ULTRASTRUCTURAL BASIS OF ACUTE RENAL ALLOGRAFT REJECTION

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PROEFSCHRIFT

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To my parents

To Radmila and Ana

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INTRODUCTION

In the past twenty years probably no other subject in medical science has been so widely investigated on a multidisciplinary basis with the use of various experimental models as the transplantation of tissue and organs. The results of these studies have often been promptly, and more or less satisfactorily, applied to clinical practice.

Today, the principle of surgical removal of irreversibly damaged organs and their replacement by healthy grafts is generally accepted. The results are increasingly satisfactory, owing to the improvement of surgical procedures, the perfecting of histocompatibility matching, and the use of a wide range of immunosuppressive agents.

This holds especially for kidney transplantation, which is now widely acknowledged to offer an alternative to dialysis as a form of treatment for renal failure.

Among the problems still requiring more detailed investigation are the precise nature and sequence of the various rejection processes and the influence of immunosuppressive agents on these processes.

It is difficult to separate rejection changes attributed to immunological factors from the changes caused by concurrent processes such as ischemia, infection, possible reoccurrence of the underlying renal disease in the graft, and the effect of immunosuppressive drug therapy (Rosenau *et al.* 1969).

Morphological characteristics of the rejection pattern altered by immunosuppression require detailed ultrastructural studies and interpretation, which would provide important information of therapeutic and prognostic value (Murray and Wilson 1966).

The present investigations were performed to obtain a detailed analysis of the electron-microscopical characteristics of the changes occurring during acute renal allograft rejection in experimental animals.

The results were compared with those in ultrastructural studies on the acute renal allograft rejection modified by immunosuppressive therapy in the same experimental model.

Special attention was given to the fine morphology of the glomerular and vascular lesions and the structural changes occurring under the influence of various immunosuppressive agents.

An attempt was made:

1. to demonstrate the evolution and the time of onset of the ultrastructural morphological changes in the renal parenchyma and blood vessels, as well as the ultrastructural feature of the interstitial cellular infiltration in acute rejection of kidney allografts;
2. to acquire more information about the nature of the rejection processes by studying the sequential development of the ultrastructural lesions in renal allotransplants; and
3. to analyse the effect of various immunosuppressive agents on the fine morphology of acute renal allograft rejection and the extent to which the various features of the rejection process are affected by the immunosuppressive treatment.

CHAPTER I

ALLOGRAFT REJECTION

Mechanism of allograft rejection

Allograft rejection, which represents a response of the host to foreign tissue transplants, is a complex of immunological manifestations to which both types of immunological processes, i.e., humoral antibody-mediated reactions and cell-mediated immune responses, contribute.

The rejection of allografts is a dynamic process that commences with recognition of graft antigenic determinants, followed by antigenic stimulation of the host lymphoid centers, central lymphoid-tissue response, proliferation of specific effector cells, and a response of the sensitized effector cells toward the transplanted organ.

Antigenic stimulation

The immune response in the graft recipient, resulting in the rejection of the grafted tissue or organ, is induced by and directed against transplantation antigens. It is widely held that transplantation antigens are controlled and determined by the individual's genetic constitution (SNELL and STIMPFLING 1966).

Within a certain interval after application of allotransplants, antigen released from the allografted organs or tissues could be detected in the host (MAY *et al.* 1966).

NAJARIAN *et al.* (1966), who studied the onset, duration, and mechanism of antigen release in canine kidney allotransplants, demonstrated the presence of antigens in plasma from blood vessels of transplanted organs only 10 minutes after establishment of the vascular anastomosis between the graft and the recipient. Release of antigen was still noticeable 2 to 3 days after the transplantation. These authors also showed that antigen is transferred to the recipient as a soluble substance in the plasma of the renal vein.

STROBER and GOWANS (1965) stressed the importance of blood lymphocytes in the system of antigen transport in rat renal allografts. The antigen is carried from the transplanted organs by means of circulating lymphocytes considered to be mediators of sensitization of the host to renal allografts.

With regard to the quantities and the time of antigen release, the studies of NATHAN (1963) indicated that the amount of sensitizing

material released from the transplanted organ during relatively short periods is sufficient to immunize the recipient strongly.

The exact chemical nature of the antigen is unknown and the pure antigen has not been isolated, but the chemical structure of a number of active substances and antigenic fractions known to induce transplantation immunity, has been determined (HASKOVA and HRUBESOVA 1958; MEDAWAR 1958).

In 1956 BILLINGHAM *et al.*, studying the role of antigens in the induction of the allograft reaction in mice carrying skin allotransplants, were the first to show that nuclear substances obtained by ultrasonic disintegration of cells, possessed antigenic properties and might be responsible for transplantation immunity. Chemically, these substances represented desoxyribonucleoproteins.

Some tissue fractions containing mucopolysaccharides, mucoid substances, and lipoproteins, may also have antigenic properties responsible for transplantation immunity (MANN *et al.* 1960). In 1959 CASTERMANS and OTH isolated from the spleen and thymus of Albino, Swiss, and C57BL mice, an active antigenic fraction that caused sensitization to skin grafts.

The chemical composition of this substance, which is closely related to a carbohydrate-protein complex, included 85% protein, 12% RNA, and 2–3% galactose-manose.

It is also of interest to note that HERZENBERG and HERZENBERG (1961) isolated from mouse liver H-2 antigens containing lipid and protein fractions and consisting mainly of nuclear and cellular membranes.

Mode of sensitization

The way in which allografted organs evoke allograft sensitivity in the host and the site where this sensitization occurs, have both been the subject of considerable debate (RUSSELL and WINN 1970).

Skin allografts

As far as skin allografts are concerned, it is widely accepted that an undisturbed lymphatic drainage of the graft is essential for host sentisization, since the antigens are carried by the afferent lymphatics to the regional lymph nodes (WILSON and BILLINGHAM 1967). Experimental evidence supporting this view was advanced by BARKER and BILLINGHAM (1968), who studied the role and significance of lymphatic drainage in the rejection of orthotopic skin allografts in guinea pigs. Sensitization of the recipient resulting in rejection of an allograft implanted into a skin flap of the host, occurred only when the flap had not only an intact vascularization but also an unimpaired lymphatic drainage. In spite of the normal

vascularization of the skin flap, the implanted skin graft showed no signs of rejection when the lymphatics were disrupted.

This is in agreement with the observations of LAMBERT *et al.* (1965), who demonstrated that the principal route of antigen transfer from the skin graft to the host is via the lymphatic pathways, since interruption of the continuity of lymph flow from the site of the skin graft to the draining lymph node delayed allograft rejection by the period of time required for alternative lymph drainage to become re-established.

In guinea pigs the onset of the immune response and the time of onset of rejection in full-thickness skin transplants coincided with the restoration of the lymphatic circulation of the graft, which occurs by the fifth day after transplantation (SCOTHORNE, 1958).

Renal allografts

The process of sensitization to renal allografts, which are vascularized organ transplants, apparently occurs in another way than in skin grafts. Contrary to the situation in skin homografts, no essential role is attributed to the presence of an intact lymphatic connection between the host and the transplanted organ.

HUME and EGDAHL (1955), who studied the rejection process in canine renal homografts, demonstrated that destruction of the transplant occurs when the transplanted kidneys are devoid of lymphatic drainage and are therefore not directly connected with the regional lymphatic tissue.

It has been postulated by MAY *et al.* (1966) that sensitization occurs centrally in the lymphoid system of the host. Antigen released from the transplanted kidney would reach the lymphoid system either by being carried free as soluble material within the plasma or attached to the host's leucocytes, accompanying them on their passage through the graft.

MEDAWAR (1958) suggested that immunologically competent cells passing through the graft's blood vessels react with the transplant's antigens and initiate the rejection reaction after returning to the host's lymphatic tissues.

On the basis of Medawar's assumption that in renal allografts sensitization of the host occurs peripherally within the graft itself, STROBER and GOWANS (1965) showed that the blood-borne small lymphocytes circulating through the vascular bed of the transplant and within the graft itself, interact with the antigen and are mediators of sensitization. It was assumed that after leaving the blood circulation and migrating to the lymphatic tissues, these lymphocytes generate a new effector-cell population after transforming into large pyroninophilic blast cells. This was confirmed by PEDERSEN and MORRIS (1970), whose electron-microscopical observations indicated that the transformation of the host's small lymphocytes to blast

cells, takes place immediately after the establishment of close contact between the lymphocytes and the endothelial layer of the blood vessels, which appears to be a source of transplantation antigens (VETTO and LAWSON, 1967).

Central lymphatic tissue response and effector mechanism

The regional lymph nodes draining the site of an allograft exhibit distinctive changes after the transplantation. The structural basis of these changes was first demonstrated by SCOTHORNE and MCGREGOR (1955), who studied the microscopical appearance of the lymph nodes in rabbits after skin transplantation. These changes are similar to those found by OORT and TURK (1965) in lymph nodes of guinea pigs treated with ^{3}H-thymidine, a substance which characteristically induces delayed-type sensitivity. At the time of early cell-mediated immunity during rejection of the graft there is a striking proliferation of large lymphoid cells, particularly in the paracortical areas of the regional lymph node, which reaches a peak by the fourth day after the transplantation. The lymphoid cells have a large amount of pyroninophylic cytoplasm and large pale nuclei.

In an electron-microscopical study done by BINET and MATHE (1962), these cells appeared to be undifferentiated blast cells rich in ribosomes and with a moderate amount of endoplasmic reticulum. The nuclei contained several nucleoli and a small amount of nuclear chromatin. The role of pyroninophilic cell proliferation in the lymph nodes seems to be the production of a new generation of immunocompetent lymphocytes.

PORTER *et al.* (1964), who studied the significance of the lymphoid cell infiltration in canine renal allotransplants, postulated that the circulating lymphocytes that become sensitized by the graft pass through lymphatic tissue of the host, where they transform into large pyroninophilic cells. The progeny of these cells invade the grafted organ via the blood and cause an ischemic destruction of the transplant by damaging peritubular capillaries.

In vitro studies have shown that the sensitized lymphocytes are able to destroy various allogeneic target cells in tissue cultures within a period of 48 hours in the absence of complement (GOVAERTS 1960). The cytotoxic effect of the immune lymphoid cells has been demonstrated to be immunologically specific (ROSENAU and MOON 1965).

These findings accentuate the significance of the lymphoid cells, which appear to be initiators and effectors of cell-mediated immune reactions, participating, together with circulating antibodies, in the rejection of allografted organs (NAJARIAN and FOKER 1969; TURK, 1967).

Two distinct types of immunocompetent lymphocytes, belonging to two different immunological systems, are known (RAFF 1973; ROITT *et al.* 1969). Both types originate from stem cells in the bone marrow. After leaving the bone marrow, these cells become immunologically competent in two different ways. The cells which mature after passing through the thymus are designated as thymus-dependent or T-lymphocytes. The other population, the cells of which migrate to the "bursa equivalent" lymphatic tissues in mammals, is indicated as bursal or B-lymphocytes.

In addition to the principal immunological function of the T-lymphocytes in the initiation of the cell-mediated immune reactions and as active participants in the effector phase of graft rejection, T-lymphocytes are known to be responsible for long-term immunological memory and to help trigger humoral antibody synthesis by cooperating with B-lymphocytes (MÖLLER 1971). When T-lymphocytes are activated by antigen, they proliferate and differentiate to become blast cells. They secrete a variety of non-antigen specific factors, such as cytotoxic factors, migration inhibition factor, and mitogenic factors, some of which play a role in cell-mediated immune responses, for which T-cells are primarily responsible (CARPENTER 1974). The cytotoxic capacity of T-lymphocytes and the various factors they may release suggest that T-lymphocytes are active participants in the effector phase of cell-mediated immune reactions. Although T-lymphocytes are called "killer cells", it is still not certain whether they themselves can directly kill target cells *in vivo*.

The main role of B-lymphocytes, which are also involved in graft rejection, is to synthetize and secrete antibody. After activation by antigen, B-lymphocytes divide and differentiate into blast cells and plasma cells. These cells remain in the lymphoid tissues and secrete a large amount of antibody which, in conjunction with complement components and various accessory cells (macrophages and mast cells), is responsible for graft rejection in cases of tissue and organ transplantation (RAFF 1973).

Both B- and T-lymphocytes can be clearly distinguished on the basis of their architecture by the use of scanning electron microscopy (SEM) (POLLIACK *et al.* 1973; BENTWICK and KUNKEL 1973). In SEM relatively smooth lymphocytes, which probably are T-lymphocytes, show a completely smooth membrane surface, in contrast to the "villous lymphocytes" (probably B-lymphocytes), whose membrane surface architecture is characterized by multiple microvilli covering almost the entire surface of the cell. However, VAN EWIJK and his associates (1975) have presented evidence that the surface structure of lymphocytes as observed by SEM *in vivo* is not a morphological criterion for distinction of the two classes of lymphocytes, but reflects the state of physiological activity of the lymphocytes.

Humoral antibodies in renal allotransplantation

The presence of humoral antibodies accompanying renal allograft rejection has been detected by various serological methods as well as by immunofluorescence techniques for light and electron microscopy.

In 1953 SIMONSON first demonstrated the presence of circulating agglutinating antibodies to donor erythrocytes in canine renal transplantation. HAGER *et al.* (1964), who used immune adherence techniques, provided additional evidence of the presence in dogs of antibodies in kidney allografts rejected by first- and second-set allograft reactions.

HOROWITZ *et al.* (1965), who investigated 34 renal transplants histologically and immunocytochemically, were able to demonstrate a deposition of gammaglobulin in the medial layer of the small blood vessels and in the glomerular tuft of the kidneys, 3 days after transplantation. Similar findings have been reported by LINDQUIST *et al.* (1968), who studied acute allograft rejection in rats with an immunofluorescence technique. The results of both studies suggest that the antibody formed by the host is an immunoglobulin-G, specific for graft antigens and that antigen-antibody complexes deposited in the blood-vessel wall cause vascular injury and occlusion of the vascular lumen, followed by ischemic necrosis in the transplanted organ.

LUBBE *et al.* (1972), using fluorescence microscopy, found IgG and β1c-globulin deposition in the arterial walls of rat renal isografts and allografts 3, 5, 7 and 9 days after transplantation. They considered the early presence of immunoglobulins in the blood-vessel walls to be non-immunological in nature and suggested that ischemic damage of the blood vessels may have been a factor in the deposition of these proteins. In contrast, later deposition of IgG and complement appears to be specific for graft rejection.

Using an original experimental model of renal allotransplantation, CLARK *et al.* (1968) demonstrated that the humoral factors do indeed participate in allograft rejection and initiate the immunological process in the absence of immunologically competent cells.

It is assumed that the very rapid rejection of renal allotransplants (hyperacute rejection) occurring within hours of transplantation is undoubtedly associated with the presence of humoral antibodies in the host. These antibodies promptly react either with the donor antigen on the surface of the vascular endothelium or with the donor erythrocytes and thrombocytes carrying transplantation antigen (JEANNET *et al.* 1970; WILLIAMS *et al.* 1968). Microscopical observations usually show characteristic necrotizing vascular alterations and widespread microthrombosis with mixed granulocytic and mononuclear cell infiltration of the vascular wall (MILGROM *et al.* 1966).

Summary

Allograft rejection is an immunological phenomenon induced by immunogenetic differences between donor and recipient, in which two types of immune response, cellular and humoral, play important parts. A cellular immune reaction is mediated by thymus-derived lymphocytes (T-lymphocytes), whereas a humoral immune reaction is mediated by B-cells (non-thymus-dependent) secreting humoral antibodies. After the establishment of vascular connection between the grafted organ and the recipient, the host lymphocytes interact with the donor tissue antigens. The stage of antigen recognition is followed by a form of lymphocytic stimulation characterized by transformation of the sensitized lymphocytes into large blast-like cells. The process of transformation takes place in the lymphatic organs of the host or in the graft itself. The transformed lymphocytes seem to have a direct cytopathogenic potential against donor cells. Humoral antibodies, mainly G-immunoglobulins, actively participate in renal allograft rejection, and are produced by the sensitized B-cells.

Morphological characteristics of allograft rejection

General remarks

Morphological characteristics of the allograft rejection were first extensively reported by MEDAWAR in 1944 and 1945, from studies on the behaviour and fate of whole-thickness skin homografts in rabbits, and later by WAKSMAN (1963), who described in detail the evolution of the histopathological pattern of rejection in rat skin allografts.

The essential structural changes in unsensitized recipients started in both studies with a diffuse cellular infiltration consisting of lymphocytes and histiocytes which invaded the upper dermis and epidermal layer, causing destruction of the epidermal cells. This phenomenon occurred by the fifth or sixth day after transplantation and reached its maximum on the 12th day, when the destructive process was most severe. In the first few days after transplantation there were signs of ingrowth of the blood vessels into the graft and the appearance of granulation tissue and acanthosis of the epidermal layer of the transplant.

Vascular lesions also seem to play an important role in allograft rejection. Luminal obstruction of small graft vessels by mononuclear cells and infiltration of these cells into the vessel wall were followed by thrombosis and destruction of the vessels. The progressive obliteration of the dermal vessels resulted in ischemic necrosis of the transplant. A complete breakdown of the skin graft occurred by the 12th day, when replacement of the necrotic remnants by host tissue had already begun, as evidenced by proliferation of young fibrous vascular tissue and re-epithelization.

The ultrastructural features of the homograft reaction and the fine morphology of the infiltrating cells in rabbit skin allografts were studied by WIENER *et al.* (1964). The initial changes consisted of hyperplasia of the graft epithelium and proliferation of fibrovascular tissue in the transplant bed. More important, however, was the mononuclear cell infiltration, which appeared first in the capillaries and around the blood vessels in the dermis and subsequently invaded the epidermal layer. Here, the mononuclear cells, which contained a large number of ribosomes, established a very intimate relationship with the adjacent epidermal cells. This occurred through what seemed to be discontinuities in the apposed cell membranes. The final stage of the homograft reaction resulted in destruction of the transplant, which showed an ultrastructural picture of epidermal cells completely devoid of cytoplasmic constituents followed by complete dissolution of the cell surface membrane and of necrosis of the epidermal layer, which represented the final phase of the rejection process.

Kidney allografts

Hyperacute rejection

Changes in allografted kidneys that are rejected and destroyed within an interval varying from a few minutes to hours after transplantation, are found mainly in the glomerular capillaries and in the interstitial blood vessels (STARZL *et al.* 1968; MYBURGH *et al.* 1969).

Light-microscopical examination reveals numerous glomerular capillary loops which are occluded by fibrin deposition associated with a marked intra-glomerular inflammatory infiltration with polymorphonuclear cells. Coagulative necrosis of the glomerular tuft and the tubuli is also a frequent feature. The cortical arterioles very often show thrombosis and fibrinoid necrosis of their wall.

A frequent finding is obliteration of the interstitial capillaries by aggregations of platelets and destruction of the vascular endothelium (SHARMA *et al.* 1972).

The phenomenon of intravascular platelet accumulation is assumed to play a very important role in the initiation of the vascular damage in the allografted organ and the onset of the hyperacute rejection (LÖWENHAUPT and NATHAN 1968).

Some investigators point to the similarity of the morphological changes in hyperacute rejection with those in the Shwartzman or Arthus reaction (MYBURGH *et al.* 1969). It is believed that hyperacute rejection of allotransplanted kidneys is due to the cytotoxic effect of humoral antibodies already formed in the host by prior sensitization (WILLIAMS *et al.* 1968; KISSMEYER-NIELSEN *et al.* 1966).

Electron-microscopical studies on hyperacute renal allograft rejection suggest that platelet aggregation is the earliest morphological feature of the blood vessels of the transplanted organ (LÖWENHAUPT and NATHAN 1968). It was noticed that vascular damage became manifest after release of the cytoplasmic content of the platelets and the occurrence of intimate contact between their cytoplasmic pseudopods and the endothelial cells.

Acute rejection

Acute rejection can occur within the first week of transplantation or, more usually, within the first month, and episodes of acute rejection crisis may develop many months after transplantation (BALCH and DIETHELM 1972).

Prominent morphological features of the acute rejection process include interstitial cell infiltration, interstitial edema, changes in the small blood vessels (capillaries, venules, and arterioles), and tubular changes. Glomerular lesions are less common (PORTER 1974).

The first structural change during acute allograft rejection is a mononuclear cellular infiltration, which appears around the blood vessels in the renal cortex as early as 2 days after transplantation. This cellular infiltration can be observed in transplanted kidneys without clinical evidence of rejection or alteration of the normal macroscopical appearance (GUTTMANN *et al.* 1967; KOUNTZ *et al.* 1963; DEMPSTER 1953; DAMESHEK 1963).

The cellular infiltration consists mainly of large mononuclear cells which have been called large lymphocytes, immunoblasts, large pyrominophilic cells, and immature plasma cells (FELDMAN and LEE 1967; DEMPSTER 1953; GUTTMANN *et al.* 1967).

Many of the cells in the renal interstitium are presumably T-lymphocytes involved in cell-mediated immune responses and B-lymphocytes involved in humoral antibody-mediated reactions.

Simultaneously with the increase in density of the mononuclear cell infiltrations in the interstitium, lesions of the vascular system become evident. Rupture or occlusion of the peritubular capillaries, fibrinoid necrosis, or intimal proliferation with acute arteritis of larger blood vessels and venous thrombosis, appear to be the main structural changes affecting the blood vessels in acute rejection (LINDQUIST *et al.* 1968; KOUNTZ *et al.* 1963; PORTER *et al.* 1963; KINCAID-SMITH 1967).

It is now widely accepted that the destruction of the peritubular capillaries, which is a very significant occurrence in the rejection process, is a structural expression of the cytotoxic effect of the lymphoid cells on the endothelial layer. A humoral mechanism also appears to be an important factor in causing endothelial disintegration (HERBERTSON 1973).

The arteriolar and arterial lesions in acute rejection are considered to be mediated primarily by humoral antibodies (PORTER *et al.* 1964). This conclusion is based on the demonstration of immunoglobulins in the wall of damaged blood vessels in the allotransplants (HOROWITZ *et al.* 1965; LUBBE *et al.* 1972).

Acute allograft rejection may be accompanied by glomerular alterations. According to LINDQUIST *et al.* (1968), two distinctive morphological features of acute glomerulopathy accompanying the rejection process are the most prominent: proliferative glomerulopathy and thrombotic glomerulopathy. The former is morphologically characterized by hypercellularity of the glomerular tuft, mesangial matrix increase, and variable thickening of the glomerular capillary loops. In the latter type of acute glomerulopathy, platelet clumping and fibrin deposition in the glomerular capillary loops predominate. Immunofluorescent studies on the glomerular lesions indicate that a pathogenetic role is played by the deposition of immune complexes consisting of transplant antigens and antibodies with bound complement in the glomerular structures (LINDQUIST *et al.* 1968; PORTER *et al.* 1968; ANDRES *et al.* 1970).

On the other hand, it is thought that the degenerative changes and necrosis of the tubuli occurring frequently in acute rejection are not directly immunologically mediated. The diminished blood flow due to obstruction of the blood vessels appears to be responsible for the tubular damage.

Chronic rejection

Chronic or late rejection of kidney allotransplants becomes manifest months or years after grafting.

Morphologically, the chronic rejection process is characterized mainly by obliterative vascular changes and a wide variety of glomerular alterations (Go 1972). Details of the vascular morphology in chronic renal rejection were provided by PORTER *et al.* (1964), KINCAID-SMITH (1964), and others, who called attention to the immunological nature of the changes caused by circulating antibodies. These changes consist of arterial intimal thickening, damage to the internal elastic lamina, and fibrinoid necrosis present in vessels ranging in size from the main renal artery to arterioles.

The glomerular lesions in chronic rejection have been considered either to be manifestations of the re-occurrence of the host's original glomerulonephropathy in the grafted organ or to be due to the rejection reaction (HAMBURGER *et al.* 1972).

Summary

Renal allograft rejection can be classified according to its clinical and morphological characteristics as hyperacute, acute, and chronic.

The histopathological features of hyperacute rejection, which is considered to be due to the presence of preexisting cytotoxic antibodies in the recipient, include extensive fibrin accumulation and microthrombosis in the peritubular and the glomerular capillaries in association with fibrinoid necrosis of the glomeruli.

Acute rejection can be divided, according to the morphological features of the changes, into two types of reactions:

1. Parenchymal rejection, consisting of lymphoid cell intestitial infiltration followed by rupture of the peritubular capillaries and progressive glomerular and tubular damage.
2. Vascular rejection, consisting of fibrinoid necrosis of larger blood vessels and vasculitis associated with thrombosis of the arteries and the veins, resulting in multiple infarctions.

The morphological characteristics of a chronic allograft rejection process are chronic obliterative alterations of the larger blood vessels and glomeruli as well as interstitial fibrosis and tubular atrophy.

Modification of allograft rejection

Immunosuppressive drugs

Depression of the immune responses to tissue and organ allografts and prolongation of the survival of the grafts can be achieved by administration of various immunosuppressive agents. In current immunosuppressive therapy the most frequently used agents are corticosteroïd hormones, azathioprine, and anti-lymphocyte serum (ALS).

Corticosteroid hormones

Corticosteroids are known to have many effects on the human organism. They possess anti-inflammatory activity, prevent both the rupture of lysosomes and release of their hydrolytic enzymes, and stabilize cell membranes.

The value of corticosteroid hormones as immunosuppressive agent first became evident from the observations of MORGAN (1951) and BILLINGHAM *et al.* (1951), who investigated the mechanism of action and the effect of cortisone on the survival of skin allografts in rabbits. The results of Morgan's experiments indicated that a daily dose of cortisone, beginning on the day of grafting, delayed the allograft rejection and modified the morphological features of the rejection process. Billingham and his associates also demonstrated that daily administration of cortisone ace-

tate had a favourable effect upon the survival time, healing, and growth of skin allotransplants in rabbits. The intensity of the inflammatory reaction accompanying allograft rejection was diminished. Morphologically, the degree of vascularization of the graft was strikingly lower than in untreated allografts. The proliferative activity of the fibrous tissue in the graft bed was retarded. Lymphoid infiltration of the skin transplant was focal and very mild. Prolongation of the survival time of the skin allograft was attributed to the effect of cortisone in suppressing the recipient's systemic immunological response and the local inflammatory reaction.

ZUKOSKI *et al.* (1965) demonstrated that Prednisolone had a very favourable influence on the survival of canine renal allografts. A survival time of 1177 days was achieved in a bilaterally nephrectomized dog with a renal allotransplant by the daily administration of 30 mg Prednisolone over a period of 428 days after transplantation. Several biopsy specimens taken from the kidney revealed mild interstitial lymphoid cell infiltration and changes resembling those seen in membranous glomerulonephritis.

It has been shown that cortisone depresses the formation of antibody to purified antigen. This aspect of corticosteroid function is probably effective in delaying the rejection of allotransplanted organs, but the non-specific anti-inflammatory action of these compounds appears to be important in this respect.

In 1964, MARCHIORO *et al.* discussed the value of steroids as supplementary therapeutic agents in kidney allotransplantation, and commented on their capacity to inhibit further progression of the rejection process and to reverse this process when administered in large doses.

It has been demonstrated that corticosteroids suppress allograft rejection most effectively when administered in combination with other immunosuppressive agents. They are used mainly for the prevention of chronic rejection and acute rejection crises (HAMBURGER *et al.* 1972).

6-mercaptopurine and derivates

In 1958 SCHWARTZ *et al.* pointed out that 6-mercaptopurine, already known as an anti-metabolite and an antileukemic agent, suppressed the immune response in rabbits when injected together with bovine serum albumin. They reported that 6-mercaptopurine depressed the antibody formation to soluble protein antigens. When administered to rabbits in a dosage of 6 mg per kg, 6-mercaptopurine produced a complete blockade of the primary immune response and partially delayed the onset of the secondary response. These observations were extended by the work of MEEKER *et al.* (1969), who clearly demonstrated the inhibitory effect of 6-mercaptopurine on the immune response to skin allografts in rabbits

and the prolongation of skin-allograft survival when 6-mercaptopurine was administered continuously after grafting.

The studies of SCHWARZ and DAMESHEK in 1960 confirmed the immunosuppressive effect of 6-mercaptopurine in the allograft reaction. These investigators obtained longer survival times of full-thickness skin allografts in rabbits with a daily doses of 10 mg and 12 mg of 6-mercaptopurine per kg.

The effect of 6-mercaptopurine on renal allografts was investigated in 1961 by ZUKOSKI *et al.*, who used the canine renal allotransplant as an experimental model. They reported a significant prolongation of the functional survival of the transplanted organs to 66 days, which was achieved with daily doses of 3, 5 or 10 mg 6-mercaptopurine per kg per day. Microscopically, the allotransplanted kidneys in 6-mercaptopurine-treated animals showed, in comparison with the untreated animals, much milder degrees of interstitial edema and tubular destruction as well as much less lymphoid cell infiltration. Marked atrophy of the central lymphoid tissue and almost complete disappearance of the germinal centers in the host's mesenteric lymph nodes were the most striking features in the treated animals (SHEHADEH *et al.* 1970).

Azathioprine (Imuran^{R}, Burroughs, Wellcome Ltd. U.K.), at present the chemical agent most commonly employed in transplantation, is an imidazole derivate of 6-mercaptopurine, and has a similar immunosuppressive effect on the rejection processes. Data on significant prolongation of kidney transplant survival, achieved with Imuran, have been presented by MURRAY *et al.* (1964), who studied the mechanism of action of immunosuppressive drugs, and also by TINBERGEN (1968) and DE BRUIN (1970).

DE BRUIN studied the immunosuppressive effect of various agents on the function of renal allografts in rats and found that the ability of Imuran to prolong renal allograft survival was increased when the drug was given in combination with Prednisolone.

The mode of action of the 6-mercaptopurines on the immune responses has not yet been elucidated, but several possibilities have been postulated. As an antimetabolite, azathioprine inhibits cell multiplication in antibody-forming tissues, inhibits DNA and RNA synthesis, and suppresses the formation of humoral antibodies (BILLINGHAM *et al.* 1951). HAMBURGER *et al.* (1972) hypothesized that "the cells which recognize antigen are extremely sensitive to antimetabolites and it may be that antimetabolites prevent contact between the antigens and the cells that recognize antigen".

From the studies of BOREL and SCHWARZ (1964) it is apparent that the 6-mercaptopurines potently inhibit antibody formation and reduce the

reactions occurring after the interaction between the antigen and sensitized cells or humoral antibodies.

Anti-lymphocyte serum

The immunosuppressive properties of antilymphocyte serum (ALS) have been very successfully employed in clinical and experimental transplantation since 1964, when WOODRUFF and ANDERSON demonstrated marked prolongation of skin allograft survival in rats after treatment with rabbit antiserum to rat thoracic-duct lymphocytes. Prior to this important study, WAKSMAN *et al.* (1961) had presented evidence of a specific effect of anti-lymphocyte serum on several types of "delayed" hypersensitivity reactions, such as tuberculin and contact sensitivity, the delayed reactions to purified proteins (diphtheria toxoid), and allergic encephalomyelitis. It was noted that administration of anti-lymphocyte serum resulted in the destruction of circulating lymphoid cells and in cell depletion of lymph nodes.

Prolongation of skin-allograft survival in mice and monkeys given anti-lymphocyte serum was reported by LEVEY aud MEDAWAR (1966) and BALNER *et al.* (1969).

A number of studies describe considerable prolongation of renal allograft function after treatment of the recipient with anti-lymphocyte serum as an immunosuppressive agent.

ABAZA *et al.* (1966) tested the immunosuppressive properties of ALS in canine renal allotransplantation, and demonstrated its effectiveness in prolonging the survival of the kidney grafts. These authors stressed the possible significance of ALS for clinical organ transplantation.

LAWSON and his associates (1967), who studied the favourable effect of ALS on canine renal allografts, described the destruction and atrophy of lymphatic tissue in the lymph nodes and the spleen of recipient animals. These studies suggested a correlation between the degree of lymphopenia produced by ALS and the degree of immunosuppression achieved.

Further evidence of the value of ALS in kidney allograft rejection has been reported by CLUNIE *et al.* (1967), who achieved a mean survival time of 68.4 days in canine renal allotransplantation by the administration of ALS prior to transplantation. These authors underlined the importance of the combination of pre-operative and post-operative ALS treatment in renal allografting for an optimal effect.

DE VRIES *et al.* (1968) studied the effect of ALS and other immunosuppressive agents on the morphology of allograft rejection. They described the allograft reaction as consisting of two main components:

1. Parenchymal rejection, affecting the small intertubular capillaries and venules, associated with lymphoid cell infiltration and progressive

glomerular changes. This reaction was postulated to be cell-mediated.

2. Vascular rejection consisting of necrotizing vasculitis of the larger renal vessels leading to multiple infarctions, which was postulated to be antibody-mediated and is associated with the deposition of antigen-antibody complexes in the wall of the blood vessels. The authors tentatively concluded that a combination of Imuran and ALS appeared to be the immunosuppressive treatment of choice in organ transplantation, since Imuran suppresses the vascular rejection and ALS the parenchymal rejection.

