J. Anat., Lond. (1964), 98, 4, pp. 545–569
With 11 *plates and* 7 *text-figures*
Printed in Great Britain

Ultrastructural and haemodynamic studies in canine renal transplants

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INTRODUCTION

It has become well established in recent years that the transplantation of tissues or organs from one individual to another results in the quick destruction of the homograft, unless the individuals concerned are genetically identical, or, unless the recipient is modified by some previous treatment (Billingham, Brent & Medawar, 1953; Calne, 1963; Shackman, Dempster & Wrong, 1963; Küss, Legrain, Mathé, Nedey & Camey, 1963; Hamburger, Vaysse, Crosnier, Auvert, Lalanne & Dormont, 1962; Murray, Merrill, Dammin, Dealy, Alexandre & Harrison, 1962).

Mammalian renal homotransplants have often been used in investigations on homograft reactions and the experiments to be described here have been carried out on canine renal transplants.

Functional arrest in the homotransplanted kidney is commonly attributed to an immunological reaction, but little is known of the changes which result in oliguria. The demonstration of the 'second set' reaction (Medawar, 1944), by Dempster (1953*a*) using transplanted kidneys, has suggested that the rejection of kidneys is the result of an immune process akin to that which follows the introduction of bacterial antigens or other foreign material. During the succeeding years, considerable effort has been directed at identifying and isolating cytotoxic antibody from the serum of the host; but in the case of 'first set' recipients these investigations have proved uniformly negative (Simonsen, 1953; Fisher & Fisher, 1959). It has also been shown (Dempster, 1955) that the histological appearance of 'first set' renal transplants differs markedly from that of kidneys destroyed by autologous or heterologous antibody.

From this point two distinct theories have developed. Dempster (1953*b*, 1955) has examined a number of physical factors and can find none to explain the onset of oliguria. Total blood flow appears to be within the range compatible with the function of the normal kidney. In view of these results, Darmady, Dempster & Stranack (1955) suggest that anuria might be initiated by a metabolic abnormality of the renal tubule cell. The limited physiological data available on the renal homotransplant (Dempster, 1953*b*; Fedor, Lee & Fisher, 1959; Tyler, Lister-Cheese, Struthers & Dempster, 1962) have given no information on the pathological processes at work during the rejection process. Biochemical and histochemical

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studies (Tyler, Williams, Kountz & Dempster, 1964; Williams, Morton, Tyler & Dempster, 1964; Janigan, Williams, Tyler & Dempster, 1964; Tyler, Williams & Dempster, 1964) suggest that patterns of enzymatic changes in the transplant are ischaemic in origin. No changes in metabolic pathways have yet been observed.

Other workers in the field of renal transplantation (Hume, Jackson, Zukoski, Lee, Kauffman & Egdahl, 1960; Fowler & West, 1961; Calne, 1963) have taken the view that renal homotransplants are destroyed by host antibody borne to the graft by cells that infiltrate from the blood stream (Porter & Calne, 1960; Dempster & Williams, 1963). This suggestion is an extrapolation from the 'endoantibody theory' of Medawar (1958), evolved to explain the rejection of mouse skin homografts. The evidence obtained by Medawar and others has suggested that the 'active' cell is the small lymphocyte. Convincing evidence has been obtained for the release of antigen from the skin graft, and the appearance of 'activated' cells in the blood stream of the host (Scothorne & MacGregor, 1955; Billingham, Silvers & Wilson, 1962). Recently it has been suggested that such antigens consist of lipoprotein probably derived from cell membranes (Brent, Medawar & Ruskiewicz, 1962; Davies, 1962).

In the case of the canine renal transplant, the evidence is far less complete. The cells infiltrating the kidney appear to be mainly pyroninophilic cells of the plasma cell series (Dempster, 1953*b*, 1955). No positive evidence has been obtained for the release of antigen from 'first set' transplants (Dempster, Calnan & Kulatilake, 1963) and no serious attempts to chemically define such an antigen are on record.

Neither, in the case of skin nor kidney, has any information been put forward to explain how infiltrating cells effect the destruction of the donor tissue. It is clear therefore that more evidence will be required before the rejection of the renal transplant can be fitted into the pattern outlined for mouse skin grafts and a great deal more information is required concerning the relation between infiltrating cells and donor cells.

The present investigation has been designed with several points in mind. In view of the lack of evidence of metabolic changes, and the difficulties of assessing such changes in a tissue containing two populations of cells of changing proportions (Janigan *et al.* 1964), a means of obtaining further information on the integrity of renal tissue is required, particularly on glomeruli, proximal and distal convoluted tubules.

The histochemical evidence of ischaemic damage (Janigan *et al.* 1964) coupled with the finding of a total blood flow of 100–200 ml. per minute in oliguric homotransplants (Dempster, 1953*b*, *c*) suggests that useful information might be obtained from a combined physiological and anatomical investigation involving measurements of effective renal blood flow, or total venous flow, and inulin clearance, together with an anatomical examination of the renal vasculature using the electron microscope. In view of the variable survival period of renal homotransplants in the dog, it is very necessary to make serial measurements or observations on individual transplants. Previous blood flow measurements (Dempster, 1953*b*, *c*; Mims, 1961; Egdahl & Hume, 1956) have been mainly limited to single observations, a fact that has limited greatly the information obtained. The taking of material for electron microscopy has afforded an opportunity for further examination of the cells infiltrating the homotransplants; and a study has been made of the cell types present,

and their relationship to the various elements of the kidney tissue. The investigation resolved itself therefore into the taking of a series of physiological measurements during the life history of a number of autotransplants and homotransplants, together with serial anatomical surveys using the electron microscope and light microscope.

Evidence will be presented that the features of the rejection process of canine renal homotransplants stem from the primary involvement of the intertubular vascular endothelium. Endothelial changes occur in association with immature plasma cells of host origin and are accompanied by intertubular oedema and a progressive dissolution of the capillary walls. Dissolution of the capillaries is followed by the occupation of the enlarged intertubular spaces by oedema fluid, immature plasma cells closely associated with groups of rounded, isolated endothelial cells and by leucocytes, red cells and cell débris. An ischaemic necrosis of the proximal tubules supervenes and is accompanied by an intense phagocytosis.

MATERIAL AND METHODS

Physiological investigations

Two series of experiments were performed using healthy greyhound bitches weighing 22–25 kg.

Series I

Experiments were carried out on 34 dogs. They were anaesthetized with sodium pentobarbitone (30 mg./kg.) and supplemented as needed. Frequently, oxygen, or a mixture of oxygen and nitrous oxide, was administrated through an endotracheal tube with an automatic positive-pressure machine. Under fluoroscopic guidance, specially designed, double-lumen, nylon catheters were introduced into the inferior vena cava percutaneously via the right and left external jugular vein (Kountz, Dempster & Shillingford, unpublished).

The abdomen was entered under sterile conditions through a mid-line incision, using care to maintain good haemostasis. After ligation of the left ovarian vein, a catheter was placed into each renal vein. Blood flow was then measured by a constant local indicator-dilution method, which has been described elsewhere (Shillingford, Bruce & Gabe, 1962). This method depended on complete mixing of indicator and blood throughout the cross-section of the vessel before sampling. The indicator used was ^{131}I-albumin, which was injected at a constant known rate through a fine hole in the tip of the double lumen catheter and the diluted blood was simultaneously sampled 7–10 mm. downstream, through the other lumen. Flow in the renal vein was then calculated using the following formula:

$$F = f\left(\frac{C}{c} - 1\right),$$

where C was the concentration of the indicator injected, c was the concentration of the indicator in the sample, and f was the rate of indicator injection. This formula accounted for the volume of injectate.

Frequently it was difficult to get the catheter tip into the right renal vein. In these cases flow was measured above and below the level of the renal veins in the

inferior vena cava. Flow in the right renal vein was calculated from the differences in inferior vena caval flow above and below the renal veins, minus the left renal vein flow. The right kidney was then removed, and transplanted to the right iliac fossa of a recipient, in whom renal blood flows and identical procedures had been carried out. The technique of renal transplantation was that described by Dempster (1954) in which the renal artery was anastomosed end-to-side with the external iliac artery and the renal vein end-to-end with the common iliac vein and the ureter implanted into the bladder. Thirty to sixty minutes after restoring the circulation, a catheter was placed into the right common iliac vein. Blood flow was measured in both the transplanted and again in the normal left kidney, before and after closing the abdomen.

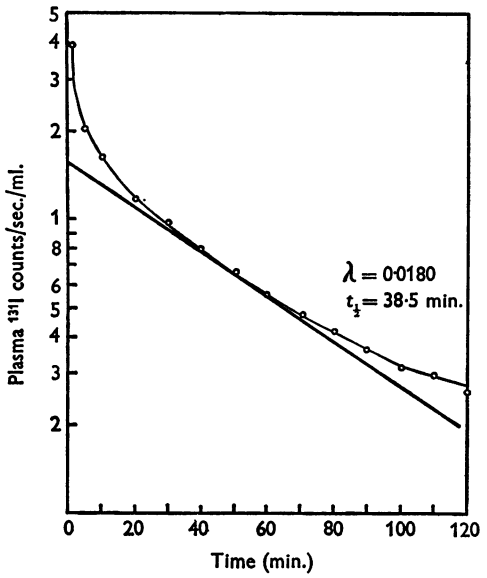
At various times following transplantation, the abdomen was re-opened and under fluoroscopic guidance the specially designed double-lumen catheters were placed into the inferior vena cava percutaneously via the external jugular veins and manually directed into the left renal vein and right common iliac vein. Due to enlargement of the transplanted kidney after 2–3 days, it was frequently difficult to get the catheter tip into the right common iliac vein. In these cases blood flow was measured in the left common iliac vein and inferior vena cava above the bifurcation and the difference taken as the flow in the transplanted kidney. Clinically the natural history of a renal homotransplant was divided into three phases—'functioning', 'oliguric' and 'anuric'. The functioning phase was characterized by the ability to maintain normal blood urea and electrolyte levels, although the ability to concentrate and acidify the urine was usually depressed. This phase varied in length, often from 5–8 days but extremes of 3–18 days were occasionally encountered. Oliguria was manifested by a sudden decrease in urine volume which was not reversed by successive intravenous infusions of 0·9% saline (500 ml.) and 2·0% saline (300 ml.). This state was accompanied by a high blood urea level and a 'toxic syndrome' (Dempster, 1953*d*). Oliguria was considered imminent when a sudden fall in the serum trypsin inhibitor level was observed (Tyler *et al.* 1962).

During placement of the catheters for blood flow measurements successive wedge biopsies were taken of the transplant cortex and fixed for subsequent electron microscopy. The first biopsy was taken from the upper pole, the second from the lower pole and the third midway between these points. The biopsy sites were closed with fine silk sutures. This procedure was followed to minimize the risk of infection and polymorphonuclear cell infiltration in the later biopsies. In four dogs the right kidney was transplanted to the right iliac fossa as an autotransplant. Renal blood flows and identical procedures as with the homotransplants were performed.

