THE EFFECT OF IMMUNOSUPPRESSION ON FUNCTION OF KIDNEY ALLOGRAFTS IN THE RAT

(with a summary in Dutch)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE AAN DE MEDISCHE FACULTEIT TE ROTTERDAM, OP GEZAG VAN DE DECAAN PROF. D.C. DEN HAAN, HOOGLERAAR IN DE FACULTEIT DER GENEESKUNDE, TEGEN DE BEDENKINGEN VAN DE FACULTEIT DER GENEESKUNDE TE VERDEDIGEN OP WOENSDAG 25 NOVEMBER 1970 TE 16.00 UUR PRECIES

DOOR

RANDOLPH WILLEM DE BRUIN

GEBOREN TE AMSTERDAM IN 1937

1970
DRUKKERIJ BRONDER-OFFSET N.V.
ROTTERDAM

PROMOTOR: PROF.DR. M.J. DE VRIES

CO-REFERENTEN: PROF.DR. D.W. VAN BEKKUM
PROF.DR. M. FRENKEL

Aan de nagedachtenis van mijn vader

The experiments were performed at the Radiobiological Institute of the Organization for Health Research TNO, 151 Lange Kleiweg, Rijswijk Z.H., The Netherlands.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to:
Prof. Dr. M.J. de Vries for introducing him into the field of transplantation research and for his help in the realization of this work.
Prof. Dr. D.W. van Bekkum for the facilities offered at the Radiobiological Institute TNO and for his continued and stimulating interest in this study.
Prof. Dr. M. Frenkel for his assistance during the preparation of this manuscript.
Dr. C.F. Hollander for his advice and for his presistent encouragement.
Mr. W.J. Kort for his essential help in gathering and processing the data for this study.
Mr. R.L. Marquet, Mr. F. Hess, Mr. G.A. Heystek and Mr. M.G. van der Ven for skilfully performing the renal transplantations and the biopsies for the experiments.
Prof. Dr. H.A. Valkenburg for his help in planning and evaluating the experiments.
Dr. M. Wijnans for his advice concerning the statistical evaluation of the results.
Dr. W.N. Eastham for his collaboration in the drafting of the English text.
Mrs. M.A. Lelieveld-Muller and Miss J.C.M. Verkleij for typing the manuscript and Miss M.W.Th. Wagener for the tables.
Mr. A.A. Glaudemans for preparing the illustrations and Mrs. W. van de Hartskamp-Roodbeen for the microphotographs.
Finally, the author thanks the technical and administrative staff of the Radiobiological Institute TNO for their kind help and advice during his work in Rijswijk.

CONTENTS

INTRODUCTION

Transplantation of living tissues is not a new concept. Greek mythology presents us with the Chimera in which a goat's body, a lion's head and a dragon's tail are joined to form a terrifying monster. Ancient Indian surgeons made use of local skin flaps for rhinoplasty, an art which has been practiced in Italy as early as the fifteenth century.

Various transplantation experiments were performed by John Hunter in the eighteenth century. However, a systematic approach to the study of transplantation biology has only been started during the present century when technical progress in the field of surgery (Carrel and Guthrie 1905, 1906; Carrel 1908) made it possible to transplant a large variety of tissues and organs.

Unfortunately, surgical problems are not the only obstacles to successful transplantation. It has been known for a long time that tissue transplants in which the donor is also the recipient (*autografts*) or transplants between animals of the same inbred strain (*isografts*) will survive. Without complications this also applies to grafts between identical twins. On the other hand, transplants between two randomly chosen individuals of the same species called *homografts or allografts*, behave like autografts for a few days only, after which progressive damage occurs, leading to loss of function and destruction of the graft. This process, called rejection, is usually complete within a few weeks following transplantation and is invariably accompanied by infiltration with inflammatory cells, predominantly of the mononuclear type. In transplants exchanged between members of different species (*heterografts*, syn. *xenografts*), a much more violent and rapid rejection may take place.

The immunological nature of the rejection process is clearly demonstrated by the "second-set phenomenon". After rejection of an allograft, a second transplant from the same donor will be rejected by the recipient in an accelerated fashion while a second transplant from another donor will survive for the same period of time as the first graft. This may be compared to the accelerated and specific response of an animal that has been immunized on subsequent exposure to the sensitizing antigen.

Being able to distinguish between "self" and "non-self", the body can recognize and react specifically against foreign antigens. Thus the process of graft rejection is part of a normal and essentially useful defense mechanism of the body against all kinds of foreign substances including microorganisms. It is for this reason, that in addition to its great practical importance for surgery, investigation of the immune response leading to rejection is also of fundamental biological interest.

Skin grafting experiments have contributed largely to our knowledge of transplantation biology. Special mention in this respect should be made of the work by Medawar (1944, 1945, 1958), leading to the formulation of the basic rules of transplantation. At present, our knowledge in the various fields of transplantation is expanding rapidly. Yet, while surgical problems are not a limiting factor any more to clinical application, insufficient understanding of the rejection process and of the ways to prevent it is still responsible for a considerable failure percentage.

An ever growing number of papers has been published during the past years on transplantation and related topics. Several review articles and books covering these subjects being available, it seems unnecessary to make an attempt at reviewing exhaustively the current state of our knowledge. For this reason only some of the subjects, pertinent to our experiments, will be discussed briefly in the following pages. The reader is referred to the following references for detailed information on:

- Immunological aspects of tissue- and organ transplantation:
  Amos, 1968; Ramseier, 1969; Russell and Monaco, 1965.
- Bone marrow transplantation:
  van Bekkum and de Vries, 1967; Mathé et al., 1965.
- Experimental and clinical renal transplantation:
  Calne, 1967; Hume, 1967; Largiadèr, 1966; Rapaport and

Dausset, 1968; Starzl, 1964.

- Pathology of human renal transplants:
  Porter, 1966a,b; Shorter and Hallenbeck, 1968.
- Chemical immunosuppression:
  Amiel, 1967; Berenbaum, 1965; Lardiadèr, 1968.
- Antilymphocyte serum:
  van Bekkum, 1969; Ciba Foundation study group, 1967; James, 1969; Pichlmayr, 1967.
- Latest developments in transplantation research:
  Advance in Transplantation. Proceedings of the First International Congress of the Transplantation Society, 1968; Proceedings of the Second International Congress of the Transplantation Society, 1969.

Genetic factors

Antigenic substances, present in donor tissues but absent in the host, give rise to allograft rejection. These substances are called histocompatibility antigens and they are genetically determined. Extensive studies in highly inbred strains of mice have demonstrated the existence of at least 15 histocompatibility loci on the chromosomes, which act independently while the corresponding antigens vary in strength. Thus skin grafts between strains of mice that differ at the strong H_2 locus will be rejected in a much shorter time than grafts between strains that differ only at one of the weaker loci. This strong H_2 system in the mouse received much interest and it proved to be very complex, at least 18 alleles occurring at this locus (Snell and Stimpfling, 1966). From the independent actions of the histocompatibility genes it follows that no rejection will occur if the recipient possesses all the dominant genes of the donor. A system similar to that in mice, has been found in other animals e.g. in the rat, and also in man. In human renal transplantation the effect of genetic factors has been demonstrated by the much higher incidence of successful grafts when related donors are used as compared to the significantly poorer results when donor and recipient are unrelated (Seventh Report of the Human Kidney Transplant Registry, 1969).

Transplant characteristics and the recipient site

Homografts are rejected by a process requiring sensitization of the host. Transplantation antigens are present in all the tissues of an

individual and hence any organ or piece of tissue can induce homograft immunity. However, the effectiveness with which different grafts do so may vary, depending on a number of factors. Organization of the graft is such a factor: it is known that sensitization resulting from injection of living dissociated cells is of a much shorter duration than sensitization from a whole organ graft. The quantity of antigen available for sensitization is an important factor and, although little is known for organ grafts in this respect, it has been demonstrated for skin that massive allografts and xenografts survive longer than small ones (Ballantyne et al., 1969). The route of administration of the antigen is also important. In general the intravenous and subcutaneous routes are known to be much less effective in producing a state of immunity than are the intradermal and intraperitoneal routes. Thus, the site of a given transplant in the recipient and the nature of its connections to the host can affect the duration of graft survival. Therefore, it is reasonable to expect that grafts of skin and grafts of kidney will behave differently. A skin allograft is comparable to an intradermal injection of antigen. Soon after grafting, large lymphoid cells have been found to appear in the lymph nodes draining the graft site and it has indeed been demonstrated that effective sensitization by a skin graft depends on adequate lymphatic drainage (Barker and Billingham, 1967). In the case of a whole kidney graft, sensitization of the host will occur by way of the venous route, while regional lymph nodes are not particularly involved. The importance of the recipient site is further illustrated by the occurrence of several privileged sites, where a defect in the afferent or the efferent pathway of the host's immune response appears to be responsible for a permanent survival of allografts and even xenografts. Examples of privileged sites are the cheeck pouch of normal, unsensitized hamsters and the anterior chamber of the eye. In the latter case vascularization of the graft will be accompanied by rejection, a phenomenon which has also been observed in corneal grafts. Incidentally, pregnancy can also be considered as an allograft that is normally exempt from rejection.

Other differences between transplants of skin and kidney can be thought to play a role. The kidney graft is provided with an adequate blood circulation from the start, while nutrition of the skin depends on the development of anastomoses of small vascular and lymphatic channels between host and donor tissue. Specific factors inherent to

the specific function of the organ involved could also contribute to differences in survival. The clearance function of the kidney could promote damage to the glomeruli by exposing them to high concentrations of macromolecules, e.g. antibodies or immune complexes. On the other hand, impairment of clearance function may result in uremia and raised blood levels of immunosuppressive drugs, both known to promote graft survival. Generally speaking, acceptance of skin grafts is more difficult to achieve than acceptance of kidney grafts. It is known even that a recipient can reject a skin graft while a kidney from the same donor, transplanted at the same time, continues to function (White and Hildemann, 1968, 1969; White et al., 1969).

The homograft reaction

Notwithstanding the overwhelming number of investigations on the subject, the whole sequence of events leading to rejection of a transplant is still incompletely understood. Strong histocompatibility antigens, while evoking the development of transplantation immunity, induce at the same time the formation of humoral antibodies (as hemagglutinins, hemolysins, cytotoxins, etc.) which can be detected with relative ease by means of serological tests in vitro. Since sensitization with cell-free extracts proved to be feasible, attempts have been directed towards establishing the chemical nature of the antigens. So far, it has been demonstrated that antigenic activity is mainly associated with lipoprotein structures on cell surface membranes. To study the effects of antigen administration it is important to isolate these substances as pure antigens.

There is no agreement about whether histocompatibility antigens are released from the graft site as free antigens or as cell-bound antigens. Little is known about how antigens are recognized and processed to give rise to proliferation of immunocompetent cells. Apparently, both the antigen reactive cell and the effector cell reside in the fraction of small lymphocytes.

It is well known that after antigenic stimulation a certain proportion of the small lymphocytes undergoes transformation into large, pyroninophilic, blast-like cells. It is probable that this change results from interaction with antigen and morphologically it is the earliest manifestation of the immune reaction. Transformed cells can be readily demonstrated in the lymph nodes draining the site of a skin

homograft. It is thought that the production of immunologically active lymphocytes represents the ultimate result of this reaction. In case of a kidney graft, lymphatic drainage is initially lacking. However, there is experimental evidence to suggest that in such a vascularized graft sensitization of blood lymphocytes can occur (Strober and Gowans, 1965). On the other hand, in the venous outflow from renal grafts, free antigens have been demonstrated as well (Najarian et al., 1966).

For a long time the invariable presence of large numbers of lymphoid cells in first-set allografts during rejection and the finding that allograft immunity could be passively transferred by means of lymphoid cells, serum being ineffective in this respect, were the main arguments to consider rejection as a cellular immune reaction. Moreover, it has been demonstrated that specifically sensitized lymphoid cells can directly destroy allogeneic and xenogeneic target cells in vitro (Ginsburg et al., 1969). Nevertheless, the question remains, whether or not only a proportion of the cells infiltrating an allograft is capable of a specific action, the rest being secondarily attracted by the damaged tissue.

Morphological lymph node changes associated with the development of cellular immunity can be distinguished from those found in humoral antibody formation (Turk and Oort, 1967). In the latter, development of germinal centres and the presence of plasma cells are prominent signs. Lymph node changes observed in experimental induction of a cellular immune response resemble closely those found after skin grafting. The changes consist of a blast-cell response in the paracortical area of the draining lymph nodes. This once more points to the importance of cellular mechanisms in the rejection of a skin graft. For kidney grafts however, this is much more difficult to demonstrate. There is no doubt that lymphoid cells play an important role in the rejection of kidney grafts as well, but recent investigations have shown that circulating antibodies directed specifically against donor kidney tissue and present within a week after transplantation, can induce lesions, comparable to those found in allografts in the course of their rejection (Clark et al., 1968). Of great practical importance in this respect is the role played by preformed antibody in a number of clinically known cases of early transplant rejection called "hyperacute rejection". Failure to demonstrate the significance of humoral antibody in earlier experiments might have been due to in-

sufficient sensitivity of the techniques used and to disappearance of antibody from the circulation as a result of cross reaction with the host's own tissues and absorption by the graft.

Prevention and modification of the immune reaction

Methods, used to prevent or to modify the immune reaction in experimental and clinical renal transplantation include such diverse procedures as histocompatibility matching, induction of tolerance, splenectomy, thymectomy, lymphoid depletion by chronic thoracic duct drainage, extracorporeal irradiation of the blood, irradiation of either the whole body or the graft and administration of various chemical agents and antilymphocyte serum (ALS). Of these, whole body irradiation and thymectomy are no longer in current clinical use, while extracorporeal irradiation of the blood and lymphoid depletion by thoracic duct fistula are still largely in the clinical experimental stage.

The induction of tolerance is of particular interest to the experimentalist. Specific immunological tolerance (Billingham et al., 1953, 1954, 1955) can be induced in neonatal animals by exposing them to large doses of an antigen before they have developed the capacity to react against it. The animals thereby permanently lose the ability to react against this particular antigen while the ability to react against other antigens is not impaired. It has been demonstrated since, that, under certain experimental conditions, tolerance can be induced in adult animals as well by prolonged treatment with large doses of soluble antigen. For insoluble antigens, the matter is less clear, while injection of lymphoid cells has been either ineffective in this respect or has resulted in chimerism. Sensitization to homografts is probably due to a mixture of antigens, which have not yet been isolated and identified as being important as sensitizing antigens. However, induction of tolerance appears to be a very promising way to alter the immune reaction against human transplants in the future.

Of great practical importance is the technique of histocompatibility testing, which is now used with increasing frequency in clinical transplantation to prevent a strong immune reaction. Using the fact that most histocompatibility antigens are present on the leucocytes, typing procedures have been developed which permit selection of a recipient possessing all known histocompatibility antigens, or all the supposedly strong ones, present in the donor (Ceppelini et al., 1967;

Dausset et al., 1967; Payne et al., 1967; van Rood et al., 1967; Terasaki et al., 1967). In this way, it is possible to select the best compatible donor-recipient combination, resulting in a relatively weak immune reaction after transplantation which can be suppressed readily with immunosuppressive therapy.

At present, the basic therapy in the management of clinical renal transplantation consists of administration of the approved drug Imuran (azathioprine) together with corticosteroids, combined during rejection episodes with actinomycin C. Local irradiation of the graft is sometimes used prophylactically or in treatment of a rejection crisis, its action being probably due to destruction of lymphocytes in the graft (Tinbergen, 1968). In some centres anti-lymphocyte serum has been used in addition to chemical agents.

Drugs known to suppress the immune response have several mechanisms of action. They have in common the capacity to interfere with the production of immunocompetent cells and/or antibody and hence an increased risk of infectious complications is inherent to their use especially with the high doses given in treatment of rejection crises. The antimetabolite Imuran (azathioprine) is an imidazolyl derivative of 6-mercaptopurine. It has the same immunosuppressive properties as 6-mercaptopurine, but it is less toxic. However, liver damage and bone marrow depression are known complications of Imuran therapy. Corticosteroids are effective if given in high doses with the risk of side effects.

The immunosuppressive action of heterologous antibodies, directed against lymphocytes has been first demonstrated in 1963 (Woodruff and Anderson, 1963, 1964). It appeared that ALS was able not only to affect the afferent and efferent pathways of the immune response, but also to erase immunological memory as evidenced by suppression of the second-set reaction. The initial concept that immunosuppressive action resulted from a direct toxic influence upon lymphocytes is not an entirely adequate explanation since it was shown that the lymphopenia resulting from ALS administration is not a prerequisite for immunosuppressive activity. However, it is now generally accepted that only a certain fraction of the peripheral lymphocytes is specifically interfered with. Alternative theories to explain the action of ALS (Levey and Medawar 1966a,b, 1967) include competition (blocking of antigen-reactive sites on lymphocytes by ALS) and blindfolding (interfering with the recognition of

antigen by lymphocytes after ALS treatment). Another hypothesis is called sterile activation: in contact with ALS, non-committed lymphocytes are transformed into blast cells, thereby losing their capacity to develop immune competence towards other antigens. Whatever its action, it has been shown in various animal species, and in man, that ALS can be a very potent immunosuppressive agent. Unfortunately, clinical use of ALS is hampered by the fact that, until recently, no reliable in vitro method was available to test its immunosuppressive potency. Moreover, experiments on ALS have been performed with sera prepared in various species, using cells from different sources as the antigen, which were administered along various routes according to different immunization schedules. As could be anticipated the resulting sera showed wide variation in immunosuppressive potency, and in toxicity (especially towards erythrocytes and thrombocytes). All this, of course, makes it difficult to compare experimental results. Particularly important from the clinician's point of view is the fact that foreign proteins are introduced by ALS treatment with the associated dangers of anaphylaxis and serum sickness, while the formation of antibodies against the agent itself will also lead to loss of immunosuppressive potency. Some of the risks of ALS treatment, as its hemolytic action and the development of thrombocytopenia can be reduced by using the purified IgG fraction (ALG) which represents the active principle and by absorption. However, toxicity may not be ruled out completely by these procedures. Contradictory results have been reported about the effect of purification of ALS in preventing hypersensitivity reactions (Cohen et al., 1970; Lindquist et al., 1969). In human transplantation, the advantage of ALG therapy has been that doses of other immunosuppressive drugs could be reduced, thus avoiding their side effects (Starzl et al., 1969).

Rationale for the experiments

In the Seventh Report of the Human Kidney Transplant Registry (1969), based on 2347 human kidney transplantations, it is estimated that kidneys from related living donors have a 1-year survival of 87% and a 2-years survival of 77% while kidneys from cadaver donors have a 1-year survival of 42% and a 2-year survival of 40%. Probably, figures for patient survival are much higher, because of the common practice to dialyse and retransplant a patient after

transplant failure. Recent results showed significant improvement over those before 1966. However impressive these results are, as compared to the situation during the early days of human renal transplantation, it is evident that there is much to be desired. Apart from the still considerable percentage of failure, it is obvious that the ethical objections to and the limited availability of kidneys from related living donors, make the use of cadaver donors preferable to the use of living donors. However, apart from improvement in storage techniques and selection methods, this would require the development of better and more specific immunosuppressive methods. Besides, experience in renal transplantation is now of great importance in management of other organ transplants, failure of which might present much more urgent problems than failure of a kidney graft. For instance, it is now realized that immunosuppression as used in renal transplantation is not equally effective in heart transplantation (van Bekkum et al., 1969, 1970; Second World Symposium on Heart Transplantation, 1969). This underlines the importance of continued studies on the action of immunosuppressive agents.

The experiments which will be discussed below are concerned with immunosuppression: Imuran and Prednisolone as the agents which are universally used in clinical practice and ALG (antilymphocyte globulin) which is now at the centre of interest, both in experimental and in clinical transplantation, have been compared in a controlled trial. It is known that both the incidence and the severity of rejection crises decrease when an allograft has resided in the host for some time. Therefore we investigated the action of immunosuppressive agents during a restricted period early after transplantation, which period is apparently of crucial importance. Because of qualitative and quantitative differences between grafts of skin and kidney, as discussed above, skin grafts were considered inadequate for the present investigation. Sofar, renal transplantation experiments have been performed mainly in larger animals such as dogs. Results from these experiments are difficult to compare for several reasons: most series produced are small and there is considerable variation in technique, maintenance and immunosuppressive methods used, as well as drug doses given. Most important, however, is the variation in histocompatibility difference between randomly selected non-inbred members of a species, which makes the unmodified rejection time greatly variable. These drawbacks, which also operate in human

transplantation, are largely overcome by the use of inbred strains of small laboratory animals now available. Since a technique for orthotopic renal transplantation in the rat* has been developed it is possible to produce large series of allogeneic transplantations in which rejection time is almost constant while isogeneic transplantations can be performed as controls. Moreover, rats are relatively cheap and easy to maintain. A disadvantage is the difficulty of the operation technique causing a high percentage of technical failures. Several reports by now have demonstrated the usefulness of the rat model system for renal transplantation studies (Feldman and Lee, 1967; Feldman et al., 1968; French and Batchelor, 1969; Gardner et al., 1968; Guttmann et al., 1967a,b, 1968, 1969a,b,c,d; Guttmann and Lindquist, 1969; Hollander, 1970; Lindquist et al., 1968a,b,c; Murray, 1969; Sakai et al., 1968, 1969; Salaman, 1968; Spong et al., 1968; Stuart et al., 1968a,b; Taguchi et al., 1968; Tinbergen, 1968; White et al., 1969; White and Hildemann, 1968, 1969). The technique for orthotopic renal transplantation in the rat as described by Fisher and Lee (1965) has been introduced into the Radiobiological Institute of the Organization for Health Research TNO in 1966 by Tinbergen who studied the effects of continuous treatment with some immunosuppressive agents on graft survival (Tinbergen, 1968). He observed marked prolongation of survival time by treatment with Imuran. Treatment with either Prednisolone or local irradiation of the graft was less effective. Mean survival time in 43 untreated allografts was 12 days (9 - 24 days) if one exceptional case of long survival (55 days) is omitted. The present study can be regarded as a continuation of this work, with special emphasis on a more precise evaluation of the condition of the grafted kidney under immunosuppressive treatment. The investigation comprises the following: groups of allografted rats, treated for no longer than 3 weeks after transplantation with various doses and combinations of Imuran, Prednisolone and ALG were compared with untreated allografted and isografted rats with respect to 50-day survival, renal function and transplant histology. The period of 3 weeks after transplantation for immunosuppressive treatment was chosen because the majority of rejection crises was known to occur during that period.

* The term orthotopic transplantation is used as opposite to transplantation in the neck, performed in earlier experimental work, and does not imply that the position of the grafted kidney is really orthotopic.

The purpose of the present investigation is mainly:

- to evaluate the usefulness of some serial function tests in assessing the condition of a grafted kidney in the rat as related to 50-day survival and microscopical appearance of the graft;
- to compare under controlled conditions the efficacy of immunosuppression obtained with different regimens, especially with regard to ALG;
- to study the effect of short-term immunosuppressive therapy on renal graft rejection.

MATERIALS AND METHODS

Experimental animals

The rats used in the experiments were derived from 2 inbred strains, Wag/Rij and BN/Bi, maintained at the Radiobiological Institute of the Organization for Health Research TNO Rijswijk. Since 1960 both strains were bred under specific pathogen free conditions by rearing of young animals in isolated quarters after delivery by cesarean section of the first few individuals. The Wag/Rij strain is an inbred Wistar albino strain, originally obtained from Glaxo Laboratories, Greenford, Middlesex, England and bred by brother x sister matings for 29 generations since 1953 at Rijswijk. Histocompatibility between members of this strain was frequently confirmed by reciprocal skin grafting, showing permanent takes in 100%. Wag/Rij males, 5 - 8 months of age and ranging in weight from 250 - 350 g were recipients of kidney grafts as well as kidney donors in the isogeneic transplantations. The other inbred strain, BN/Bi, was supplied in 1963 by Microbiological Associates Inc., Washington and also maintained by brother x sister mating. This strain was started in 1958 by Silvers and Billingham from a brown mutation obtained from King and Aptekman. Subsequent inbreeding has been directed towards developing histocompatibility resulting in 100% skin graft acceptance. Males from the BN/Bi strain, aged 3 - 6 months and weighing 200 - 300 g, were used as donors of kidney allografts. BN/Bi and Wag/Rij rats differ at the strong H-1 histocompatibility locus (Štark and Křen, 1969; Štark et al., 1969). Skin grafts between the two strains are consistently rejected within 10 - 12 days.

Rats were maintained individually or in groups of 2 in autoclavable, transparent polycarbonate cages. Temperature and

humidity of the room were kept constant at 21°C and 65% respectively. Saw dust, used as bedding material, was changed twice weekly. Tap water and food pellets (Hope Farms, Holland, crude protein content 25%) were supplied at libitum. To prevent spread of bacteria the drinking water was acidified up to pH 3 by adding HCl. Cages were sterilized twice weekly by steam heating for 20 min. at 120°C, while drinking bottles and tubes were sterilized daily for 1 hour at 120°C by dry heat.

Surgical technique

The microvascular technique for orthotopic renal transplantation in the rat as described by Fisher and Lee (1965) was used. The method has been slightly modified since Tinbergen introduced it into the Radiobiological Institute in 1966 (Tinbergen, 1968). Briefly, the procedure is as follows. After an intraperitoneal dose of Nembutal (Abbott, 10% solution in saline) containing 4 mg of Na pentobarbital/100 g body weight, duration and depth of narcosis was easily controlled by open drop ether administration during operation. After opening the abdomen of the donor animal the right kidney, together with its renal artery and vein are mobilized. The ureter is then freed and transsected near the bladder. After injection of Heparin 1500 I.U. (Vitrum, Stockholm) into the aorta, the kidney is removed from the body in such a way that elliptical cuffs of aorta and vena cava are left at the end of the renal vessels. The kidney is then kept at 4°C in Tyrode solution. Donor kidneys showing double arteries, evident hydronephrosis or dilatation of the ureter, are discarded. The aorta and vena cava of the recipient animal are clamped together below the renal vessels with a Satinsky-like curved clamp. The clamping is done in such a way as to isolate part of the vessel from the circulation. Small elliptical openings are then made in this part, to which the cuffs of the transplant vessels are anastomosed (fig.1). Bleeding at the site of anastomosis after release of the vascular clamp is controlled by pressure with a piece of folded gauze for 20 - 40 seconds. The total period of renal ischemia ranged between 20 and 40 minutes, the average being 36 minutes.

A small opening is then cut in the mid-dorsal part of the bladder. The ureter tip is taken and drawn into the bladder through this opening by means of a stitch through the low-dorsal part of the bladder wall. After the ureter is secured by this stitch, the bladder opening is

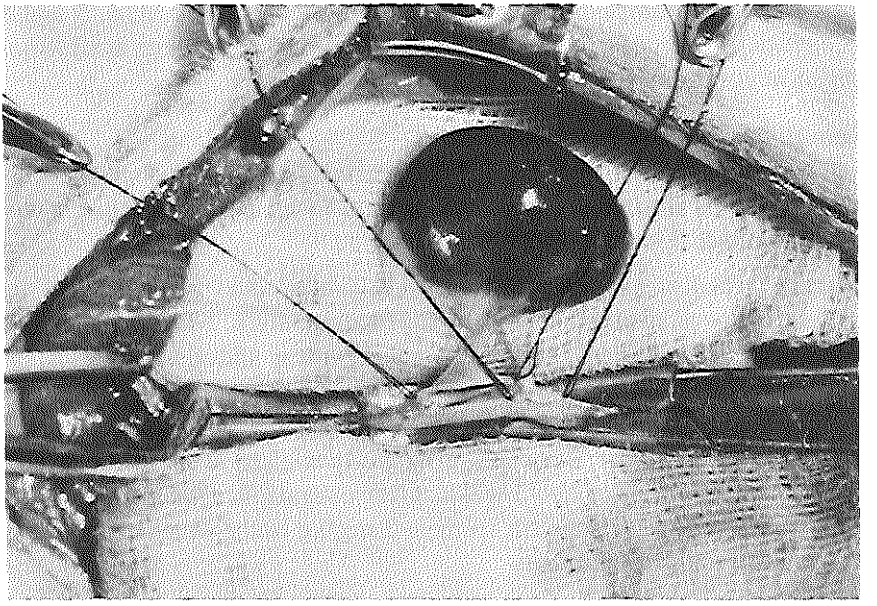


Fig.1 Anastomosis of the renal vessels. Cuffs of aorta and vena cava, left at the end of the renal artery and vein are sutured to elliptical openings in the recipient aorta and vena cava. The first stitches of the continuous sutures at both sides of the cuffs are shown. Part of the aorta and vena cava are clamped off from the circulation. In this case an accessory clamp has been used (the left one in the picture). The ureter is still loose.

closed. To facilitate differentiation between technical failure and rejection of the transplant, both kidneys of the recipient are finally removed. Atraumatic silk (Ethicon, no. 7-0) is used for the anastomoses of ureter and vessels. In general the whole procedure is completed within 2 hours.

Seven days after transplantation an open wedge biopsy was taken from the mid-lateral part of the transplanted kidney under ether anaesthesia.

Antibiotic treatment

All animals (unilaterally nephrectomized controls included) were treated with antibiotics for 8 days after operation according to a scheme that proved to be most effective in previous experiments. Penicillin and streptomycin were given i.m. on day 0, 1, 7 and 8, 10.000 I.U. and 10 mg resp. (Retromypen S, Mycofarm, Delft, Hol-

land) and chloramphenicol 10 mg i.m. daily on day 2, 3, 4, 5 and 6 (Gloveticol, Mycofarm, Delft, Holland). In no case were antibiotics given beyond day 8.

Immunosuppressive treatment

Imuran, Prednisolone and ALG in various doses and combinations were used as immunosuppressive agents. In all cases, treatment was discontinued, 3 weeks after operation.

*Imuran** (azathioprine) solutions were prepared as follows: 500 mg of Imuran was suspended in 10 ml of saline, then, while stirring and dropwise, 1.81 ml of a 1 N NaOH solution was added to pH 9. After adding saline to a total volume of 50 ml, pH was brought to 8, if necessary, with 1 N HCl. Solutions were used for no longer than one week. Imuran either 2 or 4 mg/kg body weight was administered daily intraperitoneally for 3 weeks after operation. One group of rats was treated for 4 weeks with Imuran 2 mg/kg body weight, starting 1 week before transplantation.

Prednisolone sodium succinate (Organon, Oss, Holland) was used as a 2 mg/ml solution in distilled water and prepared just before use. Rats treated with Prednisolone received a daily dose of 4 mg/kg body weight subcutaneously for 3 weeks, starting the day after operation.

*Antilymphocyte globulin*** (rabbit anti-rat thymocyte serum, containing highly purified IgG after purification) was given in 4 weekly doses of 19 mg IgG/animal subcutaneously, the first dose being administered 1 week before operation.

ALS was produced as shown schematically in fig.2. Both male and female rabbits of 7 - 10 kg, randomly bred and shown to be without naturally occurring toxins for rat lymphocytes were injected subcutaneously every 10 days with thymocytes, obtained from 6 week old Wag/Rij rats and suspended in Tyrode solution. After 4 doses of 10^9 thymocytes, 100 ml of blood was taken from the ear vein. A booster dose of thymocytes was given 10 days later and after a further 10 days the rabbits were bled out. Serum from tap I and tap II were pooled, yielding for subsequent purification a total of

* Imuran was made available by Burroughs Wellcome, London, through the courtesy of Dr. D.A. Long.

** Purification of ALS, as described, was kindly performed by Miss A.W.M. Appelman and Mrs. M.A. Graver-van der Vring.

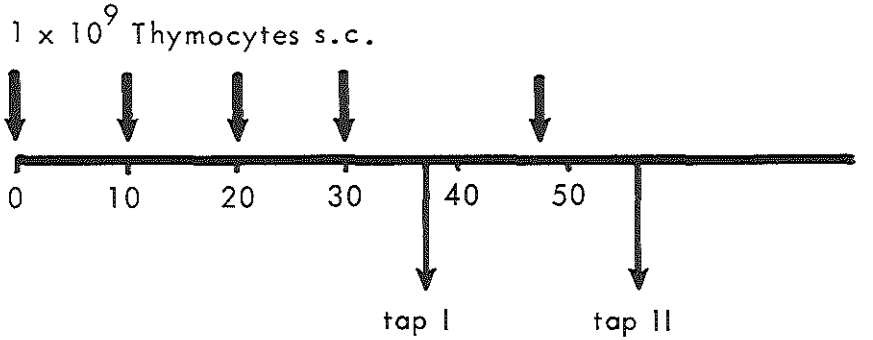


Fig.2 Immunization schedule for preparation of rabbit anti-rat thymocyte serum (ALS). Serum from tap I and tap II were pooled for isolation of the IgG fraction.

± 150 ml serum per rabbit in which γ-globulin constituted 12% of the total protein, as determined by electrophoresis. To precipitate the globulin fraction, $(NH_4)_2SO_4$ was added to the serum to a final concentration of 45%. The precipitate was suspended in distilled water and dialyzed for 24 hours against running tap water and subsequently for 36 hours against 0.01 M phosphate buffer in 0.82% NaCl, pH 7.4. The dialysate was then passed through a column of DEAE Sephadex A-50 anion-exchanger (Pharmacia, Uppsala, Sweden). The first fractions eluted from the column by the NaCl/phosphate buffer contained almost pure IgG, which was checked by electrophoresis on cellulose acetate and by immune electrophoresis. Only those fractions containing 97% or more IgG were used. The final preparations contained ± 19 mg IgG/ml. The whole procedure of purification was performed at 4°C to prevent growth of bacteria in the serum. Lymphocytotoxic titers of the crude preparations were 1:2000 (ALG I) and 1:128 (ALG II).

