

STATEMENT

FACTORS INFLUENCING ALLOGRAFT REJECTION

The experimental work for this thesis was carried out
entirely by the author. The author performed the
statistical analysis using the aid of Dr W J Cliff
provided the illustrations shown in Figure II-3.

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ABSTRACT

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ABSTRACT

The degree to which ischaemia is involved in the destruction of allografts undergoing acute rejection has not been fully elucidated and, because of this, determination of the direct effects of the immunological system on the allografts in vivo has been difficult. In vivo observations in rabbit ear chambers combined with light and electron microscopy of fixed tissues were used in the present study to evaluate the relative contributions made by these factors to the degeneration of rejecting allografts of endometrium. Vascular grafts of endometrium were transplanted to graft ear chambers and non-vascular grafts of epithelium enzymatically separated from endometrium were transplanted to graft and injection ear chambers. Rejection in the ear chambers was compared to that occurring in subcutaneous sites in the rabbits' ears.

As reported by others, the onset of spontaneous rejection of the vascular allografts in graft chambers alone was delayed (25 to 43 days after transplantation). Rejection of the vascular allografts in the chambers induced by the simultaneous subcutaneous transplantation of endometrium from the same donor occurred in a significantly shorter period of time (11 to 17 days after transplantation, $p < 0.004$). An effect of the size of the allografts on the time from transplantation to rejection was observed in subcutaneous sites. This suggested that the size of the allografts may have contributed to the delayed onset of spontaneous rejection in the ear chambers. An effect of stress on the rejection of the

allografts in subcutaneous sites or ear chambers could not be demonstrated.

When the effect of allograft size in the graft chambers was tested by the transplantation of larger vascular allografts than usual, the survival of these allografts was paradoxically further prolonged (greater than 60 days). It was also observed that when rejection in the chambers was induced by simultaneous subcutaneous transplantation of allografts, the destruction of the chamber allografts lagged behind that of the subcutaneous allografts. The prolonged survival of the larger allografts cannot as yet be explained but the results indicate that the graft chamber behaves as an immunologically privileged site where both the afferent and the efferent arms of the immune response are modified. The spontaneous rejection of non-vascular allografts of endometrial epithelium was also delayed in graft chambers but not in injection chambers indicating that some characteristic of the graft chambers in particular was responsible for the immunological privilege.

A reaction to the vascular endometrial allografts before the onset of overt spontaneous rejection was shown by the larger numbers of mononuclear cells in the lamina propria of these grafts than in that of control autografts. On average, the epithelium of these allografts was lower and had fewer secretory vacuoles than that of control autografts indicating that the reaction of the recipient was detrimental to the allografts at this stage.

Both spontaneous and induced rejection of the vascular endometrial allografts in the ear chambers, although the former was

delayed, were typical of acute rejection reactions.

The endometrial epithelial cells of the non-vascular allografts underwent similar atrophic and degenerate changes to those observed in the epithelium of the vascular endometrial allografts. The destruction of both the vascular and non-vascular allografts could not be attributed to ischaemia and, thus, must have been due to the direct effects of the response mounted by the recipient.

The initially focal appearance of damage in the vascular allografts and the earlier onset of rejection of subcutaneous than chamber allografts in individual rabbits indicated that rejection was not due to humoral antibody acting at the blood-endothelium interface.

Electron microscopic observations on both vascular and non-vascular allografts suggested that specific cell-mediated lysis of allogeneic cells occurred during rejection. However, the paucity of evidence that degenerating cells underwent apoptosis or zeiosis, the form of degeneration characteristic of T lymphocyte-mediated or antibody-dependent cellular-cytotoxicity, indicated that these mechanisms made only minor contributions to the rejection of the allografts. Most of the degenerating cells showed coagulative necrosis.

The degeneration of both donor and recipient tissues indicated that non-specific effector mechanisms predominated during the acute rejection reaction. The results also showed that other tissues as well as those of the vasculature can be directly damaged by the effector mechanism(s) during acute rejection.

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CHAPTER I

INTRODUCTION

GENERAL INTRODUCTION

A graft of cells, tissues or an organ may be classified in the following way: autograft, derived from the same individual to which it is transplanted; isograft (isogeneic or syngeneic), derived from a donor with the same genotype as the recipient; for example grafts transplanted between identical twins or between members of the same strain of highly inbred experimental animals; allograft, derived from a donor of the same species but with a different genotype from that of the recipient; xenograft, derived from a donor of a species other than that of the recipient (SNELL ET AL, 1976). Autografts and isografts are accepted but, in the normal course of events, allografts and xenografts are rejected. A first-set graft is one where the recipient has had no previous exposure to the histocompatibility antigens of the donor. A second-set graft is one that is transplanted to a recipient who has previously been sensitized to the donor's histocompatibility antigens either by a previous graft or by some other means.

Autografts are widely used in human medicine for reconstructive surgery and have also been used in the treatment of some endocrine disorders; for example, hyperparathyroidism (WELLS ET AL, 1982). Isografts are ideal for transplantation from one individual to another because rejection is not a problem but few patients have an identical twin. At the present time, allografts, together with immunosuppression of the recipient, have the greatest potential for the replacement of diseased organs in human beings.

Corneal allografts are widely used in human medicine to treat dystrophies, post-inflammatory scarring, endothelial dysfunction and other disorders of the cornea (COSTER, 1982). In the best circumstances, greater than 90% of corneal allografts are successful. Allograft rejection is considered the major obstacle to successful corneal transplantation now that technical difficulties have been largely overcome.

Kidneys are the most widely utilized vascularized allografts and their successful transplantation provides an improved quality of life for many patients with terminal renal failure who would otherwise be entirely dependent on dialysis. In recent times, patient survival rates have improved but little improvement in graft survival rates has been observed (ADVISORY COMMITTEE OF THE RENAL TRANSPLANT REGISTRY, 1977; MOORE, 1980; MARSHALL, 1981). During the period 1968 to 1975, survival rates of first transplant renal allografts derived from sibling, parent and cadaver donors up to 80%, 75% and 55% respectively at one year and 66%, 55% and 35% respectively at 5 years were recorded (ADVISORY COMMITTEE OF THE RENAL TRANSPLANT REGISTRY, 1977). Reports of graft survival rates at 10 to 13.75 years have ranged from 35% to 40% for kidneys from living-related donors and 7% to 40% for cadaveric kidneys (MARSHALL, 1981; MAHONY ET AL, 1982; from the data of KIRKMAN ET AL, 1982 and STARZL ET AL, 1977). At 15 years, graft survival rates of 39% and 18% for living-related and cadaveric or living-unrelated renal allografts respectively has been reported (from data of KIRKMAN ET AL, 1982).

The clinical problems of patients that have received renal allografts are now becoming clear. Major medical problems have been reported in 66% to 94% of longer-term survivors with functional renal allografts. These include hypertension requiring treatment (46%); infections (18% to 55%); cataracts (24% to 45%); neoplasia, mostly of lymphoid tissue and skin (14% to 47%); osteoporosis and aseptic necrosis of bone affecting predominantly the hips and often requiring hip replacement (13% to 18%); vascular disease including myocardial infarction, angina, cerebrovascular accidents and claudication of limbs (6% to 30%); hepatic dysfunction (6% to 9%) and

diabetes mellitus (4% to 6%) (KIRKMANN ET AL, 1982; MARSHALL, 1981; MAHONY ET AL, 1982). MAHONY ET AL (1982) found that 56% of patients had died within 10 years of transplantation and major causes of death were infection (34%) and vascular disease (24%). KIRKMAN ET AL (1982) reported patient mortality of 15% between 5 and 20 years after transplantation. Major causes of death were hepatic dysfunction (21%), infection (18%) and vascular disease (18%).

Success rates of cardiac allografts in man approaching that of renal allografts have been reported but only 15% of potential recipients were selected for transplantation (JAMIESON ET AL, 1982). This group had performed 221 cardiac transplants in 202 patients (15 retransplanted once, 2 retransplanted twice). Patient survival rates over the period 1974 to 1980 was 65% at 1 year and 45% to 50% at 5 years.

Bone marrow transplantation has improved the survival rates of patients with aplastic anaemia and some forms of leukaemia. This is now the preferred treatment of aplastic anaemia and apparent cure of about 60% of patients has occurred. Success in a similar proportion of leukaemic patients transplanted in remission may also be possible (HERSHKO and GALE, 1980).

The response of human recipients to skin allografts has been extensively studied but clinical application has been mainly limited to temporary cover of severely burnt patients (BALLANTYNE and CONVERSE, 1980). Study of allografts of other organs and tissues; for example, pancreas and islets of Langerhans, liver, lung-heart preparations, and cartilage, also continues because successful transplantation of these allografts has considerable potential for alleviating suffering and preserving life if problems of rejection can be safely overcome (MARSHALL, 1982).

Rejection of vascularized allografts may be hyperacute, accelerated, acute or chronic (WILLIAMS, 1979). Since the recognition of the importance of ABO blood group compatibility (STARZL ET AL, 1964)

and the development of cross-matching (PATEL and TERASAKI, 1969), hyperacute rejection can now largely be avoided (NAJARIAN and ASCHER, 1979; MORRIS, 1982). Recent refinement of the cross-match test has reduced the number of patients who would otherwise have been excluded from transplantation because of a positive reaction (TING, 1982). Accelerated rejection is relatively rare in human organ transplantation (WILLIAMS, 1979, BIEBER ET AL, 1970). Both hyperacute and accelerated rejection show little response to treatment (MORRIS, 1982). Acute rejection and the complications of the conventional immunosuppressive therapy used to treat this response are major causes of failure of renal and cardiac transplantation (SIMMONS, ET AL, 1977; JAMIESON ET AL, 1982). Chronic rejection also causes loss of many allografts and is not prevented by current immunosuppressive treatment (KIRKMAN ET AL, 1982; MORRIS, 1982).

Current immunosuppressive therapy is non-specific and based on azathioprine and steroids (prednisolone and methylprednisolone) and is sometimes supplemented with antilymphocyte globulin and irradiation of the graft (SALAMAN, 1982). The toxicity of the regime is well-known and side-effects include infection, impaired growth and wound healing, bone disease, cataracts, diabetes, obesity and Cushingoid appearance, gastrointestinal perforations, pancreatic and hepatic dysfunction, hypertension, malignancy and psychiatric disturbances. The present risks of immunosuppression permit the use of vascularized allografts only where the patients alternatives are death or restrictive and inadequate therapy such as haemodialysis (JAMIESON ET AL, 1982; STEWART, 1982). Currently, a new non-specific immunosuppressant, Cyclosporin A, is being clinically evaluated in human beings for the suppression of rejection of allografts and, in the case of bone marrow grafts, graft-versus-host disease.

The problems of non-specific immunosuppression have stimulated considerable research seeking alternative, safer means of preventing rejection. Specific immunosuppression, based on the hypothesis that the action of the lymphocyte clones that lead to the rejection of a

particular graft in a recipient can be selectively suppressed, has been actively pursued (FABRE, 1982). Passive and active enhancement of allograft survival involves treatment of the recipient with antibody against donor histocompatibility antigens and by exposure to donor histocompatibility antigens respectively. Successful enhancement has been demonstrated in some experimental systems but this approach has not been of clinical value as yet.

Another approach has been to reduce the immunogenicity of the graft and some advances have been made in this area. Matching of ABO blood group antigens is performed following the recognition that they act as histocompatibility antigens (STARZL ET AL, 1964; CEPPELLINI ET AL, 1966; GLEASON and MURRAY, 1967). Mismatching of Lewis and the P1-P2 blood group antigens may also lead to lower survival rates of allografts in man (ORIOLE ET AL, 1978; CEPPELLINI ET AL, 1966; GLEASON and MURRAY, 1967).

Much effort has been directed towards matching the major histocompatibility antigens of the donor and the recipient (TING, 1982). Renal allograft survival rates have improved with histocompatibility matching of living-related donors and recipients; for example, graft survival rates at 1 year of 90% when both haplotypes were matched and only 60% when both haplotypes were mismatched have been observed. Matching for the HLA-A and -B loci has improved survival rates of cadaveric renal allografts by 10% to 20% in some centres. Histocompatibility matching has been considered of great importance for bone marrow allografts and sibling donors matched for both haplotypes have been considered mandatory. A beneficial effect of HLA antigen matching may even occur in corneal allograft transplantation when there is pre-existing vascularization of the recipients' corneas. Improved survival rates of renal allografts have recently been demonstrated following matching at the HLA-DR locus. Incompatibility at this locus may be a major stimulus of the immune response in the recipient. Additionally the DR locus appears to be less polymorphic than the HLA-A and B loci so that the chances of matching donors and recipients would be improved if only the DR locus had to be matched. However, rejection and graft-versus-host

disease may still occur when all major histocompatibility antigens are matched and this is thought to be due to incompatibility at undefined minor histocompatibility loci.

Relationships between the ABO blood group of the recipient and HLA-A and B matching have been recognized in human cadaveric kidney transplantation (OPELZ and TERASAKI, 1977). HLA matching did not improve graft survival in blood group O-donor to O-recipient combinations but did in other matched combinations of ABO blood groups. Blood group B-donor to B-recipient combinations fared significantly worse than other combinations.

Pretreatment of the grafts may also reduce their immunogenicity by removing cells that are particularly effective at evoking a rejection response from the host. Pretreatment of renal grafts in rats with cytotoxic drugs, steroids and irradiation has shown considerable success (GUTTMANN and LINDQUIST, 1969). However, early promising results in man have not been confirmed in later better controlled studies (SALAMAN, 1982). Culture of thyroid tissue and islets of Langerhans, thought also to deplete the more immunogenic cells, has been shown to be effective in experimental situations (LAFFERTY, 1980; SUTHERLAND, 1981) but has not achieved clinical application as yet. Attempts have also been made to prevent stimulation and/or expression of the host's immune response by enclosing grafts in a membrane which prevents cellular contact between the graft and the host but which allows passage of nutrients and secretions. Some promising results have been obtained with allogeneic and xenogeneic tissues enclosed in membranes (STURGIS and CASTELLANOS, 1957; GATES and LAZARUS, 1977; ALGIRE ET AL, 1954) but more recent studies have been discouraging (GARVEY ET AL, 1979; THEODOROU ET AL, 1980; TZE and TAI, 1982).

Recently it has become obvious that recipients of renal allografts who had received prior blood transfusions have better graft survival rates than those not transfused (TING, 1982; OPELZ and TERASAKI, 1980). The reason for this beneficial effect is not well understood but may involve enhancement or the selection of patients

who are less immunologically responsive to allogeneic tissues; that is, those that do not develop a positive cross-match are transplanted.

From the foregoing it can be seen that allografts now have an established place in the treatment of human disease but there remains much room for improvement particularly in the use of vascularized allografts.

THE IMMUNOLOGY OF ALLOGRAFT REJECTION

MEDAWAR (1944) established that allograft rejection was the result of an immunological response by the recipient when he demonstrated an accelerated reaction to second-set skin allografts. A central role of lymphocytes, and more specifically T lymphocytes, in allograft rejection has been demonstrated in a number of ways. Following the study of adoptive transfer of immunity to tumors (MITCHISON, 1953), accelerated rejection of skin allografts on non-sensitized mice was shown to follow the adoptive transfer of lymph node or spleen cells from mice syngeneic with the recipients which had rejected skin grafts from the same donors (BILLINGHAM ET AL, 1954). T lymphocytes were implicated by the marked impairment of the rejection of skin allografts in nude mice and in other rodents which had been depleted of these cells (MILLER and OSOBA, 1967; ROLSTAD and FORD, 1974; WORTIS, 1971). The reintroduction of T lymphocytes to such rodents reconstituted their ability to reject allografts (SPRENT and MILLER, 1971; HALL ET AL, 1978; ROLSTAD and FORD, 1974). More recent experiments have demonstrated that T lymphocytes of the Lyt 1⁺2⁻ subclass from sensitized or naive mice can reconstitute the ability of T lymphocyte-deficient syngeneic mice to reject allografts but those of the Lyt 1⁺2⁺ subclass do not (LOVELAND and MCKENZIE, 1982). Other studies have produced similar results (DALLMAN and MASON, 1982; KIM ET AL, 1983). However, PROWSE ET AL (1983) have found that sensitized T lymphocytes of the Lyt 1⁺2⁺ subclass were required for the rejection of long-surviving pancreatic islet allografts.

The afferent arm of the immune response to allografts

The stimulation of an immune response that leads to allograft rejection is usually dependent on exposure of the recipient to alloantigen on viable cells and passenger leukocytes, contained in the blood vessels and tissues of the graft, have been considered to play an important role in this regard (SNELL, 1957; STEINMULLER and HART, 1971). More recently attention has focused on dendritic cells as potent stimulators of alloreactivity (LECHLER and BATCHELOR, 1982).

The lymphatic system into which donor cells or sensitized host cells may drain is a major route by which the recipient is sensitized to allografts. This has been shown by the delay in the rejection of allografts transplanted to sites where the lymphatics have been transected (BILLINGHAM ET AL, 1954; LAMBERT ET AL, 1965) and the absence of lymphatics in most of the immunologically privileged sites (BARKER and BILLINGHAM, 1977). Peripheral sensitization of the recipient; that is, within the allograft, also occurs and may result from recipient lymphocytes encountering alloantigens on the vascular endothelium of allografts or while migrating through the tissues of the allograft (STROBER and GOWANS, 1965; PEDERSEN and MORRIS, 1970; NAJARIAN and ASCHER, 1979; WUSTRACK ET AL, 1975).

At the cellular level, in vitro studies have indicated that the activation of T lymphocytes to mount an effective response to allogeneic cells requires that they receive 2 signals (LAFFERTY ET AL, 1983; BACH ET AL, 1976). The examination of this hypothesis has primarily centered on the generation of T lymphocyte-mediated cytotoxicity. The first signal is regarded as foreign major histocompatibility antigen. LAFFERTY ET AL (1983) maintain that the second signal is a lymphokine (Interleukin 1) released by the stimulating allogeneic cell. Proliferation of the activated T lymphocytes occurs in the presence of Interleukin II. Only certain cells of the allograft are considered able to deliver the second signal; for example, lymphocytes, macrophages and dendritic cells, but all cells of the allograft are susceptible to the subsequent immunological response. BACH ET AL (1976) have postulated instead

that class II major histocompatibility antigens activate helper T lymphocytes which provide a second signal to facilitate the activation and expansion of primed cytotoxic T lymphocytes. However, LAFFERY ET AL (1983) point out that, although the cells which stimulate a cytotoxic response usually carry class II antigens, cells without these antigens have also been shown to be effective. This indicates that the presence of class II antigens may not be mandatory for a cytotoxic T lymphocyte response. WIDMER and BACH (1983) have recently described a cytotoxic T lymphocyte clone which they consider requires only antigen to stimulate its proliferation. The relevance of this finding to the present models of T lymphocyte activation and allograft rejection remains to be established.

The efferent arm of the immune response to allografts

A number of mechanisms by which the recipient may destroy allogeneic cells have been identified (MCCLUSKY, 1980; CARPENTER, 1981; CARPENTER ET AL, 1976; FABRE and TING, 1979; STEINMULLER, 1982). These may lead to specific destruction of the allogeneic cells that sensitized the responding lymphocytes or may be non-specific in that the ultimate mediators generated during the immune response are able to damage cells whether or not they carry the same alloantigens as the cells that stimulated the response.

Specific lysis of allogeneic cells may be mediated by allo-antibody and complement (CARPENTER ET AL, 1976). Additionally, the binding of antibody and complement to antigens on cells may have other effects in vivo. The components of the complement cascade may be chemotactic for polymorphonuclear leukocytes and macrophages and induce the release of destructive products from polymorphonuclear leukocytes. Another avenue whereby antibody may lead to injury of allografts is the formation of immune complexes with antigen which localize in the graft. These complexes may also lead to the release of destructive products from polymorphonuclear leukocytes (HENSON, 1971 a and b; ARCHER and HIRSCH, 1963).

Antibody-dependent cellular-cytolysis may also cause lysis of allogeneic cells. Non-sensitized cells including lymphocytes, mononuclear phagocytes and neutrophil and eosinophil granulocytes have been shown to mediate this reaction in the presence of specific antibody (WAHLIN ET AL, 1976; GREENBERG ET AL, 1973; LOPEZ and SANDERSON, 1982; GALE and ZIGHELBOIM, 1975). Non-phagocytic mononuclear cells which mediate this type of cytolysis may express neither T nor B cell markers and they are referred to as K-cells (RAMSHAW and PARISH, 1976; GREENBERG ET AL, 1975). However, T and B lymphocytes sensitized to the target cells have also been shown to mediate this type of cytolysis (KIMURA ET AL, 1977; LAMON ET AL, 1975). Antibody-dependent cellular-cytotoxicity may be mediated by antibodies of either the IgG or IgM classes (WAHLIN ET AL, 1976; LAMON ET AL, 1975). K-cell mediated antibody-dependent cellular-cytotoxicity has been shown to occur in the presence of very low concentrations of specific antibody, concentrations which were insufficient for complement-mediated lysis (PERLMANN ET AL, 1972a and b; GALE and ZIGHELBOIM, 1975). Contact between the K-cells and target cells is required for the mediation of lysis and zeiosis (see below) is characteristically observed (SANDERSON, 1981). The K-cells appear not to be damaged during the mediation of lysis and may kill a number of target cells. However, an unexplained decline in the activity of K-cells with time is observed when they are co-cultured with target cells (ZIEGLER and HENNEY, 1975). Antibody-dependent cellular-cytotoxicity of endothelium and myocardiocytes has been demonstrated in vitro (HIRSCHBERG ET AL, 1975; PARTHENIAS ET AL, 1979).

Non-sensitized macrophages, specifically armed by a factor produced by immune cells, may also be able to prevent division of target cells to which the immune cells were sensitized (EVANS ET AL, 1972). The macrophage arming factor appeared to be too small to be an immunoglobulin and its production appeared to be dependent on the presence of thymus-derived lymphocytes.

Specifically sensitized cytotoxic T lymphocytes also represent a mechanism by which allogeneic cells may be destroyed (CARPENTER,

1981; MCCLUSKY, 1980). The events leading to the lysis of target cells in vitro can be divided into 3 stages; 1) recognition of the target cell by the cytotoxic T lymphocyte, 2) binding of the cytotoxic T lymphocyte to the target cell, and 3) programming of the target cell for lysis (SANDERSON, 1981; BERKE, 1983). Time-lapse cine studies have shown that lysis of the target cells is accompanied by a characteristic violent blebbing of the cell surface and shedding of cell fragments termed zeiosis (SANDERSON, 1976). WYLLIE ET AL (1980) consider this phenomenon to be the same as apoptosis, a form of cell death that occurs physiologically and in some pathological states. In vitro studies have shown that the effector T lymphocytes are not damaged during their interaction with the target cells and may kill a number of target cells (ZAGURY ET AL, 1975; KOREN ET AL, 1973). Most studies on T lymphocyte-mediated cytotoxicity have employed lympho-myeloid cells as targets but lymphocyte-mediated lysis of endothelium has also been demonstrated in vitro (HIRSCHBERG ET AL, 1975). Lymphotoxin has been suggested as the mediator of this type of cytotoxicity (ROSENAU and TSOUKAS, 1976) but other possible mechanisms are being actively studied (BERKE, 1983). Susceptible cells exposed to lymphotoxin show a reduced capacity to proliferate and undergo lysis during which zeiosis is observed in most of the cells (RUSSELL ET AL, 1972).

A delayed-type hypersensitivity reaction may lead to the generation of cytotoxic effector mechanisms which, although lacking specificity for the allogeneic cells, may also represent a means by which allografts are destroyed (MCCLUSKY, 1980; LIEW, 1982). The generation of such a reaction is dependent on stimulation of sensitized T lymphocytes with specificity for the alloantigens of the graft. These lymphocytes release lymphokines leading to the accumulation and activation of macrophages at the site of antigen presentation. Activated macrophages have been shown to release numerous potentially noxious substances, including hydrogen peroxide and proteases (NATHAN ET AL, 1980), which may damage cells in their immediate vicinity.

Natural killer cells are also capable of lysing cells without showing specificity for alloantigens (HERBERMAN and HOLDEN, 1978) and the involvement of these cells in allograft rejection has been suggested (NEMLANDER ET AL, 1983). Additionally, recent work has shown that cytotoxic T lymphocytes may lose their specificity and show natural killer cell-like activity under certain in vitro cell culture conditions (BROOKS, 1983).

TYPES OF ALLOGRAFT REJECTION AND IN SITU EFFECTOR MECHANISMS

Acute rejection

Skin allografts. It is well known that a blood circulation is re-established in skin allografts in man and experimental animals (TAYLOR and LEHRFELD, 1953; CONVERSE and RAPAPORT, 1956) but the manner in which this occurs has been controversial. Anastomosis of the graft and host vessels has been proposed but ingrowth of host vessels either into pre-existing vascular channels or by creating new channels has also been suggested (BALLANTYNE and CONVERSE, 1980). Evidence that donor endothelium is retained by skin allografts has been recently provided by studies of the rejection of these grafts on immunosuppressed rodents by antibody and complement (JOOSTE and WINN, 1975; GERLAG ET AL, 1980).

The time of rejection of skin allografts has been determined by various criteria but cessation of blood flow in vivo, escharification determined macroscopically, and damage to the epithelium determined by light microscopy have been those most commonly used. Where major histocompatibility differences between donor and recipient have existed as in outbred animals and man and between inbred strains of animals, rejection has usually been observed from 8 to 14 days after transplantation (CONVERSE and RAPAPORT, 1956; HENRY ET AL, 1962; MEDAWAR, 1944; WIENER ET AL, 1964; TAYLOR and LEHRFELD, 1955). An effect of the size of the allograft has also been observed with delayed rejection of small and very large grafts (TAYLOR and LEHRFELD, 1955; MEDAWAR, 1944; BALLANTYNE and CONVERSE, 1980).

Initially, auto- and allografts of skin are both infiltrated by leukocytes which may include polymorphonuclear leukocytes, lymphocytes, monocytes and macrophages. The numbers of leukocytes within autografts recedes with time but the infiltration of mononuclear leukocytes in the allografts progressively increases (HENRY ET AL, 1962; MEDAWAR, 1944; ROLLE ET AL, 1959; ROTHWELL AND PAPADIMITRIOU, 1972). The infiltration of the allografts occurs firstly around vessels and may be largely confined there and/or to the graft bed until rejection or it may spread into the allogeneic dermis (DVORAK ET AL, 1979; WAKSMAN, 1963; HENRY ET AL, 1962; DEMPSTER, 1977; MEDAWAR, 1944; SPARROW, 1953; BAUER, 1958). The epidermis may also be substantially infiltrated by the leukocytes (SCOTHORNE and MCGREGOR, 1953; SPARROW, 1953; WIENER ET AL, 1964; HENRY ET AL, 1962; GUTHY ET AL, 1974; WAKSMAN, 1963).

ROTHWELL and PAPADIMITRIOU (1972) examined the leukocytes infiltrating skin allografts on mice by electron microscopy. They found at 8 days after transplantation that the infiltrating mononuclear leukocytes were predominantly mononuclear phagocytes while lymphocytes were the next largest population. Neutrophil and eosinophil granulocytes represented up to 20% and 2% of the infiltrating cells in the allografts respectively and these levels were significantly higher than in the autografts. DEMPSTER (1977) also examined the leukocytes in skin allografts by electron microscopy and found that macrophages were the predominant cell type. However, BHAN ET AL (1982) using anti-T4 and anti-T8 monoclonal antibodies for immunohistochemistry and a non-specific esterase stain on sections of rejecting human skin allografts found that the majority of the mononuclear leukocytes were T lymphocytes while fewer B lymphocytes and macrophages were observed. T4 positive lymphocytes were found on average to be twice as numerous as T8 positive lymphocytes in the perivascular regions and the graft bed while T8 positive lymphocytes were more common in the epithelium.

With few exceptions, vascular damage has been reported as a prominent feature of the rejection of skin allografts but the role of ischaemia in the destruction of the allografts has been

controversial. CONVERSE and RAPAPORT (1956) and TAYLOR and LEHRFELD (1955), using in vivo stereomicroscopy, found that cessation of blood flow in the grafts preceded their destruction as determined by macroscopic observation. Researchers using transparent chambers in mice to make in vivo observations have observed continuing blood flow in some vessels of rejecting skin allografts for prolonged periods after damage to other tissues of the grafts (EDGERTON and EDGERTON, 1954; EDGERTON ET AL, 1957; CONWAY ET AL, 1957). EDGERTON and EDGERTON (1954) suggested on the basis of this that ischaemia was not important in the destruction of the allografts. Conclusions from histological studies have been similarly confusing with damage to blood vessels before destruction of the epidermis being cited as evidence that ischaemia leads to graft destruction (DVORAK ET AL, 1979; WAKSMAN, 1963) while others have concluded that ischaemia was not consistently the cause of graft failure or was not the predominant cause of graft failure (MEDAWAR, 1944; HENRY ET AL, 1962; WIENER ET AL, 1964). Observations on the relationship between damage to the extravascular cells of the dermis and damage to vessels have been made less frequently. DVORAK ET AL (1979) reported that as vascular damage progressed necrosis resembling infarction involved all cell types in the dermis and sometimes superficial areas of the host tissue. MEDAWAR (1944) observed in 'violent' forms of rejection that the cells of the dermis and epidermis above the band of infiltrating cells had degenerated. The conclusion made from both studies was that the cell degeneration was due to ischaemia.

Approaches combining both in vivo observations on blood flow and histological examination would appear to be the best way to determine the importance of ischaemia in the rejection of skin allografts but the conclusions of workers taking this approach have also been in conflict. SCOTHORNE and MCGREGOR (1953) used diffusion of bromophenyl blue from the blood into the tissues of skin allografts as a measure of blood supply and related this to the histology of serial biopsies. Their observations indicated that blood flow ceased simultaneously with or up to 48 hours before the destruction of the epidermis. They concluded that some other

factor, apart from ischaemia, was involved in the destruction of the allografts because the epidermis in newly transplanted grafts survived 3 days or more without evidence of blood circulation. ROLLE ET AL (1959) examined skin grafts on mice by in vivo stereomicroscopy, by diffusion of bromophenyl blue and by histology. They found that degeneration of the dermal and epidermal cells of the allografts occurred 24 to 48 hours after complete stasis of blood flow. On the basis of this and other experiments where the blood supply to skin was interrupted below the level of the panniculus carnosus, they concluded that destruction of the allografts was due to ischaemia. However, the absence of damage to the grafts during similar periods of ischaemia immediately after transplantation appeared to conflict with this conclusion. GUTHY ET AL (1974) also used in vivo stereomicroscopy and histology to study skin allograft rejection but the relationship between the observations made by the two methods was not extensively described. The authors observed two forms of allograft rejection in which technical failure appeared not to have played a part; one form was thought to be due to vascular damage and subsequent ischaemia while in the other form lymphocytes were thought to have attacked both vascular endothelium and the epidermis of the allografts.

Early attempts to cause rejection of skin allografts by humoral alloantibody mainly failed or showed inconsistent effects (STEINMULLER, 1962; BARKER and BILLINGHAM, 1968). However, more recent experiments have shown that skin allografts can be destroyed by humoral antibody and exogenous or endogenous complement but that their susceptibility varies with time after transplantation (JOOSTE and WINN, 1975; GERLAG ET AL, 1980). The allografts became susceptible to the antibody-mediated damage earlier in the experiments performed by GERLAG ET AL (1980) who suggested that susceptibility was dependent on the re-establishment of blood circulation in the allografts. JOOSTE and WINN (1975) suggested that immaturity of the vessels of the allografts explained their initial lack of susceptibility to the damage. They concluded that the involvement of humoral antibody in the destruction of skin

allografts was unlikely because the allografts were not susceptible to this type of damage at the time that acute rejection usually occurred and serum from recipients that had rejected first-set skin allografts was not effective in mediating the rejection of the allografts in their experimental system.

Apart from the morphological identification of infiltrating cells and their location in relation to damage in rejecting skin allografts, little has been done to identify further the cell types or the cellular mechanisms that may contribute to rejection except for the studies of DVORAK ET AL (1979) and BHAN ET AL (1982). Their observations led them to postulate that the rejection of skin allografts followed damage to the vasculature by either specific cell-mediated cytotoxicity or a delayed-type hypersensitivity reaction. They suggested that a delayed-type hypersensitivity reaction was the predominant mechanism because of the distribution of T4 positive cells around vessels and the non-specificity of the reaction shown by the damage to host tissues. BILLINGHAM and SPARROW (1954) had earlier suggested that a delayed-type hypersensitivity reaction led to the rejection of allogeneic epidermal epithelium because they also observed damage to the host tissues underlying these grafts.

Organ allografts. Although transplantation of a number of different organs has been studied, most experimental work has concentrated on kidney and heart allografts and only these will be considered here. Functional criteria can be used to ascertain rejection in these organs; for example, anuria and renal creatinine clearance and cardiac contraction and electrocardiographs, but technical failure and disease must still be distinguished from rejection. Acute rejection of organ allografts in non-immunosuppressed animals where there are major histocompatibility differences between donor and recipient usually occurs from 5 to 12 days (SIMONSEN ET AL, 1953; KLASSEN and MILGROM, 1969; GUTTMANN ET AL, 1967; KOSEK ET AL, 1968; TILNEY ET AL, 1976; CHRISTMAS and MACPHERSON, 1982). In immunosuppressed human recipients, acute rejection of renal allografts occurs most frequently in the first two months after

transplantation but it may also occur much later; for example, 5 years after transplantation (DUNNILL, 1979). Acute rejection episodes occur in 90% of human patients transplanted with cardiac allografts at 8 to 12 weeks after transplantation (JAMIESON ET AL, 1982). Successful transplantation of organ allografts is dependent on surgical re-establishment of blood circulation to the graft. The allografts retain donor endothelium unless there are episodes of active rejection (SINCLAIR, 1972; BURDICK ET AL, 1979; HART ET AL, 1980).

Leukocytic infiltration occurs predominantly in the cortex of first-set kidney allografts and is almost exclusively by mononuclear leukocytes (SIMONSEN ET AL, 1953; FELDMAN and LEE, 1967; GUTTMANN ET AL, 1967; LINDQUIST ET AL, 1971). The leukocytes are distributed around small vessels and the glomeruli initially and then they extend around larger vessels and become generalized throughout the interstitium. Polymorphonuclear leukocytes, plasma cells and macrophages become more obvious amongst the infiltrating leukocytes from the time of functional failure of the rejecting allografts. In first-set cardiac allografts in rats, MACSWEEN ET AL (1971) reported that the mononuclear leukocytes appeared firstly under the endo- and epicardium and around vessels and then extended into the myocardium. The intensity of the infiltrate progressively increased until rejection. Plasma cells were identified at 4 to 5 days after transplantation and polymorphonuclear leukocytes and macrophages were identified at 10 days. TILNEY ET AL (1976) made similar observations on rat cardiac allografts but, utilizing intravenous injections of India ink, observed macrophages scattered in the myocardium from 3 days after transplantation.

Recent electron microscopic studies (DEMPSTER, 1977; CHRISTMAS and MACPHERSON, 1982) have identified mononuclear phagocytes as the major cell type infiltrating both renal and cardiac allografts at the time of rejection. DEMPSTER (1977) found that morphologically typical lymphocytes were uncommon while blast-like and plasma cells formed only minor proportions of the cellular infiltrate. CHRISTMAS and MACPHERSON (1982) used a stain for endogenous peroxidase in

been prognostic of irreversible rejection. In the detailed study in conjunction with electron microscopy to study cardiac allografts in rats. They found that lymphocytes predominated at 3 days after transplantation but that mononuclear phagocytes were more than twice as numerous as lymphocytes by 5 and 7 days. These authors also found that at 7 days after transplantation control isografts had been infiltrated by fewer mononuclear phagocytes but contained equal or greater numbers of lymphocytes compared to the allografts.

The proportions of the different types of leukocytes in cells isolated from renal and cardiac allografts have also been studied but the degree to which the isolates represent the in situ population is questionable, especially where attempts to separate the leukocytes from the parenchymal cells were made (DEMPSTER, 1977; VON WILLEBRAND ET AL, 1979a). The studies have shown that T, B and null (expressing neither T nor B cell markers) cells were present in the allografts at the time of rejection (TILNEY ET AL, 1979; VON WILLEBRAND and HAYRY, 1978; VON WILLEBRAND ET AL, 1979a; STROM ET AL, 1977). However, the proportions of the different types of infiltrating cells have sometimes shown wide differences between studies; for example, STROM ET AL (1977) reported that the cells isolated from rat cardiac allografts were 68% T lymphocytes and 20% B lymphocytes while VON WILLEBRAND ET AL (1979a) found that only 31% of cells isolated from rat renal allografts were lymphocytes of which 23% and 14% were T and B lymphocytes respectively while the remainder were null cells. In both of these studies, cells isolated from isografts contained T, B and null cells in similar proportions to those observed in the cells isolated from the allografts.

Studies combining histology with in situ identification of leukocytes by monoclonal antibodies have shown larger numbers of T lymphocytes than B lymphocytes in acutely rejecting human renal allografts (PLATT ET AL, 1982; HANCOCK ET AL, 1983). These studies also showed that OKT8-positive T lymphocytes predominated over OKT4-positive T lymphocytes. However, VON WILLEBRAND (1983) found that OKT4-positive T lymphocytes outnumbered OKT8-positive T lymphocytes in renal allograft biopsies of some patients and that this may have

been prognostic of irreversible rejection. In the detailed study performed by HANCOCK ET AL (1983), renal allograft biopsies were divided into those showing mild, moderate and severe rejection, depending on the intensity of leukocytic infiltration. The mean proportion of leukocytes that were T lymphocytes was lower in severe than in mild or moderate rejection (15% versus 34% to 42%). The mean proportions of macrophages and polymorphonuclear leukocytes were higher in severe than in the less severe grades of rejection. (60% versus 38% to 52% and 22% versus 4% to 9% respectively). The mean proportions of B lymphocytes and plasma cells did not exceed 9% of the leukocytes in any grade of rejection. The distribution of the T lymphocytes was predominantly perivascular in mild rejection, but increasing numbers were diffusely distributed in the interstitial tissue in moderate rejection. The other types of leukocytes showed diffuse distribution in all grades of rejection. It has become obvious recently that the OKT4- and OKT8-positive T lymphocytes do not necessarily show strict separation of their functional specificities (HANCOCK, 1984).

At the time of functional failure in rejecting renal allografts, the endothelium of vessels of all sizes may be enlarged, proliferating or sloughing (GUTTMANN ET AL, 1967; SIMONSEN ET AL, 1953; PORTER 1965). HOBBS and CLIFF (1973) observed in vivo a characteristic form of platelet adherence to endothelium in rejecting renal allografts in the rabbit ear chamber. PORTER (1965) observed thrombi in the interlobular arteries and breakdown of peritubular capillaries and venules. Medial fibrinoid necrosis may be observed in some medium and small arteries (SIMONSEN ET AL, 1953; PORTER 1965). Glomeruli may be largely unaffected (SIMONSEN ET AL, 1953; FELDMAN and LEE, 1967) or show occlusion of capillary loops and necrosis of cells (GUTTMANN ET AL, 1967). Necrosis of tubules has been observed from 4 days after transplantation (FELDMAN and LEE, 1967). In other studies this was first noted at functional failure (GUTTMANN ET AL, 1967; LINDQUIST ET AL, 1971). SIMONSEN ET AL (1953) observed that tubular destruction occurred in areas of intense cellular infiltration but areas of coagulative necrosis, involving glomeruli, were also observed.

Rejection of cardiac allografts in dogs has been described by KOSEK ET AL (1968, 1969a). Vascular endothelial enlargement or degeneration and apparent arteriolar spasm were noted within 4 days of transplantation. At the time of acute rejection, the endothelial changes were still present and other vascular changes included engorgement, thrombosis, rupture of venules and capillaries, and medial and intimal necrosis of small arteries and arterioles. Myocytes showed necrosis and were phagocytosed by macrophages.

In the rat, TILNEY (1974) did not observe vascular damage until after total destruction of the myocardium had occurred. CHRISTMAS and MACPHERSON (1982) also noted that the vasculature of rejecting rat cardiac allografts showed little morphological abnormality. MACSWEEN ET AL (1971) found necrosis of individual myocardial fibres and minor damage to vessels between 6 and 10 days after transplantation. In contrast, FORBES ET AL (1983) reported that diffuse microvascular endothelial injury preceded widespread damage to myocytes in acutely rejecting cardiac allografts in rats.

The relationship between the damage to vessels and that to the parenchyma in acutely rejecting renal and cardiac allografts has led some workers to conclude that ischaemia was the dominant mechanism by which the allografts were destroyed (PORTER, 1965; LINDQUIST ET AL, 1971; FORBES ET AL, 1983). Others have not been convinced of the role of ischaemia and considered direct damage by immunological mechanisms an alternative possibility (FELDMAN and LEE, 1967; GUTTMANN ET AL, 1967). Blood flow rates through kidneys have been shown to decrease during acute rejection and this has been used to support the concept that rejection was due to ischaemia (KOUNTZ ET AL, 1963).

The role of antibody in acute rejection of organ allografts has been the subject of much investigation. Immunoglobulin (IgM and IgG) and complement have been demonstrated in lesions in the walls of arteries and arterioles, on the endothelium of vessels and in some of the cells infiltrating the allografts (PORTER, 1965; LINDQUIST ET AL, 1971; BIEBER ET AL, 1970; BUSCH ET AL, 1977; MACSWEEN ET AL,

1971; PAUL ET AL, 1979). However, the specificity of the immunoglobulin was not known and its presence in the walls of vessels could be due to passive diffusion following damage mediated by some other mechanism (KOSEK ET AL 1969a; MACSWEEN ET AL, 1971). Humoral allospecific haemagglutinating and cytotoxic antibodies have been demonstrated in renal allograft recipients (GUTTMAN ET AL, 1967). It was postulated that the late appearance of the antibody may indicate that it was of little importance in the rejection of the allografts or that, before its appearance in the blood, all that was available had been trapped in the allograft. The specificity of humoral antibody for donor endothelium in some human recipients of kidney allografts has been demonstrated and the appearance of these antibodies appeared to be associated with a poor prognosis for survival of the allograft (PAUL ET AL, 1979). Allospecific antibody has also been eluted from acutely rejecting kidneys and shown to be specific for donor endothelial antigens and able to mediate K-cell and complement-dependent lysis of donor cells (PAUL ET AL, 1979; JEANNET and LAMBERT, 1975). Fibrinoid necrosis of the arteries and other changes characteristic of rejection have also been shown to follow in vivo perfusion of kidneys with allospecific antibody (STRAUS ET AL, 1971).

The isolation of leukocytes from rejecting organ allografts capable of mediating lysis of donor cells has supported a role for specific cellular mechanisms in the rejection of these grafts. Specific cell-mediated cytotoxicity has been demonstrated by cells isolated from rejecting renal allografts in human beings and rejecting renal and cardiac allografts in rats (STROM ET AL, 1977; BUSCH ET AL, 1977; VON WILLEBRAND and HAYRY, 1978; VON WILLEBRAND ET AL, 1979b). STROM ET AL (1977) found that the cytotoxicity was associated with T lymphocytes and also demonstrated antibody-dependent lymphocyte-mediated cytotoxicity. Cells capable of mediating cytotoxicity of cells other than those of the donor type (non-specific cell-mediated cytotoxicity) have been isolated from acutely rejecting and non-rejecting renal allografts in rats (NEMLANDER ET AL, 1983; MASON and MORRIS, 1984). However, the susceptible target cells in all these studies were cells of lympho-myeloid or tumor origin. In

in vitro studies, leukocytes from human and rat renal allografts have shown minimal or no ability to lyse parenchymal cells from donor kidneys while leukocytes from rat cardiac allografts did mediate specific lysis of cultured heart cells from neonatal rats (VON WILLEBRAND and HAYRY, 1978; VON WILLEBRAND ET AL, 1979b; PARTHENAIS ET AL, 1979).

Other types of rejection

Hyperacute rejection. This form of rejection has a rapid onset and leads to white graft rejection of skin allografts (BALLANTYNE AND CONVERSE, 1980) and failure of kidney allografts within a period of minutes up to 2 or 3 days after transplantation (WILLIAMS, 1979).

The white graft rejection of skin allografts is so called because blood circulation to these grafts is not re-established (BALLANTYNE and CONVERSE, 1980). This type of rejection may occur in man and experimental animals following presensitization (CEPPELLINI ET AL, 1966; EICHWALD and DOLBERG, 1977). Rapid expression of the immune response is thought to preclude vascularization of the graft by causing early degenerative changes in the graft vessels and extensive necrosis of the graft (BALLANTYNE and CONVERSE, 1980). Morphological studies and in vivo tests of viability have demonstrated more rapid destruction of the tissue of white grafts than would be expected from ischaemia alone and indicates a direct effect of the recipient on the graft (HENRY ET AL, 1962; EICHWALD ET AL, 1976). The relative contributions of humoral and cellular immune mechanisms in this form of rejection remain unclear (EICHWALD ET AL, 1967; STETSON and DEMOPOULOS, 1958).

Hyperacute rejection of renal allografts in man was first described following transplantation in the face of ABO blood group incompatibilities (STARZL ET AL, 1964) and was later recognized in presensitized recipients with circulating cytotoxic antibodies to the donor tissues (KISSMEYER-NIELSEN ET AL, 1966; TERASAKI ET AL, 1968). Macroscopically, kidneys undergoing hyperacute rejection become cyanotic, flaccid and anuric and, if left in situ, cortical necrosis

develops (DUNNILL, 1979; PORTER, 1965; WILLIAMS, 1979). Ultra-structurally, the earliest change observed is the formation of platelet aggregates in glomerular and peritubular capillaries. This is followed by increased numbers of neutrophil granulocytes in capillaries and the endothelium of affected vessels shows cytoplasmic swelling and basement membrane disruption. Following this, the interstitium becomes haemorrhagic, most noticeably at the corticomedullary junction, and is infiltrated by neutrophil granulocytes.

Hyperacute rejection of organ allografts is thought to be mediated by antibody and complement (PATEL and TERASAKI, 1969; FORBES ET AL, 1979; HOLTER ET AL, 1972). Other studies have also implicated activation of the coagulation cascade and the kinin system (COLMAN ET AL, 1969; BUSCH ET AL, 1975).

Accelerated rejection. Accelerated rejection was first adequately described by MEDAWAR (1944) when he examined second-set skin allografts in rabbits. Skin allografts undergoing accelerated rejection survive long enough for blood circulation to be re-established but are rejected sooner than a first-set skin allograft (BALLANTYNE and CONVERSE, 1980). Histological features of this form of rejection include absence of epidermal proliferation, blood stagnation and disruption of the endothelium of the graft vessels. Leukocyte infiltration of the graft is usually minimal but may include lymphocytes, plasma cells and neutrophil and eosinophil granulocytes (MEDAWAR, 1944; HENRY ET AL, 1962). The leukocyte infiltration is usually most intense in the graft bed (BALLANTYNE and CONVERSE, 1980). The results of morphological studies have suggested that the destruction of grafts during accelerated rejection was due to ischaemia (BILLINGHAM ET AL, 1954; HENRY ET AL, 1962).

Second-set rejection of renal allografts was first described in the dog (SIMONSEN ET AL, 1953). Accelerated rejection of renal allografts in man is presumed to follow presensitization of the host to donor tissues (MORRIS, 1982; WILLIAMS, 1979). The striking features of kidneys rejected in this way are interstitial oedema and

haemorrhage, arteriolar fibrinoid necrosis and vascular thrombosis (SIMONSEN ET AL, 1953; MORRIS, 1982). Increased numbers of neutrophil and eosinophil granulocytes may be observed in blood vessels and the interstitium while in some of these kidneys there is an intense mononuclear leukocyte infiltration (SIMONSEN ET AL, 1953; WILLIAMS, 1979).

The immune mechanisms involved in accelerated rejection may be humoral, cellular or both (WILLIAMS, 1979).

Chronic rejection. Prolonged survival of skin allografts has been reported in human beings; for example, when exchanged between mothers and their children and between 2 haplotype identical siblings (PEER, 1957; CEPPELLINI ET AL, 1966; WARD ET AL, 1978). ROGERS ET AL (1959) using vivo stereomicroscopy and gross observation, found that some skin allografts transplanted between related rats survived for up to 200 days. Two types of chronic rejection termed scab and scar reactions were recognized while 'acute' rejection occurred up to 65 days after transplantation. In the scab reaction, the surface of grafts which were well established suddenly formed a dry scab. Allografts showing scar reactions were also initially well healed and stable but then gradually became smaller until only a thin linear scar remained. In late 'acute' rejection, stasis of blood flow and vascular breakdown was observed.

HILDERMANN and WALFORD (1960) observed chronic rejection of 93% of skin allografts exchanged between hamsters of a closed, outbred colony. Three types of chronic rejection were defined on the basis of the period of time that the grafts survived; rapid chronic rejection (19 to 61 days), intermediate chronic rejection (78 to 307 days), and prolonged chronic rejection (more than 307 days). The earliest histological changes during rapid chronic rejection were degeneration of the basal layer of the epidermis and partial destruction of epidermal appendages. An intense leukocytic

infiltration, involving mostly lymphocytes and 'reticulo-endothelial' cells, was observed around and obliterating epidermal appendages and similar leukocytes were also seen subepidermally and in the dermis. Numerous plasma cells were also found in the deep dermis. As rejection progressed, the number of epidermal appendages decreased and leukocytic infiltration of the grafts gradually declined. The epidermis was still intact at the endpoint of graft survival but it was suggested that the donor cells had been gradually replaced by host cells. The cellular reactions during intermediate and prolonged chronic rejection were milder. However, large numbers of mast cells were noted, particularly during the middle phase of prolonged chronic rejection.

The morphology of human renal allografts undergoing chronic rejection has been reviewed by DUNNILL (1979). The kidneys may be oedematous and pale or, in advanced cases, shrunken and fibrosed. Chronic rejection is characterized histologically by occlusive vascular changes. Occasionally these lesions may be present as early as 10 days after grafting and their severity increases with time after transplantation. The lesions in renal arteries include intimal proliferation, small foci of necrosis with fibrinoid change in the media, atrophy of the media and fragmentation and reduplication of the internal elastic lamina. Plentiful IgM, fibrin and complement and variable amounts of IgG can be demonstrated in the media. Changes in glomeruli may also be observed but may be due to recurrent glomerulonephritis or ischaemia. Immunoglobulin, predominantly IgM, and complement have been demonstrated by immunofluorescence in the glomeruli. Tubular atrophy is often seen but occurs particularly when arterial narrowing is severe. Interstitial fibrosis and oedema are prominent and are often accompanied by obliteration of peritubular capillaries and foci of lymphocytes and plasma cells.

Chronic rejection of renal allografts in immunosuppressed dogs has been reported by PORTER ET AL (1964). Morphological features were

similar to those described in man. The authors suggested that circulating antibody directed against the allografts damaged the arteries and led to the deposition of fibrin and platelets. If the kidney survived this damage, fibrosis of the lesions led to obliterative intimal thickening.

Cardiac allografts undergoing chronic rejection have been described in man and dogs where the recipients were immunosuppressed (BIEBER ET AL, 1970; KOSEK ET AL, 1968 and 1969b). The most consistent change in these allografts was obliterative intimal proliferation in the coronary arteries. Changes in the endothelium of vessels included hypertrophy or degeneration and sloughing. Immunoglobulin (IgG) and complement was rarely detected in the intima but was invariably present in the media of affected vessels (BIEBER ET AL, 1970). Degenerative changes in the myocardium included myocytolysis, subendocardial and papillary muscle fibrosis and infarcts. BIEBER ET AL (1970) suggested that the intimal proliferation may have been a reaction to recurrent intimal injury of immune origin and may have arisen also by organisation of thrombi on the intimal surface. KOSEK ET AL (1968, 1969b) suggested that the destructive lesions in the walls of arteries may have been due to ischaemia resulting from damage to capillaries in the adventitia during acute rejection episodes or due to penetration of the wall by cytotoxic antibody and complement.

Chronic rejection has been observed also in hearts transplanted between rats which were identical for major histocompatibility complex antigens (LADEN and SINCLAIR, 1971). The survival times of the transplanted hearts ranged from 9 to 110 days. Observations by light and electron microscopy suggested a sequence of events which led to intimal thickening of arteries. The lesions appeared to begin with endothelial cell swelling and necrosis and exposure of subjacent tissues. Inflammatory cells and fibrin accumulating on the intima were covered by regenerating endothelium. Organization of these lesions was accompanied by the appearance of myointimal cells and the deposition of collagen in the intima.

SUMMARY AND OBJECTIVES OF THE PRESENT WORK

The possible role of ischaemia in the acute rejection of allografts in vivo remains an area of considerable confusion despite a voluminous literature on this subject. Not only is the role of ischaemia unresolved but the possibility that it is a cause of cell degeneration in allografts complicates the elucidation of the immunological mechanisms that may lead directly to alteration or death of allogeneic cells in vivo. The conflicting views of the various researchers of allograft rejection may have arisen because ischaemia is a cause of damage to some acutely rejecting allografts in some donor-recipient combinations but not in others. Much confusion has also undoubtedly arisen because the degree of ischaemia can only properly be determined by direct observation in vivo. Few workers have related such in vivo observations to results obtained by other methods of examining allografts. In the few studies where this has been done, conclusions on the role of ischaemia have still been conflicting but doubts about the validity of some of the conclusions exist. It was against this background that the present work was undertaken with a view to re-examine the role of ischaemia in acute allograft rejection in technically superior preparations than had previously been used and to attempt to obtain further knowledge of the immunologically mediated mechanisms that lead to allograft rejection in vivo.

Allografts transplanted to rabbit ear chambers were studied because this preparation allows sequential, non-intrusive observation of vessels and cells in allografts in living animals. Additionally, much of the error due to the variability inherent in allograft rejection can be avoided by using rabbit ear chambers because samples for light and electron microscopy may be obtained with unparalleled precision in regard both to the stage of rejection and to location within the preparation. Furthermore technical failure of transplantation and infection can be recognized and the affected

grafts can be excluded from the analysis of rejection. Endometrium was chosen for transplantation because it is relatively accessible by surgery, is sterile, and most of the cells are readily identifiable in vivo, histologically and by electron microscopy. Additionally, grafts of this tissue could be prepared that were either vascular or non-vascular allowing further evaluation of the role of blood vessels during rejection. Acute rejection of vascular and non-vascular allografts of endometrial tissue transplanted to ear chambers and to subcutaneous sites were compared to determine how closely rejection in the ear chamber resembled that in more conventional preparations.

Ear chambers

Three types of 'round-table' ear chambers (WILLIAMS, 1954) were used in this study; graft chambers with a removable coverslip (CLIFF ET AL., 1963), a modified graft chamber more recently developed by Dr. W.J. Cliff, and an injection chamber developed during the course of this work (Figure II-2). Perspex glue (perspex dissolved in chloroform) was used to bind the bushes to the bases of the chambers and the perspex coverslip to the supporting ring of the lid of the injection chambers. Buffers 50 μ m. thick were attached to the central table of the chamber bases with Araldite (5-minute Araldite, Sulleys Chemical Co., Bankstown, Australia). The perspex ring and mica coverslip of the lids of the graft chambers were glued together with silastic glue (732 RTV, Dow Corning Corp., Midland, Michigan, U.S.A.). The injection tube of the injection chamber lids was held in

CHAPTER II

MATERIALS AND METHODS

Experimental animalsInsertion of ear chambers

Female rabbits from the outbred closed colony maintained at the John Curtin School of Medical Research, Australian National University, were used. These rabbits had large ears which facilitated the insertion and observation of the ear chambers. During the experiments, the rabbits were housed individually in metal cages in a room with alternating 12 hour periods of light and dark and received rabbit pellets (Doust and Rabbidge Pty. Ltd., Sydney, Australia) and water ad libitum. The average age of the rabbits when they received grafts was 9 months (range, 6.5 to 16 months) and their average weight was 4 kgs. (range, 3.5 to 5.25 kgs.). Except where indicated, the rabbits in each donor-recipient pair were selected to ensure that they had no parents in common.

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place with Supa Glue (Selleys Chemical Co, Bankstown, Australia) at the centre hole of the coverslip and with perspex glue at the supporting ring. The free end of the injection tube was inserted into the end of a tightly fitting polyethylene tube. Protective covers were attached to the ear chambers to prevent damage to the lids and the enclosed tissues.

Insertion of ear chambers

The rabbits were anaesthetized by intravenous injection of pentobarbitone sodium at 30 mg./kg. of body weight (Nembutal, Abbott Laboratories Pty. Ltd., Sydney, Australia) and given a subcutaneous injection of atropine sulphate at 0.12 mg./kg. of body weight (Lancet Pharmaceutical Ltd., Sydney, Australia). The fur was clipped from the ears, the pinnae were shaved and the external auricular meati were plugged with cotton wool. The surgical site was disinfected with 0.5% chlorhexidine gluconate in 70% alcohol (Hibitane, ICI Australia Operations Pty. Ltd., Sydney, Australia). A surgical plane of anaesthesia was induced by open ether inhalation (Anaesthetic Ether, Hoechst Australia Ltd., Melbourne, Australia) and aseptic surgical techniques were used.

A wooden block was placed under the ventral surface of the distal pinna and a template for the punches was positioned on the dorsal surface so that major vessels were avoided. Holes for the table and bushes of the ear chamber were punched through the pinna (Figure II-3A). A circle of skin slightly larger than the ear chamber was dissected from the perichondrium on both surfaces of the pinna (Figure II-3B). The site was flushed with saline containing 100 IU/ml. benzylpenicillin sodium (Crystapen injection, Glaxo Australia Pty. Ltd., Boronia, Australia). The ear chamber which had been disinfected in 10% neutral buffered formalin and then washed in saline containing penicillin was assembled around the perichondrium and cartilage (Figure II-3C). The free skin was cut away so that it just overlapped the edges of the ear chamber and gum tragacanth (H.B. Selby and Co. Pty. Ltd., Sydney, Australia) with acriflavine at 0.1 mg./ml. (BDH Chemicals Ltd., Poole, England) was

applied to seal and discourage infection in this region. A modified graft chamber established in the ear of a rabbit is shown in Figure II-4.

Maintenance of ear chambers

Gum tragacanth with acriflavine was applied regularly to the edges of the ear chambers. The tube of the injection chambers was cleared every 1 to 2 weeks. The procedure entailed disinfection of the ear and the surface of the chamber with 0.5% chlohexidine in water and aseptic technique throughout. Saline or Tyrode's solution was taken up into a 1 ml. syringe to which was attached a 21 gauge hypodermic needle from which the bevel had been removed. The needle was inserted into the end of the polyethylene tube and 0.2 ml. of the solution injected. The polyethylene tube was then sealed by grasping it with hot forceps and Supa Glue was also applied to the sealed end of the tube.

Hysterectomy

Premedication, preparation of the surgical site and anaesthesia were similar to that described for the insertion of ear chambers. The abdomen was opened by midline incision just caudal to the umbilicus and extending 5 to 7 cm. towards the pelvis. One horn of the uterus was exteriorized and ligatures were placed around the uterus, the oviduct and in the broad ligament to control haemorrhage. A segment of the uterine horn extending caudal from the utero-oviduct junction was removed from between the ligatures and placed in Hank's balanced salt solution containing 100 IU/ml. benzylpenicillin sodium (pH 7.4). The uterine horn was returned to the abdominal cavity and the abdominal incision was closed in layers. All ligatures were 2/0 chromic catgut (Ethicon, Ethnor Pty. Ltd., Sydney, Australia). When hysterectomies were performed on both the donor and the recipient, surgery was performed on the recipient first and separate instruments, materials and surgical gloves were used for the two operations.