Series II

Because of the surgical hazards involved in serial renal venous flow measurements, alternative less traumatic experiments were performed on 6 greyhound bitches, which allowed daily estimates of the haemodynamic state throughout the life of the transplant. After bilateral nephrectomy a kidney was homotransplanted to the right iliac vessels, using the technique described above. A right nephrectomy was performed 1 week prior to left nephrectomy and homotransplantation. One or two days before homotransplantation and after right nephrectomy, estimations of the

daily 'effective renal blood flow' and '1 hr. percentage excretion' in the urine of a single intravenous dose of 20 μc. of ^{131}I-Hippuran and 4 μc. of ^{14}C-Inulin were started. The dogs, unanaesthetized, were hydrated with 500–1000 ml. water given by stomach tube, and placed in a restraining stand. A catheter was placed in the bladder for urine collection. 500 ml. 0·9% saline was given intravenously. Samples of 4–5 ml. of venous blood were drawn from a leg vein into heparinized syringes before injection of the radioactive materials and at 40, 50 and 60 min. after injection. An aliquot of the dose was taken for counting in a well counter for ^{131}I. The radioactivity in 1 ml. samples of the plasma was also assayed in a well counter for ^{131}I. The urine collected in one hour was measured and an aliquot prepared for counting in a well counter. ^{131}I and ^{14}C were also assayed simultaneously in a Tri-carb liquid scintillation counter, model 314 Ex. using a gel scintillator. An aliquot of 0·5 ml. of plasma, the dose, and urine were used for the assay. In several experiments blood



Text-fig. 1. Method of estimating 'effective renal blood flow' from the ^{131}I-Hippuran disappearance curve (for details see text).

samples were taken every 10 min. to better define the disappearance curve for ^{131}I-Hippuran. The data were analysed by the methods of Blaufox, Frohmuller, Campbell, Utz, Orvis & Owen (1963), i.e. the radioactivity in three samples of plasma (^{131}I) collected at 40, 50 and 60 min. after injection of the dose was plotted on semi-logarithmic paper. The points on the paper were then connected by the best-fitting straight line. The disappearance rate was calculated according to the formula of Newman, Bordley & Winterntz (1944) (Text-fig. 1). Thus in the example illustrated, the line was extrapolated to the value at time zero (C_0 = 16·0 counts/sec./ml.). Half time ($t_{\frac{1}{2}}$ = 38·5 min.) and the slope of the line (λ = 0·018/min.) were determined. The dose (271,322 counts/sec.) was divided by value at C_0 yielding the theoretical volume of distribution (V = 271,322/16). The clearance (C) then

$= V\lambda = 16{,}939 \times 0{\cdot}018 = 305$ ml./min. The percentage of the injected dose remaining after 1 hr. was obtained by dividing the ^{131}I in 1 ml. plasma at 60 min. (5·73 counts/sec.) by the ^{131}I in the dose (271,322 counts/sec.) $\times 100$ and multiplying this by the theoretical volume of distribution. Therefore, percentage remaining after 1 hr.

$$= \frac{^{131}\text{I in 1 ml. plasma at 60 min.} \times 100}{^{131}\text{I in dose}} \times \text{volume of distribution},$$

$$= \frac{5{\cdot}73 \times 100}{271{,}322} \times 16{,}939 = 35{\cdot}8\,\%.$$

The amount recovered in the urine in 1 hr. in this experiment was 67·1, giving a total percentage recovery of the injected dose of 102·9 %.

Electron microscopy

For electron microscopy, small wedges of cortical tissue were removed and fixed immediately in ice-cold, 1 % buffered osmium tetroxide. The specimens were dehydrated in graded alcohols, block-stained for 2 hr. in 1 % alcoholic phosphotungstic acid and embedded in Araldite. Ultrathin sections were cut, mounted on carbon-coated grids and micrographs taken using an R.C.A., E.M.U., 3E electron microscope.

Biopsies were taken from (*a*) normal kidneys; (*b*) autotransplants (4); (*c*) functioning homotransplants at 24 hr. (2), 48 hr. (1), 72 hr. (2), 96 hr. (1), 9 days (1); (*d*) oliguric homotransplants (6); and (*e*) one anuric homotransplant.

In each case, surveys were made concerning the ultrastructure of the glomeruli, the proximal and distal convuluted tubules and the intertubular vasculature. In the homotransplants, attempts were made to categorize morphologically the cells which came to occupy the intertubular spaces and to establish the vascular relationships of these cells. Because of temporal variations between dogs in the course of the rejection process, where practicable, successive biopsies were taken from the homotransplant during the functioning period at the time indicated.

Estimation of urinary enzymes

For this part of the investigation 10 normal dogs and 7 homotransplant recipients were used. Complete 24 hr. urine samples were not available. Enzyme activity was therefore expressed against creatinine values estimated on small samples. Creatinine was measured by the Folin method (King & Wootton, 1956). All enzyme activities were expressed per unit volume and corrected to a constant creatinine value of 1 g./l. Urine samples collected by catheterization were stored at $-20°$ C. until examined. After thawing the samples were dialysed against distilled water for 3 hr., the volume changes noted and appropriate corrections were applied. Alkaline phosphatase was assayed according to the 4-amino-antipyrine method (King & Wootton, 1956) and the activity expressed as King–Armstrong units. Leucine aminopeptidase (LAP) was assayed by the method of Goldbarg & Rutenberg (1958). Glutamic-oxaloacetic and glutamic-pyruvic transaminases (GOT and GPT) were assayed spectrophotometrically as described by Wilkinson (1962). Lactic

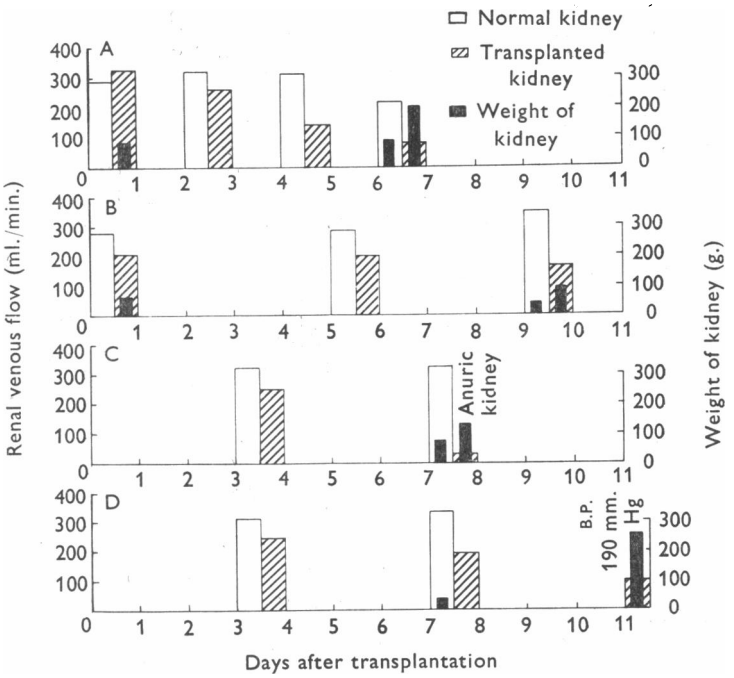
dehydrogenase (LDH) was assayed by the method of Wroblewski & LaDue (1955). Malic dehydrogenase (MDH) was assayed by the colorimetric procedure of King (1961).

RESULTS

Physiological observations

Series I

Renal venous flow estimations were carried out on 34 dogs. During the early stages of evolution of the technique it proved possible to make only a limited number of observations on each animal. Whilst the limited results from the earlier experi-



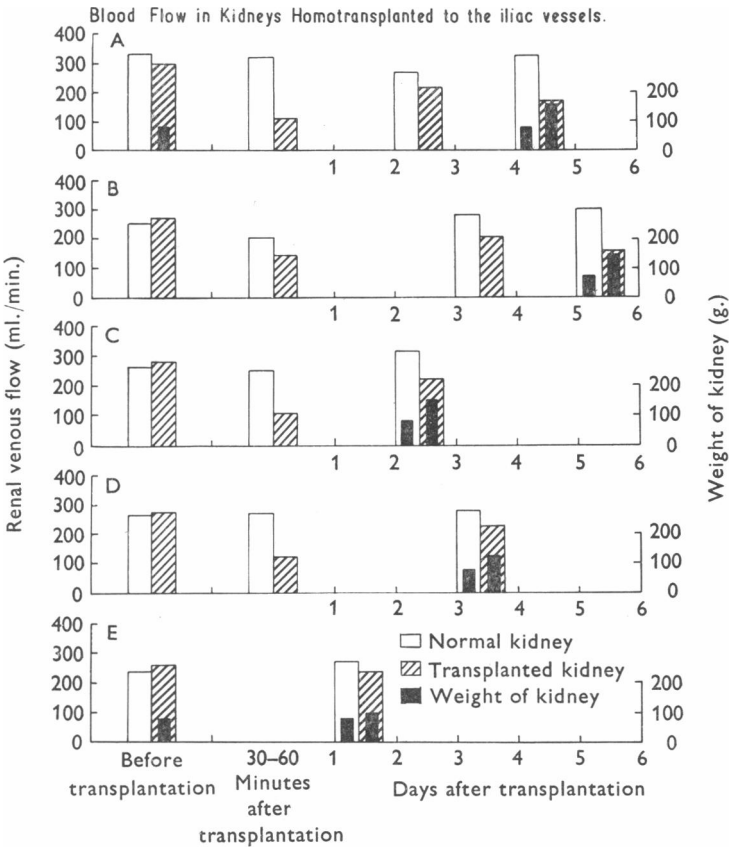
Text-fig. 2. Renal venous flow in dogs with a normal and homotransplanted kidney (see text).

ments in general supported later findings, the results reported here are based upon nine dogs on which a number of observations were made at various times up to oliguria. In Text-figs. 2 and 3, the renal venous flow estimations on these dogs, which bore a homotransplanted kidney whilst retaining a normal kidney, are shown. It is seen that there occurred a post-transplantation decrease in venous flow observed 30–60 min. after re-establishing the circulation. Twenty-four hours after transplantation flow returned to near normal. After 24–72 hr. there was a progressive fall in venous flow of the homotransplant until oliguria, after which it rapidly ceased. At oliguria, flow was frequently about one-third normal and the transplanted

kidney had more than doubled its weight. In one kidney that was anuric for only a few hours, venous flow was only 22 ml./minute. In some cases an increase in flow to the normal kidney was observed.