In the skin grafting experiments for testing ALS, best results were obtained if treatment was started before transplantation: 4 weekly doses of 12.5 mg IgG resulted in mean skin graft survival times of 116 days for ALG I (17, 18, 20, 205 and 230 days) and 66 days for ALG II (18, 73, 83 and 90 days).

Histological technique

Kidney biopsies taken on day 7 after transplantation and material obtained at autopsy were available for microscopical examination. In addition to the kidney, ureter and bladder, heart, lung, liver,

pancreas, gut, thymus, spleen, abdominal lymph node and sternum were studied. The tissues were fixed in 4% buffered formalin for a minimal time of 24 hours, then processed in an Autotechnicon 2A (Technicon Company, Chauncey, New York, U.S.A.) and imbedded in paraffin. Sections of 5 μ were cut and routinely stained with Hematoxylin-Phloxin-Saffron (H.P.S.). In some cases sections of kidney tissue were cut at 1 μ and stained with H.P.S., periodic acid-Schiff (P.A.S.) and periodic acid silver methenamine (P.A.S.M.).

Transplant function*

Transplant function was assessed from 1 week before transplantation onward by serial determination of the following parameters.

1) *Body weight*, as a general index of the health of the animal, was determined twice weekly.
2) *Hematocrit values*, reflecting possible toxic effects of immunosuppression and uremia, were also determined twice weekly. After keeping the rats for 10 minutes under an infra-red lamp, blood samples were obtained by cutting the tip of their tails. Heparinized microhematocrit tubes containing 0.075 ml of blood approximately, were centrifuged for 5 minutes at 11.500 r.p.m. before reading (International Equipment Company, Boston, Mass., U.S.A.). In 200 untreated rats a normal hematocrit value of 50% was found before transplantation, with a standard deviation of 3.6%. Standard deviation of the differences between duplicate measurements (60 pairs) was 1.3%.
3) *Blood urea* (B.U.) was measured twice weekly to assess the excretory capacity of the kidney, using the serum after hematocrit reading. Samples of 0.02 ml of serum were diluted to a volume of 1.0 ml with 0.9% NaCl. Urea concentration was determined on a Technicon autoanalyzer (diacetyl monoxime reaction)**. Mean normal value in 200 rats was 60 mg%, standard deviation 23%. In 100 duplicate determinations, standard deviation of the differences was 7%.

* Although body weight, blood pressure and hematocrit value are not direct parameters of renal function, the listed determinations will be referred to as transplant function for convenience.

** Blood urea determinations were performed at the Analytical Centre of the Central Laboratory of TNO, Delft (Head Drs. W. Brouwer).

4) *Urine osmolality* reflecting the concentrating capacity of the kidney was measured twice weekly in freshly voided specimens of morning urine, obtained between 9 and 10 a.m. If voiding did not occur spontaneously, it could be induced in most cases by squeezing the tail. Covering the test tubes with parafilm prevented evaporation. Osmolality values were determined in 0.2 ml samples of urine using a freezing point osmometer (Advanced Instruments, Inc., Mass., U.S.A., model 31 La). Reading was always done in duplicate. A mean normal value of 2190 milliosmols/kg H_2O was found (200 samples) with a standard deviation of 23%. Standard deviation of the differences between duplicate measurements was 1.5% (100 pairs).
5) *Urine production per 24 hours* was determined once weekly because of its relationship to urine osmolality in removing a certain solute load. Rats were placed in metabolic cages with free access to food and water. Mean normal value was 5 ml (40 rats) with a standard deviation of 30%.
6) *Systolic blood pressure* was measured to detect the development of hypertension during rejection episodes. Blood pressure determinations (fig.3) were performed every week in unanesthetized rats using a modification of the method described by Weinman

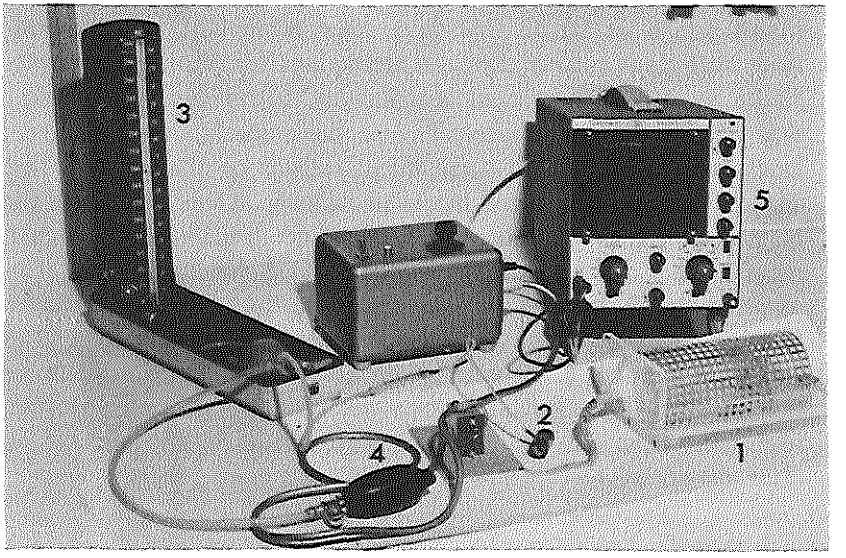


Fig.3 Equipment for blood pressure determination in the rat. For description see text.

(Weinman et al., 1960; Ben-Ziv et al., 1964). Rats were warmed under infra-red lamps for 10 - 15 minutes to ensure adequate blood flow through the tail and then immobilized in a rat holder (1). A metal tubular occluding cuff (2) (7/16" internal diameter, E and M Instrument Company Inc., Houston, Texas) connected with an ordinary blood pressure manometer (3), was placed proximally around the animal's tail. The distal part of the tail was passed through a cylindrical opening in a metal box (4), inside which a lamp and a light dependent resistor (LDR) were placed on opposite sides of the tail. Blood pulses in the tail, as detected by the LDR, were amplified and made visible as a continuous wave on an oscilloscope (5). Values for systolic blood pressure were read on the manometer after inflating the cuff up to the point where the waves flattened out to become a straight line. Three successive blood pressure readings, each taken to the nearest 5 mm Hg were averaged to yield the final value. It was found that, if handled gently, the animals were not excited by the procedure. Mean normal value in 100 rats was 125 mm Hg with a standard deviation of 10 mm Hg. Standard deviation of the differences between duplicate measurements was 6% (100 determinations).

7) *Intravenous pyelography*. Rats were sacrified for microscopical investigation at 50 or 100 days after transplantation, at which time sufficient information on transplant function was expected to be available to warrant conclusions. Intravenous pyelography was performed immediately before the animals were sacrified to assess the incidence of anatomical lesions of the urinary tract. Water was withheld for 5 hours previously. After anesthesia with Nembutal (Abbott, diluted 1:5 in saline, 5 ml/kg i.p.), each rat received a 1 ml dose of Urografin 76% (Schering A.G., Berlin), injected slowly into a tail vein. Rats were then taped on a cassette (Kodak 24 x 30 with high definition screen) and roentgenograms were obtained at 5, 10 and 15 minutes after injection of the contrast dye, using a portable roentgentube (Enraf, Delft, Holland, focus-film distance 83 cm, exposure with 55 kV and 25 mA during 0.16 sec.).

Statistical evaluation of results

In the Appendix all averages ($\bar{x}$) of the values x obtained in dif-

ferent groups of rats are given separately for each parameter, together with the number of animals from which the average was derived (n) and with the standard deviation (s) calculated by the formula

$$s = \sqrt{\frac{\Sigma(\bar{x} - x)^2}{n - 1}}$$

The average values were used to prepare the figures on renal function (figs.5 - 18).

Average values obtained during the period from the third to and including the seventh post-transplantation week were used for statistical analysis. Thus, 10 values for weight, hematocrit, blood urea and urine osmolality and 5 values for blood pressure and 24-hour urine production were available for each group, listed in the Appendix as no. 7 - 16 and no. 4 - 8, respectively. Two groups in turn were compared for one parameter. Pairs of 2 corresponding average values were tested consecutively using the test of Welch (1947) after which the t values were used in a combination test (de Jonge, 1964). This included the following steps:

A.

Welch:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1^2} + \frac{s_2^2}{n_2^2}}}$$

with a number of degrees of freedom:

$$f = \frac{\left[\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right]^2}{\frac{\left[\frac{s_1^2}{n_1}\right]^2}{n_1 - 1} + \frac{\left[\frac{s_2^2}{n_2}\right]^2}{n_2 - 1}}$$

B. Application of the combination test requires that the t value and f value found are converted to the standard normal variable Z. In order to do so, first a p_1 value is read from a table of Student's t values (Documenta Geigy, 1969) and then Z can be found from a table of the normal probability integral (Fisher and Yates, 1953, Table VIII, in which the symbol Z is expressed as x). A positive or negative sign of t is conserved.

Instead of using these tables, we have calculated Z directly from t and f, using a formula, developed by Wijnans (1969) which gives a good approximation if $f \geqslant 5$:

$$Z = t - \frac{0.588\,(t^3 + t)}{2.4195\ f + t^2}$$

C. Finally, the number of Z values (k) was combined:

$$Zc = \frac{\Sigma Z}{\sqrt{k}}$$

D. From a table of the normal distribution (Fisher and Yates, 1953, Table I) it appears that the critical values for Z (= x) at a 1% level of significance are ± 2.58.

Thus whenever treatment groups are reported to be different for a certain parameter, it means that the hypothesis, that there are no differences between the two groups of means, was rejected after two sided testing at a level of significance $x = 0.01$.

Calculation of statistical significance was performed only in those cases which were considered directly relevant to the argument. Results of statistical evaluation of differences between treatment groups is given as a survey in table IV.

RESULTS

General

Renal transplantation was performed in a total of 282 cases, including allogeneic and isogeneic transplantations. During transplantation and within 24 hours thereafter, 85 rats died. Apart from mortality during anesthesia, the main causes for this early failure were bleeding and obstruction at the site of anastomosis of the great blood vessels. It was noted that treatment with Imuran, given before operation, affected this early mortality. As is shown in table I the percentage of failure within 24 hours after transplantation was almost twice as high in the group that was pretreated with Imuran in comparison to the groups which until then had received either no treatment or one dose of ALG.

Rats, dying during the period of one to fifty days after transplantation, were separated into two groups. The first group comprises those rats in which a definite cause of death, such as infection or technical failure could be pointed out. They were excluded from the experiment. In the second group, no definite cause of death could be found, mortality therefore was attributed to rejection of the transplant. In the paragraph on renal function, the values of these animals have been included in the results obtained with the different treatment schedules.

The kind of complications found in the 102 rats making up the first group are shown in table II. Disconnection of the ureter at the site of implantation in the bladder and obstruction of urine flow by blood clots or inspissated protein accounted for death in 50% of the cases. The incidence of vascular obstruction was rather low. However, technically unsuccessful anastomoses of the great vessels gener-

TABLE I

EFFECT OF PRETREATMENT ON EARLY MORTALITY OF ISOGRAFTED AND ALLOGRAFTED RATS

Treatment	total no.	mortality within 24 hours no.	%
Untreated	165	46	28
ALG 1x19 mg/rat	86	23	27
Imuran 6x2 mg/kg	31	16	52*
Total	282	85	33

* Increased early mortality is statistically significant (chi square test, $p < 0.05$)

TABLE II

IDENTIFIED CAUSES OF DEATH IN 102 ISOGRAFTED AND ALLOGRAFTED RATS DYING BETWEEN 1 AND 50 DAYS AFTER TRANSPLANTATION

Ureter disconnected	30
Ureter obstructed	21
Vascular anastomosis obstructed	17
Infection	11
Accidents	16
Other technical failure (death before day 5)	7

ally caused massive hemorrhage and death within 24 hours after operation. Infection was regarded as the cause of death only in cases of extensive pyelonephritis and/or septichaemia. Accidents included death from bleeding or narcosis at biopsy, as well as some cases dying from injection injury during intraperitoneal administration of Imuran. As rats after bilateral nephrectomy can live for 4 - 5 days, the animals dying before day 5 were considered to be technical failures, although in these case no cause of death could be established.

The incidence of complications and of rejection during the period of one to fifty days after transplantation is shown in fig.4. The upper 2 graphs show that death from allograft rejection in treated and untreated rats occurred during the same period. Note that deaths from rejection did not occur from day 22 onward, when immunosuppressive treatment was discontinued. The 2 lower graphs in fig.4 show the incidence of complications in allografted and isografted rats. It is clear that up to the eleventh day, the mortality pattern was very much the same in both groups, while thereafter, complications continued to occur in the allografts and not in the isografts. The explanation of this is that infection as a cause of death was found almost exclusively among allografted animals following treatment with various immunosuppressive drugs. In 8 out of 11 allografted rats infection was the cause of death after the eleventh day, which could be attributed to the immunosuppressive treatment and for that reason could hardly be avoided.

After exclusion of the 85 rats dying during the first 24 hours after transplantation and of the 102 rats dying from complications a total number of 95 transplantations remained for evaluation.

Table III shows the various treatment groups together with the survival data at 50 days after transplantation. Survival in the isografted group was 100%. Quite unexpectedly, one of the allografted rats survived for 50 days without treatment. In the 3 groups treated with Imuran alone, less than 50% of the rats were alive at 50 days. Survival is appreciably higher in the last 6 groups, treated with Imuran and Prednisolone, with ALG alone and with a combination of ALG and Imuran. Considerable differences were noted in effectiveness between the two batches of ALG (ALG I and ALG II) which justifies a separate presentation of the groups treated with ALG I and II.

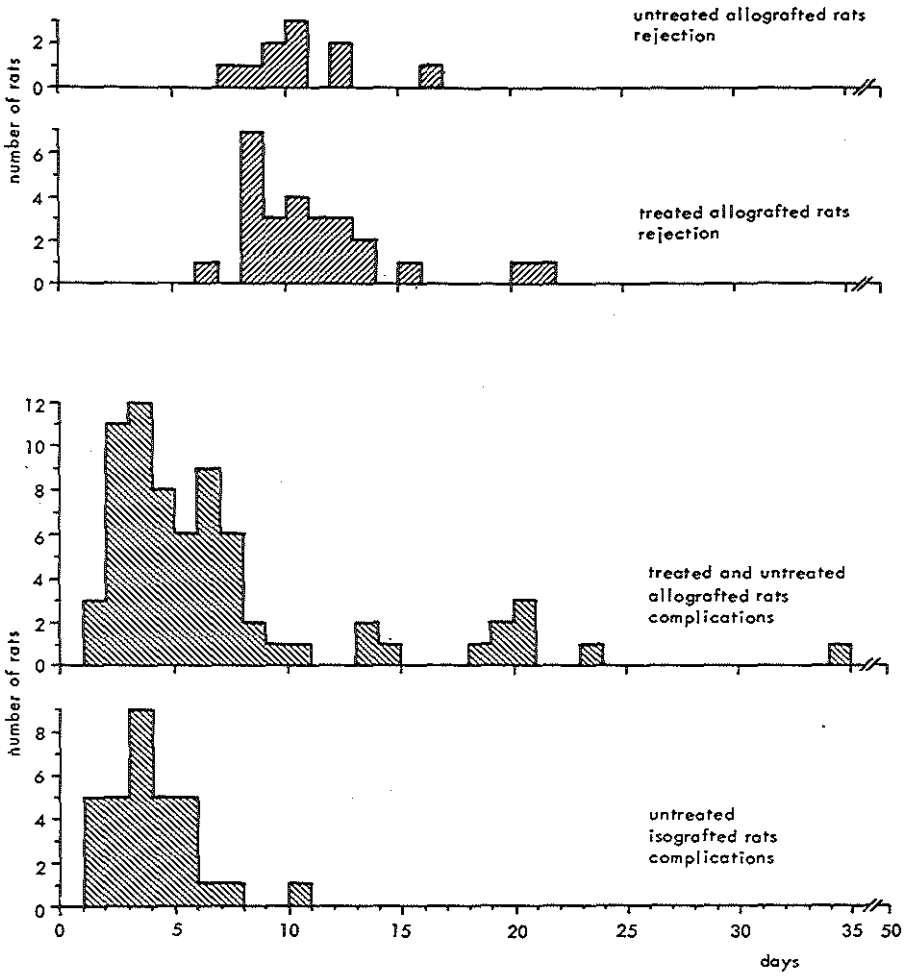


Fig.4 Mortality during the first 50 days after transplantation. The incidence of death from graft rejection and from various complications have been compared.

TABLE III

TREATMENT AND 50-DAY SURVIVAL OF 95 ISOGRAFTED AND ALLOGRAFTED RATS

	TREATMENT		50-DAY SURVIVAL	
			surviving no. / total no.	percentage
Isografted rats	Untreated		10/10	100
Allografted rats	Untreated		1/11	9
	Imuran*	4 mg/kg	3/9	33
	Imuran*	2 mg/kg	6/13	46
	Imuran pretreated°	2 mg/kg	4/10	40
	Imuran* + Prednisolone+	2 mg/kg 4 mg/kg	8/10	80
	ALG I×	19 mg/rat	7/7	100
	ALG II×	19 mg/rat	5/7	71
	ALG I× + Imuran*	19 mg/rat 4 mg/kg	4/5	80
	ALG II× + Imuran*	19 mg/rat 4 mg/kg	3/5	60
	ALG II× + Imuran*	19 mg/rat 2 mg/kg	8/8	100

* Imuran daily i.p. for 3 weeks after transplantation

o Imuran daily i.p. for 4 weeks starting 1 week before transplantation

\+ Prednisolone daily s.c. for 3 weeks after transplantation

x ALG weekly s.c. 4 doses starting 1 week before transplantation

Transplant function*

All the figures showing function of the transplant are designed in the same way. Urine production/24 hr., blood urea (B.U.), hematocrit and urine osmolality are expressed as percentages of values found in untreated rats before transplantation. Weight is expressed as percentage of pretreatment value and blood pressure is in mm Hg. Urine production and blood pressure were measured once a week; the other determinations were carried out twice weekly. Half of the animals alive were killed at random 50 days after transplantation. In the remaining animals, the function studies were continued fortnightly until they were sacrificed at 100 days after transplantation. The results obtained after day 50 are not shown in the figures but can be found in the Appendix.

Investigation of toxicity of immunosuppressive agents

First of all it was investigated whether the various immunosuppressive regimens had any direct effect on the parameters used to analyse renal function. For this purpose 6 groups of 4 rats each were unilaterally nephrectomized and, with the exception of 1 control group, were given immunosuppressive treatment. Parameters of renal function in the untreated group were compared to those of the other 5 groups, each of which received one of the immunosuppressive regimens as used in the transplantation experiments. This is shown in figs.5 and 6. In all the groups there was a temporary effect of the operation on weight and hematocrit reading, while blood pressure tended to be at a somewhat higher level than normal. One of the batches of ALG (ALG II) was probably slightly toxic as was shown by a depression of hematocrit values to 75% of normal during the first week after operation (fig.6). Apart from this no appreciable differences between treated and untreated animals were noted.

Isografts

In fig.7 a comparison is made between the isografted group and the unilaterally nephrectomized group. Neither of the groups received immunosuppressive treatment. It is clear that kidney isografts were functionally inferior to intact kidneys. All parameters except

* Although body weight, blood pressure and hematocrit value are not direct parameters of renal function, the listed determinations will be referred to as transplant function for convenience.

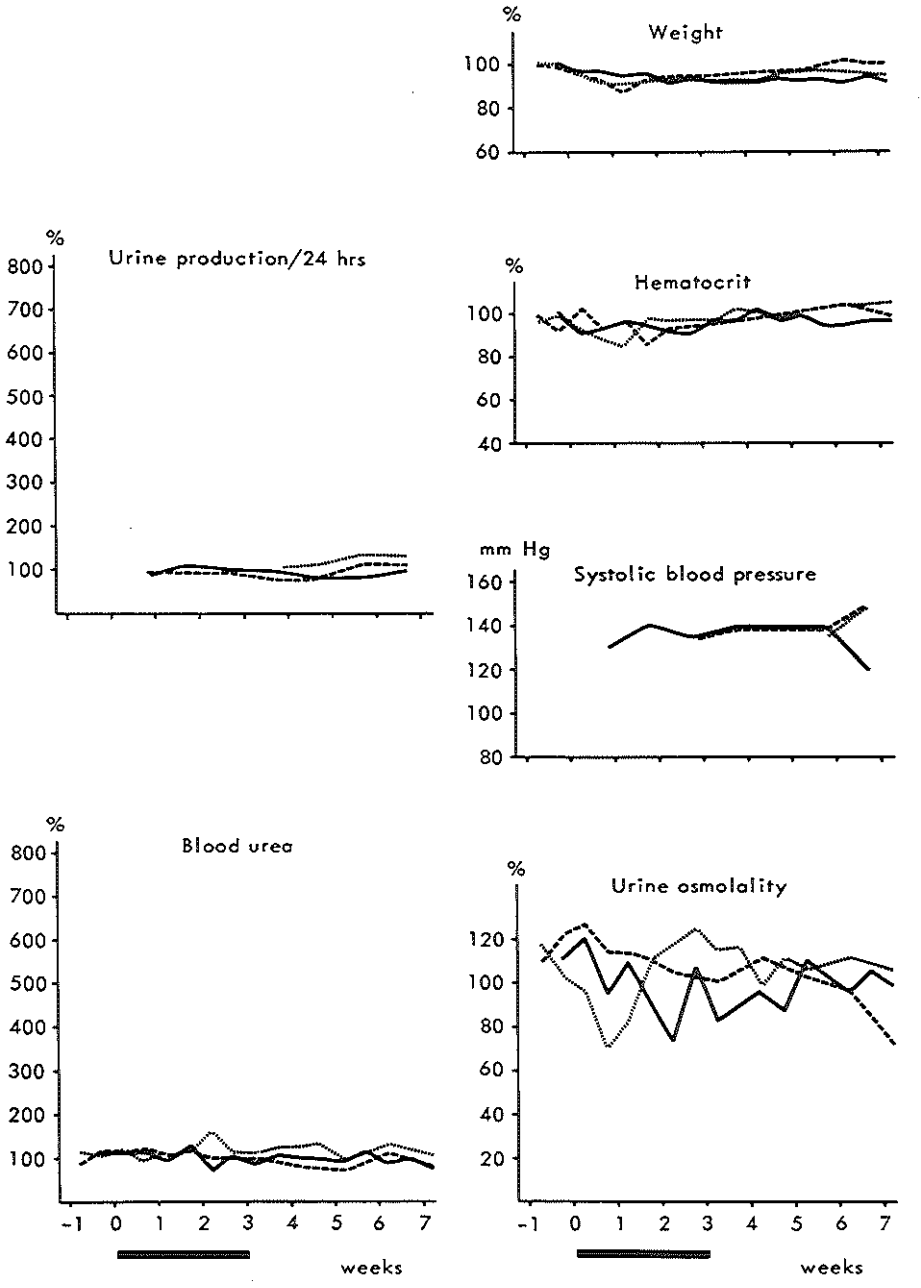


Fig.5 Effect of immunosuppressive treatment on the parameters used to analyse renal function. Groups consist of 4 unilaterally nephrectomized rats. Groups receiving Imuran 4 mg/kg (broken lines) and Imuran 2 mg/kg + Prednisolone 4 mg/kg (dotted lines) are compared with an untreated control group (solid lines). All parameters, except blood pressure are expressed as percentages of values found in untreated rats. The bars in the figure indicate the period during which Imuran and Prednisolone were given.

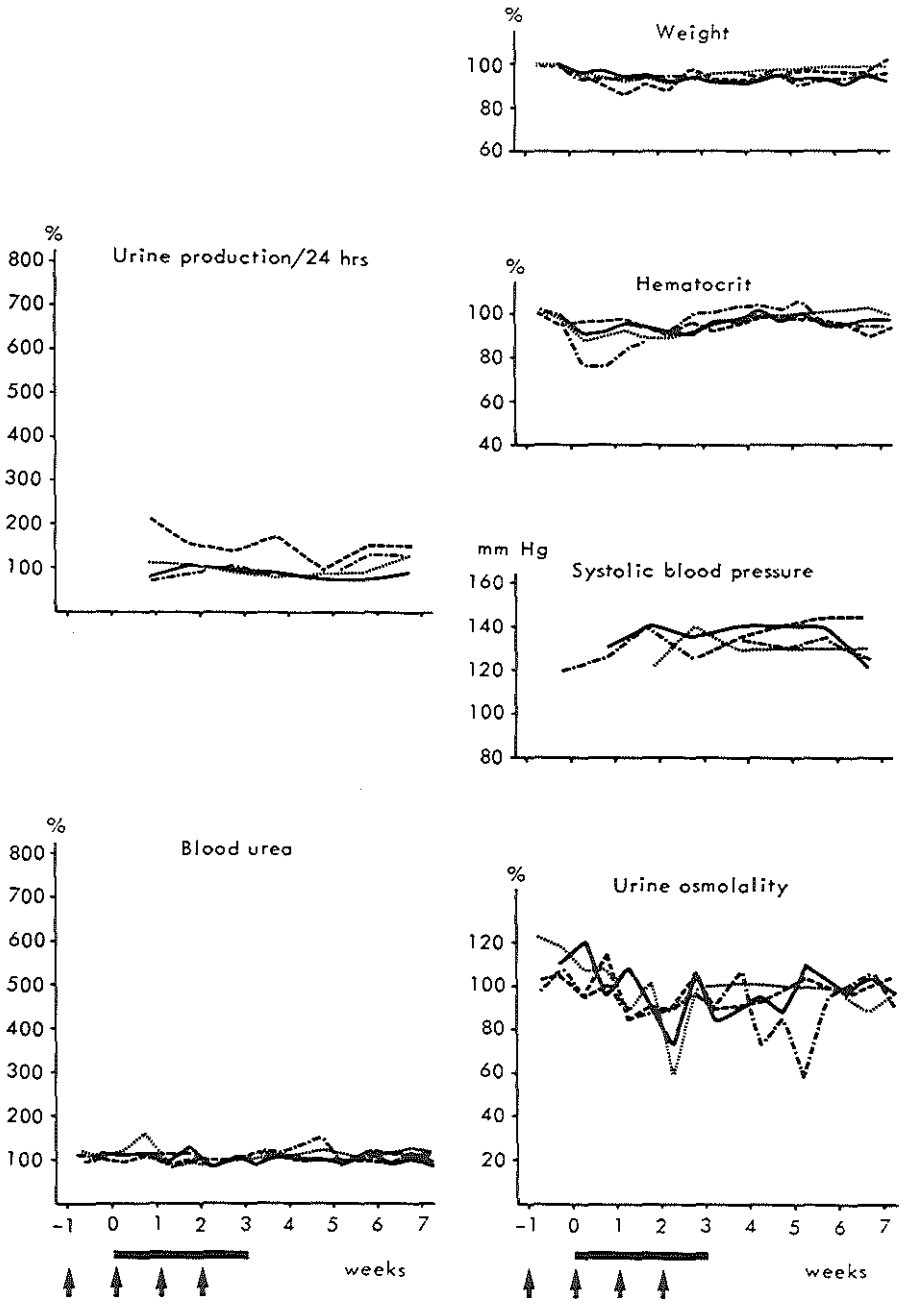


Fig.6 Effect of immunosuppressive treatment on the parameters used to analyse renal function. Groups consist of 4 unilaterally nephrectomized rats. Groups receiving ALG I 19 mg/rat (broken lines), ALG II 19 mg/rat (dot and dash lines) and ALG I 19 mg/rat + Imuran 4 mg/kg (dotted lines) are compared with an untreated control group (solid lines). All parameters except blood pressure are expressed as percentages of values found in untreated rats. The arrows in the figure indicate the days on which ALG was administered, the bars indicate the period during which Imuran was given.

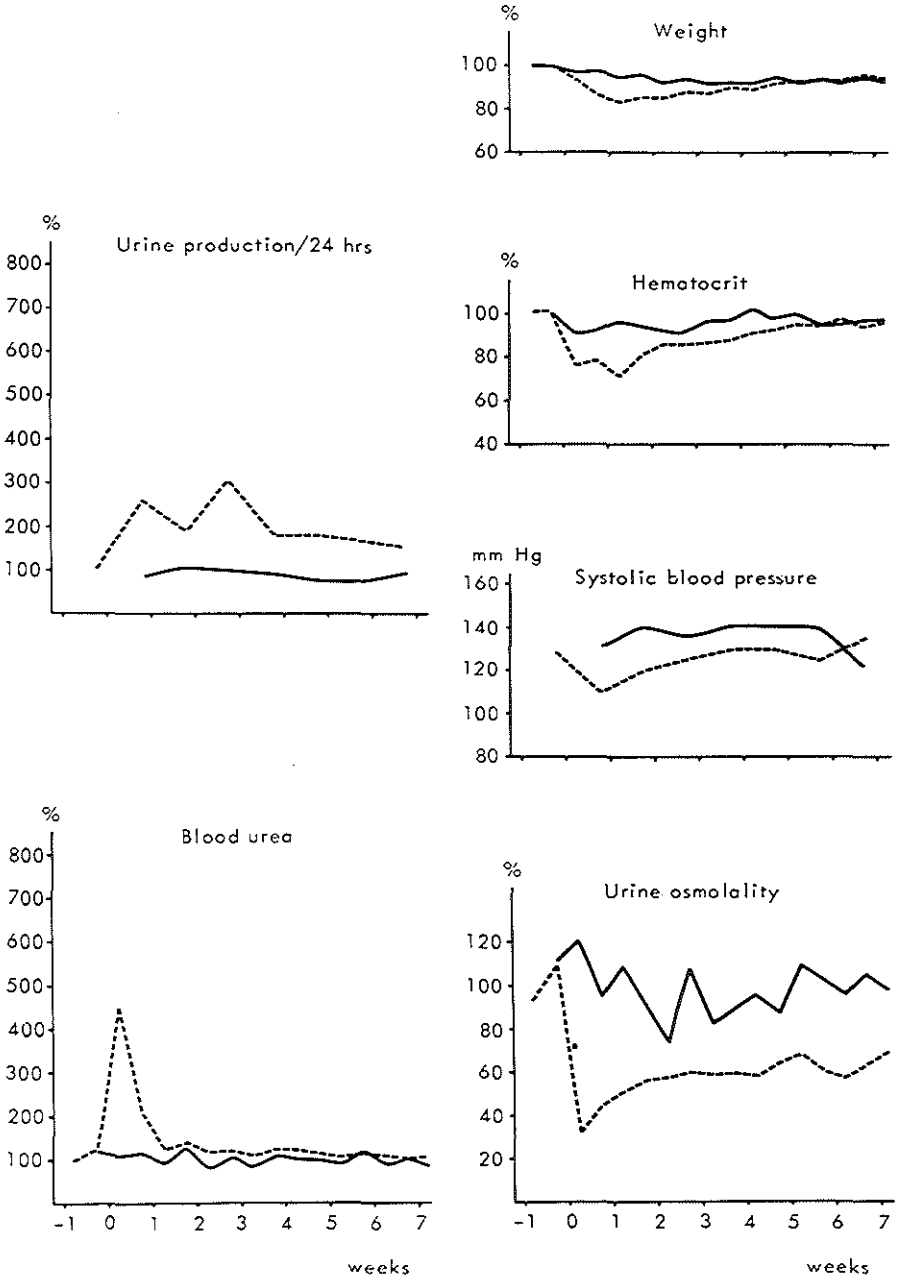


Fig.7 Comparison of function of untreated isografts (broken lines) with untreated unilaterally nephrectomized controls (solid lines). The values found in the isografted group will be shown as broken lines in the following figures for comparison.

blood pressure were significantly different. In the isografts, a high peak of B.U. was seen during the first week after transplantation and the decrease of weight and hematocrit values was more pronounced. However, practically normal values were found at 50 days. In contrast, urine osmolality, after a sharp initial drop, was only restored to a level of 60 - 70% of normal. Accordingly there was an increase in urinary output. Nevertheless, isograft function has been considered as the best result obtainable with the surgical procedure employed. Therefore, the isograft values are included in all the following figures for comparison with the various allografted groups.

Allografts

Fig.8 shows the parameters of function in the untreated allografted rats. As can be seen, impairment of function was initially the same in this group as in the isograft series, but deterioration was progressive, resulting in death of the animals in 2 weeks. The values of the untreated allografted rat, still alive at 50 days, are shown separately in fig.9. There were two very high peaks of B.U. during the first and second week after transplantation but eventually it settled down at about 300%. The bad general condition of this animal was illustrated by the severe drop in weight and hematocrit values, which tended to rise only slowly after 2½ weeks. At the same time urine production rose enormously. Urinary output per day once even exceeded 60% of the animal's body weight. This rat was exsanguinated at day 50 at which time serum osmolality was found to be 329 m Osm/l (normal serum osmolality in Wag/Rij rats is 301 m Osm/l). This osmolality value would correspond to a urinary osmolality of 15% on the graph. However, osmolality of the urine was at a constant level of 10%. Thus, the ratio of urine osmolality to serum osmolality was much less than 1, which might explain the long survival of this animal. The reason for the high water uptake is not clear.

The next group (fig.10) was treated with Imuran 4 mg/kg. Three out of 9 rats were alive at day 50 when two of them were killed, the last one died at day 80 from graft rejection. As compared to the isografts, renal function in this group was severely impaired. Weight and hematocrit values were not restored to normal. Hypertension developed in addition.