Preparation and transplantation of grafts

Only ear chamber membranes that had healed completely and were free of infection received grafts. Aseptic technique was maintained throughout.

Endometrial grafts. A piece of uterus approximately 6 mm. square taken 1.5 cm. from the utero-oviduct junction was placed in Hank's solution containing penicillin. Under a dissecting microscope, a layer of endometrium was pulled away from the muscle coat with jeweller's forceps. Grafts were cut from the separated endometrium where it had not been crushed by the forceps. The grafts were transplanted shortly after hysterectomy while the rabbits were still anaesthetized.

Initial experiments in ear chambers were done in the earlier type of graft chambers but thereafter the modified graft chambers were used exclusively. The ears containing ear chambers were disinfected with 0.5% chlorhexidine gluconate in water. The coverslip or lid was carefully removed from the ear chamber to avoid creating an excessive vacuum over the membrane. This required that a hole adjacent to the table be cut in the mica lid of the modified graft chambers. Once the membrane had been exposed it was kept moist with Hank's solution. The grafts were placed on the membrane, excess fluid was removed, and a new coverslip or lid was replaced.

Sites for subcutaneous transplantation were prepared by making a 2 mm. long incision in the ventral surface of the distal pinna. A semi-sharp probe was pushed through the incision and between the skin and perichondrium to form 2 tracts, each approximately 1.3 cm. long, to form a 'V' shape proximal to the incision. Endometrial grafts were placed under the skin on the proximal side of the incision and pushed towards the end of each tract by pressure applied externally with the probe. Sufficient pressure to express fluid was then applied to the tracts with a swab and the edges of the incision were apposed.

Separated endometrial epithelium. A 3 cm. long segment from the end of a uterine horn was placed in Hank's solution and everted by passing the tips of a pair of artery forceps through its lumen, grasping a silk ligature (size 2; Mersilk, Ethicon Ltd., Edinburgh, U.K.) placed at the furthest end of the segment, and drawing that end back through the lumen. The ligature was then removed and the endometrium overlapped the muscle at the ends of the uterine segment. The everted uterus was washed for 20 minutes with rotary agitation in Tyrode's solution containing penicillin at 37° centigrade. The segment was then placed in 10 ml. of Tyrode's solution containing penicillin and Pronase (1 mg./ml.; B grade, Calbiochem, San Diego, California, U.S.A.) and incubated with rotary agitation at 37° centigrade. A 6 hour incubation time was chosen after preliminary experiments showed that this was the shortest time that ensured a successful take of the separated epithelium when it was transplanted to subcutaneous sites in rabbits' ears (Figure II-1).

Following the incubation, the cell suspension was centrifuged and the cell pellet was separated from the enzyme containing solution. The cells were then washed 3 times by alternating resuspension in 10 ml. of fresh ice-cold Tyrode's solution containing penicillin and albumin (Rabbit Albumin, Fraction V, Sigma Chemical Co., St. Louis, U.S.A.) with centrifugation. The cells were then resuspended in 1 ml. of the Tyrode's solution and the number of live nucleated cells calculated using Trypan Blue exclusion and a haemocytometer (MISHELL and SHIIGI, 1980). The viable cell concentration was then adjusted to the desired value in the Tyrode's solution.

Transplantation to the graft chambers involved removal of the lid as described above, placing several drops of the suspension on the membrane, allowing the cells to settle and replacing a new lid. When transplanting to the injection chambers, the injection tube of the chamber was first cleared with Tyrode's solution as described above. Then 0.1 ml. of the suspension was injected over the ear chamber membrane. Following this a small air bubble was admitted to the polyethylene tube and Tyrode's solution was injected until the air bubble just appeared at the centre hole over the ear chamber

membrane. The polyethylene tube was sealed as previously described. Subcutaneous transplantation was accomplished by injecting the cell suspension between the skin and perichondrium on the ventral surface of the distal pinna using a 1 ml. syringe and a 25 gauge hypodermic needle.

In vivo observations.

Ear Chambers. The rabbits were restrained in the supine position on a bed to which they had been made accustomed over the preceding weeks (Figure II-5). The base of the ear chamber was attached to a brass mount which was then fitted to the specially designed stage of a Lietz Biomed microscope. Routine observations were made using brightfield, long working distance objective lenses (Leitz; x1, x3.5, x10, and x20), a water immersion lens (Zeiss; x40), a long working distance condensor (Leitz) and a quartz iodine light source. Interference contrast optics were also used where indicated (Leitz; objective lenses x16, x25, and x32). Photomicrographs of the ear chambers were taken with Leica cameras using 35 mm. Kodak Plus-X, Ektachrome-64 and Kodachrome-64 films (Eastman Kodak Co., New York, U.S.A.). Video recordings were made using an Ikegami camera and control unit (Ikegami Tsushinki Co. Ltd., Tokyo, Japan), VO2630 video cassette recorder (Sony Corp., Tokyo, Japan) and 3/4 inch KCA 60 tapes (Sony Corp. and Fuji Photo Film Co. Ltd., Tokyo, Japan). The video recordings were viewed on a Sony Trinitron Color Video Monitor. Time-lapse cine was performed at a rate of 30 frames per minute using Vinten scientific cine cameras (W. Vinten Ltd., London, U.K.) and 16 mm. monochromatic cine film (Pan-F; Ilford Ltd., Basildon, England).

Semiquantitative scores in the present study were based on a scale of 1 to 5, where 1 represented absence of minimal expression of a feature and 5 represented the maximum possible expression of a feature. Thus, when rolling and sticking of leukocytes in vessels was scored, 1 represented margination of only a few leukocytes in the vessels and 5 represented rolling and sticking just short of

occlusion of the lumina of the vessels. The degree of ischaemia in the regions of the allografts selected for analysis was scored in a similar fashion where

1 = no interference to blood supply

2 = blood flow static in a low proportion of vessels but unaltered in others

3 = blood flow static in a low proportion of vessels and sluggish in others or blood flow static in a moderate proportion of vessels and unaltered in others

4 = blood flow static in a high proportion of vessels and sluggish in others

5 = blood flow static in almost all vessels.

Features of the grafts and the membrane vasculature in some chambers could not be observed in vivo at every examination (see Tables III-5 and V-2). For example, the edge of the grafts may be flush with the ear chamber membrane so that the epithelium here could not be viewed edge-on, struggling of the rabbit may make blood flow to the ear chamber erratic, or the accumulation of free cells over the grafts and membrane at the time of rejection may obscure details of the underlying tissues. Where a feature could not be confidently evaluated, the ear chamber was not included in the scores for that feature at that particular time.

Subcutaneous grafts. These grafts were inspected by incident and transmitted illumination from a fibre optic light source (Schott, Wiesbaden, West Germany). The dimensions of the subcutaneous swellings containing the grafts were measured with calipers (Schnelltaster, H.C. Kroeplin, Schluechtern, West Germany). Where only one graft was present an estimated volume of the swelling was calculated by

$$EV = \frac{L \times B \times \left(T_s - \frac{T_1 + T_2}{2} \right)}{2}$$

where EV = estimated volume, L = the longest dimension of the swelling in the plane of the pinna, B = the longest dimension of the swelling perpendicular to L in the plane of the pinna, T_s = the greatest thickness of the swelling plus the pinna, and T_1 and T_2 = the thickness of the pinna on opposite sides of the swelling.

Where 2 grafts a and b were present the estimated volume of the swellings was calculated by

$$EV_{a+b} = EV_a + EV_b$$

In this case the thickness of the pinna was measured on opposite sides of the area containing the grafts so that T_1 and T_2 were the same for each graft.

A reaction to the allografts was not detected and cyst formation was not far advanced in the grafts at 7 days after transplantation (see Chapter IV). The estimated volumes calculated at this time were considered to bear a consistent relationship to the amount of tissue transplanted.

Obtaining and fixing samples.

To obtain specimens from ear chambers, the rabbits were restrained on the bed, blood flow to the ear chambers was stopped by applying external pressure to the auricular artery and the coverslip or lid of the ear chamber was carefully removed as previously described. Impression smears were taken from some ear chambers at this stage by applying a glass coverslip to the surface of the grafts and chamber membrane. The ear chamber membrane and grafts were then immediately flooded with fixative at 37° centigrade. The rabbit was killed by intravenous injection of 5 ml. of pentobarbitone sodium, the ear

chamber was disassembled, and the ear chamber membrane was cut out and immersed in the fixative. Subcutaneous grafts were punched out from the pinna immediately after the rabbit had been killed. These grafts were gently cut into narrow strips under fixative at 37° centigrade and then immersed in the fixative. After the specimens had been obtained, the reproductive tract of the rabbits was examined for evidence of pregnancy or corpora lutea. Samples were removed from only one ear of a few rabbits in which case local anaesthetic (2% lignocaine hydrochloride; Ilium, Troy Laboratories Pty. Ltd., Smithfield, Australia) was used to produce a ring-block at the base of the ear. Samples of normal endometrium obtained at surgery or termination of experiments were fixed by immersion.

The fixatives used were 4% glutaraldehyde in 0.1 M. sodium cacodylate buffer and full- or half-strength Karnovsky's fixative (KARNOVSKY, 1967). The specimens were fixed overnight and some were stored in half-strength Karnovsky's fixative at 4° centigrade. Further processing involved washing the specimens in 0.1 M. sodium cacodylate buffer and post-fixation in 2% osmium tetroxide in s-collidine buffer for 2 hours at 4° centigrade. Specimens were dehydrated through graded concentrations of ethyl alcohol and then propylene oxide and infiltrated with epon 812. At this stage, samples from selected areas of the grafts on ear chamber membranes and subcutaneous grafts were cut while viewed with a Zeiss dissecting microscope. The samples were then embedded in epon 812.

Sections 1 μ m. thick were cut on Cambridge of LKB Huxley ultramicrotomes using glass knives and stained with Richardson's stain (RICHARDSON ET AL, 1960). The sections were examined on a WL microscope or Photomicroscope (Zeiss, West Germany) and photographed using panatomic-X (Eastman Kodak Co.) or Polaroid Coaterless Land Pack Film, Type 667. Quantitative and semiquantitative analysis of the sections are described in Chapters IV and V.

Ultrathin sections were cut on the LKB Huxley ultramicrotome using a diamond knife (E.I. du Pont de Nemours and Co. (Inc)., Wilmington, U.S.A.). They were mounted on uncoated copper grids and stained

with uranyl acetate and lead citrate (VENABLE and COGGESHALL, 1965). The sections were examined in Phillips 301 electron microscopes operated at 60 KV.. Electron photomicrographs were made on 35 mm. fine grain release positive film 5302 (Eastman Kodak Co.).

Impression smears were air dried and stained with Lishman's stain.

Planimetry

The areas of grafts in ear chambers and regions of 1 μm . sections analysed were calculated from photomicrographs using a digital planimeter (Planix 7; Tamaya and Company Ltd., Tokyo, Japan) and conversion factors derived from photomicrographs of a standard grid.

FIGURE II-1

Estimated volume (EV) of enzymatically separated autogenous endometrial epithelium with time after subcutaneous transplantation to the pinnae of rabbits' ears. Two million viable nucleated cells were injected into individual sites after incubation with Pronase solution for 2 and 4 hours (●), 6 and 8 hours (■) and 10 hours (▲). Two rabbits were used in the experiment. One recieved cells separated after 2, 4 and 6 hours incubation and the other received cells separated after 6, 8 and 10 hours incubation.

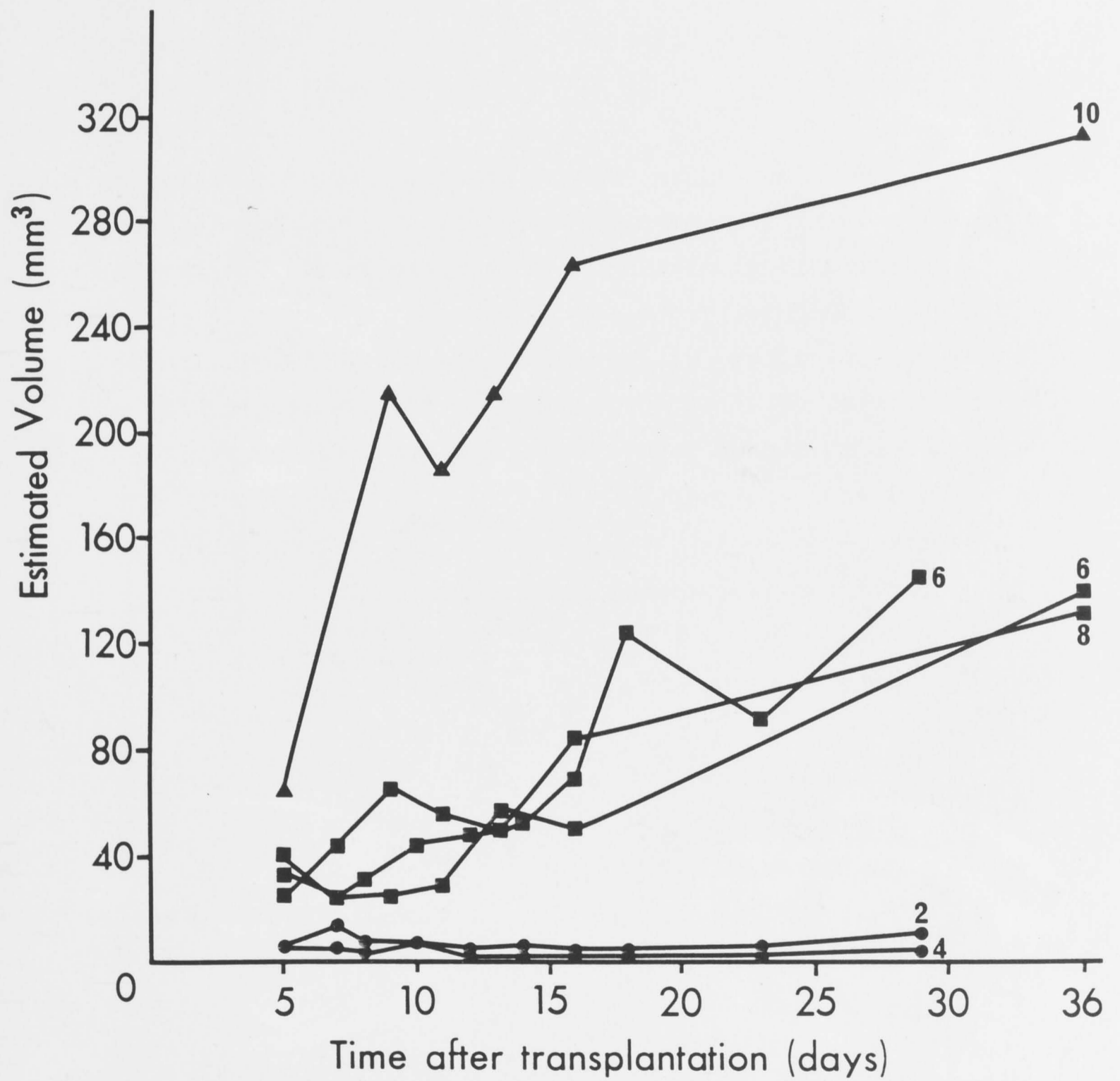


FIGURE II-2

A. Exploded view of the modified graft ear chamber.

- 1 - 10 BA brass or stainless steel screws.
- 2 - protective cover of cleared X - ray film.
- 3 - perspex supporting ring of lid. Three 10 BA nuts are glued to the surface so that the protective cover can be attached.
- 4 - mica coverslip (90 μm . thick; Best Ruby mica, Mica House, London, U.K.).
- 5 - 6 BA brass screws centre tapped to accommodate 10 BA screws.
- 6 - polystyrene basement coverslip (200 μm . thick; Plastic coverslips No. 1 $\frac{1}{2}$, Lux Scientific Corp., Newbury Park, California, U.S.A.).
- 7 - perspex base of the ear chamber with a central table (1 mm. high and 6 mm. in diameter) and 3 perspex bushes (0.8 mm. high) centre tapped to accommodate 6 BA screws.
- 8 - 6 BA brass nuts.

B. Top view of lid of injection chamber.

- a - perspex supporting ring.
- b - perspex coverslip (0.8 mm. thick).
- c - stainless steel tube (22 gauge; from Yale Disposable Hypodermic Needles, Becton, Dickinson and Co., Rutherford, New Jersey, U.S.A.).
- d - polyethylene tube (0.5 mm. internal diameter; medical grade, Dural Plastics and Engineering Pty. Ltd., Dural, Australia).

C. Assembled modified graft ear chamber without protective cover.

(Bar = 20 mm.)

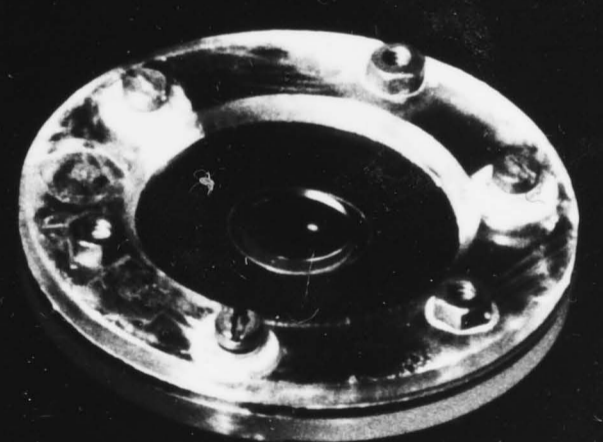


1
2
3
4
5
6
7
8

A



B



C



FIGURE II-3

Insertion of a graft chamber in the pinna of a rabbit's ear.

- A. After the template and punches have been positioned, 4 holes are punched in the pinna.

- B. The skin is dissected from the perichondrium on the dorsal and ventral aspects of the pinna.

- C. The base and the top of the chamber have been inserted and bolted into position.

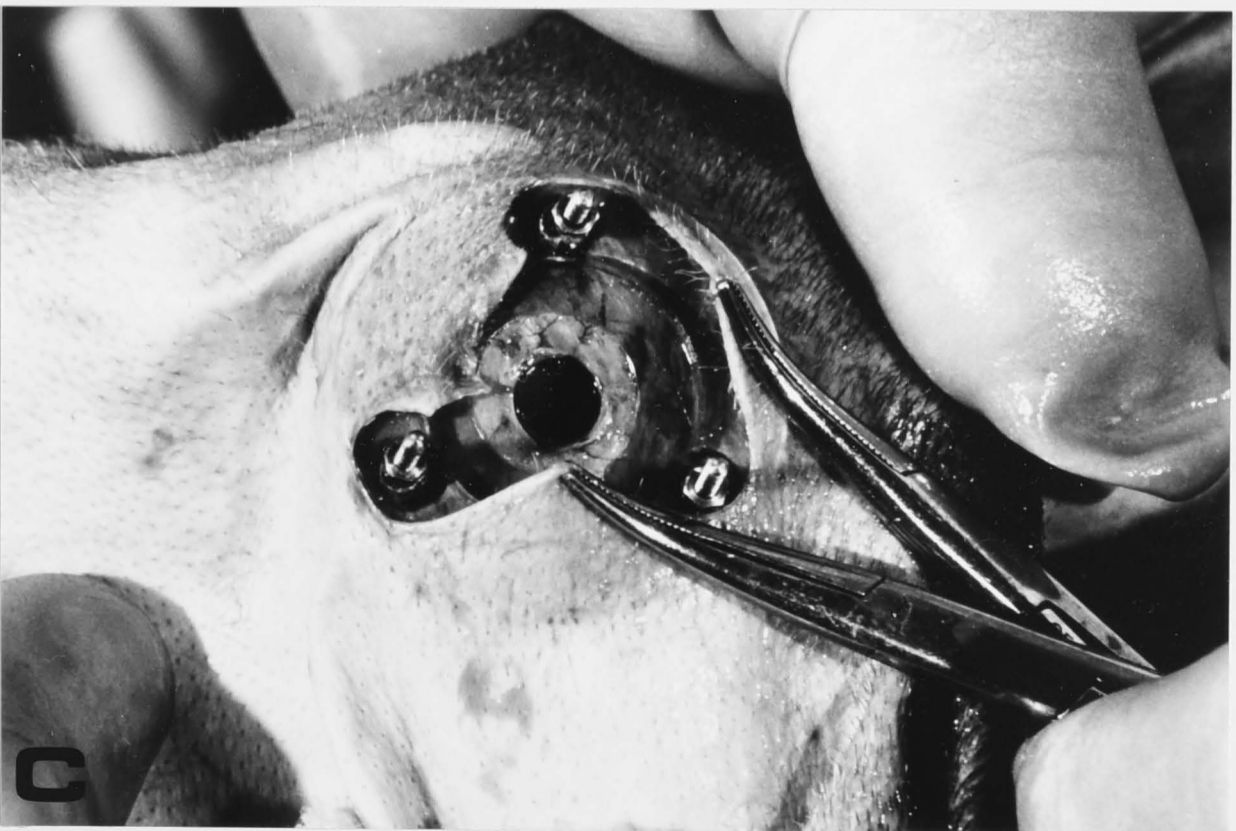
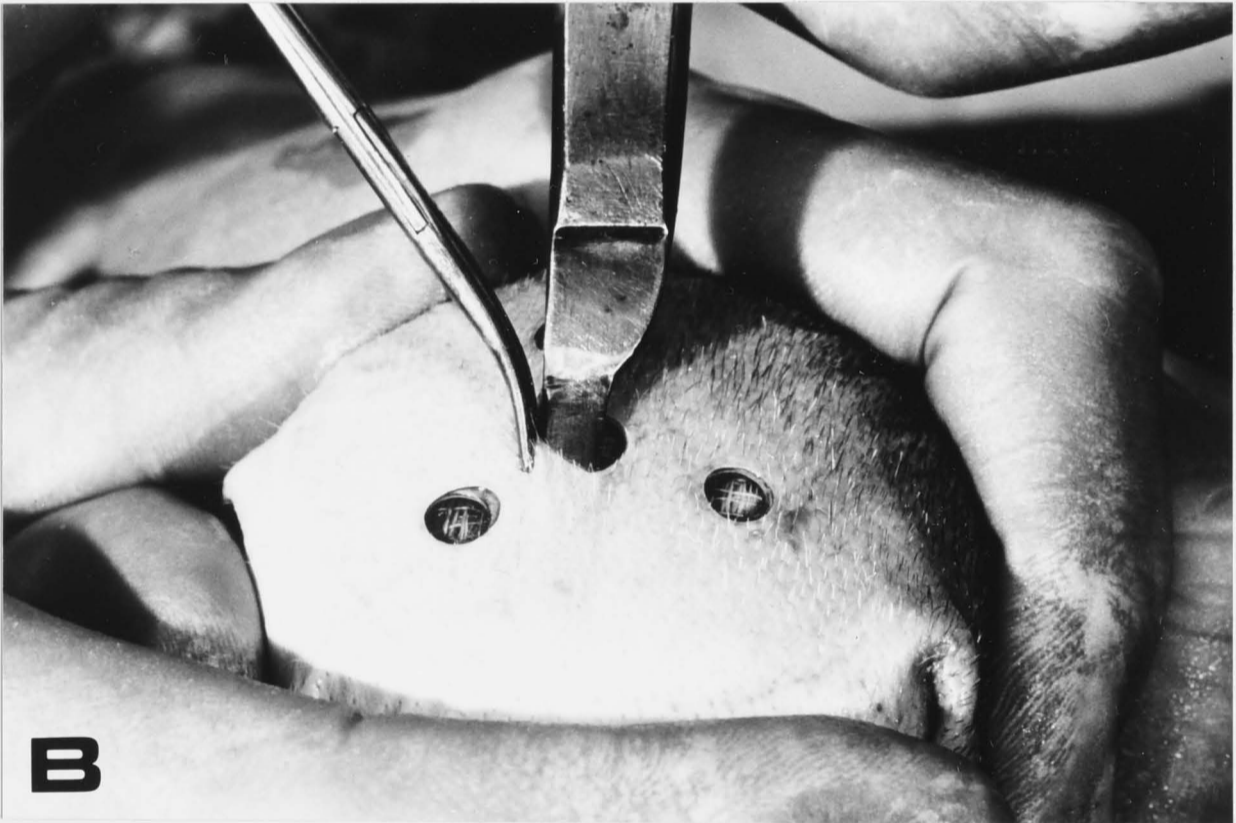
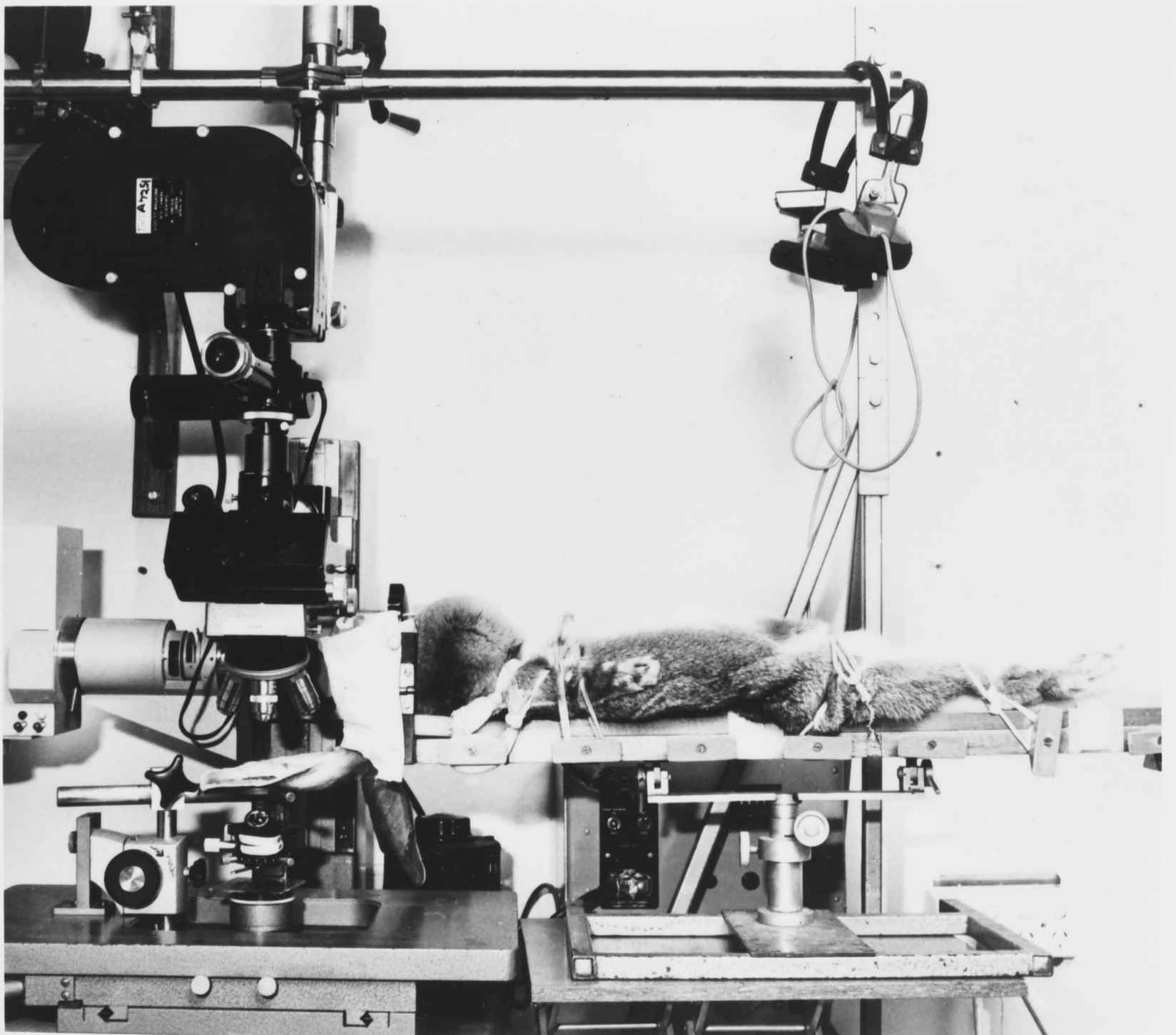
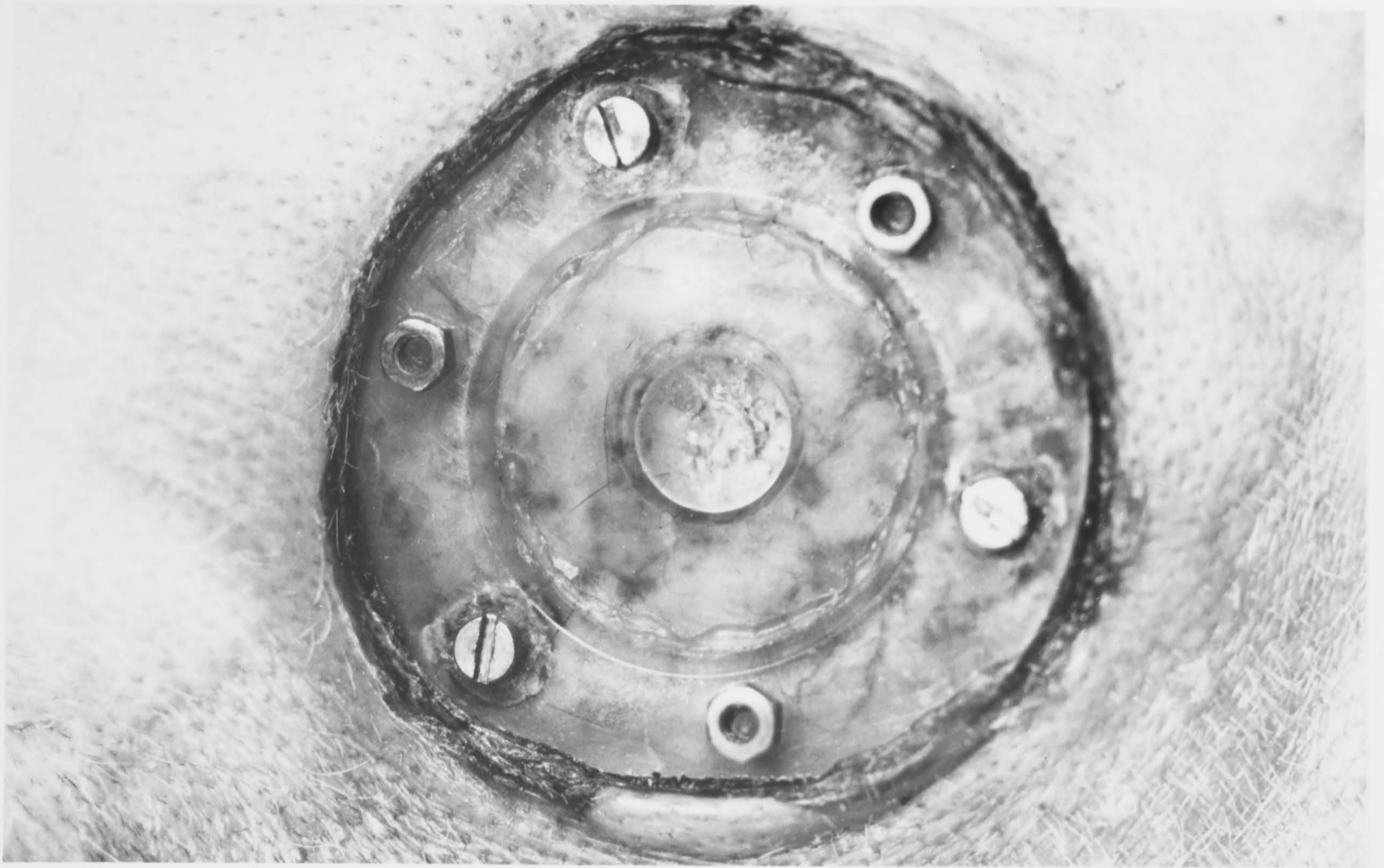


FIGURE II-4

The upper surface of a modified graft chamber 9 days after insertion. The skin flaps that overlay the edges of the chamber have sloughed. One of the 3 triangular buffers that maintain the space between the top of the table and the coverslip is obvious at the lower left edge of the table. A vascularized membrane has not yet grown into this space which remains filled with fibrin and blood. (X 3)

FIGURE II-5

Examination of the ear chamber by light microscopy. The conscious rabbit is restrained on a bed and the ear chamber has been mounted on the stage of the Lietz Biomed microscope. A time-lapse cine camera is in position over the microscope.



CHAPTER III

IN VIVO OBSERVATIONS ON ENDOMETRIAL GRAFTS

INTRODUCTION

Researchers using transparent chambers in the study of acute rejection of allografts have emphasised the prominence of vascular changes (ALGIRE, 1954; EDGERTON and EDGERTON, 1954; EDGERTON ET AL, 1957; CONWAY ET AL, 1957; ZAREM, 1969; HOBBS and CLIFF, 1973) as have many workers using other techniques (see Chapter I). However, EDGERTON and EDGERTON (1954) studying first-set skin and thyroid allografts in mice suggested that the destruction of the allografts was not due to the vascular changes observed. ALGIRE (1954) using a tumour allograft in mice also concluded that failure of the circulation did not play a primary role in the destruction of first-set allografts. These observations appear to have been largely ignored although studies in other systems have provided only circumstantial evidence for the opposing view (see Chapter I).

HOBBS and CLIFF (1971) and HOBBS (1972) found that the rejection of allogeneic tissues transplanted to the rabbit ear chamber was unusually delayed and often showed a prolonged course. This was not observed in transparent chambers in the dorsal skin fold of mice (ALGIRE, 1954; EDGERTON and EDGERTON, 1954; EDGERTON ET AL, 1957; CONWAY ET AL, 1957; ZAREM, 1969). HOBBS and CLIFF (1971) postulated that the delayed rejection in the rabbit ear chamber might be due to either the replacement of the allogeneic vascular endothelium by that of the recipient, the small size of the allografts, or immunological privilege within the ear chamber. They presented evidence that the endothelium of the allografts in the ear chambers was not replaced. The effect of size of the allografts or immunological privilege have not been investigated.

The time of rejection in immunologically privileged and non-privileged sites has been shown to be influenced by the amount of allogeneic tissue transplanted (KAPLAN and STEVENS, 1975; MEDAWAR, 1944; LEHRFELD and TAYLOR, 1953; BALLANTYNE and CONVERSE, 1980) but others have observed no effect of dosage (EDGERTON and EDGERTON, 1954; EDGERTON ET AL, 1957). A dose response in the intensity of second-set rejection of skin allografts following the injection of allogeneic cells has also been noted (MANN ET AL, 1959; STEINMULLER and WIENER, 1963). The form of rejection of allografts in privileged sites has received little attention but acute and chronic forms have been reported (BARKER and BILLINGHAM, 1971; TILNEY and FORD, 1974). Thus the observations of HOBBS and CLIFF (1971) might indeed be explained by the size of the allografts and immunological privilege acting either together or alone. Stress is another factor which may influence the rejection of allografts (WISTAR and HILDEMANN, 1960; ABEATICI ET AL, 1968) and may play a role in in vivo experiments which entail restraint or manipulation of the conscious animal (GISLER, 1974; AHMED ET AL, 1974).

In a later study, HOBBS and CLIFF (1973) demonstrated that acute rejection of allografts in rabbit ear chambers could be induced by subsequent transplantation of tissues from the donor elsewhere in the recipient.

Further studies of the mechanisms involved in acute rejection of allografts were undertaken in the rabbit ear chamber because this preparation provides superior in vivo morphological detail compared to other preparations. The rejection of endometrial allografts has not been studied in the rabbit ear chamber before so the form of rejection occurring spontaneously and that induced by transplantation of subcutaneous allografts were both investigated. In the latter, the allografts were transplanted simultaneously to ear chambers and subcutaneous sites to avoid possible modulation of the recipients' response by prior residence of the ear chamber allografts (BARKER and BILLINGHAM, 1977). The possibility that the size of grafts, stress or immunological privilege may influence rejection in the rabbit ear chamber was also studied.

Endometrial grafts transplanted to ear chambers

MATERIALS AND METHODS

The implantation of ear chambers, the transplantation of endometrium, and the methods of observation have been described in

Endometrial grafts transplanted to subcutaneous sites

Grafts of endometrium were transplanted to subcutaneous sites in the ears of rabbits as described in Chapter II. When only subcutaneous sites were transplanted, allografts were exchanged between pairs of rabbits. The transplantation of 2 autografts to one ear and 2 allografts to the other ear was attempted in each rabbit. The sizes of the grafts were chosen to cover a range that included the size of grafts that were allowed to proceed to spontaneous rejection in ear chambers alone (Table III-1). Additionally, allografts of similar sizes to those which were spontaneously rejected in ear chambers were transplanted subcutaneously in a pair of rabbits. The total areas of the allografts transplanted to each of this pair of rabbits was 5.7 and 6.2 mm². This was determined by planimetry on microphotographs taken of the grafts just before transplantation when they were placed on an ear chamber base and flattened to a thickness of 135 μm. by a coverslip. The latter thickness was determined to be representative of the in vivo thickness of grafts in ear chambers by using the fine focus microscale of the Lietz Biomed microscope. From each pair of rabbits receiving subcutaneous allografts, one was randomly chosen to test the effect of the prolonged restraint during time-lapse cine photomicrography as performed on rabbits with ear chambers. These rabbits were sham time-lapsed for 3 hours a day but with breaks from the treatment for half an hour and 1 hour after the first and second hours of the treatment respectively. The rabbits were subjected to the treatment for 7 to 9 consecutive days between 10 and 18 days after transplantation. Subsequent observation were as outlined above.

All grafts were examined on the day following transplantation and then at least at 2-day intervals (see Chapter II).

Endometrial grafts transplanted to ear chambers

The implantation of ear chambers, the transplantation of endometrium, and the methods of observation have been described in Chapter II. In the standard experiments, 2 auto- and 2 allografts were transplanted either to the same ear chamber or, in a few rabbits, the autografts were transplanted to a chamber in one ear and the allografts were transplanted to a chamber in the other ear (Table III-3). The average weight of the individual grafts determined by weighing 20 graft-sized pieces of endometrium from 2 rabbits was approximately 0.2 mg.. The total areas of the allografts in each ear chamber ranged from 1.2 to 4.54 mm². at the time of transplantation. Where both ear chambers in a rabbit were transplanted with allogeneic tissue the maximum total area of the allografts at the time of transplantation was 8.36 mm².. With few exceptions, these grafts were examined at 2 days intervals at least. In 2 other rabbits, 2 autografts of standard size and allogeneic endometrium which covered approximately 20 mm². at the time of transplantation were transplanted to single ear chambers.

Endometrial grafts transplanted to subcutaneous sites and to ear chambers in the same rabbits

In each rabbit, 2 auto- and 2 allografts were transplanted both to subcutaneous sites in the distal part of the ear and to ear chambers at the same time (Table III-4). The subcutaneous and ear chamber allografts transplanted to each rabbit were from the same donor. The estimated volumes of the subcutaneous allografts at 7 days after transplantation ranged from 2.2 to 16.4 mm³.. The techniques of transplantation and subsequent observation were as outlined above.

RESULTS

Grafts transplanted to subcutaneous sites

A summary of the experiments is presented in Table III-1. One graft in each of 3 rabbits and both allografts in another rabbit failed to take. The latter was excluded from the trial.

The establishment of grafts. Variable degrees of haemorrhage, hyperaemia and oedema were observed in and around the subcutaneous tracts following transplantation but these changes resolved in the ensuing days. The auto- and allografts became obvious as blood-filled foci by 3 to 5 days after transplantation.

Subsequently, the grafts gradually lost the blood-filled appearance and the central regions of most became translucent and then obviously cystic if not overtaken by rejection (Figure III-3A and B). The central translucency was first noted at 7 to 16 days in larger grafts (estimated volume (EV) $> 2 \text{ mm}^3$. at 7 days) but was often delayed in the smaller grafts in which it was first noted from 11 to 26 days. Examples of changes in the EV of grafts with time are shown in Figure III-1.

The rejection of allografts. All allografts were rejected. The time of rejection was taken as the day that the EV fell from peak levels (Table III-1, Figures III-1 and 2). The data on time to rejection of the allografts were analysed using Cox's regression model with Peto's treatment of the tied observations (COX, 1972) via the statistical package GLIM (BAKER and NELDER, 1978; WHITEHEAD, 1980). This analysis showed that the smaller allografts had a higher probability of long survival than did the larger allografts (Figure III-2) and that there was no significant difference between the stressed and unstressed groups.

Opacification of cysts was first noted in most allografts 1 to 2 days before the fall in EV (Table III-2). However, in some smaller allografts ($EV < 2 \text{ mm}^3$ at 7 days) this change was observed up to 13 days before the fall in the EV. Most of the allografts also acquired a haemorrhagic/hyperaemic appearance up to 4 days before the fall in EV (Table III-2, Figure III-3C). However, this change was delayed until after the fall in EV in some of the allografts and was not observed in one.

Following rejection the haemorrhage and hyperaemia resolved so that by the end of the period of observation no gross evidence of the previous existence of the allografts could be found apart from the area being marked at times by yellowish discoloration.

Grafts transplanted to ear chambers

The experiments performed are summarized in Table III-3 and -4. An example of the sequences of changes in a transplanted ear chamber is shown in Figure III-4.

The establishment of grafts. Differences in the auto- and allografts were not detected until shortly before the onset of vascular stasis in the allografts.

Immediately following transplantation, vessels containing variable amounts of blood and areas of haemorrhage were noted in the grafts (Figure III-4B). The epithelium at the edges of the grafts was most easily seen but also numerous glands in the body of the graft could be identified. The epithelium was cuboidal or columnar and numerous vigorously-beating cilia were usually present on its surface. Ear chamber membrane vessels often showed sluggish flow and a few were often in stasis; that is, blood flow had ceased and blood cells were packed tightly into the vessel lumen. Leukocyte rolling and sticking in vessels of the chamber membranes often increased to scores of 3 but scores of 4 were occasionally observed. Small

thrombo-emboli were also occasionally observed at this time. Petechial and, less often, larger haemorrhages had occurred in the chamber membranes. Lymphatics were present in the membranes of 20 of the 27 ear chambers grafted (Figure III-4A and -6A).

The first evidence of connection being established between the vasculature of the grafts and the chamber membranes was observed at 2 to 3 days after transplantation. In the grafts, the vessels became widely dilated and packed with erythrocytes and further haemorrhage was observed in the lamina propria. Additionally, blood escaped onto the chamber membrane from unanastomosed ends of the graft vessels. Blood flow in graft vessels was sometimes observed at this time but mostly commenced 1 to 3 days later. Occasionally vascular sprouts grew out from the edges of the grafts and anastomosed with the recipient vessels (Figure III-6B). Most of the haemorrhage in the grafts had cleared by 5 to 16 days after transplantation. The dilation of graft vessels receded at variable times. The epithelium at the edges of the grafts began to extend onto the surrounding chamber membrane as early as 1 day after transplantation. Ciliated cells were often observed in these extensions of the epithelium. Over the ensuing days, the epithelium continued to spread over the membrane and formed extensive monolayers (Figure III-7A). Cysts also formed at the edges of some grafts (Figure III-4C). The glands of the grafts were dilated on the day after transplantation and had disappeared at later examinations. The cilia of the epithelial cells had almost completely disappeared by 2 to 9 days after transplantation but began to re-appear by 14 to 60 days. The area of the graft bodies increased by an average of 130% by 24 to 30 days after transplantation.

The rolling and sticking of leukocytes in the vessels of the chamber membrane subsided 2 to 10 days after transplantation. Numerous leukocytes in the space above the chamber membrane and, to a lesser extent, above the grafts appeared shortly after transplantation and were present throughout the period of the experiments (Figure III-8). Leukocyte rolling and sticking in the vessels of the grafts rarely exceeded scores of 2. The numbers of leukocytes and erythrocytes

increased in the lymphatics of some chamber membranes after transplantation but subsided again by 9 to 11 days.

Some exceptions to the usual pattern of establishment occurred. Regions of 4 grafts became haemorrhagic and necrotic. This was associated with stasis of chamber membrane vessels underlying 2 of the grafts. The other 2 grafts became opaque and the degree of blood perfusion in the underlying membrane could not be determined. Additionally, an autograft showed no evidence of vascular anastomosis with the chamber membrane vessels 13 days after transplantation, although blood flow in the latter was not compromised.

The rejection of allografts. All allografts in the standard experiments were rejected unless they were removed earlier for fixation. Stasis of blood flow in the vessels of the allografts was the earliest reliable in vivo indication of rejection. Thus the first day that vascular stasis was observed was taken as the time of rejection and the time to which other changes in the allografts was related.

Allografts of standard size in the ear chambers were spontaneously rejected between 25 and 43 days while the rejection induced by subcutaneous allografts occurred between 11 and 17 days (Tables III-3 and -4). The results were significantly different ($p < 0.004$, Mann-Whitney U test; SIEGEL, 1956). When larger amounts of allogeneic tissue were transplanted only to ear chambers, rejection was not observed up to 61 days after transplantation (Table III-3).

Little difference between the in vivo features of the allografts undergoing spontaneous and induced rejection was detected and these results are combined. Often stasis was first observed in only a few vessels of one of the allografts in an ear chamber (Figure III-5). Stasis became extensive in most of the allografts 1 to 2 days following its onset. The static vessels were dilated and packed tightly with blood cells (Figures III-5B and -6D). Haemorrhage was

often present in the areas of stasis and petechiae and diffuse haemorrhage also occurred in other areas of the grafts at this time. Occasionally, blood flow in some of the vessels ceased at this time although they were not typically static. These vessels either were collapsed or contained blood or plasma that showed only back and forth pulsations. They often became static 1 or 2 days later. Blood flow continued in some allografts until 4 days after the onset of stasis but, in the later stages, in only a small proportion of vessels and rarely at more than a sluggish rate (Table III-5). Exceptionally, nearly normal blood flow persisted in one allograft until 14 days after the onset of stasis in the other allograft in the ear chamber. Stasis of blood vessels immediately below allogeneic monolayers was uncommon during spontaneous rejection but was noted more often during induced rejection.

A moderate to marked increase in blood flow within most of the ear chamber membranes and auto- and allografts preceded the onset of stasis in the allografts. This persisted within the membranes and the autografts for up to 5 to 6 days after the onset of stasis in the allografts. In some ear chambers, the blood flow within the allografts and the adjacent ear chamber membranes and autografts became intermittent with high rates of flow alternating with periods of little or no flow.

Leukocyte rolling and sticking in the vessels of the grafts was not pronounced and average scores for vessels in allografts showed little elevation over those of autografts at any time (Table III-5). Vessels of the ear chamber membranes adjacent to allografts showed higher average scores for leukocyte rolling and sticking than did those adjacent to autografts before and after the onset of stasis (Table III-5, Figure III-6C). A few thrombo-emboli were observed in the vessels of the allografts of one ear chamber from the day before to the day after the onset of stasis and partially occluded the lumen of one vessel (Figure III-6D). Thrombo-emboli were also noted in membrane vessels adjacent to the allografts in 3 other chambers on isolated occasions between 7 days before and 6 days after the onset of stasis. The growth of the grafts and

monolayers and rejection related phenomena had obscured the lymphatics in most ear chamber membranes by the time the allografts were rejected. The few observations that could be made indicated that the numbers of leukocytes in the lymphatics increased from 2 days before the onset of stasis and had begun to subside 6 days later.

The epithelium at the edges of the allografts showed more leukocytic infiltration than did that of control autografts in a high proportion of ear chambers from 4 days before the onset of stasis. In a few allografts 1 to 2 days before the onset of stasis, a reduction in the height of the allogeneic epithelium was also noted and occasionally the epithelial cells showed large vacuoles. Destruction of the epithelium of allografts began from the onset of stasis in approximately 50% of the ear chambers (Figure III-7B). Allogeneic epithelium that remained after the onset of stasis became flattened and contained leukocytes, erythrocytes, refractile clumps and occasional large vacuoles (Figure III-7C). In most cases the epithelium appeared not to persist beyond 2 days after the onset of stasis despite continued sluggish flow in vessels of some of the allografts (Table III-5). Limited definition of epithelium on the surface of the grafts and cells of the lamina propria prevented observation of their fate. Only isolated observations could be made of cysts and monolayers of the allografts at the time of rejection, because of the numbers of cells overlying them. The epithelium of cysts appeared to undergo similar changes to that at the edges of allografts. Increased leukocytic infiltration and refractile clumps in allogeneic monolayers were observed at the time of rejection (Figure III-7D). Destruction of monolayers was noted from the onset of stasis and none appeared to persist beyond 2 days thereafter. Time-lapse cine of the epithelium and the lamina propria of the allografts taken from the time that increased leukocytic infiltration of the allogeneic epithelium was noted up to 2 days after the onset of stasis and representing 36 hours real time failed to reveal any additional information on the mode of allogeneic cell death.

The numbers of cells overlying the monolayers and the grafts increased at the time of rejection (Figure III-8C). In vivo morphological features and the characteristic mode of locomotion of the cells on time-lapse cine films indicated that macrophages and polymorphonuclear leukocytes were common and that lymphocytes were also present. The presence of these cell types was confirmed on stained smears taken from ear chambers at the time of fixation.

The allografts became more opaque and granular in appearance 1 to 4 days after the onset of stasis (Figure III-4E). In 4 chambers where autografts had been transplanted with the allografts, repopulation of the monolayer areas and adjacent edges of the degenerate allografts by autograft epithelium was first noted from 2 to 11 days after the onset of stasis. The membrane also became necrotic below 2 of the 6 allografts followed after induced rejection (Figure III-4E and F). Continued blood flow was identified in the vessels of the chamber membrane underlying most other allografts but this could not be definitely established where changes in the allografts obscured the underlying membrane. The necrotic grafts were surrounded by accumulations of leukocytes, in which macrophages predominated, and were gradually resorbed. The necrotic areas of the chamber membranes healed by resorption and ingrowth of connective tissue and vessels.

Comparison of grafts in subcutaneous sites and in ear chambers in the same rabbits

Signs of rejection had developed in the subcutaneous allografts in 4 of 6 rabbits 1 to 4 days before the onset of stasis in the ear chamber allografts (Table III-4). It is not known if the subcutaneous allografts of rabbit 648 were showing in vivo signs of rejection on the day of the onset of stasis in the ear chamber allografts as they were not examined then. Histological examination of samples taken the following day revealed that rejection was more advanced in the subcutaneous than the ear chamber allografts of this rabbit (see Table IV-6).

DISCUSSION

The data obtained from grafts led to subcutaneous sites showed that the smaller allografts had a higher probability of prolonged survival (Table III-1, Figure III-2). The effect of the engorgement of vessels and subsequent blood flow in most grafts in the ear chambers occurred too quickly after transplantation to be accounted for by the ingrowth of vessels from the chamber membrane. This must have been achieved by the anastomosis of vessels of the graft and chamber membrane which was actually observed at the edges of the grafts. The manner of re-establishment of blood circulation of grafts transplanted in this way has been controversial (BALLANTYNE and CONVERSE, 1980) but the present findings are in agreement with most other studies in transparent chambers (WILLIAMS, 1954; EDGERTON ET AL, 1957; GREENBLATT ET AL, 1971; NARAYAN and CLIFF, 1981). Different parameters had to be used to determine when rejection of allografts in ear chambers and subcutaneous sites occurred and they may have identified different stages of rejection. Opacification of cysts in the subcutaneous allografts probably reflected leukocytic infiltration and destruction of the epithelium. The haemorrhagic/hyperaemic appearance of these allografts indicated vascular changes that were probably equivalent to those in the rejecting ear chamber allografts. However, these changes were unlikely to have been as obvious at their earliest stages of development in subcutaneous allografts as they were in ear chamber allografts. These parameters often identified rejection earlier than the fall in the EV of the subcutaneous allografts but they were not as reliable (Table III-2). Additionally, histological examination of subcutaneous allografts showed that most of the allogeneic tissue had been destroyed before a fall in the EV was detected (compare Table III-4 and IV-6). It was not possible to determine precisely an equivalent end-point in the rejection of ear chamber allografts in vivo. Thus, the results based on the fall in EV represent very conservative estimates for the time of rejection of subcutaneous allografts when compared to the use of the onset of stasis in ear chamber allografts.

The data obtained from grafts transplanted to subcutaneous sites showed that the smaller allografts had a higher probability of prolonged survival (Table III-1, Figure III-2). The effect of the size of allografts on the time to rejection is in agreement with the findings of MEDAWAR (1944) and LEHRFELD and TAYLOR (1953) who worked with skin allografts. LEHRFELD and TAYLOR (1953) used in vivo cessation of blood flow to identify rejection. They found that the rejection of allografts of 1 to 4 mm². occurred at 14 to 37 days while allografts of 100 to 600 mm². were all rejected within 7 to 9 days. They attributed the wide range in the rejection times of the smaller allografts to dosage variations within the group. This could not be demonstrated in the present study. However, the same principle appeared to apply in their study and the present one; the time of rejection of small allografts shows a wide range but as the amount of allogeneic tissue transplanted is increased the average time to rejection is shortened and individual variation is reduced. In the present study, the opacification of cysts long before the fall in EV and the delay or absence of haemorrhage in some smaller subcutaneous allografts suggested that their rejection was more prolonged than that of larger allografts (Table III-2).

The sham time-lapsing in the present experimental conditions failed to produce a detectable effect on the rejection of the subcutaneous allografts. This may have been because the degree of stress caused by this treatment was less than that employed in previous studies or because rabbits do not respond to stress in the same way that mice do. WISTAR and HILDEMANN (1960) stressed recipient mice for 6 hours a day until rejection occurred while ABEATICI ET AL (1968) centrifuged recipient mice for 60 minutes on each of the 5 days preceding transplantation. The rabbits in the present study were subjected to a treatment for only 3 hours a day to which they quickly become accustomed. Additionally, time-lapse cine was not used during the examination of 2 of the 5 rabbits that spontaneously rejected endometrial allografts in ear chambers (Table III-3) indicating that this stress was not responsible for the delayed rejection observed.

The endometrial allografts which were equivalent in size to those that were allowed to reject spontaneously in ear chambers showed small EVs at 7 days after subcutaneous transplantation (Table III-1, Figure III-2). The small allografts in subcutaneous sites showed a wide range of rejection times but spontaneous rejection in ear chambers, whether one or both chambers in a rabbit received allografts, began at 25 days or later (Table III-3). This suggested that the size of the allografts was not solely responsible for the delay in rejection in the ear chambers. This was confirmed by the prolonged survival of larger amounts of allogeneic tissue transplanted to the ear chambers (Table III-3). The results indicate that the rabbit ear chamber behaves as an immunologically privileged site.

The immunological privilege of many sites appears to be a consequence of the paucity or absence of lymphatic drainage (BARKER and BILLINGHAM, 1977). Lymphatic drainage has been shown to play a role in the induction of rejection of allografts transplanted to rabbit ears (LAMBERT ET AL, 1965). The ear chamber membrane lacked lymphatics in only one of the ear chambers in which allografts were spontaneously rejected. However, the functional capacity of the lymphatics in ear chambers is unclear (CLARK and CLARK, 1937; HOBBS and CLIFF, 1971). If stimulation of the rejection response was not via the lymphatics, it may have occurred by peripheral sensitization (STROBER and GOWANS, 1965; PEDERSEN and MORRIS, 1970; WUSTRACK ET AL, 1975). However, small allografts may be ineffective in eliciting rejection by this route and enhancement of allograft survival may be observed (BARKER and BILLINGHAM, 1977). Other factors may also be involved in the immunological privilege of some sites because the testes of rats have been shown to have functional lymphatic drainage (HEAD ET AL, 1983) although this site displays immunological privilege (BARKER and BILLINGHAM, 1977).

Rejection of subcutaneous allografts was observed before that of allografts transplanted at the same time to ear chambers despite the likelihood of it being detected earlier in the ear chambers (Table III-4). Dissociation of rejection times has been observed

previously when allografts were transplanted to both privileged and non-privileged sites in the same animal. WOODRUFF and WOODRUFF (1950) observed survival of a large proportion of thyroid allografts in the anterior chamber of the eye despite the rejection of allografts from the same donor transplanted simultaneously to subcutaneous sites. CONNELLY (1961) reported that skin allografts in the anterior chamber of the eye were unaffected by subsequent transplantation and rejection of orthotopic skin allografts from the same donor. However, other studies have shown that allografts in privileged sites are rejected at the same time as allografts transplanted to other sites in the recipient (BARKER and BILLINGHAM, 1971). HOBBS and CLIFF (1973) also found that rejection of renal allografts in the rabbit ear chamber mirrored rejection of the whole kidney from the same donor that was transplanted 9 to 14 days later. A delay in the rejection of allografts in privileged sites compared to that of allografts from the same donor transplanted simultaneously to non-privileged sites in the same recipient indicates a defect in the efferent arm of the immune response. This effect appears to vary depending on the immunologically privileged site and the experimental conditions.

In the present study, the efferent arm of the immune response was impeded but this was insufficient to account for the observed delay in spontaneous rejection in the ear chamber. The major effect of the ear chamber appeared to be on the afferent arm of the immune response so that the allografts either were slow to stimulate an immune response or elicited a response in which enhancement of allograft survival or suppression of effector mechanisms was the predominant feature initially. The longer survival of larger allografts in the ear chambers suggested that enhancement or suppression was elicited. The paradoxical response to the larger amounts of allogeneic tissue in the ear chamber is at variance with the results of KAPLAN and STEVENS (1975) and suggests that the immunological privilege in the rabbit ear chamber may be different from that in the anterior chamber of the eye of rats. Further study of the rabbit ear chamber under the conditions following

transplantation, including the functional capacity of the lymphatics in and around the membrane, is necessary before the basis of the immunological privilege of this site can be determined.

Spontaneous and induced rejection of the endometrial allografts in the ear chambers with epithelial destruction occurring at the same time or soon after prominent vascular changes was similar to that described in the acute rejection of most skin and organ allografts (see Chapter I). Acute forms of rejection after long survival of allografts has been observed also in immunosuppressed human beings and when transplantation was to hamster cheek pouch tissue and between related rats (DUNNILL, 1979; BARKER and BILLINGHAM, 1971; ROGERS ET AL, 1959). In contrast to the results of the present study, HOBBS and CLIFF (1971) and HOBBS (1972) found that the allografts they transplanted to rabbit ear chambers alone experienced prolonged periods of rejection. Acute rejection of the ear chamber allografts was observed when they were challenged with a whole kidney allograft from the same donor (HOBBS and CLIFF, 1973). In the present study, the only substantial difference observed in vivo between spontaneous and induced rejection of allografts transplanted to ear chambers was necrosis of the membrane underlying some allografts undergoing induced rejection.