Series II

In Text-figs. 4 and 5, and Table 1, typical experiments are presented, showing changes in the daily 'effective renal blood flow' and '1 hr. percentage excretion' in the urine of a single intravenous dose of 20 μc. of ^{131}I-Hippuran and 4 μc. of

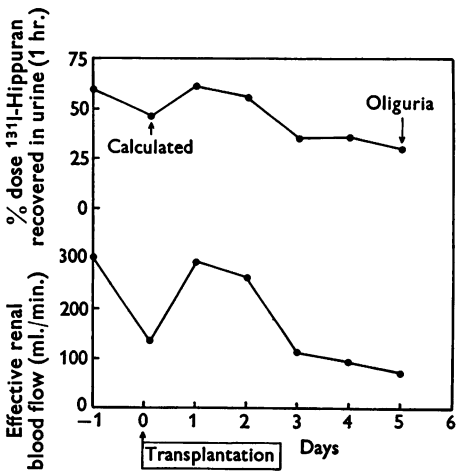


Text-fig. 3. Renal venous flow in dogs with a normal and homotransplanted kidney (see text).

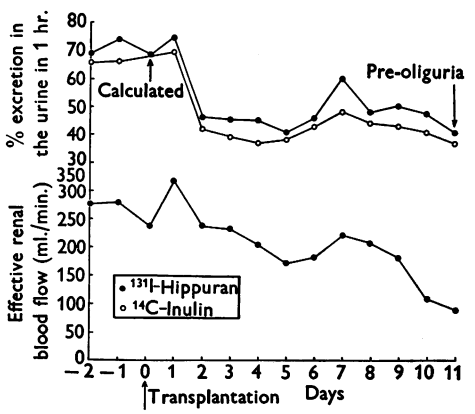
^{14}C-Inulin in two dogs bearing only homotransplanted kidneys. In general, two types of kidneys were observed: kidneys that were rejected rapidly (e.g. Text-fig. 4) and kidneys that were rejected more slowly (e.g. Text-fig. 5). The operative ischaemia during transfer resulted in a post-transplantation decrease in flow. This could be minimized by giving the low molecular weight dextran (Rheomacrodex), as was done in the dog shown in Text-fig. 5. In every case flow had returned to normal within 24 hr., after which it began to decrease. In the kidney that was rejected

Table 1. *Balance sheet showing the percentage of the daily injected intravenous dose of 20 μc. of ^{131}I-Hippuran accounted for after one hour in the dog shown in Text-fig. 5*

Days ...	−2	−1	0	1	2	3	4	5	6	7	8	9	10	11
Calculated % of the injected dose remaining after 1 hr.	35·8	23·6	28·6	24·3	50·4	53·1	51·9	54·3	50·1	34·8	48·4	44·1	53·2	54·6
Measured % of injected dose excreted in the urine in 1 hr.	67·1	73·0	—	74·0	45·8	44·0	44·0	41·8	45·4	60·4	47·2	50·3	47·1	41·2
Total	102·9	96·6	—	98·3	96·2	97·1	95·9	96·1	95·5	95·2	95·6	94·4	100·3	95·8



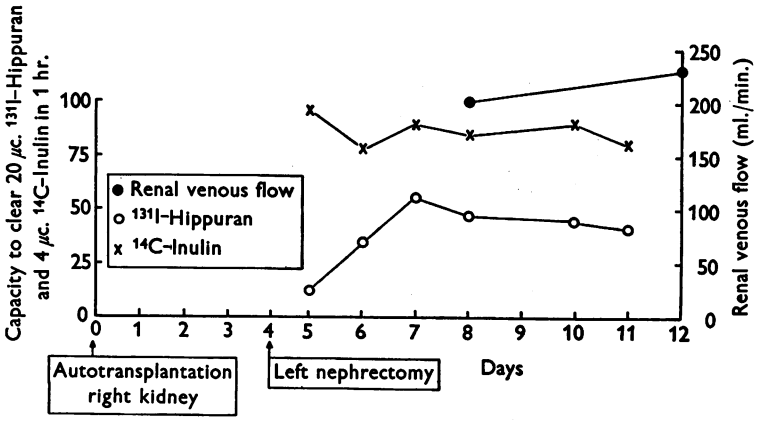
Text-fig. 4. Daily effective renal blood flows and 1 hr. percentage excretion in the urine of a single intravenous dose of ^{131}I-Hippuran in a dog bearing only a homotransplanted kidney that was rejected in a short time (see text).



Text-fig. 5. Daily effective renal blood flow and the simultaneous '1 hr. percentage' excretion in the urine of a single intravenous dose of ^{131}I-Hippuran and ^{14}C-Inulin in a dog bearing only a homotransplanted kidney that was rejected more slowly (see text).

rapidly, the fall in flow was rapid, but in the one rejected more slowly, the fall in flow was much slower. The '1 hr. percentage excretion' in the urine of the 20 μc. of ^{131}I-Hippuran and 4 μc. of ^{14}C-Inulin fell, but the magnitude of the fall was far less than falls in blood flows.

Autotransplants maintained good total venous flow, effective renal blood flow, as well as the ability to excrete a large percentage of a single intravenous dose of ^{131}I-Hippuran and ^{14}C-Inulin (Text-fig. 6). Although the amount of these substances excreted varied greatly from autotransplant to autotransplant, there was never a decrease. Occasionally, a decreased ability to excrete ^{131}I-Hippuran was observed 1–3 days after removal of the normal kidney (Text-fig. 6).



Text-fig. 6. Total renal blood flows and daily simultaneous '1 hr. percentage' excretions in the urine of a single intravenous dose of ^{131}I-Hippuran and ^{14}C-Inulin in a dog bearing only an autotransplant, immediately after removal of the left kidney.

Electron microscopic observations

Normal dog kidney

The ultrastructure of the glomeruli, proximal and distal convoluted tubules and intertubular vessels did not differ significantly from the accounts of the mammalian nephron given by previous workers (e.g. Sjostrand & Rhodin, 1954; Yamada, 1955; Pease & Baker, 1950; Pease, 1955*a*, *b*, *c*; Movat & Steiner, 1961) and it seemed unnecessary, therefore, to include a lengthy description of the normal ultrastructure of these regions in the present communication.

Autotransplants

Glomeruli. The capsular cells, podocytes, capillary endothelial cells, basement membranes, urinary spaces and capillary lumina appeared normal (Pl. 1, fig. 1).

Proximal convoluted tubules. Some tubules appeared normal whilst in others there was a slight, apparently continuous, non-progressive, shedding of the cytoplasm on the luminal aspect of some of the cells. Thus, broken microvilli, free mitochondria and rounded or irregular cytoplasmic masses were sometimes seen in the lumen of a tubule, often in the neighbourhood of a tubular cell which showed either a ragged, discontinuous, luminal aspect or a smooth membrane-bound luminal surface

devoid of microvilli (compare with Pl. 2, fig. 3). However, apart from some increase in the number of small vacuoles throughout the cell, the majority of the cells presented nuclei, basal infolding of the plasma membrane, numerous mitochondria and a basement membrane, all of which appeared normal.

The *intertubular capillaries* and *distal convoluted tubules* appeared normal but occasional distal tubules contained mitochondria and cytoplasmic masses free in the lumen presumably derived from 'shedding' proximal tubular cells and carried through the distal tubule in the urine.

Homotransplants

Glomeruli. At all stages (functioning, oliguric and even in an advanced anuric homotransplant) the capsular cells, podocytes, basement membranes and capillary endothelial cells were normal in appearance (Pl. 1, fig. 2). In one early (24 hr.) functioning homotransplant, a few free mitochondria were seen within the urinary space of otherwise normal glomeruli and it was thought likely that these had refluxed from the neighbouring proximal tubules.

Proximal convoluted tubules (Pl. 2, figs. 3–7). Throughout the functioning and most of the oliguric period many proximal tubular cells showed an increase in the number of small cytoplasmic vacuoles (Pl. 2, fig. 6) whilst some cells showed luminal shedding of cytoplasmic masses, free mitochondria and broken microvilli (Pl. 2, fig. 3). This process was qualitatively similar to, but more intense than, that occurring in autotransplants. This cytoplasmic shedding was observed in all the specimens studied. In a few instances the appearance could possibly have resulted from damage during removal of the specimen or during subsequent preparative techniques. However, for a number of reasons we regarded proximal tubular shedding as a constant feature of the transplanted kidney. Shedding was observed throughout the 'functioning' phase in all transplants studied and was never observed in normal controls. The phenomenon was always strictly localized to the proximal tubular cells—an affected tubule was often surrounded by morphologically normal tissues (e.g. glomerulus, distal tubule or collecting duct). Free mitochondria were seen within the lumina of otherwise normal distal tubules and collecting ducts. Finally, strong support for this view stemmed from the urinary enzyme assays reported below.

In all six oliguric homotransplants, large vacuoles appeared in the cytoplasm of the proximal tubular cells (Pl. 2, fig. 7). This vacuolation was variable—some tubules appeared relatively normal, others contained a few cells showing isolated large vacuoles whilst in yet others all the cells seen in a complete transverse section of the tubule were intensely vacuolated, with some of the vacuoles apparently coalescing. In the anuric homotransplant the vacuolation was even more advanced, the coalescence proceeding to complete necrosis of the tubular cells—'ghost' cells remaining consisting of a discontinuous cell membrane surrounding a few fine cytoplasmic strands indicating previous vacuolar boundaries and isolated extremely dense mitochondria (Pl. 11, fig. 34). During this terminal phase, the cell nucleus and most of the mitochondria could not be identified, but even at this advanced stage many of the proximal tubular basement membranes persisted intact (Pl. 10, fig. 32).

Distal convoluted tubules. Throughout the functioning and oliguric phases the basement membranes and cells of the distal tubules were normal in appearance

(Pl. 3, fig. 9) but some tubules contained free mitochondria and cytoplasmic masses within the lumen (presumably derived from shedding proximal tubular cells) (Pl. 3, figs. 8, 11). In the anuric homotransplant, the majority of the tubules were again normal but occasional cells showed marked vacuolation (Pl. 3, fig. 10).

Intertubular vessels and interstitium. In the normal kidney, the basement membranes of neighbouring proximal tubules occasionally approached each other closely, a few fine collagen fibres only intervening. Usually the space was more extensive and contained a capillary loop (Pl. 4, fig. 12) and where the tubules were cut approximately transversely, the capillary presented 1–3 endothelial cell profiles. External to the endothelial cells was a fine (200 Å. thick) amorphous, basement membrane which approached the tubular basement membrane closely, being separated only by a fine lamina of collagen fibres. The endothelial cell nucleus was typically elongated, often irregular in outline and presented characteristic coarse osmophilic masses along the inner aspect of its nuclear membrane. Opposite the nucleus, the cell body projected towards the lumen of the vessel (Pl. 5, fig. 18), and here the cytoplasm contained scattered mitochondria, many small vacuoles, free ribosome rosettes and an infrequent, flattened, ribosome-studded cisterna. Elsewhere the cytoplasm was attenuated and exhibited the 'fenestrations' which have been described in detail by Rhodin (1962). Sometimes terminal bars were observed between adjacent endothelial cells. It was in relation to these intertubular blood vessels that the most dramatic changes were observed in homotransplanted kidneys in the dog.