Treatment with a lower dose of 2 mg/kg Imuran resulted in a

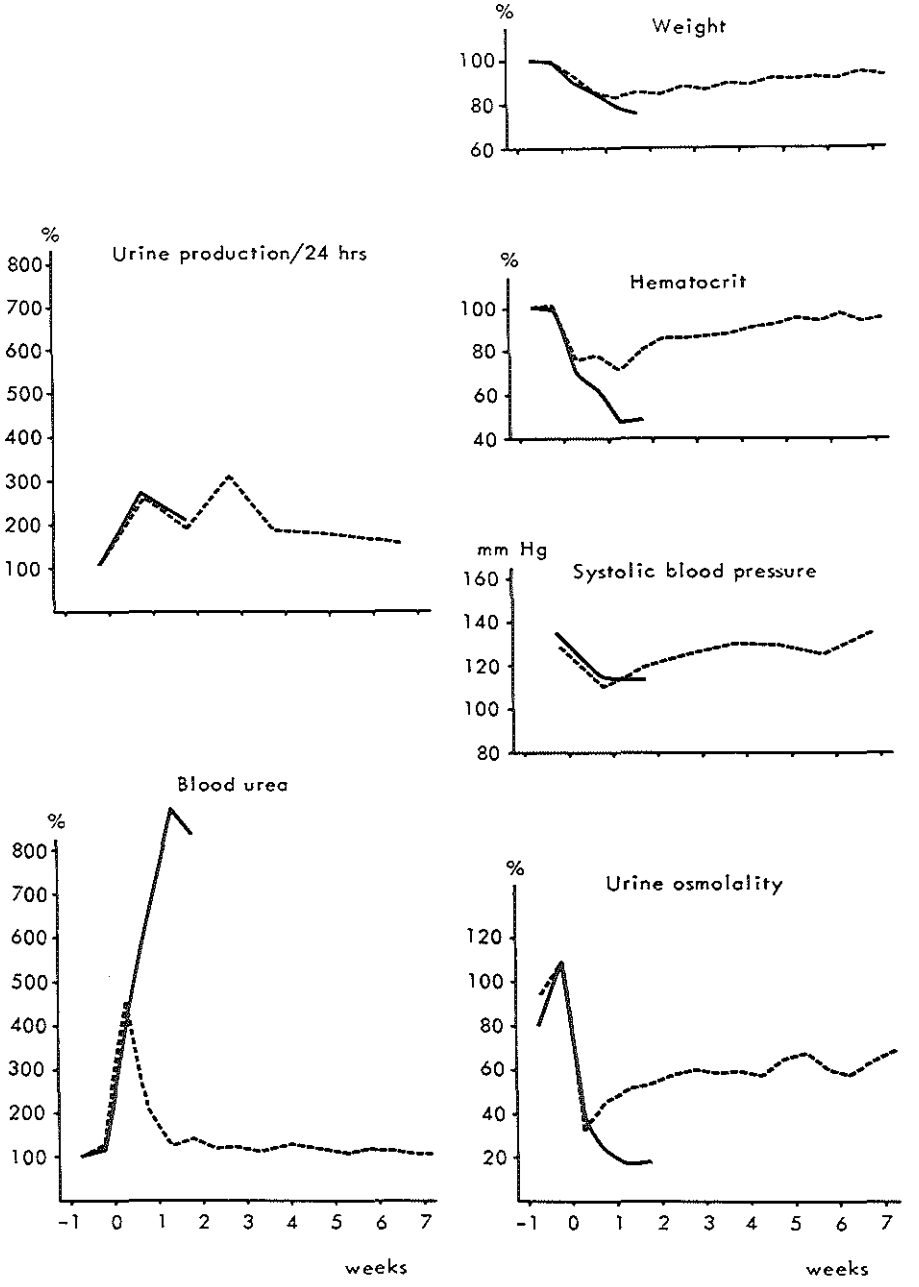


Fig.8 Function of untreated allografts with the exception of one rat surviving for 50 days after transplantation (solid lines) as compared with untreated isografts (broken lines).

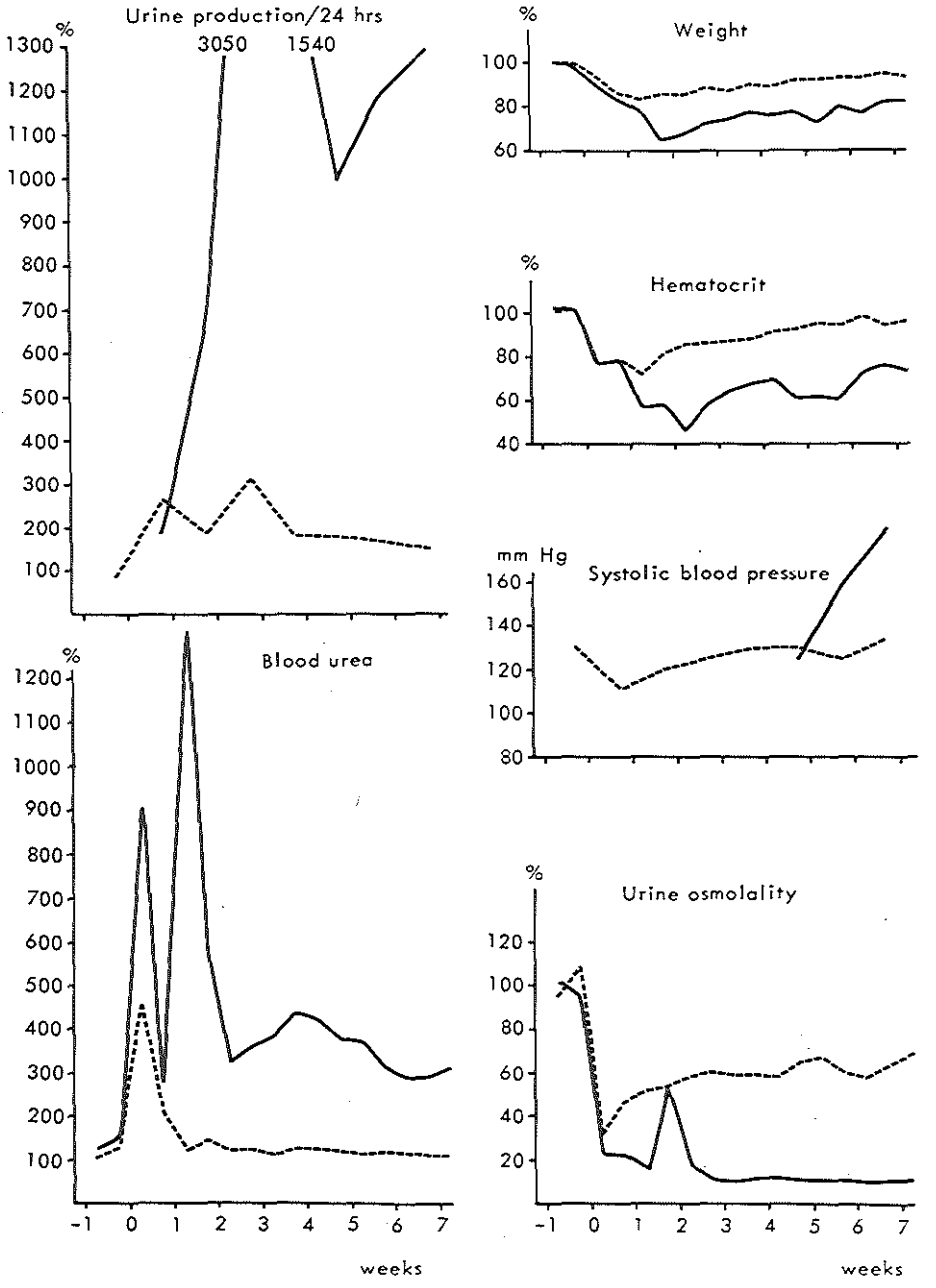


Fig.9 Function of one untreated allograft surviving for 50 days after transplantation (solid lines) as compared with function of the isografts (broken lines).

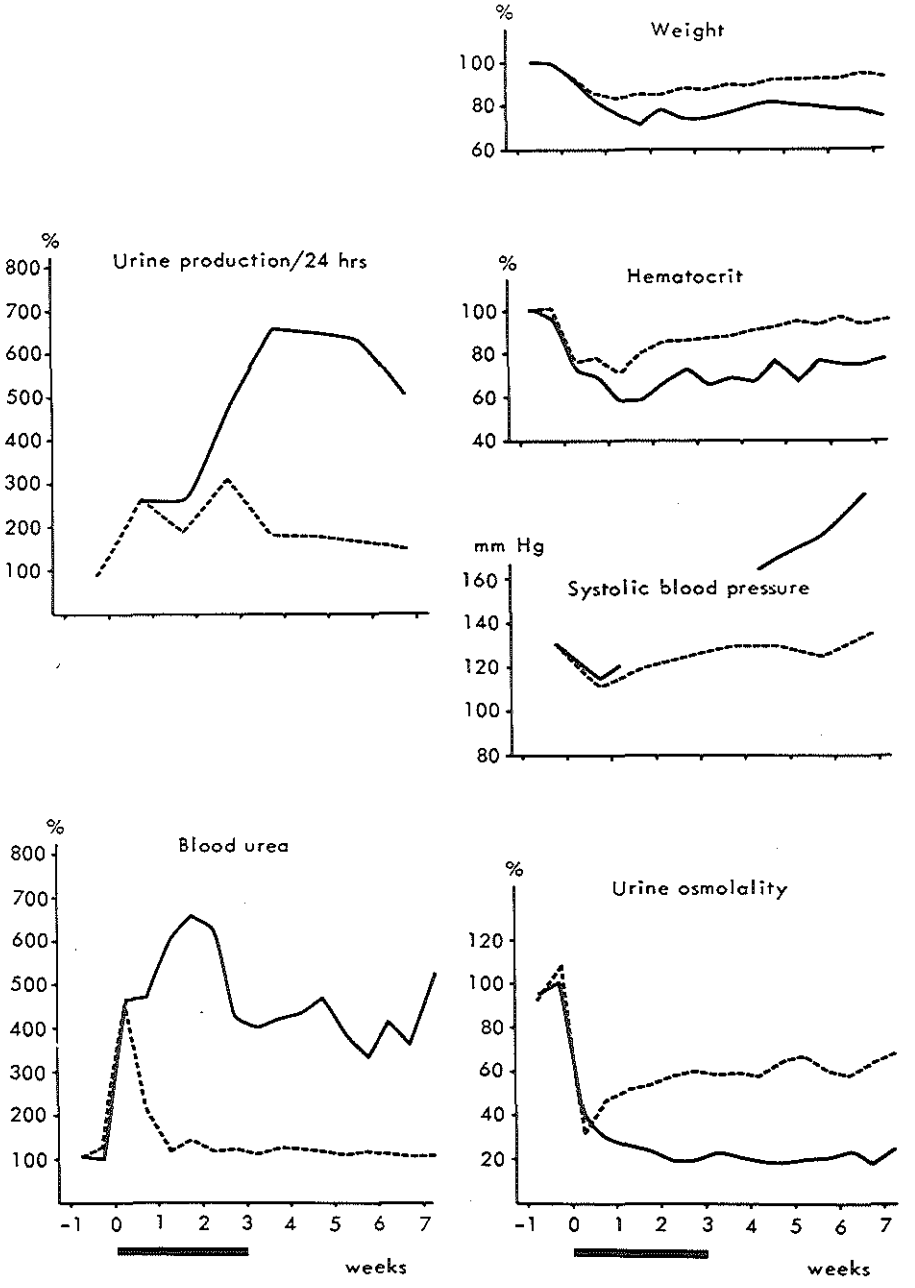


Fig.10 Function of allografts treated with Imuran 4 mg/kg (solid lines) as compared with untreated isografts (broken lines). The bars in the figure indicate the period during which Imuran was administered.

50-day survival of 6 rats out of 13. Of these, 3 were sacrificed at day 50. The other 3 showed no change in function and were killed at 100 days. Renal function was better (fig.11) than in the previous group as demonstrated by significantly lower B.U. and higher urine osmolality values. Blood pressure was only little above normal. No deterioration occurred after day 50 (see Appendix).

The values of the group, in which administration of Imuran 2 mg/kg was started one week before transplantation (fig.12) did not differ significantly from those of the group receiving Imuran 4 mg/kg. At 50 days, 4 out of 10 rats were alive, one of which was killed. The other 3 died from rejection at 52, 73 and 89 days, respectively.

When Prednisolone 4 mg/kg was given together with Imuran 2 mg/kg, the results obtained were better than with Imuran alone. Eight out of 10 rats survived for 50 days. Of the 4 animals left alive, one died at 55 days from intussusception of the ileum. The values of this group, shown in fig.13, were clearly different from those of the isografted group. Improvement over the best of the Imuran treated groups (Imuran 2 mg/kg) was demonstrated by higher urine osmolality values and also by higher weight and hematocrit values. Only blood pressure tended to be higher, although the difference was not statistically significant. During the 50 - 100 day period no appreciable changes in function were observed.

It is of interest to note in this group, and in the following groups, that discontinuation of treatment at 3 weeks after transplantation did not have an adverse effect.

It was expected that function would clearly improve over the previous groups with ALG treatment. However, this was only true for the treatment with ALG I, the group showing significant improvement over the previous one in all parameters, with the exception of hematocrit values. Results after treatment with the other batch (ALG II) were not much different from those obtained with Imuran and Prednisolone, the only significant difference being lower blood pressure.

In the group, treated with ALG I (fig.14), all 7 animals were alive at day 50. In comparison to the isografted rats, restoration of weight and hematocrit values was slower, while B.U. was somewhat higher. Blood pressure and 24-hour urine production were not significantly different. Urine osmolality was definitely lower, reaching a final level of 50% of normal. Function proved to be stable in 3 rats observed

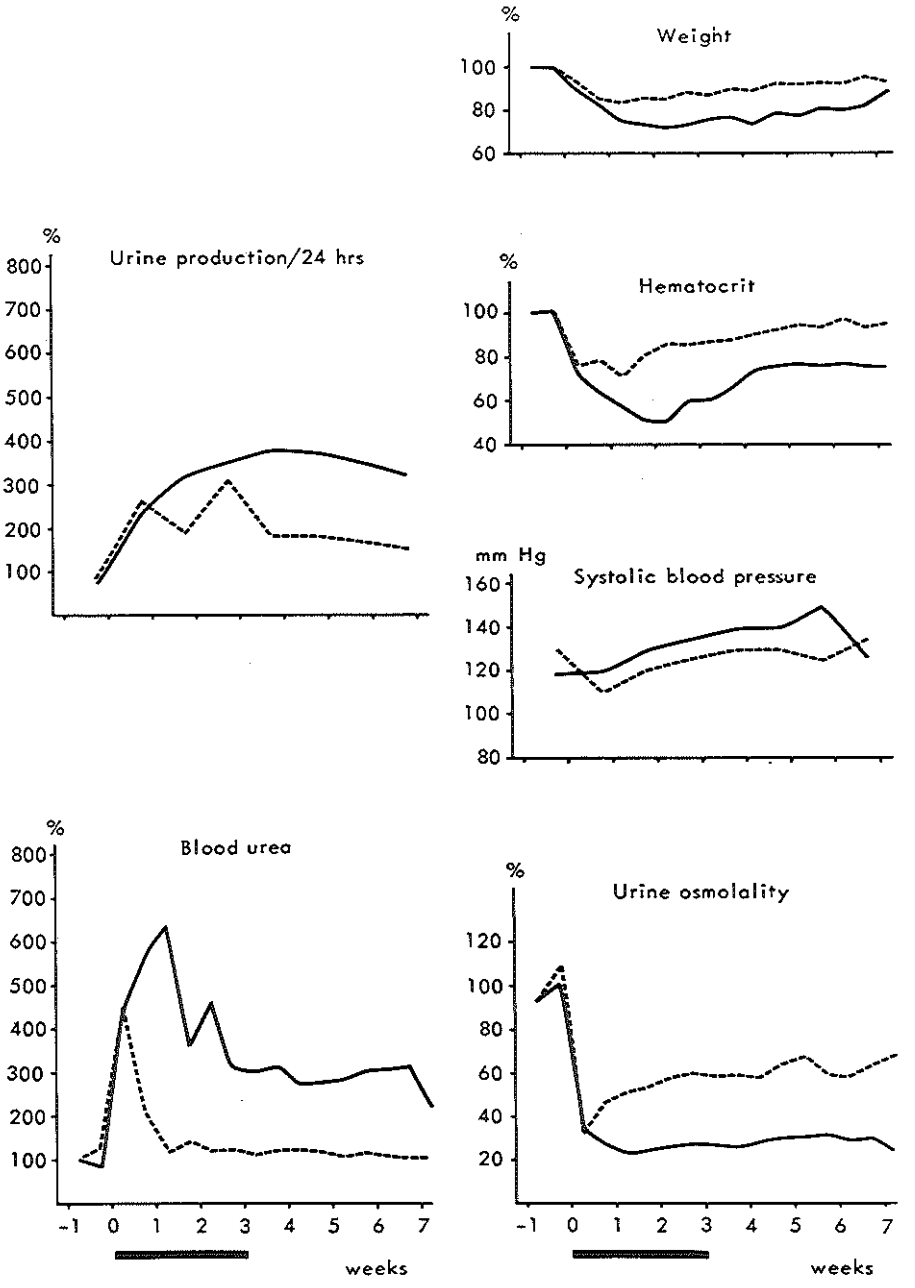


Fig.11 Function of allografts treated with Imuran 2 mg/kg (solid lines) as compared with untreated isografts (broken lines). The bars in the figure indicate the period during which Imuran was administered.

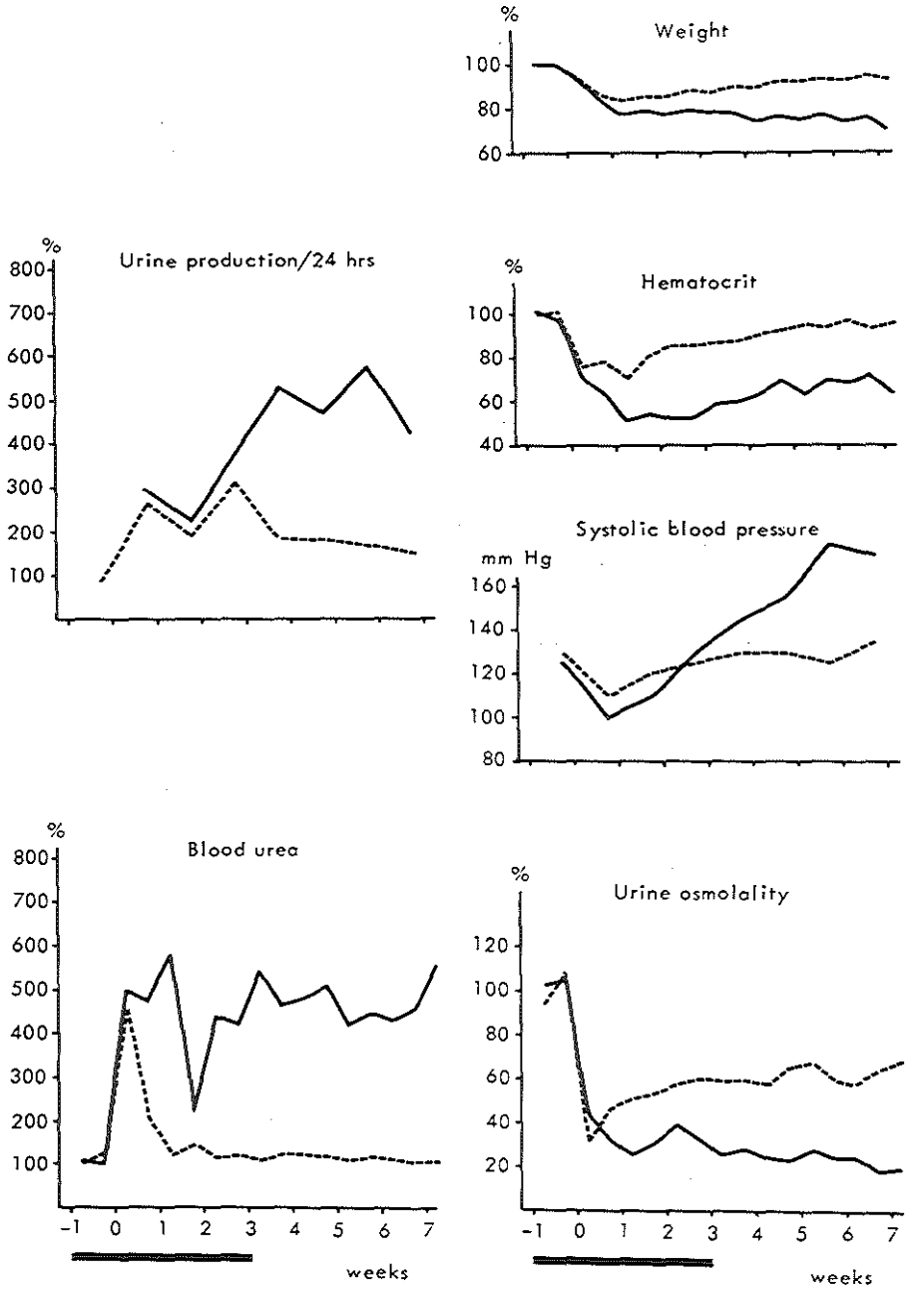


Fig.12 Function of allografts treated with Imuran 2 mg/kg for 4 weeks starting one week before transplantation (solid lines) as compared with untreated isografts (broken lines). The bars in the figure indicate the period during which Imuran was administered.

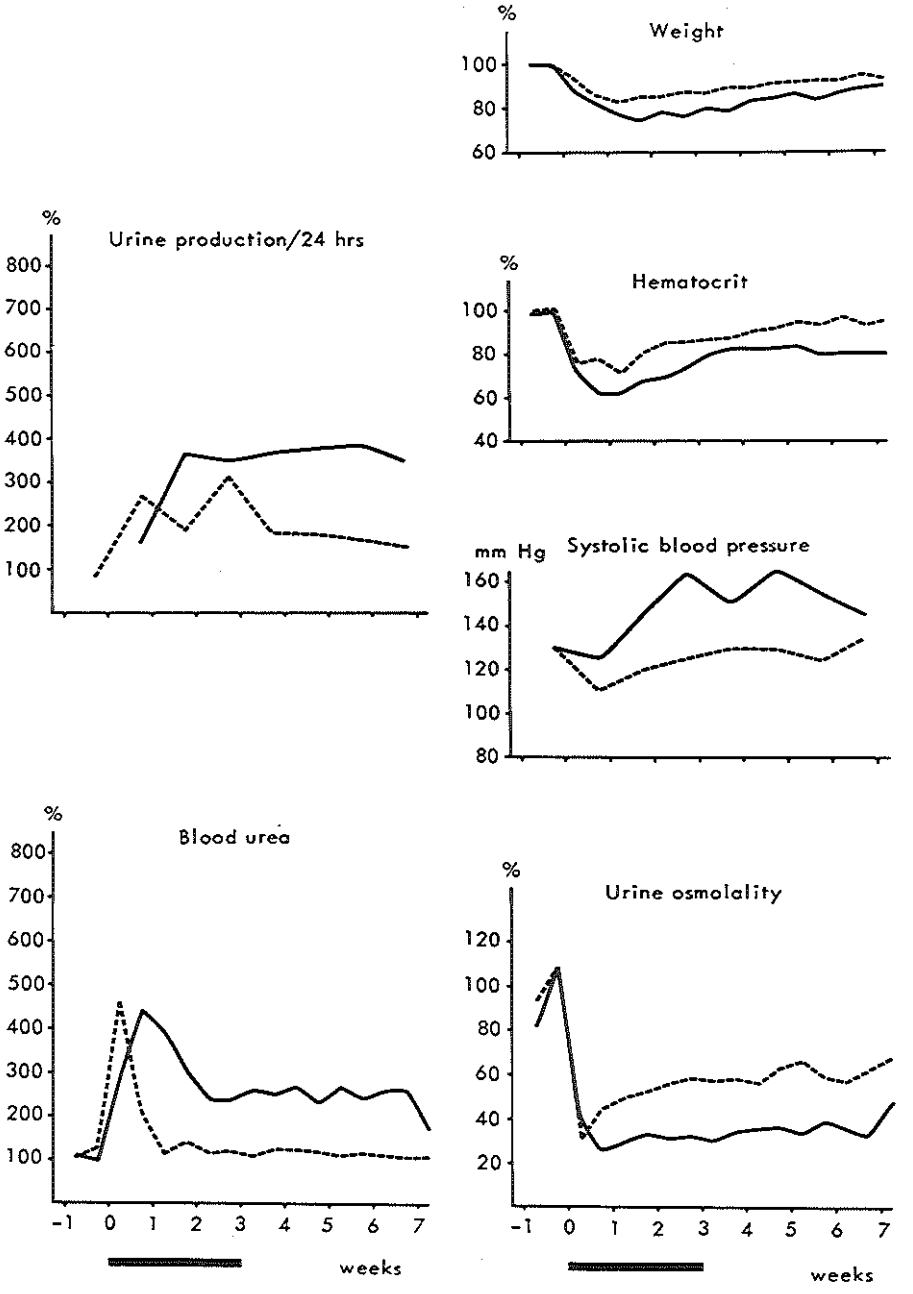


Fig.13 Function of allografts treated with Imuran 2 mg/kg and Prednisolone 4 mg/kg (solid lines) as compared with untreated isografts (broken lines). The bars in the figure indicate the period during which Imuran and Prednisolone were administered.

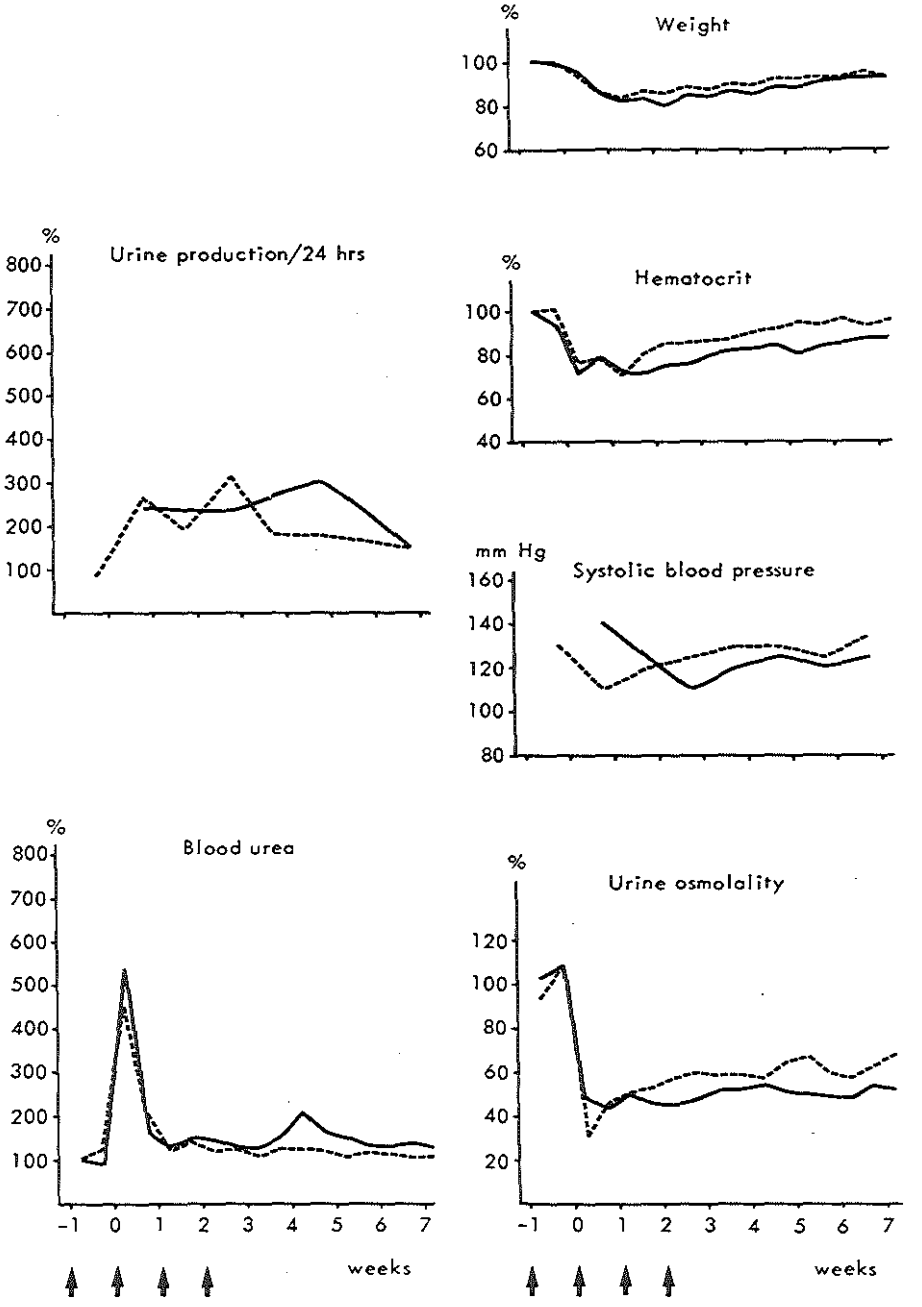


Fig.14 Function of allografts treated with ALG I 19 mg/rat (solid lines) as compared with untreated isografts (broken lines). The arrows in the figure indicate the days on which ALG was administered.

during the 50 - 100 day period.

Treatment with ALG II (fig.15) resulted in 5 out of 7 rats surviving for 50 days. One of the 3 rats observed after day 50 showed slowly progressive deterioration of function, resulting in death from graft rejection at day 95. Renal function of the other 2 rats did not change during that period. Results in this ALG II treated group clearly differed from those in the isografted group in all respects, with the exception of blood pressure.

The difference in effectiveness of the two batches of ALG became even more striking when combined with Imuran. Best recovery of function was observed in the animals treated with ALG I together with Imuran 4 mg/kg. Of the 5 animals in this group, 4 were alive at day 50, when 2 were sacrificed, the other 2 showing no deterioration of function during the follow-up until 100 days. Function, as shown in fig.16, was practically similar in this group and in the isografted group although minor differences in hematocrit and B.U. level still turned out to be statistically significant. In comparison with the group receiving ALG I only, concentrating capacity of the kidney was clearly improved as demonstrated by a higher urine osmolality level. Also weight and hematocrit values were higher.

In the last two groups, the effect of treatment with ALG II in combination with Imuran was studied. In the group treated with ALG II and Imuran 4 mg/kg, 3 out of 5 animals were alive at day 50, two of which were observed for 50 days more, when no special features were noted. In the last group, treated with ALG II and Imuran 2 mg/kg all 8 rats survived for 50 days. Renal function did not change in the 4 rats observed until day 100. Results of the tests in these last 2 groups are shown in fig.17 and fig.18. It is clear that both groups were quite unlike the isografted group. When compared with the group receiving ALG II only, neither of the 2 groups showed a significant difference in any of their parameters. Thus addition of Imuran did not improve results in the case of ALG II.

In the foregoing discussion we have not considered the parameters of function separately but it is clear that, with regard to the condition of the grafted kidney, B.U., urine osmolality and urine production/24 hr. are the most relevant. Groups have been reported to be different by a certain parameter only if statistically a difference at a 1% level of significance was found (table IV). If groups were different in more than one parameter, this was regarded as further

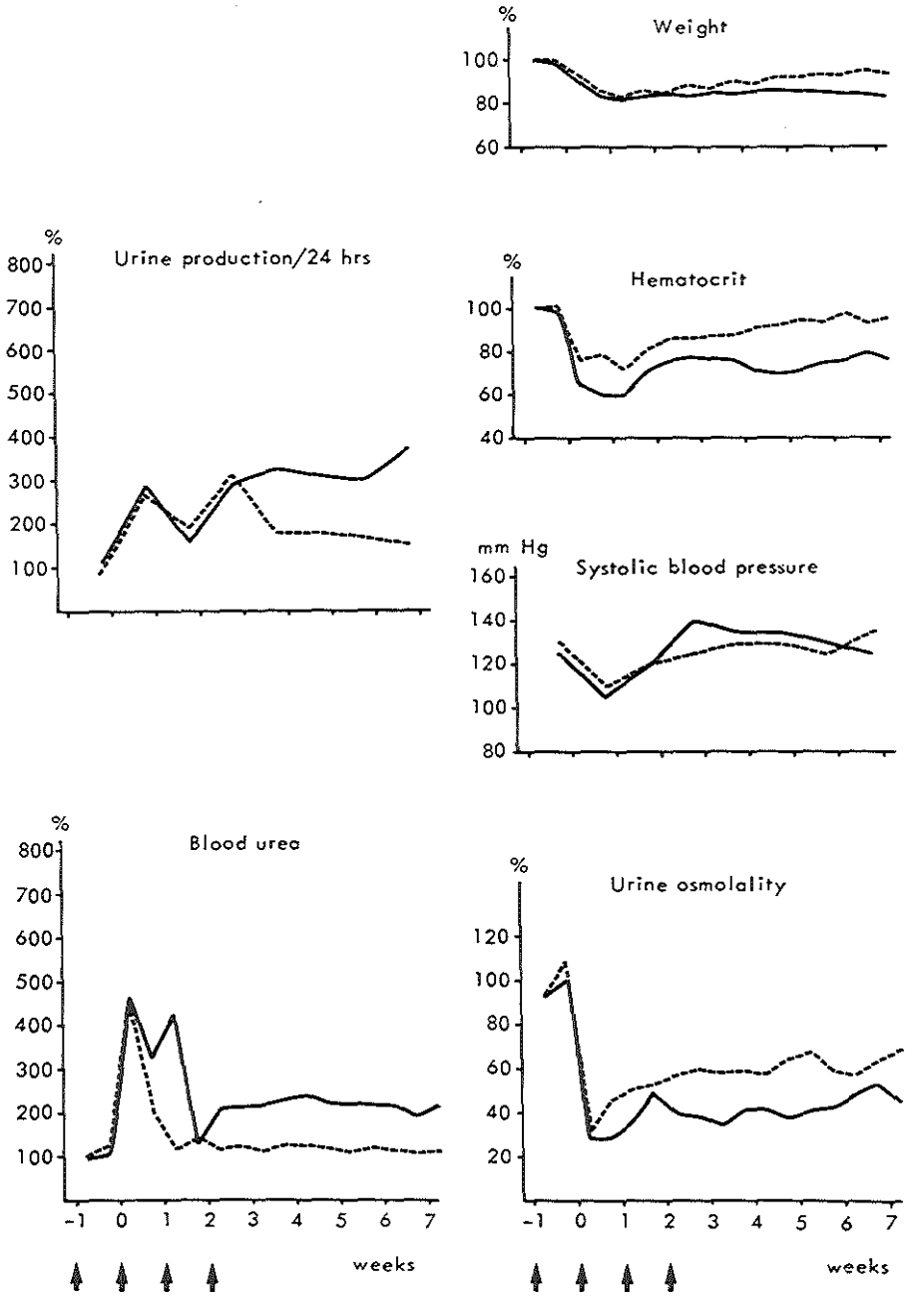


Fig.15 Function of allografts treated with ALG II 19 mg/rat (solid lines) as compared with untreated isografts (broken lines). The arrows in the figure indicate the days on which ALG was administered.