Stasis of blood flow in vessels of the allografts was a prominent feature of rejection in the present study as reported by others studying acute rejection in transparent chambers (ALGIRE, 1954; CONWAY ET AL, 1957; HOBBS and CLIFF, 1973). The narrowing and disappearance of vessels of the allografts also reported (ALGIRE, 1954; EDGERTON and EDGERTON, 1954; EDGERTON ET AL, 1957; ZAREM, 1969) may correspond to the emptying and collapse observed in some vessels of the allografts in the present study. Increased leukocyte rolling and sticking or platelet adhesion to the endothelium of allograft vessels, as reported by others (ALGIRE, 1954; ZAREM, 1969; HOBBS and CLIFF, 1973), was not observed. ZAREM (1969) and HOBBS and CLIFF (1973) both reported that leukocyte adhesion and thrombi obstructed blood flow in the allograft vessels but this was only rarely observed in the endometrial allografts

(Figure III-6D). ZAREM (1969) suggested that endothelial swelling also limited blood perfusion in the allografts they studied but no evidence for this was obtained in the present study. The many differences between the above studies; including the type of transparent chamber, the species of animal, and the type and amount of the allogeneic tissue transplanted, may explain the variation in results. More specifically, the disagreement between the present findings and those of HOBBS and CLIFF (1973) may have arisen because different tissues were transplanted and much larger amounts of allogeneic tissue were used to challenge the ear chamber allografts in the previous study. Additionally, sensitization of the recipients by the ear chamber allografts before the challenge may have occurred in the previous study (BARKER and BILLINGHAM, 1977).

A reduction in the height of the epithelium of some allografts before the onset of stasis in ear chambers indicated that the host reaction had deleterious effects on the allogeneic tissue at this stage. After the onset of stasis, recognizable edge epithelium did not persist longer than 2 days in most rejecting allografts but blood flow continued in some vessels of the allografts for longer times (Table III-5) and continued blood perfusion of the underlying chamber membrane could often be confirmed. Monolayers of allogeneic epithelium were also destroyed during rejection but stasis of blood in the underlying vessels was not commonly observed. In contrast, grafts transplanted to ear chambers routinely survived 3 to 5 days before vascular anastomosis occurred and often longer periods before blood flow was re-established. These in vivo observations show that the degree of ischaemia produced was insufficient to account for the abrupt destruction of the allografts. Thus the results indicate that the rejection reaction is directed against and is effective in destruction of both the blood vessels and the other tissues of the allografts. These findings are in agreement with those of ALGIRE (1954) and EDGERTON and EDGERTON (1954) that rejection of allografts in transparent chambers is not directly attributable to vascular damage. The findings also support the conclusions of SCOTHORNE and MCGREGOR (1953) that vascular damage was not the primary mechanism by which orthotopic skin allografts in rabbits were destroyed.

TABLE III - 1

Endometrial grafts transplanted to subcutaneous sites in rabbits' ears.

	Rabbit Identification	Allografts EV7 ¹ (mm ³)	Rejection (days) ²	Autografts EV7 ¹ (mm ³)
Untreated	603	0.5 ³	34	0.9
	659 ⁴	1.2	22	4.0
	598	1.4	25	1.4
	595	2.4	13	1.0
	590	4.8	13	7.9
	619	6.3	14	7.0
	625	10.3	16	3.3
Sham time- lapsed	658 ⁴	1.6	16	3.3
	602	2.0 ³	32	1.6
	591	2.3	23	4.2
	596	4.8	20	4.4
	618	4.8	14	6.3
	624	8.1	10	5.0

¹ EV7 = estimated volume of grafts at 7 days after transplantation.

² Time after transplantation that the estimated volume fell from the peak score.

³ Estimated volume first measured 9 days after transplantation.

⁴ The amount of endometrium transplanted was similar to that of allografts that rejected spontaneously in ear chambers (see Materials and Methods).

TABLE III - 3

Endometrial grafts transplanted to ear chambers alone.

Rabbit Identification	Grafts	Rejection (days) ¹	Termination and fixation (days) ²
560 ³ L ⁴	2 allo ⁵	-	12
560 R	3 auto	NA ⁶	12
588 L	2 auto & 2 allo	-	21
588 R	2 auto & 2 allo	-	21
575 ³ L	2 allo	-	25
575 R	2 auto	NA	25
581 ³ L	2 auto & 2 allo	25	25
581 R	2 auto & 2 allo ⁷	25	25
584 R	2 auto & 2 allo	29 ⁸	30
586 L	2 auto & 2 allo ⁷	30	34
640 L	2 auto & 2 allo	-	26
640 R	2 auto & 2 allo	28 ⁸	-
569 ³ L	2 allo	43 ⁸	-
569 R	2 auto	NA	-
629 L	large allo 2 auto	-	60
655 L	large allo 2 auto	-	61

¹ The time after transplantation when vascular stasis was first observed in allografts.

² Time after transplantation.

³ The parents of these rabbits were not identified.

⁴ L and R = left and right ears respectively.

⁵ auto = autograft, allo = allograft.

⁶ NA = not applicable.

⁷ One allograft and both autografts from 581 R and 1 allograft from 586 L were displaced from the membrane when the coverslip was replaced at transplantation. The grafts may have lodged on the fringe of vascularized fibrous tissue that extends from the membrane.

⁸ The grafts were time-lapsed before and/or during rejection.

⁹ The time after transplantation when rejection of the subcutaneous allografts was indicated by hyperaemia or haemorrhage of the grafts, opacification of cysts, or the fall of estimated volume from the peak score.

TABLE III - 4

Endometrial grafts transplanted simultaneously to ear chambers and subcutaneous sites.

Rabbit Identification	Ear chambers		Subcutaneous sites				Termination and fixation (days) ²
	Grafts	Rejection (days) ¹	Grafts	Rejection (days) ⁹ Hyperaemia, Haemorrhage/Opacification		Fall in EV	
633 R ⁴	2 auto & 2 allo ⁵	-	2 auto & 2 allo ⁵	1.7	1.8	-	3
610 L	2 auto & 2 allo	-	2 auto & 2 allo	-	-	-	7
610 R	2 auto & 2 allo	-	2 auto & 2 allo	1.8	1.8	-	9
608 L	2 allo	-	2 allo	10	-	-	13
608 R	2 auto	NA ⁶	2 auto	NA	-	NA	13
637 R	2 auto & 2 allo	13	2 auto & 2 allo	12	-	-	13
648 R	2 auto & 2 allo	11	2 auto & 2 allo	12	-	-	12
601 R	2 auto & 2 allo	12	2 auto & 2 allo	11	11	11	-
607 L	2 auto & 2 allo	17 ⁸	2 auto & 2 allo	13	15	15	-
622 L	2 allo	11	2 allo	11	11	11	-
622 R	2 auto	NA	2 auto	NA	NA	NA	-

Footnotes - see Table III - 3.

TABLE III - 5

In vivo observations of allografts undergoing spontaneous and induced rejection in ear chambers.

		Days ¹						
		-4--3	-2--1	0	1-2	3-4	5-6	
	Persistence of any allogeneic epithelium	7/7 ²	7/7	9/9	6/6	1/5	0/4	
	Persistence of any blood flow in allografts	11/11 ²	10/10	9/11	7/8	3/6	1/5 ⁴	
Leucocytic rolling and sticking in:	vessels of grafts	auto	1.6 ³ (10)	2 (8)	1.7 (7)	1.6 (8)	2.2 (6)	2.3 (1)
		allo	1.9 (11)	2 (9)	1.9 (7)	1.6 (7)	1.3 (3)	1 (1)
	vessels of chamber membrane	auto	1.9 (9)	2.7 (6)	2.2 (6)	2.4 (5)	2.4 (5)	2.3 (3)
		allo	2.8 (10)	2.8 (7)	3.4 (7)	2.9 (6)	3.1 (6)	3.3 (4)

1: Day 0 = first day that vascular stasis was observed in the allografts.

2: number of ear chambers in which feature observed
number of ear chambers where observation could be made

3: Average score and () number of ear chambers in which observations could be made.

4: Blood flow persisted in the vessels of one allograft until 14 days after the onset of stasis.

FIGURE III-1

A typical example of the changes in the estimated volume with time of endometrial grafts transplanted subcutaneously in the ears of rabbits. One of the 6 pairs of unstressed (■) and stressed (●) rabbits. The estimated volume of the allografts falls from peak values at 12 days after transplantation while the autografts continue to grow in both rabbits.

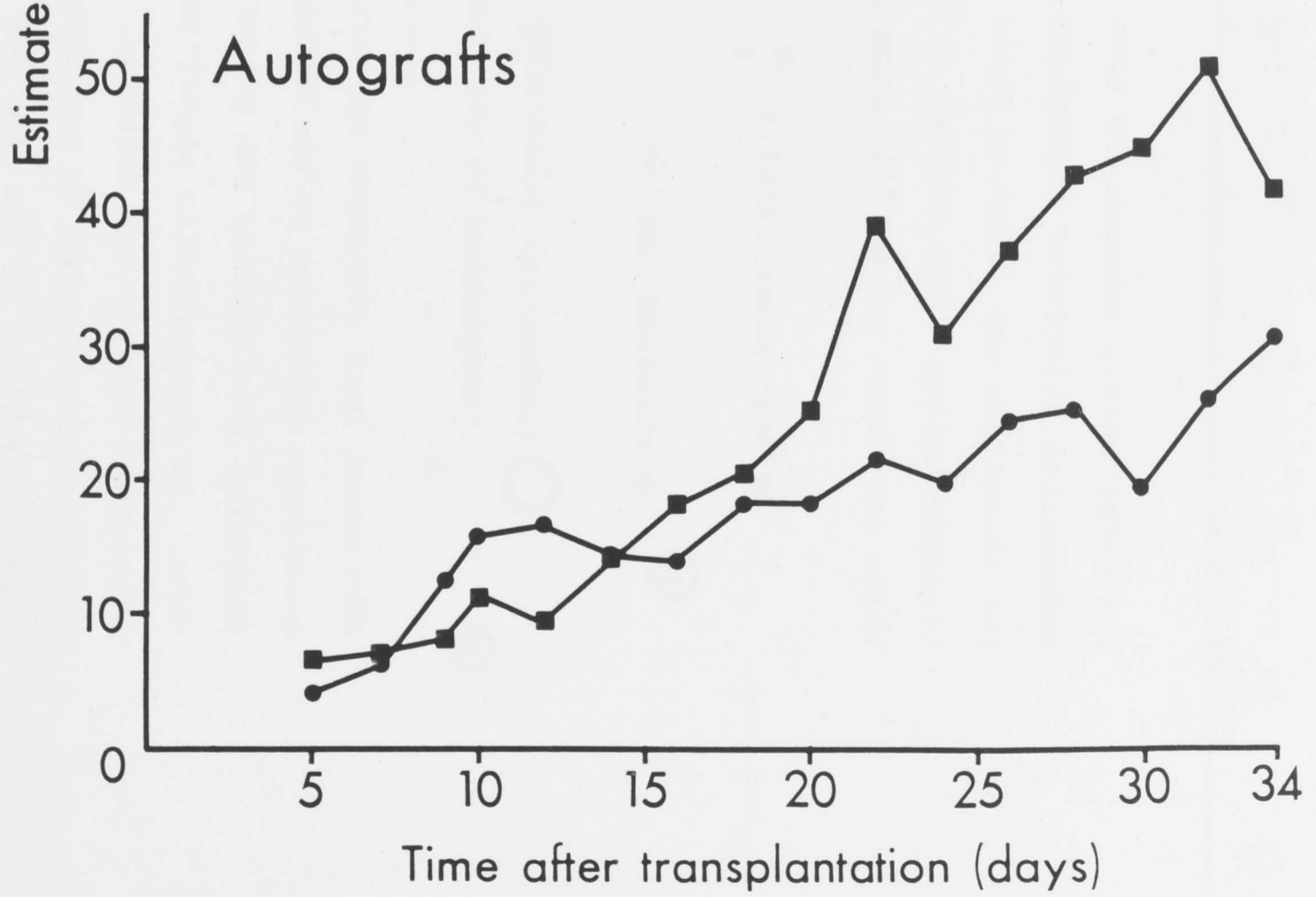
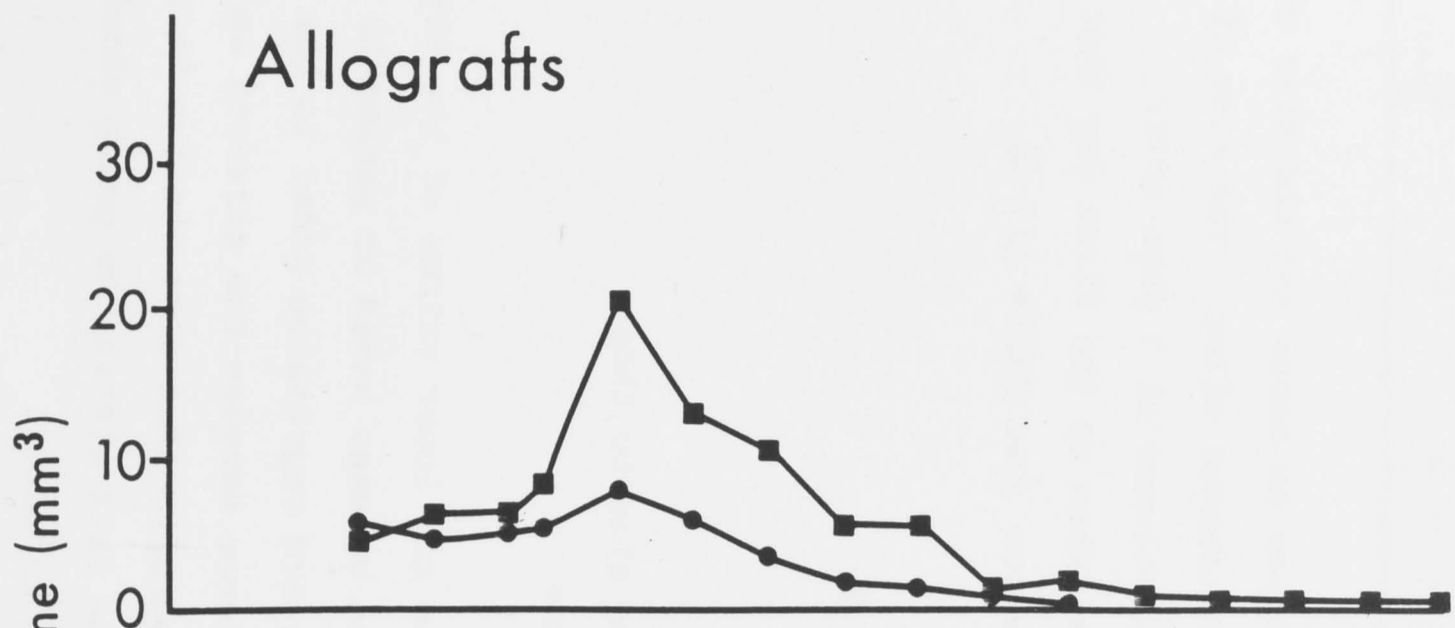


FIGURE III-2

The relationship between the time for rejection to occur and the size of endometrial allografts transplanted to subcutaneous sites. The size of the allografts was based on their estimated volumes at 7 days after transplantation. The time of rejection was taken as the first day that their estimated volume fell from its peak score (see Table III-1).

■ unstressed rabbits

● stressed rabbits

○ endometrial allografts of similar size to those transplanted to ear chambers alone.

Horizontal bars represent approximate upper and lower values of the 95% confidence intervals at the three estimated volumes based on parameter estimates from fitting the proportional hazard regression model to the data. No statistically significant difference between the stressed and unstressed rabbits was detected. The allografts that were similar in size to those transplanted to ear chambers alone fell within the group showing most variability in the time for rejection to occur.

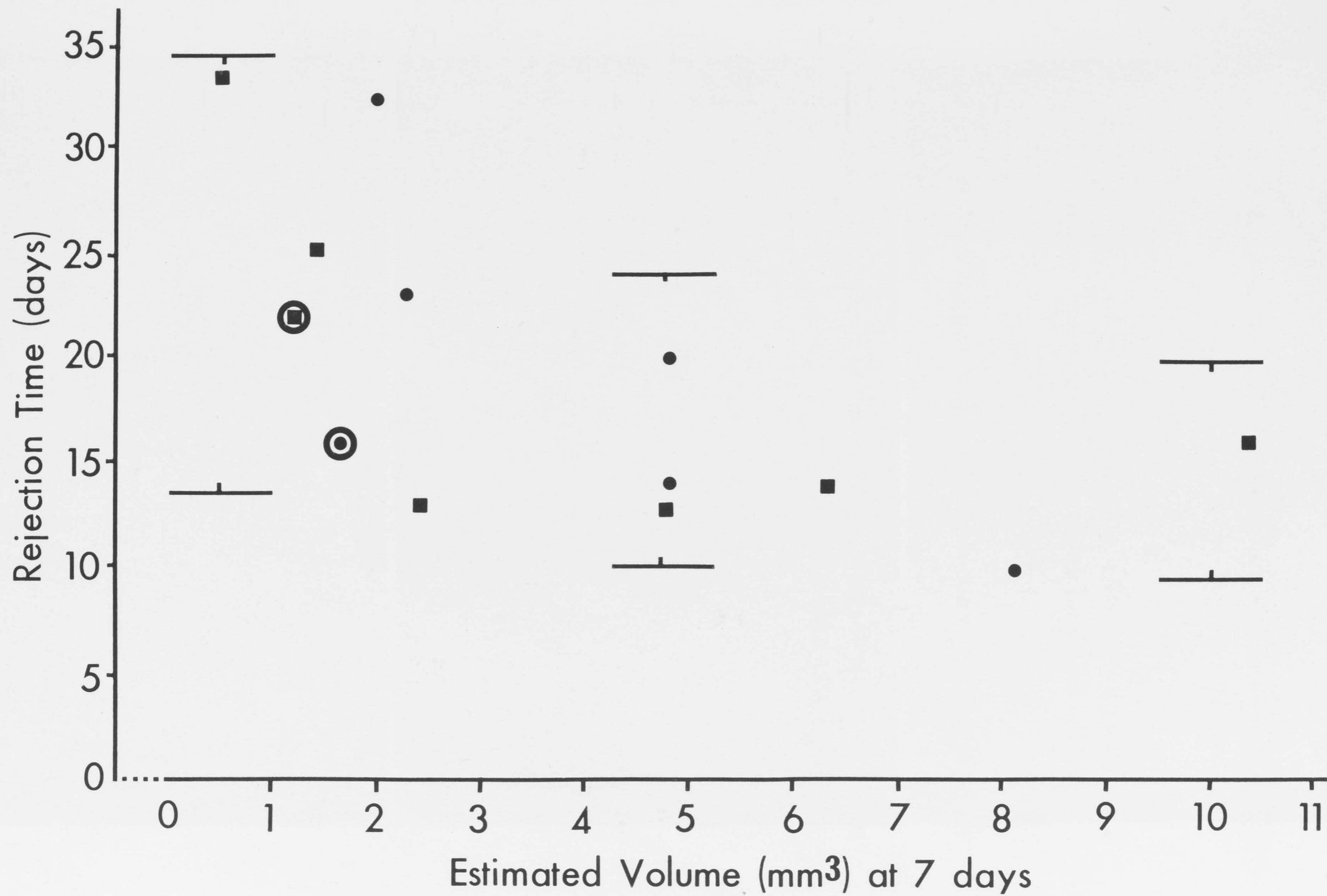


FIGURE III-3

Subcutaneous endometrial grafts transilluminated in the
pinnae of rabbits' ears. (all X 16)

- A. An autograft 7 days after transplantation. A cyst is developing and haemorrhage and congestion are resolving.

- B. A cystic autograft at 13 days after transplantation.

- C. Haemorrhage and hyperaemia in an allograft at 13 days after transplantation.

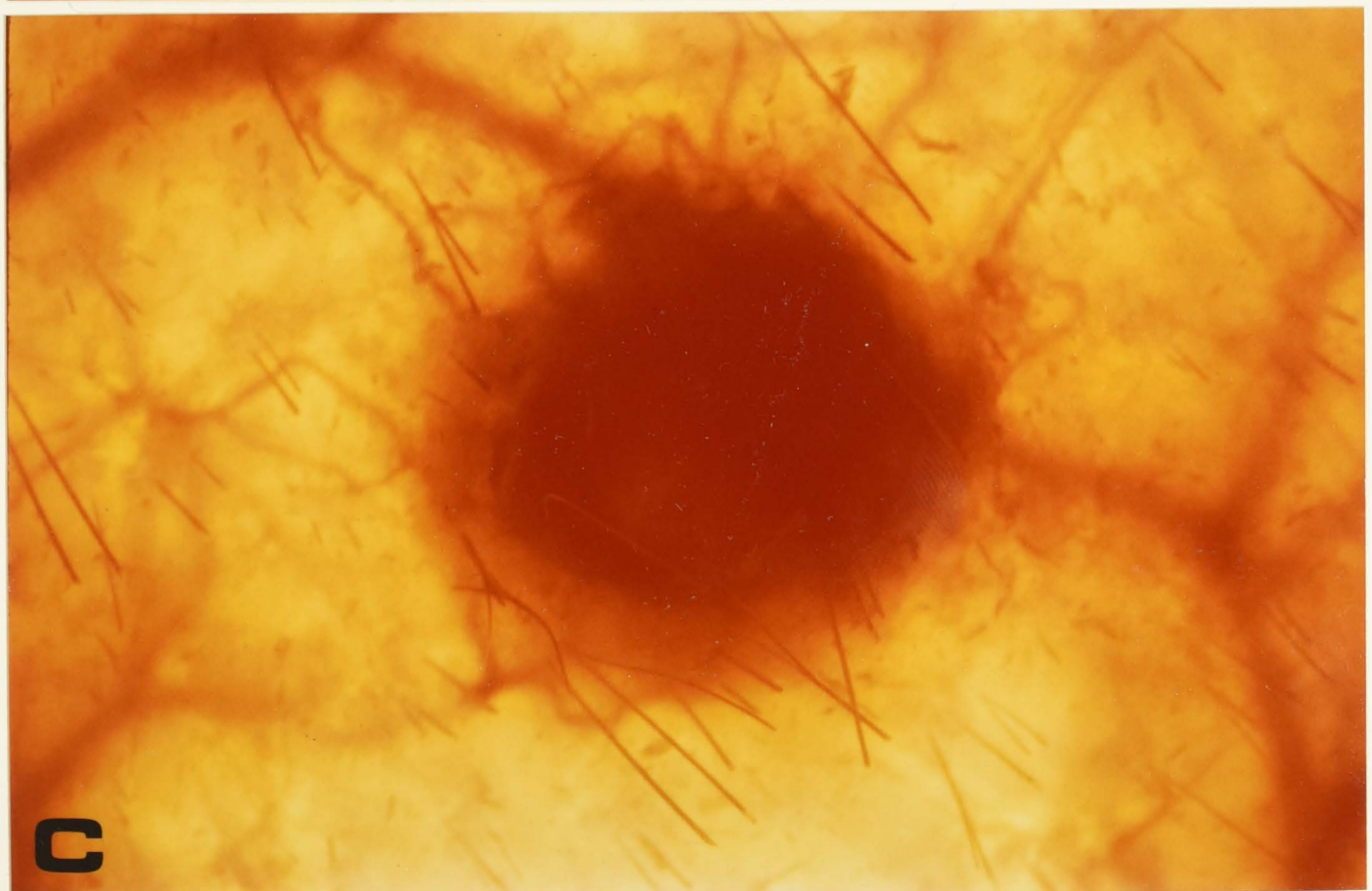
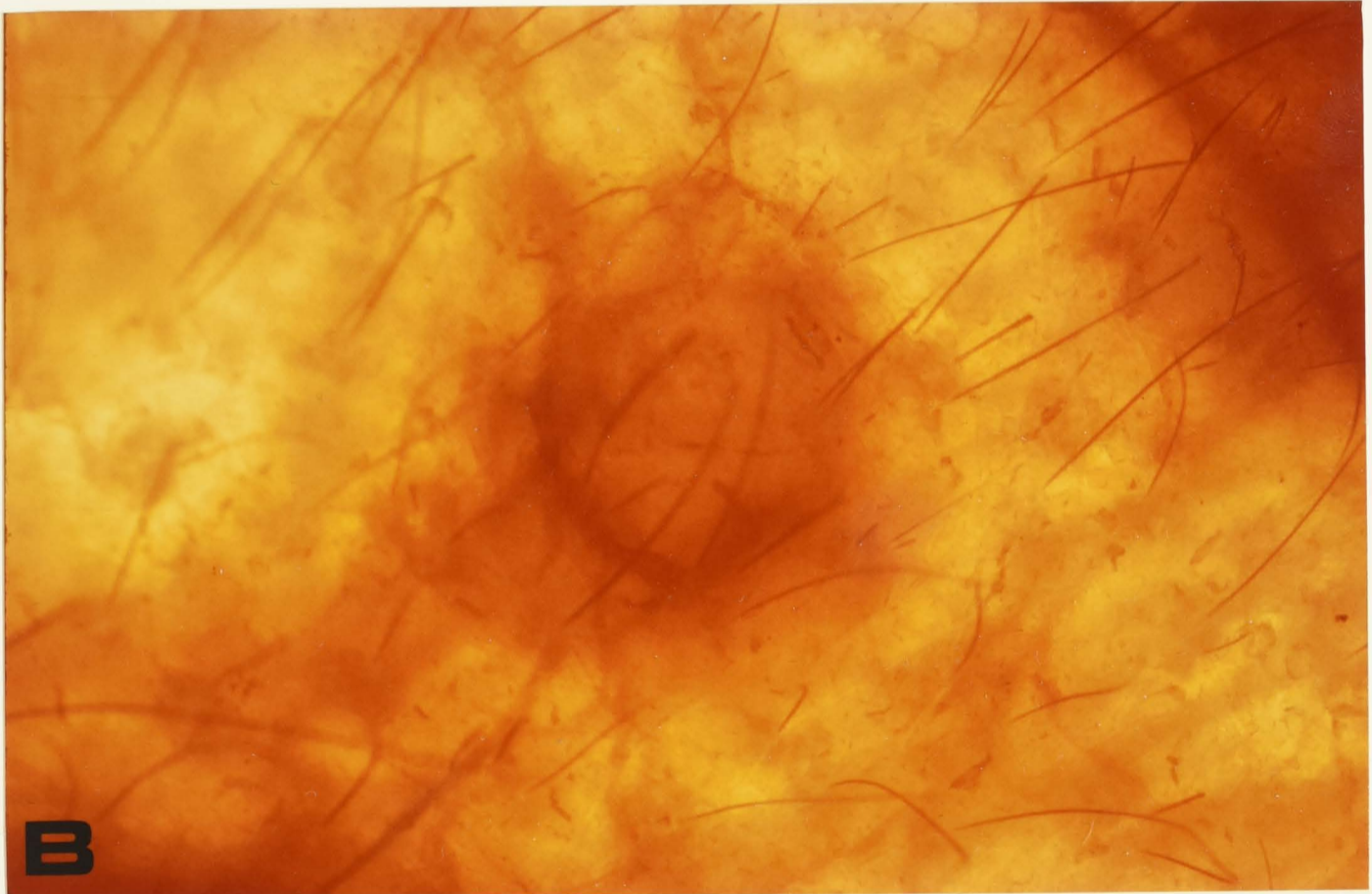
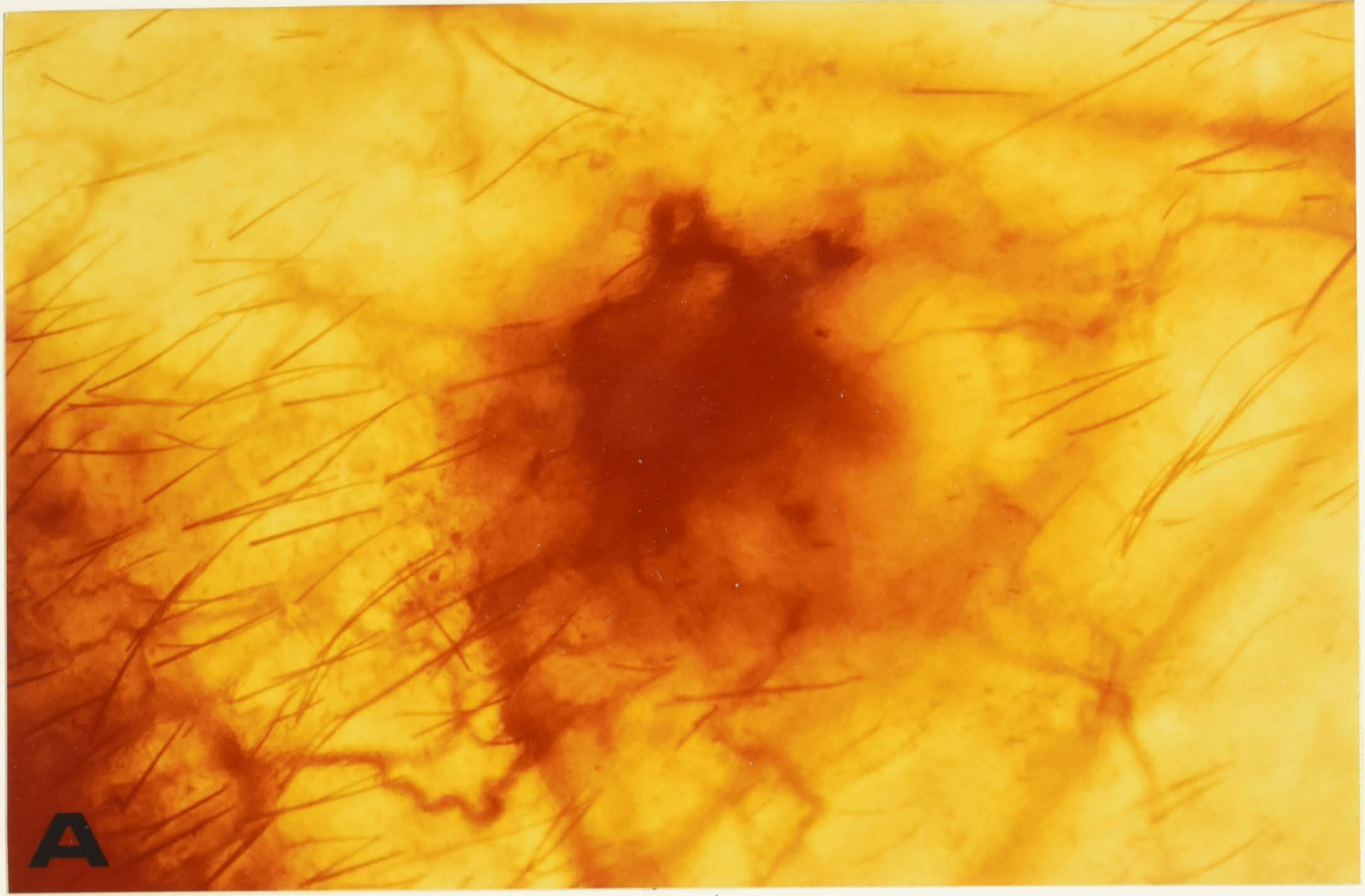
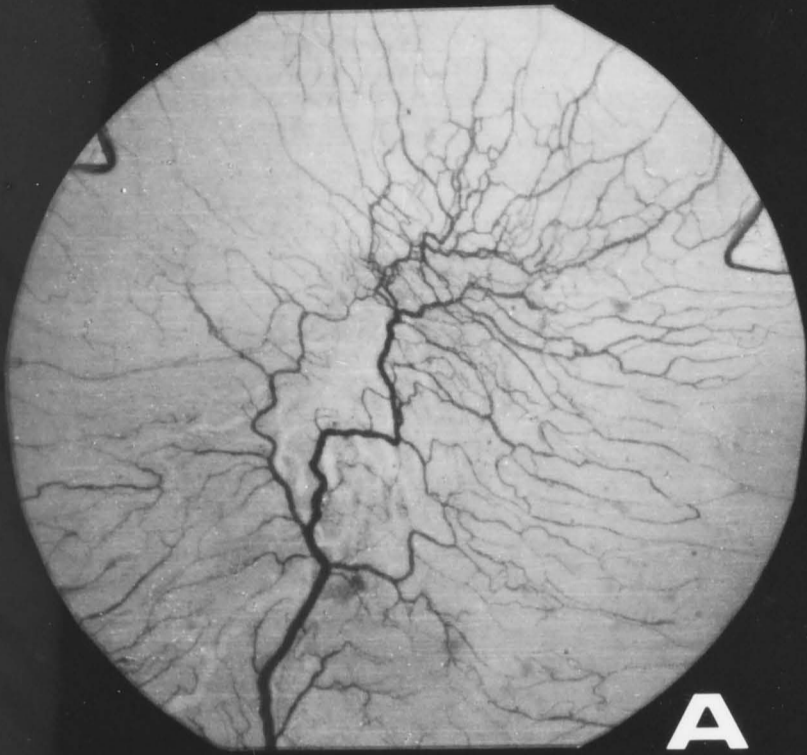


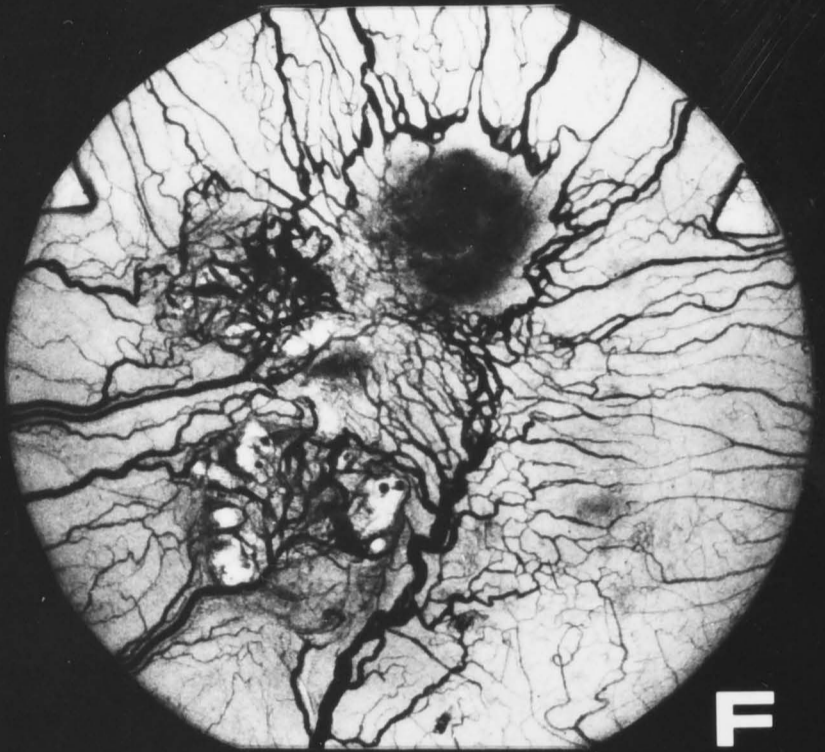
FIGURE III-4

Serial micrographs of the same rabbit ear chamber transplanted with 2 autografts (left) and 2 allografts (right) of endometrium. Rejection was induced in the ear chamber allografts by simultaneous subcutaneous transplantation of endometrial allografts from the same donor. (all X 13)

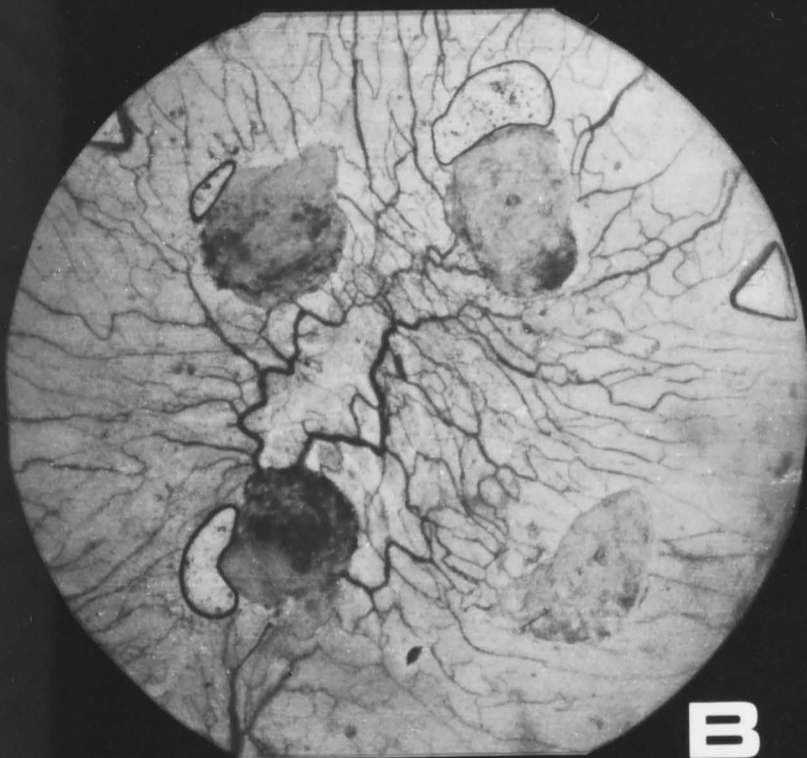
- A. The ear chamber membrane on the day before transplantation. Note the dark blood vessels and clear lymphatic vessels.
- B. One hour after transplantation and reassembly of the ear chamber. Blood is present in graft vessels and in areas of haemorrhage in the grafts.
- C. Eleven days after transplantation. The grafts are fully vascularized and blood flow to the membrane has increased. Clear cystic spaces are present in the auto- and allografts.
- D. Thirteen days after transplantation and 1 day after the onset of stasis in the allografts. Blood flow to the allografts has largely ceased with vessels collapsed or in stasis. Blood flow continues in the chamber membrane vessels below the lower allograft and in the autografts.
- E. Nineteen days after transplantation. Both allografts have been destroyed. The position of the upper allograft is marked by necrosis of the underlying ear chamber membrane. The position of the lower allograft is marked by macrophages and residual debris and vessels of the chamber membrane in this area are intact. Petechiae are present in the ear chamber membrane adjacent to the allografts.
- F. Twenty-one days after transplantation. The defect caused by necrosis of the top allograft and underlying membrane is healing by granulation. The debris from the lower allograft is being resorbed. The autografts remain healthy.



A



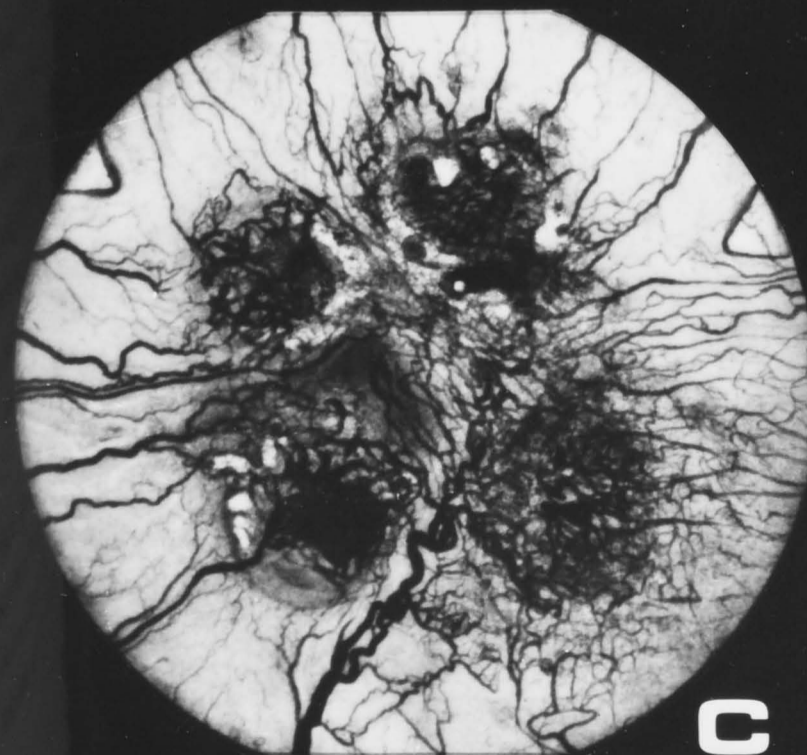
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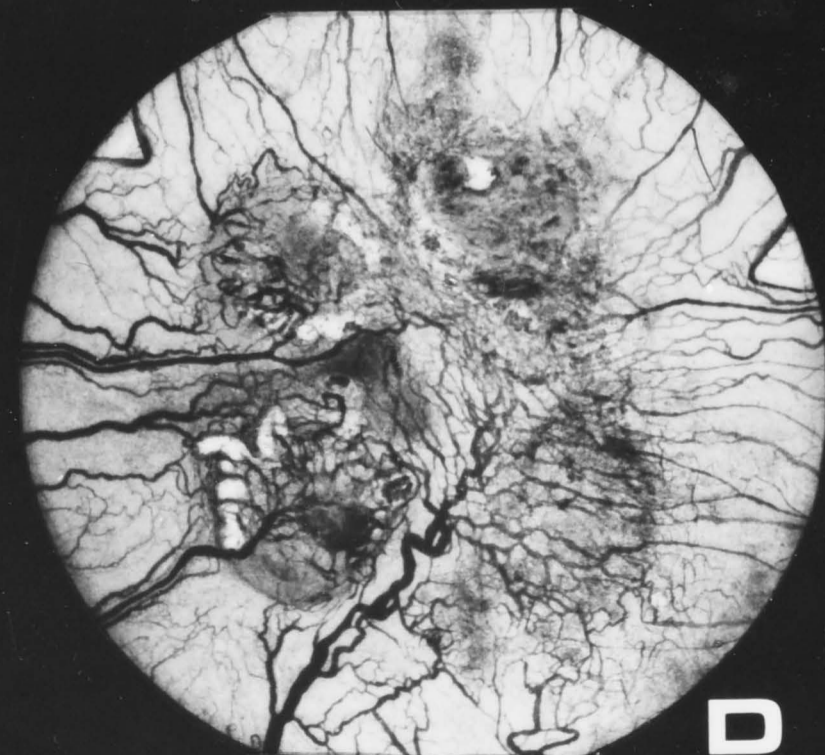
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E



C

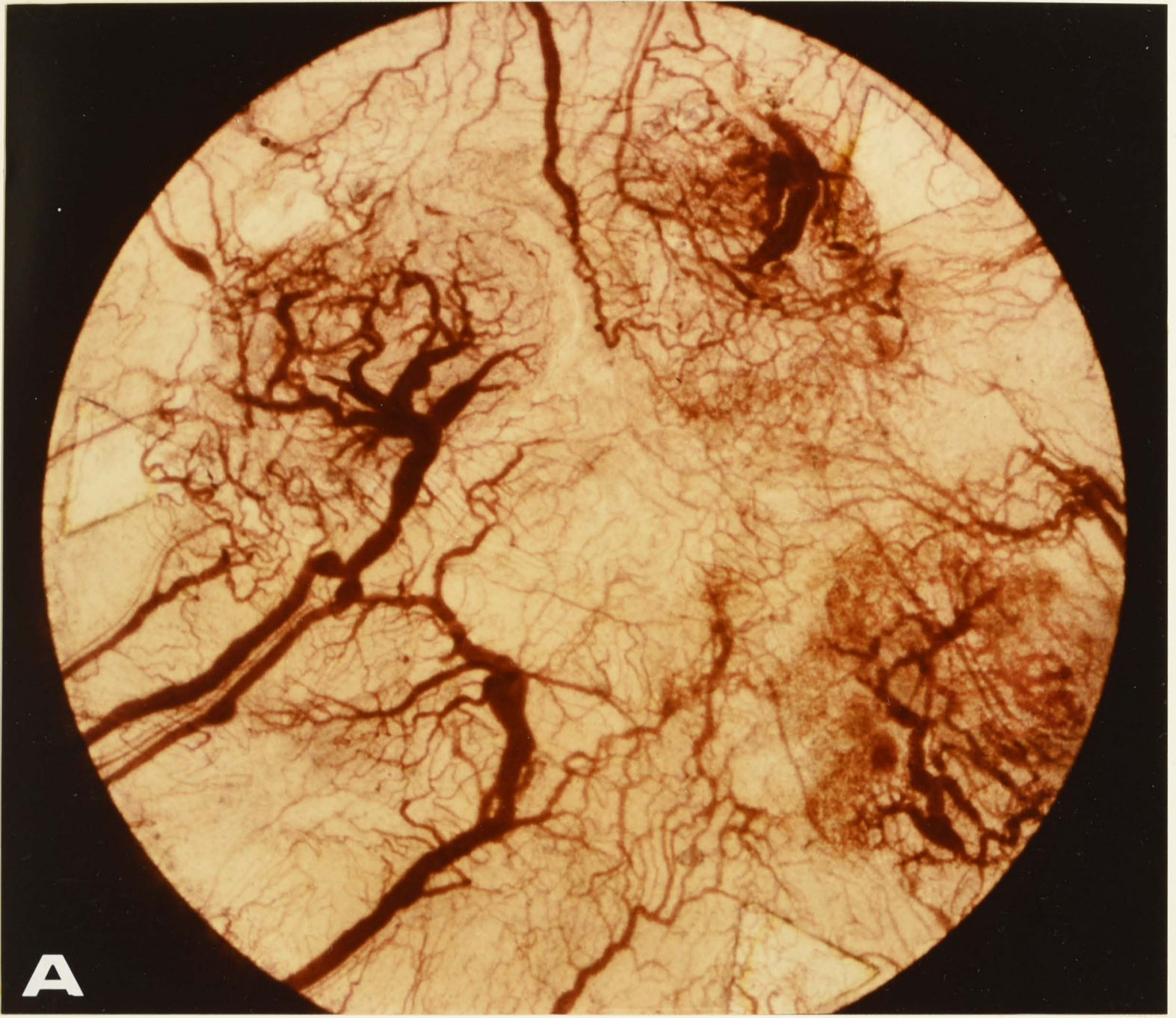


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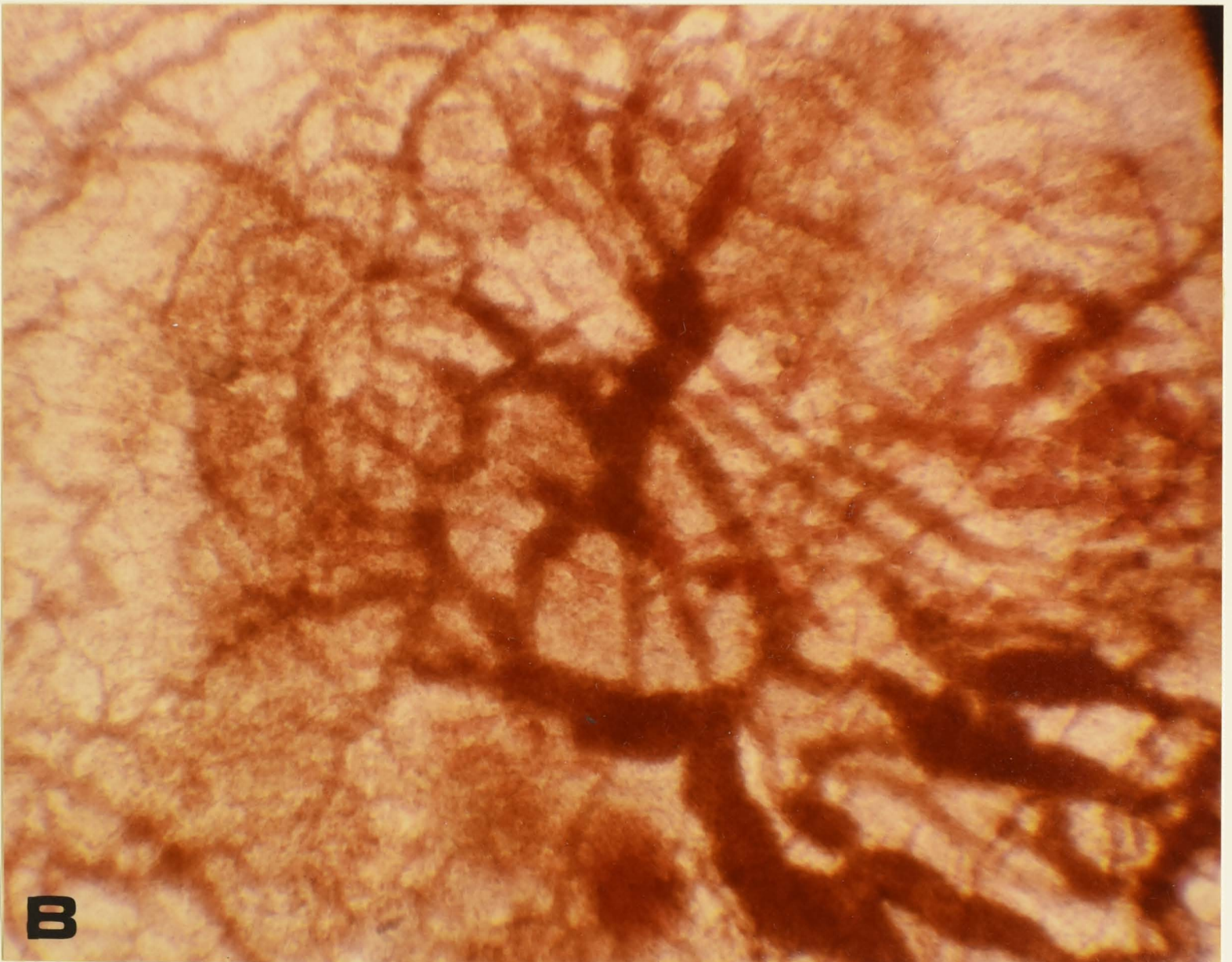
FIGURE III-5

An ear chamber with 2 autografts (left) and 2 allografts (right) of endometrium. One day after the onset of stasis in allografts undergoing spontaneous rejection.

- A. The lower allograft is showing patchy vascular stasis and petechiae. The upper allograft and the autografts show normal blood flow. (X 23)
- B. Higher magnification of the lower allograft showing detail of the vascular stasis and petechiae. The erythrocytes in the static vessels are pale pink in colour. (X 71)



A



B

FIGURE III-6

In vivo light micrographs of ear chamber preparations.

- A. Chamber membrane. A plexus of wide-caliber, thin-walled lymphatic vessels occupies most of the field. A macrophage and smaller leukocytes are in the lumina of the lymphatics. A single blood vessel crosses the lower part of the field. (X 250)
- B. The margin of an endometrial graft is present on the left of the field. Sinuous vascular sprouts are growing out from the graft. Blood vessels of the ear chamber membrane can be seen at a lower plane. (X 120)
- C. A chamber membrane 3 days after the onset of vascular stasis in adjacent endometrial allografts undergoing induced rejection. Two venules show a degree of leukocyte rolling and sticking to the endothelium that corresponds to a score of 2 on the 1 to 5 scale used in the present study. (X 490)
- D. An endometrial allograft on the day of the onset of vascular stasis during spontaneous rejection. A thrombus occludes the lumen of a venule (arrowhead). Three smaller vessels show stasis at the upper left margin of the field. (X 300)

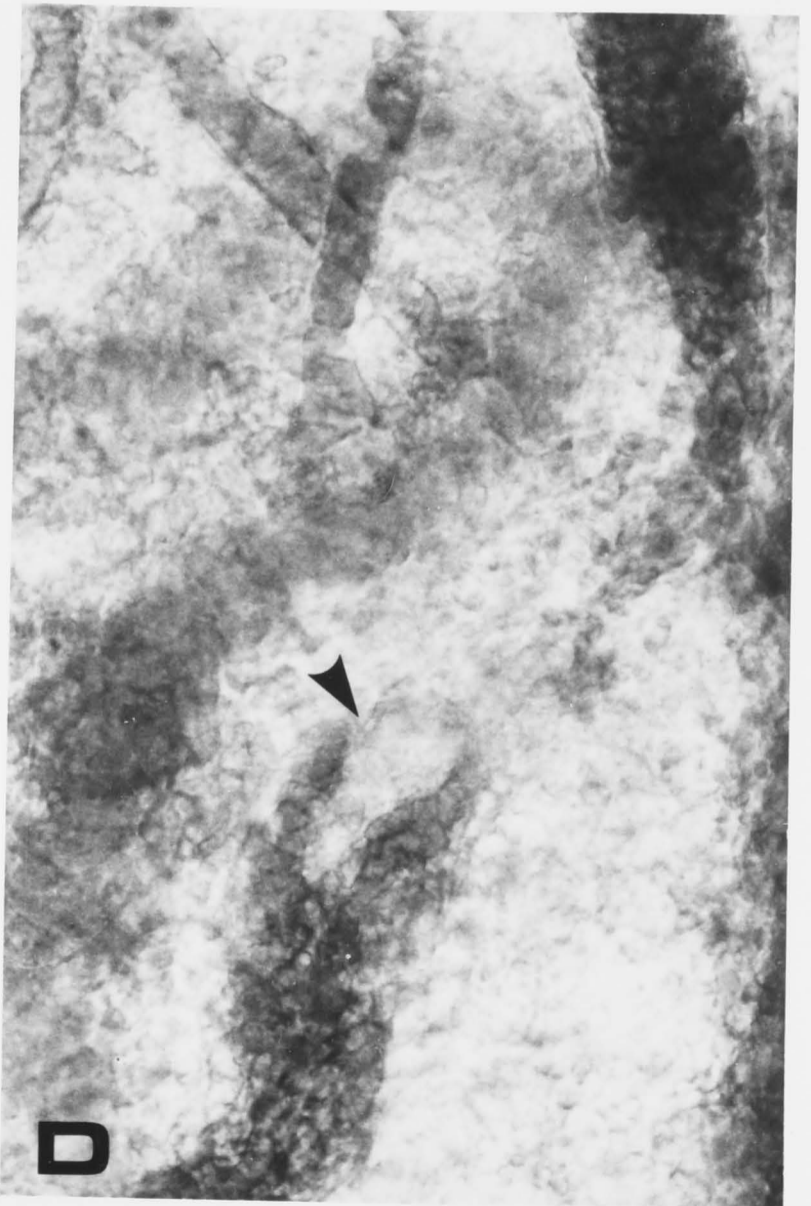
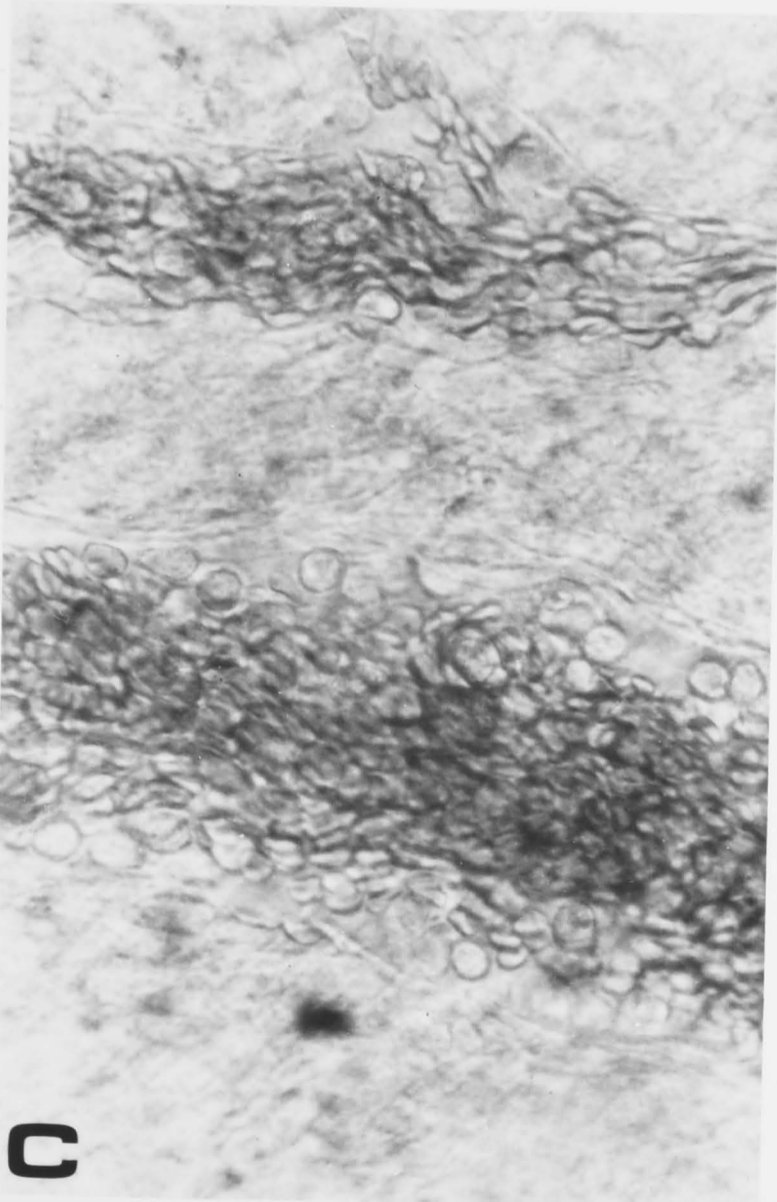
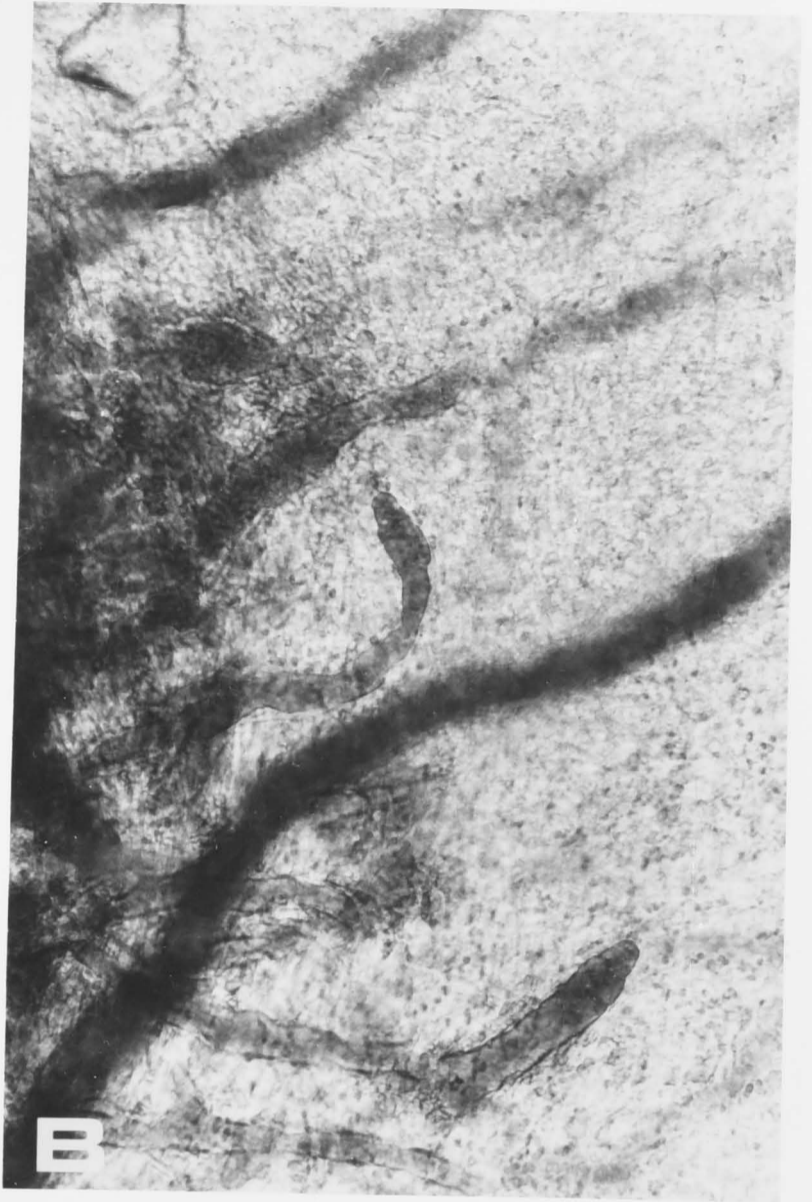
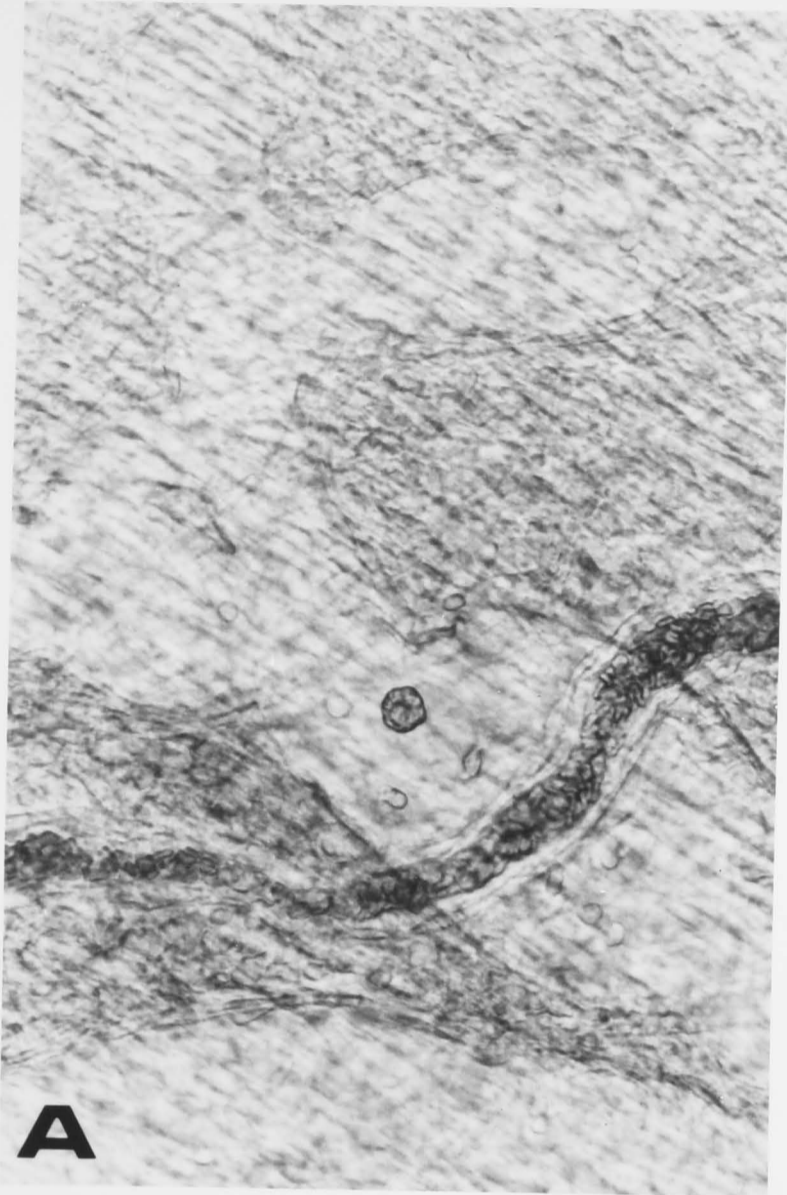


FIGURE III-7

In vivo light micrographs of endometrial allografts transplanted to ear chambers.