At 24 hr., most of the intertubular blood vessels appeared normal but close erythrocyte packing ('sludging') was observed in a few vessels.

The period 48–96 hr. was characterized by the appearance within and around the intertubular vessels of lymphocytes and plasma cells in various stages of maturity, and by the progressive dissolution of the attenuated parts of the endothelial capillary walls. Not all the intertubular vessels were affected simultaneously and the varying appearances have been placed in what we regard as a progressive series.

(*a*) Some vessels appeared normal, whilst others (still retaining an intact endothelial wall and basement membrane) showed wide separation between the basement membrane of the vessel and that of the adjacent tubule. This separation was regarded as indicative of marked intertubular oedema. Engorgement of the cortico-medullary lymphatic capillaries has been observed with the light microscope (Dempster, unpublished observations) but these could not be identified with certainty in the cortical specimens examined electron microscopically.

(*b*) The appearance of many small lymphocytes and an occasional immature plasma cell free within the lumen of an otherwise intact vessel.

(*c*) The close cell membrane apposition of immature plasma cells and endothelial cells. This was accompanied by the separation and rounding up of adjacent endothelial cells and the simultaneous extravascular appearance of lymphocytes and immature plasma cells. The various cell types seen during this process will now be described in more detail.

The *small lymphocytes* (Pl. 5, figs. 16, 17) were approximately 6–8 μ in diameter (nuclear diameter 5–6 μ), and contained a rounded nucleus which often showed a deep indentation on one side. The nucleus often contained a well marked nucleolus,

and in section the nuclear surface appeared relatively dense and homogeneous but less dense patches near the nuclear centre were not uncommon. The narrow rim of cytoplasm (0·5–1 μ thick) contained a number of quite large, well-formed mitochondria, occasional vacuoles, scattered ribosome rosettes and perhaps a single ribosome-studded cisterna but was otherwise devoid of organized ergastoplasm. The plasma membrane sometimes showed irregular microvillous projections. Such lymphocytes were seen within the lumina of intact vessels (sometimes in groups of 5–6, Pl. 4, fig. 15) or free in the interstitium in the neighbourhood of a vessel undergoing dissolution.

The plasma cell series. Previous electron microscopic investigations into the cell lineage of the plasma cell as seen in the lymph node (Thiéry, 1960; Bernhard & Granboulan, 1960) have suggested the following stages: fixed reticular cell, free reticular cell, pro-plasmoblast, pro-plasma cell and plasma cell. After the stage of the free reticular cell, the independence of the lymphocytic and plasmocytic lines is emphasized. The plasma cell progenitors are relatively large cells possessing an extensive cytoplasm (compared with lymphocytic precursors) and contain lamellar ergastoplasm in various stages or organization.

The terminology used by these authors was not wholly apposite in the present account. In homotransplants of dog kidney (72–96 hr.) a gradation of cell types with lamellar ergastoplasm was observed. These ranged from small round cells, the size of, and having the same nucleo-cytoplasmic ratio as small lymphocytes, through larger rounded cells to large stellate cells carrying extensive pseudopodia and finally to typical mature plasma cells.

The small rounded cells (e.g. Pl. 5, figs. 20, 21) were approximately 6–8 μ in overall diameter (nuclear diameter 4·5–5·5 μ) and their cut nuclear surfaces were like that of a small lymphocyte. The narrow cytoplasmic rim contained a few well-formed mitochondria and showed areas containing free ribosome rosettes. Elsewhere the cytoplasm contained well-marked ribosome encrusted cisternae, 1–3 cisternae existing between the nucleus and the cell surface. Other cells of this type often showed a well-developed nucleolus and occasionally a few microvillous extensions of the plasma membrane. Slightly larger rounded cells (e.g. Pl. 6, fig. 22) showed more electron translucent nuclei with a somewhat more extensive cytoplasm containing dilated ergastoplasmic cisternae. Cells were seen (e.g. Pl. 6, fig. 23) possessing a relatively large (7–8 μ) nucleus which was more homogeneous and electron translucent than those of the previous cells. The more extensive cytoplasm contained widely open ergastoplasmic cisternae and projected from the cell surface as short blunt pseudopodia between neighbouring rounded endothelial cells (*vide infra*).

The largest cells seen (e.g. Pl. 6, fig. 24) possessed large, round, homogeneous, electron-translucent nuclei with ergastoplasm-filled extensions which passed between and entered into intimate apposition with adjacent endothelial cells. During light microscopy such cells have been termed 'stellate cells' (Darmady *et al.* 1955).

Further stages of gradation were followed from the immature form (Pl. 6, fig. 25) until typical mature plasma cells with abundant widely open cisternae containing Russell bodies (Pl. 6, fig. 26) or extremely highly organized flattened cisternae (Pl. 6, fig. 27) were seen. These mature phases, however, were relatively infrequent

in homotransplants of 72–96 hr. but were more prevalent in biopsies taken from oliguric cases.

The small, rounded, plasma cell precursors were usually found lying free in the interstitium in company with small lymphocytes whereas the stellate cells were in close cell membrane apposition with endothelial cells. An occasional stellate cell was seen within the lumen of an intact intertubular vessel and presumably the earliest contacts were effected from the luminal aspect. Simultaneously, however, small lymphocytes and immature plasma cells appeared in the interstitium, either by migration between the endothelial cells of an otherwise intact vessel, or by release during the dissolution of the first intertubular vessels to be affected. At this time, cell membrane contacts were effected between immature (usually stellate) plasma cells and endothelial cells on either their luminal or external surface. In the latter case the capillary basement membrane was ruptured and elevated over the plasma cell. The first contacts were between discontinuous points scattered along the adjacent surfaces of the two cells often through small projections of plasma cell (Pl. 4, fig. 13) cytoplasm which reached and apparently expanded over the endothelial cell surface. More extensive contacts were soon established, the cells coming together over the distances of 3–6 μ (Pl. 4, fig. 14; Pl. 6, figs. 23, 24; Pl. 7, fig. 28). The contact area affected either the attenuated part, or the cell body of an endothelial cell. Thus a pseudopodium of a plasma cell spread as a relatively thin sheet in contact with a considerable area of the attenuated part of the endothelial cell. Sometimes two pseudopodia partially encircled the projecting endothelial cell body (Pl. 4, fig. 14). Often, when cell body contact occurred, the cell nuclei approached each other until relatively narrow rims (0·5–1 μ thick) of their respective cytoplasm remained between the nuclei (Pl. 8, fig. 29). Throughout the area of contact (Pl. 8, figs. 29, 30) the adjacent unit membranes of the cells ran an approximately parallel course with a 150–200 Å. gap between them. Over short distances this gap widened to 400–500 Å. units. Ergastoplasmic cisternae of the plasma cell were observed opening into this gap. At a few points it was impossible to resolve the unit membranes of the cells and whilst this appearance might have been a preparative artefact it was possible that cytoplasmic continuity may have existed between the cells at these points.

During contact, the attenuated part of the endothelial cell broke at multiple points and the parent cell body rounded up and showed an increase in the amount of perinuclear cytoplasm—perhaps due in part to a withdrawal towards the nucleus of some of the outlying regions of the cell. Such rounded cells—usually in contact with an immature plasma cell of stellate form at some point—often showed their endothelial origin by the persistence of a tag of typical attenuated cytoplasm (sometimes with remnants of the original capillary basement membrane nearby). The adjacent nuclei and cytoplasms of plasma cell and endothelial cell stood in sharp contrast (Pl. 8, figs. 29, 30). The immature plasma cell nucleus, often perfectly circular in profile and relatively electron translucent, was covered with a fine homogeneous osmophilic 'stippling' which was rather more pronounced along the inner aspect of the nuclear membrane. Its cytoplasm was everywhere full of partially dilated granular ergastoplasmic cisternae, between which lay innumerable free ribosome rosettes and an occasional mitochondrion. In contrast, the endothelial

cell nucleus was either rounded or elongated but always irregular in outline; it was relatively much more dense and contained large irregular osmiophilic masses, scattered along the nuclear membrane. Its now relatively voluminous perinuclear cytoplasm contained isolated flattened granular cisternae (not a prominent feature), some free ribosome rosettes, many vacuoles, dark inclusion bodies of varying size and many well-formed mitochondria. The cell surface was extremely uneven showing invaginations and protrusions of all sizes and shapes (Pl. 5, fig. 19). Sometimes club-shaped or tubular surface projections showed a constriction of the cell membrane near their base and nearby lay apparently isolated, anucleate masses of cytoplasm carrying many free ribosomes.

Finally, two other features were noted from time to time in homotransplants of 72 and 96 hr. duration. First, the occurrence of free mitochondria within the lumina of otherwise unaffected vessels—these organelles were presumably derived from neighbouring affected endothelial cells which had not been included in the plane of sectioning. Lastly, an increase in the amount of intertubular collagen was seen in some areas—this took the form of randomly scattered bundles of collagen fibres near the tubular basement membranes and also between the groups of cells now occupying the oedematous intertubular spaces.

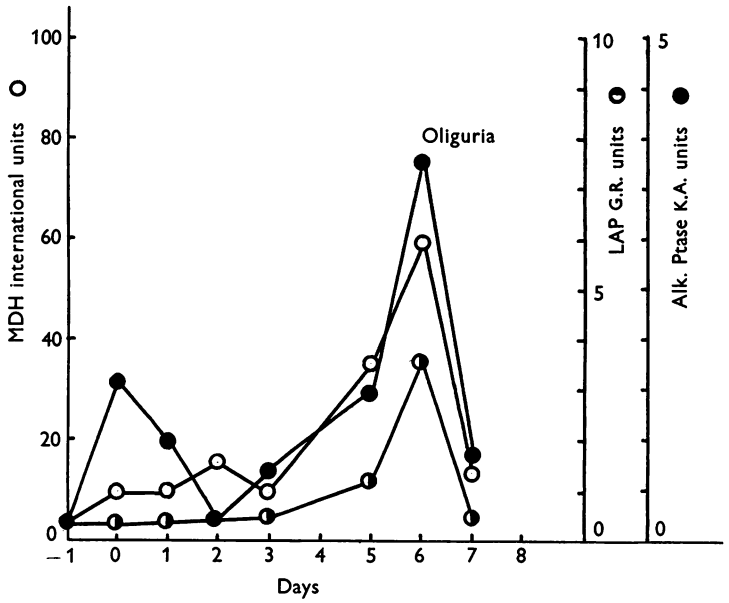
In the six oliguric homotransplants the morphological findings were similar. The majority of the intertubular vessels had been destroyed. Of the occasional vessels remaining, some of these were completely blocked by cellular débris, red blood corpuscles and polymorphonuclear leucocytes (Pl. 10, fig. 33). Where the intertubular vessels had broken down, the spaces between the tubular basement membranes were filled with masses of cytoplasmic débris, many erythrocytes (some recently extravasated, others in various stages of degradation) and numbers of persisting, rounded endothelial cells. A few plasma cells persisted and in the main these were rounded mature cells with an eccentric nucleus, paranuclear agranular Golgi membranes and cytoplasm full of regular, highly organized, flattened lamellar ergastoplasm. Between the various groups of cells rather coarse irregularly disposed bundles of collagen fibres were seen. Finally, the oliguric phase was characterized by the appearance of numbers of polymorphonuclear leucocytes at many points throughout the intertubular conglomerate.