The studies of ISRAEL and DE VRIES (1970), which were designed to evaluate the comparative effectiveness of ALS and Imuran on immune reactions in mice, indicated that ALS suppresses both antibody-mediated and cell-mediated immunity, whereas Imuran affects only antibody formation.

The evidence presented by WAKSMAN *et al.* (1961) as well as ABAZA *et al.* (1966), showing that application of ALS induced a considerable decrease in the number of circulating lymphocytes and marked atrophic changes in lymphoid organs, led to the hypothesis that ALS achieves its immunosuppressive effect by inhibition or inactivation of lymphoid immunocompetent cells (LEVEY, 1970).

EVERETT and his colleagues (1970) reported that ALS caused a selective depletion of a long-lived lymphocyte population that includes immunocompetent cells.

Subsequently, LEUCHARS *et al.* (1968) pointed out that ALS exerted its action primarily on the thymus-derived lymphocytes. These observations were confirmed by LANCE (1970), who showed that ALS exerted a much stronger effect on cell-mediated immune reactions, although it also influences humoral immune responses to a certain extent. It is to be noted that support for this conclusion could be derived from the morphology of the lymph nodes of ALS-treated animals. The characteristic morphological changes are consistent with a depletion of lymphocytes in the paracortical (thymus-dependent) areas of the lymph nodes, sites which are involved in cell-mediated immune reactions.

Summary

Various chemical agents, corticosteroid hormones, and antilymphocyte serum have been shown to prolong allograft survival in experimental allotransplantation, and are increasingly used in immunosuppressive treatment associated with clinical organ transplantation. The immunosuppressive potency of the corticosteroid hormones is due mainly to their ability to depress the formation of circulating antibodies and to their

anti-inflammatory action. The most commonly used chemical agents, i.e., the 6-mercaptopurine and derivates, which act as antimetabolites, also inhibit antibody formation and reduce the reactions resulting from the interaction between the antigen-carrying cells and sensitized cells or humoral antibodies. The usefulness of antilymphocyte serum (ALS) in renal transplantation is based on its ability to inactivate or even to destroy the immunocompetent thymus-dependent lymphocytes in the circulating blood. Antilymphocyte serum has been shown to be a powerful immunosuppressive agent with respect to both cell-mediated and humoral antibody responses.

CHAPTER II

MATERIAL AND METHODS

Experimental animals

Young adult rats of two inbred strains, WAG/Rij and BN-Bi, were used in this study. The animals were supplied by the Radiobiological Institute of the Organization for Health Research, T.N.O., Rijswijk, The Netherlands.

The WAG/Rij strain is an inbred Wistar albino strain, originating from Glaxo Laboratories, Greenford, Middlesex, England. The BN/Bi strain was obtained from the Microbiological Associates Inc., Washington. Both strains have been bred by T.N.O. Rijswijk for many generations by brother/sister mating under specific pathogen-free conditions.

BN/Bi male rats weighing 200–300 gr and aged 4–6 months were used as donors in renal allotransplantation, and WAG/Rij male rats aged from 5 to 8 months and weighing 250–350 gr served as recipients of kidney grafts. The strains WAG/Rij and BN/Bi differ at a strong H-1 histocompatibility locus, showing an almost constant rejection time (De Bruin 1971).

Surgical procedure

The microvascular technique for orthotopic renal transplantation in the rat, introduced by Fisher and Lee in 1965 and modified by Tinbergen (1971), was applied as follows. Animals were anaesthetized with ether and fixed on operating boards. Ether-soaked cotton held above the animal's nose maintained the narcotic state during the operation, which was carried out under clean but not sterile conditions. The abdominal cavity was entered via a long midline incision after cleaning of the abdominal wall with alcohol. The intestines were wrapped with wet gauze and placed laterally to provide a clear operative field. The aorta and vena cava were clamped just below the renal arteries with a curved rubber-shod clamp. The right kidney was removed together with the renal artery, renal vein, and the ureter, and quickly placed in a cold saline solution (4 °C). The dissected edges of the renal blood vessels retained small elliptical cuffs of the aortic and vena cava wall.

The recipients were prepared for kidney grafting in the same way as the donors. Small longitudinal openings having the same size as the elliptical cuffs on the donor's blood vessels were made in the host's aorta and vena cava. In these places a vascular reconstruction between the

donor's and recipient's vessels was carried out with 7–0 silk suture (Ethicon).

The ureter was inserted into the bladder lumen through the recipient's urinary bladder wall, and fixed to the posterior wall. During the same operation, both kidneys of the recipient were removed.

The normal color of the transplanted kidney was restored within a few minutes after removal of the clamp. The maximum duration of the operation was 80 minutes; the total ischemic period of the grafted kidneys was no longer than 40 minutes.

After the operation, each animal received 50.000 I.U. penicillin G/ and 50 mg streptomycin. This antibiotic therapy was continued daily until the animal was killed. The animals were kept in individual autoclavable cages at room temperature and constant humidity. Food pellets and tap water were supplied *ad libitum.*

Experimental groups

A total of 120 allogeneic transplantations were performed, yielding 98 allografts for histological and electron-microscopical examination.

The animals were divided into the following groups according to the type of transplantation performed and the immunosuppressive treatment applied:

group 1 Untreated allografts. No immunosuppressive therapy was given (28 rats).

group 2 Imuran-treated allografts. Imuran (Burroughs, Wellcome) was given intraperitoneally, in a daily dose of 8 mg/kg body weight, starting on the day of operation (20 rats).

group 3 Anti-lymphocyte serum group (ALS). Recipients of renal allografts given weekly doses of 19 mg anti-lymphocyte globulin (rabbit anti-rat thymocyte serum containing highly purified IgG) (18 rats). Anti-lymphocyte serum was prepared (TINBERGEN 1971) and supplied by the Radiobiological Institute of the Organization for Health Research, T.N.O. Rijswijk, The Netherlands.

group 4 Allotransplanted animals treated with a combination of Imuran and ALS. The animals were given daily doses of 4 mg Imuran and a weekly dose of 19 mg ALS. Imuran was administered intraperitoneally, starting on the day of transplantation; the first dose of ALS was given 1 week before transplantation (16 rats).

group 5 Allotransplants treated with a combination of Imuran and Prednisolone. The animals received 4 mg/kg body weight Prednisolone per day, starting on the day after transplantation, and 2 mg/kg Imuran daily (16 rats).

Animals of each group were killed on days 3, 5, 7, 9, and 21 after transplantation. Of the untreated allograft group, animals were killed on days 3, 5, 7, and 9. None of the animals in this group survived to 21 days.

Histological techniques

At the time of sacrifice, one half of the transplanted kidney was fixed in a cold phosphate-buffered 4% formaldehyde solution (pH 7.4), the other half being reserved for electron-microscopical studies, as described below.

The fixed tissue was dehydrated in alcohol and embedded in Paraplast, after which serial sections with a thickness of 2–4 μm were cut on a Spencer A.O. microtome. The following stains were applied: hematoxylin-azophloxin-saffron; periodic-acid-Schiff (PAS-reaction); periodic-acid-silver methenamine (Jones); pyronine-methyl green; Lendrum's stain for fibrin; Weigert's elastin stain.

Electron microscopy

Specimen sampling

Half of each transplanted kidney was placed in a drop of cold fixative (see below) on a dental-wax plate and cut into small (1 mm) cubes with an acetone-cleaned razor blade. The cubes were picked up with a strip of paper and rinsed in fresh fixative solution.

Fixation

Fixation was carried out with 3% glutaraldehyde in 0.1 M Na-cacodylate+0.05 M $CaCl_2$ in distilled water (pH 7.4) at a temperature of −4 °C. The specimens were held in the fixative for at least three days and then transferred for 24 hours to a buffer solution consisting of 0.1 M Na-cacodylate+0.05 M $CaCl_2$ in distilled water (pH 7.4) before being postfixed for 16 hours with 1% OsO_4 in 0.1 M Na-cacodylate (pH 7.3) to which 0.05 M $K_3Fe(CN)_6$ had been added (De Bruijn 1969; Glauert 1975).

Dehydration

Dehydration was performed in an acetone series of ascending concentration at room temperature: 2×10 minutes in 30% acetone followed by 2×10 minutes in 50%, 2×10 minutes in 70%, 2×10 minutes in 90%, and 2×20 minutes in 100% acetone.

Infiltration

After dehydration, the tissue was immersed in a solution of 1.2-epoxypropane for 2×10 minutes and then brought into a mixture of Epon 812 and acetone (1:1) for 1 hour at room temperature.

Embedding

Embedding was carried out in gelatine capsules containing Epon-C. The Epon-C mixture consisted of 38 ml Epon 812 + 26 ml dodecenyl succinic anhydride + 20 ml methyl nadic anhydride (Fluka).

Polymerization

A suitable degree of hardness was achieved by keeping the capsules at 37 °C for 18 hours and then at 60 °C for 2×24 hours.

Sectioning and microscopy

Of each transplanted kidney, at least five blocks were used for 1 μm thick survey sections. These sections were cut on a LKB pyramitom equipped with glass knives, and were then stained with toluidine blue. Small areas (0.20×0.20 mm) of the renal cortex, medulla, interstitial tissue, and blood vessels were selected for further ultra-thin sectioning. The blocks were trimmed into small pyramids and cut with an LKB ultramicrotome provided with glass knives. The sections, whose thickness varied between 50 and 80 μm, were mounted on Formvar-coated copper grids and stained with uranyl acetate and lead citrate. The sections were examined and photographed with a Philips E.M. 200 and a Philips E.M. 300 Electron Microscope operated at 40 and 60 kV with objective diaphragms of 20 μm diameter.

CHAPTER III

ULTRASTRUCTURAL ASPECTS OF INTERSTITIAL CELLULAR INFILTRATION

Introduction

The earliest visible morphological changes in the evolution of the unmodified acute allograft reaction are the alterations observed in the renal interstitium, consisting of perivascular cellular infiltration followed by edema and hemorrhage.

Between the 6th and 24th hour after transplantation, patchy perivascular aggregations of mononuclear cells appear in the interstitial space of the cortex. Starting on the 2nd or 3rd day, larger mononuclear cells appear (GUTTMANN *et al.* 1967; LUND and JENSEN 1970; LINDQUIST *et al.* 1968; FELDMAN and LEE 1967). The cytoplasm of these cells contains a large amount of ribonucleic acid (RNA), as shown by the methyl green pyronine strain. Some mature plasma cells are also present. At 7 days the cellular infiltration is seen over large areas of the renal parenchyma. At this time, the cellular infiltrations consist of large pyronine-positive cells and a few polymorphonuclear cells.

Immunofluorescent studies on acute allograft rejection in the rat performed by LINDQUIST *et al.* (1968) demonstrated the presence of IgG-containing mononuclear cells in the interstitial infiltrate 2 weeks after transplantation. In canine renal allotransplantation HOROWITZ *et al.* (1965) observed with immunofluorescent microscopy cellular infiltrations with gammaglobulin in the numerous cells corresponding with histologically identifiable plasma cells. Electron-microscopical examination of the kidney allografts made it possible to distinguish several varieties of mononuclear cells in the interstitial space.

In 1962, GALLE and MONTERA gave the first description of the ultrastructural morphology of the cellular infiltrations occurring in human renal allotransplants. Several ultrastructurally distinct classes of cells were distinguished, including lymphocytes, plasma cells, histiocytes, and fibroblasts as well as reticulum cells, granulocytes, and undifferentiated cells.

WILLIAMS *et al.* (1964) described two classes of cells in detail on the basis of studies on the morphology and hemodynamics of canine renal transplants. One of these classes consisted of small lymphocytes, the other included cells of the plasma-cell series. The small lymphocytes had large round nuclei and scanty cytoplasm containing mitochondria,

a few vacuoles, and scattered ribosomes. Cells of the plasma-cell series, which showed several stages of development and differentiation, included cells with a well-developed endoplasmic reticulum and a large amount of free ribosomes in a rather abundant cytoplasm.

These observations were in accordance with those of PORTER *et al.* (1967), who investigated the electron-microscopical appearance of infiltrating cells in canine renal homotransplants. Porter and his associates found two ultrastructurally different groups of lymphoid cells in the interstitial cellular infiltrates. The first was composed of lymphocytes that appeared during the first 24 hours and included cells with large indented nuclei and scanty cytoplasm with few cytoplasmic components. The second group of cells, which were pyronin-positive in light microscopy, consisted of two categories of cells, one resembling large lymphocytes or histiocytes and having little endoplasmic reticulum, the other having abundant endoplasmic reticulum, presumably being plasma cells.

Results

Untreated renal allografts

In our studies interstitial monoclear-cell infiltration in the allotransplanted kidneys was distinctly observed by the 3rd day after transplantation. The infiltration had a patchy distribution in the renal cortex around the small blood vessels and occasionally around the glomeruli. The cells possessed either very dense or pale, vesicular, oval nuclei. The cell boundaries were indistinct. The methyl green pyronine stain revealed numerous cells with a pyroninophylic cytoplasm.

By day 5 and 7 the infiltration was denser and extended through the renal cortex and medulla. The morphology of the cells appeared identical to that on day 3, although a few polymorphonuclear leucocytes were also present. Numerous pyroninophylic cells could still be demonstrated.

On the 9th day after transplantation, the infiltrating cells were diffusely distributed over the renal parenchyma. Frequent invasion of the tubuli and interstitial blood vessels and compression of the glomeruli were apparent.

The interstitial space was strikingly edematous throughout the period between the 3rd and 9th days. Seven and 9 days after the transplantation, groups of extravascular erythrocytes and larger areas of focal and widespread hemorrhages, were frequently encountered in the interstitial space of the kidneys in addition to the infiltration (Table 1).

Under the electron microscope at least four types of cells with a distinct submicroscopic morphology were discerned.

Table 1. Ultrastructural changes of the interstitium in untreated allografts

Type of change	Time after transplantation				
	3 days	5 days	7 days	9 days	21 days
Edema	+	++	+++	+++	
Cellular infiltration					
Large lymphocytes	+++	+++	+++	+++	
Immature plasma cells	++	++	++	++	
Mature plasma cells	○	○	+	+	
Macrophages	○	○	○	+	
Hemorrhages	○	○	○	+	

○ no changes
+ mild focal changes
++ moderate diffuse changes
+++ severe changes

Large lymphocytes

The large lymphocytes were either oval or elongated cells, between 8 and 12 μ in size, occurring in great numbers in the interstitial space of the kidney 3 and 5 days after transplantation. The surfaces of these cells were slightly irregular, due to numerous short thin cytoplasmic projections (Fig. 1).

The cytoplasm countained numerous small oval or elongated mitochondria and a very prominent Golgi apparatus composed of many vesicles. There were cysternae of smooth-surfaced endoplasmic reticulum. Large numbers of free ribosomes were distributed throughout the cytoplasm. In addition, small vacuoles and dense bodies were also seen.

The nucleus was rather large and irregular, and had a deep invagination. Chromatin was scattered throughout the entire nucleus, with a tendency to be denser at the periphery. A nucleolus was clearly observed.

This type of mononuclear cell was most frequently encountered invading the tubuli and establishing a close cell-membrane apposition with the endothelium of the intertubular capillaries and glomerular capillary loops.

Immature plasma cells

Two striking ultrastructural characteristics of this type of cell, which was predominant in the interstitial infiltration seen in the kidneys 5 and 7 days after transplantation, were the great number of cysternae of endoplasmic reticulum and the abundant cytoplasm. The cell had numerous cytoplasmic projections varying in thickness and length, which were cut in different directions in a given section. As a result, the cellular surface had a very rough appearance (Fig. 2). The cells were irregularly shaped, mostly oval or elongated, and measured 15 μ. They

possessed a large, oval, rather homogeneous nucleus with no indications of nucleoli. The Golgi apparatus was highly developed, and was built up of numerous vesicular components. The endoplasmic reticulum generally consisted of smooth-surfaced membranes, although in a few cases rough endoplasmic profiles were also observed. The cytoplasm included numerous free and membrane-associated ribosomes and several oval mitochondria. Often, the cytoplasm contained a few small electron-dense inclusions of unknown origin.

Mature plasma cells

In comparison with the other types of cell seen in large numbers in the interstitial space, the number of mature plasma cells was insignificant. These cells were usually observed by the 7th and 9th day after transplantation.

The cell surface was rather smooth except for a few long cytoplasmic projections. In the very abundant cytoplasm there were many rough-surfaced endoplasmic cysternae, arranged in parallel and containing homogeneous substance with a low electron density. In addition, the cytoplasm included many round mitochondria and a well-developed Golgi apparatus as well as many free ribosomes (Fig. 3).

The nucleus was small and eccentrically situated. It showed finely dispersed chromatin granules. There was no evidence indicating the presence of a nucleolus. The mature plasma cells had a diameter of 8 μ.

Macrophages

Although not numerous, macrophages were frequently observed by the 7th and 9th days. The main ultrastructural characteristic of these macrophages was the presence of intra-cytoplasmic phagocytosed foreign substances consisting of dense osmiophilic granules and large segments of foreign cytoplasmic material. These cells had a diameter of approximately 12–15 μ.

The shape of the nucleus was irregular and lobulated. The abundant cytoplasm, in addition to the ingested foreign material, was relatively rich in Golgi complexes, small mitochondria, and endoplasmic reticulum of both types.

The cell surface was very rough due to numerous cytoplasmic projections (Fig. 4).

Treated renal allografts

The results are summarized in Tables 2, 3, 4, and 5.

Group treated with Imuran

The density of the mononuclear cell accumulation in the interstitium was as great as in untreated renal allografts. Ultrastructurally, the cellular infiltration showed the same ultrastructural characteristics (Table 2).

Table 2. Ultrastructural changes of the interstitium in Imuran-treated allografts

	Time after transplantation				
Type of change	3 days	5 days	7 days	9 days	21 days
Edema	+	+	++	++	++
Cellular infiltration					
Large lymphocytes	+++	+++	+++	+++	+++
Immature plasma cells	+	++	++	++	++
Mature plasma cells	○	○	+	+	+
Macrophages	○	○	○	+	+
Hemorrhages	○	○	○	+	+

○ no changes
+ mild focal changes
++ moderate diffuse changes
+++ severe changes

Group treated with ALS

Following ALS treatment the accumulation of the interstitial mononuclear cells was very slowly progressive over the period of 3–21 days. Electron-microscopically, the cells revealed a fine morphology similar to that of the untreated allografts, and showed degenerative cytoplasmic changes (Table 3).

Table 3. Ultrastructural changes of the interstitium in ALS-treated allografts

	Time after transplantation				
Type of change	3 days	5 days	7 days	9 days	21 days
Edema	○	+	+	++	++
Cellular infiltration					
Large lymphocytes	○	+	+	+	+
Immature plasma cells	○	+	+	+	+
Mature plasma cells	○	○	+	+	+
Macrophages	○	○	○	+	+
Hemorrhages	○	○	○	○	+

○ no changes
+ mild focal changes
++ moderate diffuse changes
+++ severe changes

Group treated with ALS + Imuran

The composition of the infiltration and the ultrastructural characteristics

of the interstitial cells were similar to those of the group treated with ALS only (Table 4).

Table 4. Ultrastructural changes of the interstitium in ALS+Imuran-treated allografts

Type of change	Time after transplantation				
	3 days	5 days	7 days	9 days	21 days
Edema	○	+	+	+	++
Cellular infiltration					
Large lymphocytes	○	○	+	+	+
Immature plasma cells	○	+	+	+	+
Mature plasma cells	○	○	+	+	+
Macrophages	○	○	○	+	+
Hemorrhages	○	○	○	○	+

○ no changes
+ mild focal changes
++ moderate diffuse changes
+++ severe changes

Group treated with Imuran+Prednisolone

The cellular infiltration showed the same density and ultrastructural morphology of the cells as in the animals treated with Imuran only (Table 5).

Table 5. Ultrastructural changes of the interstitium in Imuran+Prednisolone-treated allografts

Type of change	Time after transplantation				
	3 days	5 days	7 days	9 days	21 days
Edema	○	+	+	+	++
Cellular infiltration					
Large lymphocytes	+++	+++	+++	+++	+++
Immature plasma cells	+	++	++	++	++
Mature plasma cells	○	○	+	+	+
Macrophages	○	○	○	+	+
Hemorrhages	○	○	○	○	+

○ no changes
+ mild focal changes
++ moderate diffuse changes
+++ severe changes

Discussion

The significance of the interstitial mononuclear infiltration characteristically seen in allograft reactions and the nature of the infiltrating cells have long given rise to disagreement and conflicting interpretations.

As early as 1908, CARREL was the first to report the occurrence of interstitial cell infiltration and to describe the characteristics of the plasma cells in the infiltrate. His observations were made in a study on the histological features of 9 renal transplants in cats. He designated this picture as subacute interstitial nephritis, and believed that the changes were secondary to the general condition of the animal, congestion of the transplanted organ, and the nature of the perfusion fluids used.

In 1926, WILLIAMSON expressed the view that the histological changes in allogeneic kidney transplants were those of acute "atypical glomerulonephritis" and described a significant lymphocytic interstitial infiltration accompanying the glomerular alterations. HOLLAWAY, reporting in 1926 on the effects of diuretics on transplanted kidneys in dogs, defined the leucocytic infiltration with multiple small abscesses in the renal cortex as a hemorrhagic nephritis.

Even after the establishment of the immunological nature of graft rejection by MEDAWAR in 1944, the source of the infiltrating cells seen in transplanted tissues and organs remained uncertain. DEMPSTER suggested in 1953 and SIMONSEN *et al.* in 1953 that the cell accumulations observed in allotransplanted kidneys, represented a reaction of the grafted organ against the recipient. In 1960, PORTER and CALNE used an autoradiographic technique in conjunction with tritium-labelled thymidine to trace the source of the infiltrating pyronine-positive cells in kidney and skin allografts. Their experiments convincingly demonstrated that the cells originated from the recipient; none of the cells were of donor origin.

Electron-microscopical investigation of the interstitial infiltrates has shown that the mononuclear cell population mainly consists of cells with the ultrastructural characteristics of large lymphocytes, immature plasma cells, and mature plasma cells (GALLE and DE MONTEIRA 1962).

In the present study the findings in the group of untreated renal allografts are on the whole similar to those of WILLIAMS *et al.* (1964), PORTER *et al.* (1964), and LINDQUIST *et al.* (1971). Our results, like theirs, suggest that the large lymphocytes and immature plasma cells play an important role in the rejection of a renal allograft. Populations of these cells were the most numerous in the interstitial infiltrate, accounting for more than 80% of all cells observed between the 3rd and the 9th post-transplantation day.

There were, however, differences between these two cell populations with respect to the time of appearance and their behaviour in the renal interstitium. The cell aggregates on the 3rd day after allotransplantation were composed entirely of cells with the ultrastructural characteristics of large lymphocytes. The population of immature plasma cells made its appearance by the 5th post-transplantation day. The large lymphocytes

were the only cell population observed to establish close cell-membrane apposition and cytoplasmic continuity between themselves. This phenomenon was noticed in earlier studies by LINDQUIST *et al.* (1971), which provided morphological evidence for a mechanism by which already sensitized cells could induce specific immunological competence in nonsentized cells by transferring cellular materials, not further defined by the authors, from one cell to another.

As in previous studies, no destruction of the renal parenchyme mediated by the direct cytotoxic effect of the large lymphocytes was observed, although an intimate contact between these mononuclear cells and the basement membrane of tubular cells, and the adventitia of the arterioles was seen frequently.

The role of the immature plasma cell population is more difficult to interpret. Since these cells possess rather abundant endoplasmic reticulum it seems likely that they are engaged in producing antibodies. These cells were not seen to create close cell membrane contact either with other cells in the interstitium or with blood vessels and tubules.

It should be kept in mind that the technique employed in this study has been unsuccessful in differentiating between T- and B-lymphocytes, which might be done according BENTWICK and KUNKEL (1973), by scanning electron microscopy. However, we may speculate that the lymphoid cells indicated in this study as large lymphocytes, possibly belong to the class of T-lymphocytes and that the immature plasma cells are derived from B-lymphocytes. This interpretation is based on the ultrastructural morphology of the cell surface and the cytoplasmic constituents as well as on their action in the renal interstitium.

On the basis of the results reported here, it appears that the immunosuppressive drug Imuran administered alone or in combination with Prednisolone does not play any part in modifying the ultrastructural appearance of the interstitial cellular infiltrations or influence the density of the cellular masses and the edematous changes in the renal interstitium. Of the immunosuppressive agents under study, only ALS, alone or in combination with Imuran, is capable of altering the fine morphology of lymphatic cells and of depressing significantly the number of cells in the interstitium.

In comparison with the other experimental groups, there was a significant reduction in the number of large lymphocytes, accompanied by degenerative alterations of these cells, in the group of allografts treated with ALS. In view of these results it seems very likely that the demonstration of many degenerated lymphoid cells in the renal interstitium of ALS-treated animals constitutes morphological evidence of the destructive effect of ALS on the immunocompetent lymphoid cells, as

indicated by EVERETT *et al.* (1970). Moreover, the decrease in the number of large lymphocytes in the interstitium of ALS-treated allografts is in accordance with the view of RUSSELL and MONACO (1967) that ALS selectively depletes the lymphocyte population in the peripheral blood due to marked reduction of the lymphoid elements in the lymph nodes and spleen.

CHAPTER IV

FINE MORPHOLOGY OF THE VASCULAR LESIONS

Introduction

Vascular lesions occurring in the peritubular capillaries, arterioles, and arteries of renal allotransplants are considered to be a component of the allograft rejection and to be closely related to the functional arrest and subsequent destruction of the graft.

The morphological characteristics of blood vessel damage in acute renal allograft rejection have been described by a number of investigators. In 1953, SIMONSEN *et al.* observed changes in all layers of medium-sized and small blood vessels in a series of 35 canine renal autotransplants and allotransplants. The changes consisted of extensive fibrinoid necrosis of the media, accompanied by fibroblastic cell proliferation and dense mononuclear cell infiltration around the adventitia. Morphologically, the alterations very closely resembled the blood vessel changes seen in periarteritis nodosa and malignant hypertension.

Prior to these observations, as early as 1926, WILLIAMSON transplanted kidneys of goats into dogs, which died within a few minutes after the operation and found extensive plugging of the blood vessels by tightly clumped erythrocytes.

Extensive studies on the function and the pathology of kidney transplants in humans, were carried out by HUME *et al.* in 1955. The authors noted a very close resemblance between the blood vessel damage described by DEMPSTER (1953) in experimental allografts and those seen in human allografts. They were impressed by the very severe intimal thickening occurring in the large and medium-sized blood vessels associated with thrombosis of the small arteries, and thought that these alterations were probably due to long-term high blood pressure in the recipient, who suffered from chornic glomerulonephritis.

Obstructive vascular lesions such as endothelial swelling, fibrous intimal thickening, medial atrophy, fibrinoid necrosis, and thrombosis occurring in the arterioles and interlobular arteries between the 38th and 45th day after transplantation, were also observed in 4 human kidney allografts studied by PORTER *et al.* in 1963. These authors did not agree with Hume's conclusion that hypertension in the recipient is generally responsible for the pathology of the blood vessels in transplanted kidneys, and ascribed these changes of the vascular tree to an immunological mechanism.

The studies done by KOUNTZ *et al.* in 1963 indicated that destruction of renal allotransplants can be explained by an immunological reaction mediated by immune-competent cells, which causes disintegration of the peritubular capillaries. Their electron-microscopical studies showed that the progressive damage to the peritubular capillaries was associated with a close cell-membrane apposition of lymphoid cells and capillary endothelium and by extravasation of erythrocytes and white blood cells.

FELDMAN and LEE (1967) also provided strong support for the assumption that destruction of the vessels in allografted kidneys is primarily due to an interaction between host lymphocytes and the endothelium of the blood vessels of the graft, resulting in ischemia and tubular damage. These workers studied the earliest electron-microscopical changes in the interstitium of renal allotransplants in rats. The thin-walled blood vessels showed a swelling of the endothelial-cell cytoplasm and detachment of the endothelial cells from the basement membrane. The lumina of the blood vessels contained many lymphoid cells, some of them adhering to the endothelial cell layer.

ROWLANDS *et al.* (1967) carried out ultrastructural studies of kidney heterografts. The peritubular capillaries exhibited striking changes consisting of disappearance of the endothelial layer and denudation of the basement membrane, which had increased in thickness. The arterioles, whose walls had a normal appearance, were filled with abundant lymphoid cells.

More recently, extensive studies of the electron-microscopical features of the vascular lesions in a series of 16 human renal allotransplants were undertaken by ROSSMANN *et al.* (1970). Apart from endothelial swelling and subendothelial deposits of dense granular material associated with a thickening of the basement membrane, the most striking ultrastructural feature was severe degeneration of the smooth muscles in the media and of the endothelial layer. The authors concluded that the vascular changes were "*sui generis*" and different from all lesions observed in atherosclerosis and other inflammatory conditions; but the pathogenesis remained obscure. However, many other studies support Porter's view that the vascular lesions in allograft rejection are caused by specific immunological, cell-mediated and humoral mechanisms (DUNEA, HAZARD and KOLF 1964; KINCAID-SMITH 1964; HERBERTSON 1973).

Results

Untreated renal allografts

Intertubular capillaries

Three days after the transplantation, many intertubular capillaries show-

ed complete occlusion of the lumen by aggregates of platelets, lymphoid cells, and erythrocytes. Occasionally, small accumulations of fibrinoid material between the cells were also observed (Fig. 5).

The platelets usually had an irregular shape (stellated or polygonal) and short cytoplasmic processes. They were present in larger numbers than the mononuclear and polymorphonuclear cells.

In all of the capillaries studied the endothelium had an abnormal appearance. Generally, the endothelial cells showed a marked increase in the amount of cytoplasm, with vacuoles of varying sizes and distended endoplasmic reticulum sacs containing some small dense droplets. The swollen, enlarged and rounded endothelial cells bulged into the vascular lumen.

Disintegration of the endothelial layer appeared to be a very significant feature, evident in many capillaries by the 3rd and 5th day after transplantation (Fig. 6). This phenomenon took two forms. In the first and most frequent, disintegration of the endothelial layer appeared to be associated with the presence of intravascular lymphoid cells and thrombocytes situated close to the affected endothelial cells. The second form seemed to be related to loss of cohesion between the endothelial cells and the underlying basement membrane, followed by cellular detachment (Table 6).

Table 6. Ultrastructural vascular changes in untreated allografts

Type of change	Time after transplantation				
	3 days	5 days	7 days	9 days	21 days
Intertubular capillaries					
luminal occlusion	++	+++	+++	+++	
intimate contact between lymphocytes and endothelium	++	++	++	++	
endothelial swelling	++	+++	+++	+++	
rupture	+	++	+++	+++	
Arterioles and small arteries					
endothelial swelling	○	+	+	++	
medial vacuolization	○	+	+	++	
fibrinoid necrosis	○	○	+	+	
lymphocytic invasion	+	+	++	++	

○ no changes
\+ mild changes
++ moderate changes
+++ severe changes

In many cases the mononuclear cells and the platelets were in very close contact with the endothelial cells, either along segments of their surface or via cytoplasmic projections. At these places, the cell membrane of both the endothelial and the intraluminal cells showed complete

dissolution permitting immediate cytoplasmic continuity (Fig. 7). The endothelial cells involved were severely swollen and showed increased cytoplasmic vacuolation. Less frequently, the lymphoid cells, cytoplasmic projections of lymphoid cells, and platelet pseudopods penetrated between endothelial cells, creating endothelial gaps without evidence of fusion. Here, the continuity of the wall of the blood vessels was preserved solely by the thin, delicate, capillary basement membrane.

In the few blood vessels containing neither lymphoid cells nor thrombocytes, some of the endothelial cells had lost their attachment to the underlying capillary basement membrane (Fig. 8). Although detached and lifted away from the basement membrane, these endothelial cells possessed intact cellular boundaries. At these places the basement membrane was completely nude.

Complete disintegration of the vascular wall was only seen in a few capillaries by the 3rd day after transplantation. It occurred in the areas of intimate contact between the endothelium and mononuclear lymphoid cells, apparently giving rise to extravasation of numerous lymphoid cells, platelets, and erythrocytes, which filled the surrounding edematous interstitium (Fig. 7). By the 5th day, the number of ruptured intertubular blood vessels was strikingly increased and in the kidneys of the 7th and 9th days after transplantation only disintegrated endothelial cells and fine particles of basement membranes remained.

However, all of these kidneys also had some completely intact capillaries and some with slight damage, although the number of these were small in comparison with those showing significant injury.