- A. Eighteen days after transplantation. The edge of the allograft extends over most of the left margin of the field. The epithelium at the edge of the graft is tall and columnar and where it extends over the adjacent ear chamber membrane the cells are seen end-on and present a cobblestone appearance. A few free-floating leukocytes are present at the graft margin. (X 430)
- B. One day after the onset of stasis during induced rejection. The epithelium at the edge of the allograft has been almost totally destroyed. Pleomorphic cells infiltrate the graft and are densest at the graft margin. (X 540)
- C. One day after the onset of stasis during induced rejection. The epithelial margin is indicated by the arrowheads. The epithelium is low and infiltrated by small numbers of leukocytes. The graft body is heavily infiltrated by leukocytes. The 2 blood vessels on the left of the field are still flowing. (X 540)
- D. An allogeneic epithelial monolayer overlying the ear chamber membrane 25 days after transplantation. Vascular stasis had not commenced in the allografts. An area of the monolayer contains refractile clumps (arrowheads) and leukocytes (open arrows). (X 540)

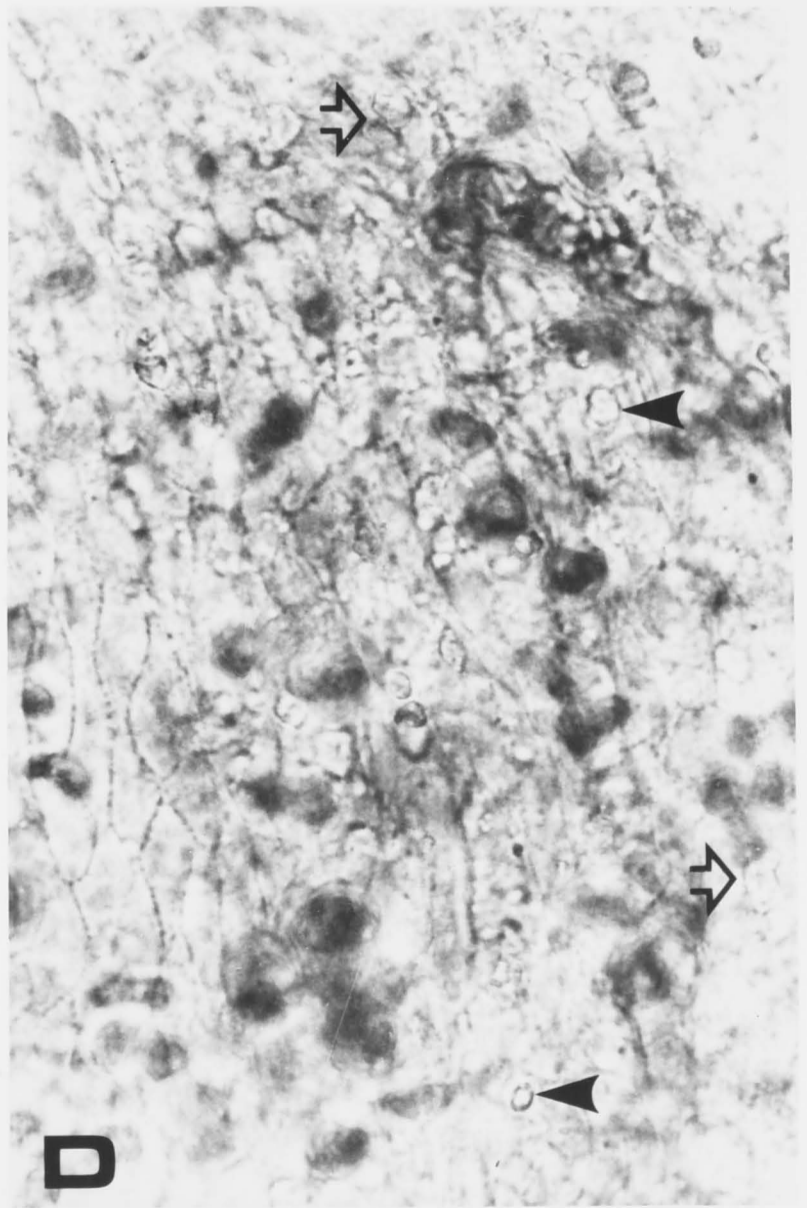
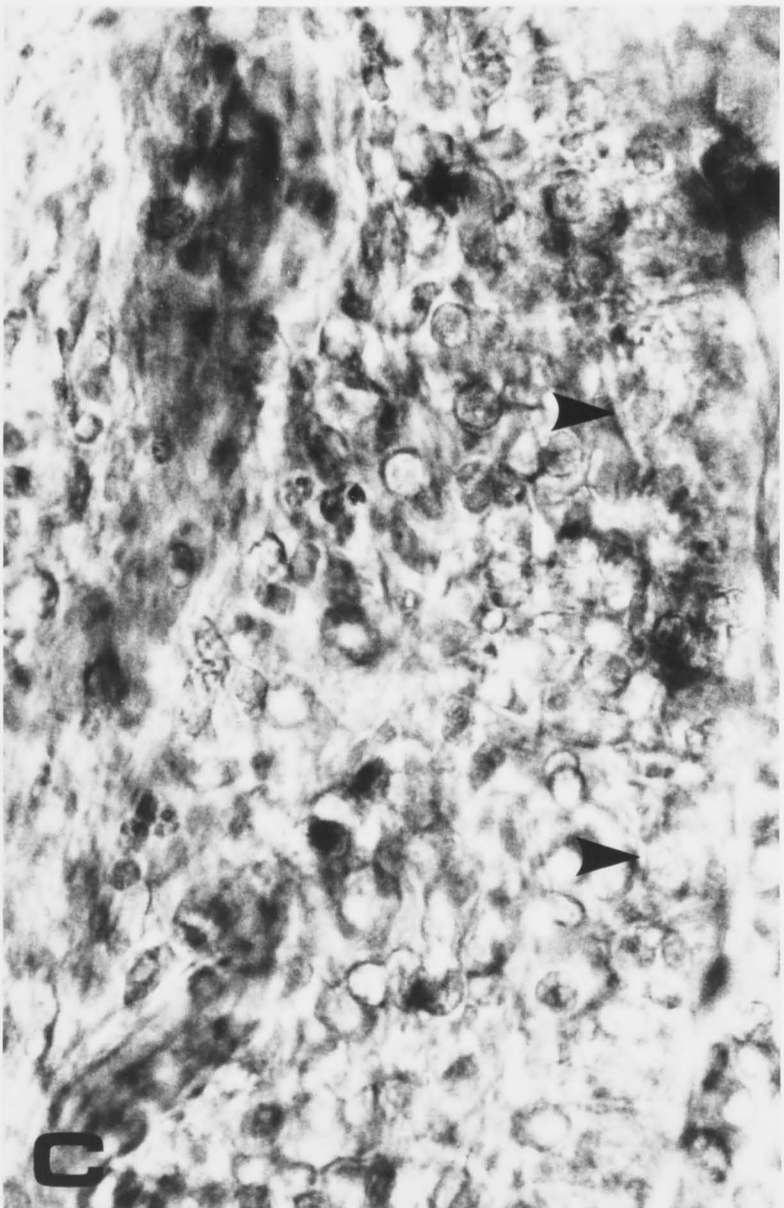
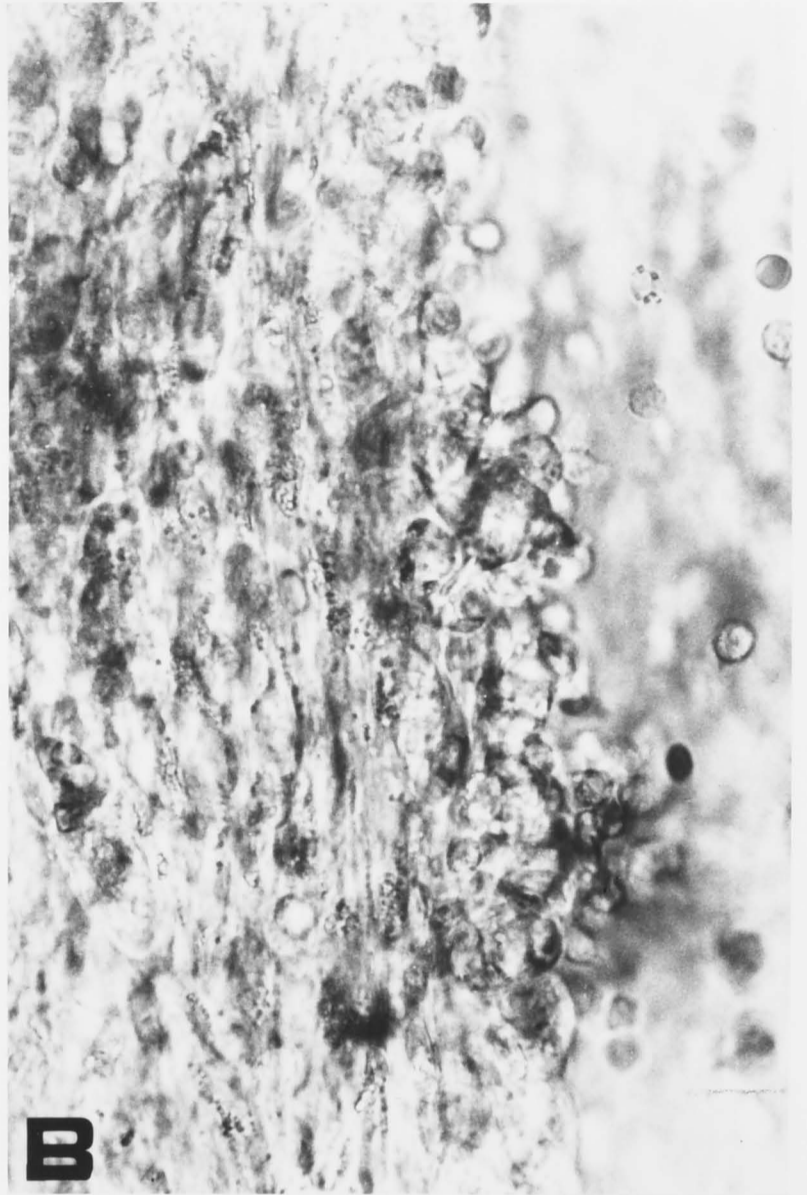
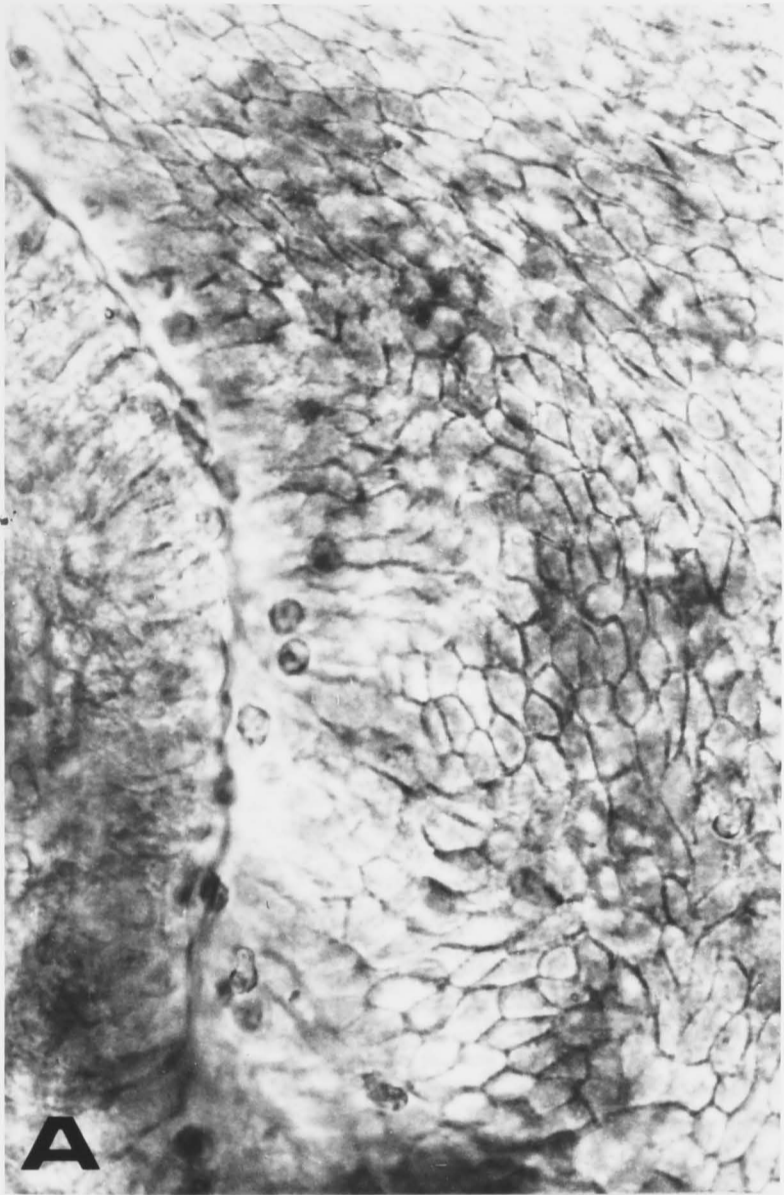
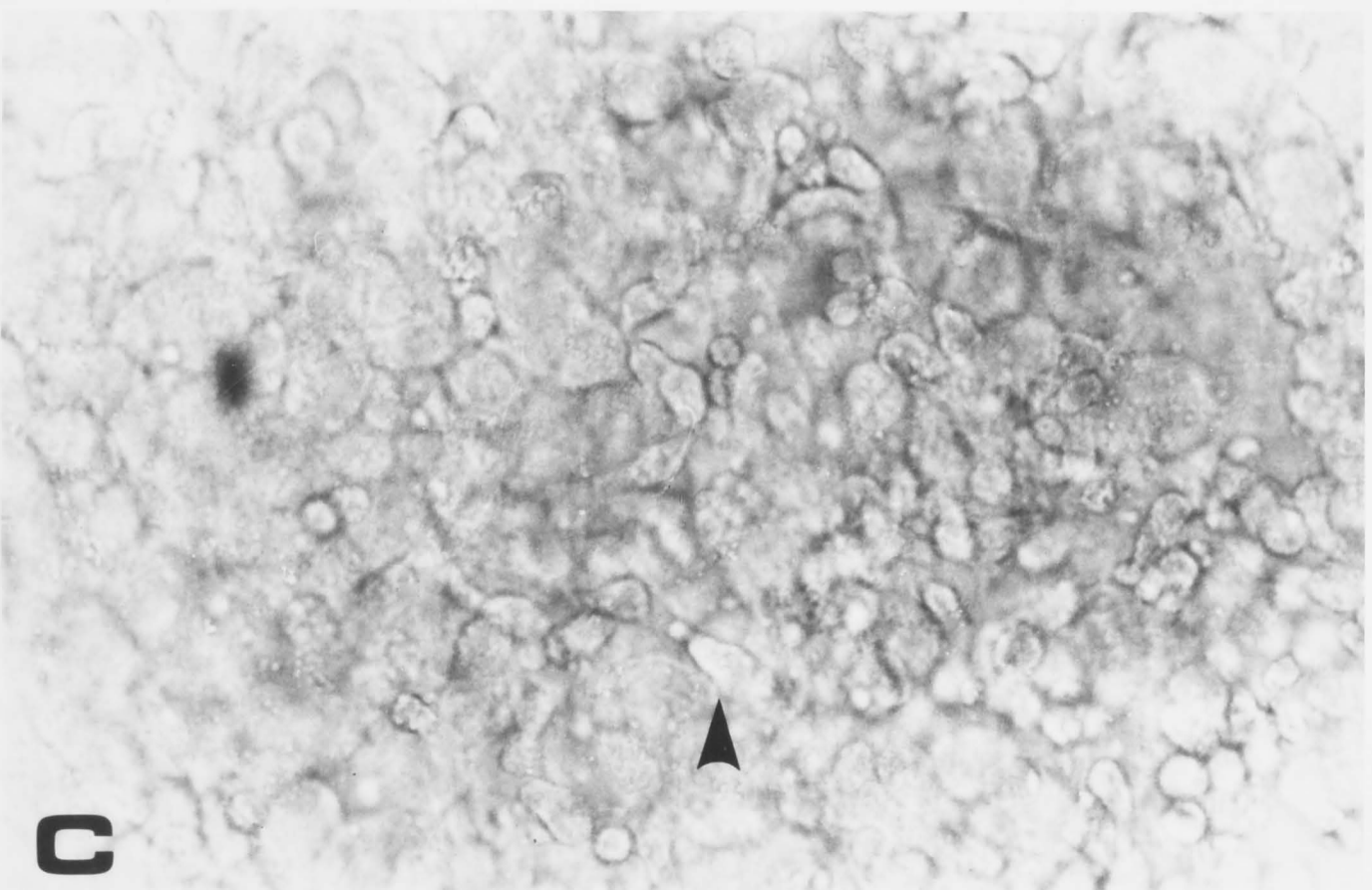
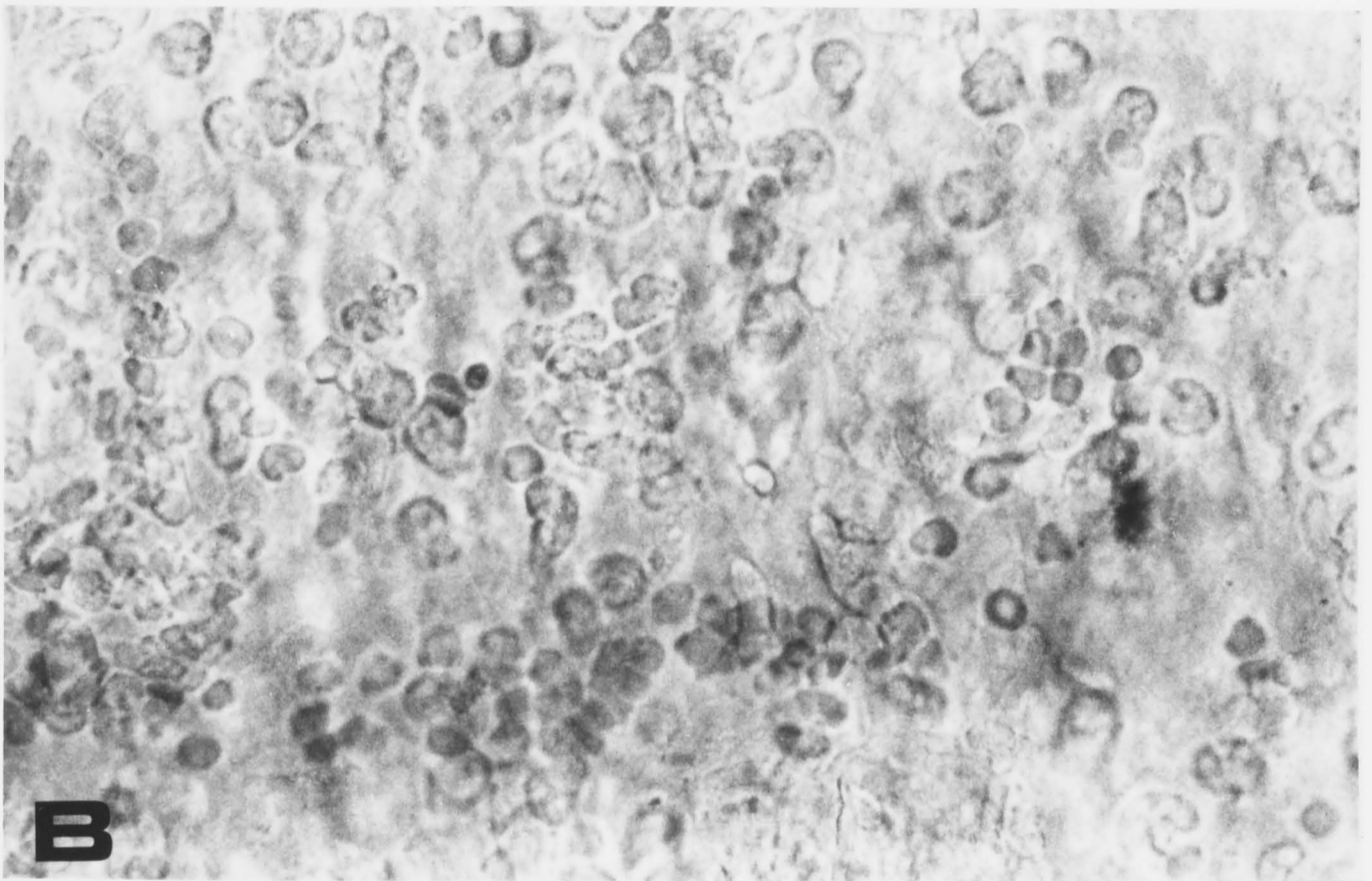
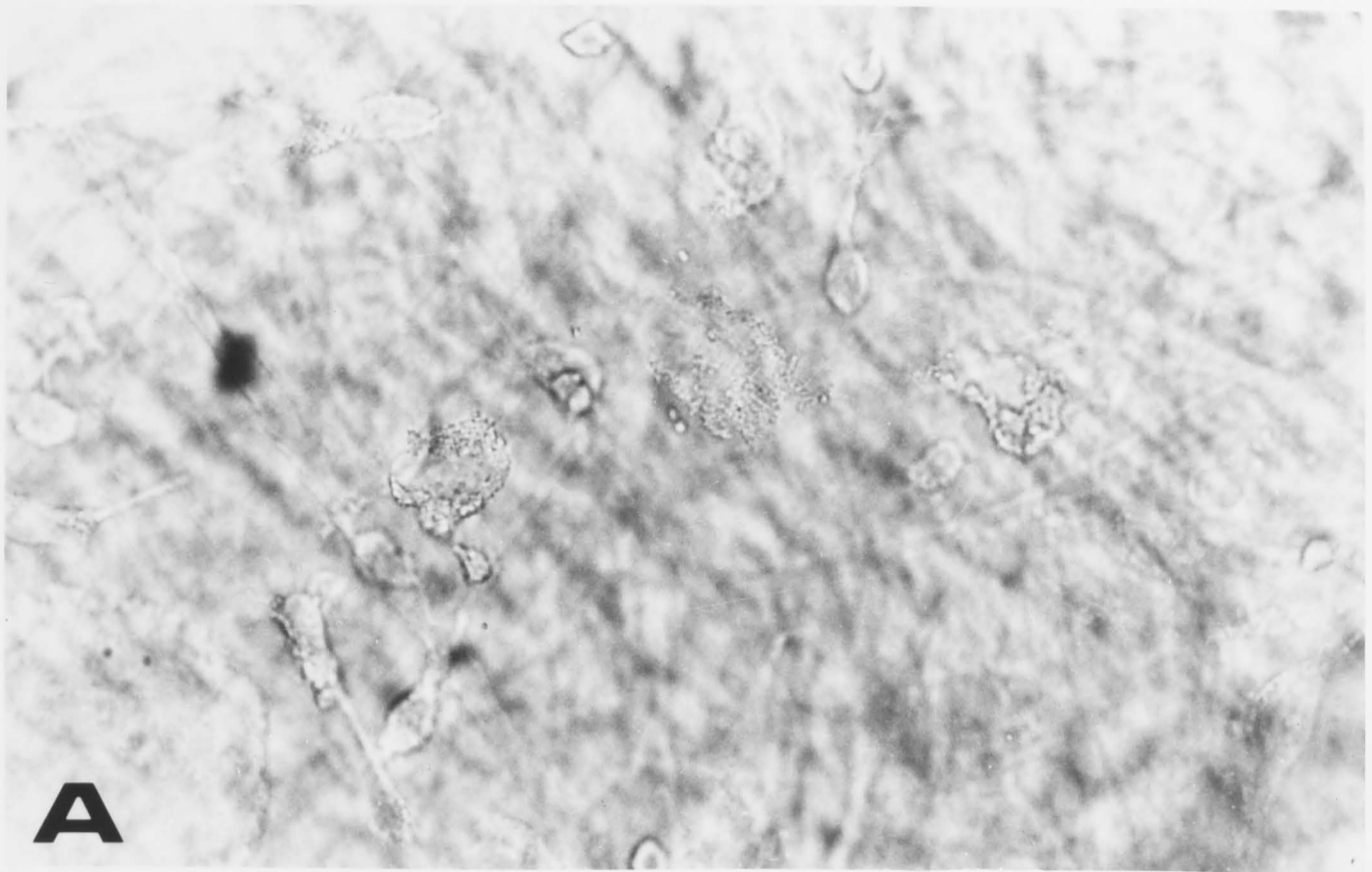


FIGURE III-8

Free cells on the surface of the ear chamber membrane.

(all X 600)

- A. A normal population of cells before transplantation. The population consists largely of macrophages some of which contain large granules.
- B. Twenty-four hours after transplantation of endometrial grafts. Erythrocytes are concentrated at the lower margin of the field and leukocytes, most of which are rounded, are numerous elsewhere.
- C. The day of the onset of stasis during induced rejection of endometrial allografts. The population of cells overlying the ear chamber membrane adjacent to an allograft. Most of the cells are large macrophages with an admixture of smaller leukocytes. A classical 'hand mirror' form of a migrating large lymphocyte is obvious (arrowhead).



CHAPTER IV

MORPHOLOGY OF FIXED ENDOMETRIUM AND ENDOMETRIAL GRAFTS

INTRODUCTION

Numerous studies on the morphology of allograft rejection by light and electron microscopy have been published (see Chapter I). However, interpretation of the results is hampered by uncertainty in assessing the in vivo blood flow from fixed specimens and the limited information on the stage of rejection in individual samples selected for study. These problems can be overcome by combining in vivo microscopic examination with post mortem study, but this approach has seldom been pursued. ROLLE ET AL (1959) used in vivo stereomicroscopy and histology as well as other techniques to study skin allografts in mice. They reported that cell degeneration in the allograft tissues commenced 24 to 48 hours after the development of complete vascular stasis. This and the results of other experiments where blood flow was interrupted to skin in situ led the authors to conclude that ischaemia was responsible for the degeneration of the allogeneic tissue. However, earlier cell degeneration in the allografts may have been overlooked because their observations on fixed tissue were limited to light microscopy. Additionally, the absence of damage to the grafts following the 48 to 72 hours of ischaemia immediately after transplantation conflicted with their conclusion that subsequent ischaemia over a similar time period caused destruction of the rejecting allografts. Furthermore, a greater degree of ischaemia may have been induced experimentally than occurred in the allografts because vessels below the panniculus carnosus were interrupted in the former but this was not reported during the rejection of allografts. GUTHY ET

AL (1974) performed similar studies but the relationship between the in vivo and post mortem observations was not extensively described. However, the authors concluded that the rejection of skin allografts may be due to either ischaemia following vascular damage or the direct action of lymphocytes on both the blood vessels and the epithelium of the allografts.

In this chapter the histological and ultrastructural features of the endometrial grafts are related to the in vivo observations.

MATERIALS AND METHODS

Samples from auto- and allografts in ear chambers were taken for light and electron microscopic examination at various times after transplantation and on the day of onset of vascular stasis in allografts or the following day (see Table III-3 and -4, samples from rabbit 586 were not examined). The samples taken after the onset of stasis covered the range of degrees of interference with blood flow observed in vivo in the allografts of each ear chamber. Grafts transplanted both to ear chambers and subcutaneous sites in the same rabbit were sampled at the same time, but the subcutaneous allografts were examined only by light microscopy. The procurement, processing and examination of samples has been described (Chapter II).

Analysis of samples from ear chambers by light microscopy. The region of either the graft body or the monolayer analysed usually included all that was present in a given section. Where sections were very large, regions were selected that showed a similar degree of damage throughout. The region of lamina propria analysed extended in all cases from the ear chamber membrane to the overlying endometrial epithelium. Cell counts included only those cells in which the nucleus had been sectioned. All blood vessels, degenerate cells, and apparently viable mononuclear and polymorphonuclear

cells in the lamina propria were counted. Degenerate cells were identified by dense clumping of chromatin in the nucleus and fragmentation of the cytoplasm. The area of lamina propria examined was determined by planimetry (see Chapter II) and the density of cells and vessels was calculated. The average area of lamina propria analysed per section was $4.7 \times 10^4 \pm 2.4 \times 10^4 \mu\text{m}^2$. ($\bar{X} \pm \text{SD}$). The average height of the endometrial epithelium of the graft body or monolayer analysed was determined from measurements at 4 equidistant points along its length but excluding those points where the epithelium had been destroyed. Infiltrating leukocytes and phagosomes in the length of epithelium examined were also counted. The proportion of surface epithelium that was destroyed represented the ratio of the length of surface denuded or covered only by degenerate cells to the total length of epithelium analysed. The average length of epithelium analysed per section was $406 \pm 76 \mu\text{m}$. ($\bar{X} \pm \text{SD}$). The number of vessels in the recipient connective tissue underlying the regions of grafts and monolayers analysed was expressed as number per length of tissue analysed. Semiquantitative determinations; for example, the degree of secretory vacuole formation in epithelium, were graded on a 5 point scale as described in Chapter II.

Electron microscopy of samples from ear chambers. While tissues from grafts at the various stages of establishment were examined, special attention was given to grafts at the time of rejection. Thus representative areas of all samples taken after the onset of stasis in allografts in ear chambers were also examined by transmission electron microscopy. The identification of monocytes, macrophages, lymphocytes and plasma cells was based on previous reports by NICHOLS ET AL (1971), WEISS (1972), SORENSON (1960), and HALL ET AL (1967). However, not all mononuclear cells could be identified. These included small or medium-sized mononuclear leukocytes with intermediate to marked peripheral chromatin condensation but which lacked other features permitting further identification. These cells were probably lymphocytes or monocytes between which morphological distinction is often not possible (ZUCKER-FRANKLIN, 1974). The features of larger mononuclear cells

with thin to intermediate peripheral chromatin condensation in the nucleus did not always permit a distinction between stromal fibroblasts and macrophages.

RESULTS

Normal endometrium

Cuboidal or columnar epithelium covered the surface of the lamina propria and formed simple or occasionally branched glands. Differentiated epithelial cells either contained secretory vacuoles in their supranuclear regions or else numerous cilia of typical structure projected from their luminal surfaces (Figure IV-1A). A membrane bounding secretory vacuoles was only rarely detected. The nuclei of these cells were large, had irregular outlines, contained condensed chromatin in a narrow peripheral zone and scattered larger clumps, and contained 1 or 2 nucleoli. The cytoplasm contained small oval mitochondria, moderate numbers of free ribosomes, several long strands of rough endoplasmic reticulum, and occasionally bundles of microfilaments and prominent Golgi apparatus. Numerous long microvilli projected from the luminal surface of the cells. The cells were joined by short desmosomes. The desmosomes were less common towards the base of the cells but here interdigitation of microvillous-like processes was often observed. The epithelium had a prominent, continuous basement membrane. Phagosomes containing unidentifiable material or cell debris were observed occasionally in the cells. Large mononuclear leukocytes were observed at times amongst the epithelial cells.

Endometrial grafts in ear chambers

Stromal fibroblasts were scattered throughout the lamina propria and often were more densely distributed in the zone subjacent to the epithelium. In some areas, pairs of stromal fibroblasts in close contact were observed (Figure IV-1B and -2B). Stromal fibroblasts had an oval nucleus with thin to intermediate peripheral chromatin

listed in Tables III-3 and -4.

condensation and, often, 1 or 2 nucleoli. The stromal fibroblasts usually showed only a moderate quantity of cytoplasm in the region of the nucleus while long cytoplasmic projections extended through the matrix of the lamina propria. The cytoplasm contained numerous free ribosomes, profiles of rough endoplasmic reticulum, low to moderate numbers of mitochondria and often several Golgi apparatus. Microfilaments and microtubules were scattered in the cytoplasm and occasionally the filaments were arranged into bundles at the periphery of the cells. The stromal fibroblasts occasionally contained fat globules and electron-dense, membrane-bound inclusions. Low numbers of macrophages containing numerous phagosomes were also observed in the lamina propria (Figure IV-1B).

Numerous venules and capillaries were observed in the lamina propria but arterioles were less common (Figure IV-2A). The endothelial cells had irregularly shaped, pachychromatic nuclei in which nucleoli were occasionally seen. The cytoplasm contained free ribosomes, moderate numbers of mitochondria, a few short profiles of rough endoplasmic reticulum and areas with numerous filaments. Numerous vesicles were observed at the luminal and abluminal surfaces and cytoplasmic vacuoles and occasional phagosomes were also noted. The endothelial cells, smooth muscle cells and pericytic cells had basement membranes.

Ultrastructurally, the matrix of the lamina propria contained patchy, finely granular material with interspersed bundles of collagen fibres (Figures IV-1B and -2A). Wavy segments of basement membrane-like material were observed adjacent to vessels and to some stromal fibroblasts (Figure IV-2A).

Endometrial grafts in ear chambers

Establishment of grafts. With few exceptions (see below) the features of the establishing auto- and allografts were the same until the onset of vascular stasis in the allografts. The time after transplantation that the grafts were fixed for examination are listed in Tables III-3 and -4.

The epithelium of the grafts examined at 3 days was flattened to low columnar in height and no glands were observed. Flocculent material, fibrin and erythrocytes were observed between and beneath the epithelial cells which largely remained adherent at their apices (Figure IV-3A). Secretory vacuoles, cilia and occasionally microvilli were absent. Disorientated ciliary basal bodies were observed in a few cells. The epithelial cells showed numerous polyribosomes and occasional fat globules, small phagosomes and dilated mitochondria. The epithelial basement membrane had disappeared in some areas. By 7 to 9 days, secretory vacuoles had reappeared in some of the epithelial cells of the grafts and monolayers. Basal separation of the cells persisted in some regions and microvillous-like processes from the lateral surfaces of the cells often projected into the spaces. In a few instances an area of monolayer had been overgrown by young fibrous tissue so that cysts had formed. However, the epithelium had also extended over the surface of the new tissue. The epithelial cells showed numerous microvilli and some contained moderate numbers of secretory vacuoles and long profiles of rough endoplasmic reticulum by 12 to 13 days (Figure IV-4A). At this time a few cells showed reduced density of the central regions of the nucleus and dilation of mitochondria and rough endoplasmic reticulum suggesting the presence of injury (Figure IV-4B). By 20 to 30 days after transplantation most of the epithelium of autografts was cuboidal or columnar but lateral overlapping of the cells was also observed (Figures IV-6A and -11A). The epithelial cells showed long surface microvilli and numerous secretory vacuoles. Ciliated cells were rare. Some epithelial cells extended small cytoplasmic processes through gaps in the basement membrane. Basal separation of the epithelial cells and lateral microvillous-like processes persisted in some regions but erythrocytes and debris in the spaces had been removed.

The lamina propria of the 3 day grafts was oedematous and contained extravasated erythrocytes and fibrin (Figure IV-3A). Some stromal fibroblasts showed prominent nucleoli and an increased volume of cytoplasm in the region of the nucleus with numerous rough

endoplasmic reticulum profiles and mitochondria (Figure IV-3B). Numerous bundles of microfilaments were observed at the periphery of some of these cells. The lamina propria at 7 to 9 days after transplantation was still oedematous, contained some haemorrhage and was infiltrated by leukocytes (Figure IV-5B). A zone of young fibrous tissue which contained small blood vessels was observed below and had sometimes overgrown monolayers. By 12 and 13 days, some of the stromal fibroblasts closely resembled those of normal endometrium but many remained hypertrophied. Some of the latter showed numerous vacuoles and vesicles, enlarged mitochondria and long profiles of rough endoplasmic reticulum (Figure IV-5A). Hypertrophy of stromal fibroblasts persisted at 20 to 30 days after transplantation and cytoplasmic vacuoles and phagosomes containing cytoplasmic fragments were observed in some of these cells.

When the graft had formed connections with the recipient vasculature 3 days after transplantation, many of its blood vessels were packed with erythrocytes or platelets. Endothelial cells in parts of some vessels had degenerated and thrombi composed of platelets and fibrin were attached at these sites. Some endothelial cells were enlarged and had large oval nuclei with reduced chromatin condensation and prominent nucleoli (Figure IV-3C). By 7 and 9 days, the vessels of only a few grafts showed damage. At later times the endothelium of the graft vessels was intact. The endothelial cells often showed hypertrophic changes and a few had large phagosomes containing cell debris (Figure IV-5C). Wavy basement membrane-like material was observed around some of the larger vessels (Figure IV-6B).

Most of the vessels in the ear chamber membrane underlying the grafts at 3 days were unaltered but others were packed with erythrocytes, contained platelet aggregates and their endothelium had degenerated. Vascular abnormalities in this tissue were rare thereafter. Fibrocytes in the ear chamber membrane and young connective tissue associated with monolayers showed hypertrophic changes, but these changes had subsided by 20 to 30 days after transplantation (Figure IV-11A).

Disturbances to the establishment of grafts

Failure of vascular connection. One autograft from ear chambers in each of 2 rabbits which showed no in vivo evidence of blood vascular anastomosis with the recipient were fixed for examination at either 3 or 13 days after transplantation.

Both grafts appeared to have been transplanted with their epithelial surfaces facing the ear chamber membrane because epithelium lined both the surface of the ear chamber membrane in the region of the grafts and the undersurface of the grafts. Epithelium had also extended onto the exposed upper surfaces of the grafts (Figure IV-8A). The epithelium on the undersurface of the grafts showed similar changes with time after transplantation to that of vascularized grafts except that secretory vacuoles were not observed at 13 days (Figures IV-7A and -8A). The epithelium extending onto the upper surface of the 3 day graft and onto the surrounding membrane had only a few short microvilli. The epithelium on the upper surface of the 13 day graft was attenuated but had numerous microvilli, moderately enlarged mitochondria and dilated rough endoplasmic reticulum (Figure IV-8B). A few epithelial cells here appeared injured with nuclei showing smooth outlines, central electron-lucency and peripheral chromatin condensation while perinuclear cisternae and mitochondria were dilated and the numbers of microvilli were reduced (Figure IV-8B and C). The epithelial basement membrane in the upper regions of both grafts was discontinuous.

The lamina propria adjacent to the ear chamber membrane of both grafts contained numerous stromal fibroblasts but these cells became sparse further from the membrane (Figures IV-7A and -8A). In the 3 day graft, the stromal fibroblasts showed similar features to those in vascularized grafts at the same stage except that some cells showed bleb-like cytoplasmic protrusions (Figure IV-7A and B). The surviving stromal fibroblasts of the 13 day graft showed increased peripheral clumping of chromatin, and dilation of perinuclear cisternae, rough endoplasmic reticulum and mitochondria. In both grafts, cell fragments containing dilated or degenerate

organelles were noted at sites furthest from the ear chamber membrane.

Vessels of the 3 day graft had intact endothelium but had collapsed and contained only small numbers of erythrocytes, and occasional platelets and degenerate cells (Figure IV-7B). Blood vessels also persisted in the 13 day graft but they were dilated with their lumina filled with cell debris and occasional erythrocyte fragments (Figures IV-8A, -9A and B). The endothelial lining of the blood vessels was intact except in the regions most distant from the ear chamber membrane. In most areas, the endothelium showed only dilation of a few mitochondria and of rough endoplasmic reticulum.

Blood vessels of the ear chamber membrane underlying the grafts were unaltered except for mitochondrial dilation in some endothelial cells.

Interference with blood circulation to the graft and to the underlying ear chamber membrane. Samples were taken for histology at 3, 7 and 13 days after transplantation from regions of 2 autografts and 1 allograft that were noted in vivo to be engorged, haemorrhagic and necrotic. Only back and forth pulsatile movement of blood in a few vessels of these regions was observed.

In the region examined at 3 days after transplantation, low surface epithelium survived in places but elsewhere only the basement membrane persisted. The grafts sampled at 7 and 13 days both appeared to have been transplanted with the epithelial surface facing the chamber membrane because low to cuboidal epithelium covered the undersurfaces of the grafts and the surfaces of the ear chamber membranes (Figure IV-10A). Epithelial cells had extended onto the surface of the region examined at 13 days but they had enlarged mitochondria, dilated rough endoplasmic reticulum and numerous phagosomes containing unidentified debris. Stromal fibroblasts had degenerated in the region sampled at 3 days except for those adjacent to the ear chamber membrane. Only a few cells, including stromal fibroblasts, neutrophil granulocytes and macrophages, were observed in the lamina propria of the regions

examined at other times (Figure IV-10B). The damaged fibroblasts showed dilation of rough endoplasmic reticulum and perinuclear cisternae while others showed only marked dilation of mitochondria. More severely damaged cells showed peripheral chromatin clumping in the nucleus, greater dilation of cytoplasmic organelles and breakdown of cell membranes (Figure IV-10C). The endothelial cells of most vessels in these regions had degenerated but some persisted close to the ear chamber membrane in the region examined at 3 days.

Many of the vessels in the ear chamber membranes underlying these regions of the grafts were engorged with erythrocytes and had degenerate endothelium, features which were sometimes obscured in vivo by haemorrhage in the grafts (Figure IV-10A). Fibrocytes and leukocytes in these regions of the chamber membrane had also degenerated.

The rejection of allografts

Light microscopy. The results of analysis of samples by light microscopy are shown in Tables IV-1 to -5. The data in Table IV-4 is listed in the order of increasing density of degenerate cells in the lamina propria of the allografts and that in Table IV-5 is listed in the order of increasing proportions of degenerate epithelium. The onset of stasis refers to the development of vascular stasis anywhere in the allogeneic tissue within one ear chamber. Samples said to be taken after the onset of stasis were therefore not necessarily from regions showing such stasis.

The leukocytes and the few degenerate cells in the lamina propria of the autografts and allografts before the onset of stasis showed a diffuse distribution. After the onset of stasis in the allografts, the distribution of these cells remained diffuse in most samples but foci containing higher densities of these cells were observed in a few samples (Figure IV-11B, C and D). Surface epithelium adjacent to the latter foci often showed greater damage than in other areas (Figure IV-11B). Degenerate cells abutted both morphologically intact and damaged vessels in the lamina propria of the allografts after the onset of stasis. In a few instances, blood flow in these

intact vessels had been identified in vivo immediately prior to fixation.

Electron microscopy of allografts after the onset of stasis. The epithelial cells of the less damaged regions of allografts and their monolayers were low and some had enlarged nucleoli and condensed mitochondria (Figure IV-12A and B). Microvilli were sparse and stubby or absent. The mononuclear leukocytes infiltrating the epithelium were lymphocytes and occasionally macrophages.

Mononuclear leukocytes, including lymphocytes and macrophages, had also formed contacts with epithelial cells of the monolayers through gaps in the basement membrane (Figure IV-12A and B). Where cysts had formed following overgrowth of the monolayers, macrophages and neutrophil granulocytes were also in contact with the luminal surface of the epithelium. Rarely phagosomes containing nuclear remnants were observed in the epithelial cells.

With increasing damage, the epithelial cells separated from each other and their basement membrane (Figures IV-13, -14 and -15). The nuclei of degenerate epithelial cells usually showed margination of chromatin and diffuse nucleoli but the chromatin in some was homogeneous and of intermediate density (Figure IV-13A and B). In the cytoplasm, mitochondria were condensed or dilated, the rough endoplasmic reticulum was vesiculated, polyribosomes had broken up and large cytoplasmic vacuoles were observed. Small numbers of secretory vacuoles persisted in some of these cells. Leukocytic infiltration of the degenerating epithelium was more pronounced than that of the intact epithelium. The leukocytes were mainly macrophages, but also included lymphocytes and neutrophil granulocytes. Some of the leukocytes also appeared to be degenerate (Figure IV-13A). Macrophages were observed extending through the basement membrane in these areas. However, the basement membrane more usually was intact although it was thrown into folds (Figures IV-13 and -15). Some flattened epithelial cells remained attached to their basement membranes (Figures IV-14 and -15).

Stromal fibroblasts, small unidentified mononuclear leukocytes, macrophages and neutrophil granulocytes were observed as well as degenerate cells in the lamina propria of the less damaged allografts. Only small numbers of lymphocytes were identified when rejection was spontaneous but these cells, especially large activated lymphocytes, were more numerous when rejection was induced (Figures IV-16 and -17). The mononuclear leukocytes and, less often, neutrophil granulocytes formed contacts with the stromal fibroblast bodies or their processes. The mononuclear leukocytes often formed broad parallel or interdigitating contacts or extended cytoplasmic projections to the allogeneic cells (Figures IV-16, -17 and -18). The neutrophil granulocytes formed predominantly broad parallel contacts. Some stromal fibroblasts in contact with the leukocytes showed swollen mitochondria. When rejection was induced, some of the stromal fibroblasts in contact with the mononuclear leukocytes had formed large pedunculated cytoplasmic protrusions and, less often, blebs of cytoplasm appeared to have been shed (Figure IV-17). In all the micrographs examined, only one cell showed nuclear fragmentation without advanced disruption of cytoplasmic organelles (Figure IV-18B). Cells of the vessels showed evidence of minor injury through to advanced degeneration. Where more damage had occurred in the lamina propria, mononuclear phagocytes were the predominant infiltrating cells and were phagocytosing degenerate cells and erythrocytes. The form of degeneration of cells was similar to that observed in the epithelium. Some degenerate cells still contained abundant rough endoplasmic reticulum indicating that they were stromal fibroblasts (Figure IV-20). Degeneration of cells immediately adjacent to intact blood vessels was often observed (Figure IV-21). Undamaged leukocytes persisted amongst the degenerating cells in all areas (Figure IV-19). Small mononuclear leukocytes with numerous narrow cytoplasmic projections, some of which could be identified as monocytes, were observed free in the lumina or in contact with the endothelium of some undamaged vessels (Figure IV-22). Evidence of a breakdown

in the integrity of vessels; for example, the presence of erythrocytes and platelets below the endothelium, was occasionally observed before degeneration of the endothelium occurred. Thrombo-emboli composed of fibrin, platelets, erythrocytes, leukocytes and degenerate cells were observed in some of the severely damaged vessels (Figure IV-23). The degenerate cells often had numerous sacs of rough endoplasmic reticulum and were probably sloughed endothelial cells. Other vessels were packed tightly with erythrocytes and all the endothelium was degenerate (Figure IV-24). Leakage of erythrocytes and fibrin into the stroma of the grafts accompanied severe vascular damage.

The same range of leukocyte types observed in the lamina propria of the allografts had infiltrated the tissues underlying the allograft bodies and their monolayers (Figures IV-12 and -14). These leukocytes often formed contacts with the host fibrocytes. Plasma cells were observed when destruction of the overlying allogeneic tissue was advanced. Fibrocytes and blood vessels showed little abnormality in these regions during spontaneous rejection. When rejection was induced, host fibrocytes and cells of the vessels showed evidence of minor injury through to advanced degeneration similar to that observed in rejecting allogeneic tissues (Figure IV-12B).

Subcutaneous grafts

The subcutaneous grafts sampled for light microscopy are listed in Table IV-6.

Establishment of grafts. Subcutaneous auto- and allografts transplanted alone or in conjunction with grafts in ear chambers showed similar features at 7 and 9 days after transplantation. Epithelium of the grafts was cuboidal or columnar and often contained moderate numbers of secretory vacuoles (Figure IV-25A). Ciliated cells were rare. Occasionally, basal separation of the cells was observed, as in ear chamber grafts. Polymorphonuclear leukocytes were often numerous immediately below the endometrial

epithelium but few were actually infiltrating this tissue. The epithelial cysts derived from the grafts contained variable numbers of polymorphonuclear leukocytes, macrophages and erythrocytes. The lamina propria of the grafts was moderately oedematous and contained foci of haemorrhage. Stromal fibroblasts were numerous but few infiltrating leukocytes were observed. Numerous thin-walled blood vessels were observed in the lamina propria. The endothelial cells of many of the vessels were enlarged. A fibroplastic response was observed in the host connective tissue surrounding the grafts and some of the vessels here also showed enlarged endothelial cells. Infiltration by moderate numbers of polymorphonuclear and fewer mononuclear leukocytes was often observed. Haemorrhage and fibrin was identified in the surrounding host tissues.

In subcutaneous autografts 12 to 13 days after transplantation, epithelial cells contained more secretory vacuoles than observed earlier and phagosomes were observed in moderate numbers of these cells (Figure IV-25B). Most cells free in the lumina of the epithelial cysts appeared viable. Vascular sprouts were still obvious and enlargement of the endothelium persisted in many of the vessels of the lamina propria. In a few samples, moderate mononuclear leukocytic infiltration was observed in the adjacent host tissue together with perivascular cuffing of occasional vessels. Enlargement of endothelial cells persisted also in a few of the host vessels.

Rejection of allografts. The subcutaneous allografts examined at 12 days after transplantation showed extensive damage. Much of the endometrial epithelium lining the host tissue had been destroyed. Epithelium persisted on the surface of the lamina propria of one allograft but was markedly attenuated, contained few secretory vacuoles and showed only minimal leukocytic infiltration (Figure IV-25C). Peripheral clumping of the chromatin and loss of density of the central regions of the nuclei of some of these epithelial cells indicated that they were damaged. The epithelium of the smaller cysts was either attenuated or occasionally, was heavily invaded by large mononuclear cells. The lamina propria of the

allografts was oedematous and contained many degenerate cells with densely clumped chromatin in the nuclei and some scattered large mononuclear cells. The allograft vessels were engorged with blood cells and the endothelium of many had degenerated. The host tissue surrounding the allografts was densely invaded by mononuclear leukocytes. Blood vessels were sparsely distributed in these regions, and they often appeared collapsed and had enlarged endothelial cells. Occasional vessels in the host tissue adjacent to the allografts showed engorgement and endothelial degeneration. Little evidence of cell damage in the host connective tissues was noted.

In the allografts examined 13 days after subcutaneous transplantation, almost all cells in the lumina of the cysts, the epithelium, the blood vessels, and the lamina propria had degenerated. The blood vessels of the lamina propria were packed with erythrocytes and some also contained fibrin thrombi and degenerate cells. Variable amounts of adjacent host tissue also showed degeneration. However, epithelium of the cysts had often been destroyed without any evidence of damage in the adjacent host tissue which was heavily infiltrated by mononuclear leukocytes. Vessels in the infiltrated host tissue showed similar changes to those in the host tissue adjacent to the 12 day allografts.

Where allografts were transplanted to both subcutaneous sites and ear chambers in the same rabbit, histology showed that rejection began earlier or was more advanced in the subcutaneous than in the ear chambers allografts in individual rabbits (Table IV-6).

DISCUSSION

Oestrus in sexually mature female rabbits is continuous and ovulation is induced by mating (SCHWARTZ, 1973). The rabbits used in the present study were sexually mature and unmated and thus changes in the endometrial grafts due to hormonal influences were excluded. Electron microscopic observations on the epithelium of

the normal endometrium were in agreement with those previously reported (LARSEN, 1962). Similar observations on the stromal fibroblasts of rabbit endometrium have not been reported but many of the features of these cells were similar to those of human endometrium in the early proliferative phase (FERENCZY and RICHART, 1974).

The changes observed in the endometrium 3 days after transplantation when vascular connection with the host vessels was proceeding normally probably represented responses both to trauma and to ischaemia. By 7 to 9 days after transplantation, the integrity of most vessels in the grafts was re-established. However, many of the effects of transplantation in the ear chambers; for example, absence of cilia on the epithelium, hypertrophy of stromal fibroblasts and increased numbers of phagosomes in vascular endothelial cells, persisted until the rejection of the allografts.

The ability of small grafts to survive in transparent chambers without establishing vascular connection with the host has been reported previously (MERWIN and ALGIRE, 1956; WILLIAMS, 1954). Epithelium, stromal fibroblasts and endothelial cells of the endometrial grafts survived up to 13 days of graft ischaemia provided blood flow was maintained in the underlying ear chamber membrane in the present study. However, degeneration of stromal fibroblasts and endothelium in the upper regions of the grafts seen at both 3 and 13 days indicated the limits to which the tissues could be maintained by blood flow in the membrane alone. In areas of the grafts that had become necrotic because of interruption to blood flow in the underlying membrane, most stromal and endothelial cells had been destroyed but some epithelium persisted for up to 13 days indicating the remarkable ability of this tissue to withstand ischaemia. This ability has previously been demonstrated with epidermal epithelium by MEDAWAR (1947) and PEPPER (1959).

The persistence of vessels characteristic of the type of tissue transplanted to ear chambers has indicated that the vessels were of graft origin (WILLIAMS, 1954; HOBBS and CLIFF, 1971). The survival

of endothelium in endometrial graft vessels despite 13 days of graft ischaemia, together with the observation that graft vessels anastomose with those of the host (see Chapter III), strongly supports this view. Experiments with allogeneic skin grafts on immunosuppressed mice have indicated that donor endothelium was retained at 12 and 35 days after transplantation (JOOSTE and WINN, 1975; GERLAG ET AL, 1980). Donor endothelium in surgically-anastomosed, organ allografts appears to be retained for prolonged periods unless episodes of active rejection occur (SINCLAIR, 1972; HART ET AL, 1980; BURDICK ET AL, 1979). These studies indicate that the donor endothelium would be retained at the time of rejection of allografts in the present study. Recent studies on the replacement of endothelium of vessels in skin xenografts on immunosuppressed recipients have given conflicting results (GERLAG ET AL, 1980; JOOSTE ET AL, 1981a and b).

The study of fixed tissues from ear chambers supported the conclusion based on the in vivo observations that the rejection of allografts was not due to ischaemia. Following the onset of stasis during spontaneous rejection, degeneration of cells in the lamina propria and, in one case, the epithelium occurred in regions where there was little histological or in vivo evidence of interference to blood flow (Table IV-4). The exclusion of a role for ischaemia in cell degeneration was further substantiated by the degeneration of cells adjacent to blood vessels in which flow was observed in vivo at the time of fixation (Figure IV-21). Severe damage to the allografts and their monolayers was also observed with virtually no evidence of interference to the blood supply in the underlying host connective tissues (Table IV-4 and -5, Figure IV-11C), conditions under which the grafts survived for prolonged periods after transplantation. The possibility appeared to exist that ischaemia played a role in the destruction of allografts during induced rejection because vessels underlying the allogeneic tissues were also affected (Tables IV-4 and -5). However, in vivo observations revealed variable degrees of blood flow persisting in many of the regions of the allografts examined histologically (Table IV-4 and -5). Thus, the degree and duration of the ischaemia (1 to 2 days) and

damage at all levels of these grafts, when compared to that in the grafts where blood supply was impeded until 13 days after transplantation, was insufficient to account solely for the damage observed during rejection. The results indicate that the damage to the vessels, rather than lead to ischaemic degeneration of the other tissues, occurs in company with the degeneration of the other tissues of the allografts.

The average scores for height and secretory vacuoles was lower in the allogeneic than the autogenous epithelium in all situations except before the onset of stasis during induced rejection (Table IV-1). The lower average scores for the allogeneic compared to the autogenous epithelium before the onset of stasis during spontaneous rejection were consistent with in vivo observations indicating that the host reaction had a deleterious effect on the allogeneic epithelium at this stage. The absence of a similar effect on the allogeneic epithelium before the onset of stasis during induced rejection may have reflected the shorter time that the allogeneic epithelium was exposed to the host reaction. Overall the degree of leukocytic infiltration of the epithelium before and after the onset of stasis was low (Tables IV-1, -4 and -5). More leukocytic infiltration of allogeneic than autogenous epithelium was not demonstrated histologically but was detected in vivo when more epithelium could be examined (see Chapter III). The allogeneic epithelium often showed more pronounced leukocytic infiltration when it became degenerate but this may have been a response to, rather than a cause of, the degeneration. Apart from infiltration, the presence of leukocytes above the epithelium (see Chapter III) and below the epithelial basement membrane (Figures IV-14 and -15), and contacts between epithelial cells and mononuclear leukocytes through gaps in the basement membrane (Figure IV-12) provided ample opportunity for short-range diffusion of substances and cell-cell interaction. In vitro studies have indicated that lymphocytes and macrophages may inhibit the proliferation of other cells (RUSSELL ET AL, 1972; KELLER, 1978; EVANS ET AL, 1972) and cause cell lysis (see Chapter I). The lower height and secretory vacuole score of the allogeneic epithelium could be due to either effect; that is, the

epithelium may flatten itself out and lose specialized features in maintaining the integrity of its cover in the face of reduced cell proliferation and/or increased cell death rates.