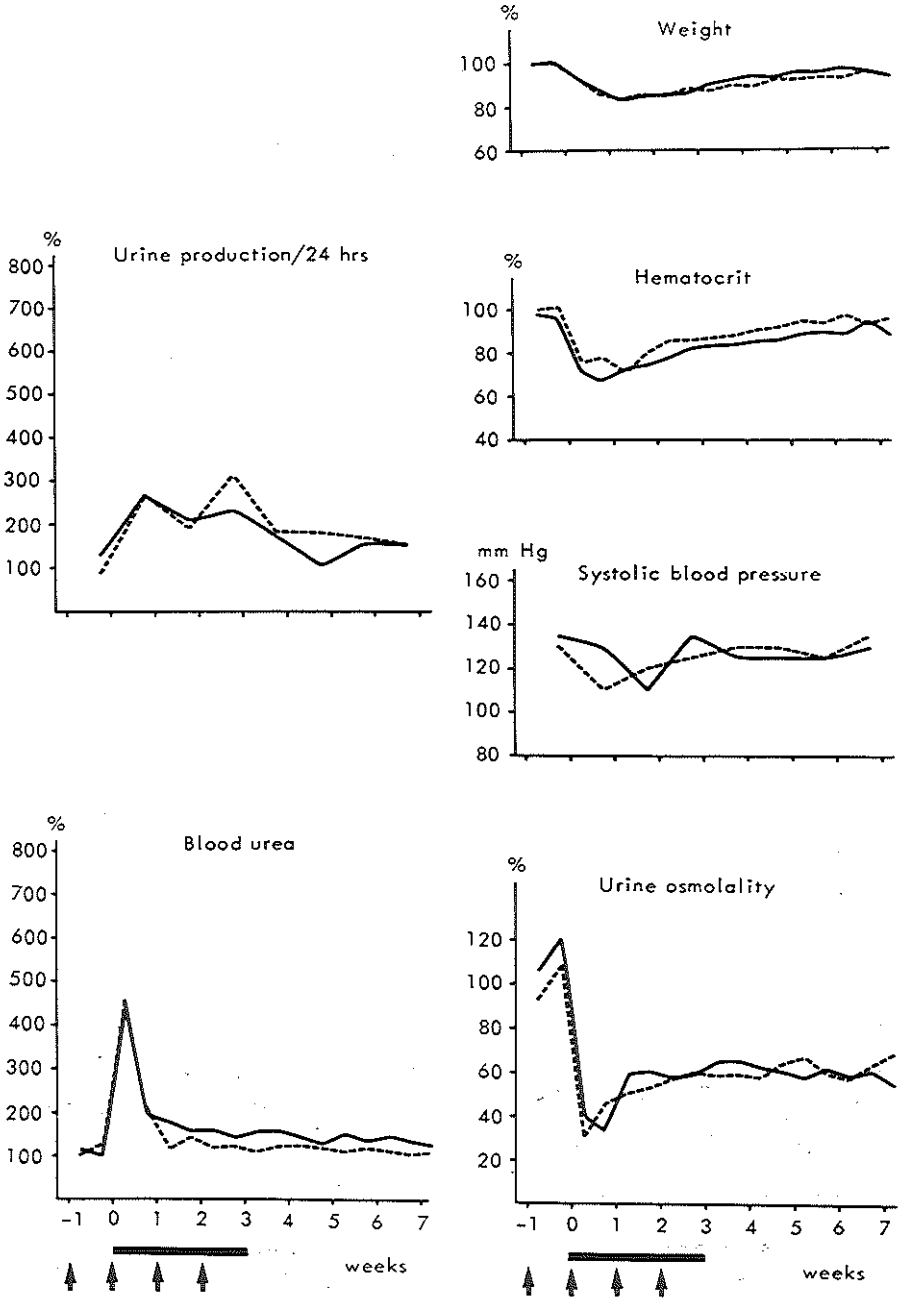


Fig.16 Function of allografts treated with ALG I 19 mg/rat and Imuran 4 mg/kg (solid lines) as compared with untreated isografts (broken lines). The arrows in the figure indicate the days on which ALG was administered, the bars indicate the period during which Imuran was given.

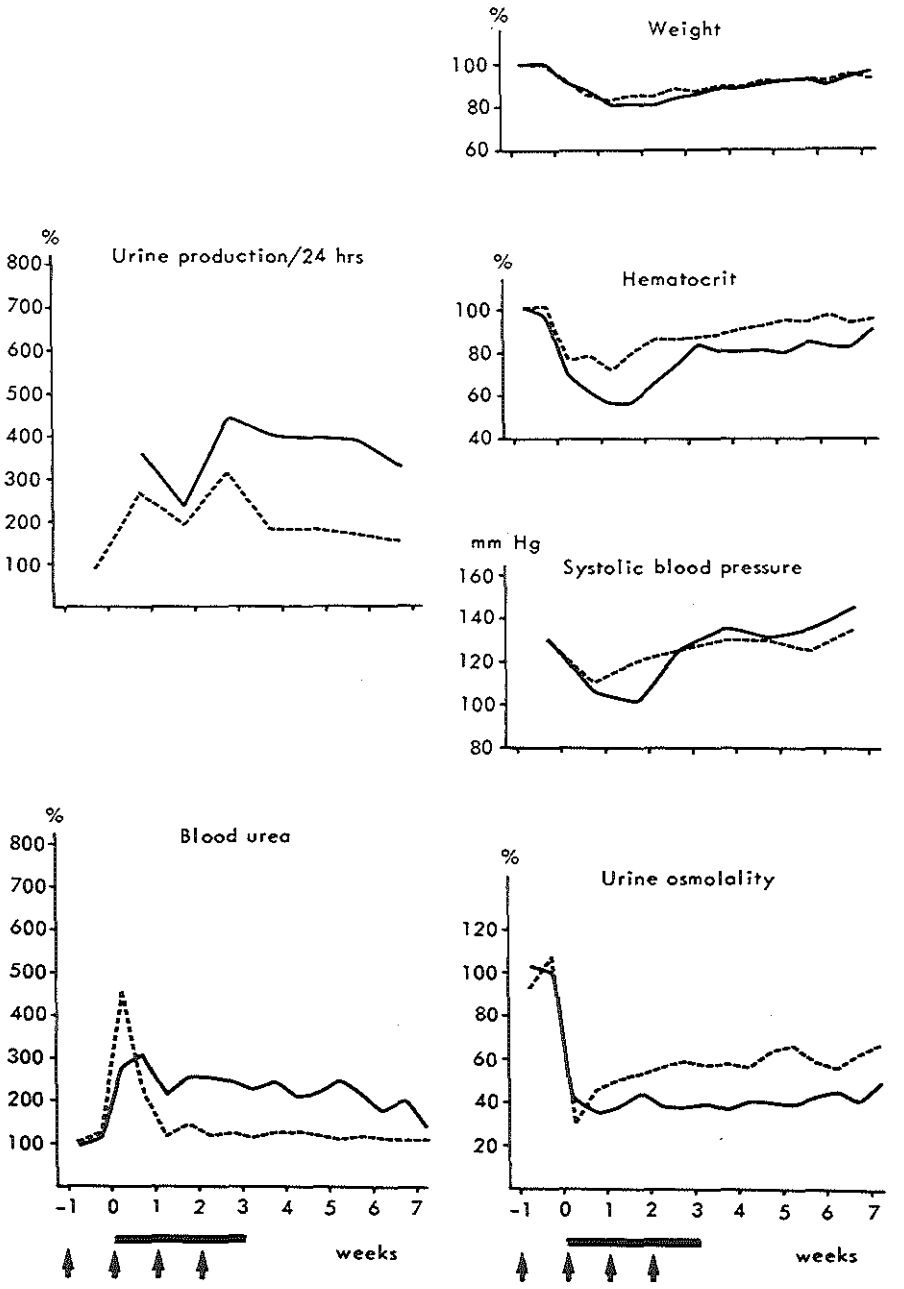


Fig.17 Function of allografts treated with ALG II 19 mg/rat and Imuran 4 mg/kg (solid lines) as compared with untreated isografts (broken lines). The arrows in the figure indicate the days on which ALG was administered, the bars indicate the period during which Imuran was given.

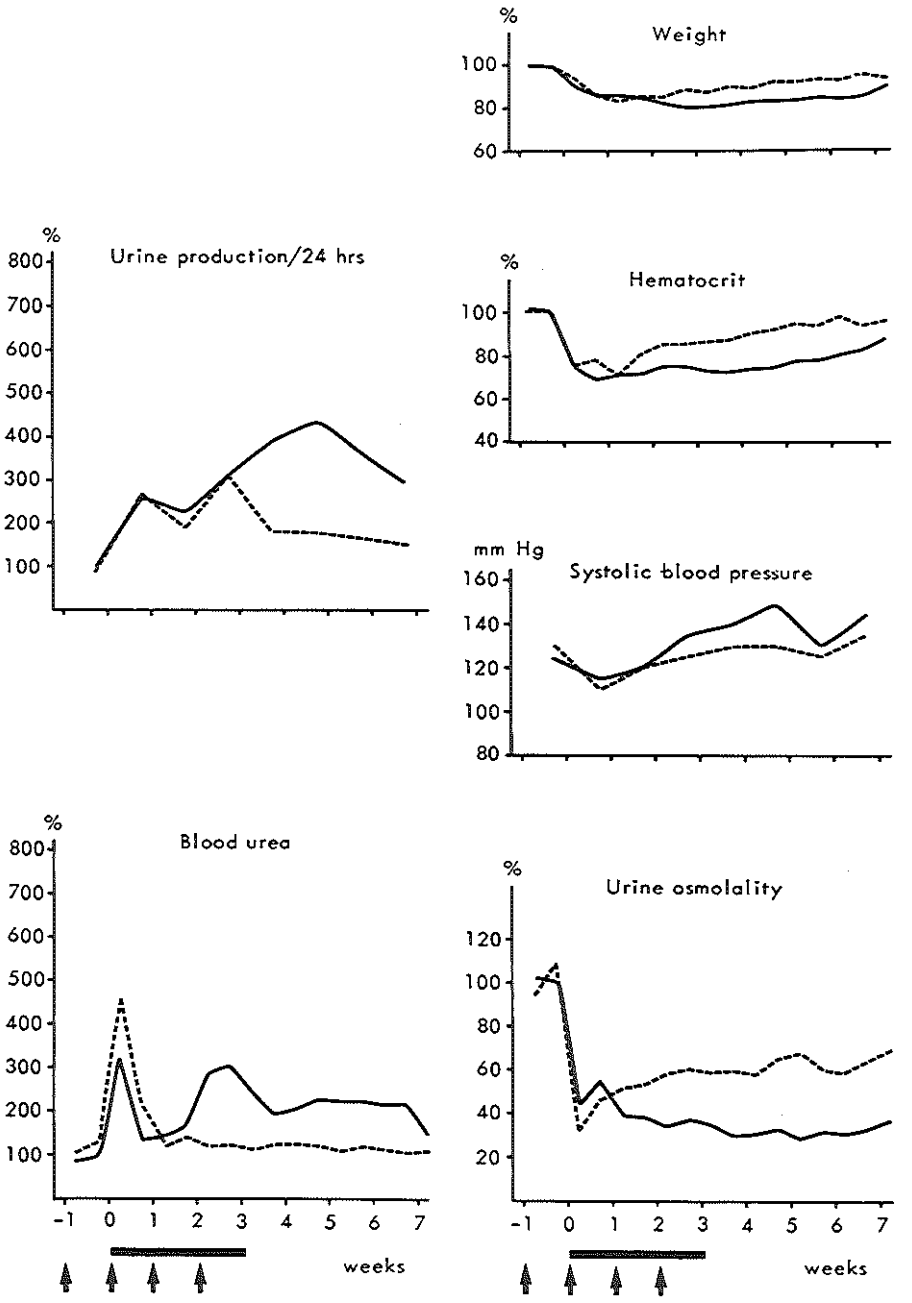
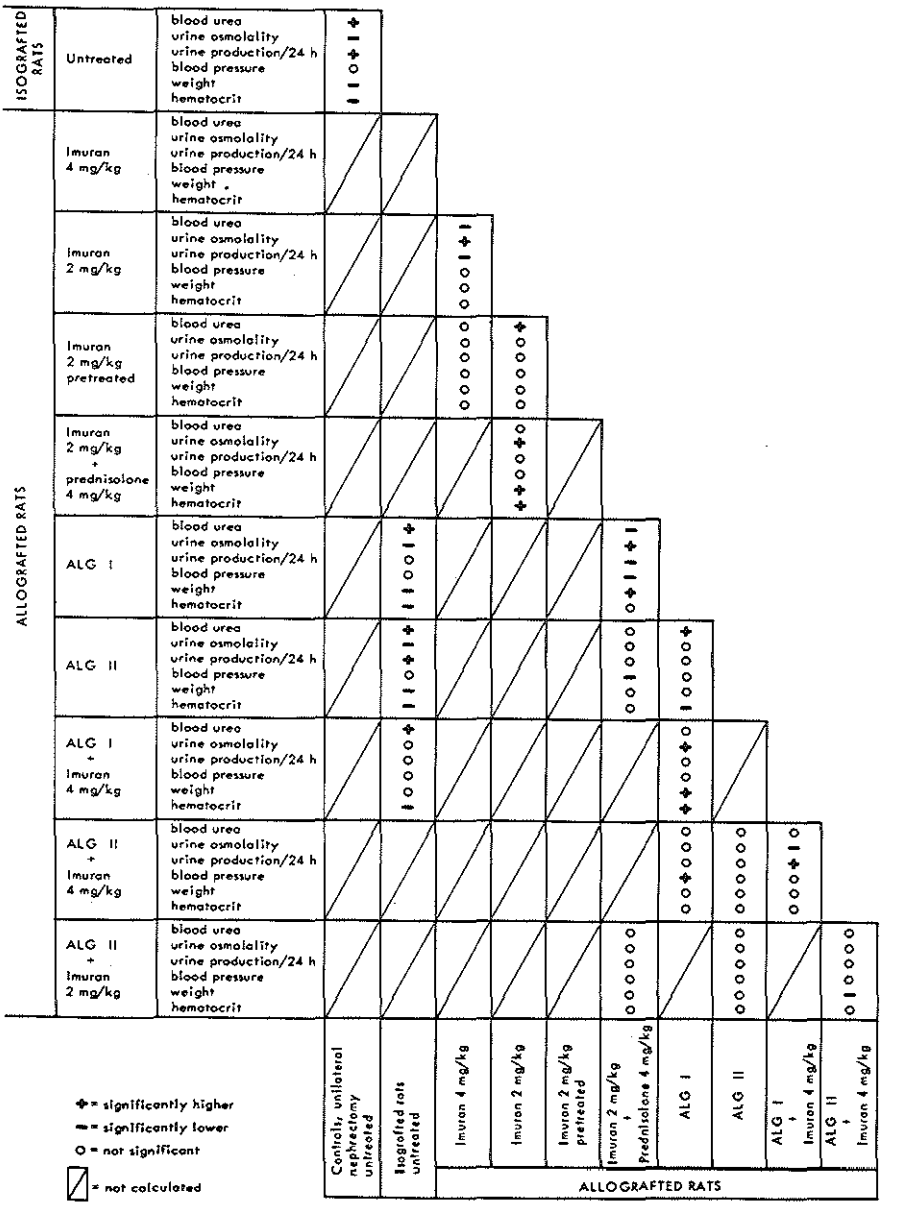


Fig.18 Function of allografts treated with ALG II 19 mg/rat and Imuran 2 mg/kg (solid lines) as compared with untreated isografts (broken lines). The arrows in the figure indicate the days on which ALG was administered, the bars indicate the period during which Imuran was given.

TABLE IV

STATISTICAL EVALUATION OF GROUP DIFFERENCES



support for the conclusions drawn. Of course the question arises whether B.U. and urine osmolality were independent parameters of renal function. To evaluate a possible interdependence of the two, a scatter diagram was made, depicting the relationship between the values of blood urea (mg%) and urine osmolality (mOsm/l) obtained before sacrificing in the isografted, allografted and unilaterally nephrectomized rats (fig.19). It is clear that both parameters are related in a way and indeed, it was not to be expected that severe renal damage would selectively affect either the concentrating or the excretory capacity of the kidney. However, there is a wide range in

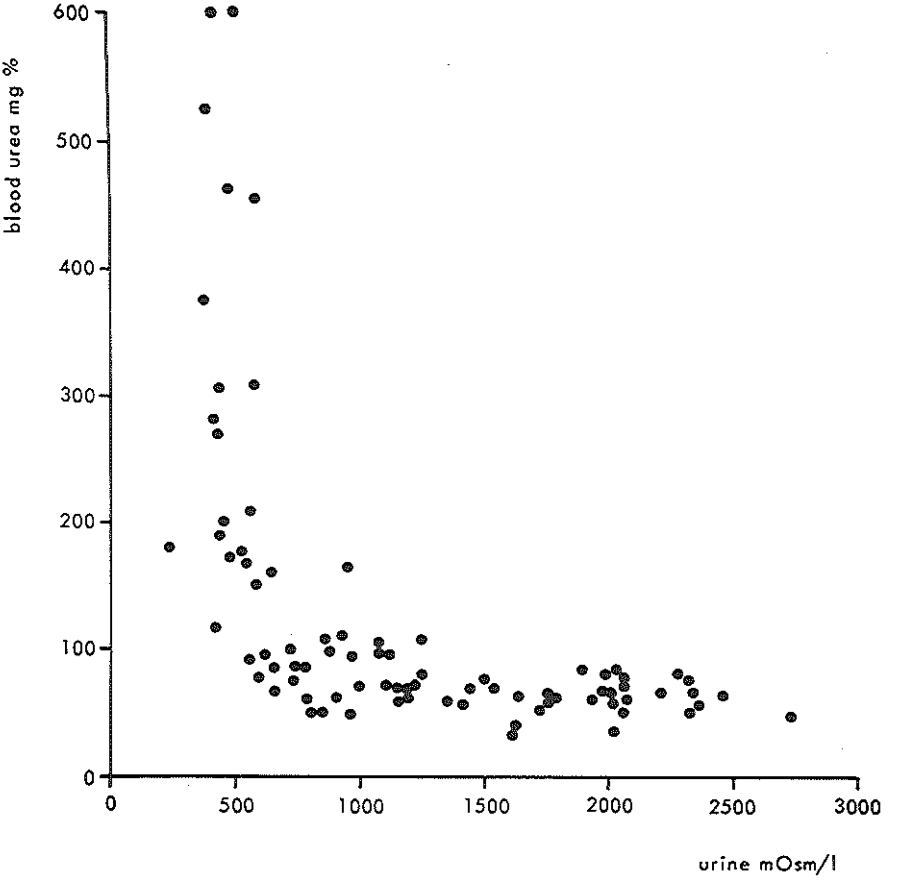


Fig.19 Scatter diagram showing the relationship between blood urea and urine osmolality. Values were obtained in isografted, allografted and unilaterally nephrectomized rats before sacrificing.

which considerable changes in urine osmolality were not accompanied by appreciable changes in blood urea, while at osmolality values of 600 m Osm/l or less there is a distinct variation in the severity of uraemia. If this is to be compared with the situation in man, one should keep in mind that the range of urine osmolality values in the rat is twice as great, thus making it a very sensitive index in the latter species. From the diagram in fig.20, in which urine osmolality and

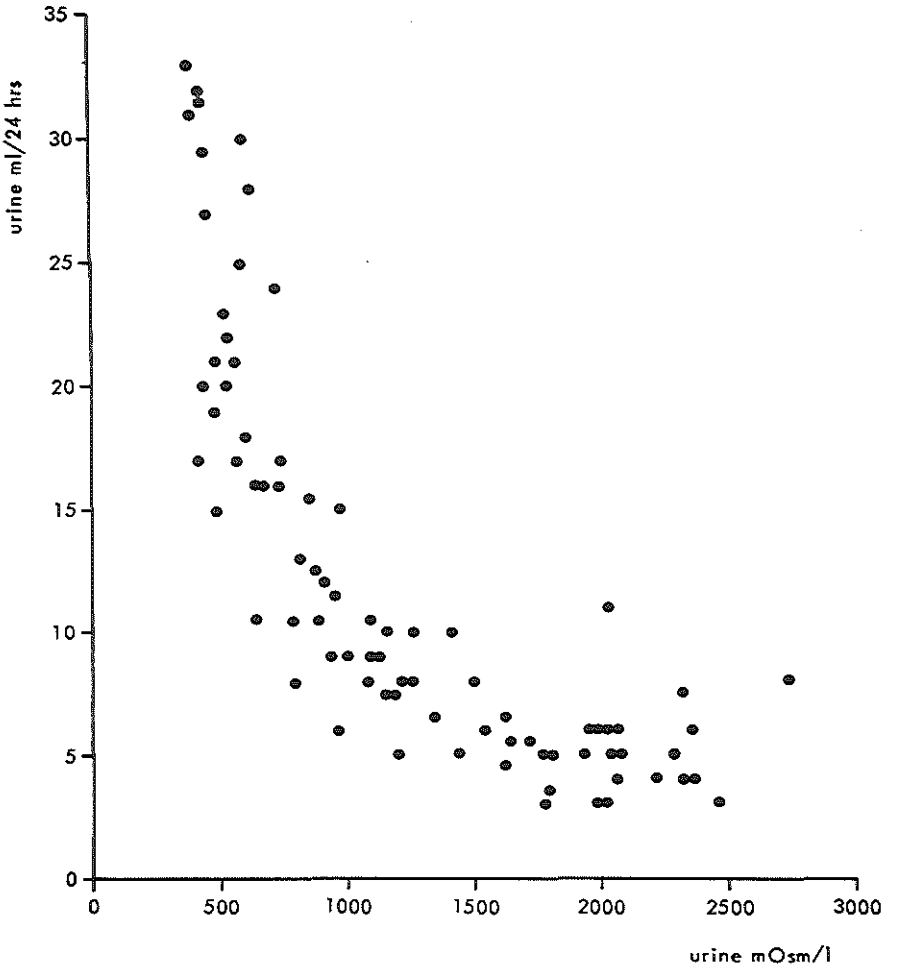


Fig.20 Scatter diagram showing the relationship between urine production and urine osmolality. Values were obtained in isografted, allografted and unilaterally nephrectomized rats before sacrificing.

urine production/24 hr. are plotted in the same fashion, it becomes apparent that these two parameters are functionally related. This is in agreement with the fact that in the various groups a decrease in urine osmolality was generally associated with a higher urinary output.

Chronic rejection

It has been discussed before (cf. fig.4) that in some cases during the period of 50 days after transplantation, death from graft rejection occurred within the first 2 - 3 weeks. However, in some of the allografted rats surviving for 50 days, a more chronic pattern of rejection was observed. Table V shows the incidence of this condition in

TABLE V

INCIDENCE OF CHRONIC REJECTION IN LONG SURVIVING ALLOGRAFTED RATS

Treatment groups	no. of rats surviving 50 days	no. of rats showing chronic rejection	onset of rejection period (in weeks)
Imuran 4 mg/kg	3	2	3
Imuran 2 mg/kg	6	1	3
Imuran 2 mg/kg pretreated	4	3	3
Imuran 2 mg/kg + Prednisolone 4 mg/kg	8	1	4
ALG I	7	0	-
ALG II	5	1	3
ALG I + Imuran 4 mg/kg	4	0	-
ALG II + Imuran 4 mg/kg	3	1	2
ALG II + Imuran 2 mg/kg	8	2	3 - 4

the various groups. In each individual case, chronic rejection could be recognized with ease from the function data: invariably there was a gradual rise in blood urea and 24-hour urine production with a gradual fall in urine osmolality and body weight, while hematocrit values remained low. In the majority of cases hypertension developed. The onset of the rejection period was sometimes marked by a temporarily high peak of blood urea, after which the progressive changes began. In other cases the onset was less conspicuous. In the group, treated with Imuran 2 mg/kg with pretreatment for one week, 3 out of 4 rats suffered from this condition. Fig.12, showing function in this group, reflects clearly the changes as observed in individual cases.

Special mention should be made of two rats in which all the changes noted above were observed without a progressive course (therefore not included in table V). The first rat, which had been treated with ALG I, had a rejection crisis during the fifth week after transplantation with complete repair within a few weeks (all parameters recovering to the same level as found before the rejection episode). The second rat, from the group treated with ALG II, showed a pattern of chronic rejection during weeks 3 and 4 after transplantation but then recovered, although not completely, during the subsequent weeks.

Intravenous pyelography

Intravenous pyelography was performed before sacrificing animals at 50 or 100 days after transplantation. In a total of 45 cases, successful roentgenograms of transplanted kidneys were obtained (10 isografts, 1 untreated allograft and 34 treated allografts). In 39 of these, definite hydronephrosis of the grafted kidney was observed, in some cases with hydroureter (figs.23, 24 and 25). The 6 kidneys having a pelvis of normal size belonged to various treatment groups: 1 isograft, the others allografts, treated with Imuran 2 mg/kg (1x), Imuran + Prednisolone (2x), ALG I (1x) and ALG II + Imuran 2 mg/kg (1x). In order to quantitate the degree of hydronephrosis the renal pelvis was measured on the roentgenograms. The greatest length in mm was measured and thereafter, along an axis perpendicular to this, the greatest width in mm. Multiplication of the two figures yielded a measure of pelvic size that could be used for the purpose of comparison. Mean pelvic size of the isografts amounted to

104.5 mm^2, of the treated allografts to 109 mm^2.

To obtain "normal" values of pelvic size, intravenous pyelography was performed in 18 male Wag/Rij rats of the same age as the recipients (and isograft donors) used. Unexpectedly 6 of these 18 rats showed hydronephrosis of the right kidney (the one which was used for isogeneic transplantation) as shown in fig.26. Pelvic size of these 6 hydronephrotic kidneys averaged 86.5 mm^2 as compared to 43.5 mm^2 for the 12 apparently normal right kidneys. More data about the incidence of this defect were published recently (Kort, 1970). It should be mentioned that further investigation of this condition in 2 month old Wag/Rij rats showed 7 out of 20 males to have hydronephrosis, occurring only at the right side, while no hydronephrosis was found in 20 females. It has been suggested that this lesion is produced by partial obstruction of the right ureter by the spermatic artery (Sellers, 1960). Observations at autopsy did not confirm the existence of such a mechanism in the Wag/Rij rat: the anatomical relationship between the spermatic artery and ureter was found to be variable but could not be correlated with the occurrence of hydronephrosis. Nonetheless, comparing renal pelvic size in the normal and in the isografted kidneys appears to justify the conclusion that severity and incidence of hydronephrosis in the rat are increased after transplantation of the kidney.

The next question concerned the incidence of hydronephrosis in the donor rat used for allogeneic transplantation. Intravenous pyelography was performed in 83 BN/Bi males of the same age as the donors used in the transplantation experiments (fig.27). Hydronephrosis of the right kidney was seen in 24 cases (29%). In the BN/Bi rat hydronephrosis also occurred on the left side (14 times in the 83 rats, 17%). Moreover, the condition was also found in female rats in which the incidence did not differ from that in the males (Cohen et al., 1970; Kort, 1970). Thus, incidence of hydronephrosis of the right kidney, which was used exclusively in the transplantation experiments, was almost as frequent in the allograft as in the isograft donors. Only in a few cases of severe hydronephrosis, seen during operation, were the kidneys discarded. Consequently, it can be stated that no difference between isografts and allografts with respect to the condition of the kidney at the time of transplantation has been introduced, so that the comparison of results obtained in treated allografted rats with those obtained in isografted rats does

not present any difficulties.

The role of the transplantation procedure itself in the development of hydronephrosis was further evaluated by comparing the severity of hydronephrosis of kidneys transplanted by 2 different persons, A and B. It appeared that in kidneys which had been grafted by A (20x, mean pelvic size 137 mm^2) there was significantly more severe hydronephrosis than in kidneys grafted by B (18x, mean pelvic size 92 mm^2), as determined by measuring the pyelograms made on day 50 and 100 (Wilcoxon's test, $p < 0.05$). No increase in severity of hydronephrosis was noted when values obtained at day 50 were compared to those obtained at day 100.

A most important question was now whether the occurrence of hydronephrosis had influenced the outcome of the tests used to determine renal function. In other words: could variations in the incidence of hydronephrosis have been responsible for the differences observed between the various treatment groups. In fig.7 renal function of isografted rats with hydronephrosis is compared with that of normal rats without hydronephrosis after right-sided nephrectomy. It can be seen that, at 50 days after operation, the 2 groups were different only in urine osmolality and 24-hour urine production. Thus, it could be, that differences in these parameters were produced by hydronephrosis of the kidney. This was checked by making scatter diagrams of the relationship between pelvic size on the one hand and the last values (obtained before sacrifice in treated allografted rats), of urine osmolality and 24-hour urine production on the other hand. This is shown in figs.21 and 22. Application of Spearman's rank correlation test showed that neither decrease of urine osmolality values nor increase in 24-hour urine production were significantly correlated with increase in pelvic size. Moreover, if treatment groups, which were not significantly different in function, were combined, 4 categories can be distinguished (figs.21 and 22) and it is clear that values for pelvic size were evenly distributed in these categories. From this, it can be concluded that the observed differences in function between treatment groups were not attributable to a variation in severity of hydronephrosis.

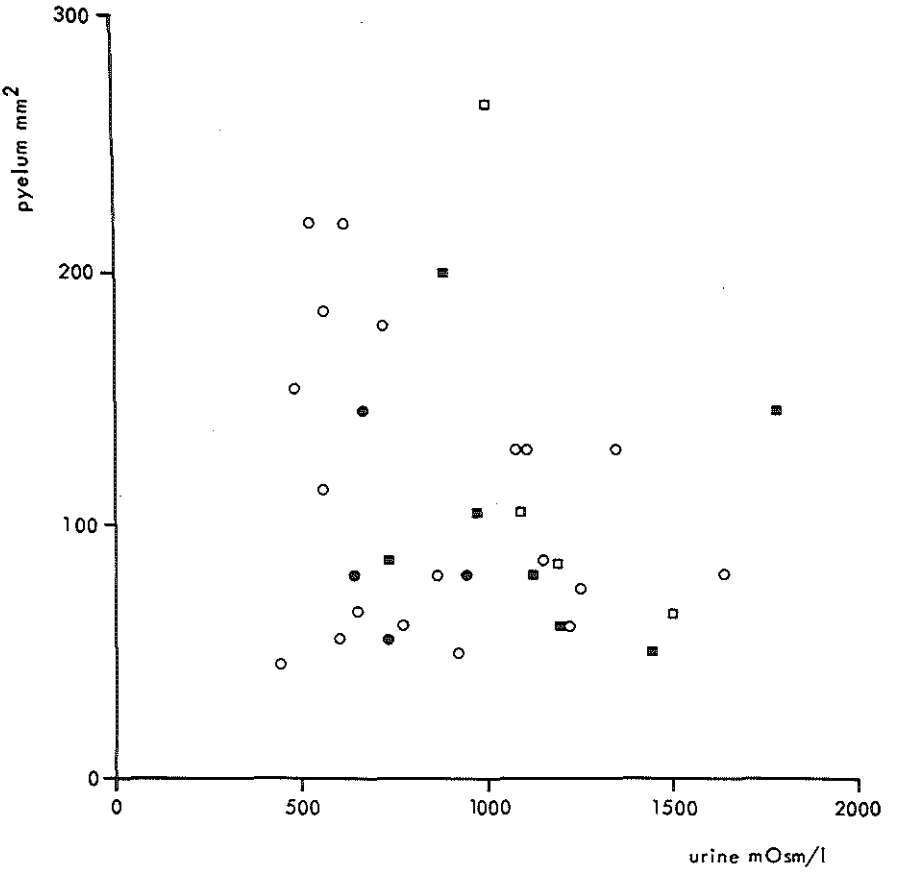


Fig.21 Scatter diagram showing the relationship between size of the renal pelvis and urine osmolality. Size of the renal pelvis was measured on the roentgenograms and the last values of urine osmolality were obtained before sacrifice in treated allografted rats. The correlation is not significant (Spearman ranking correlation coefficient – 0.188. 95% confidence limits 0.147 and –0.522).