In the *anuric homotransplant* the intertubular vasculature was completely obliterated. The intertubular spaces were filled with tightly packed erythrocytes, scattered bundles of collagen, occasional rounded endothelial cells (Pl. 10, fig. 32; Pl. 11, fig. 34) and the whole, permeated with many active phagocytes (Pl. 9, fig. 31).

Urinary enzymes

The urine from 10 normal dogs was examined on several occasions as controls. In all cases the enzyme level was either indetectable (LDH, GOT and GPT) or extremely low (MDH, 0–5 International units per ml., LAP 0–0·5 Goldbarg–Rutenberg units/ml., alkaline phosphatase 0–0·5 King–Armstrong units/100 ml.). The 7 homotransplant recipients studied gave a consistent picture (e.g. Text-fig. 7). A progressive rise of urinary enzyme level (MDH, LAP and alkaline phosphatase) occurred throughout the 'functioning' phase, becoming maximal at oliguria. Often

this was preceded by a transient, marked increase in the immediate post-operative period. Increased levels of LDH, GOT and GPT were detectable immediately post-operatively and at oliguria. These observations, on homotransplants which had not been biopsied, were interpreted as evidence that shedding of cytoplasmic material into the urine occurred from some part of the nephron, throughout the 'functional', and into the 'oliguric' phase. This was correlated with the ultrastructural observations concerning shedding of proximal tubular cell cytoplasm and organelles at these times.



Text-fig. 7. Activity of three enzymes in the urine from a dog bearing a renal homotransplant. MDH (malic dehydrogenase); LAP (leucine aminopeptidase) expressed as Goldbarg–Rutenberg (G.R.) units; alkaline phosphatase expressed as King–Armstrong (K.A.) units. All values corrected to 1 g. creatinine/l.

DISCUSSION

The data obtained in our investigations offer two separate, yet strong bodies of evidence to the effect that canine renal homotransplant rejection is an ischaemic process.

Haemodynamic studies of renal homotransplants by other workers have in the main been limited to a single observation on individual transplants (Dempster, 1953*c*; Egdahl & Hume, 1956; Mims, 1961). Unfortunately, such observations do not permit a full assessment of the pathophysiological changes occurring within the homotransplant. Because of the variable survival times of individual homotransplants, serial observations on individual transplants appear essential if meaningful concepts are to be drawn from the obtained data. Our concept of renal homotransplant rejection, as presented here, is based on serial physiological and anatomical observations on individual transplants.

Although the method used for measuring total renal blood flow in the first series of experiments is not ideal because of the high mortality from technical difficulties, repeated anaesthetics, and operative procedures, nevertheless, the successful experiments provide a definite answer to the haemodynamic changes occurring in both the normal and transplanted kidney. An error in the constant local indicator-dilution method, as used, would result in an overestimation of venous flow due to contamination of the diluted blood from collateral veins before sampling. The possibility of such an error is small due to the short separation distance of injecting and sampling lumina of the catheter. Also, the chance of such an error is much greater in the transplanted kidney than in the normal left kidney, where the site of the catheter and collateral veins cannot always be adequately documented. Nevertheless, during the life history of a homotransplant, its total blood flow measured by this method 24–72 hr. after transplantation when compared almost simultaneously with the flow in a normal kidney left in the recipient, is found to decrease progressively until oliguria. An increase in flow and weight of the normal kidney was apparent, but this was also observed, perhaps to a lesser degree, in autotransplant controls.

The reliability of the method used in the second series of experiments has been documented. In dogs, Blaufox *et al.* (1963) find that it agrees well with the standard PAH method for measuring effective renal blood flow. An error in the method as used here would tend to overestimate the effective renal blood flow, especially in the presence of decreasing renal function. Any error is believed to be small since most of the injected dose could be accounted for after one hour. The daily '1 hr. percentage' excretion in the urine of the single intravenous dose of ^{131}I-Hippuran decreases in homotransplants much more slowly than the effective renal blood flow. This is readily understandable when one takes into account the form of the ^{131}I-Hippuran disappearance curve. These experiments, as those in the first series, provide further proof that the rejection of homotransplanted kidneys is an ischaemic process.

Inulin labelled with ^{14}C is used simultaneously with ^{131}I-Hippuran with the hope of estimating the daily change in the glomerular filtration rate and to calculate the filtration fraction in homotransplanted kidneys. Because of the low efficiency in assaying ^{14}C in liquid scintillation counters, especially when the changes are small and in the presence of ^{131}I, it is not possible to determine the glomerular filtration rate by the rate of disappearance of ^{14}C-Inulin from the plasma as was done with ^{131}I-Hippuran. Since the daily '1 hr. percentage' excretion in the urine of ^{14}C-Inulin parallels that for ^{131}I-Hippuran, it is assumed that there is also a progressive fall in the glomerular filtration rate. However, further experiments are needed to better document the pattern of change of the glomerular filtration rate in homotransplanted kidneys. The decreased ability of autotransplants to excrete ^{131}I-Hippuran 1–2 days after removal of the normal kidney (Text-fig. 6) may be related to the renal counterbalance phenomenon described in such transplants.

Anatomically, the primary lesion resides among the intertubular capillaries, while physiologically decreases in 'effective renal blood flow' and renal venous flow are concurrent with the anatomical changes. Oliguria occurs at a time when renal venous flow is reduced to 50 % of the pre-operative level or less, and the onset of widespread tubular necrosis has commenced.

The pathological changes which appear to be primary in the rejection process make their first appearance within 48 hr. following homotransplantation, although not to any appreciable extent within the first 24 hr. The early changes we have described take the form of a close association between immature plasma cells and homotransplant capillary endothelial cells. This close association bears certain similarities to the adherence of leucocytes to capillary endothelial cells during the early stages of various forms of inflammation (Marchesi & Florey, 1960; Williamson & Grisham, 1961; Movat & Fernando, 1963). However, the inflammatory adherence is transitory, the leucocyte passing rapidly through or between the endothelial cells to reach the interstitium. Thereafter, the endothelial cell is substantially unaltered in appearance. In the case of the renal homotransplant however, the adherence of immature plasma cell and endothelial cell persists with radical alteration of the endothelial cell and dissolution of the vessel. Groups of rounded, isolated endothelial cells often remain in close cell membrane apposition with individual maturing plasma cells throughout the remaining life of the transplant.

The cell labelling studies of Porter & Calne (1960), Fowler & West (1961) and Dempster & Williams (1963) strongly suggest that at least a substantial proportion of the heavily pyroninophilic, infiltrating cells, originate in the host. The host population appears to consist mainly of lymphocytes of various degrees of pyronine positivity, stellate and rounded immature plasma cells and mature plasma cells. The electron microscope studies presented here, suggest that the 'attacking' population of host plasma cell forms must be distinguished from a second population of cells that stain less well with pyronine G. The second population of cells has a different nuclear morphology, very little ergastoplasm and derives from the transplant capillary endothelium. Great care would be required with light microscope observations to distinguish between the two populations. These morphological studies suggest that the cell of origin of the immature plasma cell is the small lymphocyte. A complete series of forms showing the development of ribosome-studded cisternae in the cytoplasm of small lymphocytes could be demonstrated. The cell labelling studies of Dempster & Williams (1963) indicate that the precursor cell must occur among the leucocytes of the peripheral blood. Medium sized lymphocytes were much more frequent than small lymphocytes among the cells labelled with tritiated thymidine. However, labelling studies have not been able to determine if the large or the small lymphocyte is likely to be the responsible cell type. The time course of the reaction would be in favour of a cell type existing within the peripheral blood at the time of homotransplantation.

The studies of MacGregor & Gowans (1963) on the immune response to soluble antigens, and the studies of Gowans, Gesner & MacGregor (1961), Gowans (1962) and Porter, Chapuis & Freeman (1962) on graft versus host reactions offer very strong evidence, both for the ability of the small lymphocyte to take part in immune reactions, and also to transform into a large pyroninophilic cell. The case for the immune ability of the larger lymphocyte is not strong and although Cole & Garver (1961) suggested that it may participate in graft-versus-host reactions, the work has not been confirmed (Billingham, Defendi, Silvers & Steinmuller, 1962). However, it is known that injected, labelled, large lymphocytes can appear as pyroninophilic cells in tissue sections. Recent work has demonstrated the ability of the

small lymphocyte to assume a large proliferative form (e.g. Carstairs, 1962). In view of the weight of evidence it is reasonable to assume as a working hypothesis that the precursor cell is the small lymphocyte.

Very little data are available on the fine structure of cells involved in graft versus host reactions and other homograft reactions, but anatomical data are more plentiful on classical immune responses and plasma cell structure (Bernhard & Granboulan, 1960; Bessis, 1961; de Petris, Karlsbad & Pernis, 1963). In contrast to the classical immune response, where typical plasma cells are involved, the early work available on homograft reactions suggests that the pyroninophilic, immunologically competent cell derived from the small lymphocyte is mainly histiocytic in character (Binet & Mathé, 1962). The cell portrayed by Binet & Mathé that occurs in the proliferating lymph node draining a skin homograft and in graft versus host reactions appears to be devoid of ergastoplasm. In this respect the pyroninophilic cells examined by Binet & Mathé differ markedly from those we have observed within renal homotransplants. Burwell (1962) has examined the cells proliferating in the lymph node draining bone homografts and noted two types of cell. One cell type was similar to that observed by Binet & Mathé and was interpreted as a manufacturer of cell-borne antibodies. The second cell type was of the plasma cell series and it was suggested that it synthesized serum antibodies. The relation that local lymph node cells bear to cells infiltrating homotransplants is at present unknown and it is difficult to reconcile our data with that of other authors who have examined local lymph nodes.

The speed with which the plasma cell attack is initiated contrasts with the data on local lymph node reactions (Scothorne & MacGregor, 1955; Billingham, Silvers & Wilson, 1962). In addition, the plasma cell attack appears to begin at a time when the host is unable to mount a detectable 'second set' response (Dempster *et al.* 1963). These two facts, together with the labelling data of Dempster & Williams (1963) derived for 'second set' transplants are best explained at present by the theory that antigen is not released in detectable quantities. The host reaction is, therefore, probably mounted by circulating cells which after contact with and adherence to graft endothelium, differentiate into cells capable of an immune response.