Arterioles and small arteries

Characteristic ultrastructural changes in the cortical arterioles and small arteries became evident as early as the 3rd day after transplantation. Most of the examined blood vessels were severely contracted (Fig. 9). The lumina were reduced to narrow irregular fissures lined with endothelial cells which were markedly enlarged and vacuolated. These cells contained increased amounts of cytoplasmic components. The endoplasmic reticulum, consisting of elongated sacs and cysternae to which numerous ribosomes had become attached, was very prominent. The mitochondria, although enlarged, were not more numerous. The endothelial cytoplasm was rich in vacuoles of varying size and content. Frequently, large vacuoles containing membranous material were present in the swollen endothelial cells (Fig. 10).

In a few cases a slight separation of the endothelial cell membrane from the underlying basement membrane was noticed. At these sites there was an accumulation of a fine granular material of low electron

density. The basement membrane showed no significant abnormality, although in some places small areas of swelling or rarefaction were encountered. On the 7th and 9th day after transplantation, however, the basement membrane was thickened locally and moderate amounts of a granular amorphous substance had accumulated between the membrane and the overlying endothelium. These deposits were slightly more electron-dense than the basement membrane. The first alterations of the muscle cells of the media occurred in the mitochondria, which were swollen and vacuolated and invariably showed disintegrated cristae (Fig. 11).

Five days after transplantation the cytoplasm of the smooth muscle cells showed vacuolation as well as striking prominence and dilatation of the Golgi apparatus and the endoplasmic reticulum. The number of free ribosomes was increased. Dense bodies of varying size were also seen, mostly around the nucleus. More advanced degenerative alterations were indicated by large amounts of round dense cytoplasmic inclusions scattered throughout the cytoplasm, in which only a few remnants of the normal cytoplasmic components were present (Fig. 12). The nuclei showed shrinkage and condensation of the nucleoplasm.

The occurrence of a dense homogenous or finely granular deposition between the muscle cells and below the basement membrane seemed to be a significant morphologic feature on the 7th day. The deposited material did not differ greatly in density from the basement membrane, but was more or less amorphous. This substance pushed aside and compressed the adjacent cells of the media and, although remaining circumscribed, extended over large areas of the blood vessel wall (Fig. 13).

Invasion of the arteriolar walls by mononuclear lymphoid cells was clearly observed in all groups of animals. In a few blood vessels the cells had penetrated the vascular wall very deeply. The endothelial cells showed severe cytoplasmic vacuolization.

Treated renal allografts

The ultrastructural vascular changes observed in allografts after immunosuppressive treatment are shown in Tables 7, 8, 9 and 10.

Group treated with Imuran

Electron-microscopical examination of the intertubular capillaries of 3-, 5-, and 7-day allografts treated with Imuran revealed blood vessel changes similar to those observed in untreated allografts. Most of the arterioles in this group of animals showed scanty vacuolization of the cytoplasm of the endothelial cells.

However, in animals killed on days 9 and 21, the alterations in the arterioles were less clearly expressed than in the untreated group (Table 7).

Table 7. Ultrastructural vascular changes in Imuran-treated allografts

Type of change	Time after transplantation				
	3 days	5 days	7 days	9 days	21 days
Intertubular capillaries					
luminal occlusion	+	++	++	++	++
intimate contact between lymphocytes and endothelium	++	++	++	++	++
endothelial swelling	+	++	++	++	++
rupture	+	++	++	++	++
Arterioles and small arteries					
endothelial swelling	○	○	+	+	+
medial vacuolization	○	○	○	+	+
fibrinoid necrosis	○	○	○	○	+
lymphocytic invasion	+	+	++	++	++

○ no changes
+ mild changes
++ moderate changes
+++ severe changes

Group treated with ALS

Very slight alterations in the ultrastructural morphology of the blood vessels, characterized by unsignificant vacuolization of the capillary endothelium and mitochondrial swelling of the smooth muscle cells in the arterioles, was seen infrequently in 7-, 9-, and 21-days allografts. Rupture of the peritubular capillaries appears to be strikingly reduced in this group of allografts (Table 8).

Table 8. Ultrastructural vascular changes in ALS-treated allografts

Type of change	Time after transplantation				
	3 days	5 days	7 days	9 days	21 days
Intertubular capillaries					
luminal occlusion	○	○	+	+	+
intimate contact between lymphocytes and endothelium	○	+	+	+	+
endothelial swelling	○	+	+	+	++
rupture	○	○	○	+	+
Arterioles and small arteries					
endothelial swelling	○	+	+	+	++
medial vacuolization	○	○	+	+	++
fibrinoid necrosis	○	○	+	+	+
lymphocytic invasion	○	○	+	+	+

○ no changes
+ mild changes
++ moderate changes
+++ severe changes

Group treated with a combination of ALS and Imuran

No ultrastructural alterations in the interstitial blood vessels were observed in the allografts of this group (Fig. 14) and (Table 9).

Table 9. Ultrastructural vascular changes in ALS+Imuran-treated allografts

Type of change	Time after transplantation				
	3 days	5 days	7 days	9 days	21 days
Intertubular capillaries					
luminal occlusion	○	+	+	+	+
intimate contact between lymphocytes and endothelium	○	+	+	+	+
endothelial swelling	○	○	+	+	++
rupture	○	○	○	+	++
Arterioles and small arteries					
endothelial swelling	○	○	○	+	+
medial vacuolization	○	○	○	+	+
fibrinoid necrosis	○	○	○	○	+
lymphocytic invasion	○	○	+	+	++

○ no changes
+ mild changes
++ moderate changes
+++ severe changes

Group treated with a combination of Imuran and Prednisolone

The changes in the fine morphology of the intertubular capillaries and arterioles were similar to those observed in the group treated with Imuran only (Table 10).

Table 10. Ultrastructural vascular changes Imuran+Prednisolone-treated allografts

Type of change	Time after transplantation				
	3 days	5 days	7 days	9 days	21 days
Intertubular capillaries					
luminal occlusion	+	++	++	++	++
intimate contact between lymphocytes and endothelium	++	++	++	++	++
endothelial swelling	+	++	++	++	++
rupture	+	++	++	++	++
Arterioles+small arteries					
endothelial swelling	○	○	+	++	+
medial vacuolization	○	○	○	+	+
fibrinoid necrosis	○	○	○	○	+
lymphocytic invasion	+	+	++	++	++

○ no changes
+ mild changes
++ moderate changes
+++ severe changes

Discussion

In most respects the ultrastructural vascular changes demonstrated in the present study on unmodified renal allotransplants are similar to those described by KOUNTZ *et al.* (1963), FELDMAN and LEE (1967), and ROSSMANN *et al.* (1970).

Occlusive lesions of the peritubular capillaries, accompanied by endothelial damage and followed by disintegration of the vessel wall, were the earliest alterations of the blood vessels in the allotransplanted kidney and preceded all the other fine structural changes.

The damage to the endothelial layer was generally associated with either close contact between the cell membranes of the endothelial cells and the intravascular large lymphocytes or a progressive vacuolization of the endothelial cell cytoplasm of undetermined origin.

Most frequently, large lymphocytes were seen, as early as 5 days after transplantation, adhering closely to the surface of endothelial cells. These endothelial cells showed necrotic changes. All these findings strongly suggest that the endothelial damage is caused by a direct cytotoxic effect of the lymphocytes on the capillary endothelium.

The peritubular capillaries may be the site of an immunological reaction, mediated by cell-bound antibodies directed against transplantation antigens residing on the vascular endothelium (VETTO and LAWSON 1967; KOUNTZ and COHN 1969). Evidence supporting the immunological nature of this reaction has been provided by LINDQUIST *et al.* (1968) who, using an immunofluorescent-antibody technique, demonstrated IgG deposition on the peritubular capillaries and IgG in the cytoplasm of the intravascular lymphoid cells.

There are now indications that platelet aggregation in the peritubular capillaries accompanied by blood vessel blockage and rupture play a significant role in the initiation of allograft rejection, particularly of hyperacute and acute rejection episodes (TURK 1970; STARZL *et al.* 1967).

LOWENHAUPT and NATHAN (1968) postulated that the antigen-antibody complexes formed on the vascular endothelium induce platelet aggregation in the capillaries and adhesion of the platelets to the surface of the endothelial cells, causing obstruction and vascular injury leading to ischemic death of the grafted organ.

The findings in this study correspond with the above-mentioned observations of LOWENHAUPT and NATHAN, i.e., that the platelets are the first blood elements to accumulate in the peritubular vessels of the renal allograft, forming aggregates capable of blocking the blood flow. Vascular injury often coincided with these aggregates. However, our results do not indicate whether this is the result of platelet degranulation and release of their cytoplasmic contents, as suggested by SHARMA *et al.* (1972).

The importance of arteriolar damage for the mechanism underlying the rejection of renal allografts has been documented. The lesions demonstrated by electron microscopy were characterized mainly by severe degeneration of the endothelial cells and extensive necrosis of the muscular layer with accumulation of cell fragments in the vascular wall (ROSSMANN *et al.* 1970; BUSCH *et al.* 1971). These vascular alterations have been regarded as the result of an immunological reaction mediated by circulating antigen-antibody complexes (PORTER *et al.* 1964; BUSCH *et al.* 1971; KINCAID-SMITH 1964; HOROWITZ *et al.* 1965).

In the present study the earliest ultrastructural alteration of the renal arterioles could be ascribed to the effects of severe spasm of these blood vessels. Strong blood vessel constriction in renal allografts appears to be closely associated with the rejection process and may contribute considerably to the cortical ischemia in early allograft rejection (HOLLENBERG *et al.* 1968). Vasoconstriction of the blood vessels in renal allografts might be, according to LINDQUIST *et al.* (1968), the earliest response of the vascular tree to deposition of immune complexes in the vessel wall, or is possibly due to the secretion of a vaso-active chemical agent by the periarterial accumulation of mononuclear cells (GARDNER *et al.* 1968). Arteriolar changes in allograft rejection begin with injury of the cytoplasmic membrane of the endothelium and the smooth-muscle cells of the media and alteration of the permeability of the endothelium due to binding with humoral antibodies and complement, as indicated by BUSCH *et al.* (1971). Platelets aggregated in areas with damaged endothelium might release platelet factors that activate fibrinogen, thus leading to thrombosis, complete occlusion of the blood vessels, and infarction.

HOROWITZ and his associates (1965), using a fluorescent-antibody technique, demonstrated gamma-globulin in the media of the arteries of canine renal allotransplants and ascribed the degenerative and necrotic changes of the vessels to antigen-antibody complexes formed or deposited in the vascular wall. This seems to be in agreement with the ultrastructural evidence obtained in the present study, where vascular damage consisting of vacuolization of the smooth-muscle cell cytoplasm and deposition of finely granular material between the cells of the media was observed on the 7th and 9th days after transplantation. The nature of these arteriolar lesions remains obscure, however.

Both the blood vessel changes in the modified renal allograft rejection described in this study and the site of these changes are variable, and seem to be related to the immunosuppressive agent employed.

In the *Imuran-treated group*, vacuolization of muscle cells rarely occurred and the severity of the lesions was slight as compared with the untreated group. Deposition of material in the wall of the arterioles was absent.

However, the incidence of occlusive changes and rupture of peritubular capillaries was similar to that in untreated allografts. The combination of *Imuran and Prednisolone* had more or less the same effect on the course of the vascular changes as Imuran alone. In the *ALS-treated group*, however, the incidence of peritubular capillary damage was very low but was associated with arteriolar lesions of similar severity and ultrastructural morphology as those seen in the untreated allografts.

These findings confirm the conclusion reached by De Vries *et al.* (1968), that treatment with Imuran after renal allotransplantation in the rat can almost completely prevent the so-called vascular rejection presumably mediated by humoral antibodies and characterized morphologically by necrotizing arteritis and thrombosis of the larger blood vessels, whereas the administration of ALS mainly influences the parenchymatous rejection, presumably a cell-mediated immunological reaction, by suppressing damage to the peritubular and glomerular capillaries.

De Bruin (1970), who used the same experimental rat model as was employed in the present study, demonstrated a prolongation of renal allograft survival amounting to more than 50 days, with no deterioriza-tion of the function of the grafts, in rats treated with a combination of Imuran and ALS. At autopsy these animals showed only slight to moderate vascular changes consisting of fibrous intimal thickening and some discrete foci of arteritis.

The results of this study clearly show that ultrastructural alterations in the blood vessels of allografts are very small after treatment with a combination of Imuran and ALS. These ultrastructural changes consisted of a slight to moderate enlargement of the endothelial cell, infrequent interaction between endothelium and large lymphocytes, and an almost complete absence of fibrinoid deposition in the vascular wall. These observations do not make it possible to identify the mechanism of action of the immunosuppressive agents in question. Their favourable effect on the vascular changes might be due to a combination of the favourable effects of Imuran, which is known to inhibit humoral antibody formation, and that of ALS, which mainly inactivates or destroys the effector cells (presumably lymphoid cells) in the graft (Borel and Schwartz 1964; Kountz and Cohn 1969; Israel and De Vries 1970).

CHAPTER V

ELECTRON-MICROSCOPICAL OBSERVATION OF GLOMERULAR CHANGES

Introduction

Light-microscopical observation of the glomeruli in renal allotransplantation material, both experimental and human, provides only limited information about the glomerular changes in the rejection process. Many investigators who have performed experimental studies on kidney transplants using different experimental models, even do not mention glomerular alterations (CARREL 1908; IBUKA 1926; WILLIAMSON 1926; WU and MANN 1934; SIMONSEN *et al.* 1953; WILLIAMS *et al.* 1964).

GUTTMANN and his associates (1967), who investigated the functional, immunologic, and morphologic alterations occurring during acute rejection in rat renal allotransplants, described the abnormalities seen in the glomeruli in the kidneys 5 days after the transplantation. Most of these glomeruli had a hypercellular appearance and showed enlargement of the mesangial matrix. Glomerular capillary loops were thickened and frequently obstructed.

Similar glomerular changes, particularly thickening of the capillary basement membrane, have also been observed in human renal transplants (HUME *et al.* 1955; HAMBURGER 1967; CALNE 1967; PORTER *et al.* 1967).

A wider range of glomerular alterations has been observed in electron-microscopical studies on transplanted kidneys. In 1964, HAMBURGER *et al.* investigated the fine structure of the glomeruli in patients with homotransplanted kidneys. In light microscopy, the renal changes were consistent with a histological picture of glomerulonephritis. Ultrastructurally, they included partial obliteration of the capillary lumen, proliferation of the endocapillary cells and subendothelial and intercellular hyaline deposits, as well as slight epithelial alterations.

STARZL *et al.* in 1965 briefly described the light- and electron-microscopical pictures of membranous glomerulonephritis, as seen in two renal allotransplants. The glomeruli showed marked thickening of the basement membranes and fusion of the foot processes.

Glomerular ultrastructural changes were extensively investigated by PORTER and his associates (1967) in 50 human renal allografts which were examined 43 days to 2 years and 3 months after transplantation. In all of these cases the kidneys showed subendothelial accumulation of amorphous fine granular material. The mesangial matrix was increased,

the mesangial and epithelial cells were hyperplastic. The glomerular capillary loops were either narrowed or obstructed by hypertrophic endothelial cells, subendothelial fibrin deposits, and accumulations of erythrocytes, lymphoid cells, platelets, and polymorphonuclear granulocytes. In addition, many of the transplants showed endothelial, mesangial and epithelial cells containing increased amounts of rough endoplasmic reticulum, free ribosomes, and enlarged Golgi bodies.

FISCH *et al.* (1967) presented information on the glomerular changes in long-term human homografts, which are similar to those reported by PORTER and co-workers. The ultrastructural abnormalities in the glomeruli included focal endothelial-cell proliferation and marked basement membrane thickening. Mesangium cell proliferation and increased amounts of extracellular basement-membrane-like material within mesangial areas were also noticed. The authors ascribed the glomerular changes to an imbalance in the homeostatic mechanism of synthesis and turnover of glomerular basement membrane substance.

In his survey concerning the pathology of human renal transplantation, DAMMIN (1968) reviewed the ultrastructural features of four homografted kidneys. Biopsy specimens were taken 12 days, 14 days, 28 months and 3 years after transplantation. Two of these kidneys, which were studied light- and electron-microscopically on the 12th and 14th post-transplantation day, showed narrowing of the glomerular capillary lumen due to swelling and proliferation of the endothelial cells and platelet aggregation, but basement membrane alterations were absent. The other two kidneys, examined 28 months and 3 years after transplantation, showed marked thickening of the glomerular basement membrane, which included incorporated cytoplasmic elements and fibrin fragments. The author interpreted these alterations as glomerulonephropathy of the allografts due to rejection mechanisms.

This view is in accordance with the opinion of PORTER *et al.* (1968) and ANDRES *et al.* (1970), who performed immunologic studies in a series of 34 human renal allografts one to 31 months after transplantation. Under the electron microscope the biopsy specimen exhibited deposition of basement membrane-like material in the subendothelial glomerular spaces and within the basement membrane of Bowman's capsule. The glomerular basement membranes were thickened. In these places localization of ferritin-conjugated antibody to IgG and IgM was evident. In the authors' opinion these observations indicate that the renal damage in allografts is due to circulating antigen-antibody complexes deposited in glomerular and vascular structures of the kidneys.

More recently, SCHÜRCH *et al.* (1972), who performed combined light- and electron-microscopical and immunofluorescence studies on the

recurrent lobular glomerulonephritis seen in human allotransplanted kidneys, thought that immune complexes in the glomeruli of allotransplanted kidneys play a primary role in the pathogenesis of glomerular lesions in human renal allografts. Ultrastructurally, 2, 6, and 15 months after transplantation, the altered glomeruli showed obstruction of the capillary lumens due to endocapillary cell proliferation, deposition of membranoid substance in the mesangial areas, and extensive accumulation of fibrinoid material in the subendothelial sides of the capillary loops. Prior to these cellular proliferative changes, only deposition of subendothelial electron dense fibrinoid material was noticed.

Busch *et al.* (1971) also used electron-microscopical and immunofluorescence techniques to study 27 biopsy samples from 14 long-surviving human renal allotransplants. The glomerular alterations consisted of subepithelial fine granular deposits along the glomerular basement membrane, fusion of the foot processes, and striking thickening and folding of the glomerular basement membranes. The authors considered these changes to be due to a combination of ischemia and rejection resulting from repetitive endothelial injury, progressing to thickening of the basement membrane and obliteration of capillary lumina.

Little is known about the electron-microscopical characteristics of the glomerular damage occurring during acute renal allograft rejection. Lindquist *et al.* (1971) were the first, and are still the only investigators to describe the glomerular ultrastructural alterations of the acute renal allograft rejection in experimental animals. They demonstrated that the changes in the fine structure of the glomeruli became evident between the 3rd and the 5th day after transplantation and consisted of mesangial proliferation and endothelial hypertrophy, followed by subepithelial deposition of basement membrane-like material on glomerular basement membranes. In some places the lymphoid cells established direct contact with the glomerular basement membrane through cytoplasmic pseudopods. The electronmicroscopical observations together with those of immunofluorescence studies suggest that immunological mechanisms are responsible for the glomerular lesions. It is suggested that the deposition of circulating immune complexes on glomerular basement membranes initiates the alterations of the glomeruli.

Results

Untreated renal allografts

In the kidneys of animals killed 3 days after transplantation, no ultrastructural alteration of the glomeruli could be seen (Fig. 15). The earliest changes in the fine glomerular morphology were observed 5 days after

transplantation. At that time changes were apparent in the capillary endothelium, mesangium, and the glomerular epithelial cells.

The endothelial cells were enlarged due to a considerable increase in the amount of endoplasmic reticulum and the number of Golgi bodies, ribosomes, and small cytoplasmic vesicles. Many of these cells also had large nuclei. These swollen endothelial cells had caused narrowing of the capillary lumina.

In many glomeruli most of the endothelial cells were detached from the underlying basement membrane. In a few areas the normal relationship between the endothelium and the basement membrane was preserved. The free spaces resulting from endothelial cell desquamation were filled with a fine granular substance (Fig. 16).

The glomerular basement membrane very often showed irregularities giving it a wrinkled and undulating appearance. These irregularities were associated with collapse of the glomerular capillary loops (Fig. 17). Lymphoid cells having ultrastructural characteristics similar to those observed in the renal interstitium and peritubular blood vessels, were frequently demonstrated in capillary loops. These lymphoid cells either lay free in the capillaries or were in close contact with the endothelium; at the site of this contact, disintegration of the adjacent cytoplasmic membranes was apparent and had resulted in cytoplasmic continuity between endothelium and lymphoid cells. Frequently, the lymphoid cells were in direct contact with the glomerular basement membrane via elongated cytoplasmic processes which were insinuated between the endothelial remnants and between endothelial cell and basement membrane (Fig. 18).

The mesangial matrix of most glomeruli was expanded, but without evidence of mesangial cell proliferation.

The glomerular epithelial cells were voluminous, showing enlarged mitochondria and an increased amount of cytoplasm and cytoplasmic organelles. In many cases small osmiophilic droplets were present in the cytoplasm. A very prominent Golgi apparatus was noticed in many epithelial cells. The nuclei were irregularly enlarged and showed deep invaginations. The foot processes were slightly swollen and flattened against the basement membrane (Table 11).

The kidneys of rats examined 7 days after transplantation, revealed significant alterations in all glomerular structures.

In most of the glomeruli a high proportion of the capillary loops were completely occluded by mononuclear or polymorphonuclear cells, fibrinoid material, platelets, erythrocytes, and degenerated cellular structures of unidentified origin (Figs. 19 and 20).

The glomerular epithelial cells were markedly swollen and vacuolated.

Table 11. Ultrastructural glomerular changes in untreated allografts

Type of change	Time after transplantation				
	3 days	5 days	7 days	9 days	21 days
Endothelial cells					
hypertrophy	○	++	++	+++	
hyperplasia	○	++	++	+++	
cytoplasmic changes	○	++	+++	+++	
endothelial cell detachment	○	++	++	++	
Epithelial cells					
hypertrophy	○	++	++	+++	
fusion of foot processes	○	++	+++	+++	
cytoplasmic changes	○	++	+++	+++	
Mesangium					
cellular changes	○	+	++	++	
matrix changes	○	+	++	++	
Capillary lumina					
narrowed	○	○	++	+++	
obstructed	○	++	+++	+++	
Capillary basement membrane					
wrinkling and collapse	+	++	++	++	
subendothelial deposits	○	○	+	++	
subepithelial deposits	○	○	+	+	
thickening	○	○	○	++	

○ no changes ++ moderate changes
+ mild changes +++ severe changes

The voluminous cytoplasm contained a large number of variably sized vacuoles, enlarged Golgi zones, lipid droplets, and occasional myelin-like figures. The foot processes were flattened, fused, or completely replaced by broad cytoplasmic sheets which encircled the capillary loops. Disintegrated microvillous formations and epithelial cell structures, together with fibrinoid masses, filled the urinary space of the glomerulus.

Simultaneously, marked enlargement of the mesangial space – due to the accumulation of a electron-lucent fibrilar material in the mesangial matrix and mesangial cell hypertrophy – was a constant feature in almost all observed glomeruli. The mesangial cell cytoplasm contained many small vacuoles and dense droplets. These changes resulted in protrusion of the mesangium into the capillary lumina.

The fine architecture of the glomeruli 9 days after transplantation was much more distorted than that observed on the 7th day. At this time, too, the number of affected glomeruli was increased; more than 50% of all glomeruli showed marked alterations.

Apart from the previously described morphological changes, which

had progressed, there were lesions not previously observed. The capillary lumina that had not undergone complete occlusion by fibrin and cellular structures showed very marked narrowing due to either extreme endothelial cell hyperplasia or an extensive wide-spread accumulation of electron-dense material between the basement membrane and the detached endothelial cells (Fig. 21).

The endothelial cells were greatly swollen and vacuolated. Their cytoplasm contained an abundance of mitochondrial fragments and numerous hyaline droplets.

The subendothelial deposition of material of moderate electron density had greatly increased the thickness of the glomerular capillary wall. The glomerular basement membrane appeared unaltered. The deposited material was clearly separated from the lamina interna rara. Only in a few instances was there segmental thickening of the glomerular basement membrane due to deposits with the same electron density as the basement membrane (Fig. 22).

The epithelial cell cytoplasm was very rich in rough-surfaced endoplasmic reticulum, electron-dense droplets, and osmiophilic inclusions of variable size. Vacuolization of the cytoplasm was more extensive than on day 7. Occasionally, either focal homogenization of the cytoplasm was seen near the basement membrane or homogeneous deposits of indistinctly delineated electron-dense material occurred on the epithelial surface of the basement membrane (Fig. 23). This material was clearly separated from the lamina densa. The foot processes of the overlying epithelium were irregularly broadened, swollen, or completely fused. Concurrently, mesangial areas were strikingly broadened and expanded toward the capillary lumina as a consequence of extensive swelling of the matrix fibres and the accumulation of electron-lucent finely granular material among the matrix cells (Fig. 24).

Treated renal allografts

The results are shown in Tables 12, 13, 14 and 15.

Group treated with Imuran

The glomerular ultrastructural changes in Imuran-treated animals killed 3, 5, 7 or 9 days after transplantation were similar to the alterations observed in untreated allografts.

Kidneys of rats killed 21 days after allografting and the start of Imuran treatment showed prominent glomerular alterations, encountered mainly in the basement membrane, mesangium, and the epithelial cells. These

changes were present in all of the observed glomeruli and varied only in the degree of severity (Table 12).

Table 12. Ultrastructural glomerular changes in Imuran-treated allografts

Type of change	Time after transplantation				
	3 days	5 days	7 days	9 days	21 days
Endothelial cells					
hypertrophy	○	+	++	++	+++
hyperplasia	○	+	++	+++	+++
cytoplasmic changes	○	+	++	+++	+++
endothelial cell detachment	○	+	++	++	++
Epithelial cells					
hypertrophy	○	++	++	++	+++
fusion of foot processes	○	+	++	++	+++
cytoplasmic changes	○	+	++	+++	+++
Mesangium					
cellular changes	○	+	++	++	++
matrix changes	○	+	++	++	+++
Capillary lumina					
narrowed	○	○	○	++	++
obstructed	○	+	++	++	++
Capillary basement membrane					
wrinkling and collapse	+	++	++	++	++
subendothelial deposits	○	○	+	+	++
subepithelial deposits	○	○	+	+	+
thickening	○	○	○	++	++

○ no changes
+ mild changes
++ moderate changes
+++ severe changes

The glomerular basement membrane exhibited severe diffuse irregular thickening due to subendothelial deposition of finely granular material with an electron density similar to that of the middle membrane layer. Both the middle layer and the lamina rara interna were destroyed.

Occasionally, endothelial cell fragments and fibrin were seen to be incorporated into the accumulated material (Fig. 25). The basement membrane of the glomeruli had been replaced by an irregular broad band with more than 10 times the normal thickness and consisting of branching processes of electron-dense substance encircling the incorporated cytoplasmic structures.

Another type of deposit, observed in only a few capillary loops, appeared as focal dense homogenous accumulations on the epithelial surface of the basement membrane. These deposits were sharply demarcated from the external membrane layer.

The mesangial areas were very prominent, showing abundant accumulation of amorphous electron-dense material in the mesangial matrix and considerable hyperplasia of the mesangial cells.

The alterations in the basement membrane and protrusion of the enlarged mesangium, together with the endothelial cell proliferation, greatly reduced the lumina of the capillary loops, many of which were almost completely occluded (Fig. 26).

Ultrastructurally, the cytoplasm of the endothelial cells, which were greatly increased in number and enlarged, contained a large number of free and membrane-associated ribosomes as well as many vacuoles and multi-vesicular bodies. Numerous hyaline droplets and large osmiophilic bodies were encountered very frequently.

The epithelial cells had lost their normal ultrastructural appearance almost completely. The fine morphology of the alterations consisted of severe swelling of the cytoplasm and disappearance of the endoplasmic reticulum. Except for the area in the vicinity of the basement membrane, which was homogenized and dense, the cytoplasm showed a very striking electron lucency. The presence in the epithelial cytoplasm of numerous hyaline droplets and vacuoles of different size was a common feature. The nuclei showed a very pale nucleoplasm lacking the chromatin pattern. The extreme peripheral region of the nucleus appeared much darker as the result of an accumulation of dense granules near the inner surface of the nuclear envelope. The foot processes were almost completely replaced by broad sheets of epithelial cytoplasm encircling the capillary loops almost entirely. Complete disintegration of the epithelial cells was seen frequently in many glomeruli (Fig. 27).

Group treated with ALS

The group of animals treated with anti-lymphocyte serum and killed 3, 5, 7 and 9 days after transplantation showed no ultrastructural alterations of the glomerular morphology.

Rats killed 21 days after transplantation showed slight to moderate ultrastructural changes in the glomerular endothelium and epithelium (Table 13).

The epithelial cell alterations consisted of an increase in cytoplasm, associated with swelling and apparent loss of foot processes. In addition, droplets of osmiophilic material were occasionally seen in the glomerular epithelium.

The glomerular endothelial cells showed changes characterized by an increased amount of cytoplasmic material. Fairly frequently the cytoplasm contained small vesicles and swollen mitochondria.

The glomerular basement membrane and the mesangium appeared to be normal.

Table 13. Ultrastructural glomerular changes in ALS-treated allografts

Type of change	Time after transplantation				
	3 days	5 days	7 days	9 days	21 days
Endothelial cells					
hypertrophy	○	○	+	++	++
hyperplasia	○	○	+	+	++
cytoplasmic changes	○	○	+	++	++
endothelial cell detachment	○	○	+	+	++
Epithelial cells					
hypertrophy	○	+	++	++	++
fusion of foot processes	○	○	+	++	++
cytoplasmic changes	○	+	++	++	++
Mesangium					
cellular changes	○	○	○	+	++
matrix changes	○	○	○	+	++
Capillary lumina					
narrowed	○	○	○	○	+
obstructed	○	○	+	++	++
Capillary basement membrane					
wrinkling and collapse	+	++	++	++	++
subendothelial deposits	○	○	+	+	++
subepithelial deposits	○	○	○	+	+
thickening	○	○	○	+	+

○ no changes ++ moderate changes
+ mild changes +++ severe changes

Group treated with a combination of ALS and Imuran

The kidneys of rats killed 3, 5, 7 and 9 days after the transplantation and treated with a combination of Imuran and ALS showed no alterations of fine glomerular morphology.

The glomeruli of rats killed on the 21st day showed changes similar to those seen after the same interval in the group treated with ALS only (Table 14).

Group treated with a combination of Imuran and Prednisolone

Animals killed 3, 5, 7 and 9 days after transplantation and the start of immunosuppressive treatment consisting of a combination of Imuran and Prednisolone, showed ultrastructural changes similar to those seen

Table 14. Ultrastructural glomerular changes in ALS+Imuran-treated allografts

Type of change	Time after transplantation				
	3 days	5 days	7 days	9 days	21 days
Endothelial cells					
hypertrophy	○	○	+	+	++
hyperplasia	○	○	+	+	++
cytoplasmic changes	○	○	+	+	++
endothelial cell detachment	○	○	+	+	++
Epithelial cells					
hypertrophy	○	+	++	++	++
fusion of foot processes	○	○	+	+	++
cytoplasmic changes	○	○	++	++	++
Mesangium					
cellular changes	○	○	○	+	++
matrix changes	○	○	○	+	++
Capillary lumina					
narrowed	○	○	○	○	+
obstructed	○	○	+	++	++
Capillary basement membrane					
wrinkling and collapse	+	++	++	++	++
subendothelial deposits	○	○	+	+	++
subepithelial deposits	○	○	○	+	+
thickening	○	○	○	+	+

○ no changes
+ mild changes
++ moderate changes
+++ severe changes

in rats treated with Imuran only. However, a different type of lesion was observed in the glomeruli of animals killed on the 21st day.

Mesangial lesions and severe dilatation of the glomerular capillary loops, giving them an aneurysm-like appearance, were the most common alterations in a large number of the glomeruli (Figs. 28 and 29). The mesangial changes were characterized bij severe swelling of the mesangial matrix, degeneration of the mesangial cells, and a reduction in their number. The mesangial areas were extremely electron-lucent due to dissolution of the mesangial matrix and accumulation of a pale floccular material. Fine matrix fibrils and mesangial cells could not be found. However, some cytoplasmic remnants, probably derived from destroyed mesangial cells, were noticed. The accumulated material extended between the endothelial cells and the basement membrane, resulting in separation of the endothelium from the underlying membrane (Fig. 30). This mesangial damage was in all probability responsible for the enormous aneurysmal dilatation of the glomerular capillaries (Table 15).

