the biochemical or biological theories involved, drug induced immunological tolerance is a specific form of therapy which can protect renal homotransplants. The drug treated host can lead a normal active life, be fertile and, in some instances, achieve a permanent tolerant state. The method has such valuable clinical potential that further work seems justified.

Summary

A long-term study of the mechanism of action of immunosuppressive drugs has been completed in bilaterally nephrectomized dogs with kidney homotransplants. Over 1,000 test animals with 24 different drug protocols have been analyzed. Increasing numbers of long surviving animals have posed many questions regarding the status of the long surviving kidney in the drug treated host. The current drug protocol of Imuran and azaserine has produced 90 per cent 50-day survivors and 50 per cent 100-day survivors.

The following observations have been documented: All animals on prolonged drug therapy are immunologically competent; drug therapy can be stopped successfully in some but not all animals; long surviving kidneys apparently are protected in some way in the new environment because a second donor kidney can be rejected while the first survives; retransplantation of a long surviving kidney back to its original host did not lead to a decrease in renal function; long surviving kidneys successfully retransplanted back to their original donors are rejected when transplanted to third party, non-drug treated recipients; immune paralysis does not account for the prolonged survival because the second donor kidney which constitutes a double dose of antigen is rejected while the first continues to survive; absorption or metabolism of the drug does not account for the variation in results because two kidneys, each from separate donors, can be rejected differentially in the same drug treated host; and all hosts are sensitized against the recipient and this sensitization continues even in those animals successfully weaned from drugs.

Additional analyses of the relationship of skin homografts and kidney homografts reveal the following: skin homografts are universally rejected within 20 days by hosts treated with the drug regimen which protects kidney homografts sometimes permanently. When skin and kidney homografts from the same donor are placed simultaneously skin survival is prolonged while kidney survival is shortened. This paradoxical effect probably is explained by the production of antibodies by skin which are absorbed by the rejecting kidney. Additional observations indicate that drug treated animals, male and female, are fertile and that

multiple rejection processes can produce generalized immunological picture in the host similar to an autoimmune disease process.

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