- ● = poor transplant function (treatment with Imuran alone)
- ○ = moderate transplant function (treatment with Imuran + Prednisolone, ALG II or ALG II + Imuran)
- ■ = good transplant function (treatment with ALG I)
- □ = excellent transplant function (treatment with ALG I + Imuran)

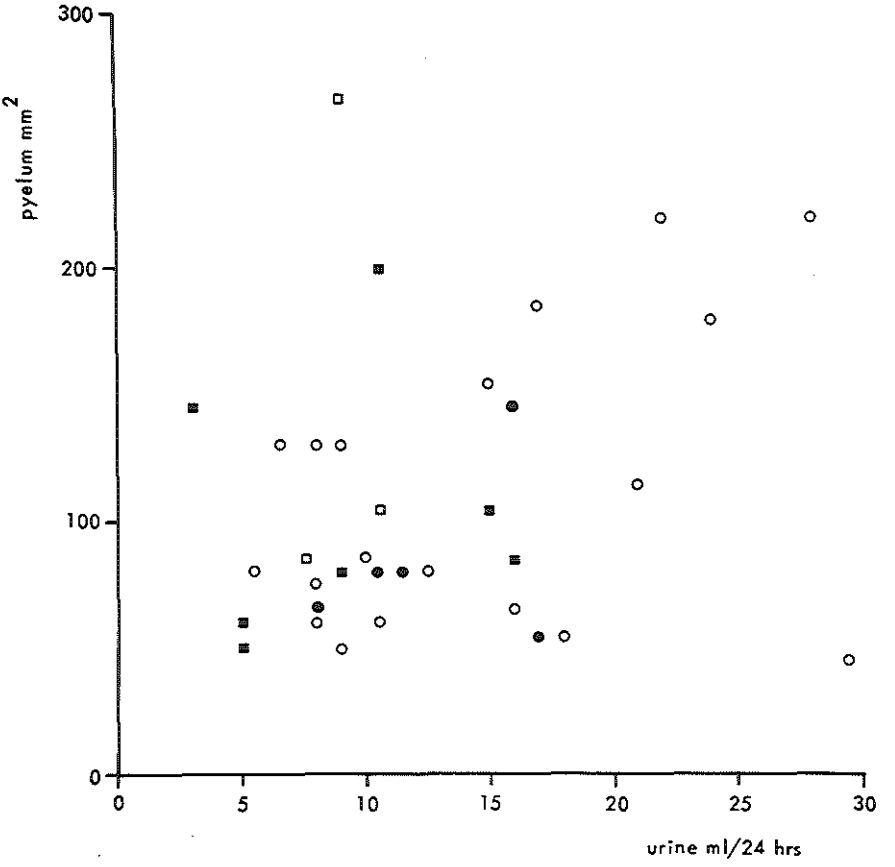


Fig.22 Scatter diagram showing the relationship between size of the renal pelvis and 24-hour urine production. Size of the renal pelvis was measured on the roentgenograms and the last values of 24-hour urine production were obtained before sacrifice in treated allografted rats. The correlation is not significant (Spearman ranking correlation coefficient +0.199. 95% confidence limits 0.548 and −0.149).

- ● = poor transplant function (treatment with Imuran alone)
- ○ = moderate transplant function (treatment with Imuran + Prednisolone, ALG II or ALG II + Imuran)
- ■ = good transplant function (treatment with ALG I)
- □ = excellent transplant function (treatment with ALG I + Imuran)

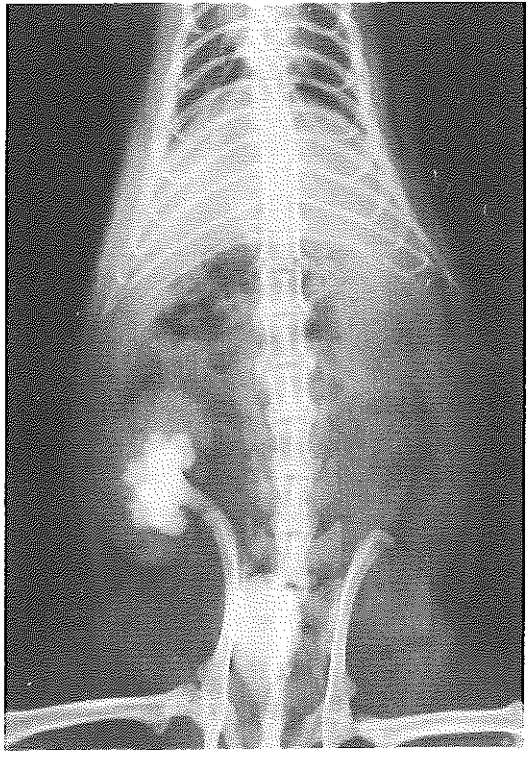


Fig.23 Pyelogram of an untreated isograft at 50 days after transplantation showing moderately severe hydronephrosis and hydroureter.

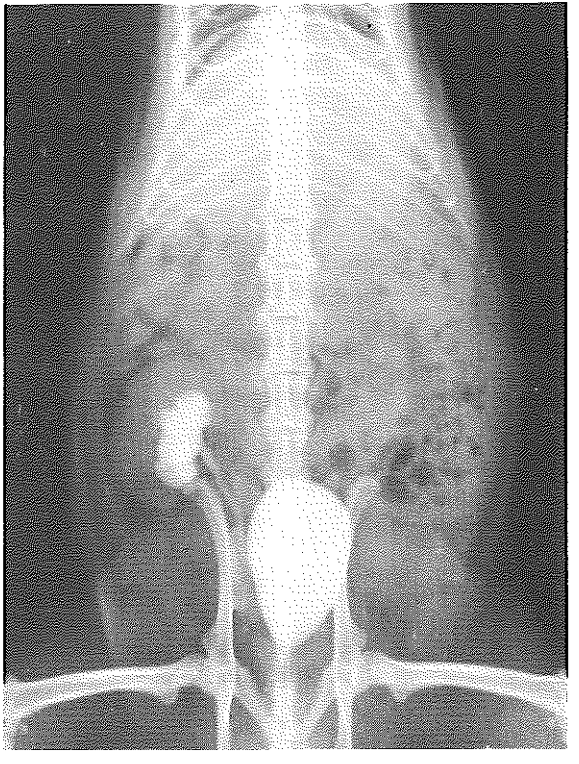


Fig.24 Pyelogram of an allograft treated with ALG I + Imuran 4 mg/kg at 100 days after transplantation showing moderately severe hydronephrosis with slightly distended appearance of the ureter.

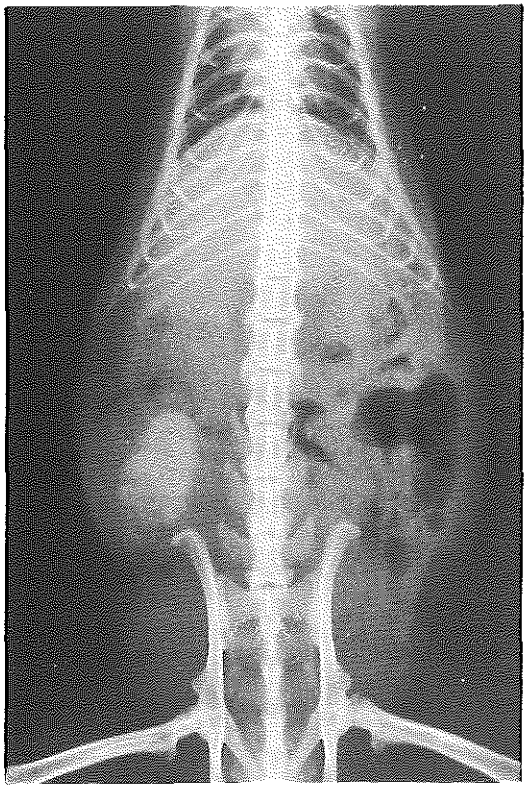


Fig. 25 Pyelogram of an untreated allograft at 50 days after transplantation showing severe hydronephrosis. Transplant function was severely impaired in this animal.

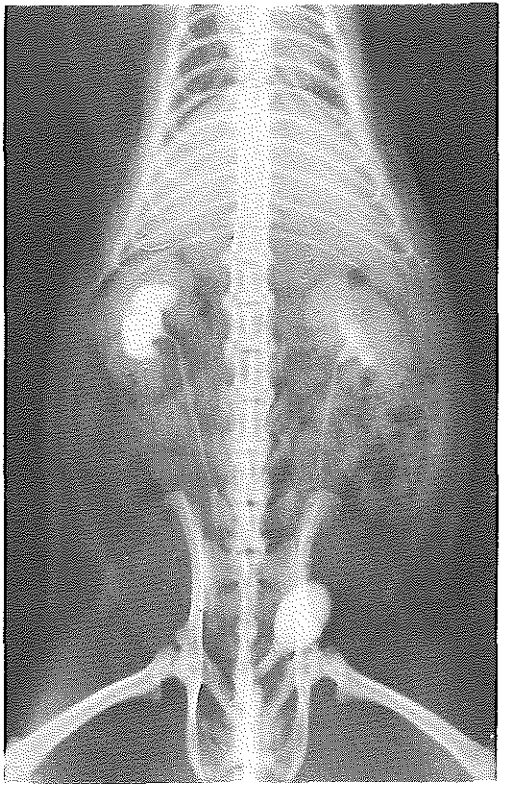


Fig. 26 Pyelogram of an untreated 8 months old Wag/Rij rat showing spontaneous right sided hydronephrosis. The left kidney and both ureters are normal.

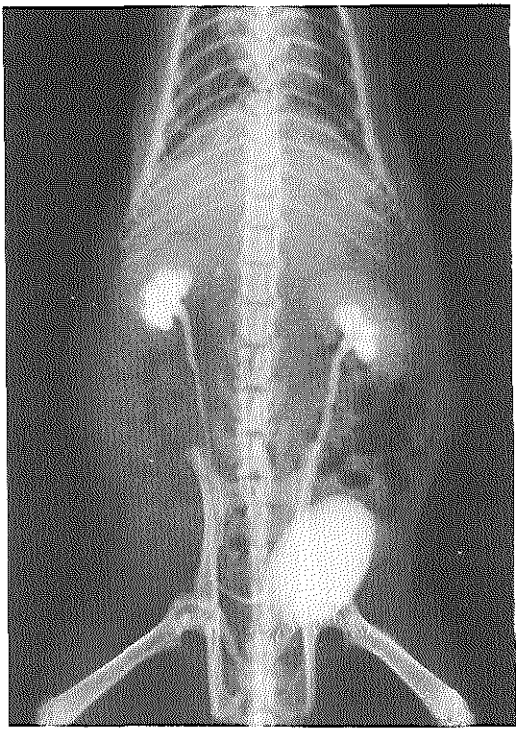


Fig.27 Pyelogram of an untreated 3 months old BN/Bi rat showing spontaneous hydronephrosis of both kidneys. The ureters are slightly distended.

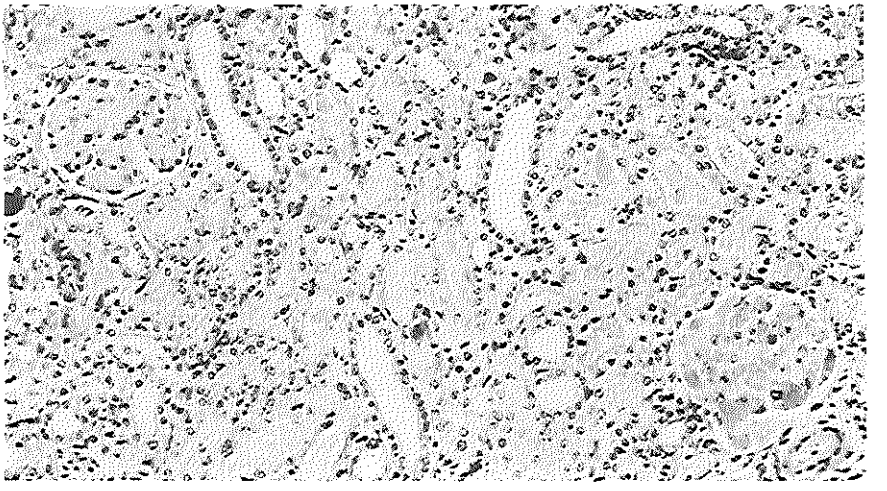


Fig.28 Rat renal allograft. Untreated renal allograft rejected at 11 days after transplantion showing some infiltration with lymphoid cells, widespread necrosis of the tubules and obliteration of the glomerular capillaries by deposition of eosinophilic material and hemorrhage. Some of the tubules contain protein casts. HPS x 140.

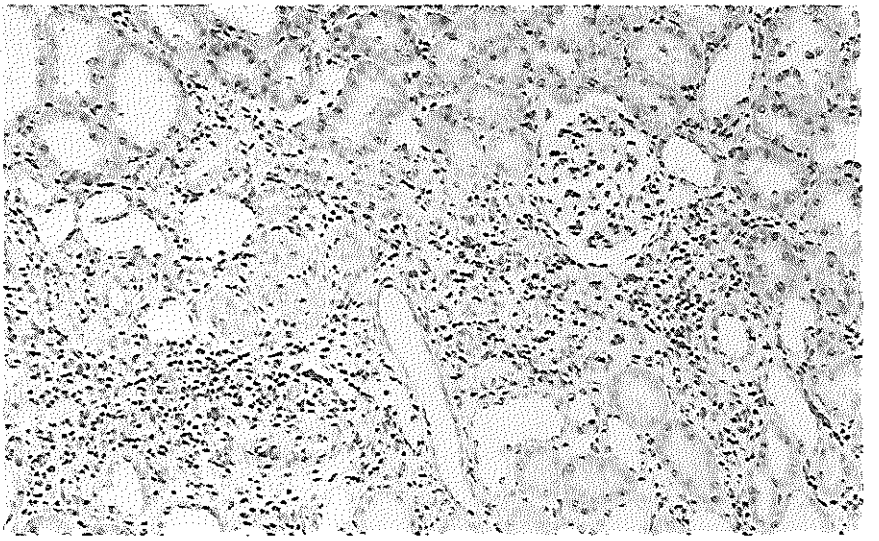


Fig.29 Rat renal allograft. Untreated renal allograft at 50 days after transplantation showing infiltration with lymphoid cells, obstruction of the glomerular capillaries and interstitial fibrosis. Some of the tubules are dilated, the others have a normal appearance. HPS x 140.

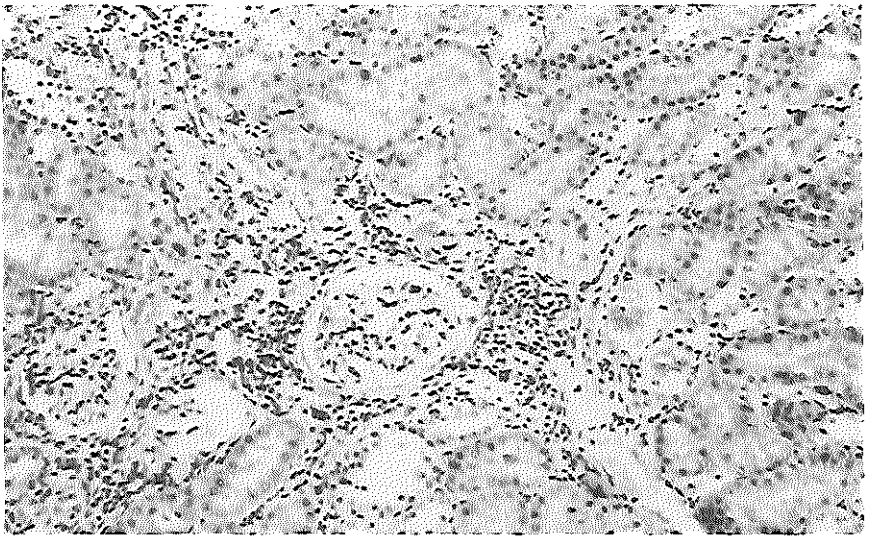


Fig.30 Rat renal isograft. Untreated renal isograft at 100 days after transplantation showing focal fibrosis with lymphoid cell infiltration. Two other such foci were observed in this kidney which looked otherwise normal. HPS x 140.

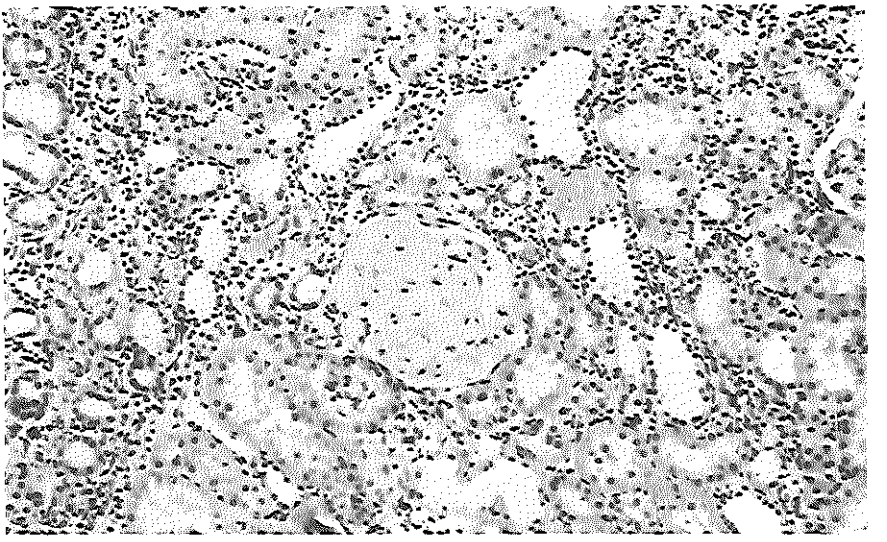


Fig.31 Rat renal allograft. Renal allograft treated with Imuran 4 mg/kg. At 50 days after transplantation the graft shows heavy infiltration with lymphoid cells, atrophy of the tubules and fibrosis. Picture shows a completely obliterated glomerulus. HPS x 140.

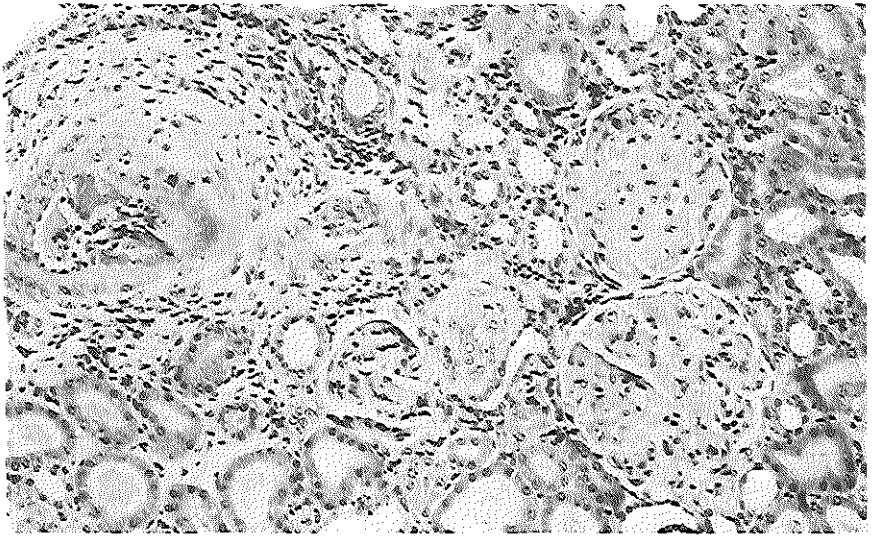


Fig.32 Rat renal allograft. Renal allograft treated with Imuran 2 mg/kg with pretreatment for one week. At 52 days after transplantation the graft was rejected, showing severe arteritis with fibrinoid necrosis of the arterial wall and perivascular fibrosis. The glomerular capillaries are obliterated, the basement membranes are thickened. Infiltration with lymphoid cells and tubular atrophy are also apparent. HPS x 140.

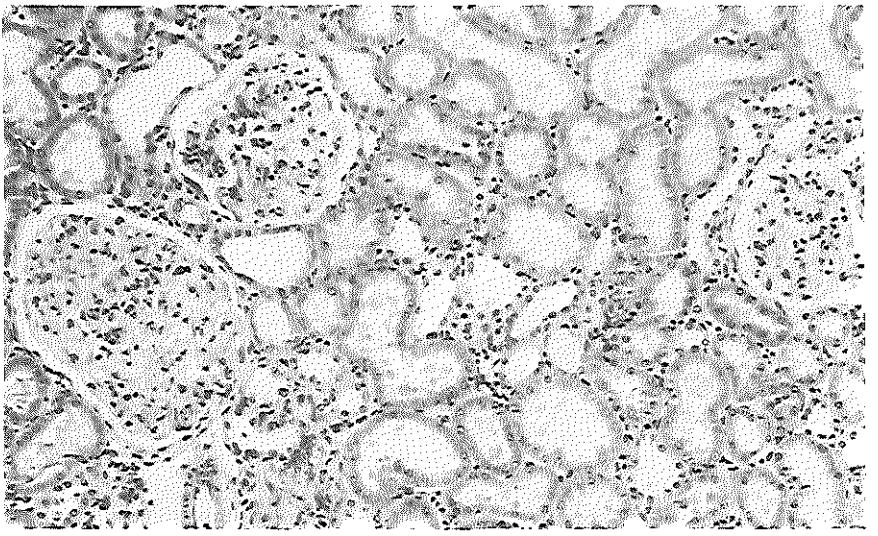


Fig.33 Rat renal allograft. Renal allograft treated with Imuran and Prednisolone at 50 days after transplantation, showing infiltration with lymphoid cells. The glomeruli are almost completely obliterated. HPS x 140.

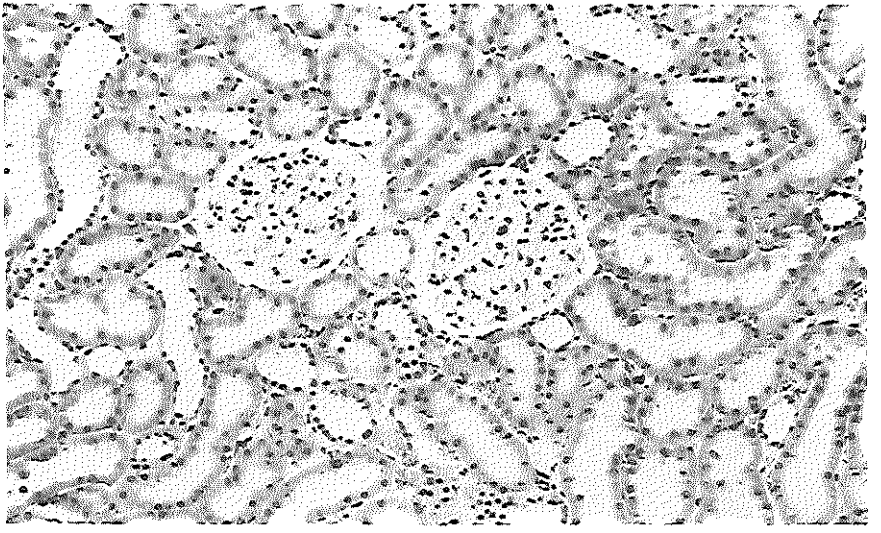


Fig.34 Rat renal allograft. Renal allograft treated with ALG I at 100 days after transplantation showing slight infiltration with lymphoid cells. Apart from this and some dilatation of the tubules the kidney is normal in appearance. HPS x 140.

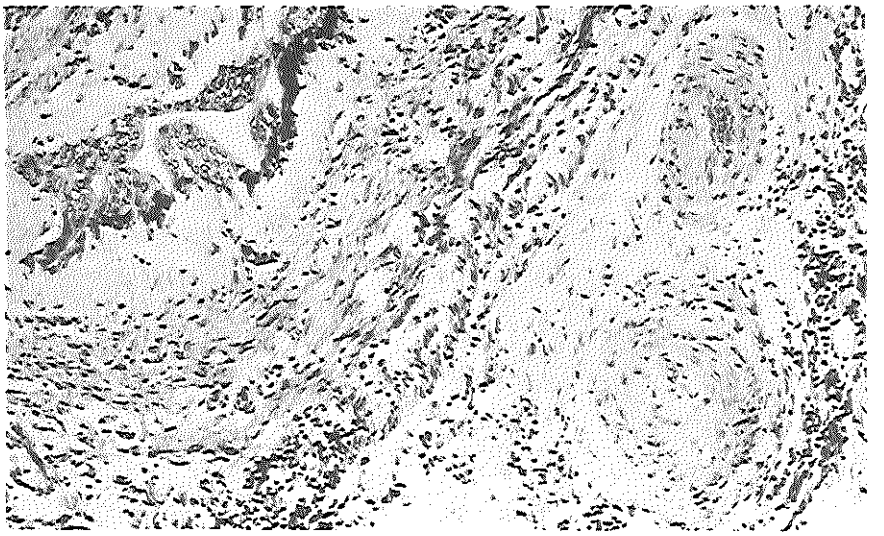


Fig.35 Rat renal allograft. Renal allograft treated with ALG II and rejected at 95 days after transplantation. A section of the ureter shows lymphoid cell infiltration in the wall. Two arteries show active arteritis and are almost completely obstructed by intimal proliferation. HPS x 140.

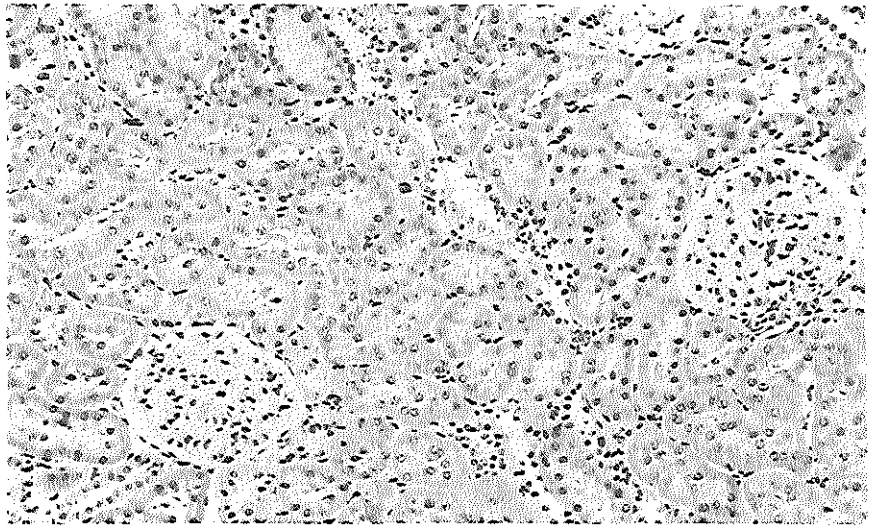


Fig.36 Rat renal allograft. Renal allograft treated with ALG I and Imuran 4 mg/kg. At 100 days no changes were observed. HPS x 140.

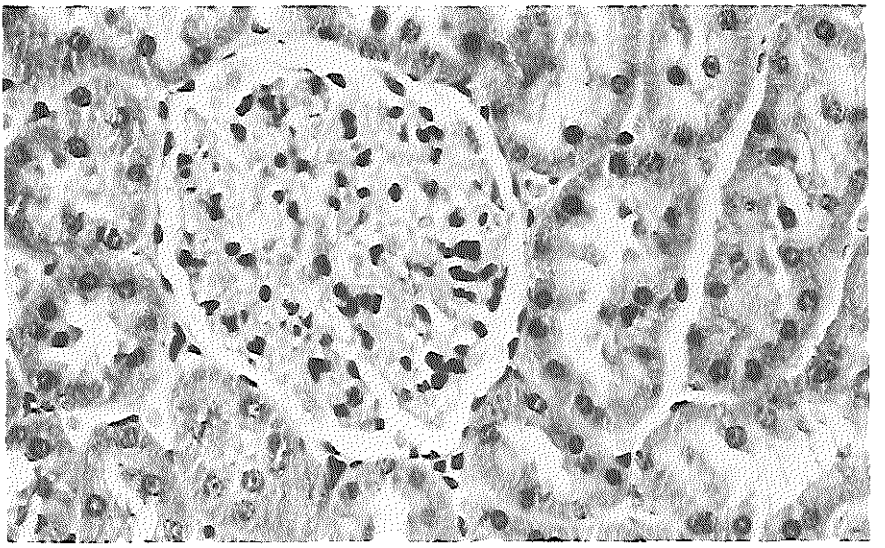


Fig.37 Rat renal allograft. Same kidney as fig.36, showing normal glomerulus and tubules. HPS x 385.

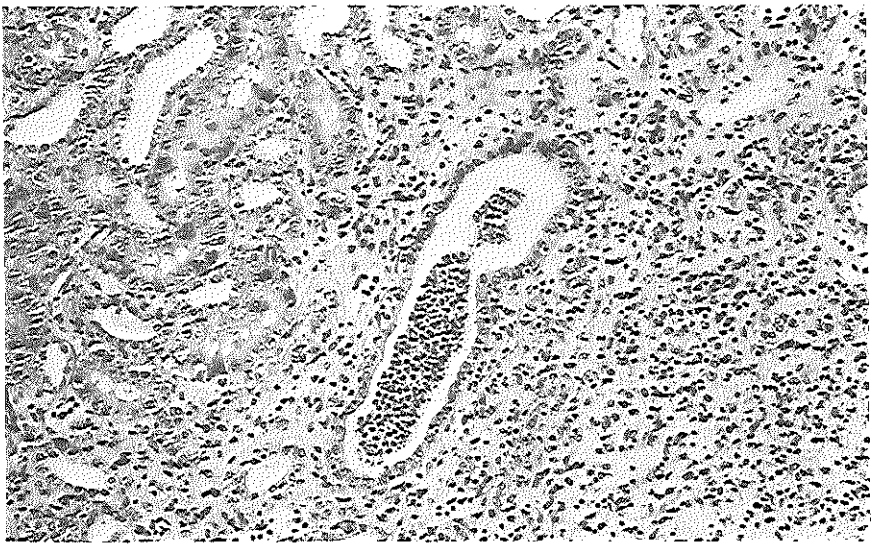


Fig.38 Rat renal allograft. Renal allograft treated with ALG II and Imuran 2 mg/kg at 50 days after transplantation showing widespread pyelonephritis: tubules containing polymorphonuclear leucocytes and debris, and focal areas of fibrosis with infiltration by inflammatory cells. HPS x 140.

Histology

Biopsy

Investigation of the material obtained by renal biopsy on day 7 after transplantation presented some difficulties, which limited the value of the information gained. Only a small piece of tissue could be taken, thus making appraisal difficult and sometimes impossible. As tissue was taken from the cortex, large blood vessels were mostly lacking. Moreover, there was considerable variability within the different groups. Because of this only a concise account of the findings will be given (table VI).

TABLE VI

SURVEY OF HISTOLOGICAL FINDINGS IN ALLOGRAFT BIOPSIES AT 7 DAYS AFTER TRANSPLANTATION

Treatment	cellular infiltration	obliteration of glomeruli	necrosis
Untreated	++	++	+++
Imuran, various doses	++	++	+++
Imuran + prednisolone	++	++	++
ALG I	++	+	++
ALG II	++	+	+
ALG I + Imuran 4 mg/kg	++	+	+
ALG II + Imuran 4 and 2 mg/kg	++	+	+

\+ = slight

++ = moderate

+++ = severe

In biopsies from the isografts, the only special feature noted in some cases was tubular atrophy, supposedly caused by edema. In the allografted kidneys, no difference in the severity of infiltration with lymphoid cells was noted between treatment groups, all biopsies showing a moderately severe cellular infiltrate. In general, deposition of moderate amounts of eosinophilic material was observed in the glomeruli of allografts that were either untreated or treated with Imuran alone, while only small amounts of this material were present after treatment with ALG I and ALG II, either alone or in combination with Imuran. Renal biopsies taken from rats, which subsequently died before day 50 from rejection of the transplant, often showed severe focal tubular necrosis. Wide-spread necrosis of the renal tissue was a bad prognostic sign. In 5 rats treated with Imuran and in one rat treated with Imuran and Prednisolone showing this, death followed within a few days.

Autopsy

In general, differences between treatment groups noted at microscopical investigation of the kidney were well correlated with the differences found by means of the function tests.

All kidneys in the unilaterally nephrectomized control groups had a normal appearance. The untreated allografts showed the picture of complete rejection: heavy infiltration with lymphoid cells, focal areas of infarct necrosis, in some cases complete infarction of the transplant, necrosis of the tubules, obliteration of the glomerular capillaries by PAS-positive material, hemorrhage, and arteritis of the large and medium-sized arteries (fig.28). The allograft that continued to function without treatment for 50 days, showed severe damage: a dense mononuclear infiltrate, partial obstruction of the glomeruli by PAS-positive material, extensive interstitial fibrosis and active arteritis (fig.29). In addition, tubular atrophy was present to a variable extent.

In order to compare the different treatment groups, microscopical examination of isografts and treated allografts was performed on all the rats which had been alive for 50 days or more after transplantation.