Electron microscopy showed that the increased numbers of mononuclear cells in the lamina propria of the allografts compared to autografts (Table IV-2) was due to the infiltration of leukocytes and confirmed that the polymorphonuclear cells were almost exclusively neutrophil granulocytes (Figures IV-16 and -17). There was little evidence of more degenerate cells in the lamina propria of allografts compared to that of control autografts in ear chambers before the onset of stasis (Table IV-2). This was despite the greater density of mononuclear cells in allografts where rejection was spontaneous and of polymorphonuclear leukocytes in allografts when rejection was of either type. Degenerate cells in the lamina propria of allografts in both spontaneous and induced rejection increased in number after the onset of stasis. This was associated overall with higher densities of mononuclear cells but a decrease in the density of polymorphonuclear leukocytes compared to allografts before the onset of stasis. Electron microscopic observations showed that the large numbers of degenerating cells in the more severely damaged allografts was due to the degeneration of both infiltrating leukocytes and stromal fibroblasts (Table IV-4). The low density of polymorphonuclear leukocytes in the less damaged regions of allografts, compared to control autografts (Tables IV-4 and -2), cannot be explained as yet. These leukocytes showed peak levels in the moderately damaged regions of the allografts. Their decline in density with progressively more severe damage to the allografts may have been due either to increased destruction of these cells or to a lower rate of entry of these short-lived cells into the allografts. Only minor differences in the degree of vascular damage were detected between the allografts and autografts before the onset of stasis (Table IV-3). After the onset of stasis, substantial vascular damage in the allografts and, when rejection was induced, in the host tissues was detected by light and electron microscopy

(Tables IV-3, -4 and -5). In contrast to the observations of DVORAK ET AL (1979) and ZAREM (1969), occlusion of vessels by endothelial hypertrophy was not observed. This may have been because the vessels in the tissue taken from ear chambers did not collapse when fixed. The initially focal distribution of the vascular damage in the allografts in ear chambers (see Chapter III, Table IV-4) together with the earlier destruction of subcutaneous compared to ear chamber allografts in the same rabbit (Table IV-6) indicate that the damage was not mediated by circulating antibody at the blood-endothelium interface because all allograft vessels would then be expected to be damaged at the same time. It has previously been noted that it may take 48 hours or longer after the onset of stasis before all vessels in allografts are similarly affected (EDGERTON ET AL, 1957; ROLLE ET AL, 1959; HOBBS and CLIFF, 1973). The slight differences observed in vivo between auto- and allografts in leukocyte rolling and sticking (see Table III-5) and the rarity of accumulations of leukocytes in the vessel lumina on histological examination also suggest that the damage to vessels was not mediated by immune cells at the blood endothelium interface. The small mononuclear leukocytes with cytoplasmic extensions identified within the endometrial allograft vessels appeared similar to those previously observed in blood vessels of rejecting kidneys (KOUNTZ ET AL, 1963). In that report, the cells were thought to be plasma cells but this was not supported by their electron micrographs. KOUNTZ ET AL (1963) postulated that these cells damaged the endothelium. However, the morphology of these cells could also be interpreted as a response of monocytes to stagnation of blood flow since the transformation of monocytes to macrophages in such situations has been reported (WEISS, 1972).

The results as listed in Table IV-4 appeared to represent the progression of damage in the allografts. Thus, during spontaneous rejection, cell degeneration usually began in the extravascular connective tissue of the lamina propria where leukocytic infiltration was most intense and then extended to the vessels and the epithelium. The earlier damage to vessels in allografts and those in the host tissues during induced rejection may reflect a

more abrupt and violent reaction during this form of rejection. DVORAK ET AL (1979) have also observed damage to host tissue during the rejection of skin allografts. The possibility that this was a consequence of damage to donor vessels which had grown into the host tissue (see Chapter III) appears to be excluded in the present study by the virtual absence of damage to vessels in this site during spontaneous rejection (Table IV-4).

The identity of leukocytes infiltrating acutely rejecting allografts has been studied extensively (see Chapter I) and the observations reported in the present study are in general agreement with those of ROTHWELL and PAPADIMITRIOU (1972) and CHRISTMAS and MCPHERSON (1982). Difficulty in distinguishing macrophages from fibroblasts in rejecting allografts has been reported previously (CHRISTMAS and MCPHERSON, 1982). In the present study, the identity of some large cells in the lamina propria of the grafts could be similarly confusing (Figures IV-15, -16 and -17). Several morphological features such as a thin layer of chromatin at the periphery of the nucleus, long profiles of rough endoplasmic reticulum and numerous Golgi apparatus in the cytoplasm indicated that most of these cells were stromal fibroblasts (Figures IV-16 and -17). Other observations that supported this conclusion were (a) the hypertrophy of stromal fibroblasts in auto- and allografts in the period following transplantation with their morphology ranging from that found in normal endometrium to that of the cells identified above, (b) the presence of such cells as the only representatives of stromal fibroblasts in some areas of grafts, and (c) the similarity of the morphological features of the hypertrophied stromal fibroblasts observed here to stromal fibroblasts in human endometrium in mid-proliferative to predecidual phases of the menstrual cycle (FERENCZY and RICHART, 1974).

Small unidentified mononuclear leukocytes as well as lymphocytes, monocytes, macrophages and neutrophil granulocytes were all observed in contact with the stromal fibroblasts in the allografts. The occurrence of intimate lymphocyte-allogeneic cell contacts in the less damaged allografts (Figures IV-17 and 18A) contrasts with the

observations of CHRISTMAS and MCPHERSON (1982) and DEMPSTER (1977). The contacts between the mononuclear leukocytes and the fibroblasts were similar to those formed during in vitro experiments on T lymphocyte-mediated cytotoxicity and antibody-dependent cellular-cytotoxicity (BIBERFELD and JOHANSSON, 1975; KALINA and BERKE, 1976; SANDERSON AND GLAUERT, 1977; GLAUERT and SANDERSON, 1979). However, the leukocyte-stromal fibroblast contacts were not necessarily related to a specific reaction to the allogeneic cells, because the leukocytes were also observed in contact with fibroblasts of autografts and the ear chamber membrane.

The allogeneic cells that were in contact with leukocytes often displayed no morphological abnormality or only mild swelling of mitochondria. The latter change in allogeneic cells has been reported by CHRISTMAS and MCPHERSON (1982) and WEISS (1968) but its relation to rejection could not be established in the present study because it was also observed in cells of autografts. On rare occasions, stromal fibroblasts in contact with activated lymphocytes and small unidentified mononuclear leukocytes showed large pedunculated cytoplasmic protrusions and apparent shedding of cytoplasmic blebs (Figure IV-17). Only one cell showed apparent nuclear fragmentation in the absence of advanced cytoplasmic disruption but its identity could not be established (Figure IV-18B). These morphological features were suggestive of zeiosis or apoptosis which occurs when cell killing is mediated by cytotoxic T lymphocytes and K cells (antibody-dependent cellular-cytotoxicity) (SANDERSON, 1981; BERKE, 1980; WYLLIE ET AL, 1980). However, it has also been reported during natural death of cultured cells (SANDERSON, 1976) and in response to ischaemia in vivo (KERR, 1971). Cells shedding cytoplasmic blebs were also observed in autografts which had failed to anastomose to the host vasculature in the present study (Figure IV-7B). Although other explanations are therefore possible, the changes in some of the stromal fibroblasts in contact with mononuclear leukocytes were suggestive of specific killing of allogeneic cell by host mononuclear leukocytes.

The short duration of zeiosis or apoptosis in affected cells (WYLLIE

ET AL, 1980; SANDERSON, 1981) makes it unlikely that cells actively undergoing this change would often be observed in the material studied here. However, evidence that this form of cell degeneration is occurring is provided by the presence of apoptotic bodies (WYLLIE, ET AL 1980). Endometrial epithelium shows apoptotic bodies in intact animals and following oestradiol withdrawal (SANDOW ET AL, 1979). However, observations by light and electron microscopy provided no evidence for a substantial increase in apoptosis in the epithelium (phagosomes, which included any recognizable apoptotic bodies, remained at low levels during rejection, Tables IV-1, -4 and -5) or the other tissues of the rejecting allografts. The vast majority of degenerate cells in the allografts and in the damaged host tissues showed densely clumped, pyknotic nuclei and disruption of cytoplasmic organelles (Figures IV-12B, -13, -19 and -20) which are changes characteristic of coagulative necrosis (WYLLIE ET AL, 1980; TRUMP and ARSTILA, 1975). Thus, although some evidence for specific cell-mediated cytolysis of allogeneic cells was obtained, this appeared to make only a minor contribution to the destruction of the allografts.

The form of cell degeneration that occurs in rejecting allografts has received scant attention but the observations reported here that the major form of cell degeneration is coagulative necrosis is in agreement with the results of WIENER ET AL (1964). However, SEARLE ET AL (1977) observed only apoptosis in liver allografts in pigs. These allografts were examined 1 week after transplantation whereas the recipients did not die until 3 weeks or later. The absence or mildness of acute rejection in this experimental system has been reported (BATTERSBY ET AL, 1974). Thus disagreement between the results of that study and those of the present one is probably because the liver allografts were not undergoing typical acute rejection.

The degeneration of leukocytes and other tissues of the host during the rejection of the allografts in the ear chambers could not be attributed to ischaemia and thus must have been a direct consequence of the rejection process. The destruction of both donor and host cells with coagulative necrosis as the main form of cellular

degeneration indicated that non-specific mechanisms predominated in the rejection of the endometrial allografts. These results are in agreement with earlier in vitro studies which showed that effector cells participating in the destruction of allogeneic cells were themselves destroyed (GRANGER and WEISER, 1964; WEISS, 1968; ABLE ET AL, 1970; CHAMBER and WIESER, 1969). In contrast, more recent studies of specific cell-mediated lysis of allogeneic cells; that is, T lymphocyte-mediated cytolysis and antibody-dependent cellular-cytolysis, have shown that the effector cells are not damaged during their interaction with the target cells in vitro (SANDERSON, 1981).

Lymph node cells from animals showing delayed-type hypersensitivity reactions are cytotoxic to allogeneic and syngeneic cells when exposed to the specific antigen in vitro (RUDDLE and WAKSMAN, 1967). Although natural killer cells may also be cytotoxic to syngeneic cells, their activity is largely confined to certain tumor cells (HERBERMAN and HOLDEN, 1978). Thus, of the two known non-specific cytotoxic mechanisms, it seems most likely that a delayed-type hypersensitivity reaction was the predominant one in the rejection of the endometrial allografts in ear chambers. This has also been postulated as the mechanism leading to the rejection of epidermal and skin allografts (BILLINGHAM and SPARROW, 1954; DVORAK ET AL, 1979; BHAN ET AL, 1982). MASON and MORRIS (1984) observed that cells obtained from rejecting and non-rejecting renal allografts were non-specifically cytotoxic in vitro and, on this basis, postulated that this form of cytotoxicity did not lead to rejection. Further study is required to evaluate the relevance of this observation to the non-specificity of cell destruction observed during acute allograft rejection in vivo in the present study and those on epidermal and skin allografts.

DVORAK ET AL (1979) and FORBES ET AL (1983) on the basis of morphological studies on fixed tissue have suggested that vascular

damage is the critical event in acute rejection of skin and cardiac allografts. In vivo confirmation that sufficient ischaemia occurred to account for the damage to the extravascular tissue of the rejecting allografts would be desirable to substantiate this conclusion. However, the possibility exists that such a mechanism may lead to allograft destruction particularly when the cellular infiltrate is confined to perivascular regions but diffuse cellular infiltration of skin and organ allografts is more often seen (see Chapter I). The results of the present study indicate that when mononuclear leukocytes are diffusely distributed in skin and organ allografts this reaction can lead directly to the degeneration of cells of all types including those of the host.

Histological examination of the subcutaneous grafts indicated that the estimated volumes of the auto- and allografts at 7 and 9 days after transplantation (Chapter III), were not biased by a greater reaction to the allografts at this stage. Additionally, the earlier destruction of subcutaneous allografts than allografts transplanted simultaneously to ear chambers in the same rabbit was histologically confirmed (Table IV-6). The degree of mononuclear leukocyte infiltration associated with rejecting allografts was far greater in subcutaneous sites than in the ear chambers. This may have been the sole cause of the 'out of phase' rejection in the two sites. The observations in the present study have shown that the expression of the rejection mechanisms is localized. The processes leading to an effective rejection reaction would be logically proceeding faster in subcutaneous sites where the accumulation of leukocytes was demonstrably greater. The reasons for the greater accumulation of mononuclear leukocytes in the subcutaneous sites remain to be precisely determined.

Assessment of the mechanism of destruction of the subcutaneous allografts in the present study suffers the same limitations that many other studies have in which in vivo microscopic and electron microscopic observations were not made. However, the destruction of epithelium immediately adjacent to regions of viable host tissue in

the subcutaneous allografts suggests that ischaemia did not lead to rejection in these regions. However, the eventual destruction of all vessels in larger allografts and a substantial proportion of neighbouring host vessels must ultimately lead to ischaemic death of cells but the results from the ear chamber experiments show that this may be a very late event.

Overall, the results presented here indicate that a non-specific cytotoxic mechanism, probably a delayed-type hypersensitivity reaction, led to destruction of the allografts and that mechanisms leading to specific killing of allogeneic cells played only minor roles. Damage due to the reaction was not confined to vessels but also involved extravascular tissues. Destruction of the allografts in the present study could not be attributed to ischaemia but ischaemic damage may occur as a late event.

TABLE IV - 1

Light microscopic observations on the epithelium of endometrial grafts and their monolayers in ear chambers.

		Height (μm)	Secretory vacuoles (1-5) ¹	Degenerate epithelium (%)	Mono- nuclear leukocytes (no/mm)	Polymorpho- nuclear leukocytes (no/mm)	Phagosomes (no/mm)
Spontaneous rejection	before stasis						
	auto ² n=13, r=4 ³	16 ⁴ (6.8-25.4)	3.5 (1-5)	0 (-)	12 (0-84)	2 (0-9)	1 (0-4)
	allo n=18, r=4	10.2 (3.8-20.4)	2.2 (1-5)	0 (-)	9 (0-38)	1 (0-9)	1 (0-6)
	after stasis						
	auto n=8, r=2	14 (7.4-32.8)	2.8 (2-4)	0 (-)	12 (0-23)	6 (0-28)	5 (0-27)
	allo n=12, r=2	6 (4.2-8.4)	1 (-)	50 (0-100)	5 (0-19)	1 (0-4)	5 (0-23)
Induced rejection	before stasis						
	auto n=11, r=2	8.1 (5.6-12)	1.3 (1-2)	0 (-)	0.2 (0-2)	1 (0-7)	0 (-)
	allo n=11, r=2	8.5 (6-14)	1.3 (1-3)	0 (-)	0.2 (0-2)	1 (0-2)	2 (0-7)
	after stasis						
	auto n=7, r=2	10 (9-12.4)	2.4 (2-3)	0 (-)	0 (-)	0 (-)	1 (0-4)
	allo n=12, r=2	6.8 (3.6-11.6)	1.1 (1-2)	27 (0-100)	1 (0-6)	1 (0-3)	3 (0-17)

1: Semiquantitative score (see Chapter II).

3: n = number of samples, r = number of rabbits.

2: auto = autograft, allo = allograft.

4: Average and () range.

5: The numbers of vessels is expressed in relation to the length of chamber membrane examined.

TABLE IV - 2 Light microscopic observations on the lamina propria of endometrial grafts and underlying tissues in ear chambers.

		Graft bodies				Monolayers				
		Lamina propria (no of cells/0.1mm ²)				Underlying membrane		Immediately underlying tissue		
		Degen- erate cells	Mono- nuclear cells	Poly- morpho- nuclear leukocytes	Total cells	Cellular degener- ation (1-5) ¹	Mono- nuclear leukocytes (1-5) ¹	Degen- erate cells	Cellular degener- ation (1-5) ¹	Mono- nuclear leukocytes (1-5) ¹
Spontaneous rejection	before stasis									
	auto ² n=8, r=4 ³	0.1 ⁴ (0-1)	98 (46-149)	16 (8-29)	114 (54-159)	1 (-)	1 (-)	n=6, r=4	1 ⁴ (-)	1.2 (1-2)
	allo n=12, r=4	1 (0-11)	198 (74-485)	34 (6-74)	233 (98-570)	1.3 (1-2)	1.3 (1-2)	n=7, r=4	1 (-)	1.9 (1-3)
	after stasis									
auto n=4, r=2	5 (3-7)	238 (167-312)	49 (23-88)	292 (210-406)	1 (-)	1 (-)	n=4, r=2	1 (-)	1 (-)	
allo n=8, r=2	51 (8-112)	381 (250-555)	22 (0-91)	454 (306-626)	1 (-)	2.5 (2-4)	n=4, r=2	1 (-)	1.8 (1-2)	
Induced rejection	before stasis									
	auto n=5, r=2	1 (0-6)	140 (73-194)	19 (2-69)	160 (76-270)	1 (-)	1 (-)	n=6, r=2	1 (-)	1.5 (1-3)
	allo n=5, r=2	1 (0-5)	144 (115-170)	32 (4-69)	176 (119-210)	1.2 (0-2)	1 (-)	n=6, r=2	1 (-)	1 (-)
	after stasis									
auto n=4, r=2	0.5 (0-2)	117 (109-130)	45 (3-113)	162 (126-223)	1 (-)	1 (-)	n=3, r=2	1 (-)	1 (-)	
allo n=7, r=2	64 (3-224)	205 (116-283)	24 (7-68)	293 (172-429)	1.7 (1-3)	2.6 (1-4)	n=6, r=2	1.7 (1-4)	4 (-)	

Footnotes - see Table IV - 1.

TABLE IV - 3

Light microscopic observations on vessels in the lamina propria of grafts and the underlying tissues in ear chambers.

		Graft bodies						Monolayers			
		Lamina propria			Underlying membrane			Underlying tissue and membrane			
		% with degenerate endothelium	% with thrombosis	Total (no/0.1 mm ²)	% with degenerate endothelium	% with thrombosis	Total (no/mm) ⁵	% with degenerate endothelium	% with thrombosis	Total (no/mm) ⁵	
Spontaneous rejection	before stasis	auto ²	6 ⁴ (0-27)	6 (0-40)	17 (6-27)	0 (-)	0 (-)	10 (2-17)	0 (-)	0 (-)	33 (22-52)
		allo	0 (-)	0 (-)	26 (8-50)	0 (-)	0 (-)	10 (0-28)	0 (-)	0 (-)	26 (13-39)
	after stasis	auto	0 (-)	0 (-)	26 (11-41)	0 (-)	0 (-)	9 (5-11)	0 (-)	0 (-)	27 (21-35)
		allo	28 (0-100)	9 (0-27)	27 (16-40)	3 (0-20)	0 (-)	7 (0-14)	0 (-)	0 (-)	12 (9-19)
Induced rejection	before stasis	auto	8 (0-37)	4 (0-18)	34 (15-62)	0 (-)	0 (-)	10 (0-15)	1 (0-8)	0 (-)	25 (13-35)
		allo	2 (0-11)	0 (-)	38 (9-50)	0 (-)	3 (0-17)	7 (2-13)	2 (0-12)	4 (0-25)	20 (2-36)
	after stasis	auto	0 (-)	0 (-)	15 (4-27)	0 (-)	0 (-)	14 (7-22)	0 (-)	0 (-)	43 (39-46)
		allo	18 (0-39)	10 (0-40)	29 (11-64)	36 (0-67)	7 (0-33)	11 (6-24)	18 (0-55)	2 (0-13)	22 (13-28)

Footnotes - see Table IV - I.

NB: The numbers of samples and rabbits were the same as in Table IV - 2.

TABLE IV - 4

Light microscopic observations on individual samples of allografts following vascular stasis in ear chambers.

	Epithelium				Lamina Propria					Underlying Membrane				Ischaemia ² (1-5) ³	
	Sample number	Height (µm)	Degenerate epithelium (%)	Mononuclear leukocytes (no/mm)	Phagosomes (no/mm)	Degenerate cells	Mononuclear cells	Polymorph. leukocytes	% with degenerate endothelium	% with thrombosis	Cells	Vessels ¹	Cells		Vessels ¹
											Cellular degeneration (1-5) ³	Mononuclear leukocytes (1-5) ³	% with degenerate endothelium	% with thrombosis	
Spontaneous rejection	584 R-7	6.6	0	6	23	8	363	0	20	0	1	2	0	0	2
	584 R-2	6.6	0	0	0	9	425	91	0	0	1	2	-	-	1
	581 L-3	-	100	-	-	27	555	43	0	0	1	4	20	0	3
	584 R-1	4.4	0	4	7	29	476	35	0	0	1	2	0	0	1
	581 L-7	8.4	0	19	4	51	376	0	0	0	1	3	0	0	3
	584 R-6	-	100	-	-	57	250	0	75	25	1	2	0	0	2
	584 R-4	4.2	41	0	4	112	314	9	30	20	1	2	-	-	4
	581 L-4	-	100	-	-	112	289	0	100	27	1	3	0	0	5
Induced rejection	637 R-5	11.6	0	0	2	3	242	7	0	0	1	1	67	0	1
	648 R-3	NR ⁴	NR	NR	NR	10	283	7	25	0	1	2	25	0	4
	637 R-4	11.2	0	6	3	18	249	68	0	0	1	4	0	0	3
	648 R-1	9.8	11	0	17	22	233	32	22	6	2	3	67	33	2
	648 R-8	5.4	30	0	0	35	116	22	20	40	3	3	36	0	3
	637 R-3	6.1	27	0	0	138	130	11	19	13	2	2	33	17	5
	637 R-1	3.7	45	0	0	224	183	22	39	8	2	3	25	0	5

1: Vessels showing both degenerate endothelium and thrombosis were included in the counts for each category of vascular pathology.

2: Based on *in vivo* observations of the vessels of grafts or below monolayers at the time of fixation.

3: Semiquantitative score (see Chapter II).

4: NR = not recorded. The epithelium appeared to have been stripped from the sample during processing.

TABLE IV - 5

Light microscopic observations on individual samples of allogeneic monolayers following vascular stasis in ear chambers.

	Epithelium				Tissues underlying monolayers				Ischaemia ² (1-5) ³	
	Sample number	Height (μ m)	Degenerate epithelium (%)	Mononuclear leukocytes (no/mm)	Phagosomes (no/mm)	Immediately underlying tissue Cellular degeneration (1-5) ³	Mononuclear leukocytes (1-5) ³	Vessels ¹ % with degenerate endothelium		% with thrombosis
Spontaneous rejection	584 R-3	7.2	0	2	0	1	2	0	0	1
	584 R-13	5.2	55	5	0	1	1	0	0	1
	581 L-3	-	100	-	-	1	2	0	0	1
	581 L-4	-	100	-	-	1	2	0	0	1
Induced rejection	648 R-8	6.4	0	0	2	1	4	17	0	3
	648 R-7	4.8	0	1	3	1	4	0	0	1
	637 R-4	6.9	28	3	2	2	4	0	0	2
	637 R-6	5.1	32	0	7	1	4	22	0	2
	648 R-1	3.6	49	0	1	1	4	13	13	2
	637 R-1	-	100	-	-	4	4	55	0	4

Footnotes - see Table IV - 4

TABLE IV - 6

Light microscopic observations on the extent of degeneration of allogeneic tissues in subcutaneous sites and ear chambers in individual rabbits.

Rabbit identification	Days after transplantation	Extent of degeneration	
		Subcutaneous allografts	Ear chamber allografts
634 L ¹	7	none	NA ²
635 L ¹	7	none	NA
610 L	7	none	none
610 R	9	none	none
648 R	12	50% of epithelium destroyed, lamina propria destroyed	minimal to moderate ³ damage to epithelium and lamina propria
608 L	13	allogeneic tissue totally destroyed	minimal damage ⁴ to allogeneic tissue
637 R	13	allogeneic tissue totally destroyed	minimal to moderate ³ damage to epithelium and lamina propria

1: Grafts transplanted to subcutaneous sites alone.

2: NA - not applicable.

3: See induced rejection, Tables IV - 4 and 5.

4: Vascular stasis had not occurred in the ear chamber allografts.

FIGURE IV-1

Biopsy of normal endometrium. The tissues shown in these and subsequent electron micrographs were embedded in epon and stained with uranyl acetate and lead citrate.

- A. The cell occupying most of the left of the figure is a secretory epithelial cell with many uniform vacuoles. Several phagosomes are present in the lower part of the cell. A ciliated epithelial cell occupies the right of the figure and its well developed Golgi zone and portion of its nucleus are present in the lower right of the field. These two cells have microvilli at their apical surfaces and are joined at several points by short desmosomes. (X 14300)
- B. Stromal fibroblasts are lying in pairs near the four corners of the field. A single fibroblast is in the centre of the field and an elongated macrophage is near the lower margin of the field. The stromal fibroblasts have large nuclei and long cytoplasmic processes extend from the bodies of the cells. The intercellular space contains bundles of collagen fibres and fine granulofibrillar material. (X 3080)

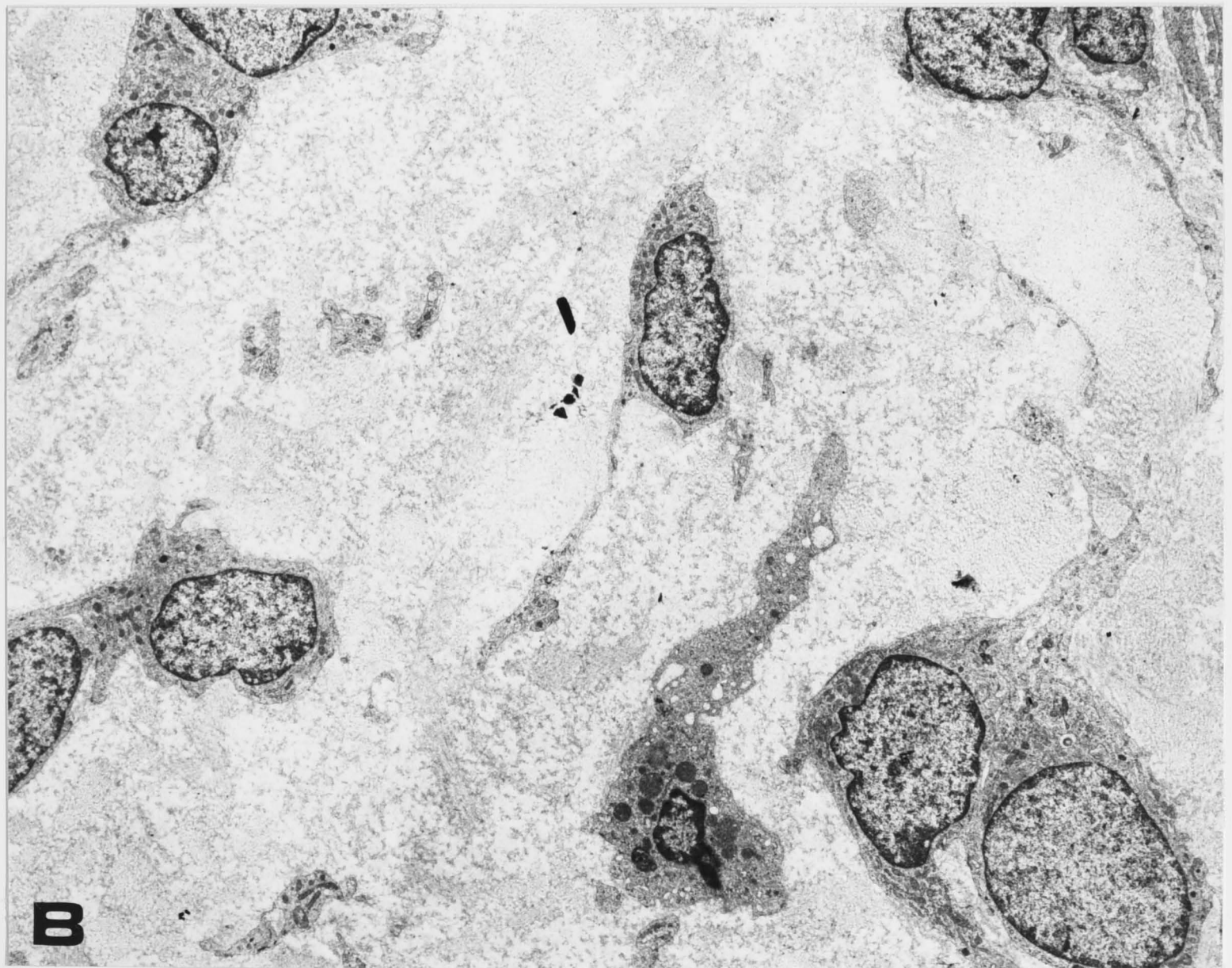
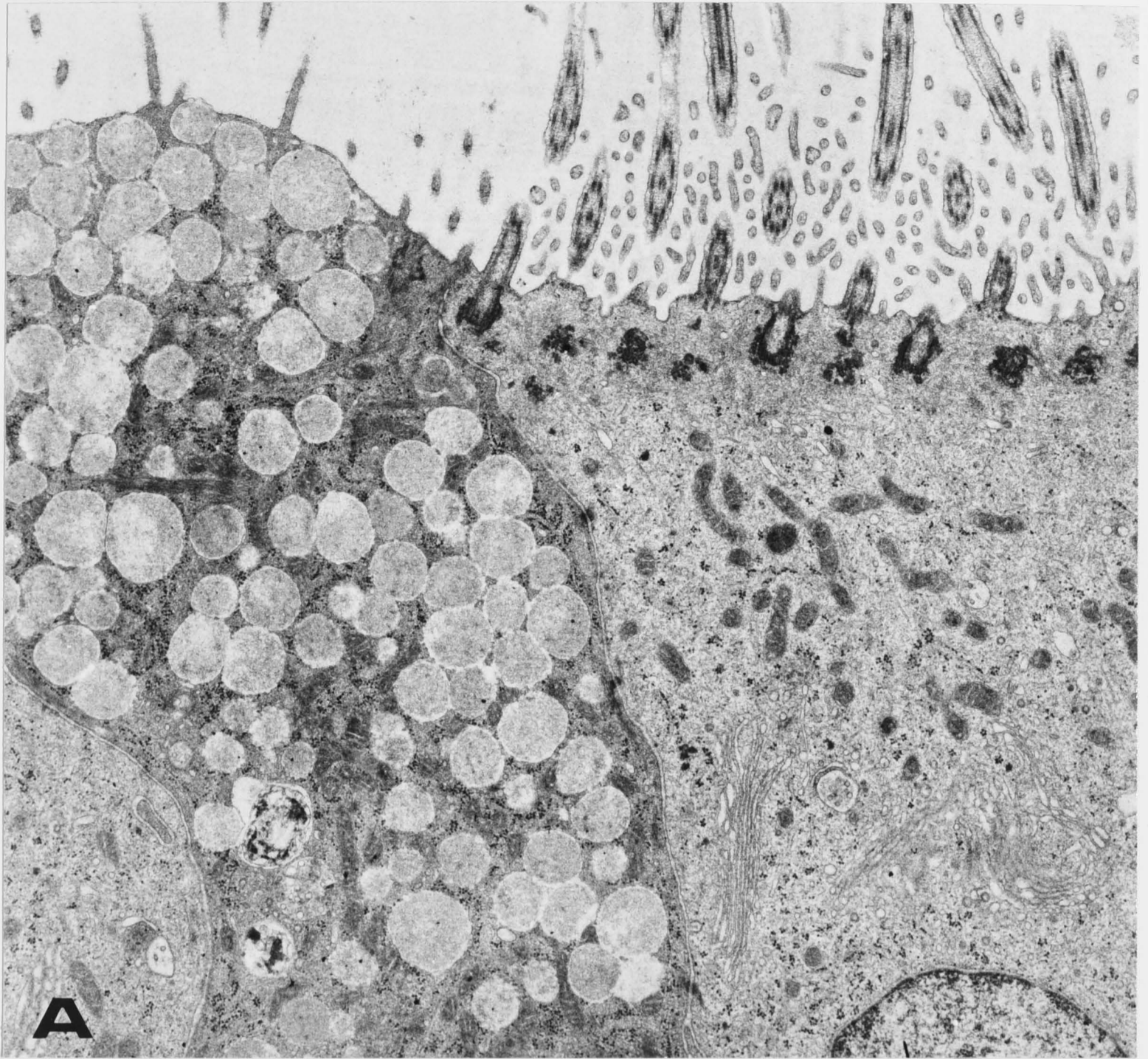
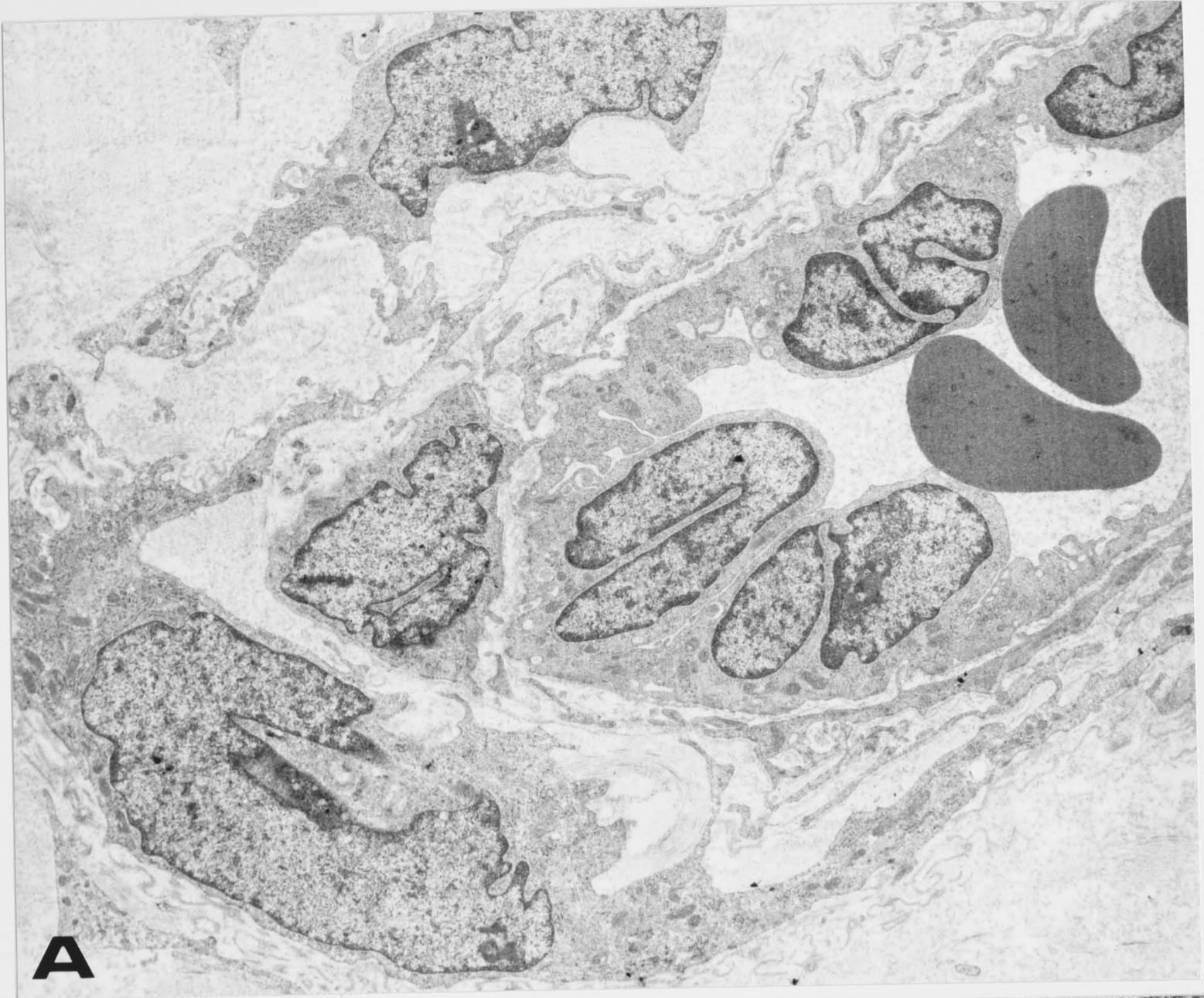


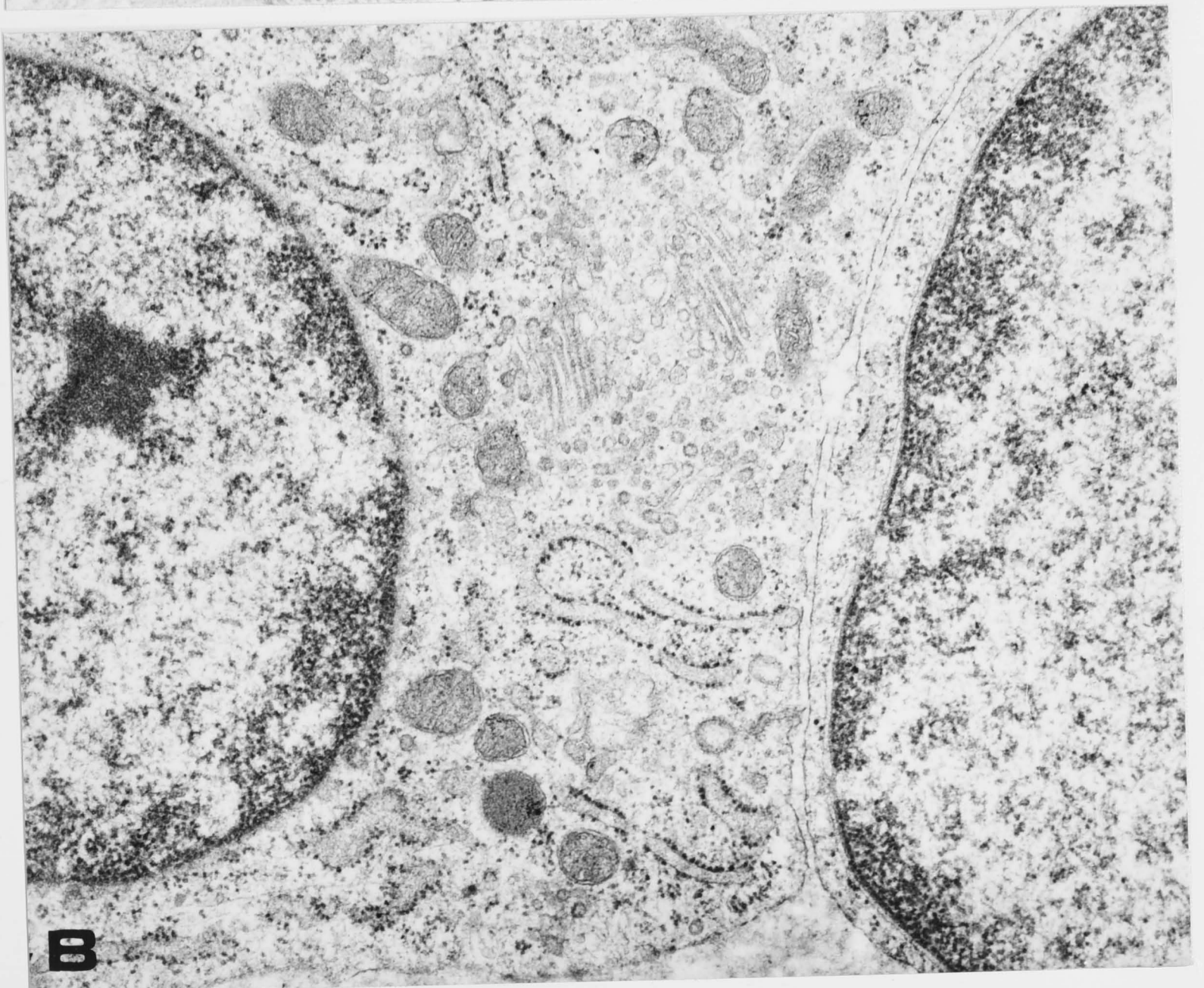
FIGURE IV-2

Biopsy of normal endometrium.

- A. A venule extends from the middle to the upper right of the field and is lined by plump endothelium. Multiple branching and discontinuous basement membrane-like condensations surround the vessel and its related cells. Particularly prominent condensations are present at the lower margin of the stromal fibroblast at the bottom of the field. (X 5300)
- B. A stromal fibroblast pair showing short oval profiles of mitochondria, a small Golgi zone and a few rough endoplasmic reticular profiles. Note the absence of basement membranes. (X 29150)



A



B

FIGURE IV-3

An endometrial allograft 3 days after transplantation to a rabbit ear chamber.

- A. Low continuous endometrial epithelium with basal separation of its cells extends obliquely across the right part of the field. A few microvilli are present at the apical surface of the epithelium. Extravasated erythrocytes lie between and below the epithelial cells and elsewhere in the body of the graft. (X 4100)
- B. A stromal fibroblast contains well-developed elongated profiles of rough endoplasmic reticulum and well-developed bundles of microfilaments at its periphery. (X 30660)
- C. A blood vessel showing haemoconcentration. The blood platelets show no evidence of degranulation. The endothelial cell nucleus has dispersed chromatin and a prominent nucleolus. (X 7300)

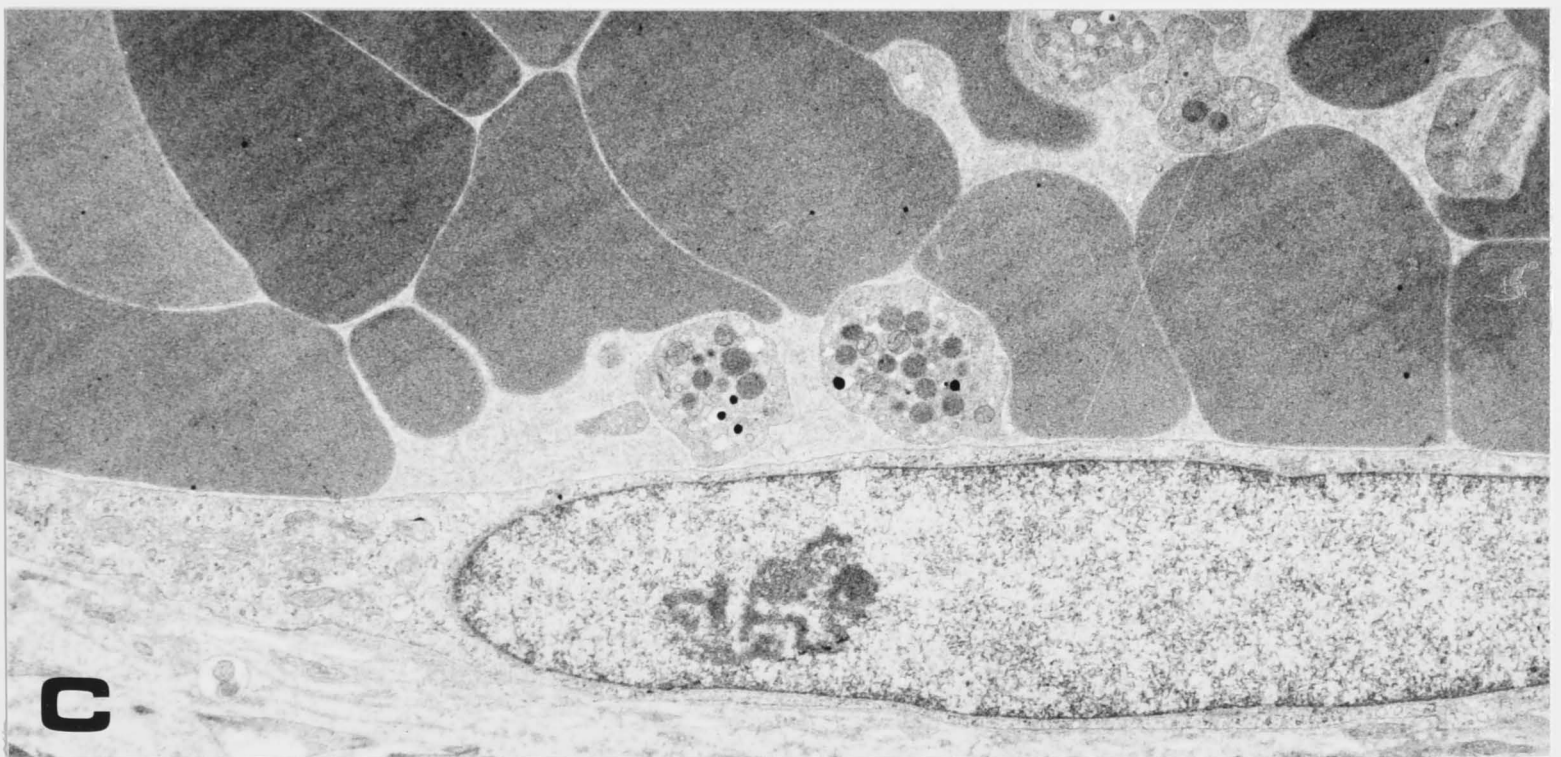
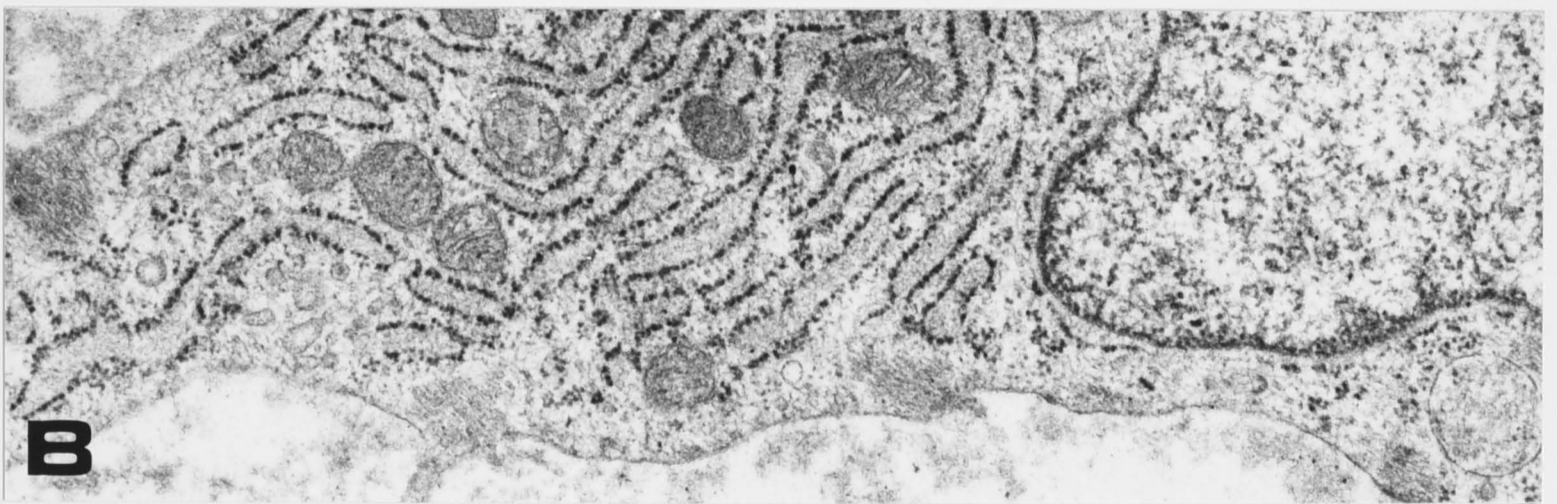
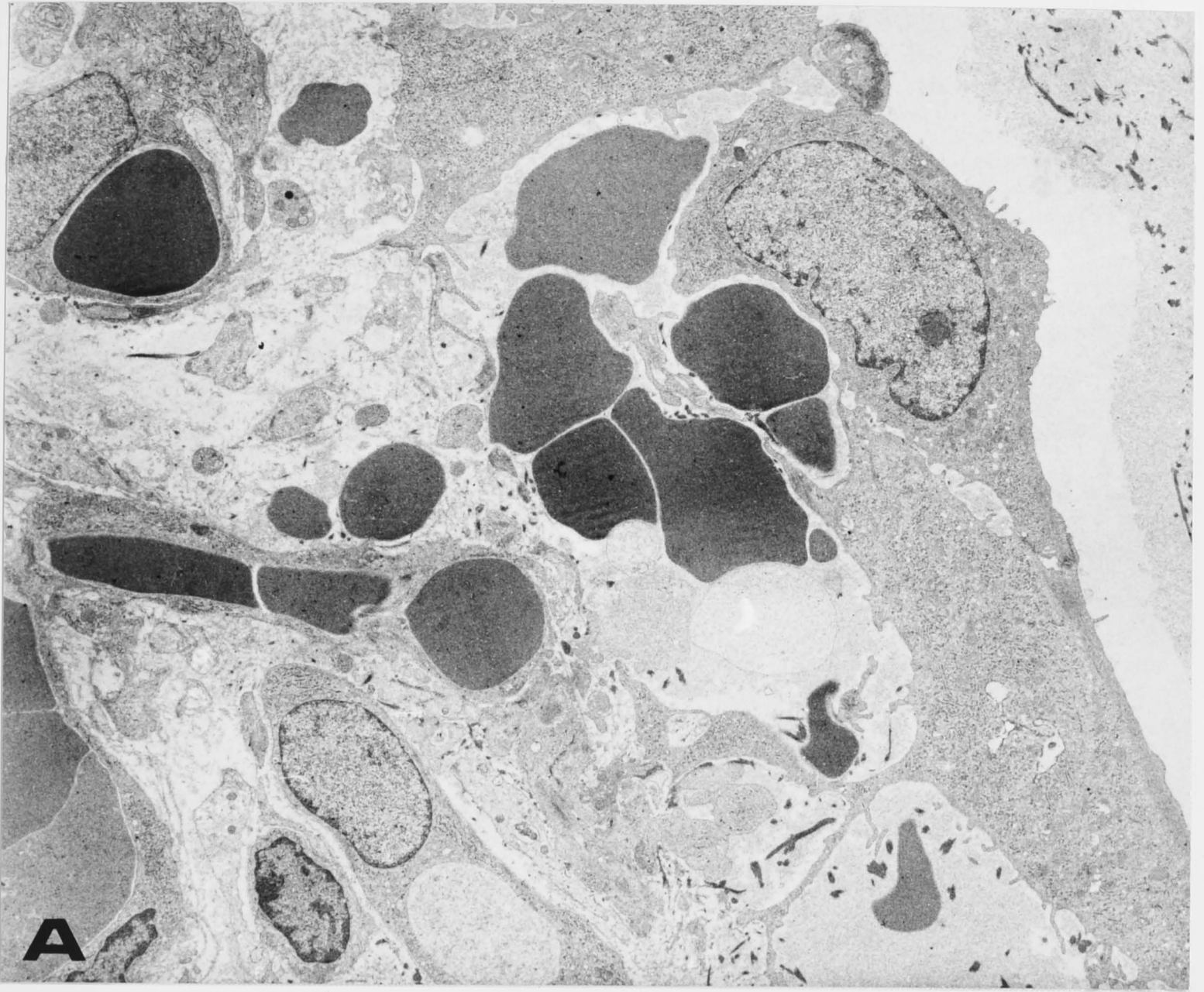


FIGURE IV-4

An endometrial allograft 13 days after transplantation to a rabbit ear chamber.

- A. The epithelium occupies the right of the figure and is moderately high with numerous microvilli and reforming secretory vacuoles in the apical regions of the cells. Rough endoplasmic reticulum is plentiful. The chromatin pattern in the nucleus of the top epithelial cell suggests incipient mitotic activity. Basal separation of the cells is still present and microvillous-like processes extend into the space. The epithelium has a well-developed basement membrane. The endothelium of the small blood vessel at the left of the field has abundant dilated rough endoplasmic reticulum. (X 6000)
- B. Two epithelial cells showing degenerative changes occupy most of the field. The mitochondria of both cells are dilated and have disrupted cristae. The rough endoplasmic reticulum is dilated. The right cell contains large vacuoles. (X 22260)

m	-	mitochondrion.
v	-	vacuole.
er	-	endoplasmic reticulum.

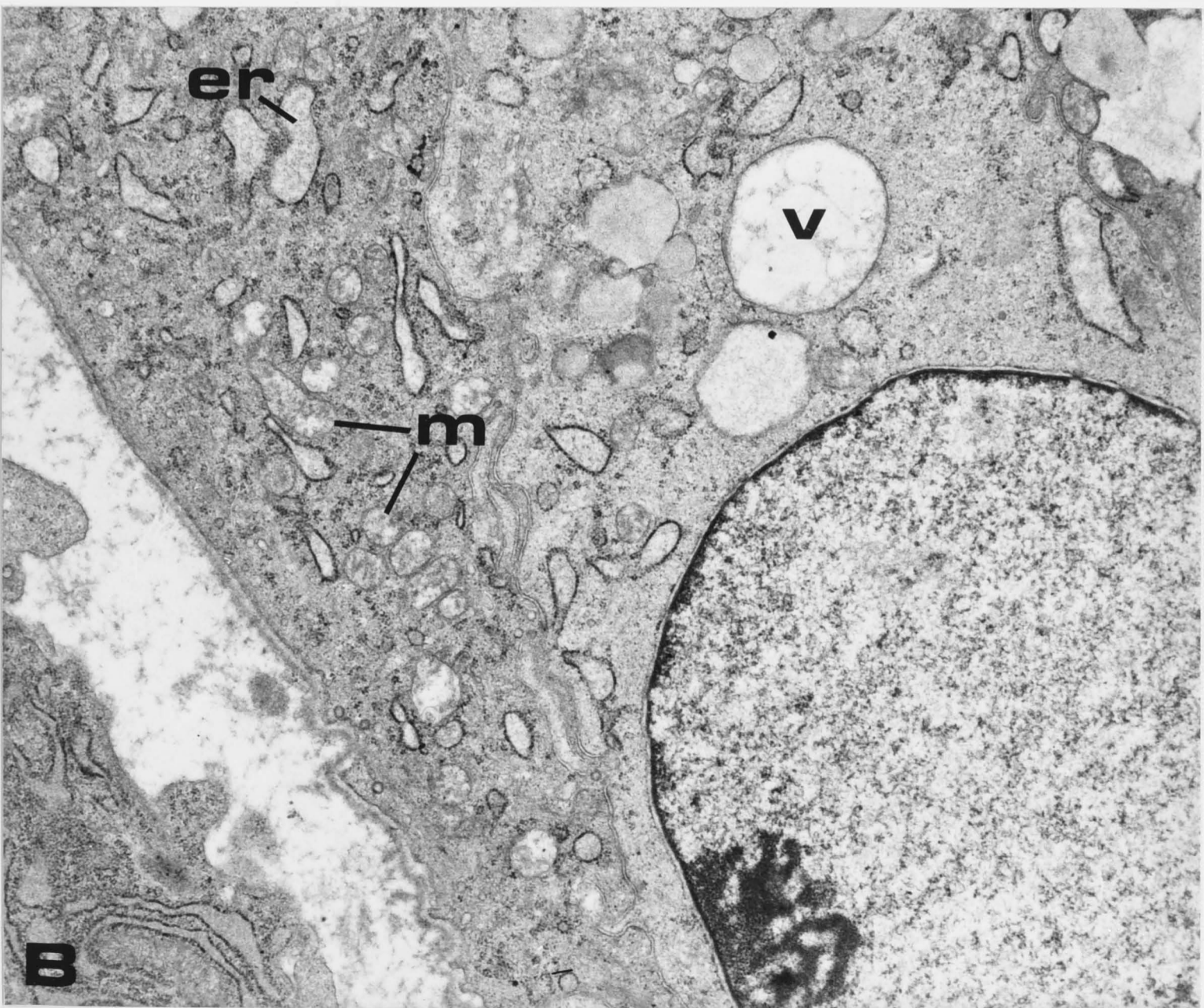
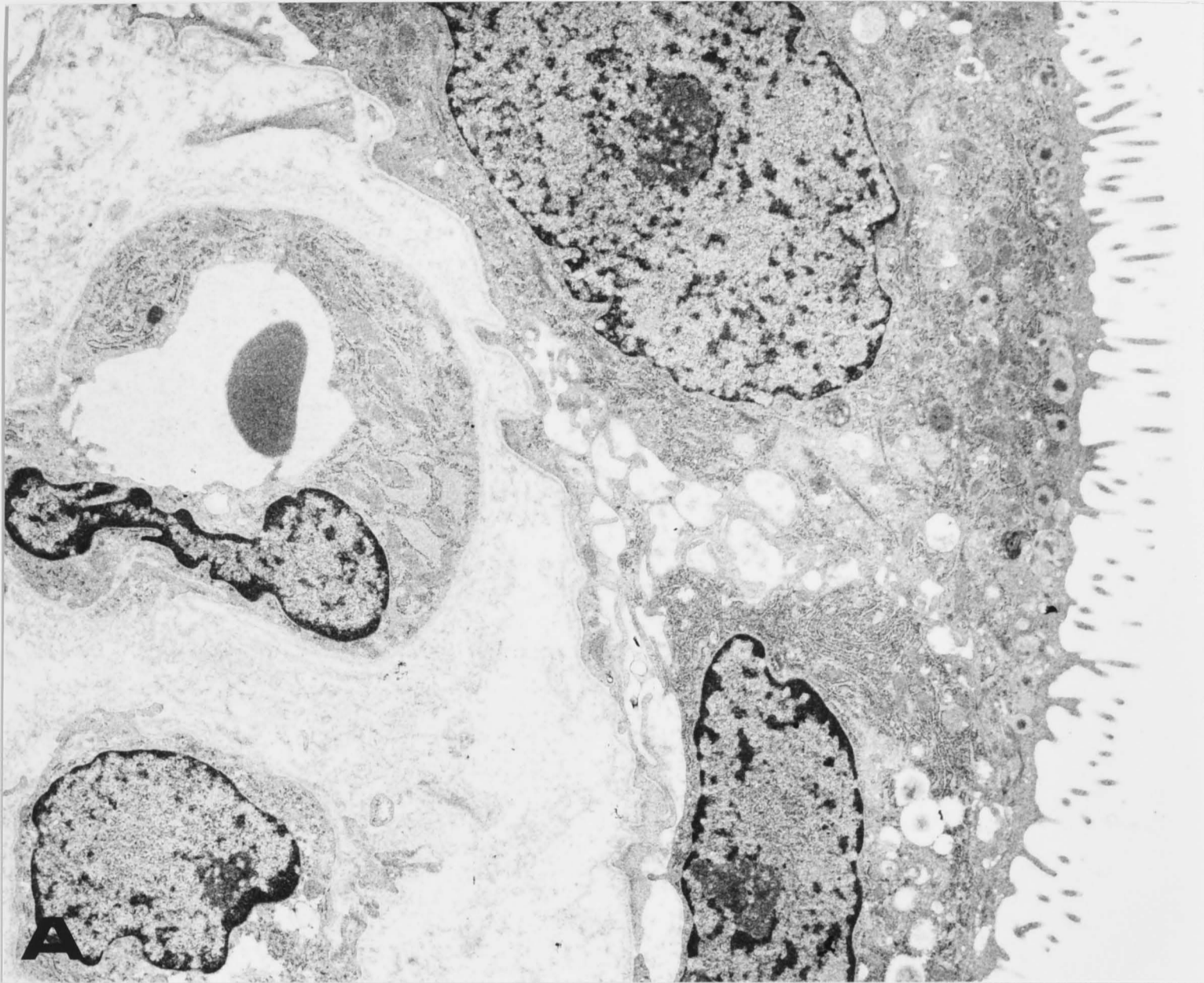


FIGURE IV-5

Endometrial autografts 13 days after transplantation to rabbit ear chambers.