The circulating cells are probably members of the lymphocyte population that are able to transform into plasma cells upon contact with a structural antigen. It has been suggested that thoracic duct lymphocytes include some forms rich in ergastoplasm (Zucker-Franklin, 1963). Other workers have demonstrated that some thoracic duct lymph cells can transform into plasma cells (e.g. Schooley & Berman, 1960).

The mechanism by which host plasma cells effect the disruption of transplant capillaries is probably a type of antigen-antibody reaction, since only such a mechanism could explain the remarkably specific localization of the damage. It has recently been shown that these cells contain gamma globulin (Horowitz, Burrows, Paronetto & Wildstein, 1963).

In recent years there have been four main theories concerning the mechanism of homotransplant rejection. These theories were those of Local Immunity (Loeb, 1945); Acquired Immunity (Medawar, 1944); Natural Immunity (reviewed by Rogers, 1950) and that of Autodestruction (Dempster, 1955).

The data obtained in the present investigations, if they are interpreted as the attack of a previously circulating uncommitted cell, have some affinity with theories of Local Immunity and that of Natural Immunity. However, the theory of Loeb implicated lymphocytes which acted locally and were presumably derived from local tissues and also would not adequately account for the occurrence of a specific attack upon one cell type in a complex organ.

The existence of uncommitted, competent cells in the blood stream would constitute a form of Natural Immunity but not of the specificity visualized in the theory.

The theory of Autodestruction of Dempster (1955) was postulated when the evidence of an immunological or vascular mechanism seemed inadequate. The results obtained in these investigations justify abandoning the theory.

The theory of Acquired Immunity clearly does not hold true for renal homotransplants if the interpretation presented here is correct. However, the theory of Acquired Immunity appears to be a plausible explanation for skin homografts. Nevertheless, since the early observations of Fasiani (1924), oedema and vascular stasis followed by disruption of vessels have been described as important features in the rejection of skin homografts (e.g. Taylor & Lehrfeld, 1953; Waksman, 1963). Large fitted skin homografts both in man and dog are clinically diagnosed as rejected when the following signs are observed—peau d'orange (indicating lymphatic obstruction), altered texture, thickening and discoloration (indicating vascular stagnation). Focal points of vascular stagnation are apparent some time before general necrosis. It is possible therefore that in skin homografts, as in kidney homotransplants, progressive focal vascular damage is the main factor involved in the rejection process.

If the release of antigen from renal transplants should be detected, or a low level of sensitization in the hosts of functioning 'first set' kidneys be discovered, then the theory of Acquired Immunity could be vindicated for renal homotransplants. Nevertheless, even if the theory of Acquired Immunity was upheld, the case for the lymphocyte bearing 'endoantibodies' is weak.

SUMMARY

The natural history of renal homotransplants and autotransplants in the dog was studied by serial physiological determinations of the renal venous flow, the daily 'effective renal blood flow' and the '1 hr. percentage excretion' of a single intravenous dose of ^{131}I-Hippuran and ^{14}C-Inulin. Parallel serial biopsies of kidney cortex were taken for electron microscopy.

It is suggested that:

1. Canine renal homotransplants are destroyed in an ischaemic process.
2. Ischaemia follows intertubular oedema and dissolution of the intertubular capillary bed.
3. The intertubular capillaries are destroyed by the immunological action of host plasma cells.
4. The host cell type responsible is probably the lymphocyte which is able to transform to a plasma cell form.
5. The relation of the data to current theories of tissue rejection is discussed.

We gratefully acknowledge support for this work provided by the Medical Research Council, The Wellcome Trust, The U.S. National Institutes of Health, The National Spastics Society and Mr W. Butlin. We are grateful for technical assistance to Miss J. Hunter, Miss V. Hanly and Miss V. Brown. The ultrathin sections were expertly prepared by Mr Graham Stevens.

REFERENCES

BERNHARD, W. & GRANBOULAN, N. (1960). In *Cellular Aspects of Immunity*, p. 92. Ciba Foundation Symposium. Ed. G. E. W. Wolstenholme and M. P. Cameron. London: Churchill.

BESSIS, M. (1961). Ultrastructure of lymphoid and plasma cells in relation to globulin and antibody formation. *Lab. Invest.* **10**, 1040–1067.

BILLINGHAM, R. E., BRENT, L. & MEDAWAR, P. B. (1953). Actively acquired tolerance of foreign cells. *Nature, Lond.*, **172**, 603–606.

BILLINGHAM, R. E., DEFENDI, V., SILVERS, W. K. & STEINMULLER, D. (1962). Quantitative studies on the induction of tolerance of skin homografts and on runt disease in neonatal rats. *J. nat. Cancer Inst.* **28**, 365–435.

BILLINGHAM, R. E., SILVERS, W. K. & WILSON, D. B. (1962). Adoptive transfer of transplantation immunity by means of blood-borne cells. *Lancet*, **1**, 512–515.

BINET, J. L. & MATHÉ, G. (1962). Optical and electron microscope studies of the immunologically competent cells during the reaction of graft against the host. *Ann. N.Y. Acad. Sci.* **99**, Art. 3, 426–431.

BLAUFOX, M. D., FROHMULLER, H. G. W., CAMPBELL, J. C., UTZ, D. C., ORVIS, A. L. & OWEN, C. A. (1963). A simplified method of estimating renal function with iodo-hippurate I^{131}. *J. Surg. Res.* **3**, 122–125.

BRENT, L., MEDAWAR, P. B. & RUSKIEWICZ, M. (1962). Studies on transplantation antigens. In *Transplantation*, p. 6. Ciba Foundation Symposium. Ed. G. E. W. Wolstenholme and M. P. Cameron. London: Churchill.

BURWELL, R. G. (1962). Studies on the primary and the secondary immune responses of lymph nodes draining homografts of fresh cancellous bone (with particular reference to mechanics of lymph node reactivity). *Ann. N.Y. Acad. Sci.* **99**, Art. 3, 821–860.

CALNE, R. Y. (1963). Biological factors in tissue transplantation. *Post Grad. med. J.* **38**, 548–559.

CARSTAIRS, K. (1962). The human small lymphocyte: Its possible pluripotential quality. *Lancet*, **1**, 829–832.

COLE, L. J. & GARVER, R. H. (1961). Homograft reactive large mononuclear leucocytes in peripheral blood and peritoneal exudates. *Amer. J. Physiol.* **200**, 147–151.

DARMADY, E. M., DEMPSTER, W. J. & STRANACK, F. (1955). Evolution of interstitial and tubular changes in homotransplanted kidneys. *J. Path. Bact.* **70**, 225–231.

DAVIES, D. A. L. (1962). The isolation of mouse antigens carrying H-2 histo-compatibility specificity. *Biochem. J.* **84**, 307–317.

DEMPSTER, W. J. (1953*a*). The relationship between antigens of skin and kidney of the dog. *Brit. J. Plast. Surg.* **5**, 228–237.

DEMPSTER, W. J. (1953*b*). Kidney homotransplantation. *Brit. J. Surg.* **40**, 447–465.

DEMPSTER, W. J. (1953*c*). The effects of cortisone on the homotransplanted kidney. *Arch. int. Pharmacodyn.* **95**, 253–282.

DEMPSTER, W. J. (1953*d*). A toxic syndrome observed in dogs with transplanted kidneys. *Acta med. scand.* **144**, 360–370.

DEMPSTER, W. J. (1954). A technique for the study of the autotransplanted kidney, adrenal and ovary of the dog. *J. Physiol. Lond.* **124**, 15*P*–16*P*.

DEMPSTER, W. J. (1955). A consideration of the cause of functional arrest of homotransplanted kidneys. *Brit. J. Urol.* **27**, 66–86.

DEMPSTER, W. J., CALNAN, J. & KULATILAKE, A. E. (1963). Time of antigen release from homografts. *Brit. med. J.* **1**, 23–25.

DEMPSTER, W. J. & WILLIAMS, M. A. (1963). Cellular infiltration in homotransplanted kidneys. *Brit. med. J.* **1**, 18–23.

EGDAHL, R. H. & HUME, D. M. (1956). Immunologic studies in renal homotransplantation. *Surg. Gynec. Obstet.* **102**, 450–462.

FASIANI, G. M. (1924). Ricerche sull'innesto omoplastico della pelle. II. Nota. Sull comportamento dei vasi nell'innesto autoplastico e nell'innesto omoplastico della pelle. *Arch. sci. Med.* **47**, 65–82.

FEDOR, E. J., LEE, S. H. & FISHER, B. (1959). Observations using diodrast I^{131} as an index of renal function in auto- and homotransplantation. *Surg. Forum.* **10**, 875–879.

FISHER, E. R. & FISHER, B. (1959). Investigation of rejection of canine renal homotransplants by fluorescent antibody technic. *Proc. Soc. exp. Biol., N.Y.*, **101**, 259–261.

FOWLER, R. & WEST, C. D. (1961). Evidence against the graft-versus-host hypothesis in renal transplantation. *Transplant. Bull.* **26**, 133–141.

GOLDBARG, J. A. & RUTENBERG, A. M. (1958). The colorimetric determination of leucine aminopeptidase in urine and serum of normal subjects and patients with cancer and other diseases. *Cancer*, **11**, 283–291.

GOWANS, J. L. (1962). The fate of parental strain small lymphocytes in F_1 hybrid rats. *Ann. N.Y. Acad. Sci.* **99**, Art. 3, 456–469.

GOWANS, J. L., GESNER, B. M. & MACGREGOR, D. D. (1961). The immunological activity of lymphocytes. In Ciba Foundation Study Group No. 10, *Biological Activity of Leucocytes*, pp. 32–40. Ed. G. E. W. Wolstenholme and M. O'Connor.

HAMBURGER, J., VAYSSE, J., CROSNIER, J., AUVERT, J., LALANNE, C. M. & DORMONT, J. (1962). Six tentatives d'homotransplantation rénale chez l'homme après irradiation du receveur. *Rev. franc. Et. clin. biol.* **7**, 20–39.

HOROWITZ, R. E., BURROWS, L., PARONETTO, F. & WILDSTEIN, W. (1963). Immunocytochemical observations on canine kidney homografts. *Fed. Proc.* **22**, 274.

HUME, D. H., JACKSON, B. T., ZUKOSKI, C. F., LEE, H. H., KAUFFMAN, H. M. & EGDAHL, R. H. (1960). The homotransplantation of kidneys and of fetal liver and spleen after total body irradiation. *Ann. Surg.* **152**, 354–373.