Table 15. Ultrastructural glomerular changes in Imuran+Prednisolone-treated allografts

Type of change	Time after transplantation				
	3 days	5 days	7 days	9 days	21 days
Endothelial cells					
hypertrophy	○	+	++	++	+++
hyperplasia	○	+	++	+++	+++
cytoplasmic changes	○	+	++	+++	+++
endothelial cell detachment	○	+	++	++	++
Epithelial cells					
hypertropy	○	++	++	++	+++
fusion of foot processes	○	+	+	++	+++
cytoplasmic changes	○	+	+	+++	+++
Mesangium					
cellular changes	○	+	+	++	++
matrix changes	○	+	+	++	+++
Capillary lumina					
narrowed	○	○	○	++	++
obstructed	○	+	++	++	++
Capillary basement membrane					
wrinkling and collapse	+	++	++	++	++
subendothelial deposits	○	○	+	+	++
subepithelial deposits	○	○	+	+	++
thickening	○	○	+	++	++

○ no changes
+ mild changes
++ moderate changes
+++ severe changes

Discussion

The present study provided detailed information on the ultrastructural morphology and the evolution of the glomerular lesions occurring during acute renal rejection in the rat. According to these findings, the fine glomerular alterations in unmodified renal allografts develop in the following sequence:

1. establishment of an intimate contact between the lymphoid cells and the capillary endothelium or the capillary basement membrane;
2. progressive endothelial damage culminating in endothelial cell desquamation;
3. occlusion of the capillary loops by platelets, fibrin, and lymphoid cells;
4. later on, the following changes occur: alterations in the mesangial matrix and the mesangial cells;
5. subendothelial and subepithelial deposition of electron-dense material followed by changes of the glomerular basement membrane.

Occlusion of the capillary loops by platelets, fibrin and lymphoid cells was the most frequent lesion in glomeruli of the unmodified allografts. This was associated with endothelial cell separation and dilatation of the subendothelial space, followed later by the subendothelial accumulation of finely granular material which was less electron-dense than the glomerular basement membrane.

Electron-microscopical studies on the glomerular alterations produced by induced intravascular clotting in rabbits have provided evidence that intracapillary fibrin aggregation, either in the extrarenal blood vessels or within the glomeruli, leads to progressive glomerular obliteration and is an important factor in the production of the glomerular damage (VASSALLI, SIMON and POULLIER 1963). Fibrin formation in the vascular tree and in the glomeruli of the allotransplanted kidney are probably the result of the deposition of immune complexes on the vascular endothelium.

The results of the ultrastructural studies on the glomerular changes in human renal transplants carried out by PORTER and his associates (1967) suggest that the most advanced state of glomerular damage is characterized by the intracapillary accumulation of platelets and fibrin, culminating in their incorporation into the glomerular walls. This process is considered responsible for the membranous glomerulopathy observed in long-surviving renal allotransplants (LINDQUIST *et al.* 1968).

The occurrence of glomerular lesions in renal allotransplants is ascribed to two distinct immunological mechanisms (DIXON 1968; HAMBURGER 1974). The first of these mechanisms is considered to be the formation of antibodies to antigen of the glomerular basement membrane. The second mechanism is thought to be a reaction between antibodies and circulating transplantation antigens derived from the graft, resulting in the formation of antibody-antigen complexes and their subsequent deposition in the glomeruli of the transplanted kidney. When studied by the immunofluorescence technique, the glomerular lesion resulting from the first mechanism is characterized by linear basement membrane deposition, whereas the second form is characterized by granular deposits (PORTER 1968; PASTERNACK and LINDER 1971).

In the present study one of the most prominent glomerular ultrastructural changes was a progressive endothelial injury, associated with intimate contact of lymphoid cells with endothelium at the damaged sites, culminating in the separation of the endothelium from the glomerular basement membrane followed by subendothelial deposition of a fine granular substance. There is a great similarity between the observations presented here and those reported by GANG and KALANT (1970), who studied the electron-microscopical features of nephrotoxic serum nephritis in the rat.

Deposition of amorphous material, consisting of IgM and complement, along the endothelial surface of the glomerular basement membrane has also been described by PORTER *et al.* (1969), who used both immunofluorescent techniques and electron microscopy after immunoferritin labeling. It has been suggested by these authors that the subendothelial depositions of IgM and complement, resembling the deposits observed in nephrotoxic serum nephritis, were due to the reaction of circulating antibodies with antigens of the capillary basement membrane. On the other hand, the large subepithelial deposits containing IgG, IgM and complement were considered to be probably derived from circulating antigen-antibody complexes. The immune complexes deposited along the glomerular basement membrane then initiate the glomerular alterations in renal allografts.

According to LINDQUIST, GUTTMANN and MERRILL (1971), the immune complexes only remain briefly on the glomerular basement membrane. They disappear simultaneously with the appearance in the glomerular capillaries of polymorphonuclear and mononuclear cells, which probably phagocytize these immune complexes.

The present study has demonstrated the occurrence of intracapillary mononuclear cells with elongated cytoplasmic processes that seem to separate the endothelium from the basement membrane, establishing a close contact with the latter. Some of these cells might be macrophages probably also phagocytizing immune complexes residing on the basement membrane.

On the other hand, the intimate lymphoid cell-endothelial contact at sites of injury might be evidence for a direct, cell-mediated, immunological reaction leading to endothelial destruction. In fact, the inability of Imuran to prevent this lesion, whereas ALS (alone or in combination with Imuran) does suppress and delay it, lends strong support to the view that the glomerular damage is primarily due to a cell-mediated immunological reaction and not to deposition of antigen-antibody complexes. These complexes might be assigned a role in the other changes of the glomeruli, such as the deposition of electron-dense material, following the endothelial damage.

With the transmission electron microscopy employed in this study it is impossible to obtain conclusive evidence of the immunological nature of the glomerular alterations in rat allografted kidneys. These alterations may, however, represent the fine-structural expression of an immunologically induced glomerulopathy.

Ultrastructural characteristics of the mesangial alterations in acute renal allograft rejection have not been reported before. In the current study distinct mesangial changes were observed in the kidneys of animals

killed on the 21st day after transplantation and immunosuppressive treatment with Imuran or Imuran in combination with Prednisolone.

A progressive widening of the centrolobular region of the glomeruli associated with dissolution of the mesangial matrix and accumulation of electron-lucent material appear to be the most common mesangial changes. This phenomenon became increasingly pronounced with time and was associated with enormous dilatation of the glomerular capillaries due to loss of coherence between the endothelial cells and the underlying basement membrane.

The light-microscopical appearance of these changes resembles the glomerular alterations described for the Prednisolone-induced nephropathy of rabbits by OGILVIE *et al.* in 1965; however, the ultrastructural features of the lesions proved to differ considerably.

The ultrastructural mesangial lesions described here closely resemble those reported by KITAMURA *et al.* (1958) and KAWAJI and OYAMA (1960) in renal lesions of rabbits produced by intoxication with "Habu" snake venom. This condition was characterized by "lysis" of the mesangial cells and matrix, progressing to loss of glomerular architecture with transformation of the glomerulus into a blood-filled cavity encircled by basement membrane and epithelial cells.

Light-microscopical mesangial lesions consisting of a broadening of the mesangial area were also seen in human and rat renal allotransplants by PIELSTICKER, EDEL and THOENES (1974), who stressed the importance of the mesangial damage and subsequent proliferation of the mesangium for the pathogenesis of transplantation glomerulopathy.

CHAPTER VI

ULTRASTRUCTURAL CHARACTERISTICS OF THE TUBULAR DAMAGE

Introduction

Tubular changes are described in almost all of the studies dealing with the morphological features of renal transplants. In 1908, CARREL noticed alterations of the tubuli in allotransplanted kindeys of cats surviving 6–12 days, i.e., vacuolization of the cytoplasm around the nucleus and the appearance of hyaline casts in the dilated secretory tubuli. He explained these minor tubular changes on the basis of hydronephrosis, which was macroscopically evident in almost every one of the transplanted kidneys.

In 1934, WU and MANN reviewed in more detail the histological observations as well as the evolution of the morphological changes in their experiments with 24 canine renal autotransplants and allotransplants. In both types of transplants the tubuli appeared normal for the first day or two. Later, the tubular epithelium usually showed a varying degree of degeneration, swelling of the cytoplasm, and hyalinization of the cells. The lumina of the collecting tubules and of the thin limb of Henle's loop were dilated and contained proteinaceous material.

In 1953, nearly 20 years later, SIMONSON *et al.* performed an extensive study on renal homotransplantation in dogs, employing several experimental surgical procedures in an attempt to find a correlation between the morphological changes of the transplanted kidneys and the biological processes responsible for the destruction of the transplants. The pathology of the homotransplanted kidney was considered to be the result of immune processes. In their study the tubuli showed considerable damage. Many of the convoluted tubules in the sub-capsular areas of the cortex had undergone coagulation necrosis. Others contained granular casts and calcium deposits. Most of the tubuli were dilated and showed disappearance of tubular epithelium.

DEMPSTER (1954), in his work on kidney allotransplants in dogs, studied and described the histological features of a first and successive allotransplants. He showed that the earliest changes in tubular structures consisted of shedding of the epithelial brush border on the third day after transplantation. In later stages he found complete absence of the brush border, vacuolar degeneration of the tubular cells, generalized or focal tubule necrosis, and widespread cast formation. Dempster dis-

tinguished four types of anuria after auto- and allotransplantation of kidneys in dogs:

Type 1 Anuria characterized by failure of the transplanted kidney to secrete urine after establishment of the new circulation. This type of anuria, which occurs in both auto- and allotransplants, appears to be related not to immunological but rather to technical factors.

Type 2 An anuria following a period of poor secretion which manifests itself between 24 and 48 hours after transplantation.

Type 3 An abrupt onset of anuria in allotransplanted kidneys at a variable interval after transplantation and following a period of good secretion. The anuria is assumed to be the result of immunological mechanisms and is probably attributable to an irreversible spasm of the intrarenal branches of the renal artery.

Type 4 Anuria associated with severe glomerular damage and generalized arterial spasm and occurring within a few hours after allotransplantation of the "second" kidney from the same donor to the recipient of the "first kidney".

In addition to the purely morphological investigations of tubular damage, results of immunofluorescence and biochemical studies of allograft rejection in experimental animals have been published (Horowitz *et al.* 1965; Lindquist *et al.* 1969; Van Breda Vriesman 1968).

Lindquist and his associates found in rat renal allografts that the altered tubular cells, which were either vacuolated or necrotic, contained IgG, β_1-globulin, fibrinogen, and α_2-macroglobulin. These proteins were scattered diffusely throughout the cytoplasm of the tubular epithelium. The earliest changes in the tubuli were noticed 5 days after transplantation, and progressed toward complete tubular necrosis at 2 weeks after transplantation. The authors emphasized the immunological nature of the reactions responsible for the morphological and functional changes of the kidney in allograft rejection, as did Horowitz *et al.* in 1965.

Van Breda Vriesman (1968) also found histochemical evidence of tubular damage, i.e., a decrease in the activity of esterase and an increase in that of glucose-C-phosphate dehydrogenase. These changes were seen in the proximal segment of the tubular system and the degree reflected the amount of cellular infiltration around the tubuli.

Kountz *et al.* (1963) and Williams *et al.* (1964), who studied the ultrastructural features of the proximal and distal tubules in allotransplanted canine kidneys, concluded that the destruction of renal allotransplants is due to ischemia. The oliguric and anuric conditions in the transplanted kidney presumably result from progressive deterioration of the intertubular blood vessels. Fine-microscopical investigation of the tubular changes occurring in well-functioning and oliguric allotransplants, reveal-

ed varying amounts of shed cytoplasm and the appearance of a large number of small basal vacuoles. In addition, anuric allotransplants showed many large vacuoles associated with abundant cytoplasmic shedding. Complete necrosis of some tubuli was also frequently encountered.

Results

Untreated renal allografts

The tubular alterations found in allotransplanted kidneys in the present study were more or less similar to those recorded previously by many investigators (DEMPSTER 1953; DARMADY *et al.* 1955; LINDQUIST *et al.* 1968).

Moderate changes consisting of cellular swelling and cytoplasmic vacuolization appeared frequently in the proximal tubuli 3 days after transplantation. The tubuli were patent and no evidence of casts was found.

More prominent vacuolization of the tubular cells, affecting a larger number of cells and larger areas of the renal cortex, were observed in sections made 5 days after transplantation.

Striking tubular alterations, consisting of advanced degeneration and necrosis of individual cells, were essential features of the kidneys studied 7 days after transplantation. These changes involved both proximal and distal tubuli, and were seen most frequently in the renal cortex. Many of the tubuli were filled with structureless granular material or hyaline casts. Nevertheless, tubular structures showing very little microscopic alteration were present among the damaged ones.

By the 9th day after transplantation the kidneys showed a varying degree of tubular damage, ranging from early vacuolar degeneration to complete necrosis of the cells and extending over large areas of the renal parenchyma.

Various alterations in the ultrastructural morphology of the epithelial cells in the proximal and distal tubules were observed during the period between the 3rd and 9th day after transplantation. During this period the fine morphological changes involved almost all structures of the tubuli and progressed from day to day in quantity and quality.

The earliest changes were those seen in the tubuli 3 days after transplantation. At this time, more than half of the tubular cells displayed slight but clearly visible abnormalities, which were localized in the apical portion of the cytoplasmic membrane of the proximal tubular cells and the mitochondria.

The brush border was moderately flattened in most tubules and a

variable swelling of the microvilli was associated with a swelling of the apical part of the tubular cytoplasm and with the occurrence of intraluminal cellular protrusions. At this time, many broken microvilli were seen in the tubular lumen. Concomitantly with the changes in the brush border, the apical invaginations of the cytoplasmic membrane were either shortened or had completely disappeared. In most of the tubules observed on day 3 after transplantation, the lateral part of the cytoplasmic membranes of adjacent tubular cells was sharply outlined.

By this time, very few of the tubules contained normal mitochondria. Many were rounded and strikingly enlarged. The cristae were irregular and occasionally disintegrated. In the matrix, which showed an increased electron density, many dark granules were often encountered. These alterations seemed in some places to have gradually progressed to rupture and disappearance of the mitochondrial cristae (Fig. 32).

Three days after transplantation, both smooth-surfaced and rough-surface endoplasmic reticulum were easily recognized in the epithelial cells of the proximal tubules. The smooth-surfaced endoplasmic reticulum was composed of a well-developed complex of membranes with a tubular or vesicular appearance, dispersed in the cytoplasm. The rough endoplasmic reticulum was observed as irregularly shaped tubular structures with closely apposed ribosomes on their outer membrane.

The Golgi apparatus appeared as well-developed accumulations of vesicles and cisternae of varying size, situated close to the nucleus. Each cell contained two or more Golgi structures.

Various kinds of cytoplasmic inclusion bodies were observed in the proximal tubules. These bodies gradually increased in number over the period between the 3rd and the 9th day after transplantation. By the 3rd day the most frequently and easily recognized cytoplasmic inclusions were cytosomes (phagosomes). These round or oval structures were generally limited by a single membrane which occasionally showed irregular thickening. The lumen of the cytosomes contained either small dense granules or membranous material with a concentric arrangement (Table 16).

The fine architecture of the tubules 5 days after transplantation was more distorted than that observed on the 3rd day. A number of proximal tubules showed disruption of the apical part of the cytoplasmic membrane with intraluminal shedding. In these cases no brush border could be recognized (Fig. 33). Frequently, the tubules contained many cytoplasmic organelles and other remnants of disintegrated tubular cells (Fig. 34).

Some of the tubules showed a loss of intercellular connection, the cells being separated by a narrow zone along their whole length. At the same time, extracellular spaces of varying size occurred between the tubular

Table 16. Ultrastructural changes of the tubuli in untreated allografts

Type of change	Time after transplantation				
	3 days	5 days	7 days	9 days	21 days
Cell membrane					
brush border flattening	++	+++	+++	+++	
disruption of the apical part	○	+	++	+++	
loss of intercellular connection	○	+	++	+++	
intercellular compartments	○	○	+	+++	
basal cell membrane separation	○	○	+	++	
Cell cytoplasm					
mitochondrial swelling and degeneration	++	+++	+++	+++	
cytoplasmic inclusions	+	+	++	+++	
osmiophilic bodies	○	+	++	+++	
vacuolization	+	++	+++	+++	
Nucleus					
chromatin condensation	○	○	+	++	
pyknosis	○	○	○	+	
Lymphoid cell invasion	○	++	+++	+++	

○ no changes ++ moderate changes
+ mild changes +++ severe changes

cells (Fig. 35). This phenomenon was clearly visibe on day 5 and reached a maximum by day 7. It proved to be the result of separation of lateral cell membranes of neighbouring cells, commencing at the base of the cells and spreading towards the apex. At the level of the brush border the cell membrane separation abruptly stopped and the normal attachment of the tubular cells was retained over a narrow zone. At the base of the cell the spaces were seen to be bounded by the tubular basement membrane. These spaces were filled with a fine granular substance of very low electron density.

A highly significant phenomenon was the invasion of tubules by lymphoid cells with the same ultrastructural characteristics as those seen in the renal interstitium. Lymphoid cell invasion was rather frequently encountered in kidneys on the 5th and 7th days after transplantation. In most cases the lymphoid cells extended into the extracellular spaces between the tubular cells, as already described. The lymphoid cells in the extracellular spaces were entirely enclosed by cell membranes and an intact tubular basement membrane (Figs. 36 and 37). Ocasionally, these cells were also seen in the tubular lumen.

The interdigitating processes at the basal part of the tubular cells were shortened and swollen. No disruption of the overlying cell membranes was seen, however.

On the 7th day after transplantation, most of the mitochondria were extremely swollen; they contained small membrane fragments and showed almost entire destruction of the cristae. Many mitochandria had changed into oval or round bodies filled with a dense homogeneous material (Fig. 38).

The smooth- and the rough-surfaced endoplasmic reticulum had completely merged with the other vacuolar structures that filled the epithelium of the proximal tubules by the 7th day. At that time the number of membrane-associated as well as free ribosomes was greatly increased.

The vesicles and the cisternae of the individual Golgi complexes were no longer connected but retained the normal perinuclear position.

The cytosomes were moderately to strongly enlarged and their number was increased. They usually contained a rather dense finely granular matrix into which large very dark granules and fragmented filamentous material were incorporated.

The cytoplasm of most of the tubule cells contained osmiophylic bodies varying in size, shape, and density. The apical part of the cytoplasmic membrane of individual tubular cells was completely disrupted. The lumina were filled with disintegrated cellular structures (Fig. 39).

At 9 days after transplantation the cytoplasm of the most proximal tubules was filled with vacuoles of varying size and large dense bodies (Fig. 40). These vacuoles were generally empty, but a few contained either remnants of destroyed membranes or a small number of dense granules. Infrequently there were very large single vacuoles entirely replacing the cell cytoplasm (Fig. 41). Mitochondria were very difficult to distinguish from the numerous other dense cytoplasmic inclusions.

At this time, separation of the basal cell membrane from the tubular basement membrane was clearly evident in some places. The basement membrane, which had a normal thickness 3, 5 and 7 days after transplantation, now showed small focal accumulations of finely granular material along the side of the cell membrane. Infrequently, these accumulations of deposited material were very dense and large (Figs. 42 and 43).

The nuclei of the tubular cells had a normal appearance for a long time after transplantation of the kidney. Clearly visible ultrastructural alterations became evident, mainly on the 9th day, in tubules showing very severe degenerative changes. These nuclear changes started with condensation of the chromatin network in the region of the nuclear envelope, followed by a decrease in the size of the nucleolus. The nucleo-

lus showed decreased electron density. In very advanced deterioration of the tubular cells, the nuclei were pyknotic with an irregular infolded nuclear membrane, often resembling the large intra-cytoplasmic inclusions of unknown origin seen so often in transplanted kidneys on the 9th day (Figs. 44 and 45).

Treated renal allografts

The effects of the immunosuppressive treatment (Imuran, ALS, Imuran and ALS, Imuran and Prednisolone) on the tubular ultrastructural morphology in renal allotransplantation are summarized in Tables 17, 18, 19 and 20.

Group treated with Imuran

The electron-microscopical appearance of the tubular damage in this group was similar to that of untreated allografts (Table 17).

Table 17. Ultrastructural changes of the tubuli in Imuran-treated allografts

	Time after transplantation				
Type of change	3 days	5 days	7 days	9 days	21 days
Cell membrane					
brush border flattening	++	++	++	+++	+++
disruption of the apical part	○	+	++	++	+++
loss of intercellular connection	○	+	++	++	+++
intercellular compartments	○	○	+	++	+++
basal cell membrane separation	○	○	+	++	+++
Cell cytoplasm					
mitochondrial swelling and degeneration	++	++	++	+++	+++
cytoplasmic inclusions	+	+	++	+++	+++
osmiophilic bodies	○	+	++	++	+++
vacuolization	○	+	+	++	+++
Nucleus					
chromatin condensation	○	○	+	++	++
pyknosis	○	○	+	+	++
Lymphoid cell invasion	○	++	+++	+++	+++

○ no changes ++ moderate changes
+ mild changes +++ severe changes

Group treated with ALS

Most of the tubuli in these ALS-treated animals showed very slight to

moderate alterations in the fine morphology as compared with the untreated group and the group treated with Imuran (Table 18).

Table 18. Ultrastructural changes of the tubuli in ALS-treated allografts

Type of change	Time after transplantation				
	3 days	5 days	7 days	9 days	21 days
Cell membrane					
brush border flattening	+	+	++	++	++
disruption of the apical part	○	○	+	++	++
loss of intercellular connection	○	○	+	++	++
intercellular compartments	○	○	+	++	++
basal cell membrane separation	○	○	○	+	++
Cell cytoplasm					
mitochondrial swelling and degeneration	+	+	++	++	++
cytoplasmic inclusions	+	+	++	++	++
osmiophilic bodies	○	○	+	+	++
vacuolization	○	+	+	++	++
Nucleus					
chromatin condensation	○	○	○	+	+
pyknosis	○	○	○	○	+
Lymphoid cell invasion	○	○	+	+	+

○ no changes
+ mild changes
++ moderate changes
+++ severe changes

Group treated with ALS + Imuran

As indicated by Table 19, the tubuli of allografts in animals treated with a combination of ALS and Imuran showed ultrastructural changes more or less similar to those observed in the group treated with ALS alone (Table 19).

Group treated with Imuran + Prednisolone

The tubular changes in this group were the same in extent and ultrastructural morphology as in the animals treated with Imuran alone (Table 20).

Discussion

Acute renal allograft rejection is associated with clinical signs of oliguria or anuria and shows the morphological features of degenerative and

Table 19. Ultrastructural changes of the tubuli in ALS+Imuran-treated allografts

Type of change	Time after transplantation				
	3 days	5 days	7 days	9 days	21 days
Cell membrane					
brush border flattening	+	+	++	++	++
disruption of the apical part	○	○	+	++	++
loss of intercellular connection	○	○	+	++	++
intercellular compartments	○	○	+	+	++
basal cell membrane separation	○	○	○	+	++
Cell cytoplasm					
mitochondrial swelling and degeneration	+	+	++	++	++
cytoplasmic inclusions	+	+	++	++	++
osmiophilic bodies	○	○	+	+	++
vacuolization	○	+	+	++	++
Nucleus					
chromatin condensation	○	○	○	+	+
pyknosis	○	○	○	○	+
Lymphoid cell invasion	○	○	+	+	+

○ no changes ++ moderate changes
+ mild changes +++ severe changes

necrotic changes in the cells of the proximal and distal tubules. The cause of this tubular damage is still unknown.

DARMADY *et al.* (1955), who used microdissection techniques to study lesions in anuric canine renal allotransplants, considered certain "metabolic biochemical alterations" in renal tubule cells – which in turn initiate generalized intrarenal vascular spasm and cortical ischemia – to be responsible for the anuric state in renal allografts.

DEMPSTER (1954) held a similar view and suggested a number of non-immunological factors that might be involved in the etiology of the tubular damage and anuria.

The studies of KOUNTZ *et al.* (1963) indicate that destruction of renal allografts is brought about by progressive, immunologically induced damage of peritubular capillaries. The destruction of these blood vessels, together with massive interstitial cellular infiltration and edema, results in reduction of the total blood flow and inadequate tubular perfusion, which appear to be directly responsible for the degenerative changes of the tubules and the oliguria and terminal anuria. These investigators did not find any morphological evidence of destruction of the tubules by a

Table 20. Ultrastructural changes of the tubuli in Imuran+Prednisolone-treated allografts

Type of change	Time after transplantation				
	3 days	5 days	7 days	9 days	21 days
Cell membrane					
brush border flattening	++	++	++	+++	+++
disruption of the apical part	○	+	++	++	+++
loss of intercellular connection	○	+	++	++	+++
intercellular compartments	○	○	+	++	+++
basal cell membrane separation	○	○	+	++	+++
Cell cytoplasm					
mitochondrial swelling and degeneration	++	++	++	+++	+++
cytoplasmic inclusions	+	+	++	+++	+++
osmiophilic bodies	○	+	+	++	+++
vacuolization	○	+	+	++	+++
Nucleus					
chromatin condensation	○	○	○	++	++
pyknosis	○	○	○	+	++
Lymphoid cell invasion	○	++	+++	+++	+++

○ no changes
\+ mild changes
++ moderate changes
+++ severe changes

direct and local effect of immunocomponent cells, as was observed for the capillary blood vessels.

Subsequent reports in the literature have not provided further evidence indicating a destructive effect of lymphoid cells on the tubules, although tubular invasion by such cells has been described (JEANNET *et al.* 1970; LINDQUIST *et al.* 1971).

At present, it might be concluded that cell-mediated immune reactions do not contribute directly to the tubular damage in acute renal allograft rejection. In the present study an attempt was made to obtain electron-microscopic evidence supporting this view. The characteristics of the tubules observed in acute renal allograft rejection have basic similarities to the electron-microscopic changes reported in experimental ischemic renal infarction and acute tubular necrosis as well as the ultrastructural tubular alterations in shock kidney.

Ischemic renal infarction produced by ligation of a branch of the renal artery of the rat was accompanied by ultrastructural changes in the tubules, consisting of mitochondrial swelling, alterations in the brush

border and the cell membrane of the basal cells, vacuolization of the ground plasma, and pyknosis of the nucleus (TOTOVIĈ 1966).

Alterations in the fine morphology of the mitochondria in combination with cytoplasmic edema and an increased number of cytoplasmic membranous inclusions are considered early changes of the tubular epithelium in acute tubular necrosis (SUZUKI and MOSTOFI 1966).

According to DALGAARD and PEDERSON (1961), the most characteristic tubular change observed by electron-microscopy in acute anuria of the human kidney is a striking dilatation of the intercellular spaces of the proximal tubules.

The electron-microscopical changes observed in the tubules in acute renal allograft rejection in the present study, confirm the resemblance between the ultrastructural changes in acute renal allograft rejection and those reported for the early stages of ischemic renal infarction, acute tubular necrosis, and acute anuria. Mitochondrial changes, cytoplasmic vacuolization, dilatation of the spaces between the tubule cells, and invasion of tubules by lymphoid cells, are the most frequently observed ultrastructural alterations in all groups of allografts, differing only in degree and in rate of development.

The variable changes in the mitochondria, ranging from a slight enlargement to severe vesiculation or homogenization, have been described by earlier investigators (OLSEN 1967; LATTA *et al.* 1965; STONE *et al.* 1961) and attributed to "imbalance of the cellular metabolism" (DELGAARD and PEDERSEN 1961). It is highly probable that the mitochondrial changes occurring simultaneously with cytoplasmic swelling and vacuolization are not very, if at all, specific for acute renal allograft rejection. Mitochondrial lesions similar in type to those found in allografts have been described in many pathological conditions which do not have the same pathogenetic basis (LATTA *et al.* 1965). Of all cell elements, mitochondria are the most sensitive to injury in general.

The changes in the cell membrane, consisting of swelling of the microvilli followed by disruption of the apical part of the membrane and luminal cytoplasmic shedding, were the most constant early tubular alterations observed in the present study. As a rule, these changes were accompanied by a decrease in the electron density of the cellular cytoplasm. In 1962, LATTA and his associates saw similar alterations in cortical tubules during autolysis and suggested that they are probably due to enzymatic changes, not further defined by the authors, occurring in the damaged tubular cells and reflecting a disturbed resorption of the tubular fluid.

The loss of intercellular coherency and the appearance of large spaces between the tubular cells were the fine structural features we encountered most frequently in tubules on the 5th and 7th days after transplantation.

The exact nature of these changes is not clear. The possibility that they bear some relationship to disturbed fluid transport has been suggested by STONE *et al.* (1961), who observed a similar alteration in a study on the ultrastructure of the renal tubules during reaction to uranium injury.

In polyuria kidneys LATTA *et al.* (1962) demonstrated intercellular spaces identical to those in our study and ascribed these changes at the base of the cells to a failure of the cellular resorptive mechanism.

The present work throws little light on the pathogenesis and significance of the interstitial spaces in acute renal allograft rejection. A relationship between these changes and polyuria is suggested by the finding of DE BRUIN (1970) in a study on the function of kidney allografts in the rat. Using the same experimental model of renal allotransplantation as was used in the present study, he noticed an enormous rise in urine production within the first two weeks after transplantation.

The phenomenon of invasion of the tubules by lymphoid cells, as revealed by electron-microscopy, appears to be the only characteristic tubular change that might be specific for renal allograft rejection. In the present study the invading cells were identified as large lymphocytes. It is noteworthy that in spite of the massive lymphoid invasion of the tubuli, no morphological evidence was found of tubular cell destruction brought about directly by large lymphocytes and resembling the injury done to the capillary endothelium.

Ultrastructural analysis of the tubular changes occurring in renal allografts treated with various immunosuppressive agents, showed that the tubular alterations did not differ qualitatively from those seen in the untreated animals.

Imuran administered alone or in combination with Prednisolone had no influence on the frequency or severity of the tubular alterations described.

Treatment with ALS only or in combination with Imuran was significantly more effective, as demonstrated by the moderate severity and very slow evolution of the tubular changes.

The present study yielded no morphological evidence that could clarify the mechanism by which ALS reduces the tubular damage. The most likely explanation of this action is that ALS exerts a beneficial effect on the tubules indirectly by suppressing the destruction of the glomerular and peritubular capillaries, since the tubular alterations proved to be closely associated with, and are probably caused by, damage to the peritubular capillaries.

CHAPTER VII

SUMMARY AND CONCLUSIONS

The experimental study reported here concerned the electron-microscopical characteristics of the renal changes occurring during acute rejection of kidney allotransplants in the rat, as well as the ultrastructural features of allograft rejection modified by different immunosuppressive regimens and their significance in relation to the nature and mechanisms of the rejection process.

An experimental model of renal allogeneic transplantation between two highly inbred strains of rats BN/Bi and Wag/Rij, was employed. A microvascular technique for orthothopic renal transplantation in the rat introduced by FISHER and LEE (1965) and modified by TINBERGEN (1971) was applied. Modification of the immune response of the host was achieved by means of immunosuppressive treatment with Imuran, Prednisolone, and ALS (rabbit anti-rat thymocyte serum) administered alone or in combination.

The observations clearly show the wide range of ultrastructural changes occurring in the grafted organ, involving the parenchyma and the vascular tree in untreated recipients with increasing frequency and intensity after transplantation. The results also elucidate various aspects of the modification of the fine morphological features of rejection under the influence of various immunosuppressive regimes.

The rejection process is initiated by alterations in the intrarenal vasculature, including endothelial damage, thrombosis, and rupture of the peritubular capillaries, which, together with invasion of the renal interstitium by lymphoid cells, represent the earliest and the most constant ultrastructural changes occurring in acute allograft rejection.

Ultrastructural analysis of the interstitial cellular infiltration occurring in unmodified renal allografts revealed two predominating types of cells which appear in the renal interstitium as early as the 3rd day after transplantation: smooth-surfaced and rough-surfaced mononuclear lymphoid cells. The smooth-surfaced cells showed a scanty cytoplasm filled with many ribosomes, whereas the cytoplasm of the rough-surfaced cells had an abundance of endoplasmic reticulum. On the basis of these findings, it is postulated that the smooth-surfaced cells, which were also seen to invade the peritubular capillaries and to establish a close cyto-

plasmic association with the capillary endothelium, belong to the population of T-lymphocytes. The rough-surfaced lymphoid cells probably belong to the B-lymphocyte population.

The submicroscopical observations on the vascular lesions occurring during acute renal allograft rejection showed that the primary site of the blood vessel damage is the vascular endothelium. The damage to the blood vessels seems to be directly caused by an intimate interaction between the intravascular smooth-surfaced lymphoid cells and the endothelium, which underlines the importance of the peritubular capillary damage with respect to the rejection of a renal allograft. It may be assumed that these findings represent the fine-structural manifestations of the vascular damage and that they are primarily due to a direct cytotoxic effect of immunologically competent lymphoid cells (T-lymphoid cells) on the vascular endothelium. On the other hand, platelet aggregations and thrombosis are in all likelihood related to the previous damage inflicted on the endothelial cells, to which the formation of immune complexes on the endothelial cell surface might contribute. Alterations in the ultrastructural morphology occur much later in the larger renal vessels than in the peritubular and glomerular capillaries. These changes are characterized by degenerative alterations in the endothelial and smooth-muscle cells, which progress to necrosis and accumulation of a fibrinoid substance in the vascular wall.