In the isografted series, some of the kidneys were slightly infiltrated with lymphoid cells. In two cases pyelonephritis was present and in two other cases a few foci of streaky fibrosis associated with

lymphoid cell infiltration were noted (fig.30). Glomeruli, tubules and blood vessels were normal.

The 3 groups treated with Imuran alone were quite similar in histological appearance, which makes separate discussion unnessary. All kidneys were severely damaged (figs.31 and 32). In these groups the whole renal tissue was heavily infiltrated with mononuclear cells. The glomerular capillaries were obliterated by deposition of eosinophilic, PAS-positive material and thickening of the basement membranes was prominent. Many of the glomeruli showed necrosis of the capillary loops often accompanied by hemorrhage. Although tubular atrophy was not equally severe in all cases, all kidneys showed substantial loss of tubules with marked interstitial fibrosis. In 12 out of 13 kidneys lesions in the large and medium-sized arteries were observed: active arteritis with fibrinoid necrosis of the vessel wall and foci of partly healed arteritis with intimal proliferation and medial fibrosis. Arteriolar necrosis was found in 6 cases. Some of the kidneys also showed hyperplastic arteriolosclerosis. Pyelonephritis was noted once.

In the group treated with Imuran and Prednisolone, the one rat that died at 55 days was distinguishable from the others on its renal histology, glomerular damage and arteritis being more severe. In the allografts of this group (fig.33) cellular infiltration appeared to be somewhat less dense than in the previous groups. The amount of eosinophilic material in the glomeruli was the same as in the allografts treated with Imuran alone, but necrosis and hemorrhage occurred less frequently. Tubular atrophy also was less severe, while interstitial fibrosis was evident only in 3 out of the 8 cases. In all kidneys the large and medium-sized arteries showed evidence of active arteritis. In 5 of these rats fibrosis of the arterial wall and intimal fibrosis indicated that the process was of longer duration. Arteriolonecrosis was observed in two of the kidneys. In all other cases the arterioles appeared normal. No pyelonephritis was found in this group.

In the 7 allografts that had been treated with ALG I (fig.34), the density of cellular infiltration was about the same as in the previous group. However, the condition of the glomeruli and tubules was much better, only minor changes being present. No arterial or arteriolar changes were noted in any of these kidneys. Interstitial fibrosis and pyelonephritis were not observed in this group.

After treatment with ALG II, one rat died from rejection of the transplant at 95 days after transplantation. In this case the glomeruli contained large amounts of eosinophilic material with necrosis and hemorrhage. Also, severe arteritis (fig.35), marked interstitial fibrosis and pyelonephritis were observed in this kidney. The remaining 4 kidneys in this group were largely similar to those treated with ALG I with the exception of the large and medium-sized arteries. In these kidneys evidence of active arteritis, sometimes partly healed, was found. The arterioles appeared normal.

Kidney grafts from the rats, treated with a combination of ALG I and Imuran 4 mg/kg were practically similar to the isografts in appearance (figs.36 and 37), except in one respect: signs of active arteritis were observed in 3 of the 4 cases. Pyelonephritis was not found in this group.

In the last two groups, treated with ALG II and either 2 or 4 mg/kg of Imuran, cellular infiltration was moderately severe. In only 3 out of 11 kidneys there were notable changes in the glomeruli and tubules, the others appeared normal. These 3 kidneys were those whose function had been most severely impaired, as demonstrated by higher B.U. and lower urine osmolality values. All 3 had evidence of arteritis, while arteriolonecrosis was observed in one case and interstitial fibrosis in another. None had pyelonephritis. Of the other 8 kidneys, 5 showed active arteritis of the large and medium-sized arteries. The arterioles looked normal and no interstitial fibrosis was noted. The incidence of pyelonephritis was found to be high, 5 of the 8 rats suffered from this condition (fig.38).

Hypertension (systolic blood pressure over 180 mm Hg on at least two occasions) has been observed in 8 allografted rats, treated with Imuran (5x), Imuran + Prednisolone (1x) and ALG II + Imuran 2 mg/kg (2x). In 7 of these, microscopical investigation revealed necrosis of the arterioles and hyperplastic arteriolosclerosis, changes which were observed in 4 other cases from the same groups without evidence of preexisting hypertension.

More or less severe atrophy of the lymphatic tissues of spleen, thymus and lymph nodes as well as moderate atrophy of the bone marrow was a common finding in the rats which died from rejection, irrespective of their immunosuppressive treatment. These rats were all in a uremic state. It was not found in rats sacrificed at 50 or 100 days after transplantation.

Slight changes in the liver were noted occasionally: slight vacuolar degeneration of liver cells in one unilaterally nephrectomized rat, treated with ALG I and in one allografted rat treated with ALG II + Imuran 2 mg/kg as well as liver cell necrosis in one unilaterally nephrectomized rat treated with ALG II and in one allografted rat treated with Imuran + Prednisolone. All other tissues examined were normal in appearance.

DISCUSSION

The results of the present investigation confirm the advantage of using inbred rats in a model system for renal transplantation. Allogeneic transplantations across a strong histocompatibility barrier were highly reproducable, while isogeneic transplantations were available as controls. A major disadvantage of the model system was the difficulty of the technique, resulting in a high mortality. This has also been reported by others (Murray 1969, Sakai et al., 1968). In our experiments approximately 50% of the rats surviving the first 24 hours after transplantation eventually died from causes other than graft rejection, the main cause being technical failure. Mortality as a result of complications was found to be higher in the isografted than in the allografted rats, a fact which was due to the ureteric tissue being of a softer consistency in the Wag/Rij as compared to the BN/Bi rat. Because of this, successful ureter-bladder anastomoses were rather difficult to perform in the isografted group, resulting in a high incidence of either leakage or obstruction at this site. On the other hand, severe infection causing death of the animal was found almost exclusively among the various allografted groups, probably as a result of immunosuppressive therapy.

In the present study, comparing the efficacy of short-term treatment with various immunosuppressive agents, transplantations were performed across a strong histocompatibility barrier. The Wag/Rij and BN/Bi strain differ at the major H-1 (AgB) locus (Štark and Křen 1969; Štark et al., 1969) and both skin and kidney grafts between the two strains are rejected within 2 weeks. As demonstrated by White et al. (1969), renal allografts transplanted between rat strains sharing the same H-1 allele, while differing by several weaker histo-

compatibility genes, are subject to a much slower rejection process. In their experiments renal allografts transplanted from Fisher to Lewis rats had survival times up to 32 weeks, although skin grafts in the same combination were rejected as promptly as H-1 incompatible ones (within 12 days).

In the present experiments serial determinations of body weight, hematocrit value, blood urea, urine production per 24 hours, urine osmolality and systolic blood pressure together with intravenous pyelography before sacrifice, were chosen as the parameters to study the grafted rats. The amount of urinary protein was also measured but, in our experience, this was a poor indicator of transplant function in the rat (data of this determination have not been presented).

Several other investigators, instead of using survival as the sole criterion, have employed tests to assess function of the transplanted rat kidney. In the experiments of White et al. (1969), mentioned above, a detailed study was made of transplant function. Body weight, blood urea nitrogen (BUN) and serum creatinine levels, 24-hour volume, specific gravity and protein concentration of the urine were regularly determined. Results of histological examination of biopsy and autopsy material was also presented. In these experiments renal function was found to be similar in isografted and in unilaterally nephrectomized control rats. This is in contrast to our observation of a decreased concentrating capacity together with an increased urinary output of isografted as compared to normal kidneys. In another publication White and Hildemann (1969) reported a slightly impaired kidney function in renal isografts with regard to urine osmolality values. In this context, it should be pointed out that variability in urine osmolality values may occur, depending on the nutritional state of the animal. Bauman et al. (1964) showed that normal rats at the end of a 24-hour fasting period concentrated urine to only about half the osmolality of urine obtained from rats fed ad libitum. In our experiments rats always had free access to food during urine collection periods. Thus urine osmolality was measured over a rather wide range which made it a very sensitive expression of renal concentrating ability.

Guttmann et al. (1967a,b; 1969b,c; Guttmann and Lindquist, 1969) measured effective renal plasma flow (ERPF) in anesthetized rats on day 7 after transplantation by analyzing the disappearance rate of injected ^{125}I-ortho-iodo hippurate (Blaufox et al., 1967).

They found that rats, living for 7 days on kidneys grafted across a strong histocompatibility barrier, were oliguric or anuric with ERPF values comparable to those of anephric rats (Guttmann et al., 1967b). The day 7 animal served as a functional and morphological control in later experiments of this group. Determination of ERPF is an accurate way of measuring renal function in the rat. However, the technique is laborious and requires prolonged anesthesia, which makes it unsuitable for repeated use over a longer period of time.

Stuart et al. (1968b) observed greatly prolonged survival of H-1 incompatible kidney grafts (73 - 142 days) with near normal function after treatment with donor spleen cells and/or antidonor serum prepared in rats of the recipient strain, while rejection episodes could easily be recognized by a rise in blood urea nitrogen (BUN), 24-hour urine volume and protein concentration together with a fall in urine specific gravity.

The determinations carried out in the present study, in combination with microscopical investigation, proved very useful to evaluate differences between the various treatment schedules. Differences between groups, with respect to the various parameters, were confirmed by statistical testing, using the stringent criterion of significance at the 1% level. Changes in body weight clearly reflected the general condition of the animals. Hematocrit values were found to decrease in all groups during the first 2 weeks after transplantation, irrespective of the immunosuppressive treatment given and of the occurrence of uremia. Hematocrit values slowly returned to normal during subsequent weeks. This may reflect impaired erythropoietin production by the grafted kidney. The development of hypertension always indicated severe renal damage. Blood urea and urine osmolality levels were found to be the most useful and reliable parameters of transplant function. With respect to urine osmolality values, it was found that considerable variation occurred when blood urea was within the normal range.

Intravenous pyelography revealed a high incidence of hydronephrosis in the transplanted kidneys, a fact which has also been reported by others (Feldman and Lee, 1967; Feldman et al., 1968; Guttmann et al., 1967b; Sakai et al., 1968, 1969). It is generally assumed that the occurrence of hydronephrosis represents failure to perform an adequate ureter-bladder anastomosis. In our experiments, the importance of the operation technique was clearly demonstrated

by the statistically significant difference in severity of hydronephrosis, as measured on the pyelograms, between 2 series of kidneys transplanted by 2 different persons. However, both the donor and the recipient strain showed a tendency to develop hydronephrosis as demonstrated by the spontaneous occurrence of this defect in the right kidney of approximately one third of the rats in the same age groups as those used in the experiments.

The first 2-3 weeks after transplantation appeared to be of crucial importance in the rat model system. Death from graft rejection in treated as well as in untreated animals was observed during that period, after which time the chance of graft rejection appeared to be reduced. When no rejection occurred within 3 weeks, prolonged survival for more than 50 days was the rule, in most cases without changes in transplant function, even in the absence of immunosuppressive treatment. A few cases of similarly prolonged renal allograft survival in the rat have been reported by others: French and Batchelor (1969) following administration of antibody against donor lymphocytes for 4 days, Guttmann et al. (1969c) following treatment with 10 mg of rabbit anti-rat thymocyte IgG intravenously before or shortly after transplantation and Taguchi et al. (1968) following treatment with donor antigen for 4 weeks. With regard to the existence of a critical period after renal transplantation, the situation resembles that found in man. According to Moore and Hume (1969) the major period of hazard in man is during the early months after transplantation when kidneys from related living donors are used. For cadaver donor transplants this period is considerably longer (1-2 years). Furthermore it appears that the pattern of transplant function for kidneys from cadaver donors as well as from related living donors during the first month after transplantation, is of particular importance in determining the ultimate success or failure of the graft.

Treatment for 3 weeks after transplantation did not prevent rejection in all rats. In a proportion of the cases (especially in those treated with Imuran as the sole immunosuppressive agent) a pattern of slowly progressive rejection was observed starting about 3 weeks after transplantation and resembling that found in larger animals and in man. Microscopical investigation in these cases, either after spontaneous death or after sacrifice, confirmed that active rejection was taking place in the graft. In 2 other cases a rejection crisis was fol-

lowed by spontaneous recovery.

Differences between groups with respect to transplant function were well correlated with those observed at microscopical examination while differences in 50-day survival per group were largely in agreement. However, it should be realized that the histological differences observed between the groups may not have been entirely due to the immunosuppressive regimens, since treatment had been discontinued for at least 4 weeks by the time of investigation.

The fact that concentrating ability in the isografted rats remained below that found in untreated unilaterally nephrectomized control rats was probably the result of incomplete recovery from the ischaemic injury at the time of transplantation. The occurrence of hydronephrosis in the isografts might have been a contributing factor but could not fully explain it, since it was found that in the allografts, increase in size of the renal pelvis was not correlated with a decrease in renal concentrating capacity.

Although rejection time of untreated allografts was fairly constant (mean survival time 11.3 days) one rat managed to survive 50 days after transplantation. Hyposthenuria and massive polyuria developed in this animal. It is not clear whether this diabetes insipidus was caused by the immune reaction, affecting the distal tubules in particular, by the severe degree of hydronephrosis present, or by other causes. When this rat was sacrificed, at 50 days after transplantation, severe histological damage of the kidney with wide-spread interstitial fibrosis was observed. Exceptional cases of long survival in untreated allografted rats have also been reported by Stuart et al. (1968b) and by Tinbergen (1968).

Detailed morphological studies of the rejection process in unmodified rat kidney grafts have been performed by Guttmann et al. (1967b), by Feldman and Lee (1967) and by de Vries et al. (1968). In our experiments, untreated allografts were always completely destroyed by the time the animal died.

Immunosuppressive treatment with Imuran alone was not particularly effective. Day 7 biopsies of Imuran treated allografts were similar to those from untreated allografts with respect to the severity of tubular necrosis and obstruction of the glomeruli. It is of interest that treatment with the lowest dose (2 mg/kg) resulted in better transplant function, higher 50-day survival percentage and lower incidence of chronic rejection than treatment with the higher doses,

although the histological picture was similar in both groups. When chronic rejection occurred, the process was already evident during the third week after transplantation, prior to the cessation of treatment. Reports about Imuran treatment in the rat are contradictory: with intraperitoneal doses of 8 and 4 mg/kg, Tinbergen (1968) obtained 50-day survival figures of 60% and 45% respectively, while intraperitoneal doses of 6 mg/kg in the experiments of French and Batchelor (1969) were ineffective (mean survival time 8.5 days). It has been shown in dogs, that Imuran therapy is most effective when started 7 to 30 days before transplantation (Starzl 1964, p.133). In our experience with the rat, pretreatment with Imuran for one week before transplantation (in addition to posttransplant treatment) did not improve function of the graft. On the contrary, after this pretreatment mortality from postoperative hemorrhage was twice as high as in the other groups.

Treatment with a combination of Imuran and Prednisolone, which is the standard therapy in clinical practice, was clearly more effective than Imuran alone as demonstrated by better transplant function and less histological damage in the former group.

It is of interest that the two batches of ALG (I and II) produced and administered in the same fashion, were not equally effective. The lymphocytotoxic titer and the ability to prolong skin graft survival were higher for ALG I as compared to ALG II. ALG I also proved more effective in suppressing the immune reaction after renal transplantation. Our data on transplant function and histology clearly show that, while treatment with a potent batch of ALG may be superior to the usual therapy with Imuran and corticosteroids, treatment with a less potent batch may not be so. This once more illustrates the need of a reliable method to assess the immunosuppressive potency of a given ALG preparation, especially when it is to be used in human transplantation. At present anti-human lymphocyte sera must be tested in vivo using sub-human primates (Balner, 1969).

Guttmann et al. (1967a) treated rats prior to renal allotransplantation intravenously with heterologous rabbit anti-rat thymocyte serum (ATS 0.7 and 4.0 ml/rat), its IgG fraction (6 and 10 mg/rat) as well as its pepsin digested fragments (23 and 30 mg/rat) and obtained effective renal plasma flow (ERPF) values within the normal range at day 7. A severe immune complex nephritis developed after treatment with the crude unadsorbed material. The IgG preparation contained

antibody to glomerular basement membrane antigens but its nephrotoxicity could be reduced by subcutaneous instead of intravenous administration (Guttmann et al., 1967a; Lindquist et al., 1969). A total of 10 mg of ALG, administered intravenously around the time of transplantation was also found to be effective in prolonging graft survival with normal renal function and histology, while subcutaneous administration of 10 mg ALG (in 2 rats) was hardly effective (Guttmann et al., 1969c). The data of the present investigation failed to support the suggestion given by the above-cited experiments that subcutaneous administration is hardly effective. However, the total dosage of 4 weekly injections of 19 mg of IgG, as given in our study, was higher than in the experiments discussed above (and, incidentally, much higher than the doses given to human renal transplant recipients). On the other hand, from our observations with light microscopy, there was no evidence of nephrotoxicity from either of the 2 ALG batches.

Although day 7 biopsies from ALG treated allografts showed much less tubular necrosis and obliteration of the glomeruli than biopsies from Imuran treated allografts, all showed the same amount of lymphoid infiltration, suggesting that not all of the cells present were engaged in an actual immunological attack against the graft.

Our interest in treatment with a combination of ALG and Imuran was based on earlier work (de Vries et al., 1968) in which continued treatment with Imuran was seen to be very efficient in suppressing the vascular component of graft rejection, while not preventing the glomerular damage. In these studies crude, unpurified ALS, also administered over longer periods of time, was found to be very effective in preventing the glomerular damage, while incompletely preventing the vascular lesions. It has been postulated that the necrotizing arteritis is caused by an antibody-mediated rejection process, while the glomerular damage is mainly due to cell-mediated rejection. It has been shown by Israel and de Vries (1970) that ALS suppresses both antibody-mediated and cell-mediated immunological processes, while Imuran, under the conditions of their experiments, only suppresses antibody-mediated immunological processes. In view of these results the finding that Imuran does not prevent glomerular damage can be explained. It should be stressed, however, that the present experiments differ from those discussed above in that treatment was discontinued after 3 weeks. In the present experiments results of

treatment with the potent batch ALG I were indeed improved by addition of Imuran. For the less potent batch ALG II no such effect on function and histological changes of Imuran addition was observed, though this might have been due to the high incidence of pyelonephritis in this group, not only as a finding at autopsy, but also as a complication leading to early death. Allografts, treated with ALG I and Imuran 4 mg/kg were practically similar to isografts in function as well as histology. It was quite remarkable that, although transplant function in this group did not reveal signs of chronic rejection, histological evidence of a discrete but active arteritis was observed, a feature which was totally absent from the allografts treated with ALG I alone. It is possible that ALG and Imuran have synergistic actions, which could explain the improved results after combined treatment but not the discrepancy in histological findings. Another possibility is that antibody formation, in particular against ALG protein, was suppressed by simultaneous administration of Imuran thus avoiding a rapid immune elimination and thereby a reduced efficacy of ALG. The early arteritis seen at 50 and 100 days after transplantation would then be the result of humoral antibody formation against the graft following cessation of Imuran treatment at 3 weeks after transplantation. Suppression of antibody formation to ALG might also have the advantage of preventing the immunological complications of ALG treatment, since it has been shown that the partial suppression of humoral antibody formation by ALG may lead to serum sickness (Cohen et al., 1970).

Jeejeebhoy et al. (1968) found that the ability of antilymphocyte plasma to prolong skin allograft survival in mice was improved when given in conjunction with 6-mercaptopurine. On the other hand, Starzl et al. (1967) observed only a slight potentiation of the effect of ALG with respect to renal allograft survival in dogs by the simultaneous use of Imuran, although the degree of histological injury seemed to be reduced. In the treatment of human renal allograft recipients, ALG has been given as an adjunct to the conventional immunosuppressive drugs, not because combined therapy was expected to be more effective but because of a reluctancy to replace the usual therapy altogether in favor of ALG alone for which clinical experience was lacking and particularly when no reliable means to diagnose rejection at an early stage were available. Pioneer work in clinical use of ALG has been done by Starzl and his coworkers (Starzl et al.,

1967, 1969) who have at present the largest series of patients, treated with horse anti-human lymphocyte globulin. During the early postoperative months, recipients of kidneys from related living donors received amounts of 14 to 50 mg/kg per week by intramuscular injection. As compared with previous groups a reduction in mortality was achieved by ALG therapy while lower doses of Imuran and Prednisone than before were required to maintain the same level of renal function. After discontinuing ALG, renal function did not deteriorate. The experience with ALG in cadaver renal transplantation was still insufficient to justify conclusions.

In human renal transplant recipients, sporadic attempts to discontinue immunosuppressive treatment entirely have generally been followed by irreversible deterioration of transplant function. In our experiments, cessation of treatment in rats with stable transplant function did not have any adverse effect. However, histological signs of active rejection were found in a proportion of these rats. The vascular lesions observed at 50 and 100 days after transplantation in some of the rats with excellent renal function after combined treatment with ALG I and Imuran, were essentially the same as those observed in biopsies from long functioning human renal transplants. Our results in the rat suggest that treatment with high doses of potent ALG during the early period after transplantation is highly effective and that ALG therapy can be discontinued without deterioration of transplant function. However, the low grade rejection process occurring at a later stage indicates that complete omission of Imuran therapy is unwarranted. Possibly, adaptation of the graft to the cell-mediated component of graft rejection, initially suppressed by ALG, is easier obtained than adaptation to the antibody-mediated component.

In human renal transplantation, the well known morbidity associated with clinical ALG therapy as reported by Kashiwagi et al. (1968) and the fact that, after a 2 - 3 weeks course of ALG, appreciable amounts of circulating antibody against ALG protein can be detected (Iwasaki, 1967) suggest that short term treatment with high doses of ALG might be more efficient than prolonged courses with this material. As long as infections and sepsis are the major cause of death in human renal transplantation, the fact that doses of other immunosuppressive drugs can be reduced when given as an adjunct to ALG is of great advantage (Starzl et al., 1967, 1969). Besides the risk

of prolonged treatment with high doses of immunosuppressive agents promoting the development of malignant tumors (Balner, 1970) might be reduced. Though cessation of immunosuppressive therapy is possible in the rat, it is not clear whether this may be extrapolated to man. More research in this direction appears indicated.

SUMMARY

In the introduction, some subjects which are relevant to the experiments are briefly discussed. Skin grafting experiments, having contributed largely to our knowledge of transplantation biology, are, for several reasons, inadequate for studying the effect of immunosuppressive treatment on renal transplants. Results of experimental renal transplantation in larger animals such as dogs have until recently been difficult to evaluate, mainly because of the variation in histocompatibility difference between randomly selected members of the same species. Transplantations between highly inbred strains of animals are not subject to this variation.

In the experiments, a microvascular technique modified after Fisher and Lee, was used for orthotopic transplantation of the rat kidney. Untreated allogeneic transplants between two highly inbred strains, BN/Bi and Wag/Rij, have an almost constant rejection time. Isogeneic transplantations between rats of the Wag/Rij strain were performed as controls. Both host kidneys were removed during operation. On day 7 an open wedge biopsy was taken from the transplant. Immunosuppressive treatment with various regimens of Imuran, Prednisolone, and ALG (rabbit anti-rat thymocyte globulin) was given to groups of allografted rats for a limited period of 3 weeks after transplantation. The groups were compared with respect to 50-day survival, renal function and histology of the transplant. Half of the survivors were sacrificed at 50 days after transplantation, the remainder after 100 days. Serial determinations of body weight, hematocrit value, blood urea, urine osmolality, 24-hour urine production and systolic blood pressure were chosen to assess function of the transplant. In addition, intravenous pyelography was performed before sacrificing the animals.

After excluding those animals which died as a result of a technical failure, a total of 95 transplants remained. Survival percentage at 50 days after transplantation was 100% in the untreated isografted group. Survival percentage was lower in 3 allografted groups treated with Imuran alone than in the various groups treated with Imuran + Prednisolone or with ALG. Death from graft rejection was not observed between 22 and 50 days after transplantation when the immunosuppressive therapy was discontinued.

In the isografted group blood urea rose to 450% of normal, returning to normal values after one week. Urine osmolality fell to 30% of normal with recovery to a level of 65% after 4 weeks. After an initial decrease, weight and hematocrit values returned to normal in 6 weeks. In the untreated allografted group deterioration of function was progressive, resulting in 80% mortality within 2 weeks. After treatment for 3 weeks with Imuran values for blood urea and urine production stabilized at high levels while urine osmolality was very low. Weight loss and decreased hematocrit values were not completely restored. A high incidence of chronic rejection was observed in 2 of the Imuran treated groups. Results were improved when Prednisolone was given together with Imuran. When this regimen or ALG, either alone or in combination with Imuran, was used it was noted that cessation of the treatment at 3 weeks after transplantation was not followed by deterioration of transplant function. Two different batches of ALG (I and II) were given. Results of treatment with ALG II were similar to those of treatment with Imuran + Prednisolone. When ALG II was given together with Imuran, results were not appreciably better. However, transplant function was clearly improved by treatment with ALG I as compared to the previous groups. Treatment with ALG I in conjunction with Imuran resulted in transplant function which was similar to that of isografts.

Intravenous pyelography revealed hydronephrosis in 39 out of 45 cases, a condition which was also found to occur in a proportion of the normal donor and recipient animals.

Histologically, obliteration of the glomeruli and tubular necrosis was most severe in biopsies taken from allografts treated with Imuran alone.

At autopsy, the untreated allografts were completely destroyed. Advanced destruction of glomeruli and interstitial fibrosis were observed in all Imuran treated allografts. Changes were slightly less

severe in the allografts treated with Imuran + Prednisolone. Only minor changes were present in allografts treated with ALG I. In appearance, allografts treated with ALG I + Imuran were practically the same as isografts, except for the presence of active arteritis in the majority of the animals. Arteritis of the large and medium-sized arteries was the main lesion in the allografts treated with ALG II either alone or in combination with Imuran.

In the discussion it is concluded that:

- The rat model system is highly suitable for screening of different regimens of immunosuppressive treatment in renal transplantation.
- The efficacy of immunosuppressive regimens can be evaluated in this model system by performing some rather simple function tests. Of these, blood urea and urine osmolality are particularly valuable to quantitate transplant function while results correlate well with the histological findings.
- A potent ALG is needed to improve the results of treatment with Imuran + Prednisolone.
- Results, similar to those obtained in isografts, can be obtained by treatment with a potent ALG in conjunction with Imuran.
- The cessation of immunosuppressive therapy at 3 weeks after transplantation in this model system is not followed by a deterioration of transplant function during a period of at least 100 days after transplantation. Nevertheless, arteritis occurred in the majority of animals after cessation of treatment, which was not reflected in the function tests. An exception to this was the group treated with ALG I, which remained free from arteritis.

SAMENVATTING

In de inleiding worden enkele onderwerpen met betrekking tot de experimenten kort besproken. Huidtransplantatie experimenten, die onze kennis van de transplantatiebiologie belangrijk hebben verrijkt, zijn om verschillende redenen ongeschikt om het effect van immunosuppressieve behandeling op niertransplantaten te bestuderen. Resultaten van experimentele niertransplantatie bij grotere dieren, zoals honden, waren tot voor kort moeilijk te interpreteren, voornamelijk door de variatie in histocompatibiliteitsverschil tussen willekeurig gekozen leden van dezelfde soort. Transplantaties tussen sterk ingeteelde dierstammen zijn niet aan deze variatie onderhevig.

In de experimenten werd gebruik gemaakt van een gewijzigde microvasculaire techniek volgens Fisher en Lee voor orthotope transplantatie van de rattenier. Onbehandelde allotransplantaten tussen twee sterk ingeteelde rattestammen, BN/Bi en Wag/Rij, hebben een vrijwel constante afstotingstijd. Als controles werden isotransplantaties tussen ratten van de Wag/Rij stam verricht. Beide nieren van de ontvanger werden tijdens operatie verwijderd. Op de 7e dag werd een open wigbiopsie uit het transplantaat genomen. Immunosuppressieve behandeling met verschillende schema's van Imuran, Prednisolon en ALG (konijn anti-rat thymocyten globuline) werd gedurende een beperkte periode van 3 weken na transplantatie aan groepen allogeen getransplanteerde ratten toegediend. De groepen werden vergeleken wat betreft 50-dagen overleving, nierfunctie en histologie van het transplantaat. De helft van de overlevenden werd 50 dagen na transplantatie opgeofferd, de rest na 100 dagen. Herhaalde bepalingen van lichaamsgewicht, hematocriet, ureumgehalte van het bloed, osmolaliteit van de urine, 24-uurs urineproductie en systolische bloeddruk werden gekozen om de functie van het transplantaat te testen.

Tevens werd, alvorens de dieren op te offeren, intraveneuze pyelografie verricht.

Na uitsluiting van de door technische fouten omgekomen dieren, bleef een totaal van 95 transplantaten over. Vijftig dagen na transplantatie was het overlevingspercentage 100% in de onbehandelde isogeen getransplanteerde groep. In 3 allogeen getransplanteerde groepen, behandeld met alleen Imuran, was het overlevingspercentage lager dan in de verschillende groepen behandeld met Imuran + Prednisolon of met ALG. Sterfte ten gevolge van afstoting van het transplantaat werd niet gezien van de 22e tot de 50e dag na transplantatie, toen de immunosuppressieve behandeling was gestaakt.

In de isogeen getransplanteerde groep steeg het ureum tot 450% van normaal met terugkeer tot de norm na 4 weken. De osmolaliteit van de urine daalde tot 30% van normaal met herstel tot een niveau van 65% na 4 weken. Na een aanvankelijke daling, herstelden lichaamsgewicht en hematocriet zich in 6 weken. In de onbehandelde allogeen getransplanteerde groep ging de functie in toenemende mate achteruit, met 80% sterfte binnen 2 weken als gevolg. Na behandeling met Imuran gedurende 3 weken bleven de waarden voor ureum en urineproductie hoog, die voor osmolaliteit van de urine zeer laag. Wat betreft het gewichtsverlies en de hematocrietdaling, trad geen volledig herstel op. Chronische afstoting werd frequent gezien in 2 van de met Imuran behandelde groepen. Betere resultaten werden bereikt door Prednisolon samen met Imuran te geven. Met dit schema, alsook met ALG, hetzij alleen hetzij in combinatie met Imuran, bleek het staken van de behandeling 3 weken na transplantatie niet te worden gevolgd door achteruitgaan van de functie van het transplantaat. Twee verschillende partijen ALG (I en II) werden gegeven. Met ALG II werden gelijke resultaten bereikt als met Imuran + Prednisolon. Wanneer ALG II samen met Imuran werd gegeven, waren de resultaten niet noemenswaardig beter. De functie van het transplantaat verbeterde echter duidelijk ten opzichte van de vorige groepen door behandeling met ALG I. Behandeling met ALG I en Imuran samen had een zelfde functie als van isotransplantaten ten gevolge.

Bij intraveneuze pyelografie werd hydronefrose gevonden in 39 van de 45 gevallen, een toestand die eveneens werd aangetroffen bij een gedeelte van de normale donor en ontvanger dieren.

Histologisch was obliteratie van de glomeruli en tubulusnecrose het ernstigst in biopsieën van allotransplantaten, behandeld met

Imuran alleen.

Bij sectie bleken de onbehandelde allotransplantaten volledig vernietigd te zijn. Ver voortgeschreden destructie van glomeruli en interstitiële fibrose werd gezien in alle met Imuran behandelde allotransplantaten. Wat minder ernstig waren de veranderingen na behandeling met Imuran + Prednisolon. Met ALG I behandelde allotransplantaten hadden slechts geringe veranderingen. Allotransplantaten, behandeld met ALG I + Imuran, waren microscopisch vrijwel gelijk aan isotransplantaten met uitzondering van de actieve arteritis die bij het merendeel voorkwam. Arteritis van de grote en middelgrote arteriën was de voornaamste laesie in de allotransplantaten die met ALG II, hetzij alleen hetzij gecombineerd met Imuran, waren behandeld.

In de discussie wordt het volgende geconcludeerd:

- Het modelsysteem bij de rat is zeer geschikt voor vergelijkend onderzoek van verschillende immunosuppressieve behandelingsschema's bij niertransplantatie.
- De werkzaamheid van immunosuppressieve schema's kan in dit modelsysteem met behulp van enkele vrij eenvoudige functiebepalingen worden beoordeeld. Hierbij zijn het ureumgehalte van het bloed en de osmolaliteit van de urine bijzonder waardevol bij de kwantificering van de functie van het transplantaat, terwijl de resultaten goed correleren met de histologische bevindingen.
- Een sterk werkzaam ALG is nodig om de resultaten van behandeling met Imuran + Prednisolon te verbeteren.
- Door behandeling met een sterk werkzaam ALG gecombineerd met Imuran kunnen resultaten worden verkregen die gelijk zijn aan die bij isotransplantaties.
- Het staken van de immunosuppressieve behandeling 3 weken na transplantatie heeft in dit modelsysteem geen verslechtering van de functie van het transplantaat ten gevolge gedurende tenminste 100 dagen na transplantatie. Niettemin kwam bij het merendeel van de dieren arteritis voor na het staken van de behandeling, hetgeen niet werd weergegeven door de functiebepalingen. Een uitzondering hierop was de met ALG I behandelde groep, die vrij bleef van arteritis.

REFERENCES

Advance in Transplantation. Proceedings of the First International Congress of the Transplantation Society, Paris 1967.
Munksgaard, Copenhagen 1968.

Amiel, J.L.: Les immuno-dépresseurs (V). Immuno-dépresseurs chimiques et transplantations d'organes.
La Presse Médicale 75: 1801-1806, 1967.