JANIGAN, D. T., WILLIAMS, M. A., TYLER, H. M. & DEMPSTER, W. J. (1964). A biochemical approach to the study of rejection of canine renal homotransplants. III. Histochemical studies. *Brit. J. exp. Path.* (in the Press).

KING, E. J. & WOOTTON, I. D. P. (1956). *Microanalysis in Medical Biochemistry.* London: Churchill.

KING, J. (1961). A routine method for the estimation of serum malate dehydrogenase activity. *J. Med. Lab. Techn.* **18**, 168–175.

KÜSS, R., LEGRAIN, M., MATHÉ, G., NEDEY, R. & CAMEY, M. (1963). Homotransplantation renale chez l'homme hors de tout bien de parenté. Survie jusqu'au 17e mois. *Rev. franc. Et. clin. biol.* **7**, 1048–1066.

LOEB, L. (1945). *The Biological Basis of Individuality.* Springfield, Illinois: C. C. Thomas.

MACGREGOR, D. D. & GOWANS, J. L. (1963). The antibody response of rats depleted of lymphocytes by chronic drainage from the thoracic duct. *J. exp. Med.* **117**, 303–320.

MARCHESI, V. T. & FLOREY, H. W. (1960). Electron microscopic observations on the emigration of leucocytes. *Quart. J. exp. Physiol.* **45**, 343–348.

MEDAWAR, P. B. (1944). The behaviour and fate of skin autografts and skin homografts in rabbits. *J. Anat., Lond.*, **78**, 176–199.

MEDAWAR, P. B. (1958). The Croonian Lecture. The homograft reaction. *Proc. Roy. Soc.* B, **149**, 145–166.

MIMS, M. M. (1961). Haemodynamics in renal transplantation. *J. Urol.* **86**, 493–500.

MOVAT, H. Z. & FERNANDO, N. V. P. (1963). Acute inflammation. The earliest fine tissue changes at the blood-tissue barrier. *Lab. Invest.* **12**, 895–910.

MOVAT, H. Z. & STEINER, J. W. (1961). Studies of nephrotoxic nephritis. I. The fine structure of the glomerulus of the dog. *Amer. J. Clin. Path.* **36**, 289–321.

MURRAY, J. E., MERRILL, J. P., DAMMIN, G. T., DEALY, J. B., ALEXANDRE, G. W. & HARRISON, J. H. (1962). Kidney transplantation in modified recipients. *Ann. Surg.* **156**, 337–355.

NEWMAN, E. V., BORDLEY, J. & WINTERNTZ, J. (1944). The interrelationships of glomerular filtration rate (mannitol clearance), extracellular fluid volume, surface area of the body and plasma concentration of mannitol. *Johns Hopk. Hosp. Bull.* **75**, 253–268.

PEASE, D. C. & BAKER, R. F. (1950). Electron microscopy of the kidney. *Amer. J. Anat.* **87**, 349–390.

PEASE, D. C. (1955*a*). Electron microscopy of the vascular bed of the kidney cortex. *Anat. Rec.* **121**, 701–722.

PEASE, D. C. (1955*b*). Electron microscopy of the tubular cells of the kidney cortex. *Anat. Rec.* **121**, 723–744.

PEASE, D. C. (1955*c*). Fine structures of the kidney seen by electron microscopy. *J. Histochem. Cytochem.* **3**, 295–308.

DE PETRIS, S., KARLSBAD, G. & PERNIS, B. (1963). Localization of antibodies in plasma cells. *J. exp. Med.* **117**, 849–862.

PORTER, K. A. & CALNE, R. Y. (1960). Origin of the infiltrating cells in skin and kidney homografts. *Transplant. Bull.* **26**, 458–464.

PORTER, K. A., CHAPUIS, G. & FREEMAN, H. K. (1962). Responsibility of small lymphocytes for the killing effect of blood-bone marrow mixtures on irradiated rabbits. *Ann. N.Y. Acad. Sci.* **99**, Art. 3, 456–469.

RHODIN, J. A. (1962). The diaphragm of capillary endothelial fenestrations. *J. Ultrastruct. Res.* **5**, 171–185.

ROGERS, B. O. (1950). A brief review. The problem of skin homografts. *Plast. reconst. Surg.* **6**, 269–282.

SCHOOLEY, J. C. & BERMAN, I. (1960). Morphologic and autoradiographic observations of H^3-thymidine labelled thoracic duct lymphocytes cultured in vivo. *Blood*, **16**, 1133–1144.

SCOTHORNE, R. J. & MACGREGOR, I. A. (1955). Cellular changes in lymph nodes and spleen following skin homografting. *J. Anat., Lond.*, **89**, 283–292.

SHACKMAN, R., DEMPSTER, W. J. & WRONG, O. (1963). Kidney homotransplantation in the human. *Brit. J. Urol.* **35**, 222–255.

SHILLINGFORD, J., BRUCE, T. & GABE, I. (1962). The measurement of segmental venous flow by an indicator dilution method. *Brit. Heart J.* **24**, 157–165.

SIMONSEN, M. (1953). Biological incompatibility in kidney transplatantion in dogs. *Acta path. microbiol. scand.* **32**, 36–84.

SJOSTRAND, F. S. & RHODIN, J. (1954). The ultrastructure of the proximal convoluted tubule of the mouse kidney as seen by high resolution electron microscopy. *Exp. Cell Res.* **4**, 426–456.

TAYLOR, A. C. & LEHRFELD, J. W. (1953). Determination of survival time of skin homografts in the rat by observations of vascular changes in the graft. *Plast. Reconst. Surg.* **12**, 423–431.

THIÉRY, J. P. (1960). In *Cellular Aspects of Immunity*, p. 59. Ciba Foundation Symposium. Ed. G. E. W. Wolstenholme and M. P. Cameron. London: Churchill.

TYLER, H. M., LISTER-CHEESE, I. A. F., STRUTHERS, N. W. & DEMPSTER, W. J. (1962). A humoral change accompanying homograft rejection. *Lancet*, **2**, 432–434.

TYLER, H. M., WILLIAMS, M. A. & DEMPSTER, W. J. (1964). Metabolism of canine renal transplants. *Nature, Lond.*, **201**, 84–85.

TYLER, H. M., WILLIAMS, M. A., KOUNTZ, S. L. & DEMPSTER, W. J. (1964). A biochemical approach to the study of rejection of canine renal homotransplants. I. Some quantitative assessments of enzyme activity. *Brit. J. exp. Path.* **45**, 219–225.

WAKSMAN, B. H. (1963). The pattern of rejection in rat skin homografts and its relation to the vascular network. *Lab. Invest.* **12**, 46–57.

WILKINSON, J. H. (1962). *An Introduction to Diagnostic Enzymology.* London: Arnold.

WILLIAMS, M. A., MORTON, M., TYLER, H. M. & DEMPSTER, W. J. (1964). A biochemical approach to the study of rejection of canine renal homotransplants. II. A chemical analysis of kidney homogenates. *Brit. J. exp. Path.* **45**, 235–241.

WILLIAMSON, J. R. & GRISHAM, J. W. (1961). Electron microscopy of leukocytic margination and emigration in acute inflammation in dog pancreas. *Amer. J. Path.* **39**, 239–256.

WROBLEWSKI, F. & LADUE, J. S. (1955). Lactic dehydrogenase activity in blood. *Proc. Soc. exp. Biol., N.Y.*, **90**, 210–213.

YAMADA, E. (1955). The fine structure of the renal glomerulus of the mouse. *J. biophys. biochem. Cytol.* **1**, 551–556.

ZUCKER-FRANKLIN, D. (1963). The ultrastructure of cells in human thoracic duct lymph. *J. Ultrastruct. Res.* **9**, 325–329.

EXPLANATION OF PLATES

PLATE 1

Fig. 1. Autotransplant—part of a morphologically normal glomerular tuft. e, Fenestrated endothelium; c, capillary lumen; b, basement membrane; p, podocyte cytoplasm; u, urinary space.

Fig. 2. Anuric homotransplant—part of a normal glomerular tuft. Labels as for Fig. 1.

PLATE 2

Fig. 3. Functioning homotransplant showing 'luminal shedding' of proximal tubule cell. One cell carries normal microvilli whilst the neighbouring cell is devoid of microvilli and has a smooth plasma membrane (pm) on its luminal aspect. lt. Lumen of tubule. Débris in lumen includes: m, free mitochondria; c, membrane bound cytoplasmic masses; db, dense bodies of uncertain origin.

Fig. 4. Functioning homotransplant—proximal tubular cell bearing normal microvilli.

Fig. 5. Functioning homotransplant—proximal tubular cell showing normal basal infolding of plasma membrane.

Fig. 6. Functioning homotransplant—proximal tubular cell showing an increased number of small vacuoles.

Fig. 7. Oliguric homotransplant—large vacuoles (lv) are present in the basal regions of some proximal tubular cells.

PLATE 3

Fig. 8. Oliguric homotransplant—substantially normal distal tubular cell (dtc) with slight paranuclear vacuolation (v). tl, Tubular lumen; dl, débris in lumen; id, intertubular cytoplasmic débris; rbc, extravascular erythrocyte.

Fig. 9. Oliguric homotransplant—normal distal tubule showing: lc, 'light' cell; dc, 'dark cell'. int, Intertubular space containing: e, rounded endothelial cells; p, polymorphonuclear leucocytes; r, extravascular red blood cell.

Fig. 10. Anuric homotransplant—distal tubular cell showing marked vacuolation (v), this is exceptional.

Fig. 11. Functioning homotransplant—normal distal tubule showing débris in lumen: v, isolated granular vesicles; c, cytoplasmic masses; m, free mitochondria.

PLATE 4

Fig. 12. Normal intertubular vessel. cl, Lumen of capillary; fe, fenestrated endothelium; cf, capillary basement membrane; tb, tubular basement membrane. Note fine lamina of collagen fibres between tubular and capillary basement membranes.

Fig. 13. Functioning homotransplant (72 hr.)—intravascular immature plasma cell with cytoplasmic feet (f) extending towards attenuated capillary endothelium (e) which has broken through at a number of points (g).

Fig. 14. Functioning homotransplant (72 hr.). en, Endothelial cell body still forming part of the wall of an intact intertubular versel. pc, Cytoplasm of immature plasma cell which has approached the endothelial cell closely and is partially surrounding it with pseudopodia (p).

Fig. 15. Functioning homotransplant (72 hr.). l, Lymphocytes within lumen of intact intertubular blood vessel.

PLATE 5

Fig. 16. Lymphocyte.

Fig. 17. Lymphocyte.

Fig. 18. Normal endothelial cell.

Fig. 19. Rounded, isolated endothelial cell.

Figs. 20 and 21. Immature plasma cell—neighbouring sections of a small round cell with general form and nucleo-cytoplasmic ratio of a small lymphocyte but containing well-formed ergastoplasm in its narrow cytoplasmic rim.