The glomerular pathology is a prominent feature of acute renal allograft rejection. The main ultrastructural characteristics of the glomerular changes, which develop very rapidly, are occlusion of the glomerular capillary loops associated with alterations of the mesangium and fibrinoid deposits along the glomerular basement membrane resulting in narrowing of the capillary lumina.

Prior to these alterations a close relationship and cytoplasmic continuity are established between lymphoid cells and the glomerular capillary endothelium, followed by the disintegration of endothelial cells. The electron-microscopical pattern and the sequence in which the earliest glomerular lesions occur are similar to those observed in the peritubular capillaries. The more advanced glomerular alterations resemble, to a certain extent, the changes seen in the glomeruli in nephrotoxic nephritis (GANG and KALANT 1970). The analogy between these changes suggests the participation of immune mechanisms in the genesis of the glomerular lesion. In this respect it is postulated that the glomerular capillary endothelium, like the endothelium of the peritubular blood vessels, is the initial site of the immunological attack, and is at least partially mediated

by a direct effect of (T-)lymphoid cells. The additional glomerular alterations, which occur primarily along the glomerular basement membrane and in the mesangial area, presumably develop as the result of the deposition of circulating immune complexes, following the endothelial damage which is the primary cause of the glomerular changes.

The ultrastructural features of the tubular damage include mitochondrial swelling, cytoplasmic vacuolization, rupture of the apical part of the cellular membrane, loss of coherence between the tubular cells, and the appearance of intercellular spaces. These changes in the tubuli develop and progress rather slowly. They are considered to be non-specific changes accompanying renal allograft rejection, since similar change have been described in a number of pathological conditions of the kidneys, and are mainly related to ischemia resulting in acute tubular necrosis. One feature frequently seen in the electron micrographs of renal allograft rejection material is invasion of the tubuli by lymphoid cells. This kind of alteration cannot be interpreted as an indication for a possible role of a cell-mediated immune reaction in the pathogenesis of the tubular damage, because there is no evidence of an intimate contact between the lymphoid cells and the tubular cells or of dissolution of the tubular cell membrane, such as is observed in the interstitial capillaries and the glomeruli. It is therefore postulated that tubular damage in acute allograft rejection is secondary to progressive renal ischemia resulting from injury of the intrarenal blood vessels.

Despite the wealth of detail provided by the electron microscope, the nature of the mechanisms underlying acute allograft rejection cannot be definitely established from the present results. In the absence of relevant immunohistochemical information, the evidence provided by these observations can at present only support the view that immunological mechanisms, both cell-mediated and antibody-mediated, are involved in the pathogenesis of the structural lesions in acute renal allograft rejection.

Each of the immunosuppressive agents employed in the present study, i.e., anti-lymphocyte serum, Imuran, and Prednisolone, influenced the morphological features of the ultrastructural lesion in the allotransplanted kidneys in a different way.

The administration of anti-lymphocyte serum strongly depressed and delayed the cellular accumulation in the renal interstitium and prevented the peritubular capillary damage almost completely. Anti-lymphocyte serum therapy reduced and delayed the occurrence of ultrastructural

glomerular lesions and also strongly diminished both tubular changes and arteriolar alterations.

Imuran was efficient in modifying the pathological changes in the larger blood vessels and some of those in the tubuli, but was markedly inferior to anti-lymphocyte serum in the suppression of the peritubular capillary damage and glomerular injury. Imuran had no effect on the density of interstitial accumulation of mononuclear cells.

Prednisolone administered in combination with Imuran gave only a very slight retardation of the progression of the glomerular changes and the peritubular capillary damage, but was more effective in suppressing the arteriolar and tubular changes.

Treatment with anti-lymphocyte serum in combination with Imuran, which almost completely prevented parenchymal and vascular alterations in the allografted kidney, appears to be the most efficient immunosuppressive regime.

The relevance of these findings for clinical kidney transplantation is evident. Imuran or Imuran and Prednisolone treatment, widely used at the moment, may effectively prevent acute rejection by antibody-mediated damage to large vessels leading to massive infarction and acute renal failure. The persistence of glomerular damage and damage to the intertubular vessels may, however, lead to a more slowly progressive (chronic) deterioration of the transplant, which is less evident clinically, because of the large functional reserves of this organ. Apart from histocompatibility matching, at present only the combination of ALS and Imuran, initiated early and preferably prior to transplantation, may succeed in suppressing these latter components of graft rejection.

It is also evident that renal biopsy in the early phase after transplantation, might provide the only reliable indication of the effectiveness of the immunosuppressive therapy and histocompatibility matching in this respect.

SAMENVATTING

In dit proefschrift worden de resultaten besproken van een onderzoek over de ultrastructurele veranderingen die in rattenierweefsel ontstaan gedurende de acute afstoting van allotransplantaten. Voorts worden de electronen-microscopische beelden beschreven van de allograft-afstotingsreactie bij de rat onder verschillende immunosuppressieve behandelingsschema's. Hierbij wordt ingegaan op de aard en het mechanisme van het afstotingsproces.

In de experimenten werd gebruik gemaakt van nierallotransplantatie tussen twee sterk ingeteelde rattestammen (BN/Bi en Wag/Rij). Voor orthothope transplantatie van rattenieren werd de microvasculaire techniek volgens FISHER and LEE (1965), gemodificeerd door TINBERGEN (1971), toegepast. De immuniteitsreacties van de gastheer werden beinvloed door Imuran, Prednisolon en ALS (konijn-anti-rat thymocytenserum), die alleen of in combinatie werden toegediend.

Het onderzoek toont duidelijk aan dat er een grote variatie in de ultrastructurele veranderingen in de getransplanteerde organen bestaat. Zowel parenchymateuze als vasculaire veranderingen nemen in onbehandelde ontvangers met de tijd na de transplantatie toe. Verschillende aspecten van de ultrastructurele beelden van afstoting onder invloed van verschillende immunosuppressieve behandelingsschema's worden eveneens verduidelijkt.

Het afstotingsproces begint met veranderingen in de intra-renale vaten. Zij bestaan uit endotheelbeschadiging, thrombose en rupturen van de peritubulaire capillairen. Daarnaast wordt een infiltratie van het renale interstitium met lymfoïde cellen gezien. De vaatveranderingen en de celinfiltratie zijn de vroegste en meest constante ultrastructurele veranderingen bij de acute allograft-afstoting.

Bij ultrastructureel onderzoek van het interstitiële cellulaire infiltraat in onbehandelde niertransplantaten worden twee typen van cellen waargenomen, n.l. mononucleaire lymfoide cellen met glad celoppervlak en mononucleaire cellen met ruw celoppervlak. Zij verschijnen voor het eerst op de derde dag in het nier-interstitium. De cellen met glad celoppervlak bezitten zeer weinig cytoplasma, dat gevuld is met ribosomen. Het cytoplasma van de lymfoide cellen met ruw celoppervlak daarentegen heeft een sterk ontwikkeld endoplasmatisch reticulum. Op grond van deze bevindingen wordt verondersteld dat de cellen met een glad oppervlak tot de populatie van T-lymfocyten behoren. Deze cellen infiltreren de wanden van de peritubulaire capillairen en treden in nauw contact met het endotheel van glomerulus en peritubulaire capillairen.

De lymfoide cellen met ruw oppervlak vertegenwoordigen waarschijnlijk B-lymfocyten.

De submicroscopische waarnemingen over de vasculaire veranderingen tijdens de acute afstotingsreactie wijzen erop, dat de eerste afwijkingen in de bloedvaten plaatsvinden en wel in het endotheel. De beschadiging van deze bloedvaten blijkt te worden veroorzaakt door een interactie tussen de intravasculair liggende lymfoide cellen met glad celoppervlak en de endotheel-cellen. Dit onderstreept het belang van de peritubulaire capillaire beschadiging voor de afstoting van niertransplantaten. Verondersteld wordt dat de vasculaire beschadiging door een direct cytotoxisch effect van immunologisch competente lymfoide cellen (T-lymfoide cellen) op het vasculaire endotheel wordt veroorzaakt. Thrombocyten-aggregaties en thrombose zijn naar alle waarschijnlijkheid betrokken bij deze endotheliale beschadiging. De aanwezigheid van immuuncomplexen op de endotheliale cellen kan hiertoe bedragen. In de grote niervaten verschijnen ultrastructurele veranderingen pas veel later dan in de peritubulaire en glomerulaire capillairen. Deze veranderingen, die tenslotte leiden tot necrose en ophoping van fibrinoid materiaal in de vaatwand, worden gekarakteriseerd door degeneratie van het endotheel en de gladde spiercellen.

De glomerulaire pathologie is eveneens een in het oog springende karakteristiek van de acute nierafstotingsreactie. De belangrijkste ultrastructurele veranderingen van de glomeruli, die reeds vroeg waarneembaar zijn, bestaan in afsluiting van de glomerulaire lissen, veranderingen van het mesangium en neerslag van fibrinoid langs de glomerulaire basaalmembraan. Aan deze veranderingen gaan een zeer nauw contact en cytoplasmatische continuiteit tussen lymfoide cellen en het endotheel van de glomerulus-capillairen vooraf. Hierop volgt de desintegratie van de endotheelcellen. Het electronen-microscopische beeld en de volgorde waarin de vroegste glomerulaire laesies worden gezien zijn gelijk aan die welke worden waargenomen in de peritubulaire capillairen. De glomerulaire veranderingen in een later stadium gelijken in zeker opzicht op de veranderingen welke worden gezien in de glomeruli bij nefrotoxische nefritis (GANG en KALANT 1970). De analogie tussen deze veranderingen doet een immunologische genese van deze glomerulaire afwijkingen vermoeden. Hierbij wordt verondersteld dat het endotheel van de glomerulus, evenals het endotheel van de peritubulaire bloedvaten, het primaire aangrijpingspunt vormt voor een directe invloed van T-lymfoide cellen. De glomerulaire veranderingen, die in eerste instantie plaatsvinden langs de glomerulaire basaalmembraan en in het mesangium zijn waarschijnlijk een gevolg van uit de circulatie neergeslagen immuuncomplexen die tot beschadiging van het endotheel leiden.

Ultrastructureel bestaan de tubulaire laesies in mitochondriale zwelling, cytoplasmatische vacuolisatie, desintegratie van de celmembraan van het apicale deel van de tubuluscellen, verlies van samenhang tussen de tubulaire cellen onderling en het ontstaan van intercellulaire ruimten. Deze veranderingen in de tubuli ontstaan echter langzaam. Zij worden beschouwd als niet-specifieke veranderingen welke gepaard gaan met de afstotingsreactie; dergelijke veranderingen worden immers eveneens beschreven bij een aantal nierziekten waar zij een gevolg zijn van ischemie en aanleiding geven tot tubulaire necrose. Een kenmerk dat vaak bij de afstoting van een nierallotransplantaat wordt gezien is de infiltratie van tubuli met lymfoide cellen. Deze verandering kan echter niet worden beschouwd als een aanwijzing voor een „cell-mediated" immuunreactie in de pathogenese van de tubulaire beschadiging en wel omdat een duidelijk contact tussen de lymfoide cellen en het tubulus-epitheel, zoals bij de interstitiële capillairen en de glomeruli, niet wordt gezien. Verondersteld wordt derhalve, dat de tubulaire laesies in de acute allotransplantatie-afstotingsreactie secundair zijn aan de progressieve renale ischemie, die een gevolg is van intrarenale vaatveranderingen.
De veranderingen die bij het ultrastructurele onderzoek naar voren komen geven nog niet een duidelijke verklaring voor het mechanisme dat aan de afstotingsreactie ten grondslag ligt. Door het ontbreken van relevante immunohistochemische informatie kan dit onderzoek slechts een bijdrage leveren tot de opvatting dat immunologische mechanismen, zowel van het celgebonden als het antilichaam-gebonden type, van betekenis zijn bij de afstoting van niertransplantaten.

De immunosuppressieve stoffen die bij dit onderzoek werden gebruikt, n.l. anti-lymfocyten serum, Imuran en Prednisolon, beïnvloeden de ultrastructurele laesies in de nierallotransplantaten op een verschillende wijze.

De toediening van *anti-lymfocyten serum* heeft een sterke vermindering en uitstel van de cel-infiltratie in het interstitium tot gevolg en voorkomt de peritubulaire vaatbeschadiging vrijwel geheel. Anti-lymfocyten serum veroorzaakt daarnaast een sterke vermindering en uitstel van de ultrastructurele glomerulaire afwijkingen en vermindert eveneens zowel de tubulaire als de arteriolaire laesies.

Imuran veroorzaakt een duidelijke vermindering van de veranderingen in de grotere bloedvaten en de tubuli; het onderdrukken van de peritubulaire capillaire en glomerulaire beschadiging is echter duidelijk minder dan bij toediening van anti-lymfocyten serum. Imuran heeft geen invloed op de dichtheid van het interstitiële mononucleaire infiltraat.

Prednisolon geeft in combinatie met Imuran slechts een geringe vermindering van de snelheid waarmee de glomerulaire veranderingen en de

peritubulaire capillaire beschadigingen plaats vinden. De invloed op het onderdrukken van de arteriolaire en tubulaire laesies is echter veel duidelijker. Behandeling met ALS in combinatie met Imuran heeft tot gevolg dat parenchymateuze en vasculaire veranderingen in de niertransplantaten vrijwel niet worden gezien; deze immunosuppressieve therapie blijkt de meest efficiënte te zijn.

Het belang van deze resultaten voor de niertransplantatie is duidelijk. De therapie met Imuran of Imuran in combinatie met Prednisolon, die op het ogenblik het meest wordt gebruikt, kan de acute afstoting tengevolge van beschadiging van grote vaten door antilichaam-gebonden immuunreacties, waarvan massieve infarcering en acute nierinsufficiëntie het gevolg zijn, voorkomen. De blijvende glomerulaire beschadiging en de laesies van de intertubulaire vaten kunnen echter leiden tot een langzaam progressieve desintegratie van het transplantaat. De grote functionele reserve van het nierweefsel maakt dat deze afwijkingen klinisch minder duidelijk zijn. Naast de histocompabiliteitsmatching kan op dit moment alleen de combinatie-therapie met ALS en Imuran, die vroeg en liefst voor de transplantatie moet worden aangevangen, de nieraf-stotingsreactie onderdrukken. Het is eveneens duidelijk dat een nierbiopsie in een vroege fase na de transplantatie uiterst waardevol is voor een beoordeling van de effectiviteit van het histocompabiliteitsonderzoek en de immunosuppressieve therapie.

CURRICULUM VITAE

Vojislav Dusan Vuzevski werd 11 december 1932 te Kladovo, Yugoslavia, geboren.

Hij doorliep het stedelijk gymnasium van Skopje, Yugoslavia en studeerde aansluitend geneeskunde aan de Universiteit van Skopje.

In juni 1960 werd bij bevorderd tot arts.

Na zijn militaire dienstplicht te hebben vervuld, was hij van october 1962 tot juni 1966 als wetenschappelijk medewerker verbonden aan de afdeling Algemene Pathologie van de Medische Faculteit te Skopje (Hoofd: Prof. Dr. D. L. Milelić). Gedurende het academisch jaar 1966/1967 was hij als researchfellow, op een grant van het USA Government, verbonden aan de Medische Faculteit te Pittsburg, Pennsylvania, Department of Pathology (Hoofd: Prof. Dr. Edwin R. Fisher).

Na zijn terugkeer naar Yugoslavia werd aan de Universiteit van Skopje in 1967 met goed gevolg het specialisten-examen Pathologie afgelegd.

In 1968, tijdens een bezoek aan de afdeling Pathologie van de Medische Faculteit te Rotterdam, solliciteerde hij naar een plaats op die afdeling (Hoofd: Prof. Dr. H. E. Schornagel).

Sinds februari 1969 is hij als wetenschappelijk hoofdmedewerker verbonden aan de afdeling Klinische Pathologie (Hoofd: Prof. Dr. G. Wielenga) van de Erasmus Universiteit te Rotterdam. In 1971 werd V. D. Vuzevski ingeschreven als patholoog-anatoom in het specialistenregister van de Koninklijke Nederlandsche Maatschappij ter Bevordering der Geneeskunst.

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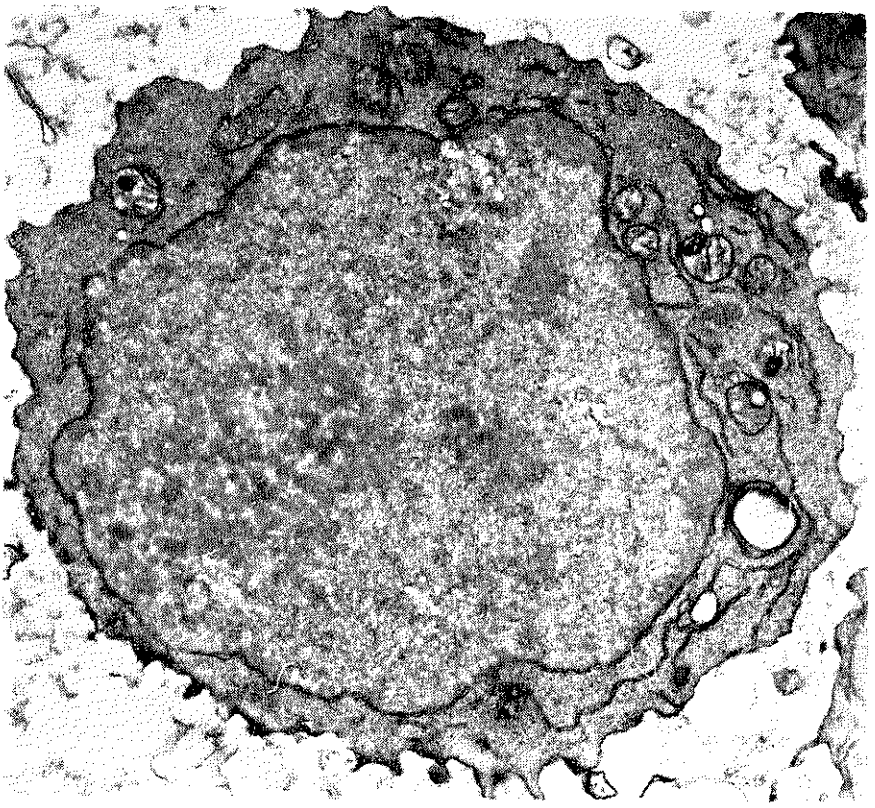
ELECTRONMICROGRAPHS AND LEGENDS

Abbreviations used in the figures

BC	Bowman's capsule
BM	Basement membrane
Cap	Capillary loop
Ep	Epithelial cell
End	Endothelial cell
Mes	Mesangium
US	Urinary space
Int	Interstitium
Lu	Lumen of vessel
D	Deposit
F	Fibrin
Lc	Lymphocyte
PMN	Polymorphonuclear leucocyte
Er	Red blood cell
Pl	Platelets
M	Macrophage
n	nucleus
nu	nucleolus
g	Golgy apparatus
r	ribosomes
er	endoplasmic reticulum
v	vacuoles
m	mitochondrion

Fig. 1. Large lymphocyte. The cell has a rather smooth surface. In the cytoplasm a scanty amount of endoplasmic reticulum and a few mitochondria.
Magnification × 16.500.

Fig. 2. Immature plasma cell. The cell surface is very rough due to numerous cytoplasmic projections. The cytoplasm shows many endoplasmic reticulum cyternae, mitochondria, and Golgi structures.
Magnification × 14.000.



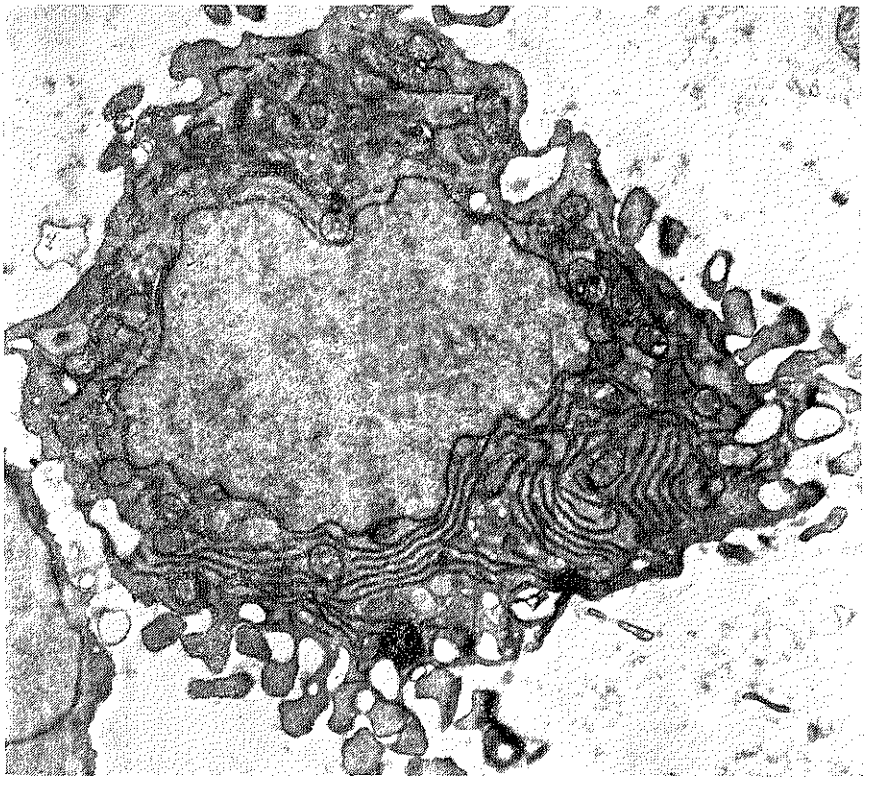


Fig. 3. Mature plasma cell. Note abundance of rough endoplasmic reticulum in the cytoplasm. Magnification × 18.000.

Fig. 4. Macrophage with ingested foreign cytoplasmic material of unknown cellular origin. Magnification × 10.500.

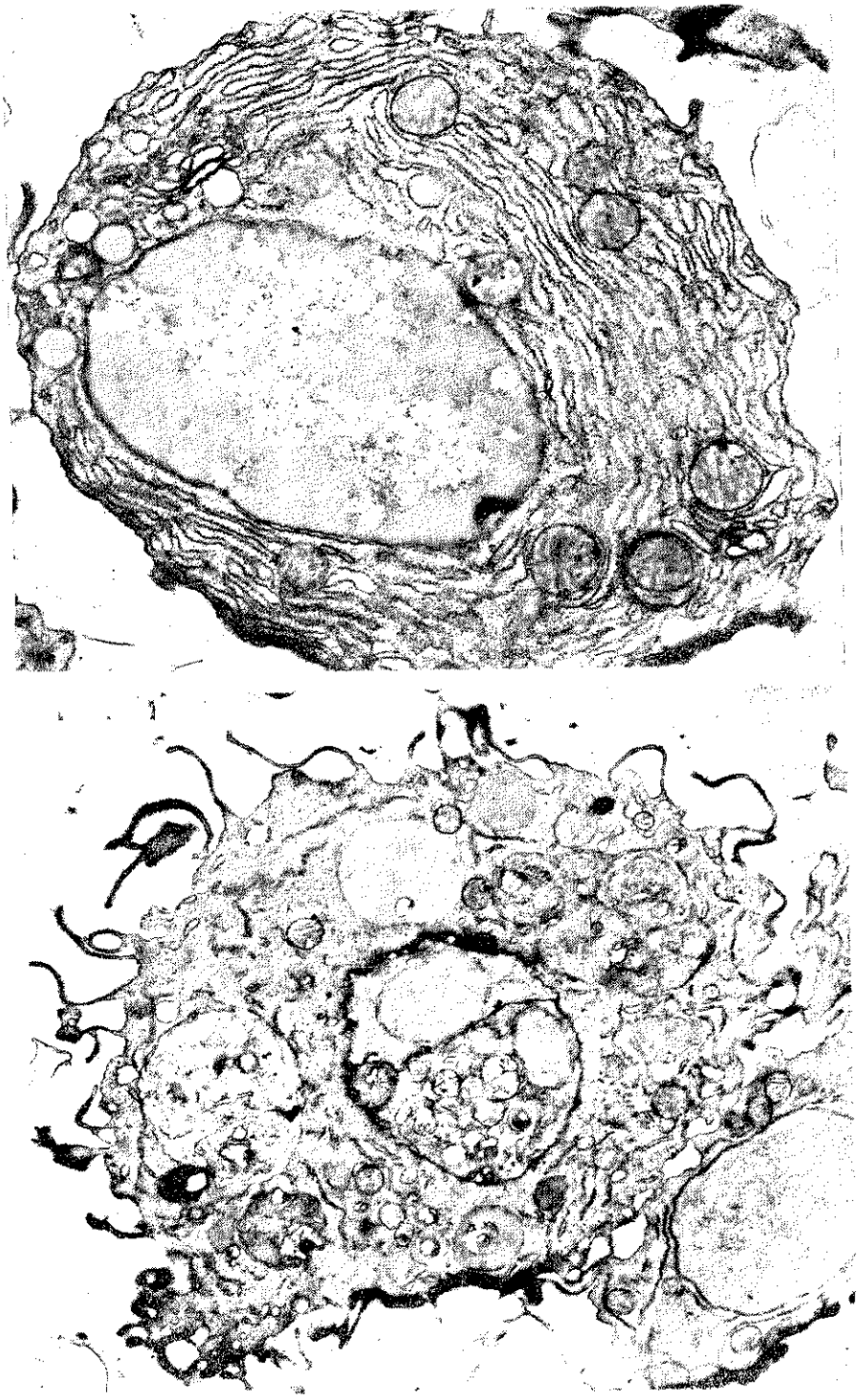
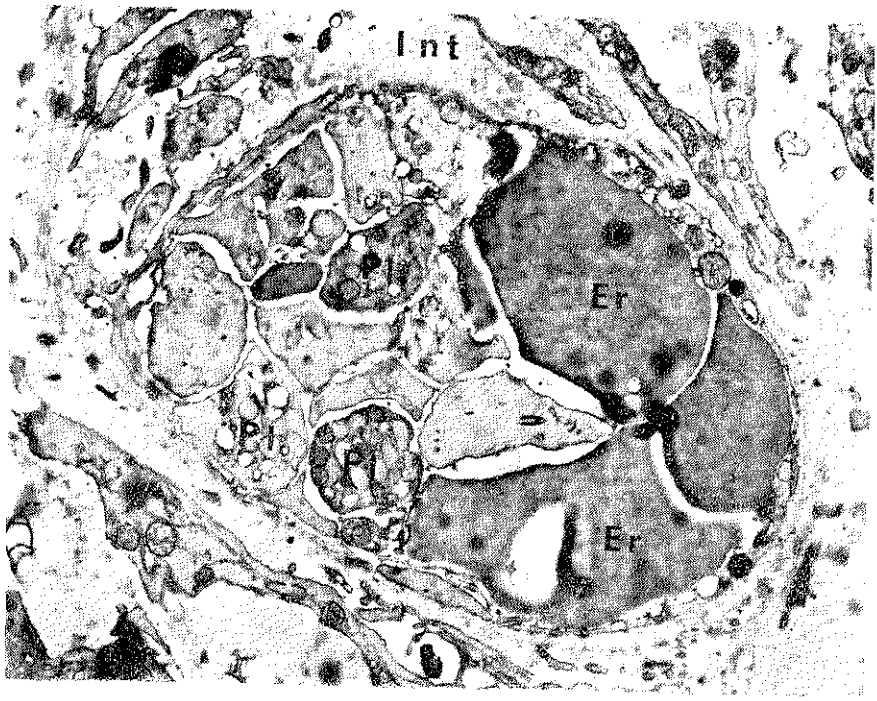


Fig. 5. Renal allotransplant on day 3. Cross-section of a capillary blood vessel obstructed by platelets, cytoplasmic projections of unidentified origin, and erythrocytes.
Magnification × 8.650.

Fig. 6. Renal allotransplant on day 3. Intertubular capillary showing rupture of the wall and release of the luminal contents composed of platelets and erythrocytes.
Magnification × 9.200.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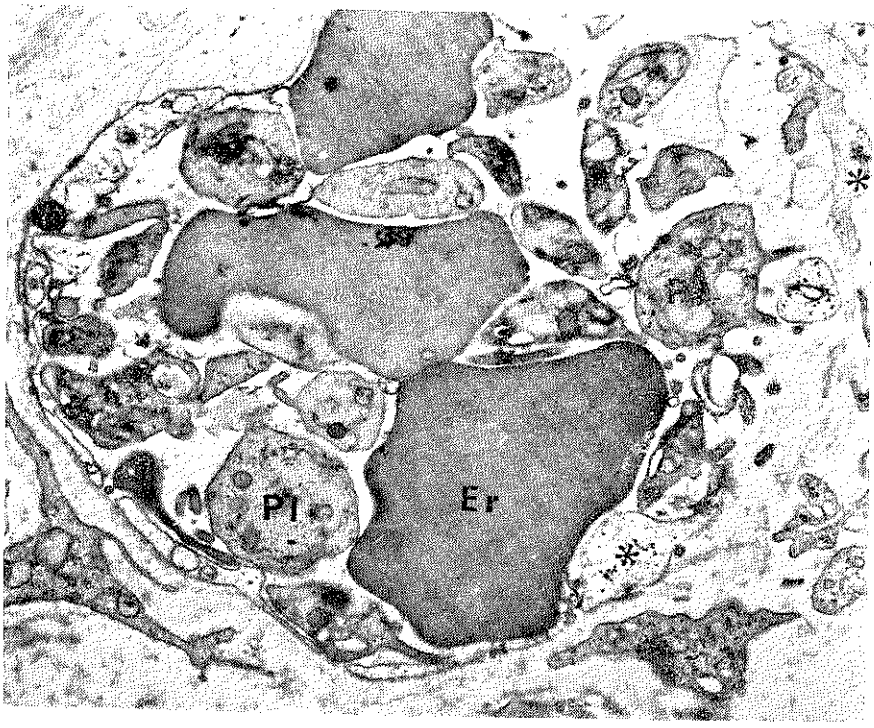
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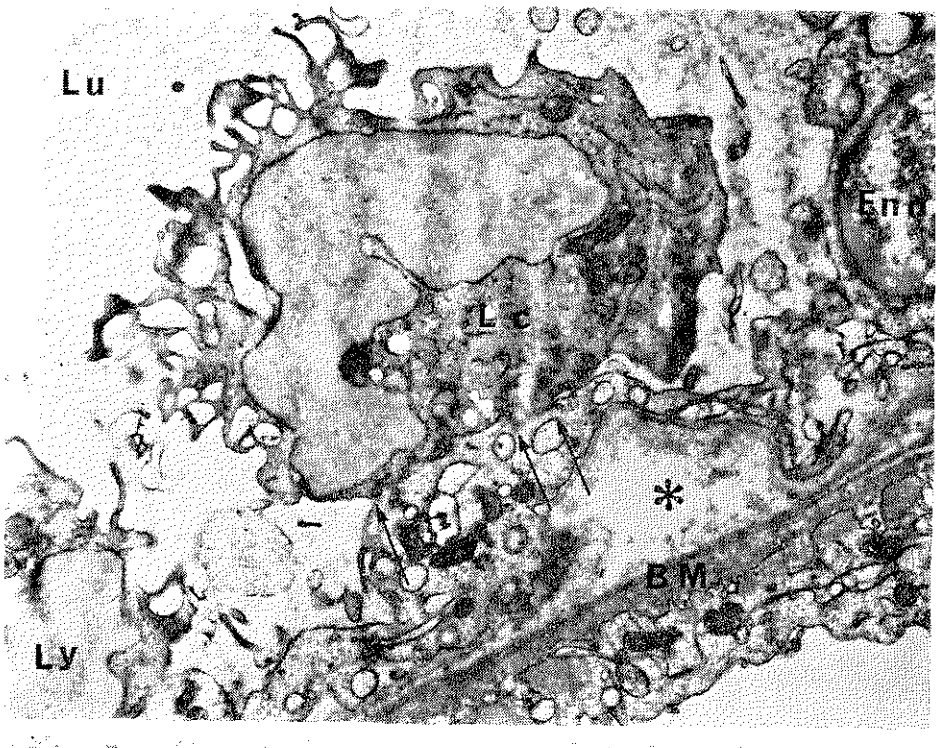
Fig. 7. Renal allotransplant on day 3, showing close apposition between the endothelial layer and a lymphoid cell (arrows). Note dissolution of the adjacent membranes and establishment of cytoplasmic continuity.
Magnification × 12.800.