Amos, B.: Immunologic factors in organ transplantation.
Amer. J. Med. 44: 767-775, 1968.

Ballantyne, D.L., jr., Uhlschmid, G.K. and Converse, J.M.: Massive rabbit skin xenografts in rats.
Transplantation 7: 274-280, 1969.

Balner, H.: Immunosuppression and neoplasia.
Rev. Europ. Études Clin. et Biol. 15: 599-604, 1970.

Balner, H., Dersjant, H. and Bekkum, D.W. van: Studies in immuno-suppression. Methods to evaluate anti-human lymphocyte sera.
Transpl. Proceedings 1: 629-637, 1969.

Barker, C.F. and Billingham, R.E.: The role of regional lymphatics in the skin homograft response.
Transplantation 5: 962-966, 1967.

Bauman, J.W., Guyot-Jeannin, C. and Dobrowolski, J.: Nutritional state and urine concentrating ability in the rat.
J. Endocrin. 30: 147-148, 1964.

Bekkum, D.W. van: Anti-lymfocytenserum.
Ned. T. Geneesk. 113: 22-28, 1969.

Bekkum, D.W. van, Heystek, G.A. and Marquet, R.L.: Effects of immunosuppressive treatment on rejection of heart allografts in rats.
Transplantation 8: 678-688, 1969.

Bekkum, D.W. van, Heystek, G.A., Marquet, R.L., Bruin, R.W. de and Tinbergen, W.J.: Comparative studies on immunosuppression of heart and kidney allograft rejection in rats.
In: Pharmacological treatment in organ and tissue transplantation. Proceedings of an International Symposium, Milan, 1969.
Excerpta Medica. International Congress Series No.197: 14-19, 1970.

Bekkum, D.W. van and Vries, M.J. de: Radiation Chimaeras.
Logos Press Ltd., London, 1967.

Ben-Ziv, G., Weinman, J. and Sulman, F.G.: A photoplethysmographic method for measurement of systolic blood pressure in the rat.
Arch. int. Pharmacodyn. 149: 527-536, 1964.
Berenbaum, M.C.: Immunosuppressive agents.
Brit. med. Bull. 21: 140-146, 1965.
Billingham, R.E., Brent, L. and Medawar, P.B.: Actively acquired tolerance of foreign cells.
Nature 172: 603-606, 1953.
Billingham, R.E., Brent, L. and Medawar, P.B.: Quantitative studies on tissue transplantation immunity. II The origin, strength and duration of actively and adoptively acquired immunity.
Proc. roy. Soc. B. 143: 58-80, 1954.
Billingham R.E., Brent, L. and Medawar, P.B.: Acquired tolerance of skin homografts.
Ann. N.Y. Acad. Sci. 59: 409-416, 1955.
Blaufox, M.D., Guttmann, R.D. and Merrill, J.P.: Measurement of renal function in the rat with single injection clearances.
Amer. J. Physiol. 212: 629-632, 1967.
Calne, R.Y.: Renal Transplantation, 2nd ed.
Edward Arnold Ltd., London, 1967.
Carrel, A.: Transplantation in mass of the kidneys.
J. exp. Med. 10: 98-140, 1950.
Carrel, A. and Guthrie, C.C.: Function of a transplanted kidney.
Science 22: 473, 1905.
Carrel, A. and Guthrie, C.C.: Successful transplantation of both kidneys from a dog into a bitch with removal of both normal kidneys from the latter.
Science 23: 394-395, 1906.
Ceppelini, R., Curtoni, E.S., Mattiuz, P.L., Miggiano, V., Scudeller, G. and Serra, A.: Genetics of leucocyte antigens: a family study of segregation and linkage.
In: Histocompatibility testing 1967, Munksgaard, Copenhagen, 149-186, 1967.
Ciba Foundation Study Group no. 29: Antilymphocytic serum.
Churchill Ltd., London, 1967.
Clark, D.S., Foker, J.E., Good, R.A. and Varco, R.L.: Humoral factors in canine renal allograft rejection.
Lancet I: 8-11, 1968.
Cohen, B.J., Bruin, R.W. de and Kort, W.J.: Heritable hydronephrosis in a mutant strain of Brown Norway rats.
Laboratory Animal Care, 1970, in press.
Cohen, B.J., Vries, M.J. de, Noord, M.J. van and Lubbe, F.H.: Strain-specific renal toxicity of heterologous antilymphocyte γ-globulin in mice.
Transplantation 10: 1-19, 1970.
Dausset, J., Ivanyi, P., Colombani, J., Feingold, N. and Legrand, L.: The Hu-1 system.
In: Histocompatibility testing 1967, Munksgaard, Copenhagen: 189-202, 1967.
Documenta Geigy. Wissenschaftliche Tabellen. 7. Auflage.
J.R. Geigy, A.G., Basel, 32-35, 1969.
Feldman, J.D. and Lee, S.: Renal homotransplantation in rats. I Allogeneic recipients.
J. exp. Med. 126: 783-794, 1967.
Feldman, J.D., Pick, E., Lee, S., Silvers, W.K. and Wilson, D.B.: Renal homotransplantation in rats. II Tolerant recipients.
Amer. J. Path. 52: 687-698, 1968.
Fisher, B. and Lee, S.: Microvascular surgical techniques in research with special reference to renal transplantation in the rat.
Surgery 58: 904-914, 1965.

Fisher, R.A. and Yates, F.: Statistical tables, 4th ed.
Oliver and Boyd, Edinburgh and London: 39 (table I) and 59 (table VIII), 1953.
French, M.E. and Batchelor, J.R.: Immunological enhancement of rat kidney grafts.
Lancet II: 1103-1106, 1969.
Gardner, L.B., Guttmann, R.D. and Merrill, J.P.: Renal transplantation in the inbred rat. IV Alterations in the microvasculature in acute unmodified rejection.
Transplantation 6: 411-418, 1968.
Ginsburg, H., Ax, W. and Berke, G.: Graft reaction in tissue culture by normal rat lymphocytes.
Transpl. Proceedings 1: 551-555, 1969.
Guttmann, R.D., Carpenter, C.B., Lindquist, R.R. and Merrill, J.P.: Renal transplantation in the inbred rat. III A study of heterologous anti-thymocyte sera.
J. exp. Med. 126: 1099-1126, 1967a.
Guttmann, R.D., Carpenter, C.B., Lindquist R.R. and Merrill, J.P.: Immunological studies on the mechanism of immunosuppression by antithymocyte immunoglobulin G. (AT IgG).
In: Advance in Transplantation, Munksgaard, Copenhagen: 141-142, 1968.
Guttmann, R.D. and Lindquist, R.R.: Renal transplantation in the inbred rat. XI Reduction of allograft immunogenicity by cytotoxic drug pretreatment of donors.
Transplantation 8: 490-495, 1969.
Guttmann, R.D., Lindquist, R.R. and Merrill, J.P.: Renal transplantation in the inbred rat. VIII Quantitation of cell proliferation during unmodified rejection.
Transplantation 8: 19-27, 1969a.
Guttmann, R.D., Lindquist, R.R. and Ockner, S.A.: Renal transplantation in the inbred rat. IX Hematopoietic origin of an immunogeneic stimulus of rejection.
Transplantation 8: 472-484, 1969b.
Guttmann, R.D., Lindquist, R.R. and Ockner, S.A.: Renal transplantation in the inbred rat. XII A mechanism of long-term survival of allografts after antilymphocyte immunoglobulin treatment.
Transplantation 8: 837-845, 1969c.
Guttmann, R.D., Lindquist, R.R., Ockner, S.A. and Merrill, J.P.: Mechanism of long term survival of renal allografts after treatment with antilymphocyte antibody.
Transpl. Proceedings 1: 463-466, 1969d.
Guttmann, R.D., Lindquist, R.R., Parker, R.M., Carpenter, C.B. and Merrill, J.P.: Renal transplantation in the inbred rat. I Morphologic, immunologic and functional alterations during acute rejection.
Transplantation 5: 668-681, 1967b.
Hollander, C.F.: Functional and cellular aspects of organ ageing.
Exp. Geront. 1970, in press.
Hume, D.M.: Renal homotransplantation in man.
Ann. Rev. Med. 18: 229-267, 1967.
Israel, D.E. and Vries, M.J. de: Comparative effectiveness of antilymphocyte serum (A.L.S.) and Imuran with respect to suppression of hemagglutinin formation and skin homograft rejection.
Rev. Europ. Études Clin. et Biol. 15: 102-104, 1970.
Iwasaki, Y., Porter, K.A., Amend, J.R., Marchioro, T.L., Zühlke, V. and Starzl, T.E.: The preparation and testing of horse antidog and antihuman antilymphoid plasma or serum and its protein fractions.
Surg. Gyn. Obst. 124: 1-24, 1967.
James, K.: The preparation and properties of anti-lymphocytic sera.
Progr. Surg. 7: 140-216, 1969.

Jeejeebhoy, H.F., Rabbat, A.G. and Vela-Martinez, J.: Studies on the mode of action of heterologous antilymphocyte plasma (ALP) 3. The effects of 6-mercaptopurine and cortisol on the immunosuppressive properties of ALP.
Transplantation 6: 765-770, 1968.

Jonge, H. de: Inleiding tot de medische statistiek II.
Nederlands Instituut voor Praeventieve Geneeskunde, afd. Statistiek, Leiden: 486-488 and 768-770, 1964.

Kashiwagi, N., Brantigan, C.O., Brettschneider, L., Groth, C.G. and Starzl, T.E.: Clinical reactions and serologic changes after the administration of heterologous antilymphocyte globulin to human recipients of renal homografts.
Ann. Int. Med. 68: 275-286, 1968.

Kort, W.J.: Hydronefrose in een ingeteelde rattestam.
Biotechniek 9: 1-6, 1970.

Largiadèr, F., ed.: Organ Transplantation.
Georg Thieme Verlag, Stuttgart, 1966.

Largiadèr, F.: Immunosuppression bei klinischer und experimenteller Organtransplantation.
Naturwissenschaften 55: 429-434, 1968.

Levey, R.H. and Medawar, P.B.: Some experiments on the action of antilymphoid antisera.
Ann. N.Y. Acad. Sci. 129: 164-177, 1966a.

Levey, R.H. and Medawar, P.B.: Nature and mode of action of antilymphocytic antiserum.
Proc. nat. Acad. Sci. 56: 1130-1137, 1966b.

Levey, R.H. and Medawar, P.B.: Further experiments on the action of antilymphocytic antiserum.
Proc. nat. Acad. Sci. 58: 470-477, 1967.

Lindquist, R.R., Guttmann, R.D., Carpenter, C.B. and Merrill, J.P.: Nephritis induced by antilymphocyte serum.
Transplantation 8: 545-557, 1969.

Lindquist, R.R., Guttmann, R.D. and Merrill, J.P.: Mechanism of renal allograft rejection.
In: Advance in Transplantation, Munksgaard, Copenhagen: 611-616, 1968a.

Lindquist, R.R., Guttmann, R.D. and Merrill, J.P.: Renal transplantation in the inbred rat. II An immunohistochemical study of acute allograft rejection.
Amer. J. Path. 52: 531-545, 1968b.

Lindquist, R.R., Guttmann, R.D. and Merrill, J.P.: Renal transplantation in the inbred rat. V Histochemical studies of acute renal allograft rejection.
Amer. J. Path. 52: 1145-1168, 1968c.

Mathé, G., Amiel, J.L. and Schwarzenberg, L.: L'aplasie myelolymphoide de l'irradiation totale.
Gauthier-Villars, Paris, 1965.

Medawar, P.B.: The behaviour and fate of skin autografts and skin homografts in rabbits.
J. Anat. 78: 176-199, 1944.

Medawar, P.B.: A second study of behaviour and fate of skin homografts in rabbits.
J. Anat. 79: 157-176, 1945.

Medawar, P.B.: The immunology of transplantation.
Harvey Lect. (1956-1957) 52: 144-176, 1958.

Moore, T.C. and Hume, D.M.: The period and nature of hazard in clinical renal transplantation.
Ann. Surg. 170: 1-29, 1969.

Murray, D.E.: Rejection time of kidneys following transplantation in inbred rats.
Nature 223: 87-88, 1969.

Najarian, J.S., May, J., Cochrum, K.C., Baronberg, N. and Way, L.W.: Mechanism of antigen release from canine kidney homotransplants.
Ann. N.Y. Acad. Sci. 129: 76-87, 1966.

Payne, R., Perkins, H.A. and Najarian, J.S.: Compatibility for seven leucocyte antigens in renal homografts: utilization of a micro-agglutination test with few sera.
In: Histocompatibility testing 1967, Munksgaard, Copenhagen: 237-247, 1967.

Pichlmayr, R.: Antilymphocytenserum – heutiger Stand der Entwicklung.
Schweiz.med. Wschr. 97: 1624-1628, 1967.

Porter, K.A.: Renal Transplantation.
In: Pathology of the Kidney, 1st ed. R.H. Heptinstall, ed., Little, Brown and Company, Boston: 601-642, 1966a.

Porter, K.A.: Tissue Transplantation.
In: Recent Advances in Pathology, 8th ed. C.V. Harrison, ed., J. and A. Churchill Ltd.: 242-318, 1966b.

Proceedings of the Second International Congress of the Transplantation Society.
Transpl. Proceedings 1: 1-678, 1969.

Ramseier, H.: Die Transplantationsreaktion als zelluläre Immunität.
S. Karger, Basel, Schweiz/New York, 1969.

Rapaport, F.T. and Dausset, J. editors: Human Transplantation.
Grune and Stratton, New York/London, 1968.

Rood, J.J. van, Leeuwen, A. van, Schippers, A.M.J., Pearce, R., Blankenstein, M. van and Volkers, W.: Immunogenetics of the group four, five and nine systems.
In: Histocompatibility testing 1967, Munksgaard, Copenhagen: 203-219, 1967.

Russell, P.S. and Monaco, A.P.: The biology of tissue transplantation.
J. and A. Churchill Ltd., London 1965.

Sakai, A., Festenstein, H. and Simonsen, M.: Does syngeneic preference operate in kidney transplantation?
In: Advance in Transplantation. Munksgaard, Copenhagen: 269-272, 1968.

Sakai, A., Simonsen, M. and Jensen, E.: Kidney transplantation in siblings. Is immunosuppressive treatment invariably needed?
Transplantation 7: 444-446, 1969.

Salaman, J.R.: Renal transplantation between two strains of rats.
Nature 220: 930-931, 1968.

Second World Symposium on Heart Transplantation. June 6, 7, 8, 1969. Montreal, Canada, in press.

Sellers, A.L., Rosenfeld, S. and Friedman, N.B.: Spontaneous hydronephrosis in the rat.
Proc. Soc. exp. Biol. Med. 104: 512-515, 1960.

Seventh Report of the Human Kidney Transplant Registry.
Transplantation 8: 721-728, 1969.

Shorter, R.G. and Hallenbeck, G.A.: The histopathologic features of rejection in the allogeneically transplanted kidney in dog and man: a review.
J. Lancet 88: 162-167, 1968.

Snell, G.D. and Stimpfling, J.H.: Genetics of tissue transplantation.
In: Biology of the laboratory mouse, 2nd ed. McGraw-Hill, New York: 457-491, 1966.

Spong, F.W., Feldman, J.D. and Lee, S.: Transplantation antibody associated with first-set renal homografts.
J. Immun. 101: 418-425, 1968.

Štark, O. and Křen, V.: Five congenic resistant lines of rats differing at the Rt H-1 locus.
Transplantation 8: 200-203, 1969.

Štark, O., Křen, V., Křenová, D. and Frenzl, B.: Serologically detected antigens in two sublines of the WAG rats.
Folia Biol. 15: 259-262, 1969.

Starzl, T.E.: Experience in renal transplantation.
W.B. Saunders Company, Philadelphia and London, 1964.

Starzl, T.E., Brettschneider, L., Penn, I., Schmidt, R.W., Bell, P., Kashiwagi, N., Townsend, C.M. and Putnam, C.W.: A trial with heterologous antilymphocyte globulin in man.
Transpl. Proceedings 1: 448-454, 1969.

Starzl, T.E., Marchioro, T.L., Porter, K.A., Iwasaki, Y. and Cerilli, G.J.: The use of heterologous antilymphoid agents in canine renal and liver homotransplantation and in human renal homotransplantation.
Surg. Gyn. Obst. 124: 301-318, 1967.

Strober, S. and Gowans, J.L.: The role of lymphocytes in the sensitization of rats to renal homografts:
J. Exp. Med. 122: 347-360, 1965.

Stuart, F.P., Saitoh, T. and Fitch, F.W.: Rejection of renal allografts: specific immunologic suppression.
Science 160: 1463-1465, 1968a.

Stuart, F.P., Saitoh, T., Fitch, F.W. and Spargo, B.H.: Immunologic enhancement of renal allografts in the rat.
Surgery 64: 17-24, 1968b.

Taguchi, Y., MacKinnon, K.J. and Dossetor, J.B.: Renal allograft modification by donor antigen in the rat: evidence for significance of this principle in man.
In: Advance in Transplantation. Munksgaard, Copenhagen: 393-398, 1968.

Terasaki, P.I., Mickey, M.R. and McClelland, J.D.: Serotyping for homotransplantation XIV Provisionary evaluation of various antisera in predicting clinical outcome of kidney transplantation.
In: Histocompatibility testing 1967. Munksgaard, Copenhagen: 231-236, 1967.

Tinbergen, W.J.: The effects of some immunosuppressive agents on kidney graft survival in rats.
Transplantation 6: 203-207, 1968.

Turk, J.L. and Oort, J.: Germinal centre activity in relation to delayed hypersensitivity.
In: Germinal centres in immune responses. Springer Verlag, New York: 311-316, 1967.

Vries, M.J. de, Tinbergen, W.J. and Westbroek, D.L.: The effect of various immunosuppressive agents on the histology of the homograft reaction.
In: Abstracts of the Seventh International Congress of the International Academy of Pathology, Milan, Italy: 72-73, 1968.

Weinman, J., Bicher, C. and Levy, D.: Application of a photoconductive cell to the study of peripheral circulation in limbs of animals and man.
J. Appl. Physiol. 15: 317-320, 1960.

Welch, B.L.: The generalization of "Student's" problem when several different population variances are involved.
Biometrika 34: 28-35, 1947.

White, E. and Hildemann, W.H.: Allografts in genetically defined rats: difference in survival between kidney and skin.
Science 162: 1293-1295, 1968.

White, E. and Hildemann, W.H.: Kidney versus skin allograft reaction in normal adult rats of inbred strains.
In: Transpl. Proceedings I: 395-399, 1969.

White, E., Hildemann, W.H. and Mullen, Y.: Chronic kidney allograft reactions in rats.
Transplantation 8: 602-617, 1969.

Woodruff, M.F.A. and Anderson, N.A.: Effect of lymphocyte depletion by thoracic duct fistula and administration of antilymphocytic serum on the survival of skin homografts in rats.
Nature 200: 702, 1963.

Woodruff, M.F.A. and Anderson, N.F.: The effect of lymphocyte depletion by thoracic duct fistula and administration of antilymphocytic serum on the survival of skin homografts in rats.
Ann. N.Y. Acad. Sci. 120: 119-128, 1964.

Wijnans, M.: Personal communication, 1969.

APPENDIX

TABLE A

WEIGHT, AVERAGE VALUES WITH STANDARD DEVIATIONS AND NUMBERS IN DIFFERENT TREATMENT GROUPS; AVERAGES ARE EXPRESSED AS PERCENTAGES OF WEIGHT AT ONE WEEK BEFORE TRANSPLANTATION

	controls, uniloteral nephrectomy																	isografts			allografts			
	untreated			Im 4mg/kg			Im 2/Pred 4			ALG I			ALG II			ALG I/Im 4			untreated			untreated			
	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	
1	-	-	-	100.0	0.0	4	100.0	0.0	4	100.0	0.0	4	100.0	0.0	3	100.0	0.0	4	100.0	0.000	7	100.0	0.0	10	1
2	100.0	0.0	4	99.2	0.9	4	100.5	1.2	4	100.0	1.1	4	99.5	0.5	4	99.2	0.9	4	99.8	0.788	10	100.0	1.1	10	2
3	95.5	0.5	4	94.5	0.5	4	95.0	1.8	4	95.0	3.7	4	92.5	3.1	4	97.2	1.2	4	93.8	4.917	10	90.1	3.6	10	3
4	97.0	0.8	4	93.0	3.3	4	91.5	2.3	4	90.7	9.8	4	92.5	6.2	4	95.0	0.8	4	86.3	5.207	10	86.2	3.7	10	4
5	93.5	0.5	4	86.7	4.8	4	91.2	1.5	4	86.2	12.2	4	93.5	4.5	4	92.0	0.8	4	82.8	5.072	10	78.5	3.3	9	5
6	95.5	0.5	4	93.0	3.5	4	93.2	0.9	4	90.7	10.5	4	94.0	4.2	4	93.7	1.5	4	85.9	4.357	10	75.6	1.5	3	6
7	91.2	0.5	4	94.0	2.9	4	93.7	1.2	4	87.0	10.6	4	95.0	3.1	4	90.7	1.7	4	85.4	4.948	10	-	-	-	7
8	94.2	0.9	4	-	-	-	92.5	1.2	4	98.0	0.0	2	95.0	3.1	4	94.7	0.9	4	88.2	5.452	10	-	-	-	8
9	91.0	1.4	4	94.7	1.5	4	92.0	2.8	4	93.0	8.7	4	95.7	2.0	4	-	-	-	87.1	4.863	10	-	-	-	9
10	91.7	0.9	4	-	-	-	94.0	1.4	4	-	-	-	96.2	2.2	4	96.0	0.8	4	90.0	5.537	10	-	-	-	10
11	91.0	2.1	4	97.2	2.7	4	91.2	2.3	4	93.0	8.7	4	94.2	0.9	4	-	-	-	88.9	5.404	10	-	-	-	11
12	94.2	0.9	4	-	-	-	96.2	1.7	4	-	-	-	95.0	2.1	4	97.5	0.5	4	92.1	4.954	10	-	-	-	12
13	92.0	0.8	4	97.7	2.2	4	96.5	1.0	4	96.7	5.1	4	88.5	3.7	4	-	-	-	91.8	4.848	10	-	-	-	13
14	93.2	1.5	4	-	-	-	-	-	-	-	-	-	91.7	2.2	4	98.7	1.2	4	93.3	5.143	10	-	-	-	14
15	91.2	1.2	4	101.5	1.7	4	96.5	1.2	4	95.7	3.2	4	92.0	1.8	4	-	-	-	92.2	5.356	9	-	-	-	15
16	94.0	0.8	4	-	-	-	-	-	-	95.5	9.1	2	95.2	2.5	4	99.2	0.9	4	94.8	3.457	10	-	-	-	16
17	91.7	2.6	4	100.0	2.8	2	94.0	1.4	2	102.0	0.0	1	93.7	3.2	4	98.5	0.7	2	93.2	4.086	5	-	-	-	17
18	93.5	0.7	2	107.0	2.8	2	100.5	0.7	2	91.0	16.9	2	102.0	1.4	2	103.0	0.0	2	96.8	6.220	5	-	-	-	18
19	96.5	0.7	2	110.0	1.4	2	99.5	0.7	2	88.5	28.9	2	103.0	0.0	2	104.5	0.7	2	99.0	7.778	5	-	-	-	19
20	95.5	0.7	2	112.0	1.4	2	105.0	0.0	2	87.5	33.2	2	102.0	1.4	2	105.5	0.7	2	98.8	7.049	5	-	-	-	20

allografts

	Im 4mg/kg			Im 2			Im 2 pretreated			Im 2/Pred 4			ALG I			ALG II			ALG I/Im 4			ALG II/Im 4			ALG II/Im 2			
	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	
1	100.0	0.0	6	100.0	0.0	9	100.0	0.0	10	100.0	0.0	7	100.0	0.0	6	100.0	0.0	5	100.0	0.0	5	100.0	0.0	5	100.0	0.0	7	1
2	99.5	1.5	9	99.6	2.8	13	99.5	1.7	10	100.1	1.1	10	99.1	1.4	7	99.4	1.1	7	100.6	2.0	5	100.5	2.5	4	99.7	1.0	8	2
3	92.2	4.8	9	90.0	3.3	13	92.6	5.4	10	87.5	3.5	10	95.8	3.4	7	91.4	6.3	7	93.6	4.8	5	91.6	8.3	5	90.1	3.5	8	3
4	82.8	8.7	8	83.2	5.3	12	83.5	3.1	10	82.9	3.7	10	85.5	2.2	7	84.0	5.8	7	88.0	5.7	5	87.8	5.1	5	85.7	4.2	8	4
5	77.3	10.2	8	75.0	4.9	10	77.0	5.1	10	77.7	6.2	9	81.7	3.8	7	81.7	7.2	7	82.6	8.3	5	79.7	8.2	4	85.8	2.4	8	5
6	70.0	13.4	6	73.8	3.8	7	79.4	5.4	5	74.1	8.0	8	83.0	5.5	7	82.6	6.1	5	84.5	5.0	4	81.0	8.5	4	84.5	3.8	8	6
7	78.3	5.6	3	71.5	6.9	7	77.4	7.9	5	77.7	5.2	8	79.1	5.2	7	84.0	4.1	5	86.0	7.7	4	80.0	10.3	4	82.1	3.7	8	7
8	74.3	7.2	3	73.1	2.2	6	78.7	3.8	4	76.0	5.8	8	84.8	7.2	7	83.2	7.2	5	85.2	7.7	4	83.6	8.3	3	80.3	5.9	8	8
9	74.3	8.9	3	76.0	4.4	6	78.0	5.4	4	79.5	2.8	8	84.0	7.7	7	84.8	3.7	5	91.0	9.3	4	85.0	7.5	3	80.2	8.2	8	9
10	77.3	8.1	3	77.1	4.1	6	78.2	5.3	4	78.7	6.1	8	86.8	9.0	7	84.4	7.3	5	91.7	10.2	4	89.0	7.2	3	81.2	7.8	8	10
11	79.6	8.5	3	74.1	8.8	6	74.0	8.8	4	82.7	4.3	8	84.8	8.6	7	85.0	6.5	5	94.0	9.2	4	88.3	6.4	3	82.6	8.0	8	11
12	81.6	7.6	3	78.8	4.5	6	75.5	6.6	4	84.1	5.5	8	89.1	9.5	7	85.8	6.3	5	92.7	9.9	4	91.3	8.0	3	82.8	7.7	8	12
13	79.6	6.1	3	76.8	9.0	6	75.0	7.5	4	85.8	4.9	8	88.1	7.8	7	86.2	7.2	5	96.2	9.6	4	90.6	8.3	3	82.7	6.8	8	13
14	79.6	4.1	3	80.5	5.5	6	76.5	8.2	4	83.1	7.4	8	90.7	8.1	7	85.4	8.2	5	95.0	9.2	4	92.6	10.2	3	84.7	6.6	8	14
15	78.3	5.6	3	79.8	9.4	6	74.0	10.8	4	86.0	3.4	8	91.8	8.1	7	85.2	9.2	5	96.7	10.2	4	90.3	10.6	3	83.8	7.6	8	15
16	78.0	1.0	3	81.8	7.6	5	75.7	9.4	4	87.5	6.5	8	93.2	7.9	7	84.4	9.7	5	96.0	8.7	4	93.6	9.4	3	85.0	6.6	8	16
17	74.0	0.0	1	-	-	-	68.6	8.9	3	90.0	2.6	3	93.2	9.0	5	83.2	12.0	4	92.6	9.0	3	96.5	2.1	2	89.7	2.5	4	17
18	71.0	0.0	1	90.3	4.7	3	73.5	10.6	2	95.6	3.5	3	94.0	13.1	3	88.3	17.0	3	100.5	12.0	2	101.5	2.1	2	95.2	3.5	4	18
19	71.0	0.0	1	90.6	1.1	3	67.0	0.0	1	95.6	3.5	3	98.6	14.7	3	88.6	19.0	3	103.5	10.6	2	104.0	7.0	2	91.7	5.7	4	19
20	-	-	-	93.0	5.2	3	-	-	-	98.3	3.5	3	99.0	16.3	3	83.6	23.4	3	108.0	9.8	2	106.5	6.3	2	94.7	3.5	4	20

TABLE B

HEMATOCRIT, AVERAGE VALUES WITH STANDARD DEVIATIONS AND NUMBERS IN DIFFERENT TREATMENT GROUPS; AVERAGES ARE EXPRESSED AS PERCENTAGES OF NORMAL VALUE (50%) FOUND IN UNTREATED RATS

	controls, unilateral nephrectomy																	isografts			allografts			
	untreated			Im 4mg/kg			Im 2/Pred 4			ALG I			ALG II			ALG I/Im 4			untreated			untreated			
	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	
1	-	-	-	99.0	1.154	4	96.0	0.0	3	101.0	7.3	4	102.0	2.0	3	101.0	1.1	4	100.8	1.9	7	101.0	4.2	10	1
2	100.5	1.9	4	92.0	9.380	4	97.5	4.4	4	94.5	3.0	4	99.3	4.1	3	99.0	2.5	4	101.6	2.4	10	100.2	4.3	10	2
3	90.5	5.7	4	102.0	5.656	4	91.5	7.5	4	96.5	1.0	4	76.0	20.4	4	87.5	18.4	4	75.8	5.2	10	69.4	12.0	10	3
4	92.0	7.4	4	93.0	12.806	4	88.0	18.0	4	95.5	1.9	4	76.0	20.4	4	91.0	7.5	4	79.2	4.9	10	62.6	6.1	10	4
5	95.5	2.5	4	96.5	12.476	4	84.5	14.2	4	98.0	4.6	4	83.0	11.8	4	93.0	1.1	4	71.0	11.1	10	46.8	11.7	9	5
6	94.0	3.6	4	86.5	8.698	4	96.5	4.7	4	94.0	10.8	4	87.5	3.4	4	89.0	8.0	4	81.0	5.9	10	48.6	13.3	3	6
7	92.0	0.0	4	92.5	6.403	4	95.5	7.1	4	91.0	15.3	4	92.0	4.8	4	89.5	9.2	4	85.6	5.8	10	-	-	-	7
8	90.0	2.8	4	-	-	-	96.5	6.6	4	97.0	4.2	2	98.5	1.0	4	92.0	8.4	4	86.0	10.5	10	-	-	-	8
9	97.0	2.0	4	95.0	2.581	4	97.0	5.0	4	92.0	12.3	4	100.0	1.6	4	-	-	-	87.0	7.7	10	-	-	-	9
10	96.0	2.3	4	-	-	-	100.5	1.9	4	-	-	-	102.0	1.6	4	98.0	4.0	4	88.4	6.3	10	-	-	-	10
11	102.0	2.8	4	97.5	8.386	4	101.0	2.0	4	98.0	4.3	4	102.5	5.2	4	-	-	-	90.6	9.4	10	-	-	-	11
12	96.5	2.5	4	-	-	-	97.5	1.9	4	-	-	-	101.5	3.4	4	100.0	1.6	4	91.6	6.8	10	-	-	-	12
13	99.5	3.4	4	101.5	4.434	4	101.0	3.4	4	97.5	6.6	4	105.7	3.3	4	-	-	-	95.0	7.4	10	-	-	-	13
14	93.5	4.4	4	-	-	-	-	-	-	-	-	-	96.0	0.0	4	101.0	3.4	4	94.2	6.8	10	-	-	-	14
15	94.5	4.1	4	105.0	2.581	4	103.5	2.5	4	95.5	12.7	4	94.0	4.6	4	-	-	-	97.5	9.5	9	-	-	-	15
16	97.0	3.4	4	-	-	-	-	-	-	89.0	7.0	2	94.0	5.2	3	102.5	5.2	4	93.4	7.9	10	-	-	-	16
17	97.0	3.8	4	100.0	0.000	2	106.0	2.8	2	94.0	0.0	1	94.0	5.1	4	100.0	0.0	2	95.6	6.5	5	-	-	-	17
18	101.0	1.4	2	103.0	1.414	2	96.0	0.0	2	93.0	7.0	2	98.0	2.8	2	99.0	4.2	2	98.4	1.6	5	-	-	-	18
19	95.0	4.2	2	106.0	2.828	2	97.0	4.2	2	97.0	4.2	2	99.0	4.2	2	99.0	1.4	2	95.6	4.3	5	-	-	-	19
20	103.0	7.0	2	107.0	1.414	2	101.0	1.4	2	89.0	15.5	2	98.0	0.0	2	99.0	7.0	2	93.6	3.8	5	-	-	-	20

allografts

	Im 4mg/kg			Im 2			Im 2 pretreated			Im 2/Pred 4			ALG I			ALG II			ALG I/Im 4			ALG II/Im 4			ALG II/Im 2			
	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	
1	100.6	1.6	6	101.4	2.9	10	101.0	3.4	10	99.4	3.7	7	100.3	4.0	6	101.2	3.0	5	98.4	2.9	5	101.0	1.1	4	100.5	3.9	7	1
2	96.8	7.3	9	101.5	2.5	12	98.4	3.3	10	100.6	3.6	10	93.7	7.6	7	99.4	2.5	7	96.8	5.4	5	98.0	4.2	5	101.0	3.0	8	2
3	71.7	8.2	9	72.6	8.6	13	70.8	8.4	10	72.8	14.7	10	71.4	6.3	7	63.7	9.6	7	71.2	6.4	5	69.6	3.8	5	74.2	6.5	8	3
4	70.0	6.9	8	65.0	7.9	12	63.2	18.2	10	62.4	17.3	10	79.4	4.5	7	59.1	21.3	7	66.8	6.4	5	62.0	13.9	5	68.0	7.3	8	4
5	58.5	15.2	8	57.6	10.7	10	50.6	16.9	10	61.5	11.1	9	72.0	10.4	7	58.8	16.4	7	73.2	8.3	5	55.5	19.3	4	70.7	7.7	8	5
6	59.0	19.7	6	51.4	13.9	7	53.6	8.4	5	67.7	10.8	8	70.5	18.9	7	70.4	14.1	5	74.0	10.4	4	56.0	16.8	4	70.5	5.4	8	6
7	66.6	4.6	3	50.0	13.0	7	51.6	21.4	5	68.7	16.4	8	75.1	22.7	7	75.2	7.1	5	77.5	10.6	4	66.0	5.2	3	74.5	9.3	8	7
8	73.3	14.0	3	60.0	9.2	6	52.0	12.5	4	73.5	15.3	8	74.8	17.9	7	77.2	5.7	5	82.5	5.5	4	84.0	19.0	3	74.5	9.4	8	8
9	65.3	20.0	3	59.6	12.6	6	58.0	15.4	4	80.2	10.1	8	80.0	11.1	7	76.4	9.9	5	84.0	2.8	4	84.6	13.0	3	72.7	10.9	8	9
10	68.6	14.0	3	65.6	16.8	6	58.5	17.0	4	83.0	8.1	8	82.2	7.9	7	76.0	8.9	5	83.5	5.2	4	80.6	15.2	3	71.7	13.7	8	10
11	66.0	15.8	3	75.3	7.5	6	63.0	15.0	4	83.2	7.9	8	83.4	10.0	7	69.6	18.8	5	86.0	6.7	4	80.6	16.1	3	73.7	12.0	8	11
12	77.3	11.3	3	76.3	11.1	6	69.0	15.1	4	83.0	6.8	8	84.2	5.3	7	69.2	15.4	5	85.5	10.5	4	82.0	17.4	3	73.7	10.0	8	12
13	67.3	17.0	3	76.6	14.4	6	63.0	16.3	4	84.0	9.9	8	80.0	13.9	7	70.8	14.0	5	89.0	5.2	4	80.0	21.1	3	78.0	13.8	8	13
14	77.3	1.1	3	75.6	9.5	6	68.5	17.5	4	80.2	16.0	8	84.2	10.4	7	74.4	9.2	5	90.0	2.8	4	86.0	14.4	3	77.2	14.1	8	14
15	74.6	3.0	3	77.3	7.4	6	67.5	15.6	4	80.7	11.1	8	85.4	6.5	7	76.0	12.5	5	89.0	2.0	4	83.3	9.8	3	80.5	13.7	8	15
16	74.6	4.1	3	75.6	12.7	5	71.5	14.4	4	81.2	14.5	8	88.2	3.9	7	78.8	15.0	5	94.5	7.5	4	82.6	3.3	3	82.7	11.9	8	16
17	78.0	0.0	1	76.0	0.0	1	63.3	15.2	3	90.6	10.0	3	88.4	3.2	5	76.0	17.0	4	89.3	8.0	3	91.0	4.2	2	88.0	11.4	4	17
18	62.0	0.0	1	87.3	6.4	3	61.0	4.2	2	94.6	4.1	3	89.3	2.3	3	80.6	14.7	3	90.0	11.3	2	92.0	5.6	2	90.5	5.7	4	18
19	36.0	0.0	1	82.0	5.2	3	60.0	0.0	1	95.3	9.4	3	88.6	4.1	3	81.3	22.0	3	90.0	5.6	2	93.0	7.0	2	91.5	3.4	4	19
20	-	-	-	84.6	4.1	3	-	-	-	91.3	7.0	3	88.6	6.1	3	71.3	24.1	3	89.0	7.0	2	95.0	1.4	2	93.0	6.2	4	20