- A. Hypertrophied stromal fibroblasts. Regions of the cytoplasm of these cells contain numerous polyribosomes and elongated profiles of rough endoplasmic reticulum. Coated vesicles and coated pits are obvious in the peripheral regions of the central cell. Numerous smaller coated vesicles are obvious in the large 'hoff' region of this cell. (X 18800)

cp - coated pit.

cv - coated vesicle.

- B. A large lymphocyte is forming an extensive region of contact with the process of a stromal fibroblast. (X 6200)

- C. Numerous vacuoles and phagosomes containing cytoplasmic remnants are present in the endothelial cells of a graft blood vessel. The wall of this vessel is composed of an endothelial layer and a basement membrane with no other cell types present. (X 11400)

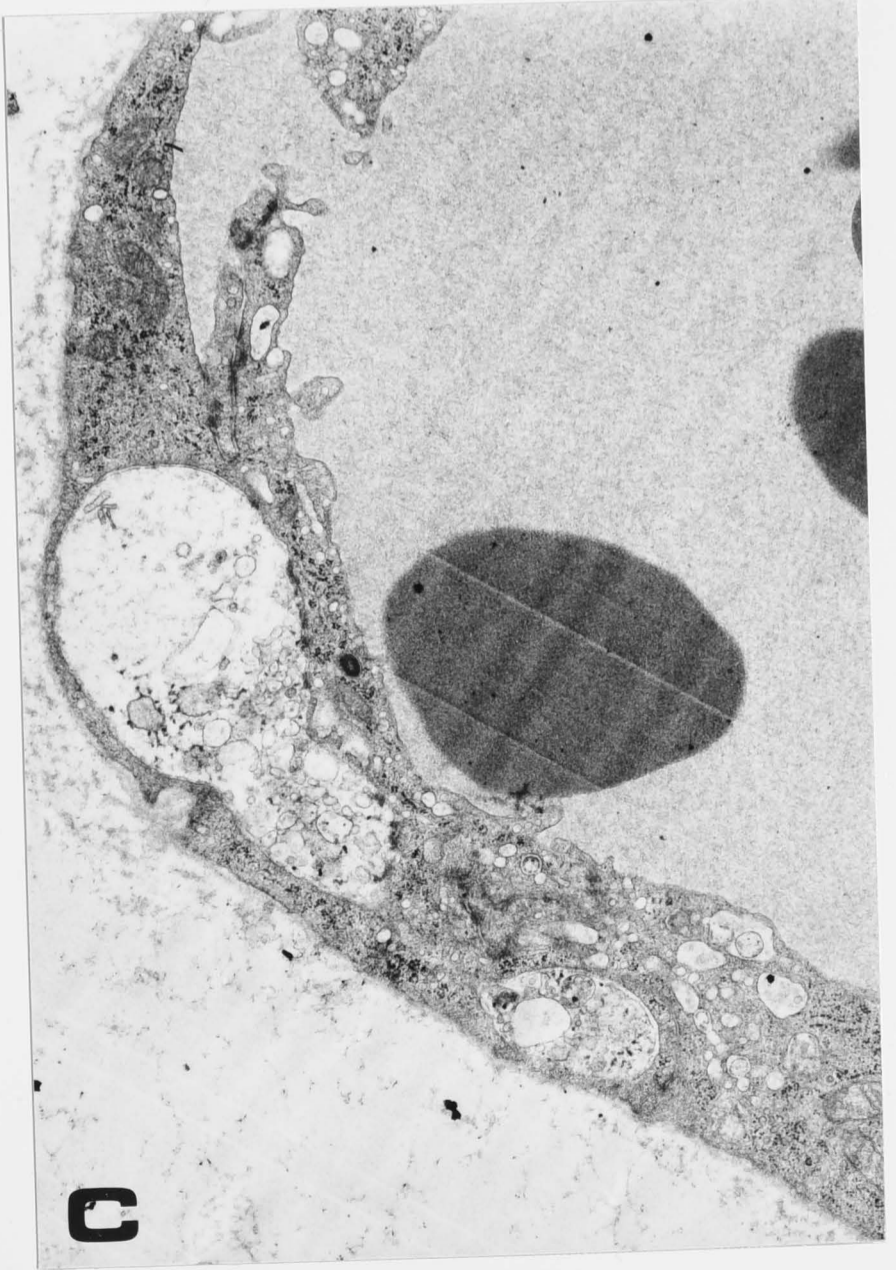
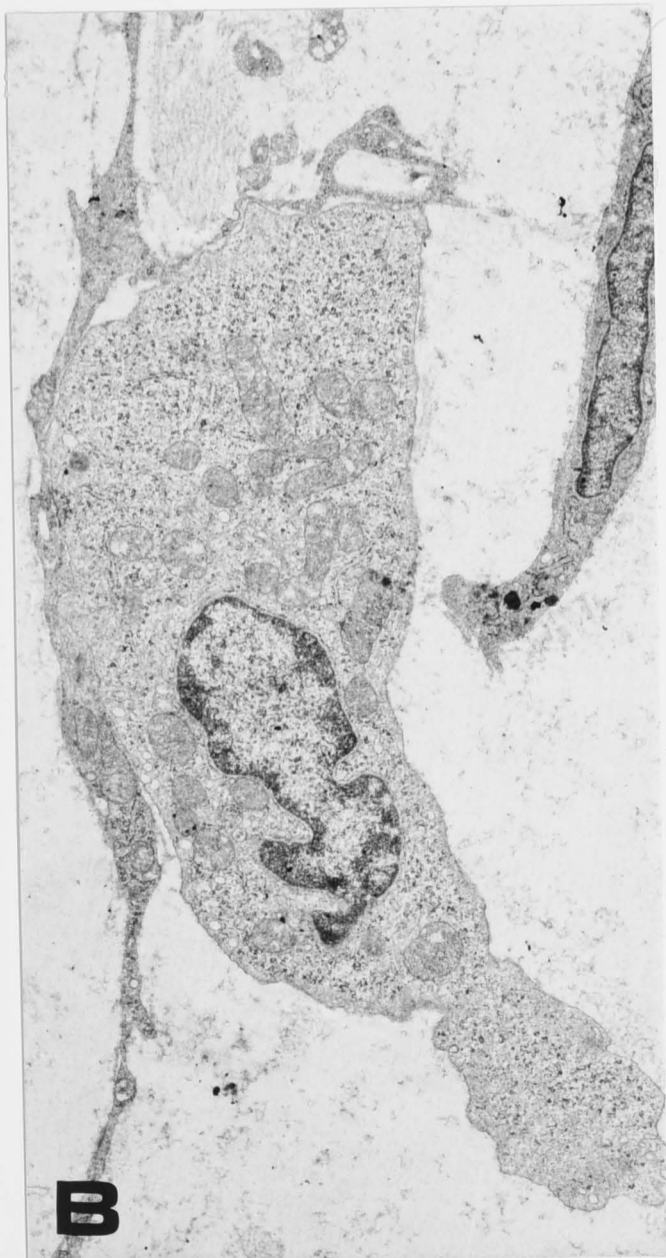
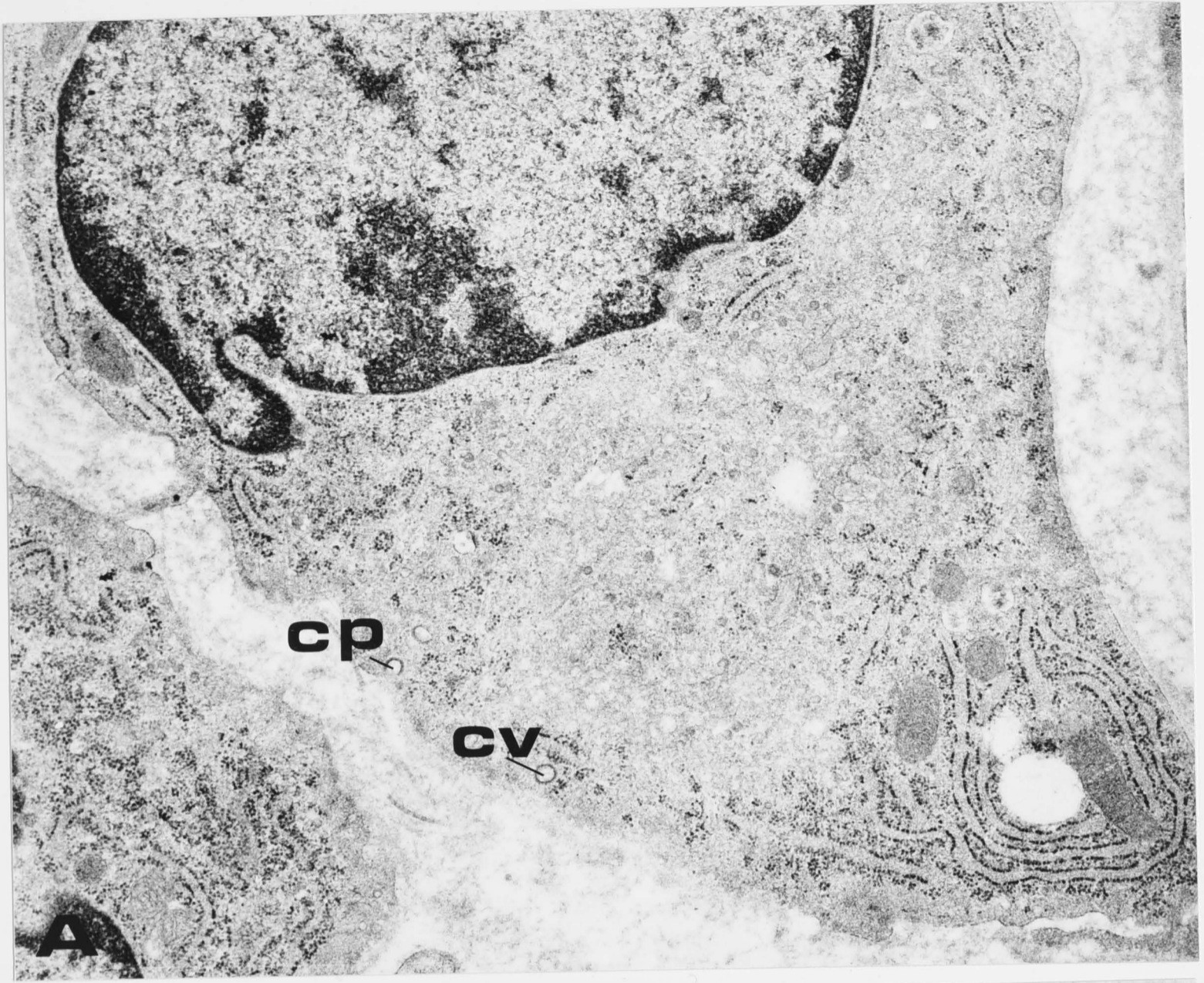


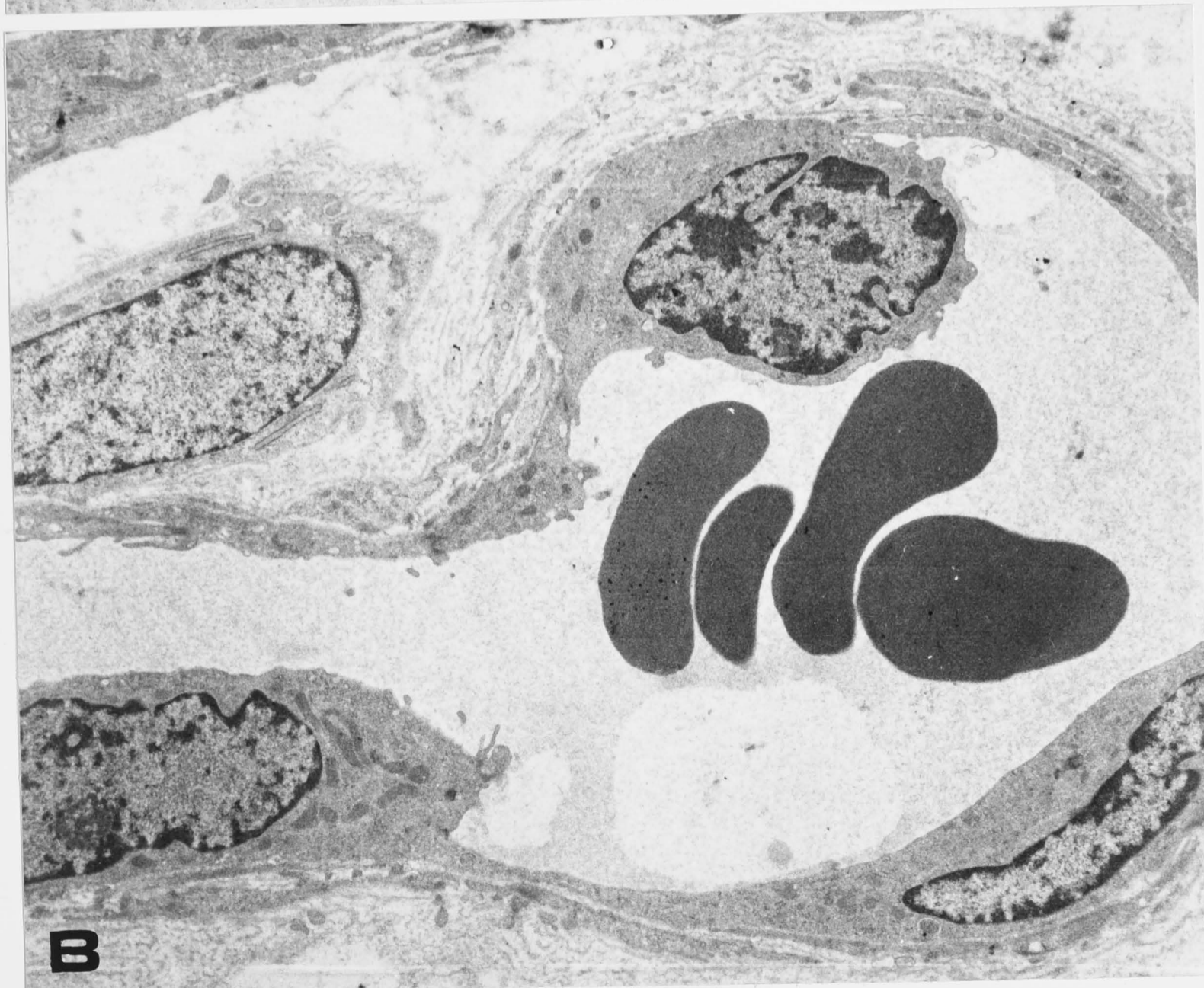
FIGURE IV-6

Endometrial autografts 25 to 30 days after transplantation to rabbit ear chambers.

- A. The graft body is at the right margin and the ear chamber membrane is at the lower margin of the field. The endometrial epithelium is extending from the graft to cover the ear chamber membrane. The epithelium has numerous microvilli and secretory vacuoles. A neutrophil granulocyte lies in the ear chamber membrane below the epithelium. (X 3400)
- B. A venule is enclosed in multiple basement membrane-like condensations which are characteristic of rabbit endometrium. (X 5200)



A



B

FIGURE IV-7

An endometrial autograft 3 days after transplantation to a rabbit ear chamber. The graft has not established connection with the recipient blood circulation.

- A. The epithelial surface facing the ear chamber membrane runs obliquely across the lower part of the figure. The epithelial cells are low and show minimal basal separation. Microvilli are short and secretory vacuoles are virtually absent. In the upper part of the field, stromal fibroblasts are numerous and have abundant cytoplasm. (X 3000)
- B. A blood vessel contains tightly packed erythrocytes and some platelets within its lumen. The endothelial lining is intact. Cells related to each end of the vessel show cytoplasmic blebbing and fragmentation. (X 3900)

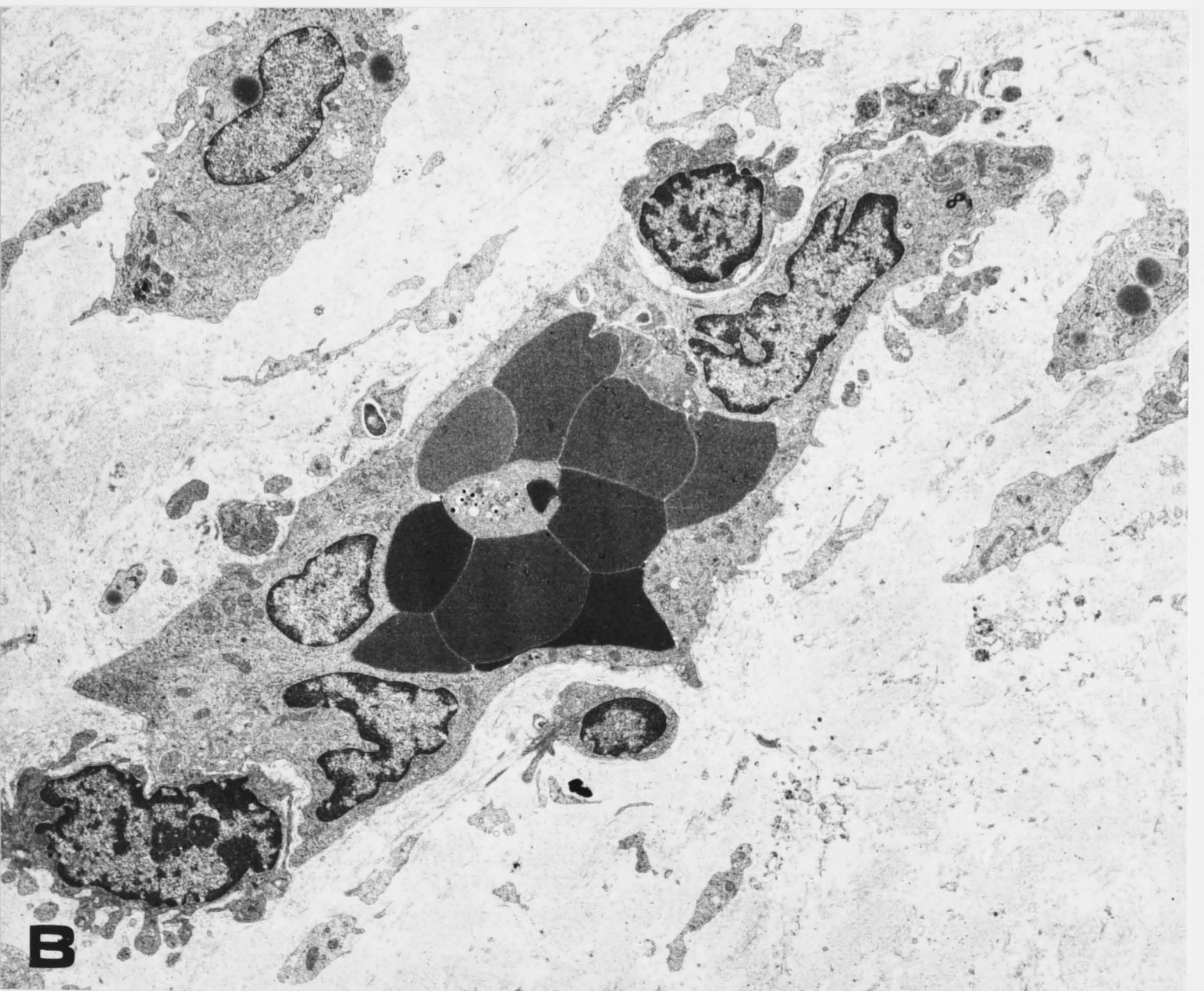
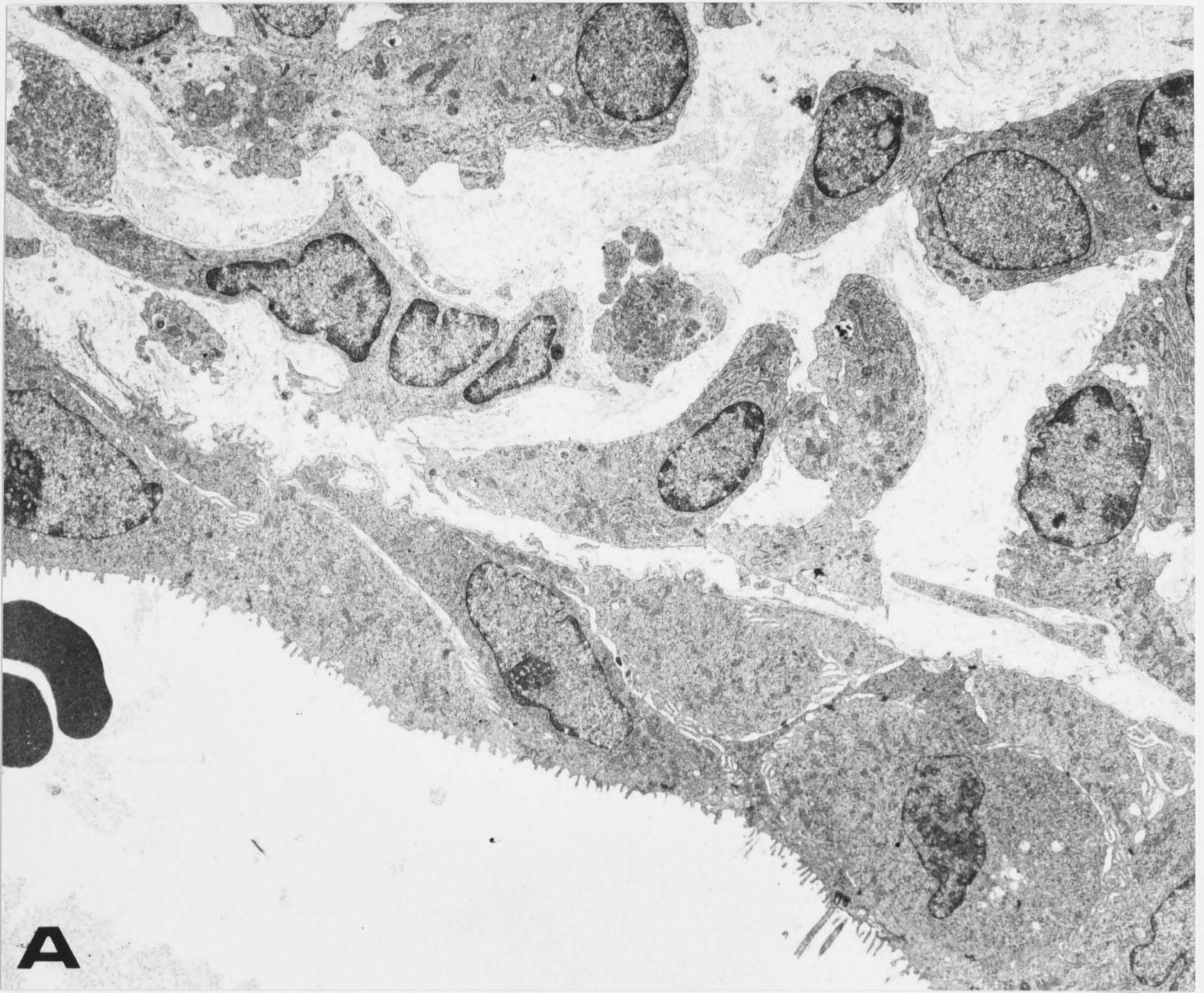


FIGURE IV-8

An endometrial autograft 13 days after transplantation to an ear chamber. The graft has not established connection with the recipient blood supply.

- A. Light micrograph of a 1 μ m. epon section. The graft was folded on itself and its epithelium faced the ear chamber membrane situated below the lower margin of the field. The epithelium, stromal fibroblasts and non-flowing blood vessels of the graft nearest to the ear chamber membrane are best preserved.

(X 230)

- B. The region most distant from the ear chamber membrane shown in figure A. A flattened continuous epithelium covers the surface of the graft. One epithelial cell has numerous stubby microvilli while the other has few and shows evidence of degeneration.

(X 3000)

- C. Higher magnification of the degenerate cell in figure B. The perinuclear cisterna is widely dilated and the rough endoplasmic reticulum is vesiculated. Secretory vacuoles are absent. A well-formed desmosome is still present at the basal region of the epithelium and fragments of basement membrane persist.

(X 41000)

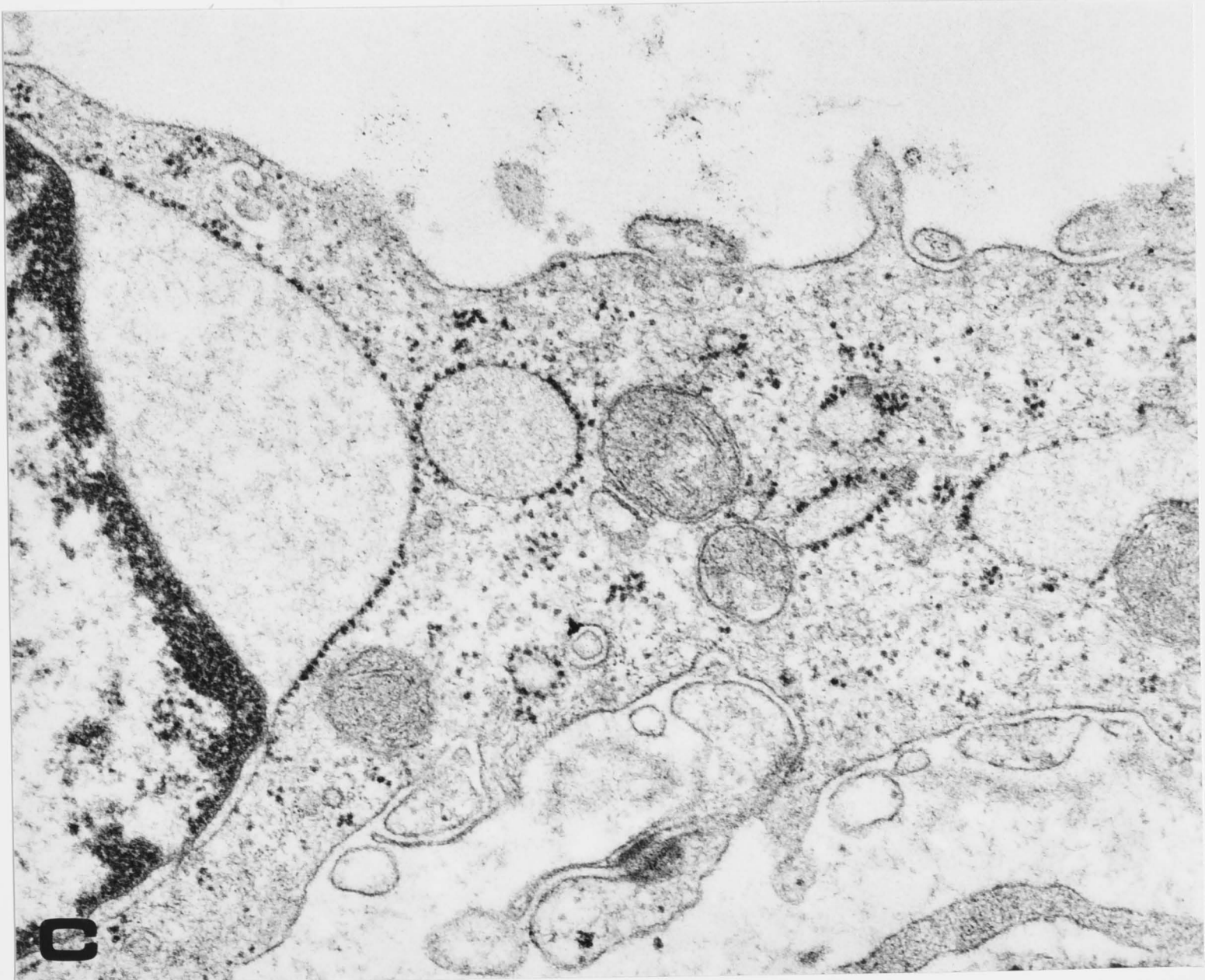
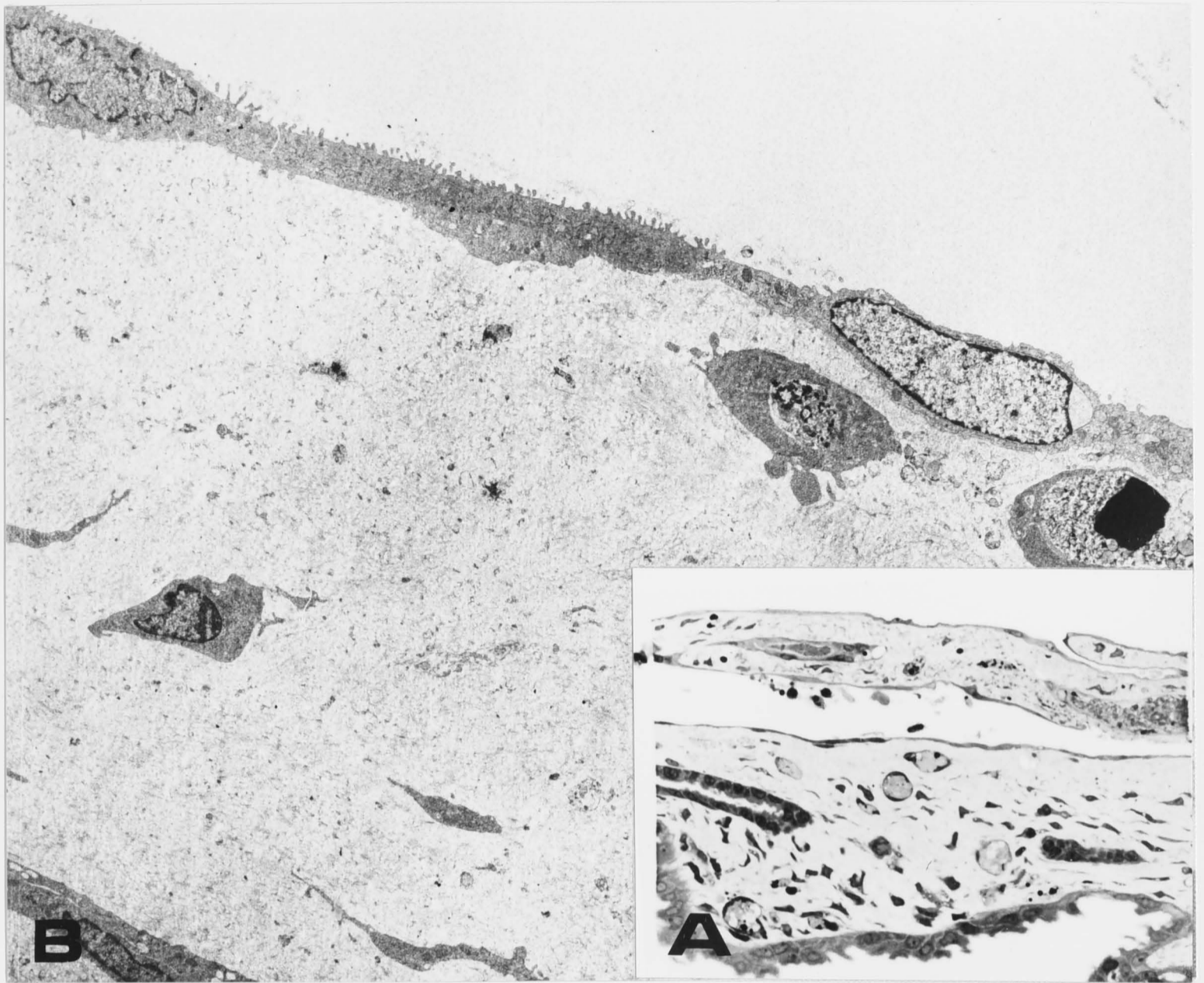


FIGURE IV-10

Necrotic endometrial grafts with vascular engorgement in rabbit ear chambers.

- A. An allograft 7 days after transplantation. Light micrograph of a 1 μ m. epon section. The lamina propria of the graft contains engorged vessels and widespread haemorrhage. Low epithelium persists on the undersurface of the graft and on the adjacent chamber membrane. Vessels and fibrocytes in the underlying chamber membrane have degenerated. (X 230)
- B. An autograft 13 days after transplantation. Electron micrograph of the lamina propria. A stromal fibroblast shows marked dilation of mitochondria but otherwise appears well preserved. A neutrophil granulocyte is present in the lower portion of the field. (X 14500)
- C. An allograft 3 days after transplantation. A cell in the lamina propria shows dilation of the rough endoplasmic reticulum, the perinuclear cisterna and mitochondria. The plasma membrane has broken down and the nuclear chromatin is condensed. (X 13000)

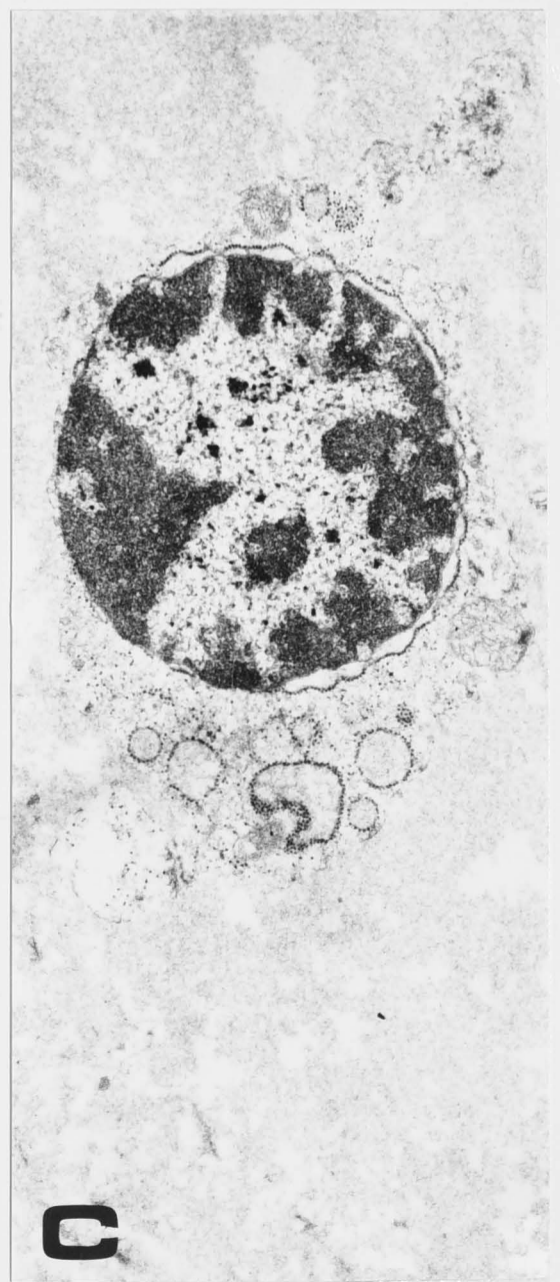
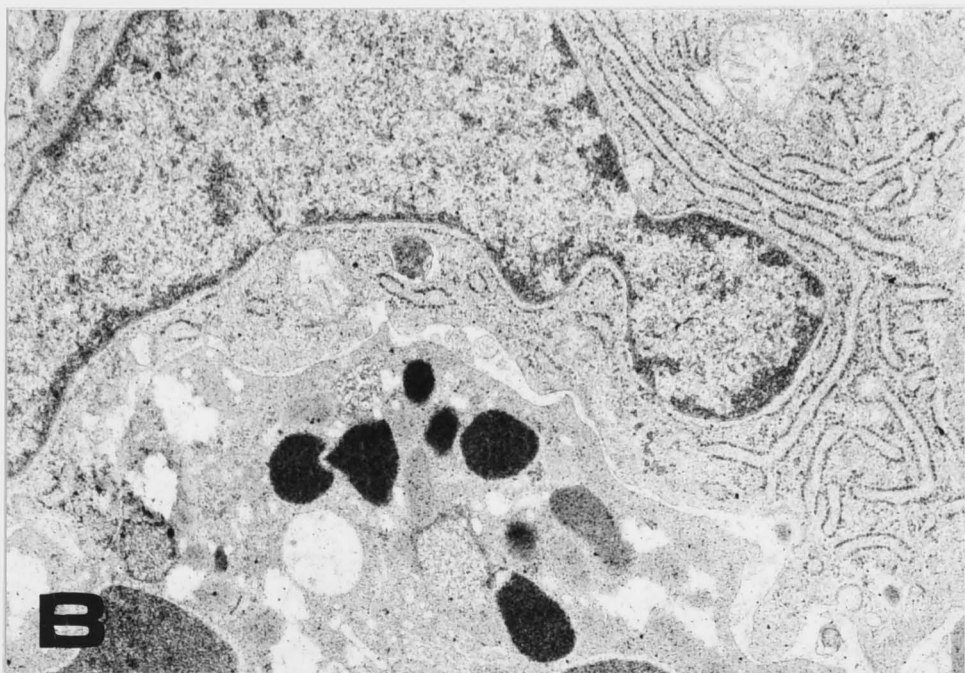
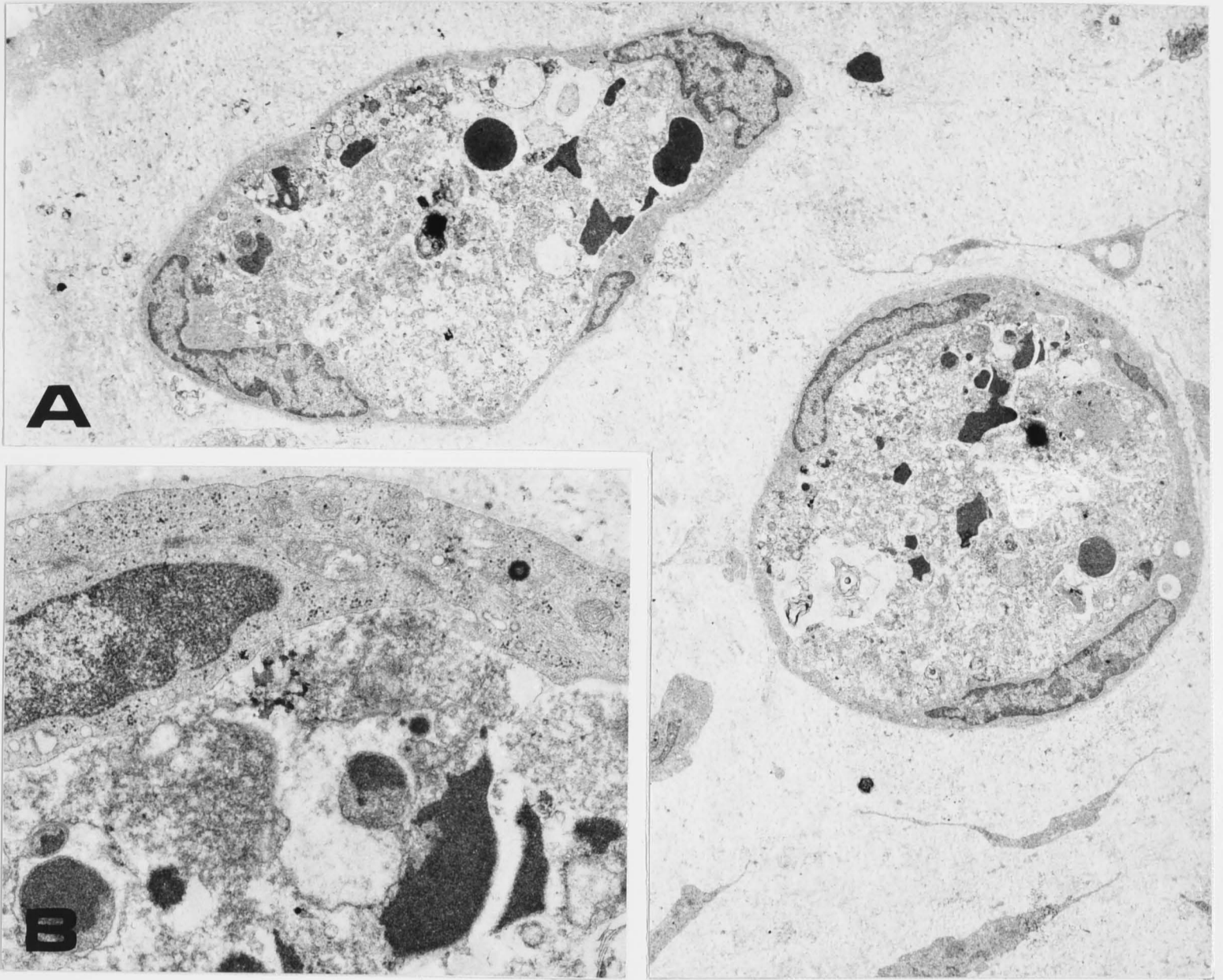


FIGURE IV-11

Endometrial grafts in rabbit ear chambers. Light micrographs of 1 μ m. epon sections. The graft is towards the left side and the chamber membrane is towards the right side of each figure.

- A. An autograft 25 days after transplantation. Endometrial epithelium covers the surface of the autograft body (g) and extends over the surface of the ear chamber membrane (m) and the new connective tissue (c) derived from the membrane. The epithelium contains numerous collections of secretory vacuoles and shows basal separation at the edge of the autograft. The lamina propria contains scattered stromal fibroblasts and leukocytes. Thin-walled blood vessels are present both in the autograft and in the underlying recipient tissues. (X 200)
- B. An allograft on the day of the onset of stasis during induced rejection. The epithelium has been destroyed in areas both on the surface of the allograft and of the ear chamber membrane. The epithelium that remains is attenuated. In the lamina propria of the graft, leukocytic infiltration and cell degeneration are more obvious adjacent to degenerate epithelium (arrowheads). Some vessels in both the graft and the chamber membrane are engorged with blood. (X 200)

FIGURE IV-11 continued.

C. An allograft on the day of the onset of stasis during spontaneous rejection. The vascular endothelium and the epithelium of the allograft have been destroyed but the blood vessels of the ear chamber membrane are undamaged. The lamina propria of the allograft is infiltrated by leukocytes and contains haemorrhage and degenerate cells. Leukocytes have infiltrated the ear chamber membrane and have accumulated adjacent to vessels near the upper margin of the field.

(X 200)

D. An epithelial monolayer on the day of the onset of stasis during induced rejection. The epithelium at the upper left of the field has been destroyed. The epithelium that remains elsewhere is flattened. The related connective tissue is heavily infiltrated by mononuclear leukocytes and contains degenerate cells (arrowhead). Blood vessels appear intact.

(X 310)

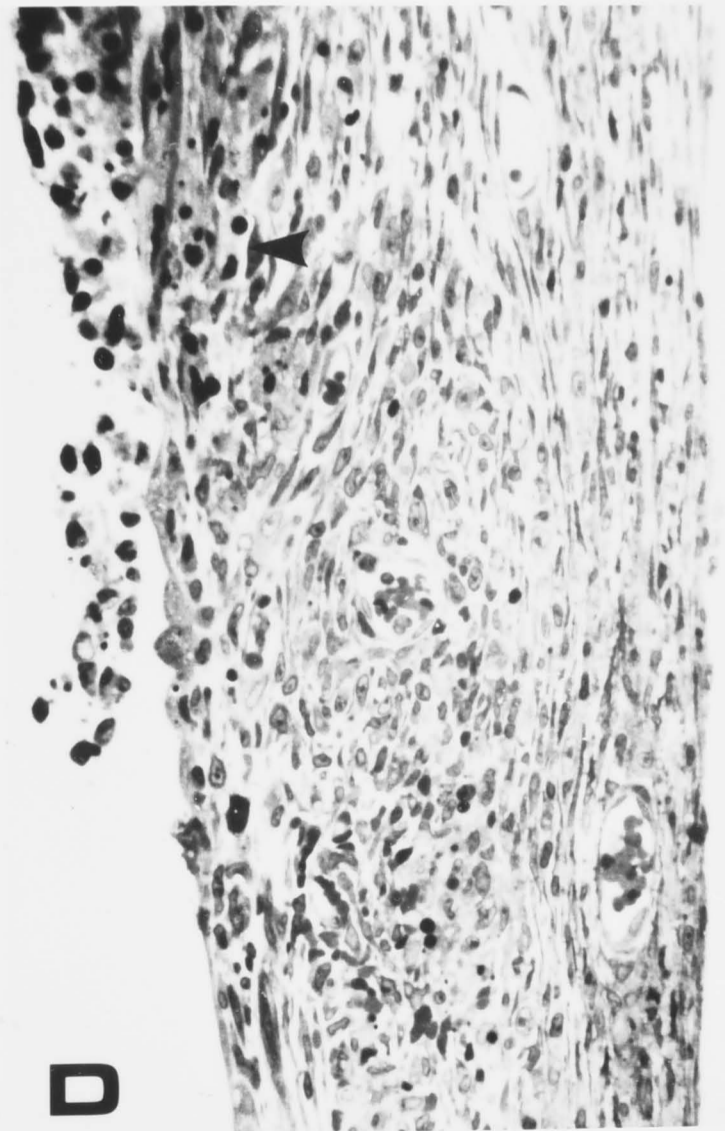
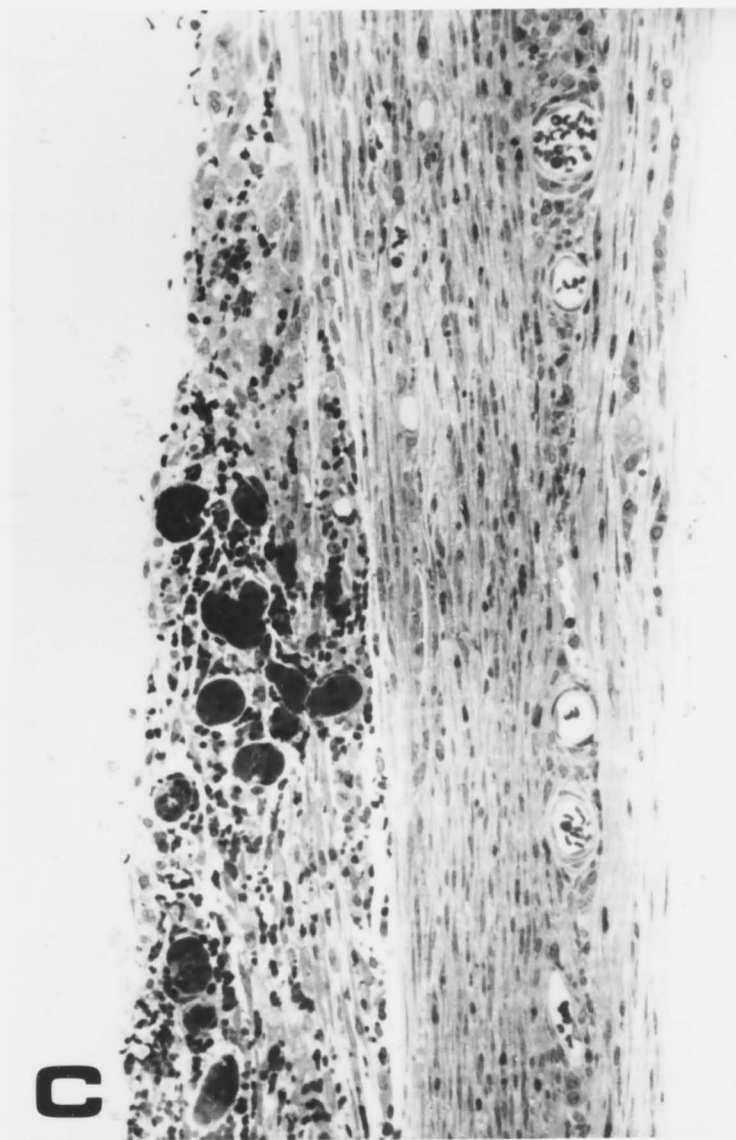
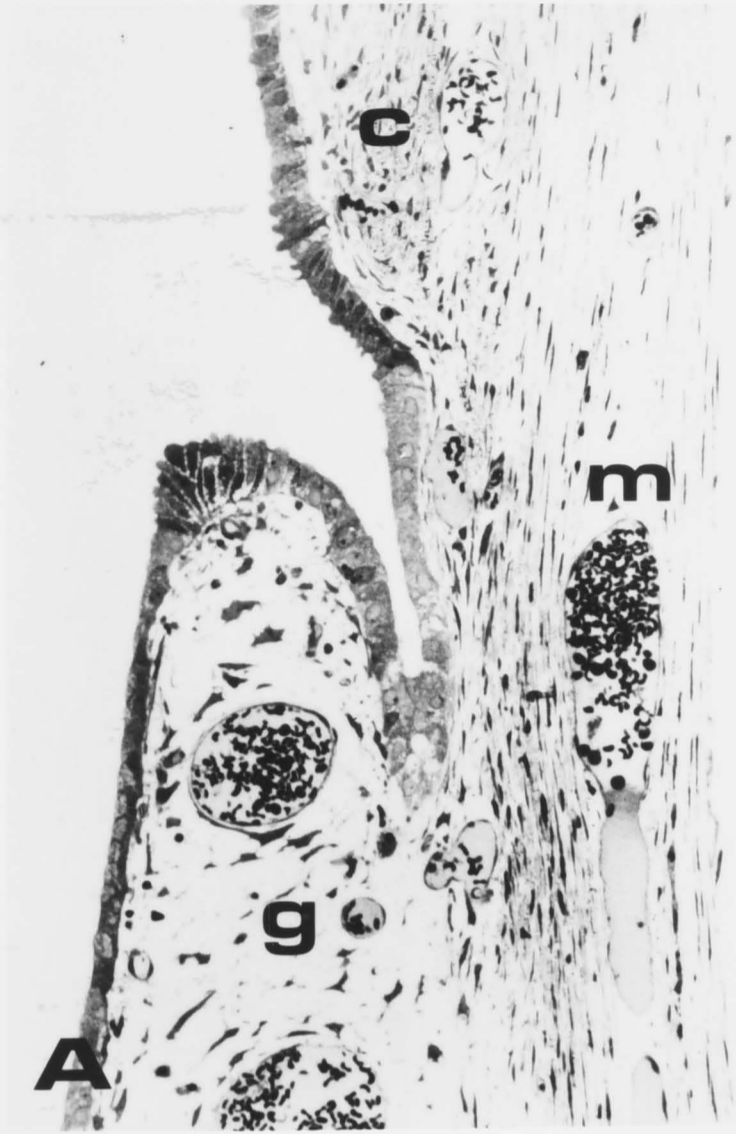


FIGURE IV-12

Endometrial allografts in ear chambers on the day of the onset of stasis during induced rejection.

- A. The epithelium (upper half of the field) is low and has a patchy distribution of stubby microvilli. The cells contain small numbers of secretory vacuoles and mitochondria are condensed. A mononuclear leukocyte is in contact with the epithelial cells. Note that cytoplasmic processes from both the epithelial cells and the leukocyte extend through gaps in the basement membrane (arrowheads). (X 7800)
- B. A cyst formed when the host connective tissue overgrew a monolayer extending over the chamber membrane. The epithelium runs from left to right in the upper part of the field. Macrophages are present in the lumen of the cyst at the upper margin of the field. The low epithelium has sparse stubby microvilli, small numbers of secretory vacuoles and contains a phagosome. The basement membrane is discontinuous. A cytoplasmic extension of a large lymphocyte lies between the abluminal portions of 2 epithelial cells (arrowhead). Neutrophil granulocytes and mononuclear leukocytes are present in the tissue underlying the epithelium. Degenerate cells showing disruption of the nuclear chromatin pattern, vesiculation of cytoplasmic organelles and cytoplasmic fragmentation are present in the lower portion of the field (arrows). (X 3900)

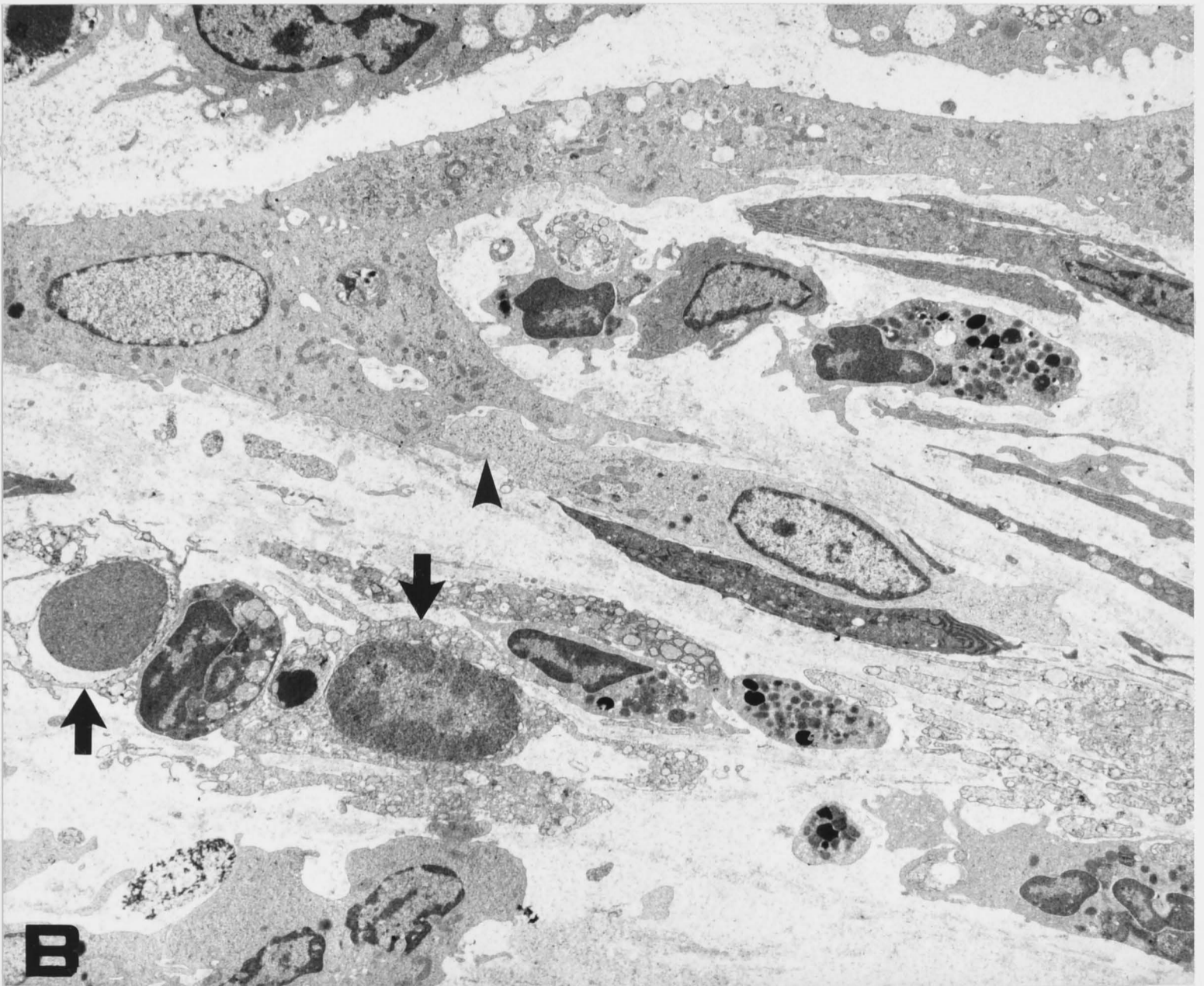


FIGURE IV-13

An endometrial allograft in a rabbit ear chamber. The day of the onset of stasis during induced rejection.

- A. The graft surface facing the ear chamber membrane. The basement membrane of the epithelium (arrowheads) is intact but is thrown into folds. Most of the cells of the epithelium (ep) and those of the lamina propria are degenerating. The chromatin of the degenerating cells is condensed at the periphery of the nuclei or fills the nuclear profile. Their cytoplasm contains vacuoles and vesiculated organelles. Epithelial cells contain abundant rough endoplasmic reticulum while related cells, often with a pachychromatic nucleus, are probably degenerating leukocytes. Apparently viable cells persist in the lower left corner and at the right margin of the field.

(X 2900)

- B. An epithelial cell showing early degenerative changes. Chromatin is clumped at the periphery of the nucleus. Mitochondria are condensed, rough endoplasmic reticulum is vesiculated and the cytoplasm contains vacuoles and phagosomes in which cytoplasmic contents are obvious. The cell is separating from its basement membrane (arrowhead).