Fig. 8. Renal allotransplant on day 3, showing severe swelling of an endothelial cell. The cytoplasm is rich in small vacuoles. There is loss of coherence between the endothelial cell and the basement membrane. (*)
Magnification × 14.750.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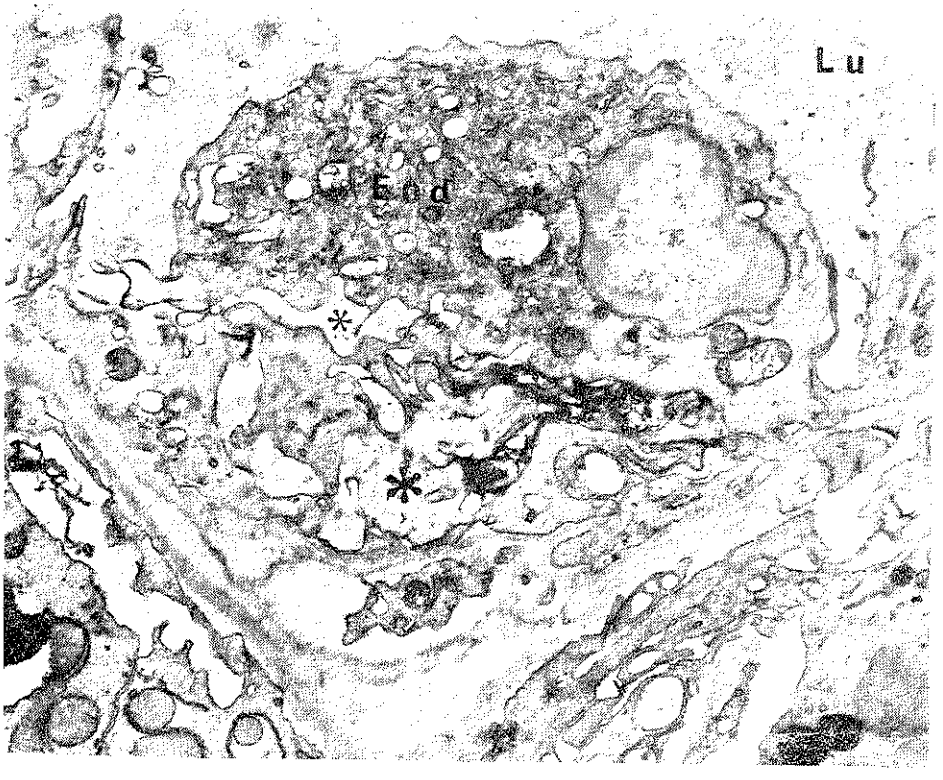
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Fig. 9. Renal allotransplant on day 5. A small interstitial arteriole shows strong contraction and vacuolization of the endothelium.
Magnification × 5.200.

Fig. 10. Renal allotransplant on day 9, showing severe vacuolization of the endothelial cells, and dense inclusion bodies in the media (arrows).
Magnification × 7.350.

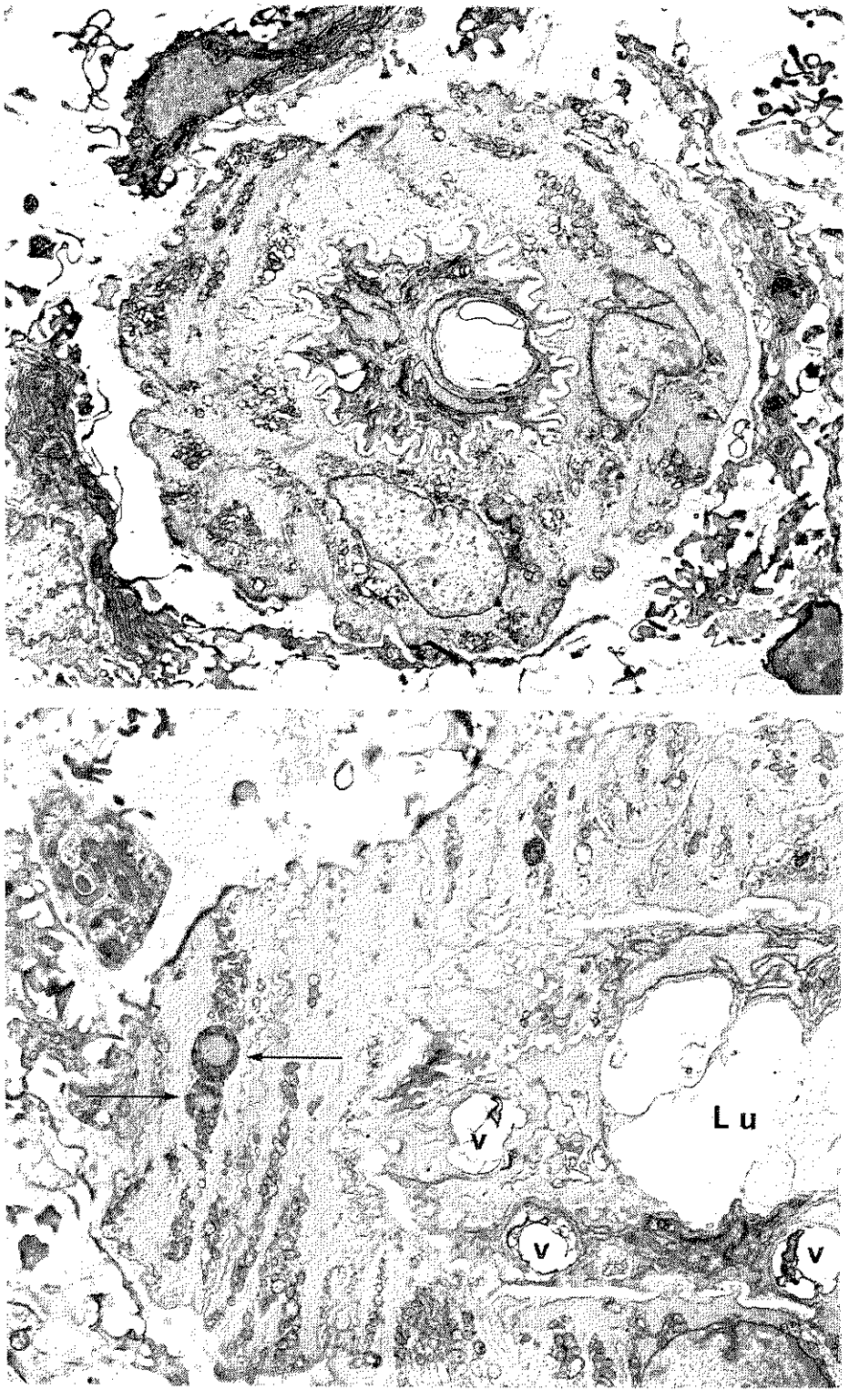
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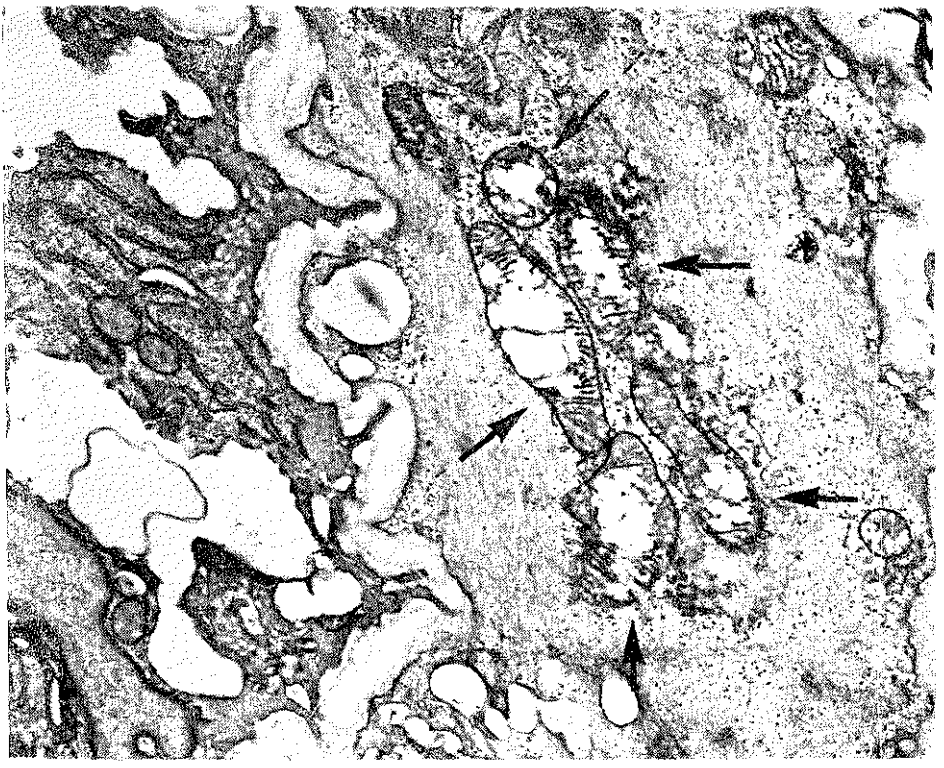
Fig. 11. Renal allograft on day 9, from animal treated with IMURAN. There is marked swelling and disintegration of the mitochondria in a medial layer cell of an artery (arrows).
Magnification × 24.000.

Fig. 12. Renal allotransplant on day 5. The endothelial layer of one arteriole shows degenerative alteration seen as vacuoles of different size.
Magnification × 12.250.



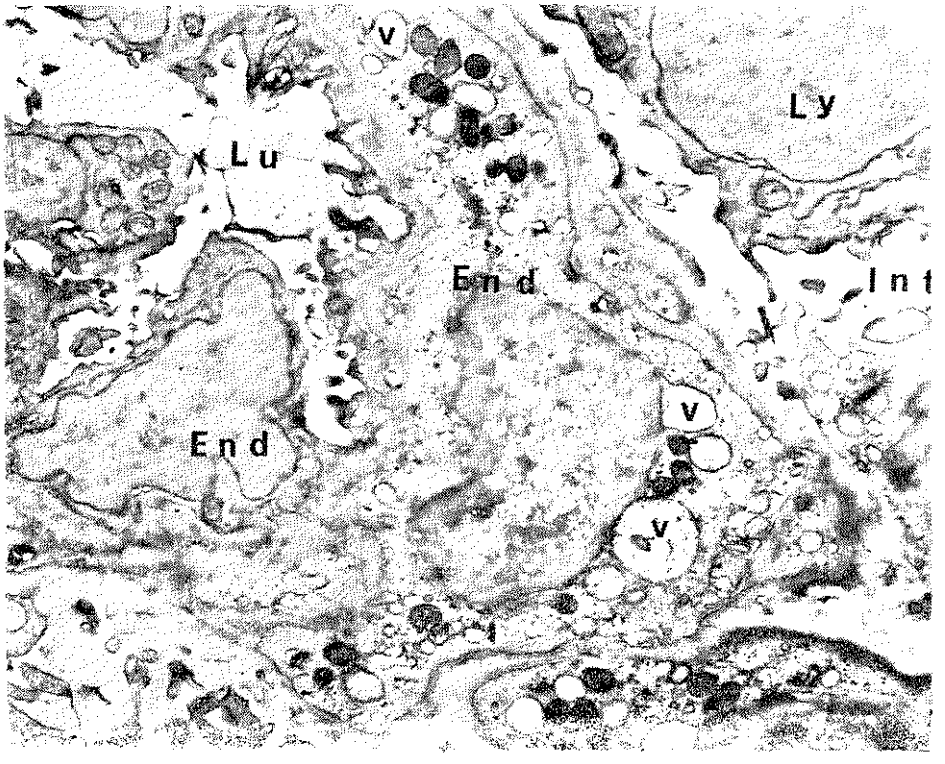

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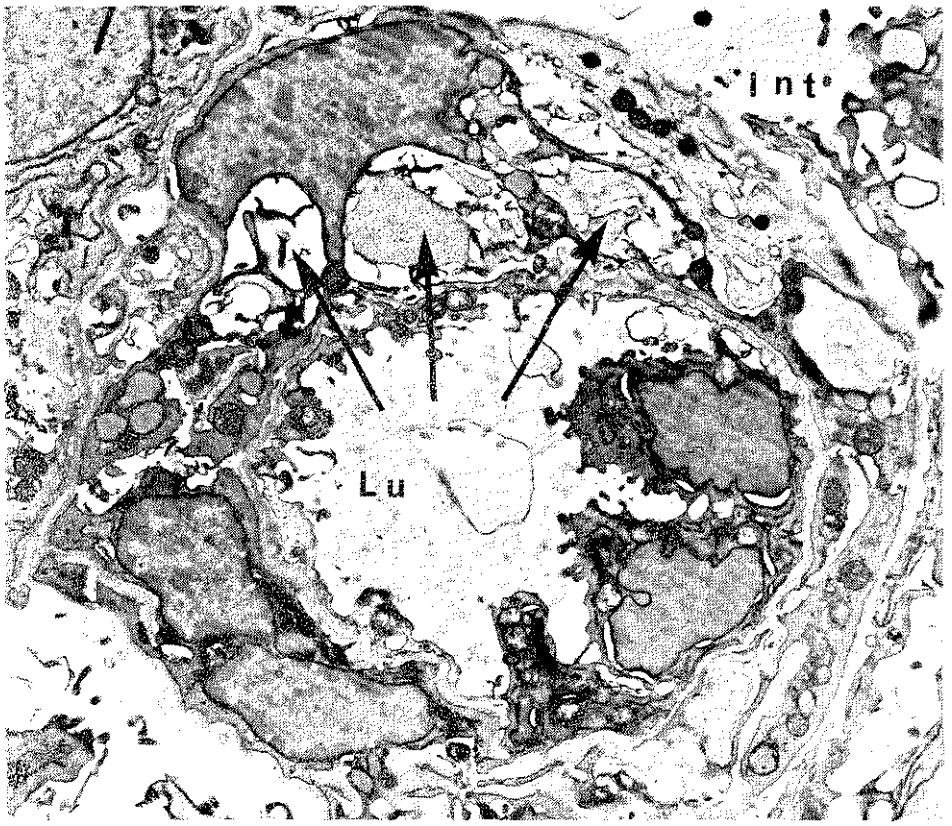
Fig. 13. Renal allotransplant on day 9, showing changes of the media as the result of severe vacuolization and deposition of amorphous substance between the smooth muscle cells, (arrows).
Magnification × 8.000.

Fig. 14. Renal allograft on day 7, from rat treated with ALS and IMURAN. Note arteriole showing a normal ultrastructure.
Magnification × 6.800.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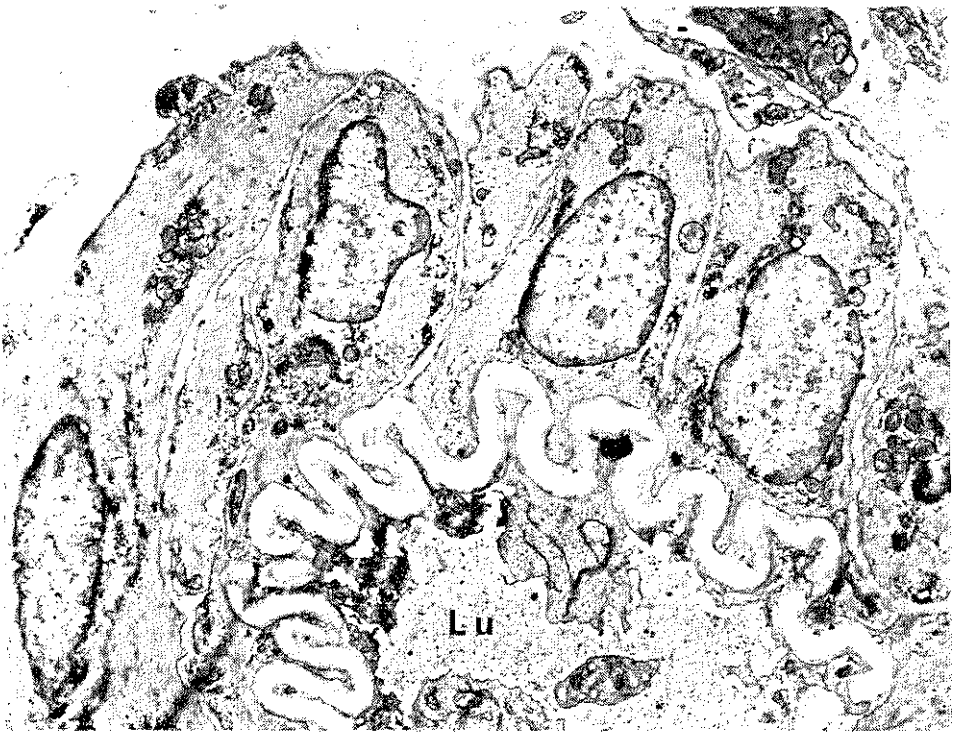
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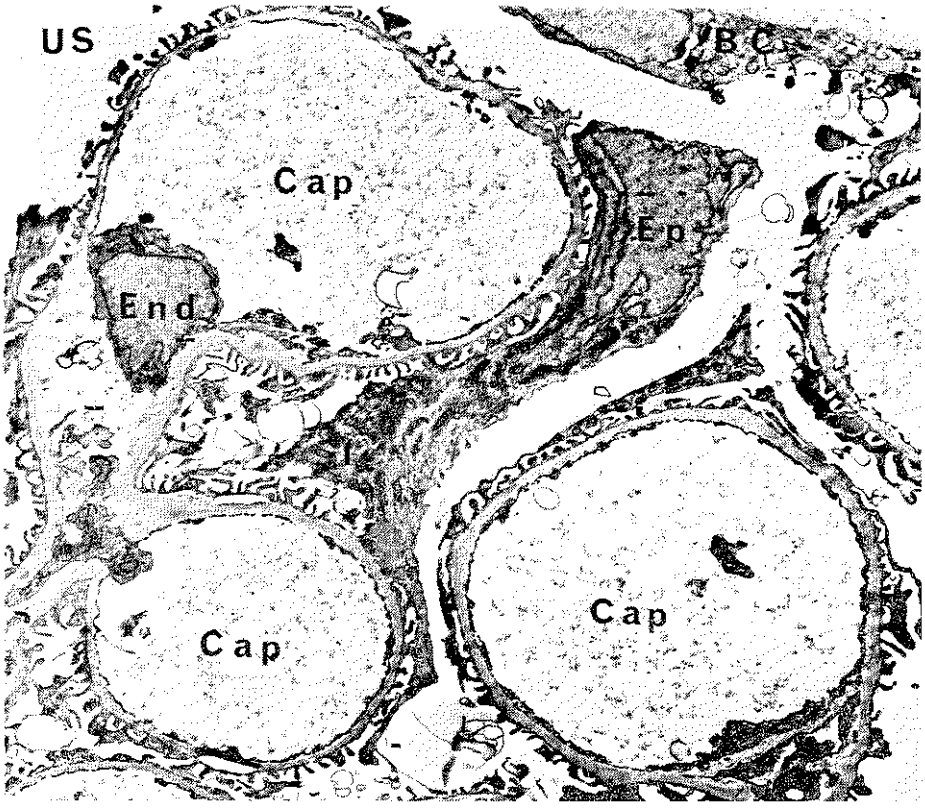
Fig. 15. Renal allograft on day 3, showing glomerulus with no alteration of the electronmicroscopical structure.
Magnification × 6.100.

Fig. 16. Renal allograft on day 5. Wide areas of separation of the endothelium from the basement membrane. (*) Note vacuolization of the endothelial cytoplasm and fusion of the foot processes. Lymphocytic cells lie free in the capillary lumen.
Magnification × 6.800.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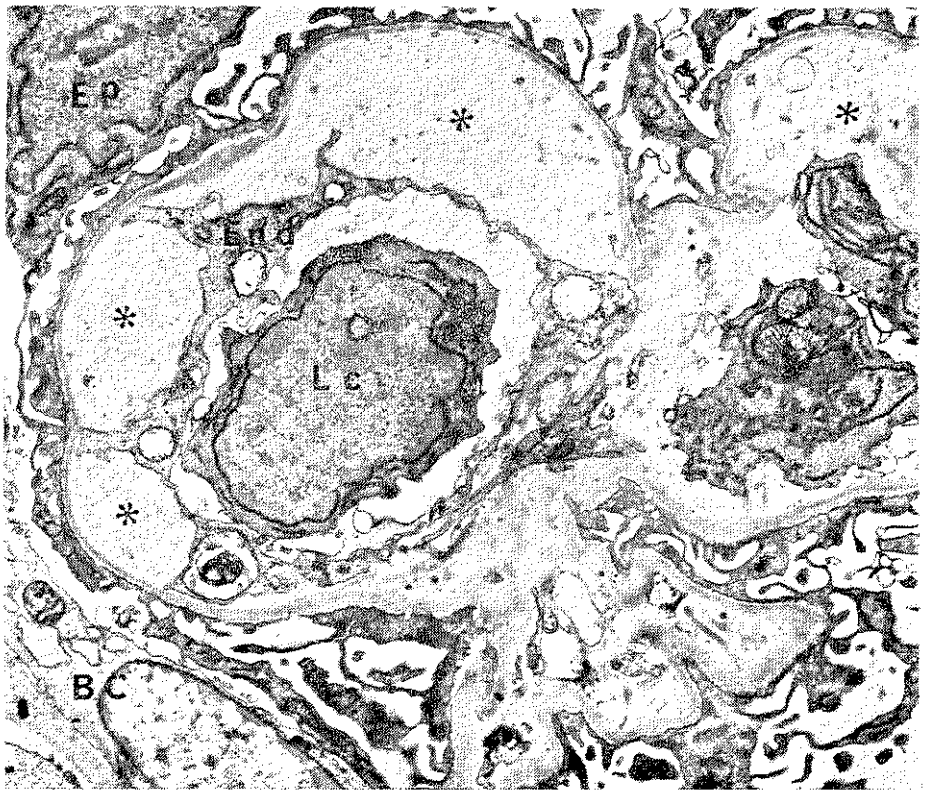
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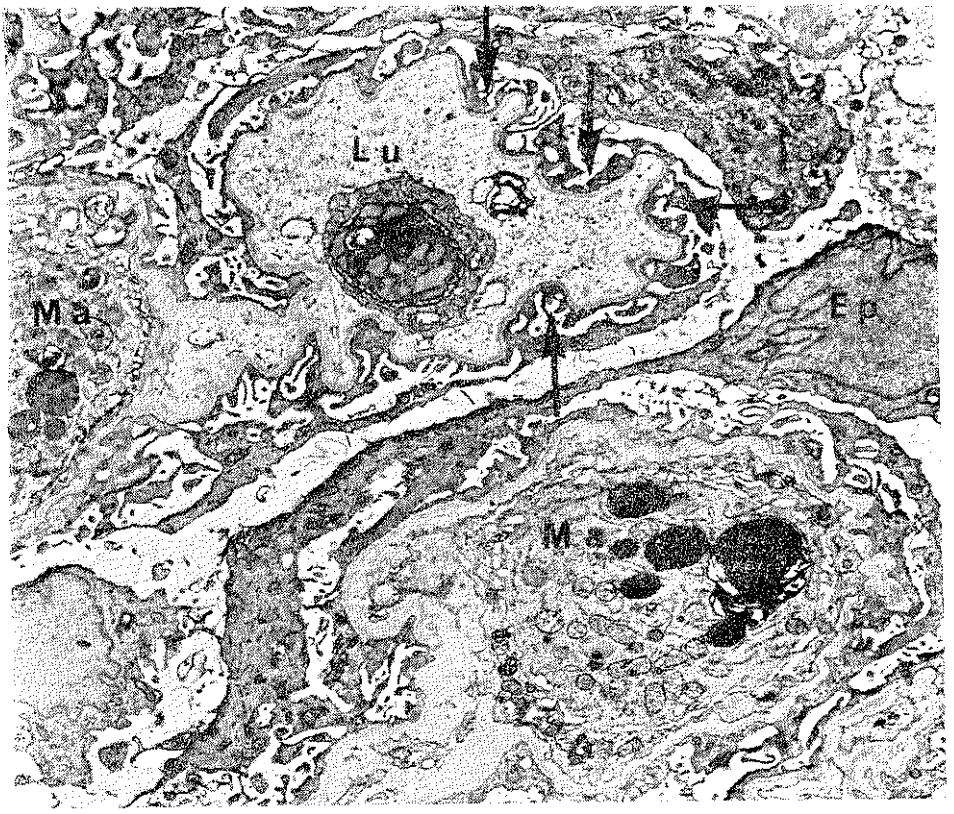
Fig. 17. Allograft on day 7. Glomerular capillary loops are collapsed due to pressure of undulating basement membrane (arrows). In the lumens there are cellular structures of unidentified origin (macrophages?).
Magnification × 5.200.

Fig. 18. Renal allograft on day 7. Complete obstruction of the glomerular capillary lumen by a single lymphocytic cell. At several places cytoplasmic extensions of the cell enter into direct contact (arrows) with the glomerular basement membrane. Note subendothelial deposits (*).
Magnification × 12.600.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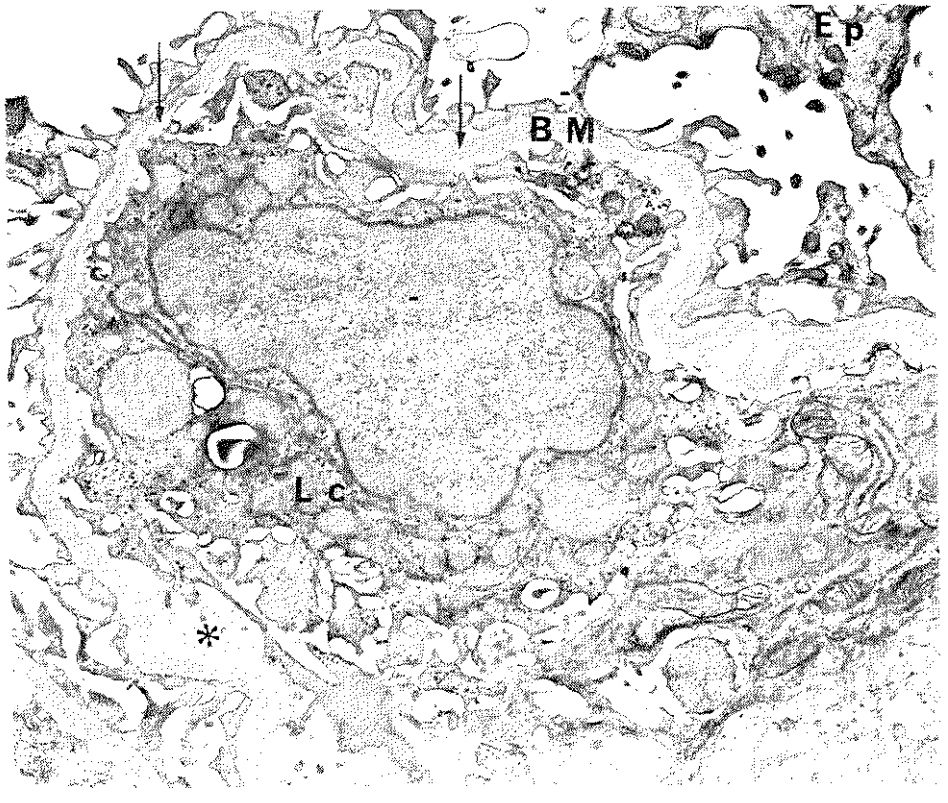
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Fig. 19. Renal allograft on day 7. Distortion of the normal glomerular architecture. Complete obliteration of capillary lumen with disintegrated cellular structure (*). The endothelial foot processes are completely fused. The urinary space contains much cellular debris, many lipid droplets, fibrin, and prominent microvilli. The epithelial cell is rich in cytoplasmic organelles and myelin figures. The basement membrane has a wrinkled appearance.
Magnification × 9.400.

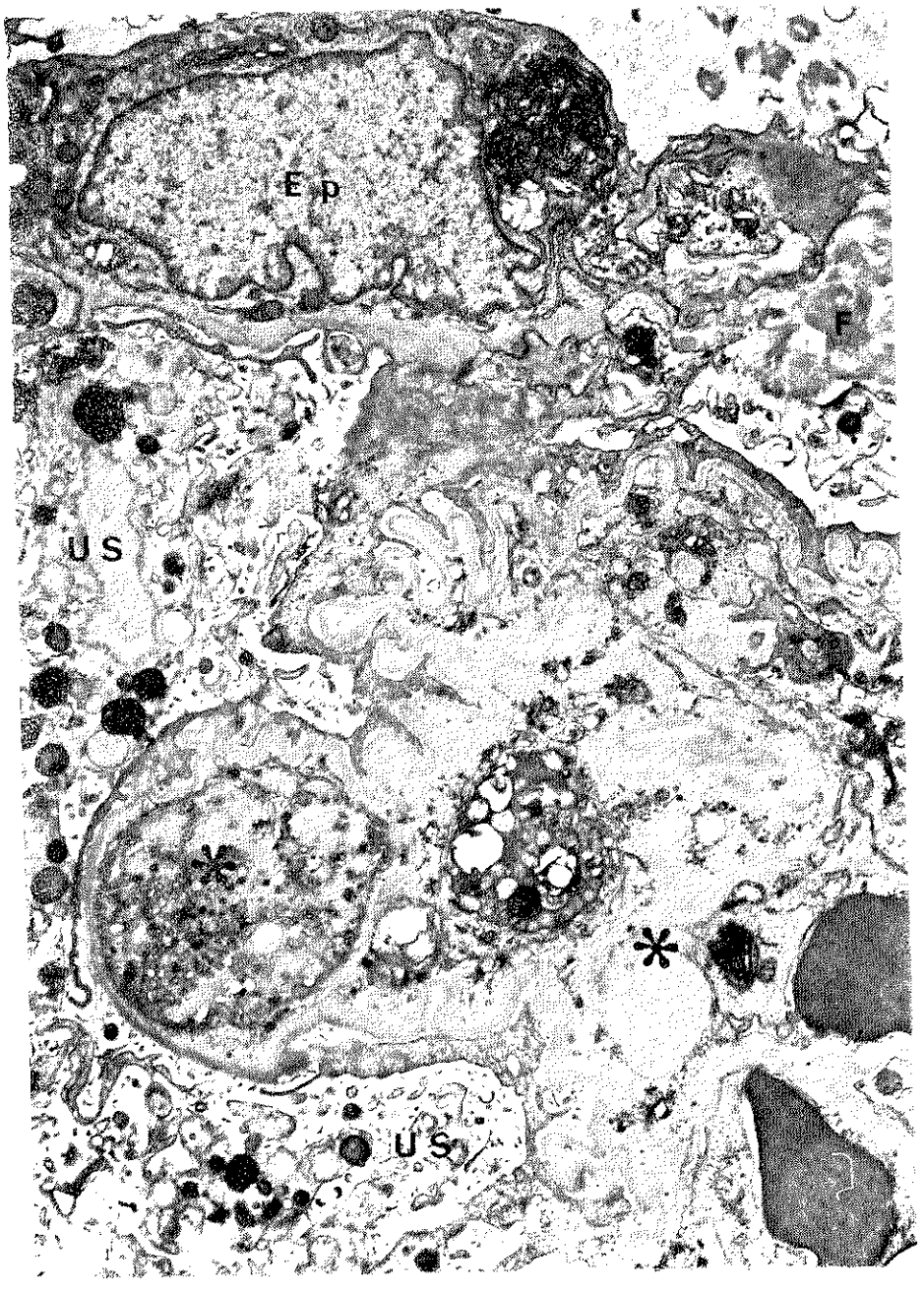
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Fig. 20. Renal allograft on day 7, showing glomerular capillary loop completely obstructed by fibrin accumulation and a hypertropic endothelial cell. Note complete fusion of the epithelial foot processes (arrows).
Magnification × 8.150.

Fig. 21. Renal allograft on day 7. Severe swelling of the endothelial cells, which together with a lymphoid cell almost completely obstruct the capillary lumen. The endothelial cytoplasm, which is increased in amount, contains many endoplasmic reticular sacs, free ribosomes, a few hyaline droplets and dense osmiophilic bodies. Note subendothelial deposits (arrows).
Magnification × 8.250.