TABLE C

BLOOD UREA, AVERAGE VALUES WITH STANDARD DEVIATIONS AND NUMBERS IN DIFFERENT TREATMENT GROUPS; AVERAGES ARE EXPRESSED AS PERCENTAGES OF NORMAL VALUE FOUND IN UNTREATED RATS (100% = 60 mg % blood urea)

	controls, unilateral nephrectomy																	isografts			allografts			
	untreated			Im 4mg/kg			Im 2/Pred 4			ALG I			ALG II			ALG I/Im 4			untreated			untreated			
	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	
1	-	-	-	91.2	6.2	4	113.7	19.7	4	95.0	31.8	4	110.0	22.9	3	126.2	24 6	4	102.5	13.322	6	103.5	21.7	10	1
2	115.0	0.0	1	117.5	11.9	4	103.7	10.3	4	98.7	13.1	4	-	-	-	106.2	13.1	4	126.0	40.674	10	114.5	21.2	10	2
3	116.6	5.7	3	115.0	10.8	4	120.0	28.2	4	96.2	4.7	4	90.0	10.8	4	118.7	11.0	4	458.0	154.240	10	408.5	257.2	10	3
4	113.7	11.0	4	120.0	26.4	4	90.0	10.8	4	110.0	9.1	4	108.7	19.3	4	160.0	24.1	4	209.5	247.940	10	647.5	282.2	10	4
5	88.7	8.5	4	106.2	19.3	4	110.0	7.0	4	86.2	10.3	4	110.0	24.1	4	77.5	20.6	4	120.5	34.111	10	906.1	253.0	9	5
6	131.2	27.1	4	118.7	7.5	4	111.2	14.9	4	101.2	22.1	4	107.5	22.5	4	101.2	13.7	4	147.0	126.780	10	838.3	280.0	3	6
7	70.0	10.8	4	96.2	18.8	4	166.2	6.2	4	95.0	5.7	4	96.2	8.5	4	83.7	8.5	4	119.5	28.230	10	-	-	-	7
8	103.7	8.5	4	-	-	-	113.7	11.0	4	100.0	0.0	2	91.2	7.5	4	98.7	11.0	4	124.3	36.098	8	-	-	-	8
9	86.2	6.2	4	98.7	20.5	4	108.7	25.6	4	116.2	8.5	4	115.0	7.0	4	-	-	-	109.4	25.055	9	-	-	-	9
10	105.0	7.0	4	-	-	-	125.0	16.8	4	-	-	-	112.5	18.9	4	105.0	7.0	4	124.0	39.355	10	-	-	-	10
11	-	-	-	81.2	11.0	4	126.2	23.9	4	96.2	22.8	4	127.5	15.5	4	-	-	-	122.5	15.500	10	-	-	-	11
12	97.5	6.4	4	-	-	-	133.7	2.5	4	-	-	-	148.7	8.5	4	117.5	9.5	4	122.0	19.321	10	-	-	-	12
13	88.7	6.2	4	71.2	4.7	4	93.7	7.5	4	97.5	21.7	4	82.5	8.6	4	-	-	-	110.5	14.230	10	-	-	-	13
14	118.7	13.7	4	-	-	-	-	-	-	-	-	-	113.7	4.7	4	96.2	4.7	4	121.6	47.236	9	-	-	-	14
15	85.0	4.0	4	111.2	6.2	4	133.7	6.2	4	87.5	15.0	4	111.2	2.5	4	-	-	-	113.3	18.618	6	-	-	-	15
16	101.2	9.4	4	-	-	-	-	-	-	115.0	0.0	1	107.5	3.5	2	123.7	4.7	4	104.0	16.124	10	-	-	-	16
17	87.5	8.6	4	90.0	35.3	2	115.0	0.0	2	92.5	60.1	2	108.7	2.5	4	115.0	14.1	4	112.0	27.294	5	-	-	-	17
18	77.5	3.5	2	115.0	0.0	2	97.5	3.5	2	105.0	0.0	2	102.5	10.6	2	115.0	7.0	2	114.0	5.477	5	-	-	-	18
19	65.0	28.2	2	107.5	3.5	2	67.5	3.5	2	177.5	88.3	2	105.0	0.0	2	132.5	17.6	2	103.0	24.899	5	-	-	-	19
20	87.5	38.8	2	137.5	3.5	2	97.5	3.5	2	120.0	28.2	2	85.0	0.0	2	60.0	7.0	2	97.0	9.082	5	-	-	-	20

allografts

	Im 4mg/kg			Im 2			Im 2 pretreated			Im 2/Pred 4			ALG I			ALG II			ALG I/Im 4			ALG II/Im 4			ALG II/Im 2			
	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	
1	110.8	32.0	6	102.2	25.6	9	103.7	23.5	8	109.2	20.7	7	97.8	18.2	7	94.0	11.9	5	117.0	46.3	5	96.2	17.9	4	85.8	8.0	6	1
2	101.8	13.0	8	85.5	23.2	10	99.4	12.6	9	92.5	14.5	10	94.0	23.8	5	102.8	24.1	7	105.0	24.2	5	112.0	30.5	5	93.1	16.6	8	2
3	468.3	266.0	9	440.0	304.3	13	505.6	244.4	8	285.0	153.4	10	550.7	286.7	7	470.7	199.5	7	456.2	147.2	4	278.7	50.0	4	327.1	93.8	7	3
4	472.5	272.1	8	576.2	285.3	12	467.5	153.8	10	442.5	239.7	10	157.5	79.1	6	325.7	316.8	7	193.3	85.0	3	309.0	196.4	5	135.6	30.5	8	4
5	610.6	300.5	8	645.0	348.7	10	580.5	338.0	10	397.2	304.7	9	130.8	30.4	6	428.3	443.2	6	179.0	118.7	5	217.5	118.4	4	140.0	24.9	8	5
6	660.8	287.9	5	364.1	157.3	6	216.2	29.2	4	303.7	174.0	8	147.8	21.5	7	123.7	18.8	4	156.2	19.7	4	255.0	190.3	4	164.3	57.4	8	6
7	633.3	282.2	3	458.5	322.3	7	445.0	338.7	5	235.6	69.3	8	143.5	26.8	7	208.0	136.8	5	157.5	13.2	4	253.3	161.7	3	290.0	269.5	8	7
8	425.0	132.5	3	309.1	162.9	6	420.0	131.3	4	239.3	58.4	8	130.7	12.3	7	211.0	124.2	5	140.0	47.4	4	243.3	174.8	3	307.1	309.1	7	8
9	398.3	107.7	3	305.0	151.9	6	547.5	24.7	2	260.0	86.8	8	125.0	17.7	7	215.0	110.1	5	156.2	35.6	4	226.6	163.2	3	243.1	134.2	8	9
10	420.0	54.0	3	321.6	184.5	6	465.0	165.3	4	251.2	101.5	8	155.8	52.8	6	228.0	128.3	5	155.0	24.4	4	246.6	164.0	3	187.8	63.9	7	10
11	430.0	72.6	3	273.3	173.1	6	480.0	151.0	4	271.8	113.7	8	211.4	158.7	7	242.0	112.2	5	137.5	9.5	4	210.0	113.0	3	205.6	91.3	8	11
12	468.3	145.0	3	280.0	188.9	5	508.7	131.6	4	231.4	106.2	7	162.1	53.2	7	221.0	127.0	5	126.2	29.5	4	215.0	122.8	3	227.5	163.4	8	12
13	391.6	52.5	3	284.0	158.5	5	417.5	143.1	4	268.5	84.9	7	151.4	33.8	7	218.0	136.9	5	147.5	50.7	4	250.0	195.2	3	223.7	147.2	8	13
14	335.0	82.6	3	312.5	181.4	6	451.2	163.7	4	241.8	104.9	8	136.4	16.7	7	215.0	134.4	5	127.5	33.0	4	213.3	142.6	3	223.7	158.6	8	14
15	416.6	58.5	3	308.3	185.8	6	431.2	165.5	4	261.2	115.7	8	130.7	13.6	7	214.0	157.5	5	142.5	18.4	4	168.3	88.9	3	217.1	148.4	7	15
16	366.6	161.2	3	322.0	250.9	5	452.5	140.5	4	262.5	118.1	8	140.0	21.2	7	191.0	112.9	5	130.0	16.8	4	206.6	125.7	3	219.3	149.5	8	16
17	515.0	0.0	1	225.0	0.0	1	558.3	205.9	3	170.0	22.9	3	128.0	32.9	5	216.2	129.6	4	123.3	15.2	3	135.0	56.5	2	151.2	17.0	4	17
18	540.0	0.0	1	173.3	27.5	3	557.5	123.7	2	162.5	31.8	2	117.5	3.5	2	251.6	210.9	3	147.5	45.9	2	120.0	28.2	2	130.0	17.7	4	18
19	1000.0	0.0	1	225.0	113.1	2	750.0	176.7	2	163.3	44.8	3	120.0	18.0	3	326.6	307.4	3	130.0	21.2	2	130.0	49.4	2	148.7	37.0	4	19
20	-	-	-	198.3	119.3	3	-	-	-	166.6	23.0	3	131.6	30.5	3	426.6	497.7	3	135.0	35.3	2	137.5	31.8	2	127.5	24.6	4	20

TABLE D

URINE OSMOLALITY, AVERAGE VALUES WITH STANDARD DEVIATIONS AND NUMBERS IN DIFFERENT TREATMENT GROUPS; AVERAGES ARE EXPRESSED AS PERCENTAGES OF NORMAL VALUE FOUND IN UNTREATED RATS (100% = 2190 mOsmol/l)

	controls, unilateral nephrectomy																	isografts			allografts			
	untreated			Im 4mg/kg			Im 2/Pred 4			ALG I			ALG II			ALG I/Im 4			untreated			untreated			
	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	
1	-	-	-	108.5	2.1	2	117.7	6.9	4	103.7	10.6	4	97.0	7.0	2	122.2	3.774	4	93.6	16.9	6	80.5	36.4	8	1
2	110.0	1.4	2	122.5	4.9	2	103.3	6.4	3	106.0	6.5	3	107.6	10.0	3	119.5	8.962	4	111.1	7.9	7	111.0	6.2	9	2
3	120.7	4.1	4	127.0	0.0	1	96.5	10.6	4	95.0	21.8	4	93.7	10.0	4	106.6	3.054	3	31.8	10.4	5	38.0	8.4	8	3
4	95.7	8.6	4	114.0	5.7	4	70.0	23.8	3	116.0	24.0	3	99.6	5.5	3	108.5	10.723	4	46.8	19.3	8	24.0	5.5	6	4
5	109.7	2.6	4	114.2	9.2	4	82.2	27.9	4	85.0	32.1	3	84.5	4.9	2	88.7	12.311	4	52.3	15.3	8	17.6	4.9	3	5
6	-	-	-	110.7	11.5	4	111.0	11.4	4	91.5	13.0	4	88.0	17.2	4	104.2	15.195	4	53.5	23.1	8	18.5	2.1	2	6
7	72.7	9.5	4	105.0	14.9	4	117.5	2.1	2	89.2	6.1	4	89.0	14.1	2	58.7	14.682	4	58.3	22.9	10	-	-	-	7
8	108.0	0.0	1	-	-	-	125.2	8.3	4	106.0	7.0	2	97.0	19.2	3	99.6	9.712	3	60.6	27.9	9	-	-	-	8
9	82.5	10.9	4	101.2	2.6	4	116.3	8.6	3	89.2	28.6	4	88.7	17.6	4	-	-	-	58.6	23.9	10	-	-	-	9
10	90.3	1.5	3	-	-	-	117.0	11.3	3	-	-	-	108.0	38.1	2	102.7	7.228	4	60.2	20.6	9	-	-	-	10
11	96.2	26.8	4	110.5	5.6	4	98.5	22.7	4	94.2	20.6	4	71.7	12.4	4	-	-	-	57.8	18.0	9	-	-	-	11
12	88.0	6.7	4	-	-	-	110.5	10.1	4	-	-	-	86.6	12.2	3	101.2	5.377	4	64.5	18.7	8	-	-	-	12
13	110.0	10.6	4	102.7	9.1	4	107.0	5.4	4	105.3	29.2	3	59.3	17.6	3	-	-	-	67.7	22.2	7	-	-	-	13
14	102.5	3.4	4	-	-	-	-	-	-	-	-	-	94.6	9.6	3	100.0	10.148	3	59.7	17.4	7	-	-	-	14
15	95.5	6.3	4	97.0	4.8	4	111.0	6.4	4	96.7	32.6	4	-	-	-	-	-	-	58.4	22.5	9	-	-	-	15
16	104.6	9.8	3	-	-	-	-	-	-	-	-	-	106.0	6.0	3	89.2	5.852	4	64.3	19.2	10	-	-	-	16
17	98.3	6.6	3	71.0	2.8	2	104.5	6.3	2	104.5	20.5	2	92.0	15.0	3	96.5	7.778	2	68.6	23.4	5	-	-	-	17
18	71.0	11.3	2	82.0	0.0	1	109.0	0.0	1	96.0	11.3	2	-	-	-	84.5	0.707	2	66.2	28.4	5	-	-	-	18
19	96.0	12.7	2	97.5	2.1	2	79.0	12.7	2	66.5	17.6	2	57.0	25.4	2	95.5	13.435	2	55.8	21.4	5	-	-	-	19
20	91.5	0.7	2	86.5	6.3	2	108.0	0.0	1	93.5	2.1	2	38.0	1.4	2	73.5	0.707	2	59.2	24.1	5	-	-	-	20

allografts

	Im 4 mg/kg			Im 2			Im 2 pretreated			Im 2/Pred 4			ALG I			ALG II			ALG I/Im 4			ALG II/Im 4			ALG II/Im 2			
	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	
1	96.6	11.7	5	94.8	33.9	9	102.8	10.6	7	81.7	24.1	7	103.0	17.0	5	93.0	21.2	2	107.2	6.0	5	103.6	6.0	3	103.0	10.3	7	1
2	102.8	15.8	6	102.6	17.4	9	106.1	10.7	6	110.6	23.4	6	108.7	13.3	4	100.7	14.8	7	121.5	4.2	4	101.6	11.2	3	101.0	10.0	8	2
3	41.7	13.2	4	35.2	15.0	11	45.4	27.6	7	41.3	11.3	6	49.0	32.5	2	27.0	8.2	4	39.5	10.7	4	41.6	8.1	3	45.1	17.4	7	3
4	31.0	8.6	8	27.4	7.2	5	31.7	6.5	8	25.7	12.5	9	43.3	15.7	6	29.4	13.3	5	34.6	10.8	5	35.2	26.7	4	54.7	27.3	8	4
5	26.6	12.8	5	22.5	7.0	9	25.7	7.7	8	29.7	9.6	8	50.6	11.7	5	37.4	13.9	7	60.4	16.2	5	37.0	7.2	3	39.1	10.6	7	5
6	25.4	8.5	5	24.5	6.2	7	31.4	5.5	5	33.7	7.5	7	46.0	14.8	5	49.5	14.0	4	62.2	19.6	4	45.3	24.3	3	38.8	13.7	5	6
7	20.3	2.3	3	25.8	8.9	7	39.5	15.8	4	31.7	8.3	8	44.8	13.1	7	39.0	22.7	4	58.0	20.2	4	39.0	28.7	3	34.2	12.0	8	7
8	20.3	4.5	3	27.6	9.6	6	33.3	18.9	3	32.7	9.5	7	47.1	10.3	7	39.0	17.0	5	59.6	14.5	3	37.6	25.4	3	37.8	12.3	6	8
9	24.0	3.6	3	27.0	9.6	5	26.2	11.2	4	30.8	10.3	8	52.4	15.9	7	35.0	15.8	4	65.5	18.2	4	40.3	30.8	3	34.5	14.4	7	9
10	22.3	4.1	3	26.2	9.0	5	28.3	13.5	3	34.5	11.3	8	51.7	19.0	7	41.8	12.6	5	66.6	18.9	3	37.3	23.1	3	29.5	8.2	8	10
11	20.0	2.6	3	29.3	6.4	5	25.2	10.5	4	35.7	13.5	7	54.6	23.4	6	41.6	16.3	5	62.5	19.0	4	41.3	27.4	3	31.1	16.3	7	11
12	18.5	3.5	2	29.8	11.2	5	23.2	8.8	4	36.5	14.2	8	50.5	18.5	7	38.2	15.8	4	62.0	5.1	3	25.0	0.0	1	33.3	15.2	8	12
13	20.5	0.7	2	29.6	7.5	6	27.7	10.8	4	33.5	12.1	8	50.1	20.0	7	41.6	21.0	3	57.5	20.0	4	39.0	21.7	3	29.0	6.8	7	13
14	20.6	0.5	3	32.0	9.6	5	25.0	11.4	4	39.8	15.2	7	49.4	17.3	7	43.2	19.5	5	62.7	16.8	4	43.3	30.8	3	32.4	11.5	7	14
15	24.6	1.5	3	28.5	8.1	6	24.5	12.5	4	36.4	18.0	7	47.5	15.8	7	49.5	20.5	4	59.0	11.8	4	45.0	32.9	3	31.1	11.7	6	15
16	19.0	0.0	2	30.2	9.0	5	18.6	1.1	3	33.2	13.3	8	54.0	18.3	6	44.4	22.4	5	61.7	10.5	4	40.3	26.5	3	32.8	13.8	7	16
17	22.0	0.0	1	24.0	0.0	1	20.0	2.0	3	49.0	11.5	3	52.4	18.9	5	46.0	16.6	3	54.5	12.0	2	51.0	35.3	2	36.7	12.9	4	17
18	-	-	-	29.0	7.0	3	16.0	1.4	2	47.0	20.4	3	43.6	18.7	3	48.3	25.6	3	60.5	13.4	2	50.5	34.6	2	39.5	15.1	4	18
19	23.0	0.0	1	25.6	4.0	3	18.0	0.0	1	45.0	10.8	3	43.0	15.1	3	37.0	15.7	3	57.0	7.0	2	49.0	32.5	2	36.0	15.6	3	19
20	-	-	-	28.0	7.2	3	-	-	-	44.3	11.1	3	42.6	11.2	3	47.6	28.0	3	52.0	2.8	2	39.0	15.5	2	42.5	17.1	4	20

TABLE E

URINE PRODUCTION PER 24 HOURS, AVERAGE VALUES WITH STANDARD DEVIATIONS AND NUMBERS IN DIFFERENT TREATMENT GROUPS; AVERAGES ARE EXPRESSED AS PERCENTAGES OF NORMAL VALUES FOUND IN UNTREATED RATS (100% = 5 ml)

	controls, unilateral nephrectomy																		isografts			allografts			
	untreated			Im 4mg/kg			Im 2/Pred 4			ALG I			ALG II			ALG I/Im 4			untreated			untreated			
	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	86.6	23.0	3	95.0	21.2	2	1
2	80.0	27.0	4	90.0	14.1	2	-	-	-	220.0	226.2	2	70.0	25.8	4	112.5	12.5	4	263.3	160.3	6	271.1	135.6	9	2
3	105.0	20.8	4	90.0	25.8	4	-	-	-	155.0	95.7	4	82.5	22.1	4	107.5	55.0	4	188.7	74.9	8	210.0	229.6	6	3
4	95.0	19.1	4	90.0	8.1	4	-	-	-	137.5	33.0	4	105.0	23.8	4	95.0	41.2	4	317.1	323.6	7	-	-	-	4
5	87.5	22.1	4	72.5	25.0	4	97.5	17.0	4	172.5	89.9	4	80.0	14.1	4	77.5	25.0	4	181.0	93.9	10	-	-	-	5
6	72.5	15.0	4	72.5	9.5	4	106.6	11.5	3	100.0	46.9	4	80.0	8.1	4	90.0	25.8	4	178.0	61.0	10	-	-	-	6
7	75.0	17.3	4	110.0	25.8	4	127.5	25.0	4	155.0	99.8	4	125.0	33.1	4	95.0	25.1	4	171.0	64.0	10	-	-	-	7
8	87.5	22.1	4	105.0	7.0	2	122.5	33.0	4	150.0	38.2	4	132.5	61.8	4	130.0	77.4	4	153.0	38.0	10	-	-	-	8
9	95.0	7.0	2	85.0	7.0	2	145.0	21.2	2	120.0	70.7	2	220.0	42.4	2	80.0	14.1	2	138.0	34.9	5	-	-	-	9
10	90.0	14.1	2	80.0	14.1	2	120.0	28.2	2	140.0	113.1	2	225.0	7.0	2	90.0	14.1	2	166.0	80.8	5	-	-	-	10
11	90.0	42.4	2	80.0	28.2	2	110.0	14.1	2	160.0	84.8	2	285.0	35.3	2	110.0	28.2	2	147.5	71.8	4	-	-	-	11

	allografts																											
	Im 4mg/kg			Im 2			Im 2 pretreated			Im 2/Pred 4			ALG I			ALG II			ALG I/Im 4			ALG II/Im 4			ALG II/Im 2			
1	-	-	-	73.3	23.0	3	-	-	-	-	-	-	-	-	-	110.000	0.0	1	130.0	14.1	2	-	-	-	100.0	0.0	1	1
2	262.0	154.3	5	232.3	160.4	13	295.0	70.1	8	162.0	99.5	5	237.5	224.8	4	282.8	181.9	7	266.0	79.8	5	358.0	127.3	5	260.0	196.1	8	2
3	260.0	219.0	4	325.5	218.9	9	227.0	184.3	10	366.6	125.0	3	236.6	82.1	6	158.3	109.8	6	207.5	133.0	4	236.6	155.3	3	223.7	107.1	8	3
4	480.0	226.2	2	357.1	214.2	7	378.0	138.6	5	348.7	97.8	8	232.5	68.9	4	292.0	177.3	5	235.0	114.7	4	440.0	253.5	3	312.5	116.9	8	4
5	666.6	195.0	3	383.3	181.6	6	527.5	270.4	4	367.5	157.0	8	268.3	225.9	6	326.0	202.4	5	170.0	34.6	4	396.6	256.9	3	395.0	190.7	8	5
6	656.6	40.4	3	375.0	238.8	6	470.0	231.2	4	377.5	162.8	8	305.7	272.5	7	308.0	160.2	5	102.5	41.9	4	396.6	236.2	3	441.2	212.9	8	6
7	633.3	227.4	3	355.0	146.7	6	577.5	142.4	4	382.5	282.5	8	237.1	215.6	7	302.0	156.1	5	155.0	46.5	4	390.0	180.2	3	365.0	153.9	8	7
8	510.0	115.3	3	326.6	157.9	6	422.5	156.7	4	347.5	231.3	8	151.6	67.0	6	368.0	194.6	5	147.5	42.7	4	323.3	180.0	3	295.7	100.4	7	8
9	-	-	-	410.0	236.4	3	560.0	84.8	2	160.0	72.1	3	256.6	120.5	3	303.3	291.6	3	105.0	35.3	2	265.0	190.9	2	307.5	149.5	4	9
10	360.0	0.0	1	356.6	212.2	3	640.0	28.2	2	210.0	52.9	3	270.0	151.3	3	356.6	232.4	3	75.0	35.3	2	305.0	233.3	2	332.5	206.2	4	10
11	460.0	0.0	1	403.3	127.4	3	-	-	-	185.0	35.3	2	210.0	110.0	3	303.3	292.6	3	180.0	42.4	2	375.0	275.7	2	267.5	128.4	4	11

TABLE F

BLOOD PRESSURE, AVERAGE VALUES WITH STANDARD DEVIATIONS AND NUMBERS IN DIFFERENT TREATMENT GROUPS; AVERAGES ARE EXPRESSED IN mm Hg

(normal blood pressure, found in untreated rats is 125 mm Hg)

	controls, unilateral nephrectomy																			isografts			allografts			
	untreated			Im 4mg/kg			Im 2/Pred 4			ALG I			ALG II			ALG I/Im 4			untreated			untreated			
	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	
1	-	-	-	-	-	-	-	-	-	-	-	-	120.0	5.0	3	-	-	-	130.0	14.142	2	133.7	4.7	4	1
2	127.5	8.6	4	-	-	-	-	-	-	-	-	-	125.0	10.8	4	-	-	-	107.5	4.183	6	113.3	11.5	3	2
3	137.5	11.9	4	-	-	-	-	-	-	-	-	-	137.5	6.4	4	118.3	2.8	3	120.0	12.247	6	113.3	20.8	3	3
4	135.0	10.0	4	133.7	9.4	4	-	-	-	125.0	0.0	2	123.7	7.5	4	141.6	2.8	3	124.1	9.174	6	-	-	-	4
5	137.5	6.4	4	140.0	13.2	3	-	-	-	132.5	5.0	4	133.7	20.5	4	130.0	10.8	4	127.5	14.053	6	-	-	-	5
6	141.2	6.2	4	141.2	4.7	4	-	-	-	140.0	4.0	4	131.2	19.3	4	127.5	3.5	2	129.2	7.867	7	-	-	-	6
7	138.7	4.7	4	141.6	7.6	3	136.6	5.7	3	143.7	6.2	4	132.5	9.5	4	128.7	8.5	4	127.1	8.591	7	-	-	-	7
8	121.2	9.4	4	150.0	14.1	2	150.0	15.0	3	143.3	12.5	3	125.0	10.8	4	127.5	15.5	4	136.6	9.013	9	-	-	-	8
9	145.0	7.0	4	145.0	0.0	1	162.5	3.5	2	157.5	10.6	2	127.5	10.6	2	130.0	0.0	2	127.0	20.796	5	-	-	-	9
10	137.5	3.5	4	150.0	0.0	2	165.0	7.0	2	152.5	3.5	2	-	-	-	152.5	3.5	2	131.2	4.787	4	-	-	-	10
11	130.0	0.0	1	160.0	0.0	2	147.5	24.7	2	152.5	17.6	2	-	-	-	130.0	0.0	2	137.0	6.708	5	-	-	-	11

	allografts																									
	Im 4mg/kg			Im 2			Im 2 pretreated			Im 2/Pred 4			ALG I			ALG II			ALG I/Im 4			ALG II/Im 4			ALG II/Im 2			
1	127.5	3.5	2	122.2	9.3	9	126.2	4.7	4	131.2	6.2	4	-	-	-	123.0	5.7	5	-	-	-	130.0	8.6	3	125.0	10.6	8	1
2	112.5	3.5	2	118.3	7.5	9	101.8	21.7	8	126.6	5.7	3	137.5	5.5	2	104.1	15.9	6	135.0	0.0	1	102.5	11.9	4	115.0	16.5	7	2
3	-	-	-	130.0	32.1	9	111.6	19.6	6	146.6	5.7	3	122.5	10.6	2	121.0	18.1	5	111.2	29.5	4	100.0	18.7	4	121.8	15.1	8	3
4	-	-	-	135.7	23.3	7	130.0	27.9	4	166.6	15.2	3	111.6	16.0	3	140.0	11.7	5	135.0	12.9	4	123.3	15.2	3	136.2	20.6	8	4
5	-	-	-	139.1	57.3	6	145.0	12.9	4	148.3	20.2	3	121.2	24.6	4	137.0	16.4	5	123.3	20.2	3	132.5	17.6	2	141.8	25.4	8	5
6	170.0	0.0	1	140.8	28.0	6	152.5	20.6	4	163.3	15.2	3	123.0	10.9	5	132.5	13.2	4	126.2	16.5	4	130.0	0.0	2	149.2	22.0	7	6
7	180.0	26.4	3	150.0	39.3	6	178.7	20.9	4	155.0	29.0	7	120.8	13.5	6	132.0	17.5	5	126.2	21.3	4	133.3	7.6	3	128.5	19.0	7	7
8	200.0	0.0	1	125.0	22.9	3	173.7	39.8	4	145.0	10.9	6	124.2	9.3	7	127.0	14.4	5	131.2	22.5	4	142.5	3.5	2	147.1	35.9	7	8
9	230.0	0.0	1	160.0	42.4	2	185.0	21.2	2	148.3	17.5	3	136.6	5.7	3	150.0	30.0	3	142.5	31.8	2	135.0	0.0	1	133.3	15.2	3	9
10	180.0	0.0	1	148.3	38.1	3	210.0	0.0	2	158.3	32.5	3	138.3	15.2	3	127.5	10.6	2	152.5	10.6	2	127.5	17.6	2	133.3	10.4	3	10
11	-	-	-	162.5	53.0	2	-	-	-	130.0	22.9	3	135.0	5.0	3	130.0	0.0	1	132.5	24.7	2	140.0	14.1	2	126.6	5.7	3	11

CURRICULUM VITAE

Schrijver dezes werd op 7 november 1937 te Amsterdam geboren.

Ter afsluiting van zijn middelbare schoolopleiding te Amsterdam, legde hij in 1956 te Amersfoort het Staatsexamen Gymnasium β af. In hetzelfde jaar werd hij als medisch student aan de Universiteit van Amsterdam ingeschreven. In 1963 legde hij aldaar met goed gevolg het doctoraal examen af en in 1965 werd hij, eveneens te Amsterdam, bevorderd tot arts.

Van november 1965 tot april 1967 vervulde hij zijn militaire dienstplicht bij de Koninklijke Luchtmacht.

Van april 1967 tot oktober 1969 was hij als wetenschappelijk medewerker verbonden aan de afdeling Algemene Pathologie van de Medische Faculteit te Rotterdam (Hoofd Prof. Dr. M.J. de Vries) met als standplaats het Radiobiologisch Instituut van de Gezondheidsorganisatie TNO te Rijswijk Z.H. (Hoofd Prof. Dr. D.W. van Bekkum). Gedurende deze periode werden de gegevens, die de basis vormen van dit proefschrift, verzameld en bewerkt.

Sedert oktober 1969 is hij in opleiding tot internist bij de afdeling Inwendige Geneeskunde II van het Academisch Ziekenhuis Dijkzigt te Rotterdam (Hoofd Prof. Dr. M. Frenkel).

Cover design by H.J. van Westbroek