(X 22700)

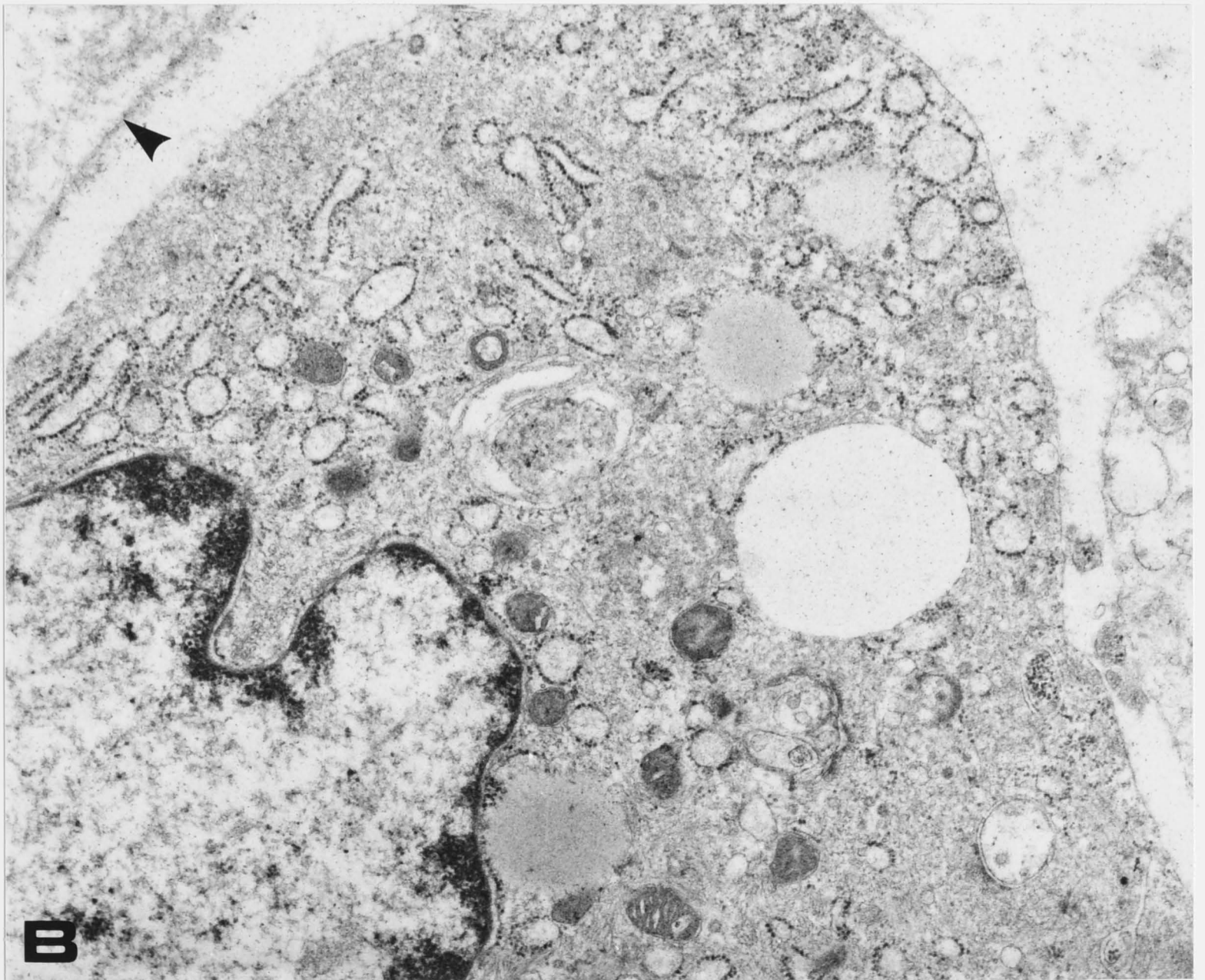
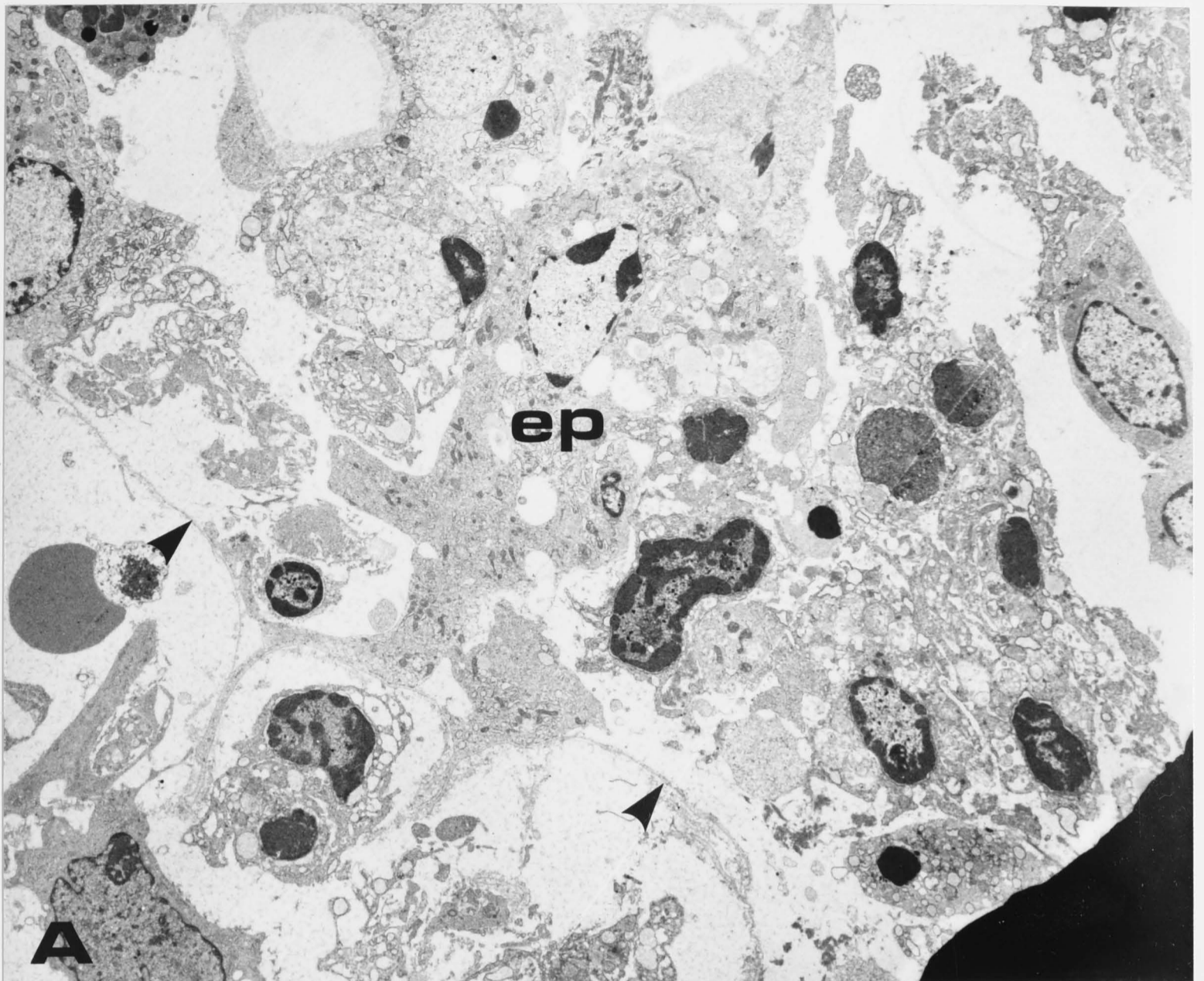


FIGURE IV-14

A cyst formed from epithelium extending from the edge of an endometrial allograft. The day of the onset of stasis during induced rejection. The epithelium (ep) is low and discontinuous and a basement membrane is absent. Macrophages are present in the lumen of the cyst above the epithelium (together with cell debris) and they are in contact with the surface of the epithelium. Small mononuclear leukocytes and macrophages are also in close proximity to the abluminal surface of the epithelium and are in contact with cells in the host connective tissue. (X 3500)

FIGURE IV-15

The surface of an endometrial allograft undergoing spontaneous rejection in a rabbit ear chamber. One day after the onset of stasis. The basement membrane of the epithelium (arrowheads) is largely intact but is wavy and diffuse. Remnants of degenerate cells are present in the upper portion of the field. Flattened cells, possibly epithelial, are in close relation to the basement membrane. A large mononuclear cell in the underlying lamina propria contains phagocytosed degenerate cells. Part of a damaged blood vessel is present in the lower left corner of the field. (X 3900)

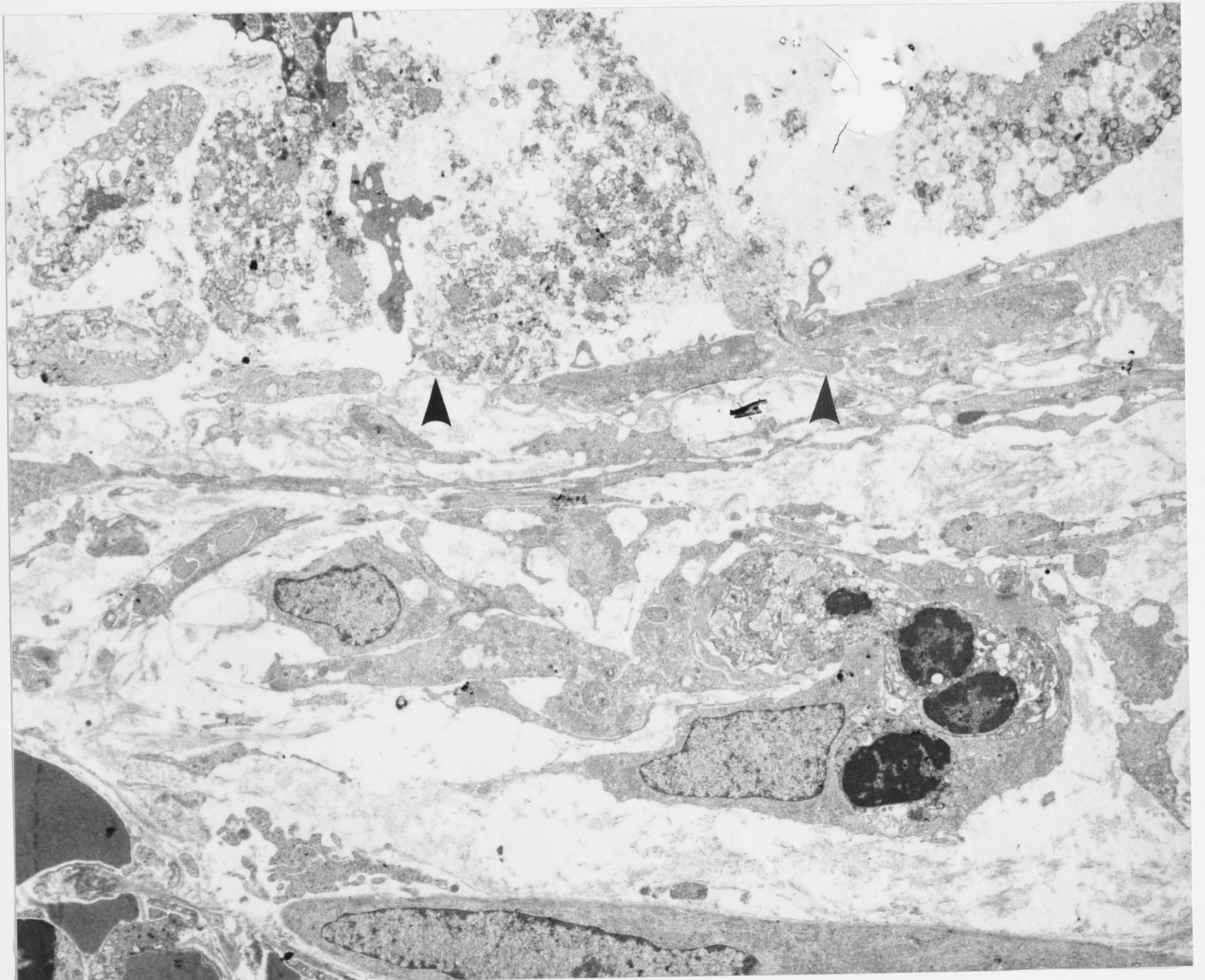
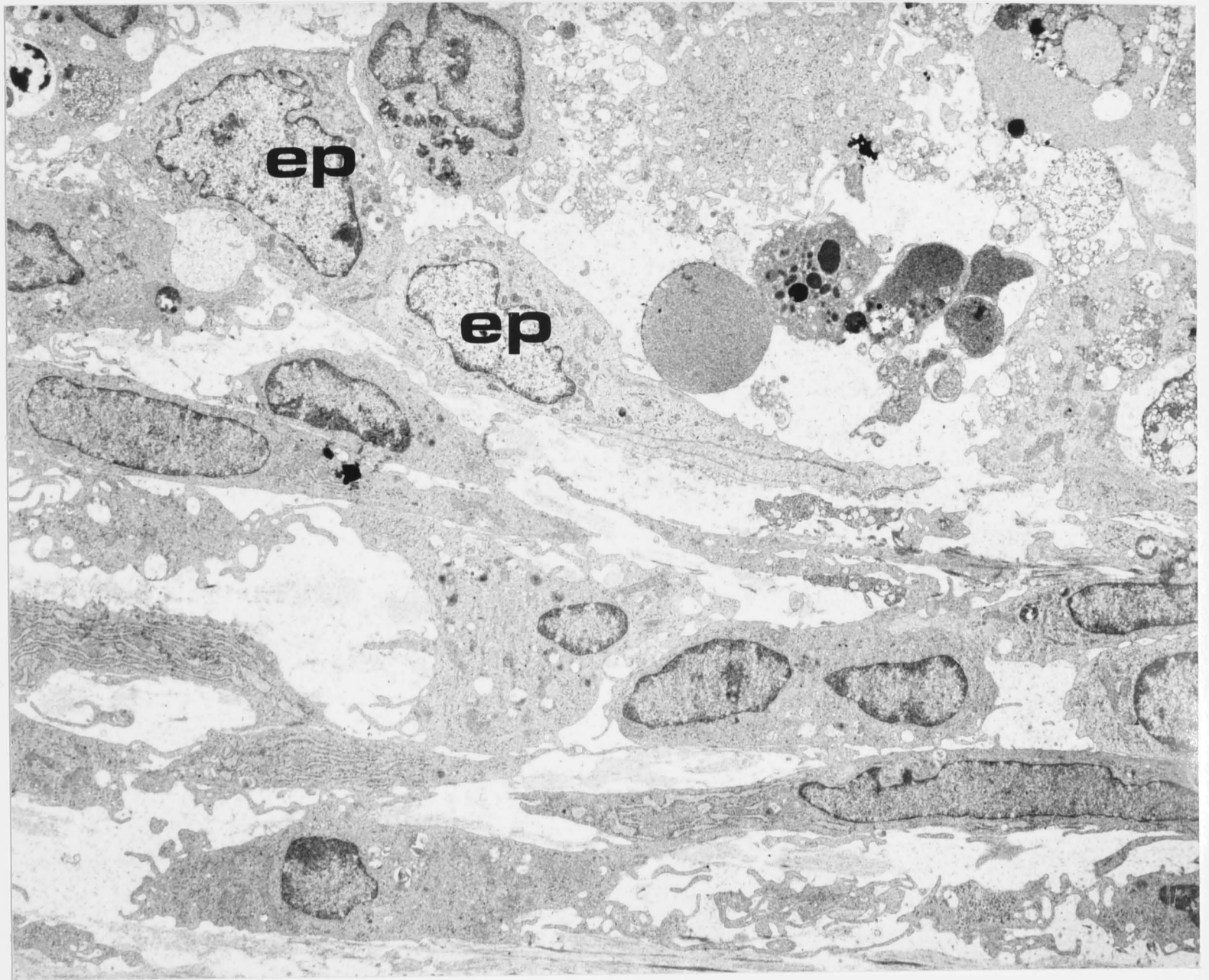


FIGURE IV-16

The lamina propria of an endometrial allograft undergoing spontaneous rejection in a rabbit ear chamber one day after the onset of stasis. Neutrophil granulocytes (n), monocytes (m) and a fibroblast (f) are identified. The monocytes are in contact with the fibroblast. A cytoplasmic process extending from one of the monocytes indents the surface of the fibroblast (arrowhead). (X 7200)

FIGURE IV-17

The lamina propria of an endometrial allograft undergoing induced rejection in a rabbit ear chamber one day after the onset of stasis. Three lymphocytes (l) form extensive contact regions with stromal fibroblasts. One stromal fibroblast near the middle of the field has numerous vacuoles in its cytoplasm adjacent to one of the lymphocytes. Two large pedunculate cytoplasmic processes extend around this lymphocyte. Blebs of cytoplasm (b) appear to have been shed from cells. The blood vessel (upper left corner of the field) shows no evidence of injury. (X 3100)

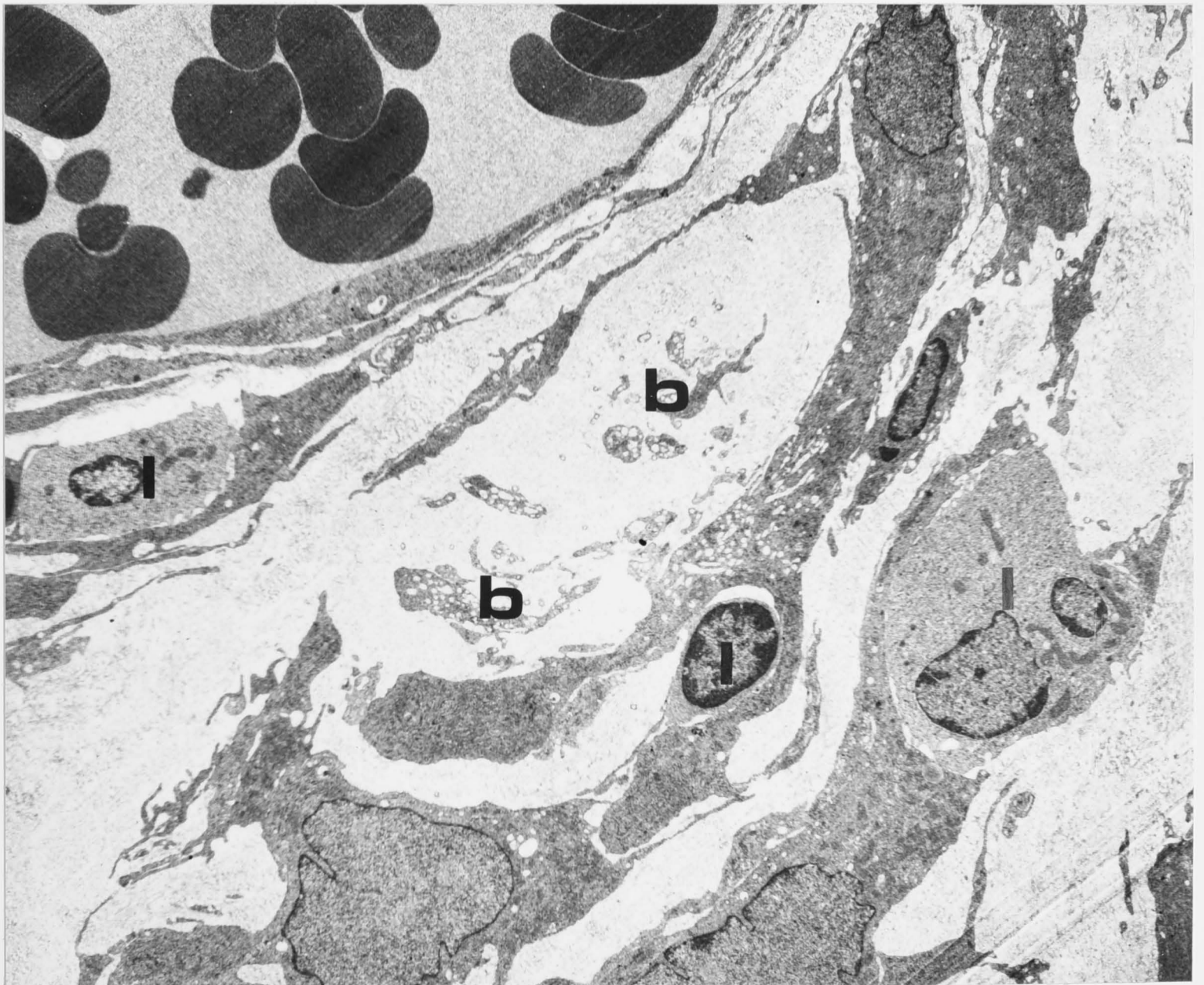
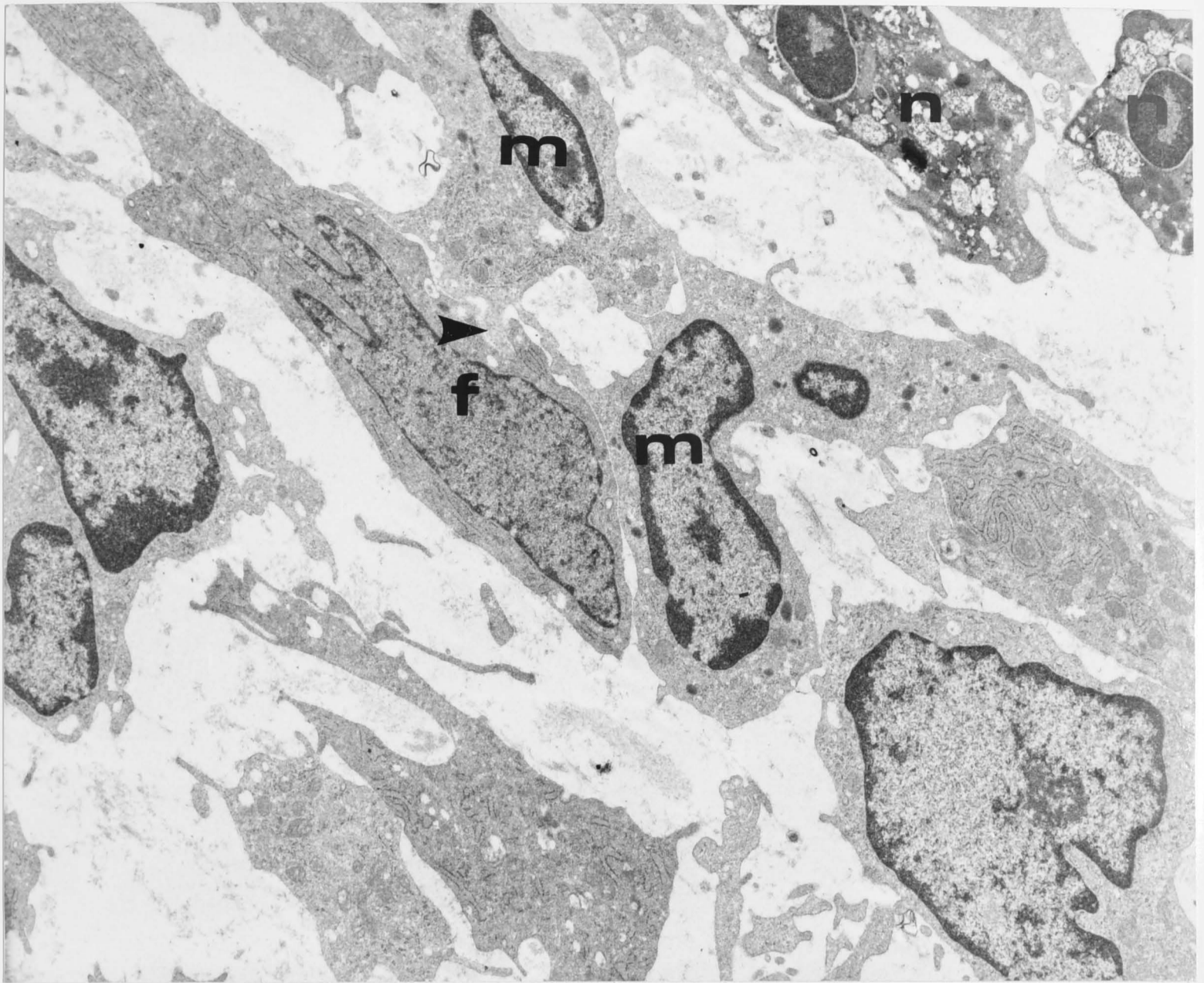


FIGURE IV-18

The lamina propria of an endometrial allograft undergoing spontaneous rejection one day after the onset of stasis.

- A. A region of contact between a large lymphocyte (left) and a stromal fibroblast (right). Short protrusions of the lymphocyte cytoplasm interdigitate with others of the stromal fibroblast. The cytoplasm of the lymphocyte contains abundant free ribosomes both single and in clusters while numerous microfilaments are present in the cytoplasm of the fibroblast.

(X 40300)

- B. The cell at the centre of the field shows fragmentation of the nucleus (fn). The nuclear chromatin is condensed and the cytoplasm of the cell is vacuolated. Large lymphocytes are obvious at the upper left and lower right corners of the figure. A cytoplasmic process (arrowhead) from one lymphocyte contacts the central cell.

(X 11800)

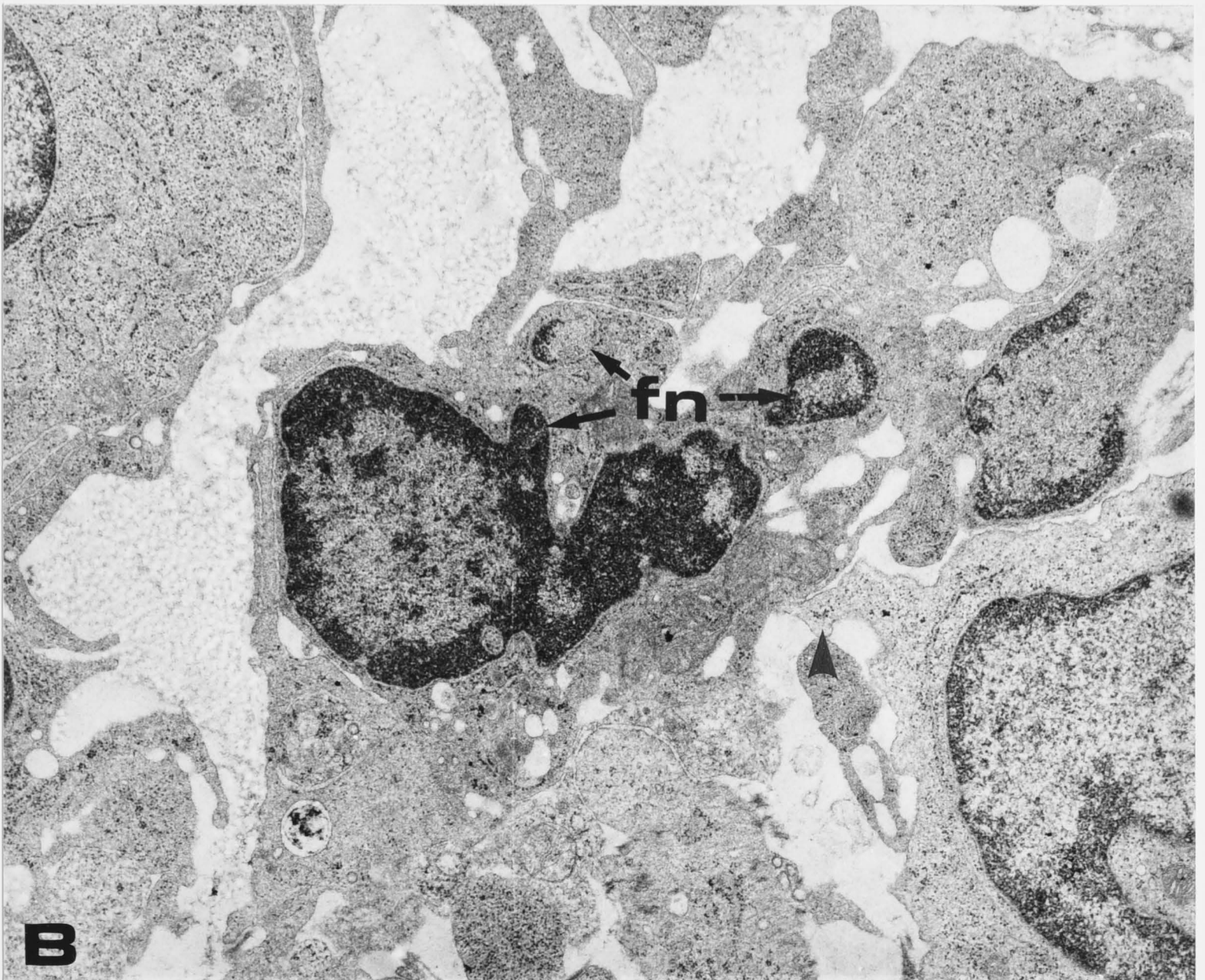
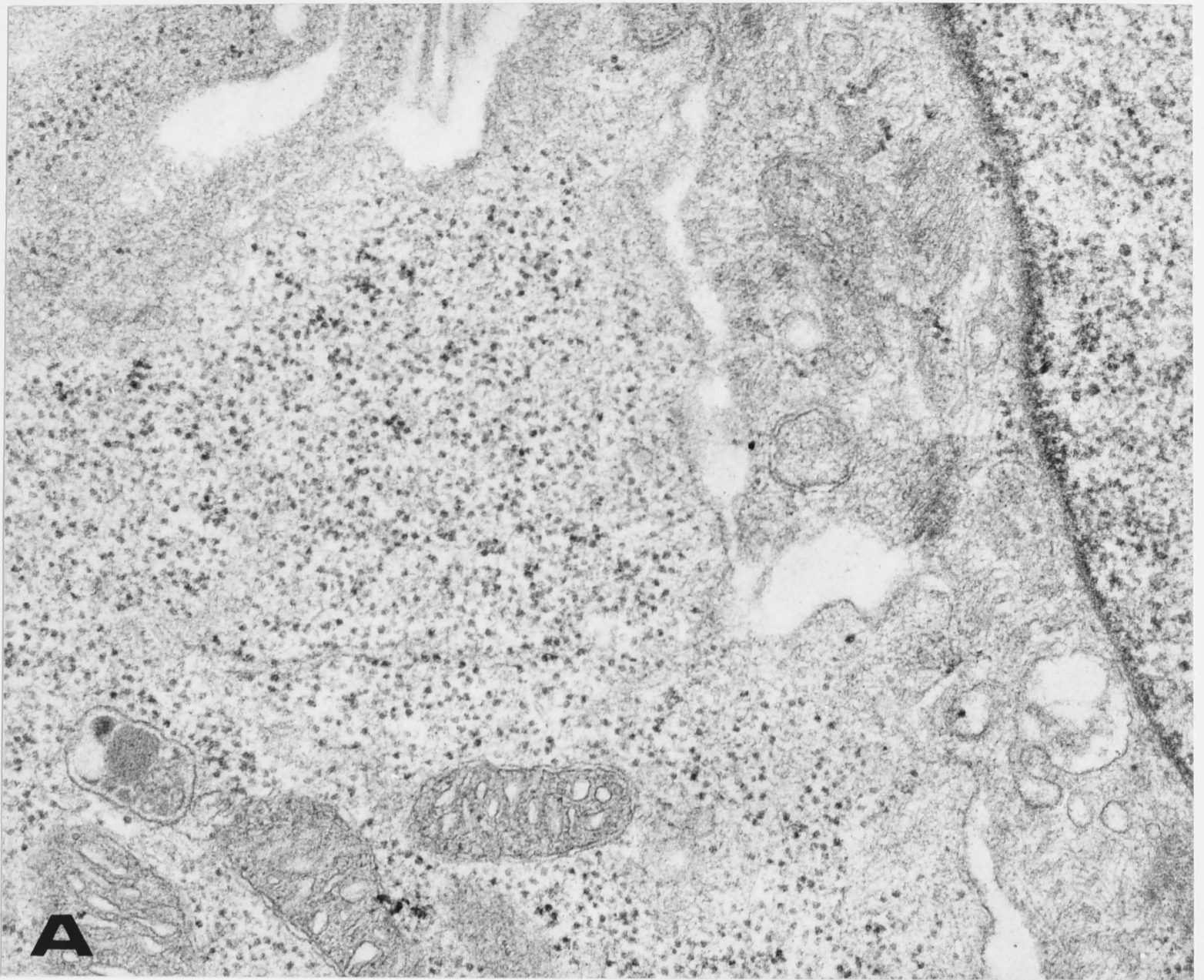


FIGURE IV-19

The lamina propria of an endometrial allograft undergoing spontaneous rejection in a rabbit ear chamber one day after the onset of stasis. Many of the cells in the field are degenerating while other cells appear undamaged. (X 4000)

FIGURE IV-20

The lamina propria of an endometrial allograft undergoing spontaneous rejection in a rabbit ear chamber on the day of the onset of stasis. A degenerate cell shows clumping of the nuclear chromatin, vesiculation of cytoplasmic organelles and breakdown of the plasma membrane. The abundant remnants of rough endoplasmic reticulum in the cytoplasm of the cell indicate that it was a stromal fibroblast. Extravasated erythrocytes are present in the field. (X 6600)

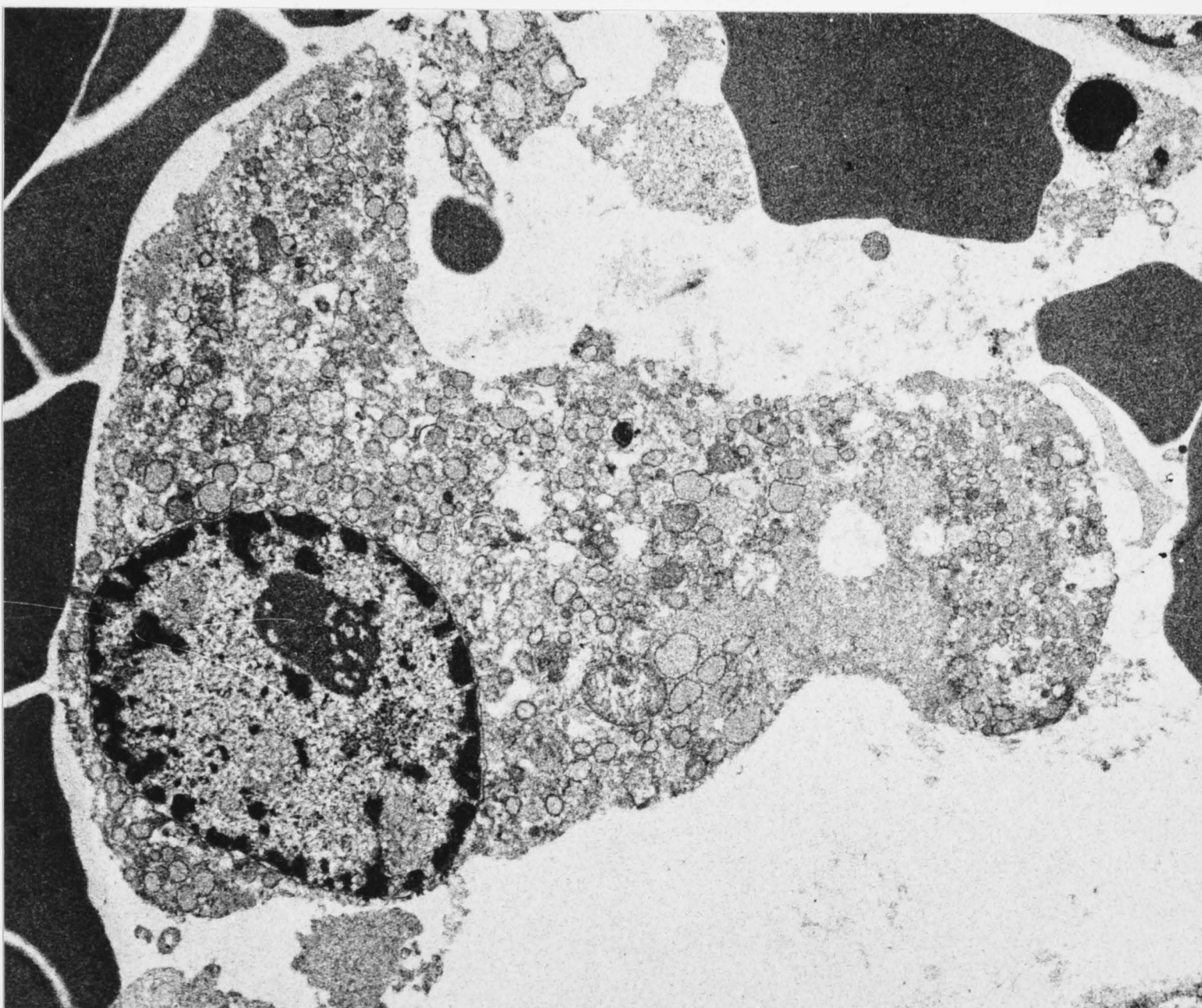
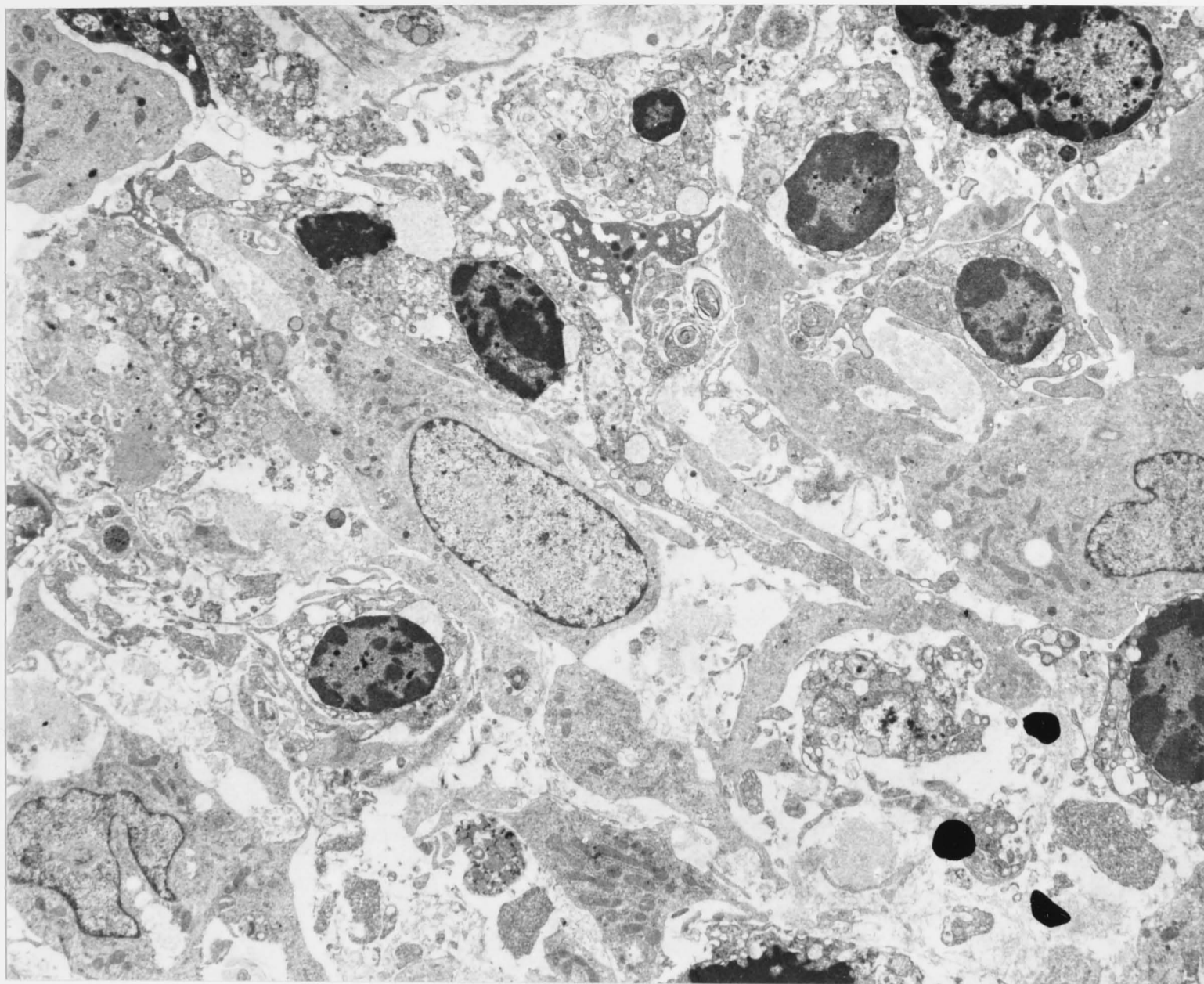


FIGURE IV-21

The lamina propria of an endometrial allograft undergoing spontaneous rejection in a rabbit ear chamber one day after the onset of stasis. A projection of the connective tissue indents the wall of a large vessel in which blood was flowing at the time of fixation. Degenerate cells are present in the connective tissue but the endothelium of the vessel appears largely undamaged. (X 4300)

FIGURE IV-22

The lamina propria of an endometrial allograft undergoing induced rejection in a rabbit ear chamber on the day of the onset of stasis. Small mononuclear leukocytes with veil-like processes are present in the lumen of a vessel and in the connective tissue. Cytoplasmic features identify the cell at the upper margin of the field as a monocyte. The endothelial cell in contact with this leukocyte has dilated mitochondria (arrowheads). Platelets in the lumen of the vessel show pseudopod formation (open arrows). (X 4800)

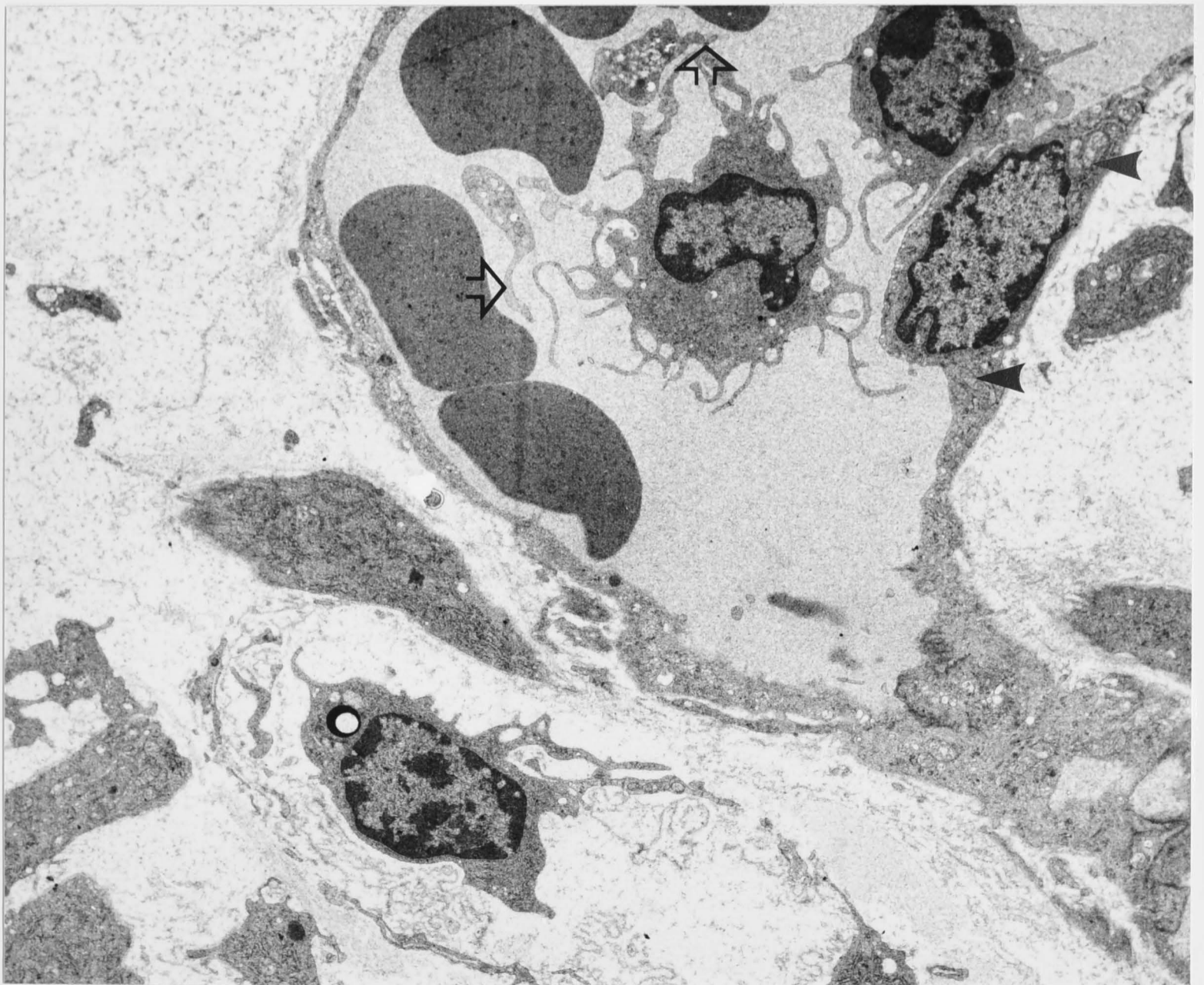
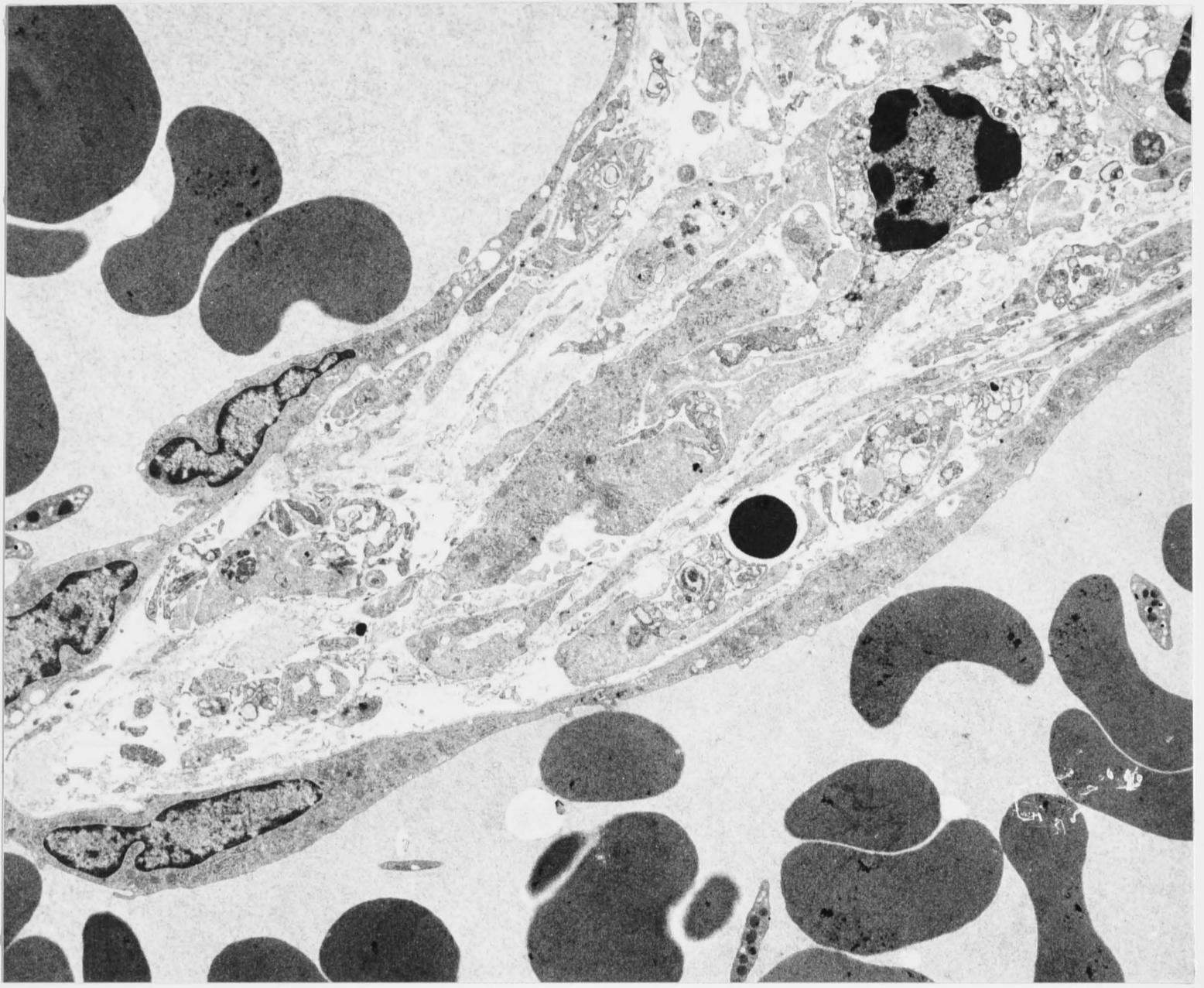


FIGURE IV-23

The lamina propria of an endometrial allograft undergoing induced rejection in a rabbit ear chamber one day after the onset of stasis. The remnants of a vessel contain a neutrophil granulocyte, various degenerate cells, erythrocytes and fibrin. The integrity of the endothelium lining the vessel is breaking down (arrowhead). A cell in the extravascular lamina propria (top left corner) also shows degenerative changes. (X 5600)

FIGURE IV-24

The lamina propria of an endometrial allograft undergoing spontaneous rejection on the day of the onset of stasis. Erythrocytes are packed tightly into the lumen of a blood vessel in which the endothelial lining has been almost completely destroyed. A degenerate endothelial cell with densely condensed nuclear chromatin remains at the upper left corner of the field. Over much of the vessel circumference the wall consists only of the basement membrane (arrowheads). The tissue surrounding the vessel contains mononuclear leukocytes, extravastated erythrocytes and cell debris. (X 4400)

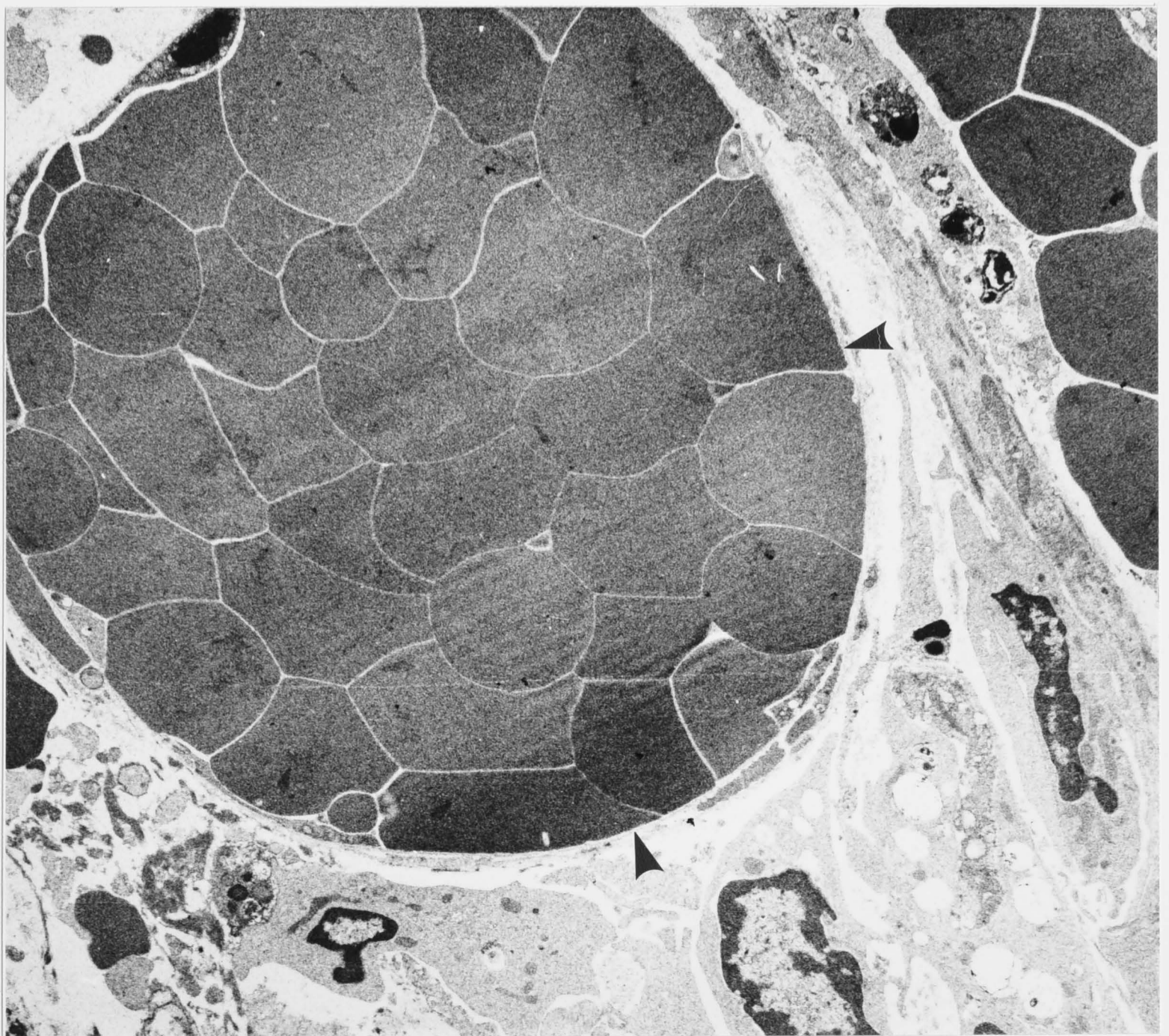
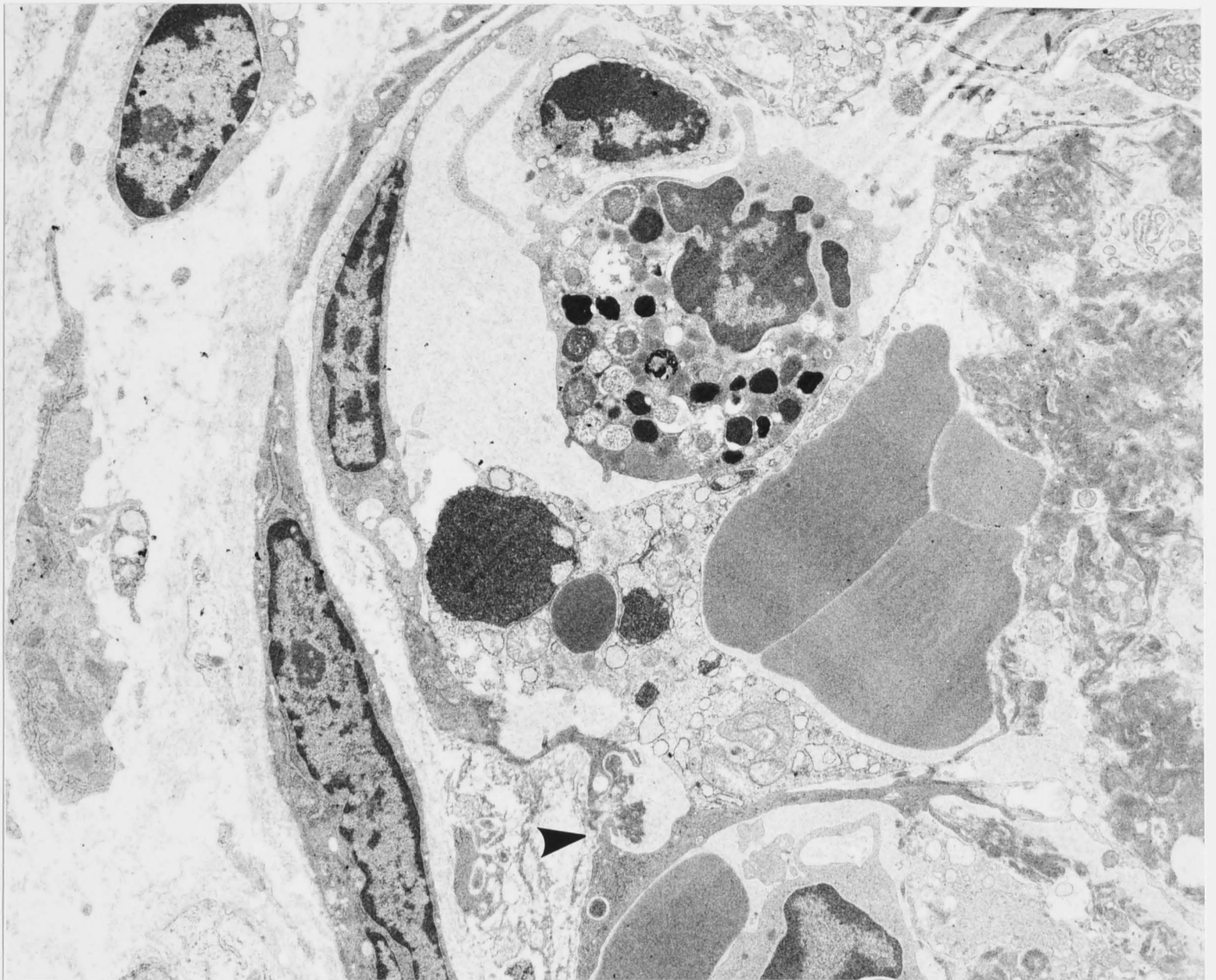
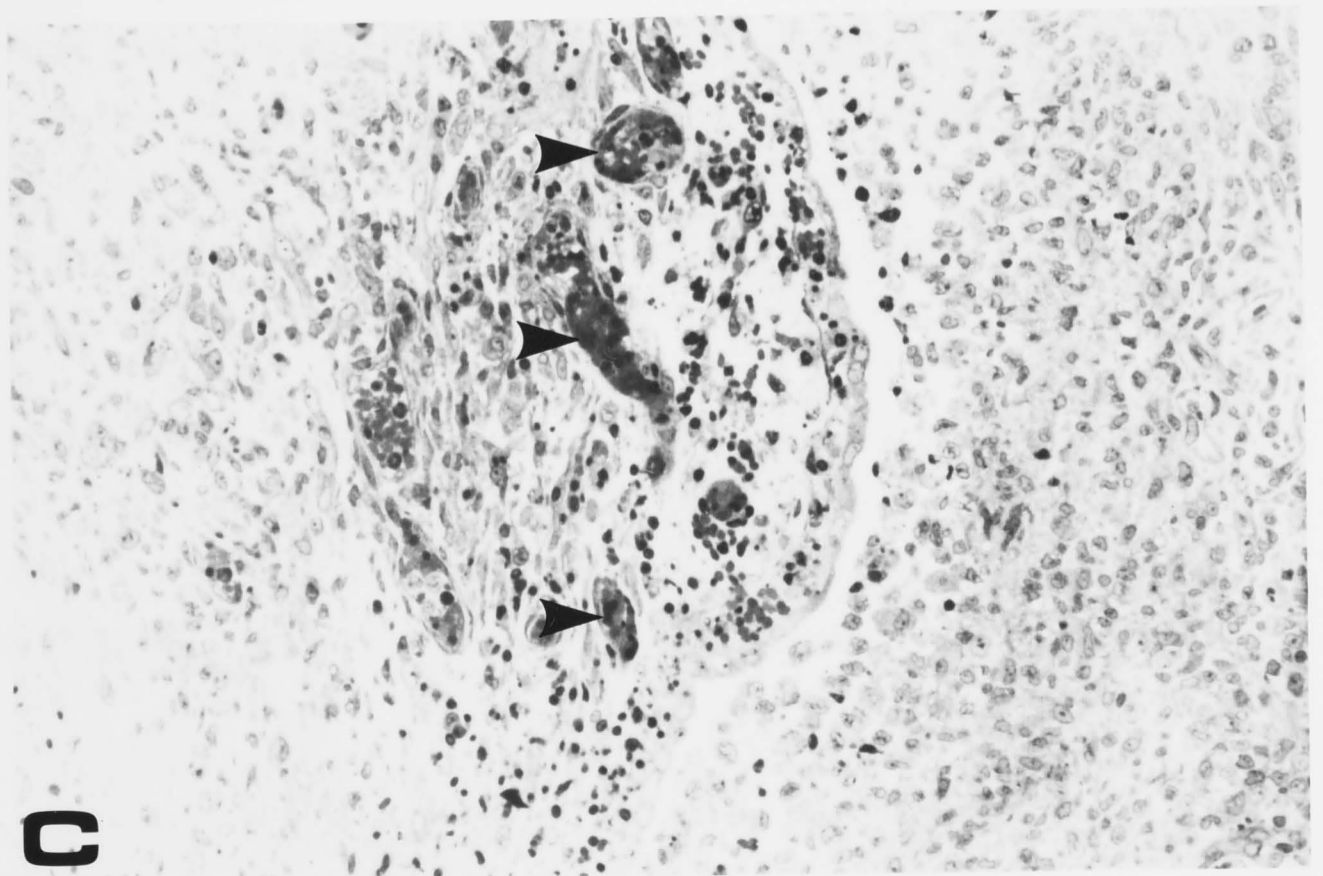
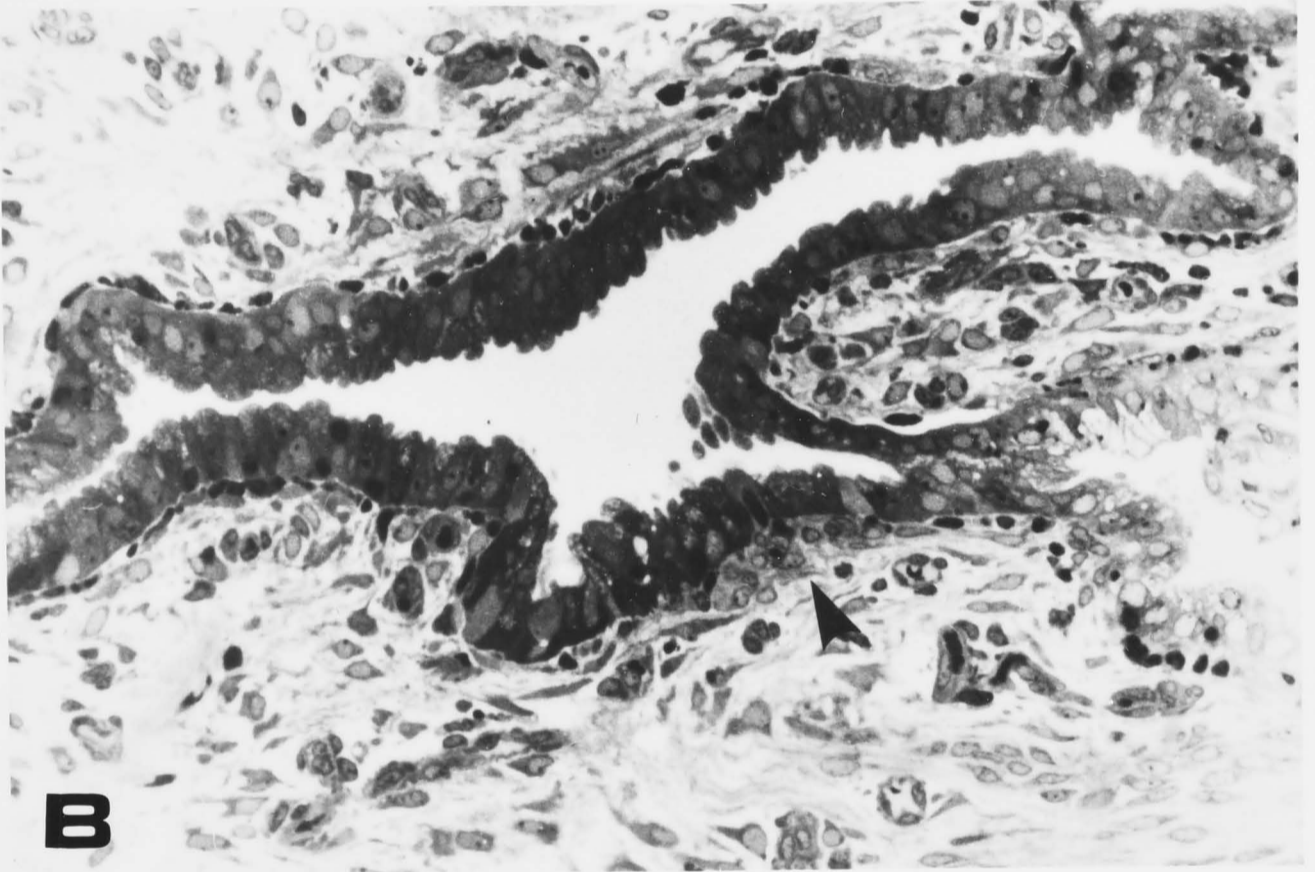
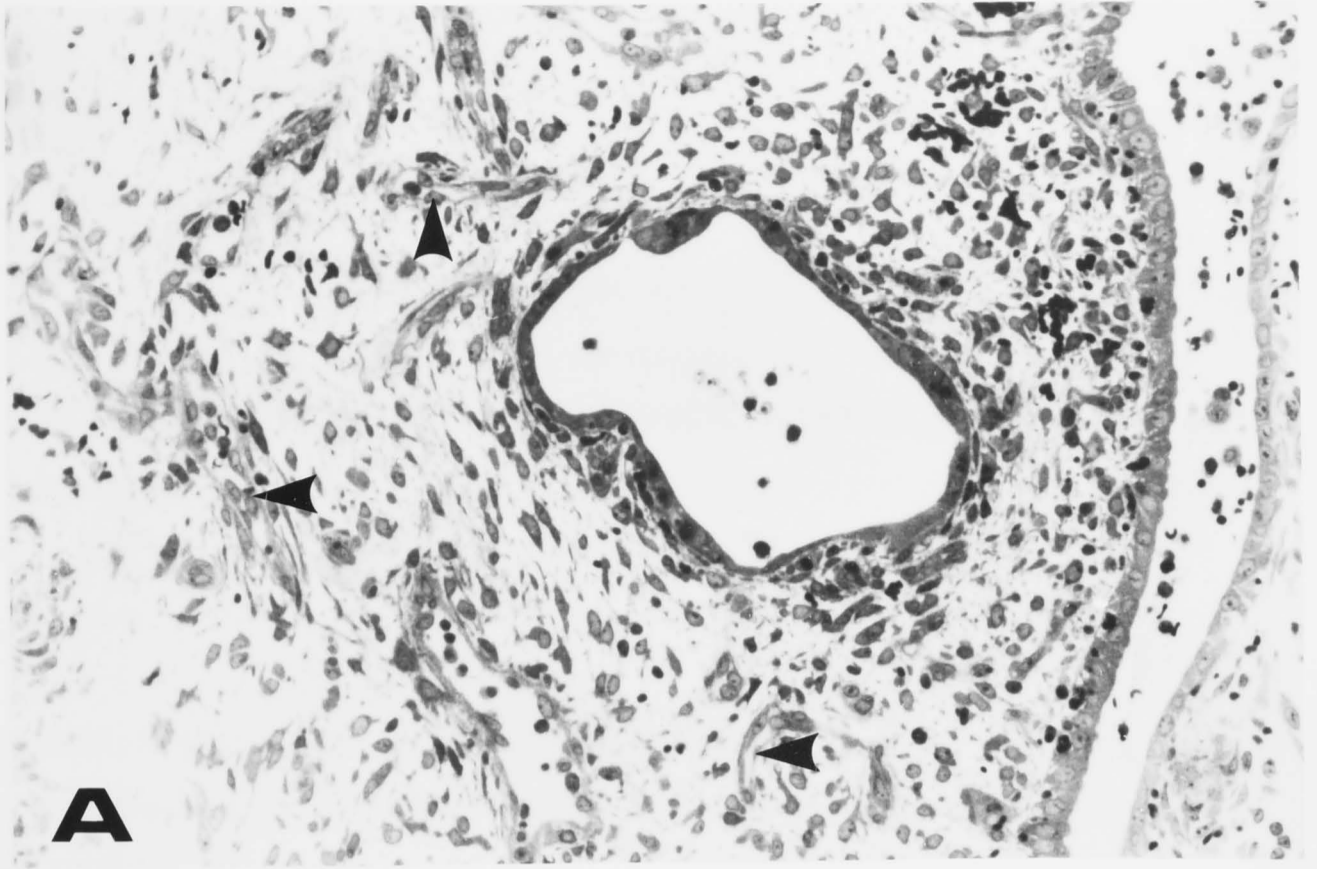


FIGURE IV-25

Endometrial grafts transplanted subcutaneously in rabbits' ears. Light micrographs of 1 μ m. epon sections.