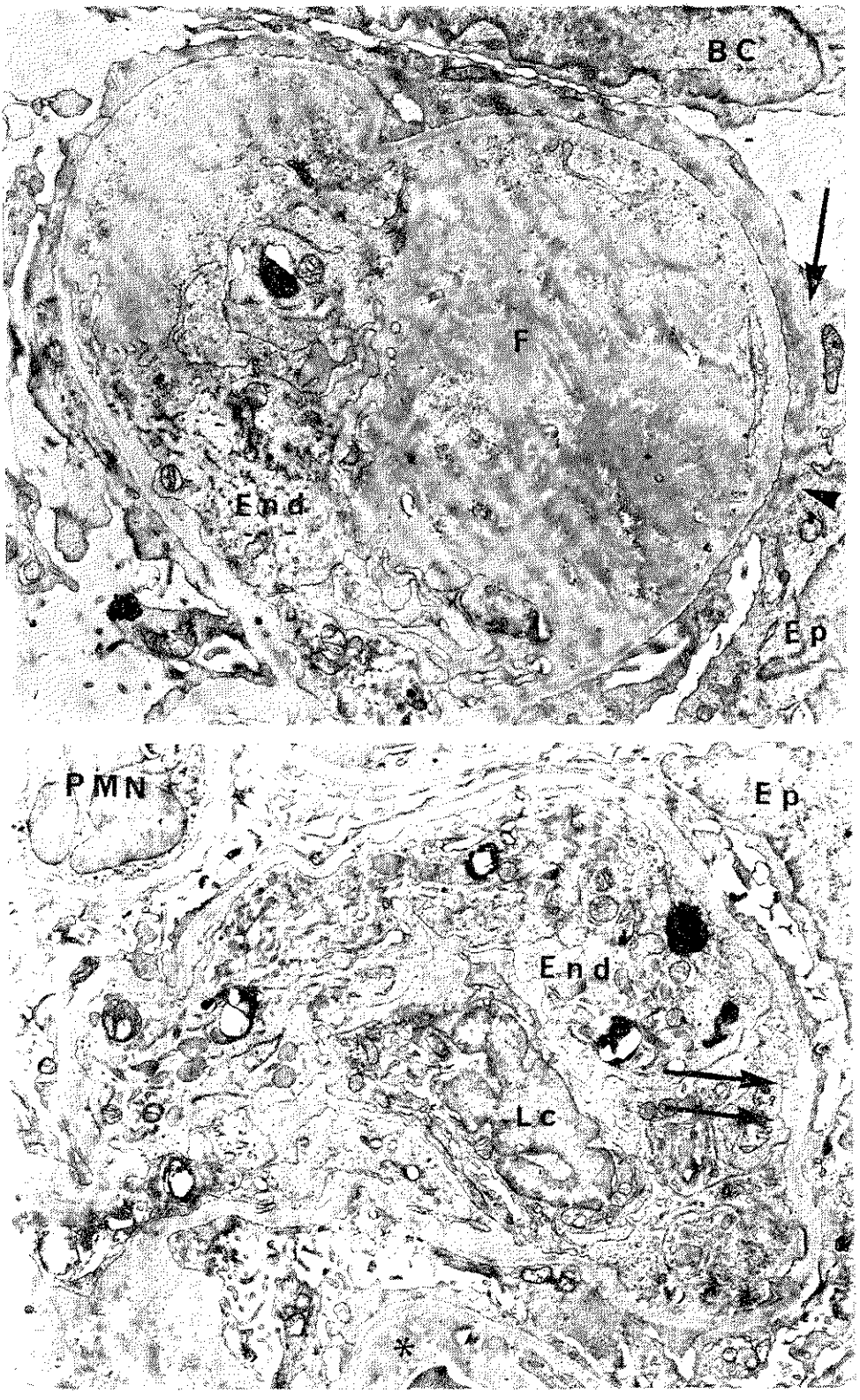
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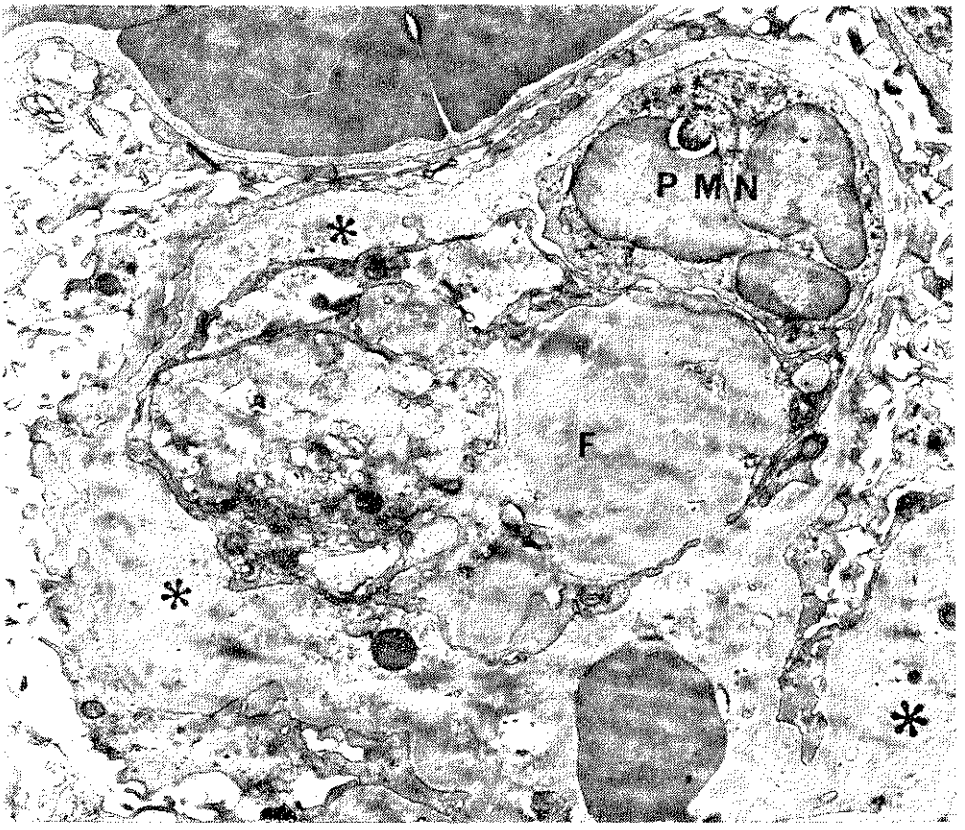
Fig. 22. Renal allograft on day 9. A glomerular loop completely obstructed by polymorphonuclear leucocytes, cell debris, and fibrin masses. Irregular thickening of the basement membrane is caused by the deposition of homogeneous basement membrane-like material (*).
Magnification × 8.400.

Fig. 23. Renal allograft on day 9. Epithelial cell with enlarged endoplasmic reticulum and Golgi complexes. Deposition of homogeneous material is evident on the epithelial side of the basement membrane (arrow), as well as thickening of the basement membrane (*).
Magnification × 10.400.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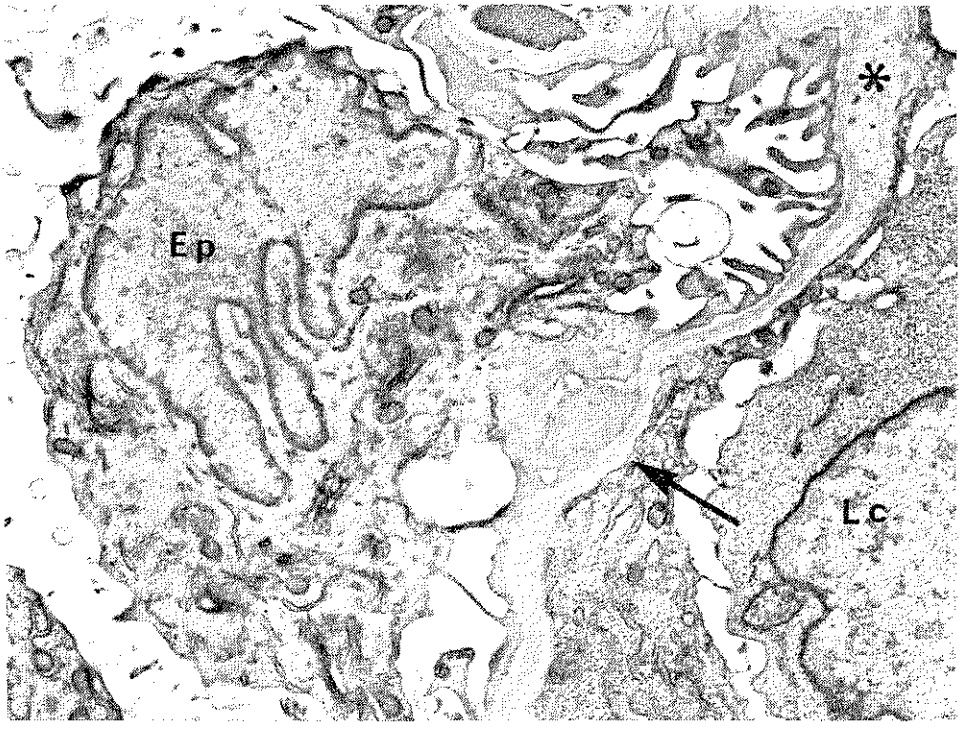
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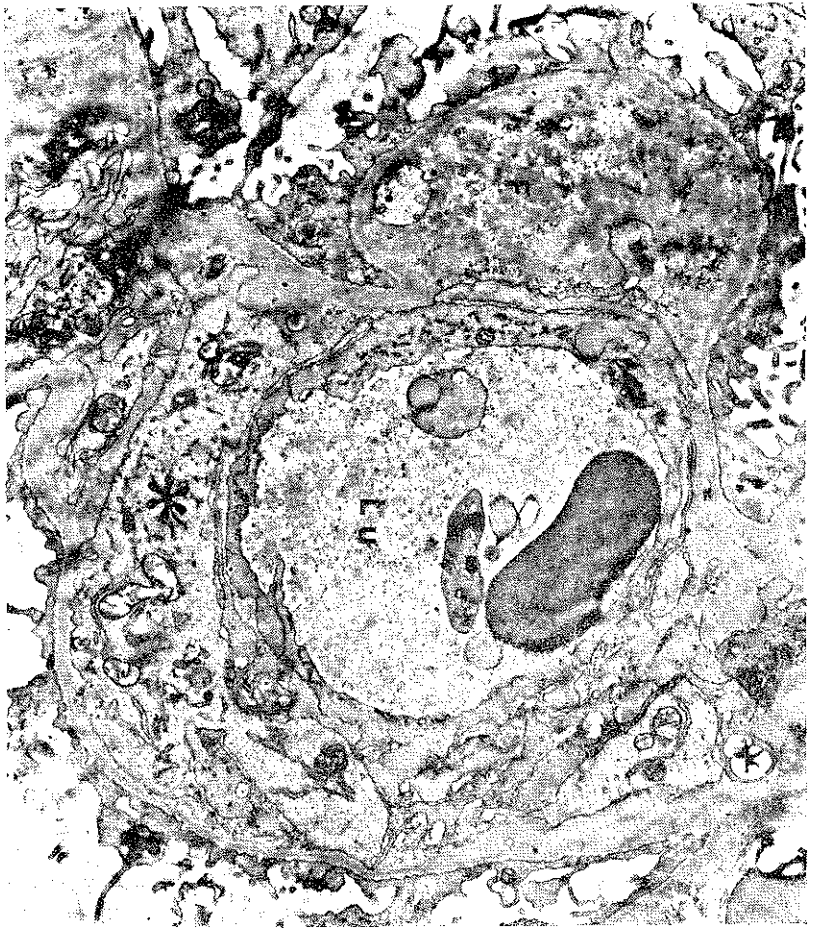
Fig. 24. Renal allograft on day 9. Mesangial changes consisting of dissolution of the mesangial matrix and accumulation of amorphous material (*).
Magnification × 21.600.

Fig. 25. Renal allograft, 21 days after treatment with IMURAN. Enormous thickening of the glomerular capillary wall due to subendothelial depositions (*) incorporating endothelial cell debris and fibrin. The capillary lumen is stikingly reduced.
Magnification × 8.200.



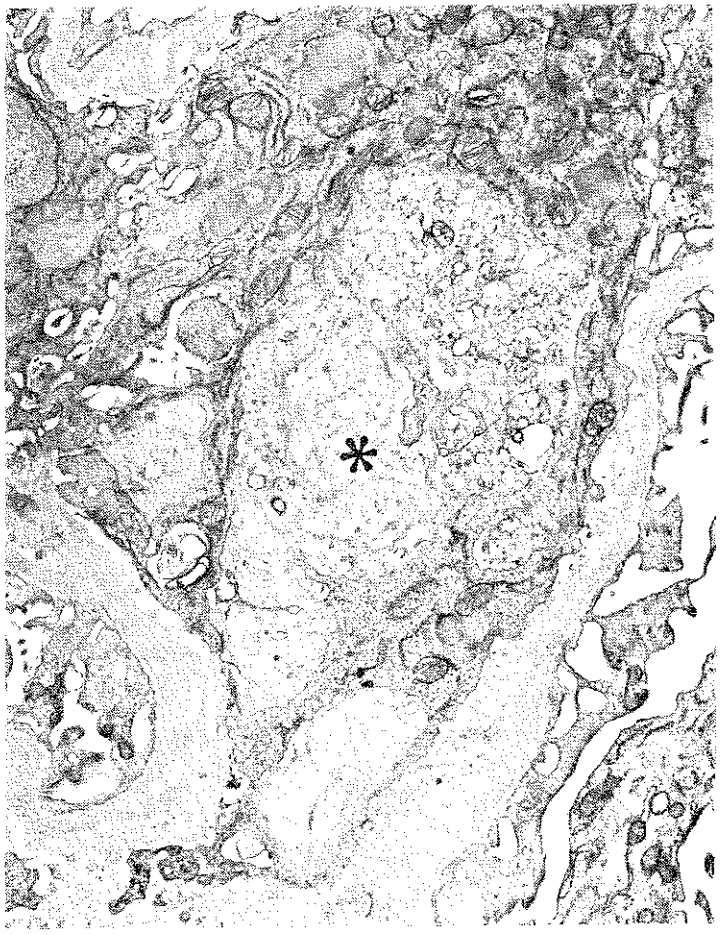
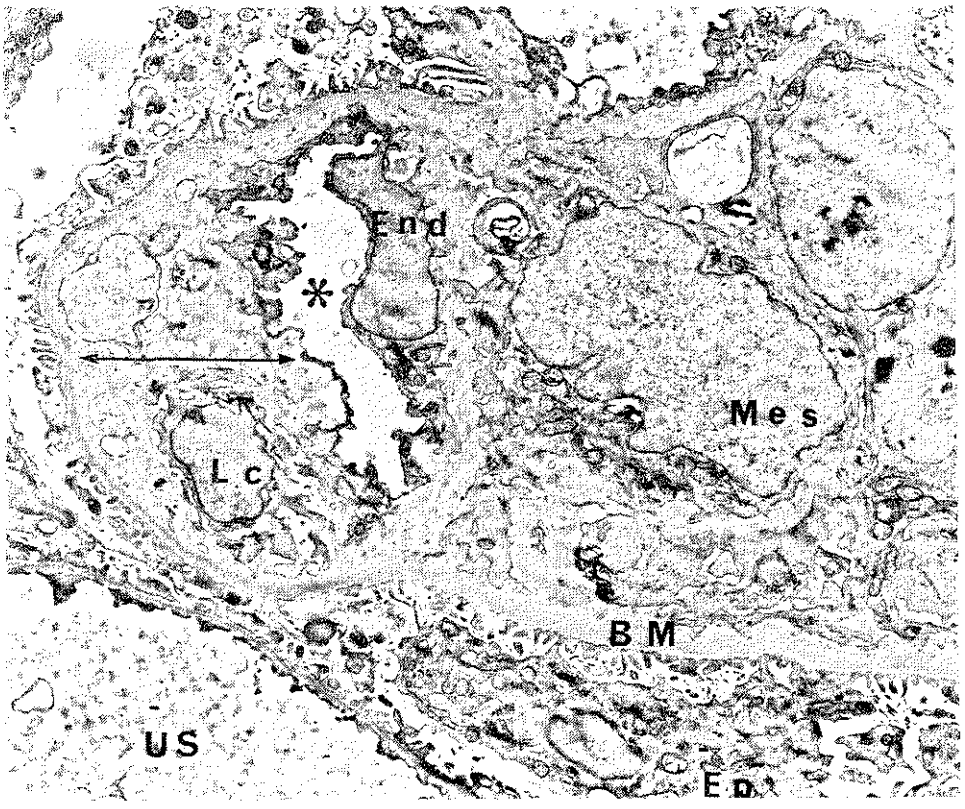


Fig. 26. Renal allograft 21 days after treatment with IMURAN showing striking hypercellularity of the mesangium and thickening of the glomerular capillary wall due to subendothelial deposition of dense homogeneous material (arrow). Note marked reduction of the capillary lumen (*).
Magnification × 6.400.

Fig. 27. Renal allograft 21 days after treatment with IMURAN. Appearance of part of a glomerulus, showing several epithelial cells, part of one capillary loop, and mesangium. The epithelial cytoplasm is voluminous and contains many dense cytosomes and vacuoles. The capillary lumens are considerably reduced due to irregular thickening of the basement membranes and endothelial cell protrusion.
Magnification × 5.800.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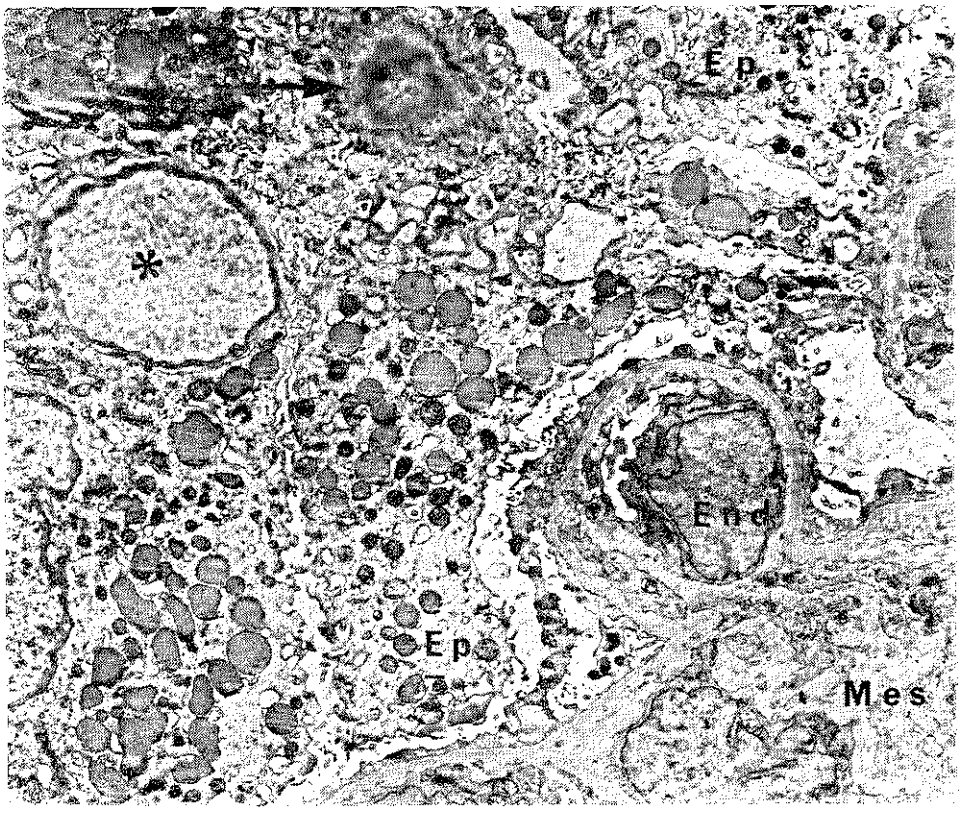
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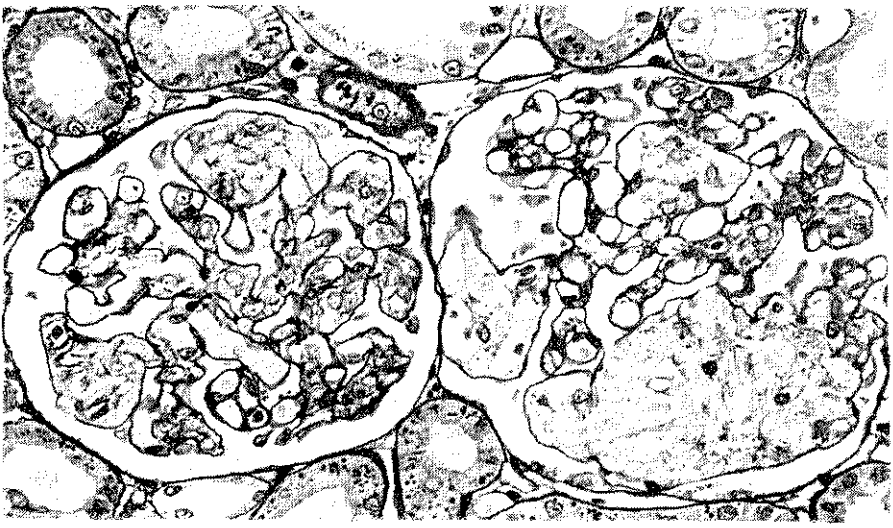
Fig. 28 and 29. Renal allograft, 21 days after treatment with a combination of IMURAN and PREDNIZOLONE. Light microscopy: three glomeruli showing various stages of dilatation of the glomerular capillary loops. Note cystic appearance of the most advanced lesions.
Magnification × 300 and 460, respectively.



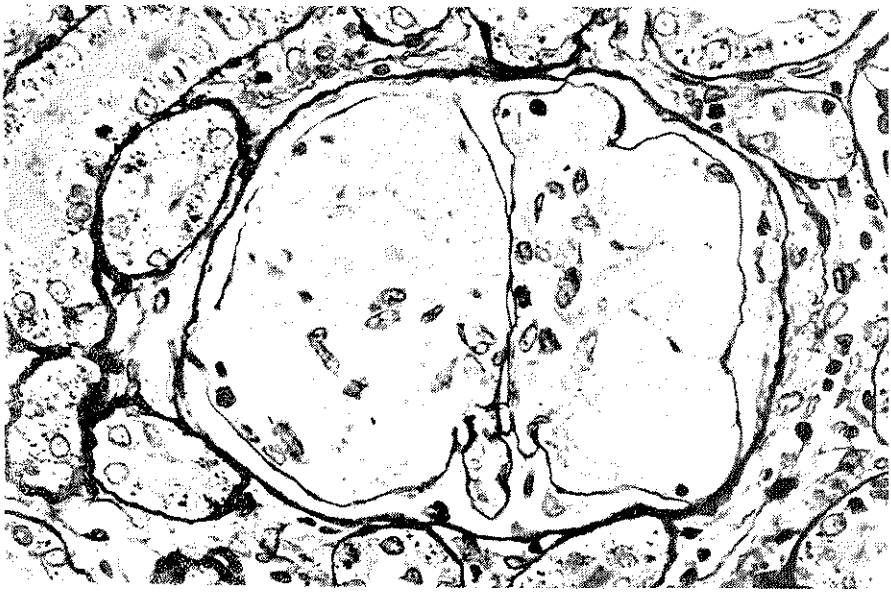


Fig. 30. Renal allograft 21 days after treatment with a combination of IMURAN and PREDNIZOLONE. Complete dissolution of the mesangial matrix and accumulation of finely granular material which extends between the endothelial cells and the basement membrane, causing separation of the endothelial cells from the underlaying basement membrane(*) Magnification × 6.400.

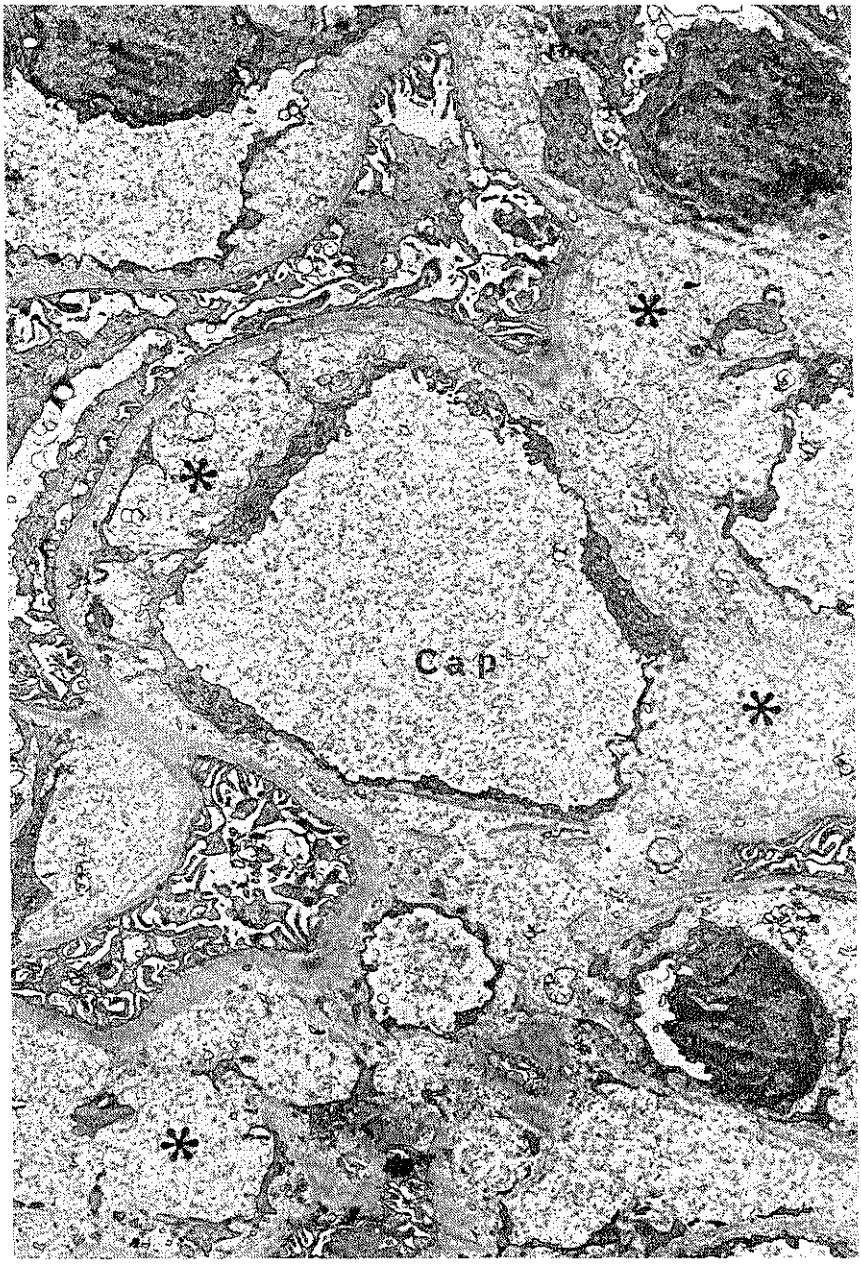

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Fig. 31. Renal allotransplant 7 days after treatment with ALS. Distal convoluted tubuli showing a normal ultrastructural morphology. Magnification × 7.800.

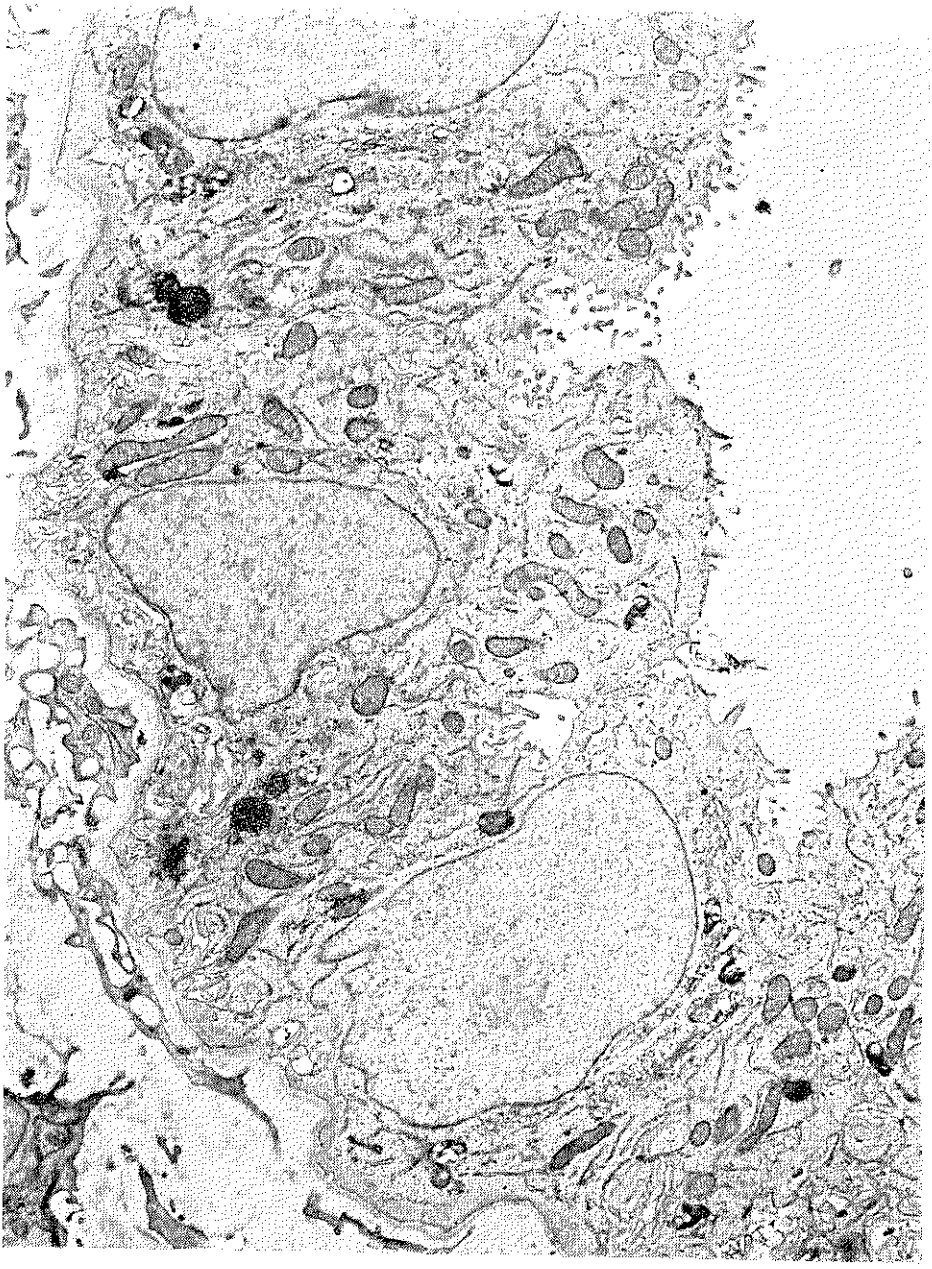


Fig. 32. Renal allograft on day 3. The mitochondria of one tubular cell show swelling and degenerative changes.
Magnification × 9.200.

Fig. 33. Renal allotransplant on day 7. Disintegration of the apical plasma membrane of one individual tubular cell. In the cytoplasm there are many dense osmiophilic bodies.
Magnification × 8.200.

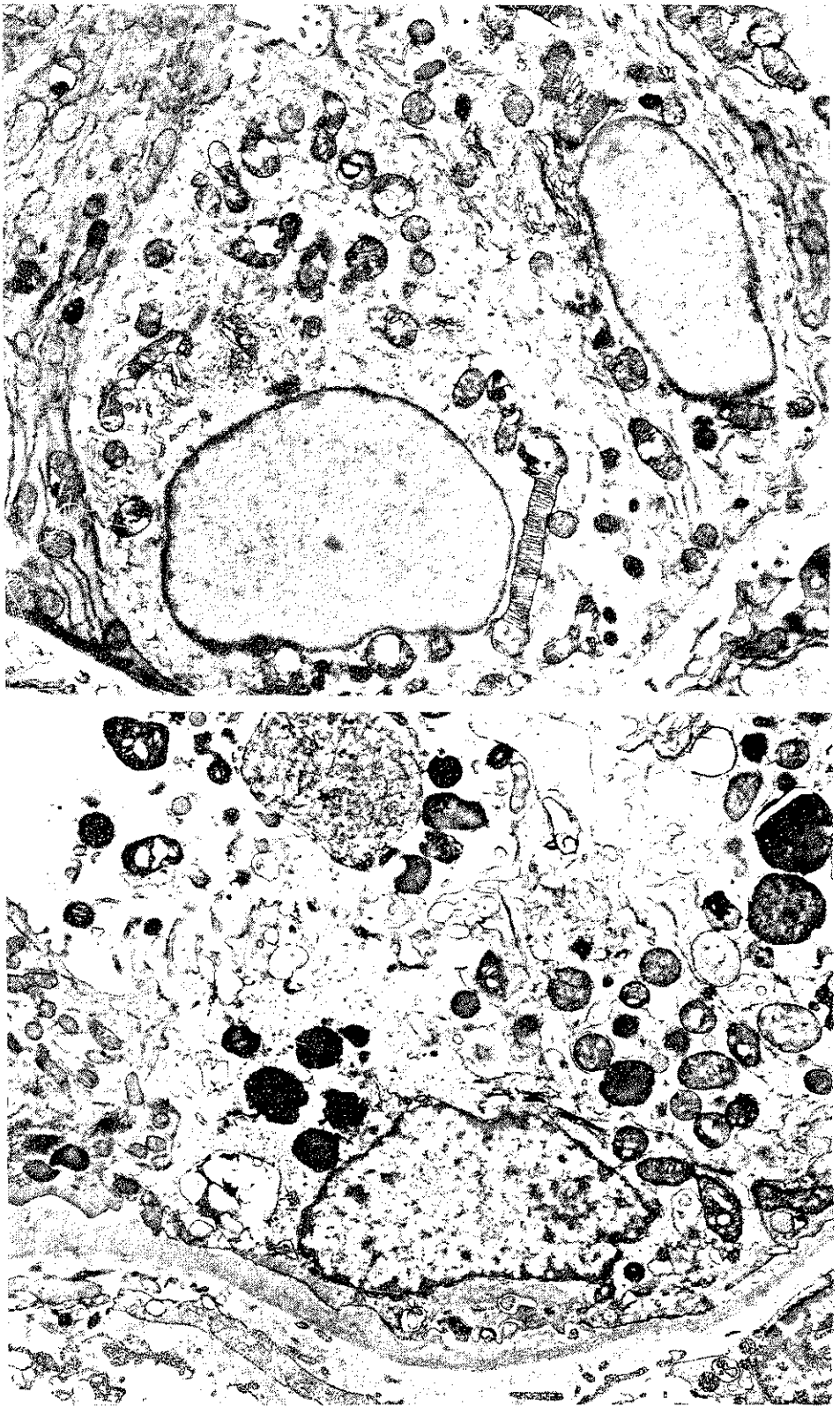
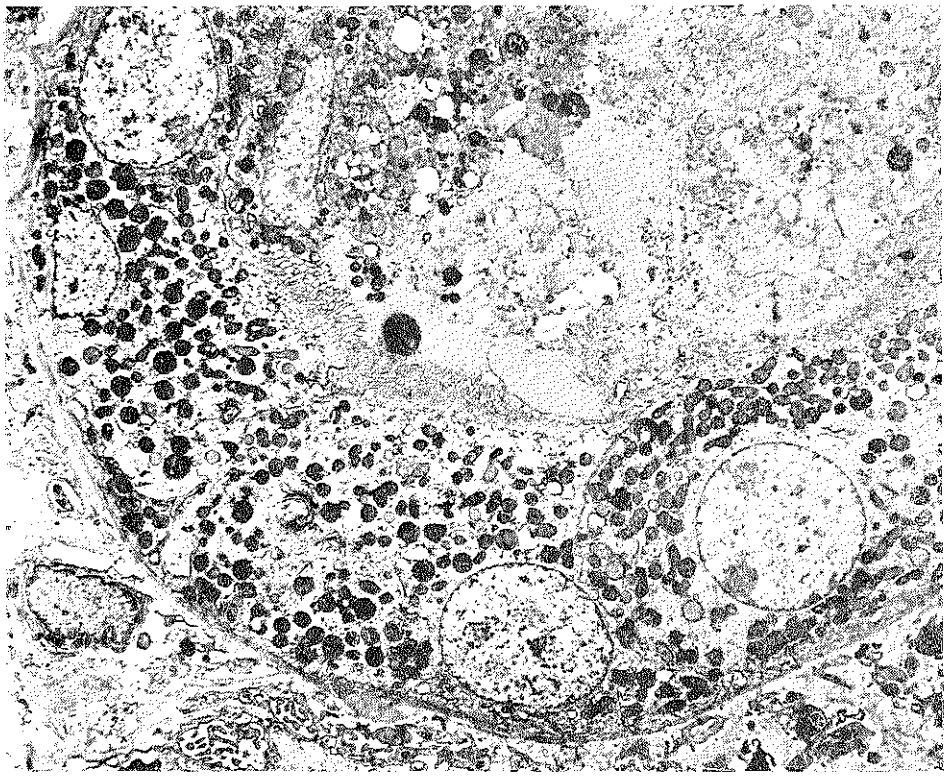


Fig. 34. Renal allotransplant on day 7. The tubular lumen is filled with necrotic, structureless material and tubular cell debris.
Magnification × 6.800.

Fig. 35. Renal allotransplant on day 5. The tubular cells are separated from each other and there is a large intercellular compartment at the base of the cells.
Magnification × 8.000.



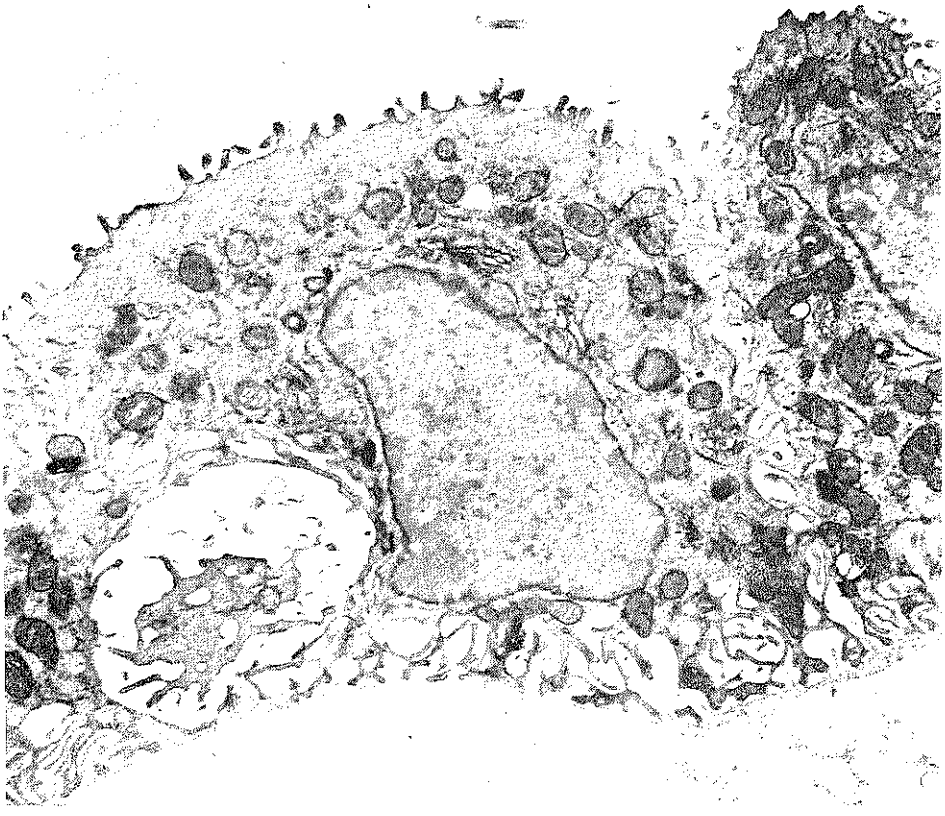
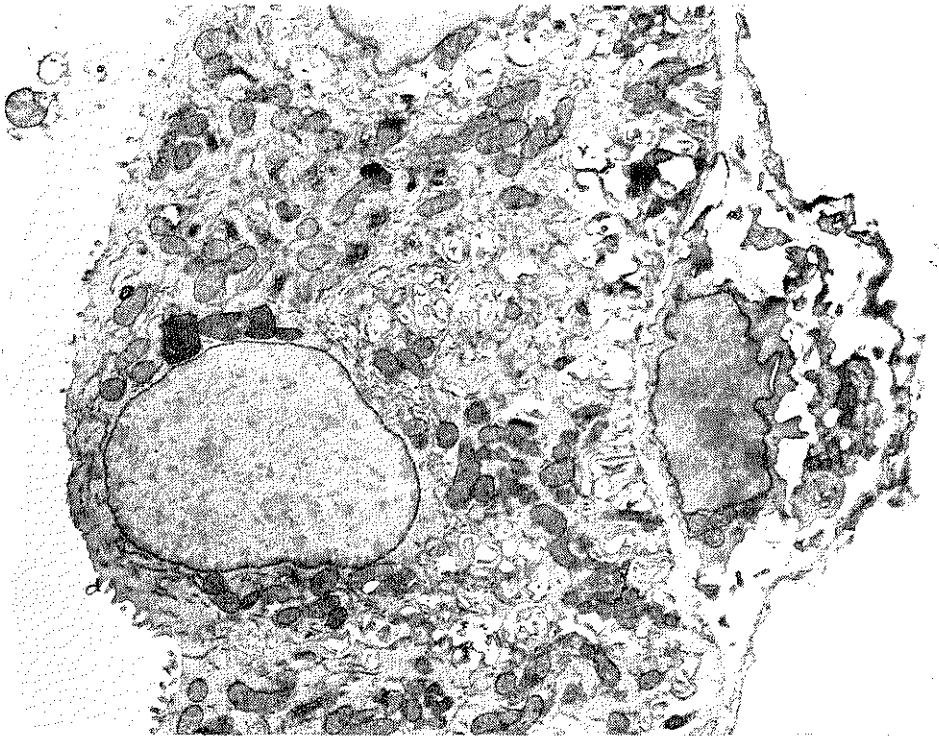


Fig. 36. Renal allograft on day 5, showing invasion of a tubule by a lymphoid cell seen lying between the tubular basement membrane and the tubular cell.
Magnification × 7.400.

Fig. 37. Renal allotransplant on day 5, showing invasion of the proximal tubule by lymphoid cells.
Magnification × 6.400.



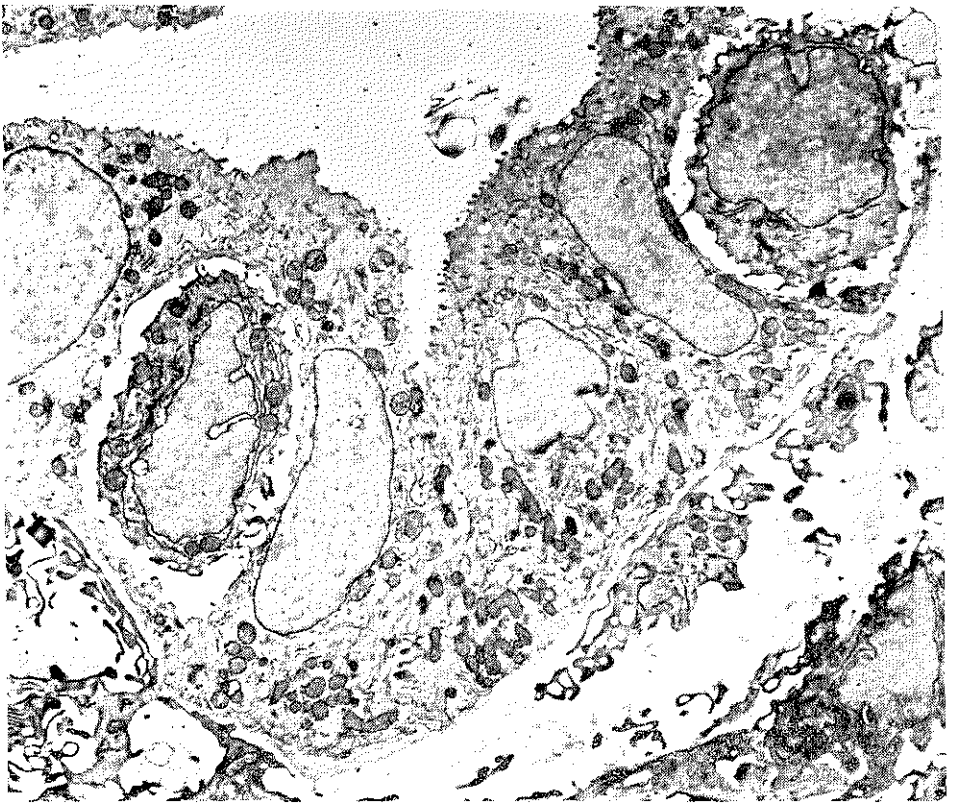
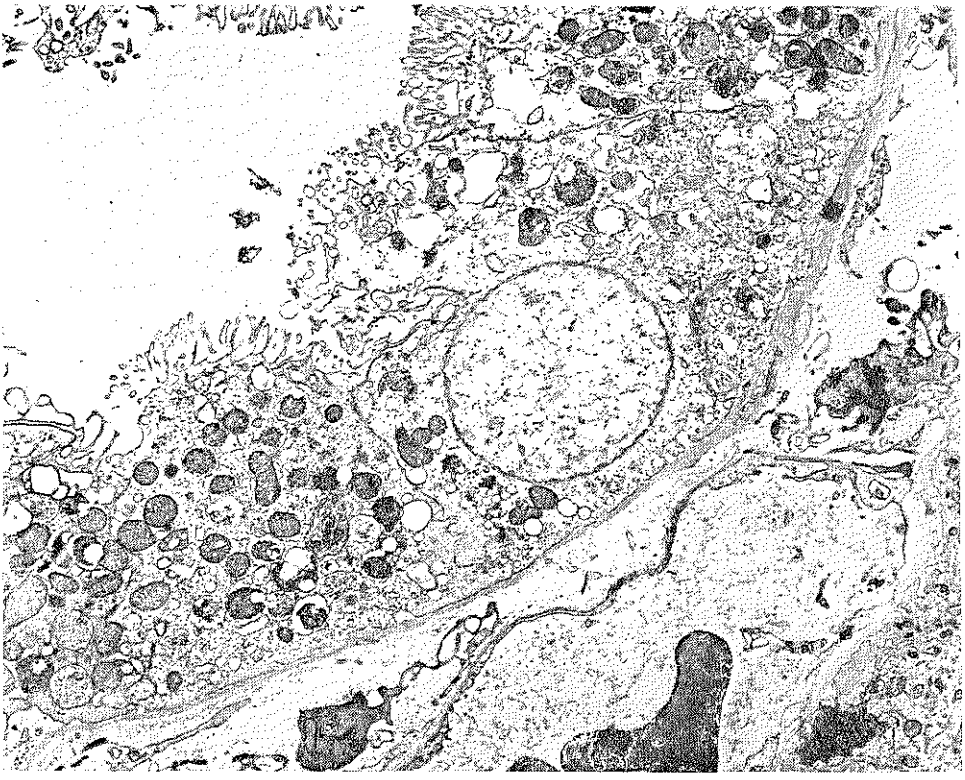


Fig. 38. Renal allotransplant on day 7. The cells are filled with cytoplasmic bodies of different size and electron density and show many vacuoles.
Magnification × 7.250.

Fig. 39. Renal allotransplant on day 7. Disintegration of the normal tubular architecture. A few tubular cells which have become detached from the basement membrane lie free in the lumen together with a lymphoid cell.
Magnification × 7.800.



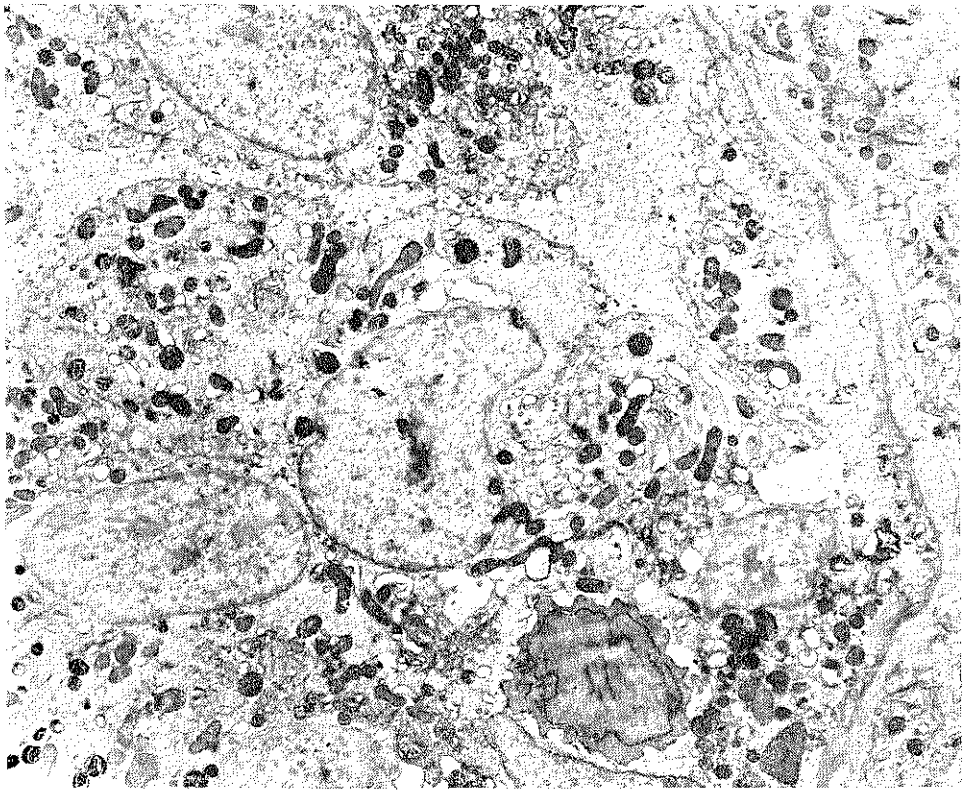
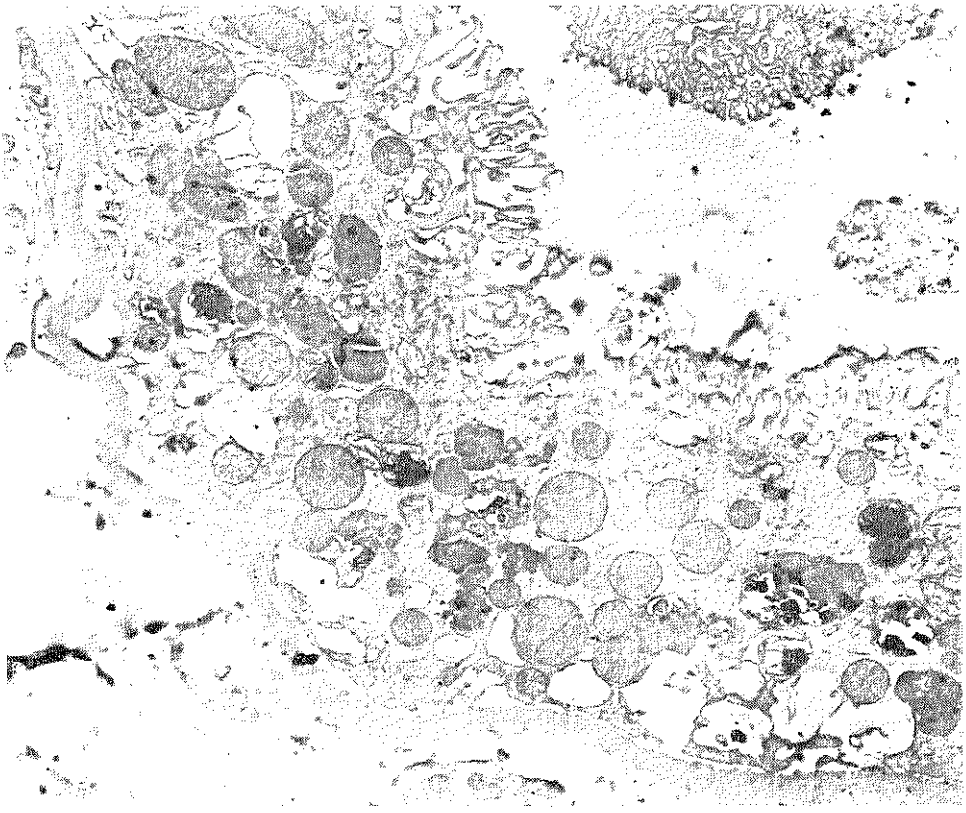


Fig. 40. Renal allotransplant on day 7. Severe vacuolization of the tubular cytoplasm. The mitochondria show enlargement and homogenization.
Magnification × 8.250.

Fig. 41. Renal allotransplant on day 7. The tubular cytoplasm contains variously sized vacuoles and electron-dense inclusions, the tubular lumen is obstructed by a plug of necrotic material.
Magnification × 8.400.



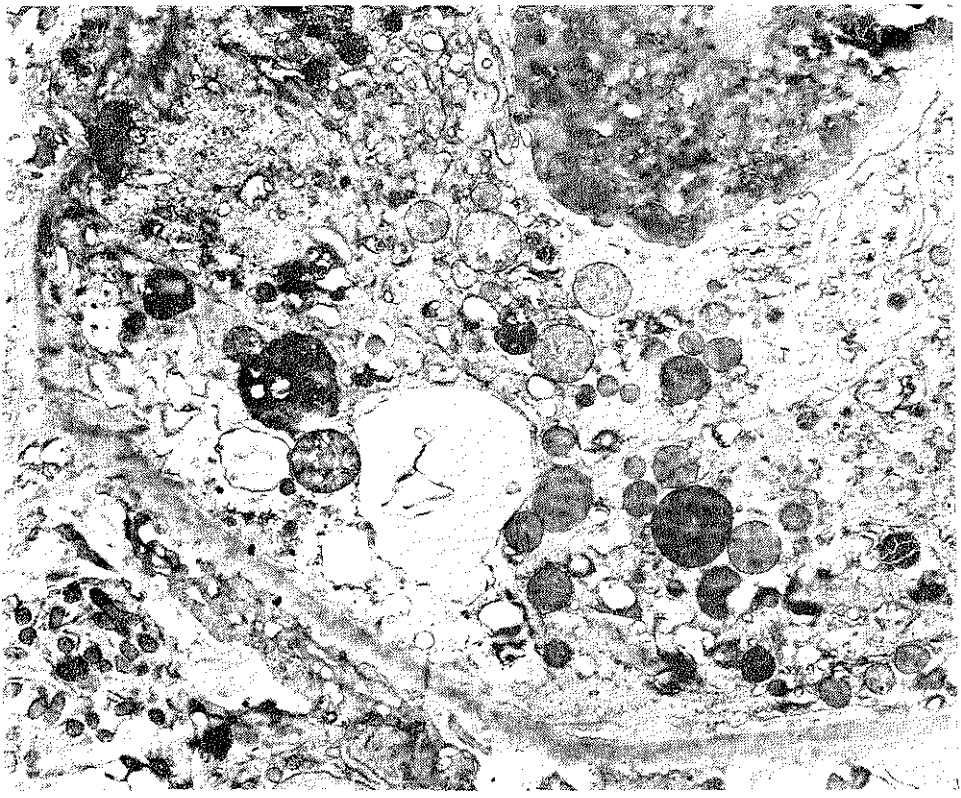
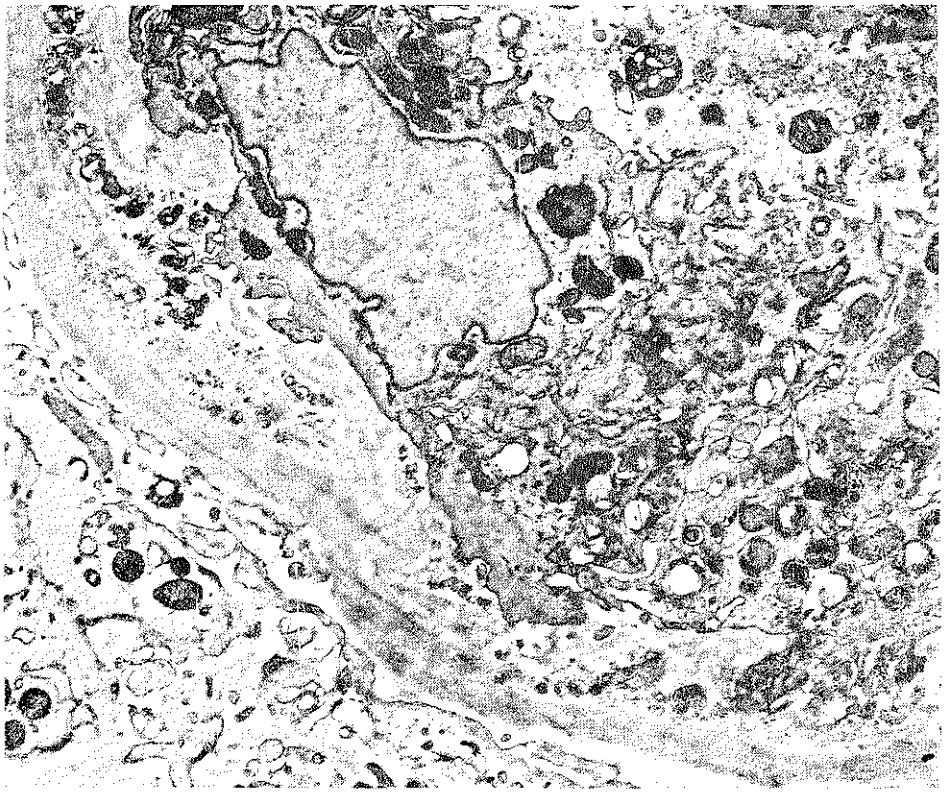


Fig. 42. Renal allotransplant on day 7, showing deposition of finely granular substance and small cytoplasmic organelles between the tubular basement membrane and basilar portion of the cell membrane. In the lumen there are remnants of cytoplasmic structures.
Magnification × 8.400.

Fig. 43. Renal allograft on day 9. The tubular cell contains numerous dense inclusions and vacuoles and shows marked thickening of the tubular basement membrane.
Magnification × 8.200.



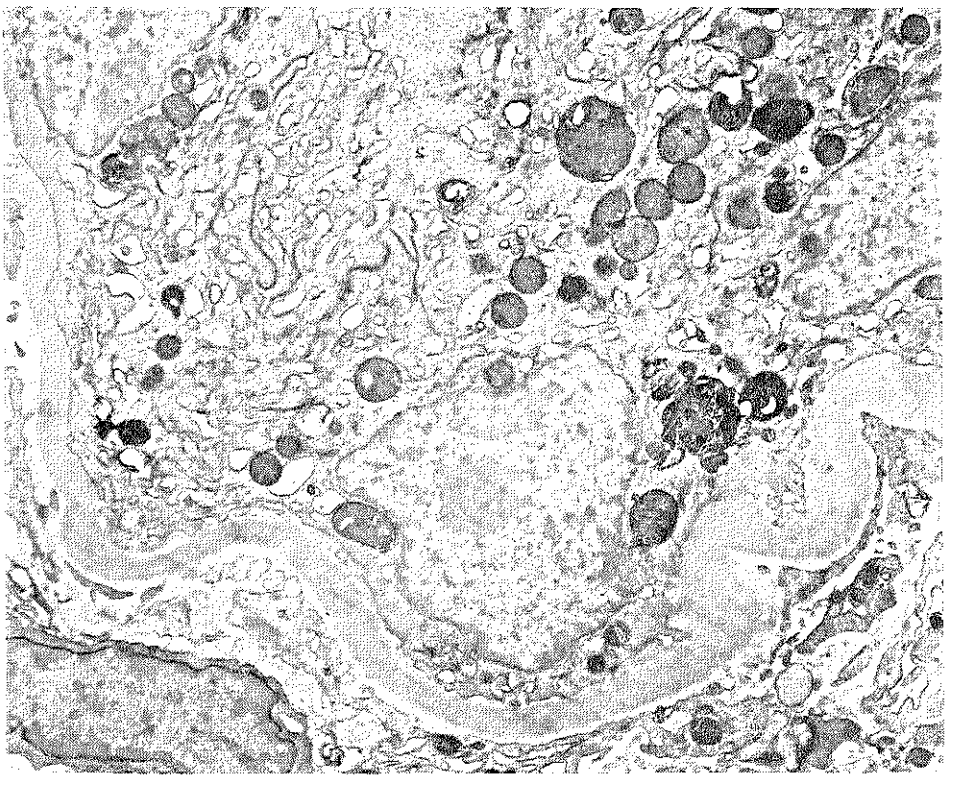
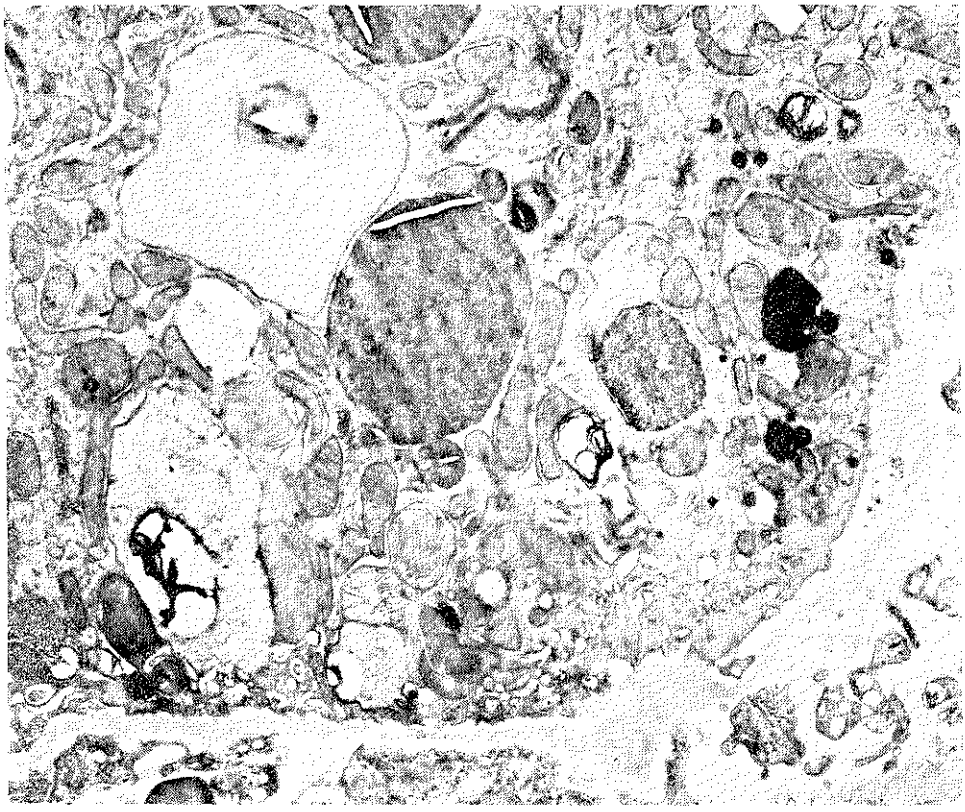
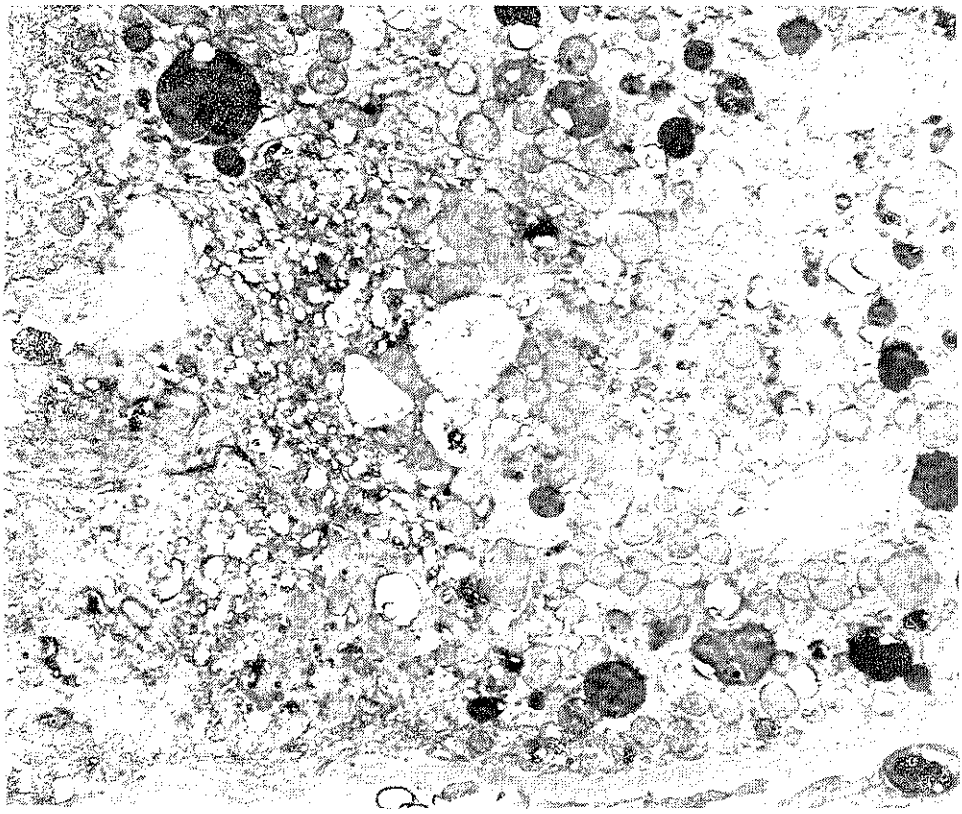


Fig. 44. Renal allotransplant on day 9, showing advanced degeneration of the tubular cell. The cytoplasm contains a large number of variably dense inclusions, as well as cytosomes and osmiophilic granules.
Magnification × 10.500.

Fig. 45. Renal allograft on day 9, showing necrotic changes in the tubular cells.
Magnification × 8.200.





De figuur op het omslag is een ornament uit de 14de eeuwse Biblia van Divoš, Servië.