- A. An allograft 7 days after transplantation. A large cyst forming at the surface of the graft is at the right margin of the field and a smaller cyst present in the lamina propria is near the centre of the figure. The epithelium of the cysts is mainly cuboidal. The lamina propria contains numerous stromal fibroblasts and thin walled vessels (arrowheads). Extravasated erythrocytes are present in the lamina propria, between epithelial cells and in the lumina of the cysts. Leukocytic infiltration is minimal. (X 290)
- B. An endometrial autograft 13 days after transplantation. The epithelium lining the cyst is tall and contains numerous secretory vacuoles. Polymorphonuclear leukocytes and a small accumulation of mononuclear leukocytes (arrowhead) lie immediately adjacent to the epithelium. Leukocytic infiltration of the surrounding lamina propria is minimal. (X 570)
- C. A rejecting endometrial allograft 12 days after transplantation. The epithelium of the cyst on the right side of the figure has been largely destroyed but adjacent host tissue appears undamaged. The epithelium on the side of the cyst nearest the centre of the figure is extremely attenuated. The lamina propria of the graft underlies this epithelium and is oedematous, haemorrhagic and contains degenerating cells. The blood vessels of the lamina propria are engorged and the endothelium of many has degenerated (arrowheads). The host tissue surrounding the allograft is heavily infiltrated by heterogeneous mononuclear cells. (X 220)



CHAPTER V

TRANSPLANTATION OF ENDOMETRIAL EPITHELIUM

INTRODUCTION

The rejection of enzymatically separated sheets of allogeneic epidermis has been studied in rabbits by BILLINGHAM and SPARROW (1954) and WIENER ET AL (1964). Using gross and histological examination, BILLINGHAM and SPARROW (1954) found that the rejection of allogeneic epidermis was accompanied by congestion and stagnation of blood in small vessels, extensive haemorrhage, eventual breakdown of host vessels and degeneration of leukocytes in the graft bed. The damage to the host's tissue led the authors to suggest that a delayed-type hypersensitivity reaction may have been involved in the rejection of the allogeneic epidermis. The possibility that ischaemia led to the degeneration of the tissues did not appear to have been excluded. WIENER ET AL (1964) using electron microscopy observed mononuclear leukocytes infiltrating the rejecting allogeneic epidermis. They observed no degenerative changes in the underlying connective tissue and the infiltrating mononuclear leukocytes remained intact until the final stages of the allogeneic cell destruction. These authors suggested that a specific cell-mediated immunological attack on the allogeneic epidermis had occurred. However, the authors did not report vascular damage during the rejection of full-thickness skin allografts in the rabbits used. This suggested that the rejection of both types of allograft may have been atypical (see Chapter I).

Because of the conflicting results of these previous studies, the rejection of epithelium separated from allogeneic endometrium was also examined in the ear chambers where in vivo evaluation of the blood supply could be related to light and electron microscopic

observations on the fixed tissues. Additionally, the possibility that degeneration of the epithelium during rejection was due to secondary effects other than ischaemia following damage to the donor lamina propria and blood vessels could be tested. It was also anticipated that more information on the mode of destruction of the epithelium would be obtained because resolution of the allogeneic cells in vivo was better than in endometrial grafts.

MATERIALS AND METHODS

The preparation and the examination of the grafts has been described in Chapters II and IV. By histological examination, the enzymatic digestion appeared to remove only the epithelium from the surface and from some of the glands of the endometrium indicating that contamination of the cell suspension with stromal fibroblasts and vascular endothelial cells was minimal. Contamination of the suspension with erythrocytes, and thus probably blood leukocytes, was variable. The experiments performed are shown in Table V-1. In each ear of the rabbits receiving subcutaneous grafts, 0.1 ml. of the suspension of either autogeneous or allogeneic epithelium was injected at each of 2 sites. Drops of the cell suspensions were placed on the membrane of the graft chambers or the suspension was injected over the membrane of injection chambers.

Samples for analysis by light microscopy were taken from injection chambers 1 and 5 days after the onset of rejection of allogeneic epithelium and were selected to cover the range of features observed in autogeneous and allogeneic follicles in vivo. The average length of epithelium examined per sample, including the full circumference of the follicles, was $970 \pm 620 \mu\text{m}$. ($\bar{X} \pm \text{SD}$).

RESULTS

Transplantation to subcutaneous sites

The results of the experiments performed in 2 rabbits are shown in

Table V-1. The time of rejection was taken as the first day that the estimated volumes of the allografts fell from their peak scores.

Transplantation to ear chambers

In vivo observations

Establishment of endometrial epithelium. Inflammation of the membrane of both graft and injection chambers following transplantation was manifest by increased rolling and sticking of leukocytes in blood vessels (up to scores of 3), occasional thrombo-emboli, increased numbers of free-floating leukocytes above the chamber membranes and increased numbers of leukocytes and erythrocytes in the lymphatics. Most of these features had returned to pre-transplantation levels by 7 to 9 days after transplantation. However, moderate rolling and sticking of leukocytes in vessels reappeared sporadically in some chambers and the increased numbers of free leukocytes above the chamber membranes persisted until termination of the experiments whether autogeneous or allogeneic epithelium had been transplanted. Lymphatics were present in all but one ear chamber membrane (597R).

On the day after transplantation, epithelial cells on the surface of the membranes could be identified by their large size, beating cilia and the presence of occasional large vacuoles. In the graft chambers, epithelium was not identified again until follicles began to appear at 5 days after transplantation. Autogeneous epithelium did not take in one graft chamber (597L, Table V-1). In the injection chambers, formation of follicles was occasionally observed by 1 day and was identified in almost all chambers by 3 days after transplantation. These early follicles were small and flattened, had no lumen or else a very small one, and occasional cells forming their walls were ciliated. As the follicles grew, their lumina enlarged while the lining epithelium remained predominantly cuboidal or columnar. Cilia were not observed beyond 3 days after transplantation. Fibrous tissue overgrew and formed a laminated border of variable width around the follicles (Figure V-1A).

Vascular sprouts derived from the membrane vessels grew around and over many of the follicles from 5 days after transplantation. The growth of the sprouts was accompanied by petechial haemorrhages and erythrocytes were also found in the lumina of some follicles.

Regression of a low number of the smaller follicles derived from either autogenous or allogeneic epithelium was noted in 4 chambers from 13 days after transplantation. Only a few small autogenous follicles formed in another graft chamber and they all regressed during the period of the experiment (600R, Table V-1). Regression was characterized by a slow, progressive reduction in the diameters of the follicles and their lumina but with maintenance of the height of the epithelium. Eventually the lumina of the follicles were obliterated, and the epithelial cells and laminar pattern of the surrounding fibrocytes disappeared. The majority of follicles of the autogenous epithelium persisted until the termination of the experiments; that is, up to 41 days after transplantation (Table V-2).

The rejection of allogeneic endometrial epithelium. A reduction in the size or number of follicles accompanied by increased cellular infiltration around or within the follicles was taken as evidence of the onset of rejection. The time of onset and a summary of the features of rejection are shown in Tables V-1 and -2.

As rejection progressed, the numbers of cells infiltrating around and within the follicles increased (Table V-2, Figure V-1B). Occasionally, moderate cellular infiltration of the epithelium was observed. A reduction in the height of the epithelium lining the follicles was often noted. This was followed by disruption of the epithelium so that the follicles appeared to contain only free cells and cell debris. Finally the follicles were represented by collections of granular material which was phagocytosed by macrophages. In one ear chamber all the allogeneic follicles were destroyed within 2

days of the onset of rejection but in the others allogeneic epithelium persisted up to 6 days after the onset of rejection (Table V-2). Destruction of individual follicles in the latter chambers occurred within 1 to 4 days of their first showing signs of rejection. As rejection progressed, the extent of damage to the allogeneic follicles in individual ear chamber membranes ranged from unaffected to complete destruction. Time-lapse cine movies of the allogeneic epithelium around the time of rejection and representing 6 hours real time were examined. Although resolution of the epithelial cells was better than when endometrial allografts were transplanted (see Chapter III), no additional information on the mode of cell death was obtained.

Slight increases in the average score for leukocyte rolling and sticking in vessels were associated with rejection of the allogeneic epithelium (Table V-2). Around the time of rejection, occasional thrombo-emboli were observed in the vessels of 2 of the ear chamber membranes transplanted with allogeneic epithelium. The rate of blood flow in the ear chamber membranes showed no consistent change in relation to the rejection of the allogeneic epithelium. A few small superficial vessels in the membranes showed stasis or stagnation of blood flow but there was little difference between membranes transplanted with autogenous or allogeneic epithelium. However, petechiae in the ear chamber membrane and haemorrhages into follicles were more common where allogeneic epithelium had been transplanted (Table V-2).

Light microscopic observations

The results of analysis by light microscopy are summarized in Table V-3. Interference with blood flow was not evident in vivo in the regions examined.

The leukocytes infiltrating the tissues were predominantly mononuclears irrespective of whether autogenous or allogeneic epithelium had been transplanted. The infiltration was most pronounced in the

region of the allogeneic epithelium and was much more intense at 5 days than at 1 day after the onset of rejection (Figure V-2). Small numbers of mononuclear leukocytes were distributed diffusely in the allogeneic epithelium but foci of up to 5 cells were sometimes observed (Figure V-2A). The lumina of some of the follicles with low epithelium and of those showing destruction of the epithelium were packed with mononuclear cells. The epithelium of other follicles was attenuated although cellular degeneration was not observed and the numbers of mononuclear leukocytes within the epithelium or their lumina were small. Alterations to blood vessels or their contents were not detected in the samples examined.

Electron microscopic observations

Autogenous endometrial epithelium. The autogenous epithelium was similar in appearance to that of the endometrial autografts (see Chapter IV) except that the rough endoplasmic reticulum showed greater dilation and secretory vacuoles had fused to form large spaces in some cells (Figure V-3). The basement membrane was often discontinuous. Fibrocytes surrounding the follicles were of the type found normally in the ear chamber membrane and they showed moderate degrees of hypertrophy. Some of these cells also showed moderate swelling of mitochondria. Infiltrating leukocytes included medium and large lymphocytes, macrophages, unidentified mononuclears, neutrophil and occasional eosinophil granulocytes.

Rejection of allogeneic endometrial epithelium. The allogeneic epithelium showed few secretory vacuoles and microvilli were stubby and sparse or absent (Figure V-4). Apoptotic bodies were only rarely observed. In damaged areas, a small proportion of the cells showed marked cytoplasmic condensation, dilation of perinuclear cisternae and, occasionally, numerous vacuoles in the cytoplasm. Swelling of the mitochondria was noted in epithelium with and without condensed cytoplasm (Figure V-4 and -5). More severely damaged cells showed marked clumping of nuclear chromatin, dilation

and disruption of cytoplasmic organelles and breakdown of the plasma membrane (Figure V-6). Most of the leukocytes infiltrating the epithelium were medium to large lymphocytes.

A low proportion of fibrocytes in the region of the epithelium showed degenerative changes at both 1 and 5 days after the onset of rejection. The changes included condensation of chromatin and dilation of perinuclear cisternae, rough endoplasmic reticulum and mitochondria (Figure V-6). The cells infiltrating the connective tissue in the region of the epithelium were predominantly small to medium mononuclear leukocytes some of which could be identified as lymphocytes. Macrophages with phagosomes containing cell debris or unrecognizable material formed only a small proportion of the infiltrating cells. Rarely a plasmablast was observed. The mononuclear leukocytes frequently formed long, simple contacts with the host fibrocytes.

DISCUSSION

Subcutaneous transplantation of the separated epithelium demonstrated that the allogeneic cells were promptly rejected and that the autogenous cells survived at this site (Table V-1). The rejection of the allogeneic cells occurred at times after transplantation that were comparable to the shortest times recorded for subcutaneous endometrial allografts (see Table III-1). It cannot be determined from the present data whether this rapid rejection was related to the amount of tissue transplanted or was a characteristic of the cell preparation.

The period of time to the onset of the rejection of the allogeneic epithelium in the ear chambers appeared to be related to the type of ear chamber used (Table V-1). The longer time period before rejection in the graft chambers compared to the injection chambers could not be related to the amount of allogeneic tissue that established in the respective chambers or to whether or not the rabbits had been

subjected to time-lapsing (Table V-1). It seems possible that the removal of the top cover as required during transplantation to the graft chambers may have led to this difference perhaps by damaging tissues in the ear chamber membrane. If this was the case it also may have contributed to the immunological privilege observed in graft chambers with respect to the spontaneous rejection of endometrial allografts (see Chapter III).

There was no evidence that stromal fibroblasts or vascular endothelium from endometrium became established in the ear chambers when the enzymatically separated cells were transplanted. Epithelial follicles were surrounded by fibrocytes of the type normally observed in the ear chamber membranes and vascular sprouts were seen to be derived from the host vessels. This indicates that changes in the allogeneic epithelium during rejection were not secondary to initial effects on donor vessels or stromal fibroblasts of the lamina propria. Additionally, the follicles were rejected in the absence of ischaemia. Thus the results indicate that the changes observed in the separated endometrial epithelium, and the epithelium of the endometrial allografts, were due to the direct effect of the host's reaction. The salient features of rejection of the separated allogeneic epithelium were similar to those observed during the rejection of endometrial allografts and included; (a) the epithelium became attenuated and lost specialized features before evidence of cell degeneration, (b) adjacent host tissues were damaged during rejection with greater haemorrhage in the membranes containing allogeneic epithelium indicating that vascular damage had occurred and with fibrocytes showing morphological evidence of injury, (c) the predominant form of cell degeneration was coagulative necrosis while apoptosis was rare, (d) the expression of rejection was highly focal as demonstrated by the destruction of some follicles while others appeared undamaged in the same chamber membrane.

The pattern of leukocytic infiltration and epithelial damage in some of the follicles examined 1 day after the onset of rejection (Figures V-2A, 4 and 5) suggested that lymphocytes had injured epithelial cells, perhaps as an expression of specific cell-mediated immunity. Such findings are in agreement with those of WIENER ET AL (1964). However, follicles showing overt destruction of epithelium were packed with mononuclear leukocytes and, at 5 days after the onset of rejection, intense infiltration of the connective tissue surrounding the follicles had also occurred (Figure V-2B). Additionally, the non-specificity of the reaction was demonstrated by damage to both host and donor tissues in the absence of ischaemia. These findings support the conclusions of BILLINGHAM and SPARROW (1954) that a delayed-type hypersensitivity reaction led to the destruction of the allogeneic epithelium during acute rejection. Thus, as in endometrial allografts (see Chapter III and IV), the results suggest that the allogeneic cells may be damaged by specific cell-mediated mechanisms but that the predominant mechanism leading to the destruction of the allografts was a delayed-type hypersensitivity reaction.

-
- 1: SC = subcutaneous, MGC = modified graft chamber, IC = injection chamber.
 - 2: Cell numbers refer to viable nucleated cells.
 - 3: auto = autogenous, allo = allogeneic.
 - 4: The time after transplantation that rejection was first detected (see text).
 - 5: L & R = left and right ears respectively.
 - 6: A hernia through the abdominal wound necessitated a partial enterectomy two days after transplantation.
 - 7: The grafts were time-factored before and/or during rejection.

TABLE V - 1

Transplantation of epithelial cells separated from endometrium.

Rabbit Identific- ation	Transplant- ation site ¹	Cell concentration in suspensions ² (no/0.1ml)	Origin of cells ³	Rejection (days) ⁴	Fate of cells grafted
612 R ⁵	SC	1x10 ⁶	auto	-	Good take.
L	SC	1x10 ⁶	allo	11	Good take, complete rejection.
613 R	SC	1x10 ⁶	auto	-	Good take.
L	SC	1x10 ⁶	allo	9	Good take, complete rejection.
597 ⁶ L	MGC	0.1x10 ⁶	auto	-	Did not take.
R	MGC	2x10 ⁶	allo	34	Good take, complete rejection.
600 R	MGC	2.2x10 ⁶	auto	-	Poor take, all follicles regressed.
L	MGC	2x10 ⁶	allo	33 ⁷	Good take, complete rejection.
643 R	IC	1x10 ⁶	auto	-	Good take.
L	IC	1x10 ⁶	allo	13 ⁷	Good take, complete rejection.
609 R	IC	2x10 ⁶	auto	-	Good take, fixed at 19 days.
L	IC	0.7x10 ⁶	allo	18 ⁷	Good take, fixed at 19 days.
642 L	IC	1x10 ⁶	auto	-	Good take, fixed at 20 days.
R	IC	1x10 ⁶	allo	15 ⁷	Good take, fixed at 20 days.

1: SC = subcutaneous, MGC = modified graft chamber, IC = injection chamber.

2: Cell numbers refer to viable nucleated cells.

3: auto = autogenous, allo = allogeneic.

4: The time after transplantation that rejection was first detected (see text).

5: L & R = left and right ears respectively.

6: A hernia through the abdominal wound necessitated a partial enterectomy two days after transplantation.

7: The grafts were time-lapsed before and/or during rejection.

TABLE V - 2

In vivo observations in ear chambers on epithelial cells separated from endometrium.

		Days ¹						
		-4--3	-2--1	0	1-2	3-4	5-6	7-8
Persistence of epithelium	auto ²	4/4 ^{3,4}	4/4	3/3	4/4	3/3	2/2	1/2
	allo	5/5	5/5	4/5	4/5	3/4	3/3	0/3
Cellular infiltration associated with epithelium								
around follicles	auto	0/4	1/4	1/3	1/4	1/3	1/2	NA ⁵
	allo	1/5	2/5	4/4	4/4	3/3	3/3	NA
in lumina of follicles	auto	1/4	1/4	1/3	0/4	0/3	0/2	NA
	allo	0/5	1/5	2/4	4/4	3/3	3/3	NA
Average score of leukocyte rolling and sticking in membrane vessels	auto	2 (4) ⁶	2 (4)	1.7 (3)	1.8 (4)	2 (3)	2.5 (2)	2 (2)
	allo	2.2 (5)	2.6 (5)	2.6 (5)	2.5 (5)	2.4 (4)	2 (3)	2 (3)
Haemorrhage in membrane or follicles	auto	1/4	2/4	1/3	2/4	1/3	0/2	1/2
	allo	5/5	5/5	4/5	4/5	4/4	3/3	3/3

1: Day 0 = day that evidence of rejection was first noted.

2: auto = autograft, allo = allograft.

3: number of chambers with feature
number of chambers examined4: Transplantation of autogenic epithelium to one chamber was unsuccessful.
Follicles in another chamber regressed after an initial poor take.

5: NA = not applicable.

6: () = number of ear chambers examined.

TABLE V - 3

Light microscopic observations on epithelial cells separated from endometrium and transplanted to ear chambers after the onset of rejection of the allogeneic epithelial cells.

	Epithelium					Connective tissue ¹		
	Height (μm)	Secretory vacuoles (1-5) ²	Degenerate epithelium %	Mononuclear leukocytes (no/mm)	Polymorpho- nuclear leukocytes (no/mm)	Phagosomes (no/mm)	Degenerate cells (1-5) ²	Mononuclear leukocytes (1-5) ²
auto ³ n=5, r=2 ⁴	11.8 ⁵ (9-14.1)	2.1 (1-3)	0 (-)	0.2 (0-1)	0 (-)	3 (0-11)	1 (-)	1.4 (1-2)
allo n=5, r=2	5 (3.4-6.2)	1 (-)	3.7 (0-19)	10 (2-20)	0.4 (0-1)	2 (0-3)	1.4 (1-2)	3 (2-4)

1: Connective tissue surrounding epithelial follicles.

2: Semiquantitative score (see Materials and Methods).

3: auto = autogenous, allo = allogeneic.

4: n = number of samples, r = number of rabbits.

5: Average and () range.

FIGURE V-1

In vivo light micrographs of endometrial epithelium transplanted to ear chambers.

- A. Autogenous epithelium. A follicle is situated at the centre of the field and is lined by relatively tall epithelium. Its lumen contains several leukocytes. The fibrous tissue surrounding the follicle has a laminated appearance and contains a small number of refractile granules. (X 530)
- B. Allogeneic epithelium. One day after the onset of rejection (see text). The epithelium lining the follicle is low. Macrophages and smaller leukocytes are moderately numerous in the lumen of the follicle. The fibrous tissue surrounding the follicle is heavily infiltrated by leukocytes. (X 420)

FIGURE V-2

Allogeneic endometrial epithelium transplanted to ear chambers. Light micrographs of 1 μ m. epon sections.

- A. One day after the onset of rejection. Much of the epithelium of the follicle closest to the chamber membrane (m) has been destroyed and its lumen is filled with large mononuclear leukocytes and cell debris (open arrow). Large mononuclear leukocytes also invade the epithelium of follicles towards the right of the field (arrowheads) and are present in the connective tissue surrounding the follicles. Extravasated erythrocytes are also present in the connective tissue and between the epithelial cells. Erythrocytes fill the lumen of a follicle in the upper portion of the field (solid arrow). (X 530)

FIGURE V-1

In vivo light micrographs of subcutaneous epithelium transplanted by ear windows.

- A. Autogenous epithelium. A follicle is observed at the centre of the field and is lined by relatively tall epithelium. Its lumen contains several leukocytes. The fibrous tissue surrounding the follicle has a lobulated appearance and contains a small number of infiltrating leukocytes. (X 530)

- B. Allogeneic epithelium. One day after the onset of rejection (see text). The epithelium lining the follicle is low. Macrophages and smaller leukocytes are moderately numerous in the lumen of the follicle. The fibrous tissue surrounding the follicle is sparsely infiltrated by leukocytes. (X 420)

FIGURE V-2 continued.

FIGURE V-1

- B. Five days after the onset of rejection. The epithelial follicles (arrowheads) are surrounded by a dense infiltrate of heterogenous mononuclear leukocytes. Blood vessels in the chamber membrane (m) and in the new connective tissue appear to be intact. (X 530)

- A. The follicle shown in the previous panel (A) has been destroyed and its lumen is filled with large mononuclear leukocytes and cell debris (open arrow). Large mononuclear leukocytes also invade the epithelium of follicles towards the right of the field (arrowheads) and are present in the connective tissue surrounding the follicles. Extravasated erythrocytes are also present in the connective tissue and between the epithelial cells. Erythrocytes fill the lumen of a follicle in the upper portion of the field (solid arrow). (X 530)

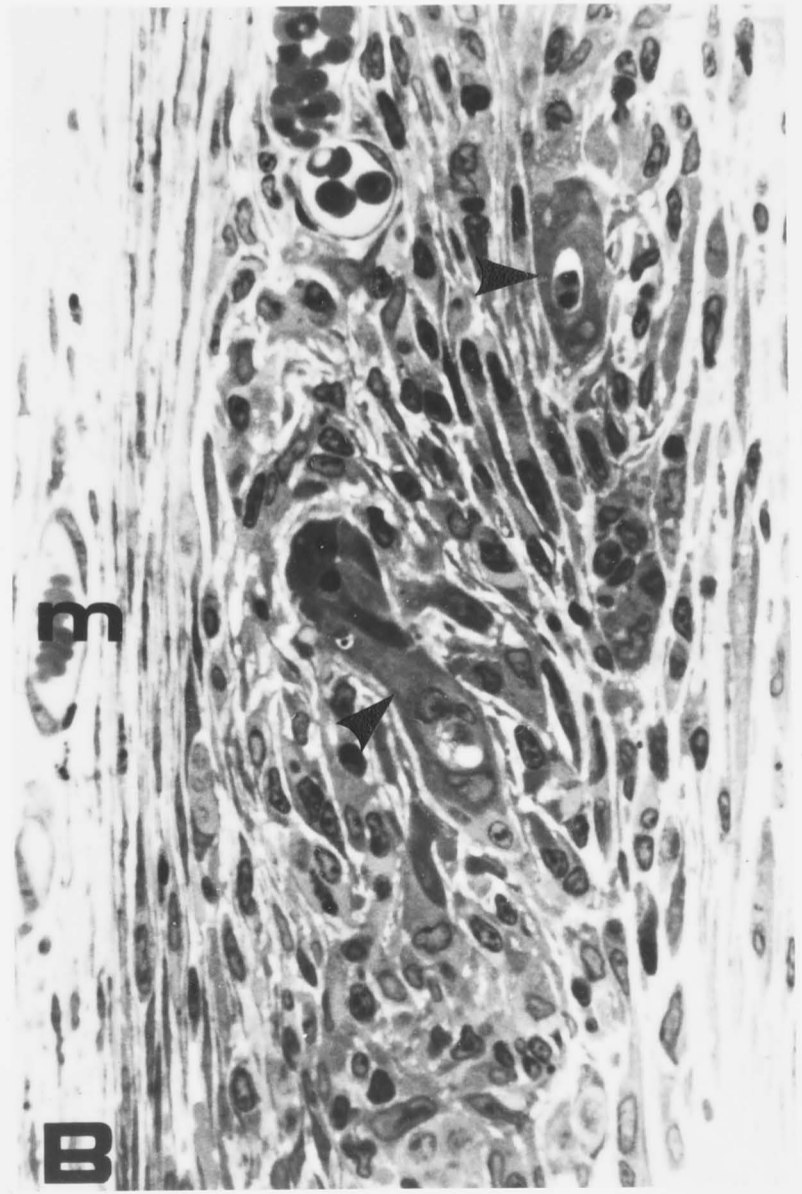
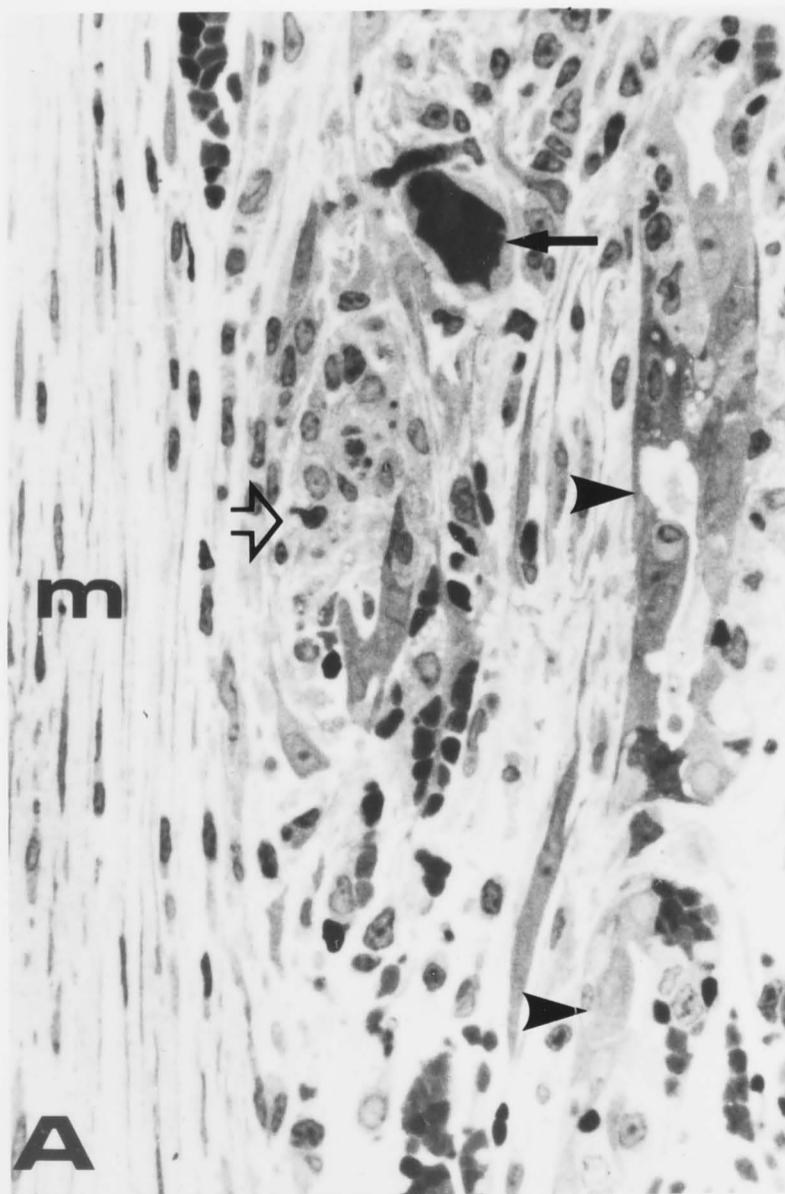
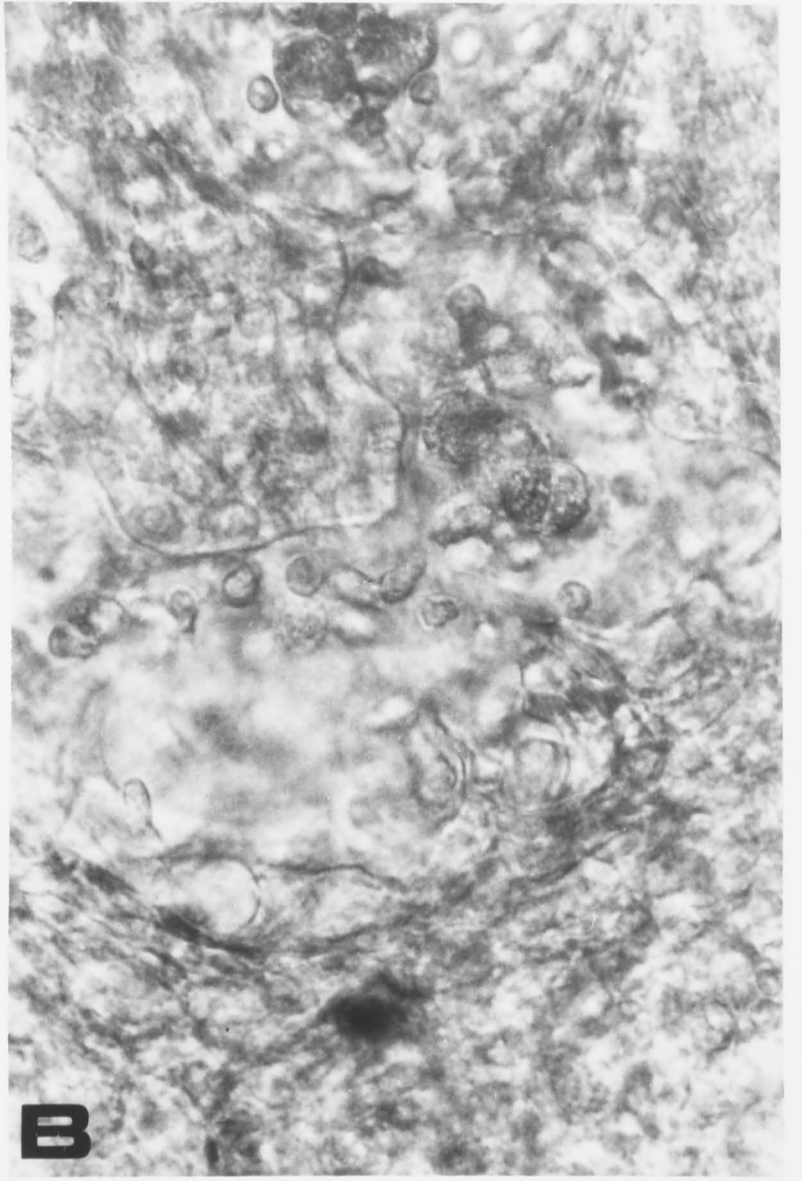
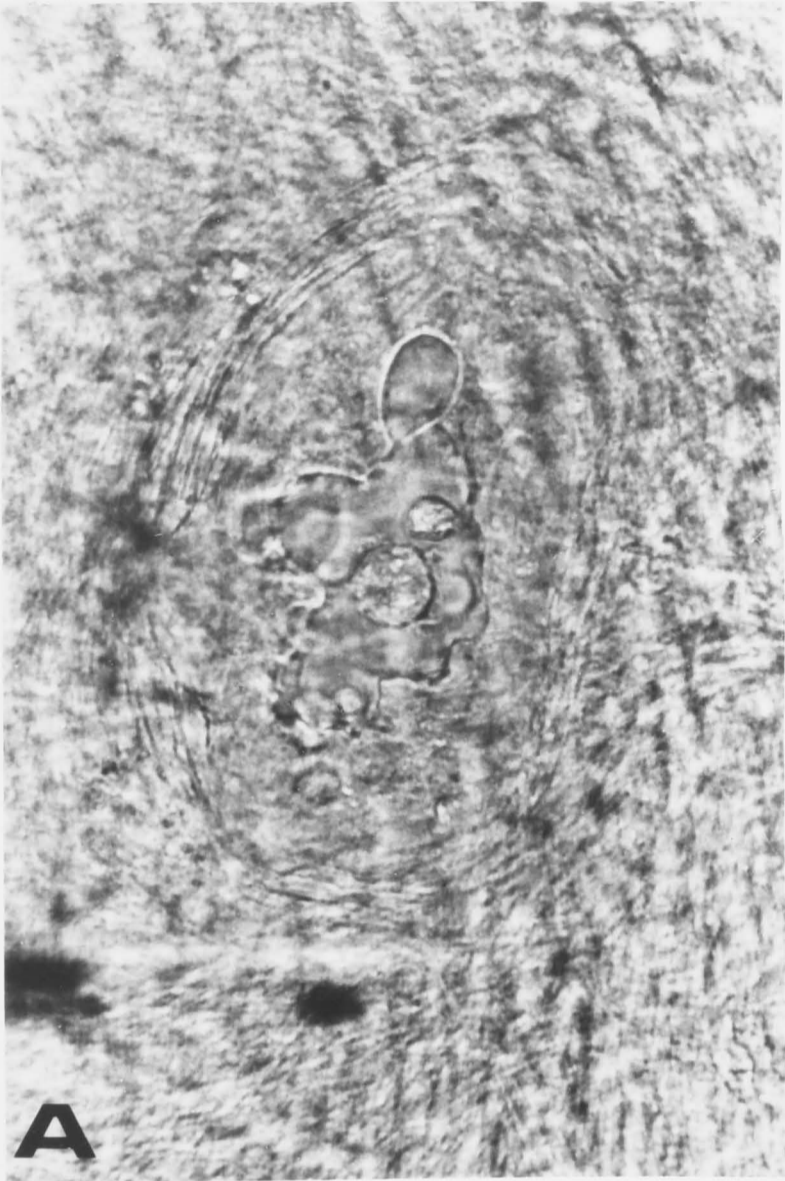


FIGURE V-3

Autogenous endometrial epithelium has formed closely packed follicles 20 days after transplantation into an ear chamber. Most of the epithelial cells are tall and have numerous microvilli. Secretory vacuoles are numerous and have coalesced in some cells. The rough endoplasmic reticulum in some of the epithelial cells is widely dilated. A basement membrane cannot be resolved. (X 3100)

FIGURE V-4

A follicle of allogeneic endometrial epithelium in an ear chamber one day after the onset of rejection. The walls of the follicle run from the upper middle to the lower right corner of the figure. The epithelium is low and has a patchy distribution of microvilli and few secretory vacuoles. An epithelial cell in the lower wall of the follicle shows condensation of the cytoplasm and the nucleus (*). A lymphocyte has invaded the epithelium (arrowhead) and erythrocytes lie between epithelial cells and in the lumen of the follicle. A mononuclear leukocyte is in contact with the abluminal surface of the epithelium (upper left corner) and several others are scattered in the fibrous tissue surrounding the epithelium. The epithelium has a discontinuous basement membrane. (X 3100)

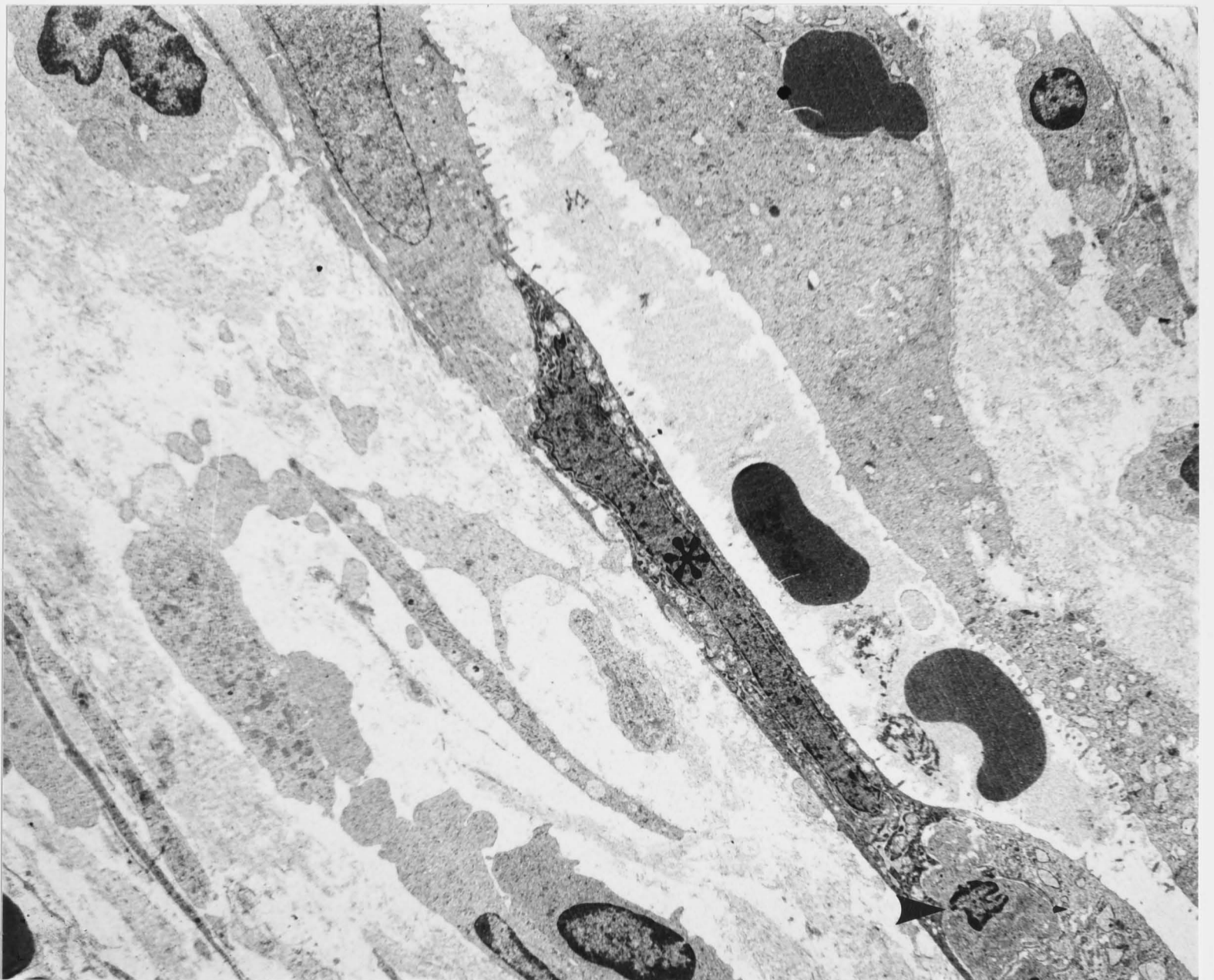
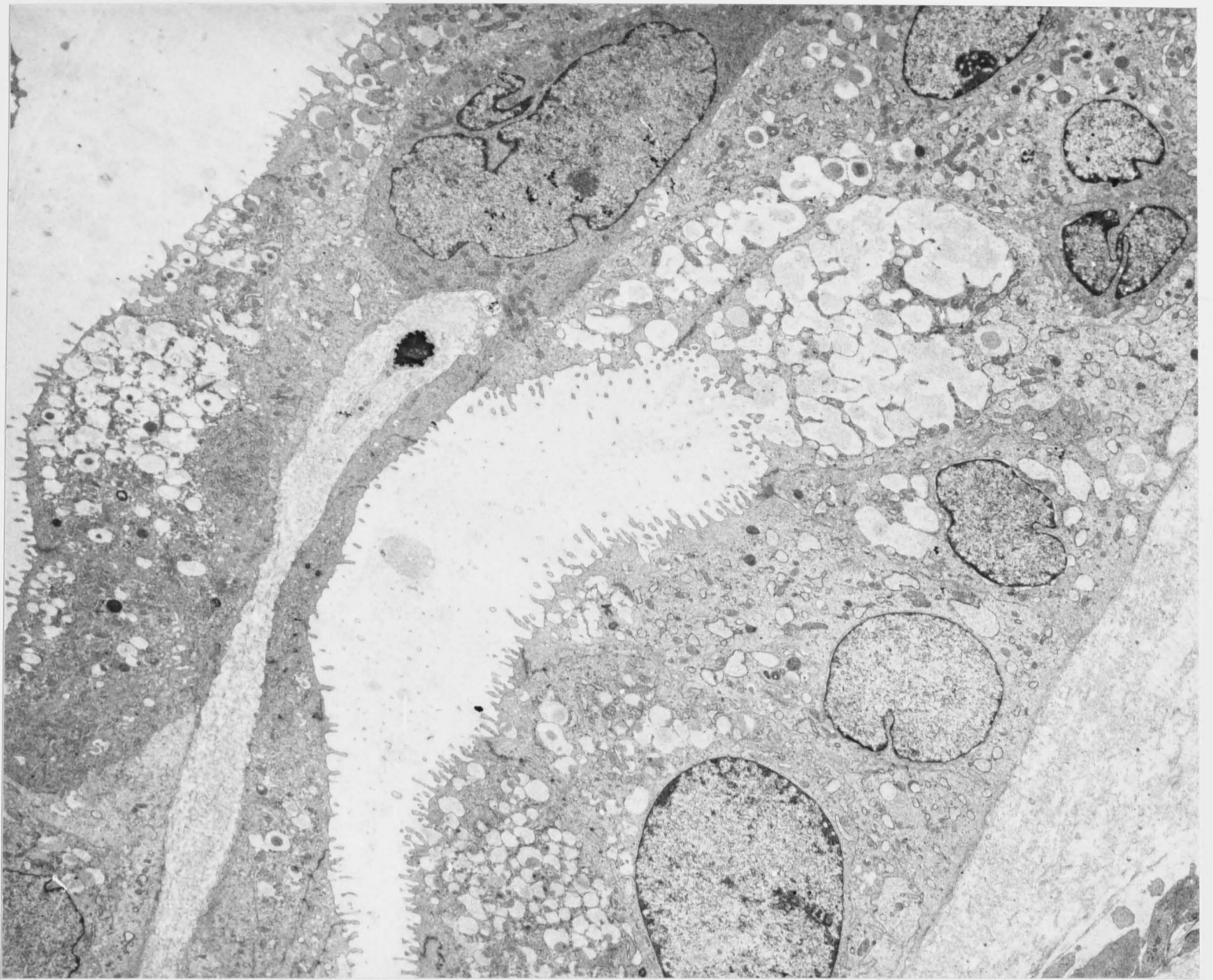
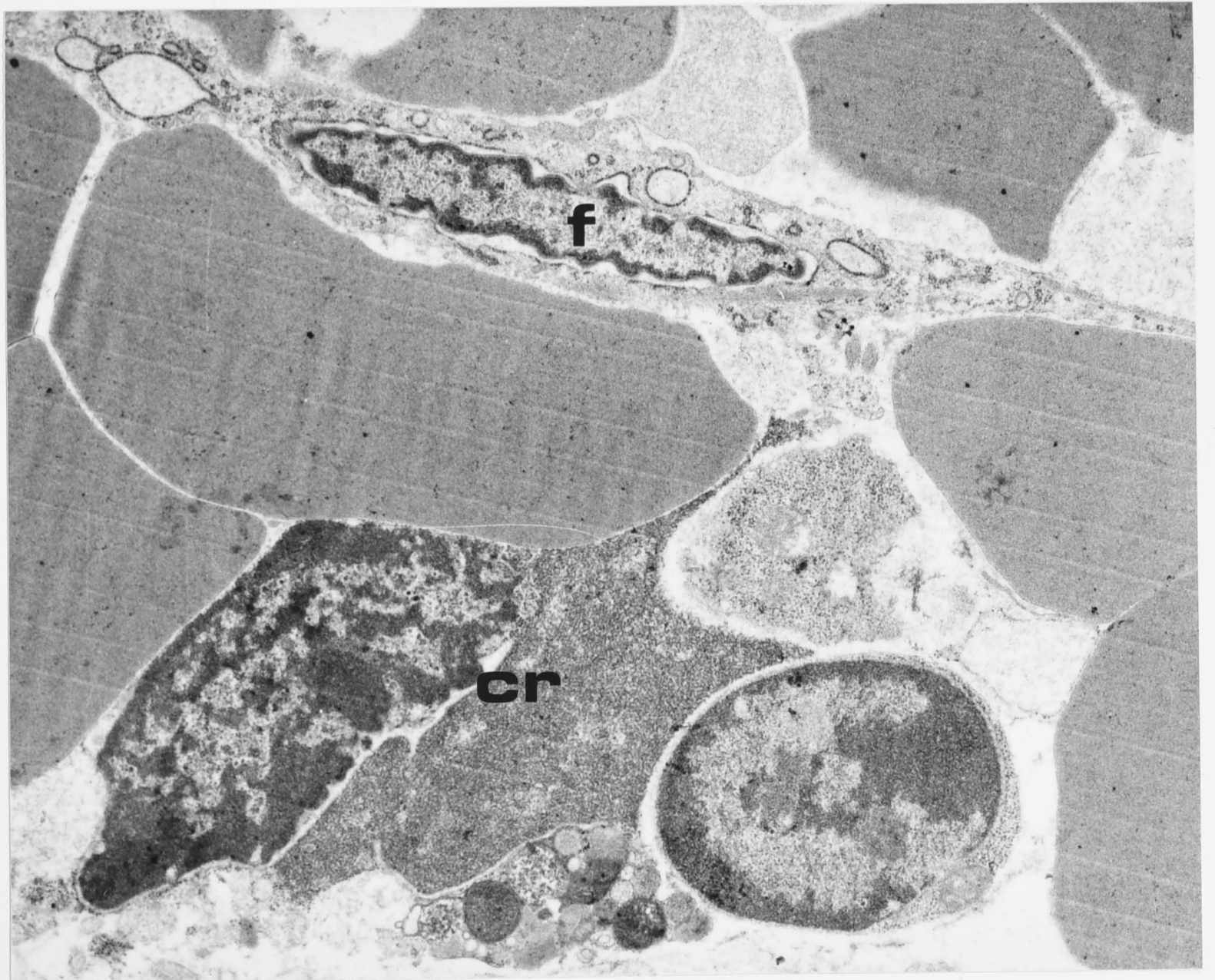
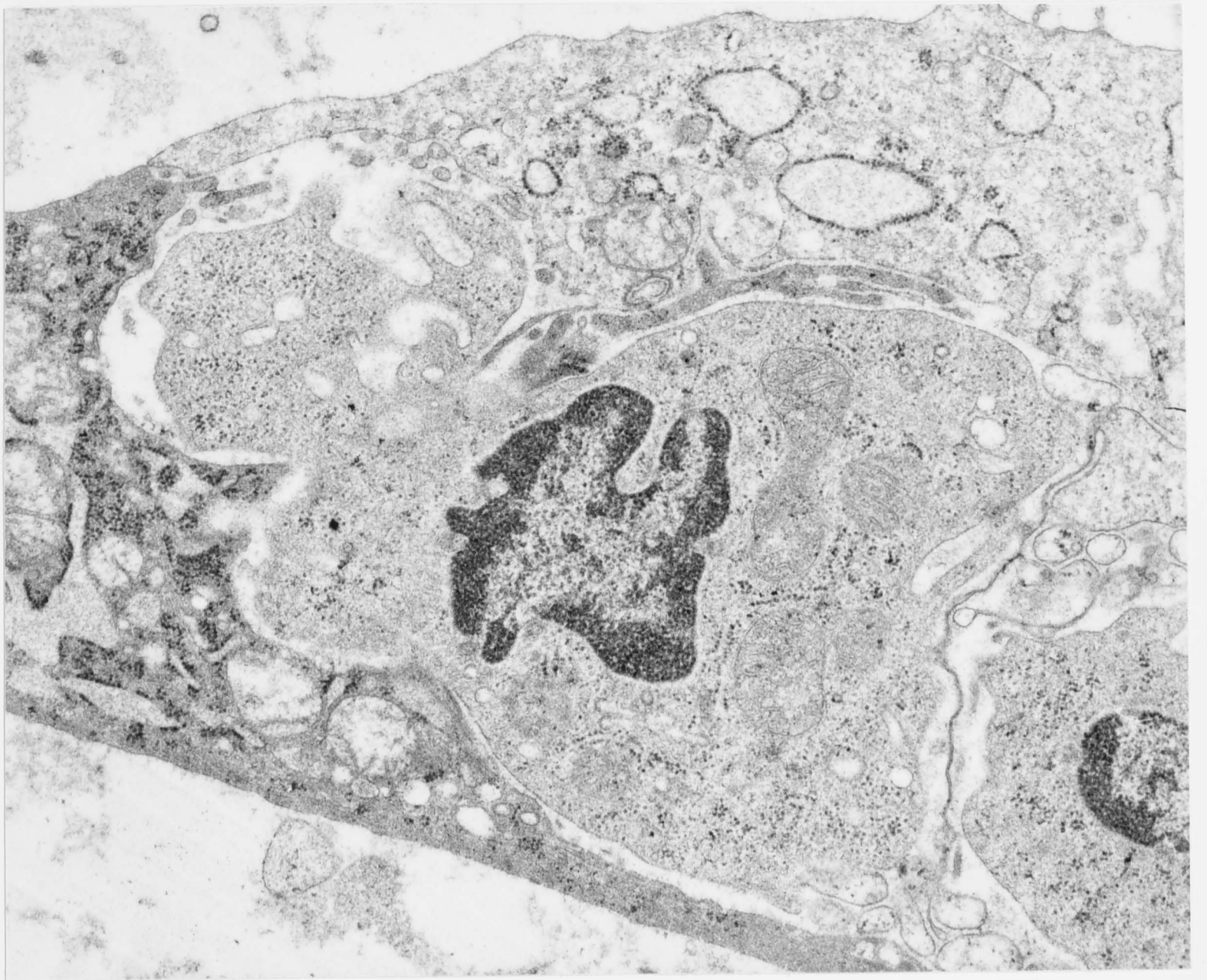


FIGURE V-5

High power detail of the lower right corner of Figure V-4. A large lymphocyte (centre) and a mononuclear leukocyte (lower right) have infiltrated the allogeneic epithelium. Mitochondria and rough endoplasmic reticulum of both epithelial cells are widely dilated. (X 23500)

FIGURE V-6

Tissue from a rabbit ear chamber transplanted with allogeneic endometrial epithelium one day after the onset of rejection. Degenerating cells in the field include a fibrocyte (f) and remnants of unknown cells (cr). The fibrocyte contains bundles of microfilaments and shows dilation of the perinuclear cisterna and of the rough endoplasmic reticulum. The cell remnants in the lower portion of the field show clumping or homogeneity of the nuclear chromatin and fragmentation of the cytoplasm. Numerous extravastated erythrocytes are included in the field. (X 14600)



CHAPTER VI

CONCLUSIONS AND FURTHER AVENUES OF RESEARCH

1. Variation in the size of endometrial allografts transplanted to subcutaneous sites in rabbits' ears influenced their survival time before rejection. Smaller allografts showed a higher probability of prolonged survival than did larger allografts.
2. Stress caused by procedures to which rabbits with ear chambers are often subjected; that is, restraint and time-lapse cine, did not have a significant effect on the survival time of the subcutaneous endometrial allografts.
3. In graft-type ear chambers, spontaneous rejection of endometrial allografts was significantly delayed in comparison to induced rejection. Rejection of the allografts whether it was spontaneous or induced was of the acute form.
4. The delay in the spontaneous rejection of endometrial allografts in graft-type rabbit ear chambers was due to immunological privilege in this site which arose mainly because of a defect in the efferent arm of the immune response. Further study of the characteristics of rabbit ear chambers is necessary to better understand the behaviour of tissues in this useful preparation. Such study, particularly in regard to the paradoxical extended survival of larger allografts, may also provide insights that would be useful in prolonging the survival of allografts in other situations.
5. The epithelium of endometrial allografts and enzymatically-separated allogeneic epithelium transplanted to ear chambers alone showed atrophic changes before overt cell degeneration and in the absence of ischaemia. The appearance of the tissue

affected in this way suggested that it was incapable of optimal function. Such changes may be of importance where the amount of tissue available for transplantation is limited; for example, in the transplantation of islets of Langerhans. Further work is required to elucidate the mechanism(s) by which these changes in the epithelium were brought about.

11. Further identification of the potential effector cells at sites

6. The destruction of the allogeneic endometrium or separated endometrial epithelium could not be attributed to ischaemia nor could the degeneration of the allogeneic endometrial epithelium be attributed to a secondary effect following destruction of endometrial blood vessels or stromal fibroblasts. The evidence indicates that the degeneration of the allografts was a direct effect of the immune response of the recipient on cells of all tissues of the allografts.
7. The initially focal damage in the endometrial allografts and the earlier onset of rejection of subcutaneous compared to chamber allografts in individual rabbits indicated that rejection was not due to humoral antibody acting at the blood-endothelium interface.
8. Electron microscopic observations provided support for the specific killing of allogeneic cells by lymphocytes and, possibly, by mononuclear phagocytes.
9. The predominant form of cellular degeneration in all tissues during rejection was coagulative necrosis while apoptosis or zeiosis was uncommon. This finding suggests that specific cell-mediated killing of allogeneic cells was not a prominent mechanism in the acute rejection of the allografts.
10. The degeneration of host leukocytes, vessels and connective tissue at the same time as the allogeneic cells indicated that a non-specific mechanism of cellular destruction predominated in the acute rejection of the allografts. Of the two non-specific mechanisms that have been postulated to be involved in acute

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11. Further identification of the potential effector cells at sites where tissue injury is actually occurring during acute allograft rejection would increase our understanding of the effector mechanisms mediating this reaction in vivo. This would be facilitated by combining the advantages of the transparent chamber technique with the identification of lymphocyte subsets and mononuclear phagocytes in inbred mice and rats. *Transplant.* 9: 9-26.
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