PLATE 6

Fig. 22. Immature plasma cell—a rounded cell with nuclear patches of low electron density and sub-angular cytoplasmic projections filled with dilated ergastoplasmic cisternae.

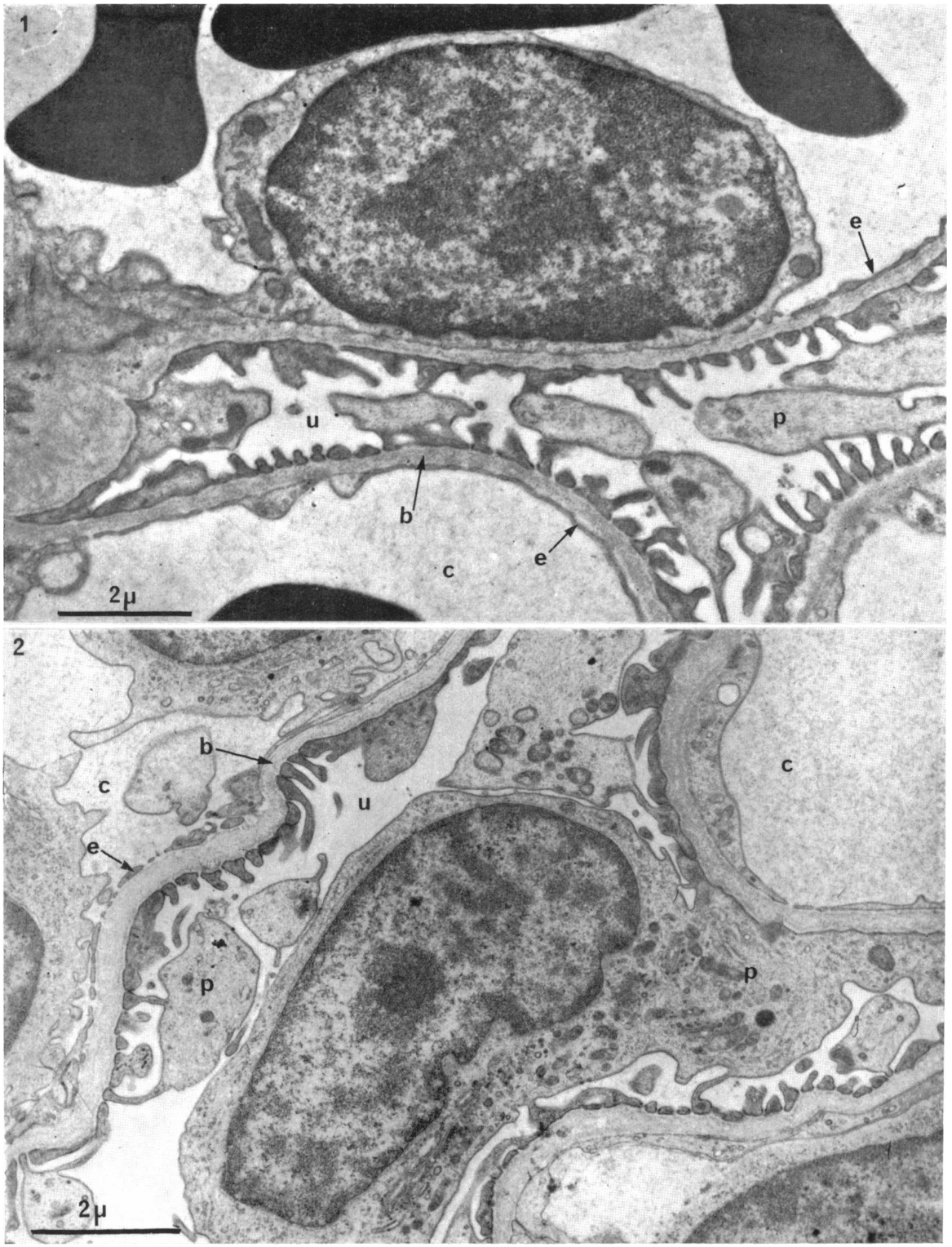
Fig. 23. Immature plasma cell—relatively large electron translucent nucleus. The cell body plasma membrane and that of short blunt pseudopodia (p) enter into intimate apposition with neighbouring rounded endothelial cells (e).

Fig. 24. Immature (stellate) plasma cell—large homogeneous nucleus with well formed nucleolus. Extensive ergastoplasm-filled pseudopodia (p) pass between, and partially surround, rounded endothelial cells (e).

Fig. 25. Ergastoplasm (granular endoplasmic reticulum) characteristic of immature plasma cells.

Fig. 26. Ergastoplasm of mature plasma cell—dilated cisternae containing Russell bodies.

Fig. 27. Ergastoplasm of mature plasma cell in highly organized lamellar form.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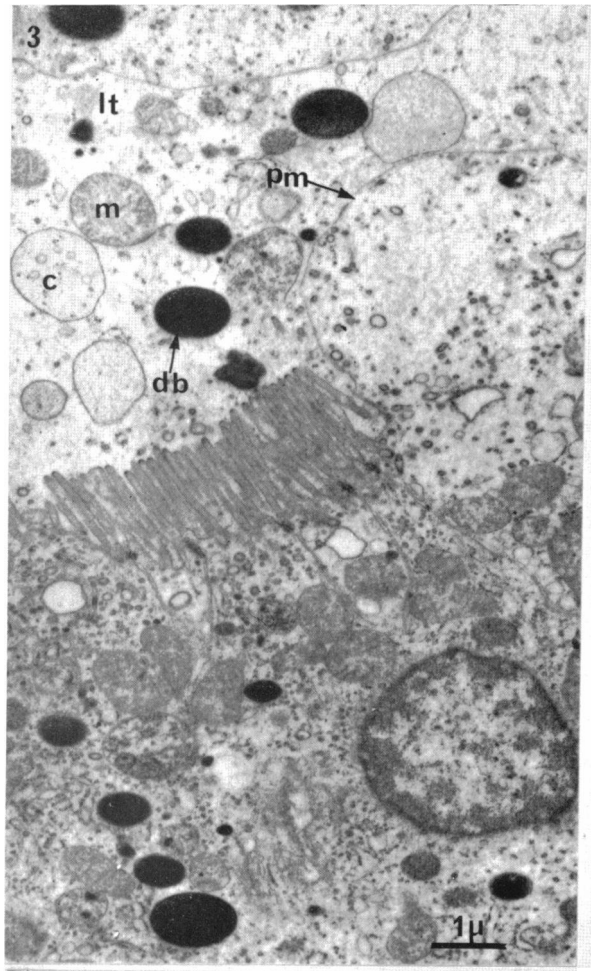
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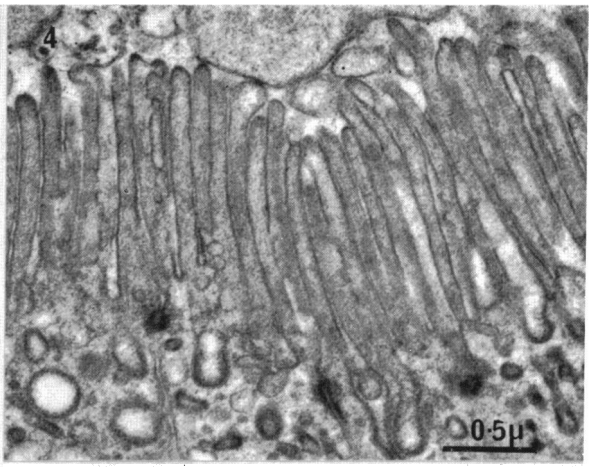
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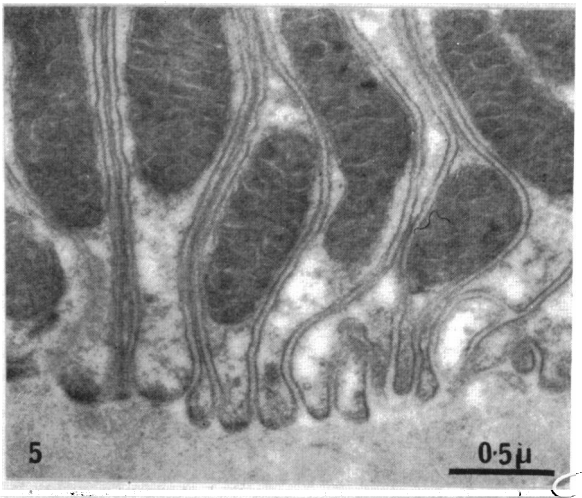
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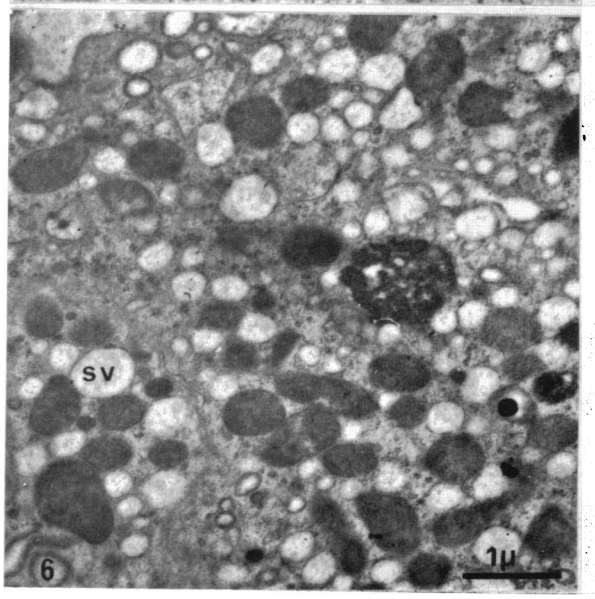
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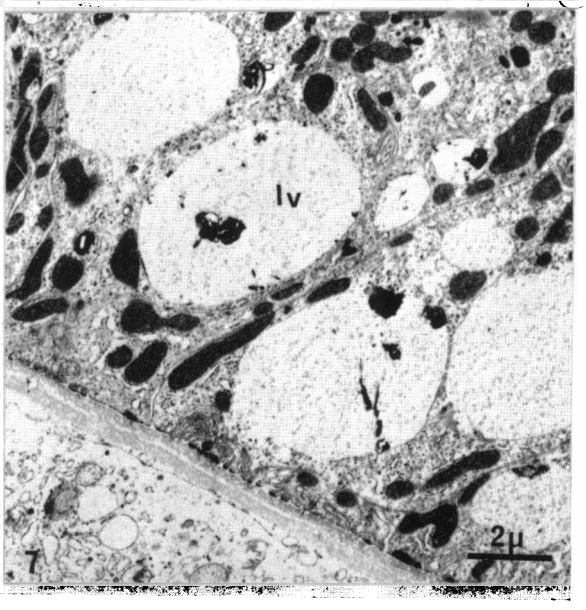
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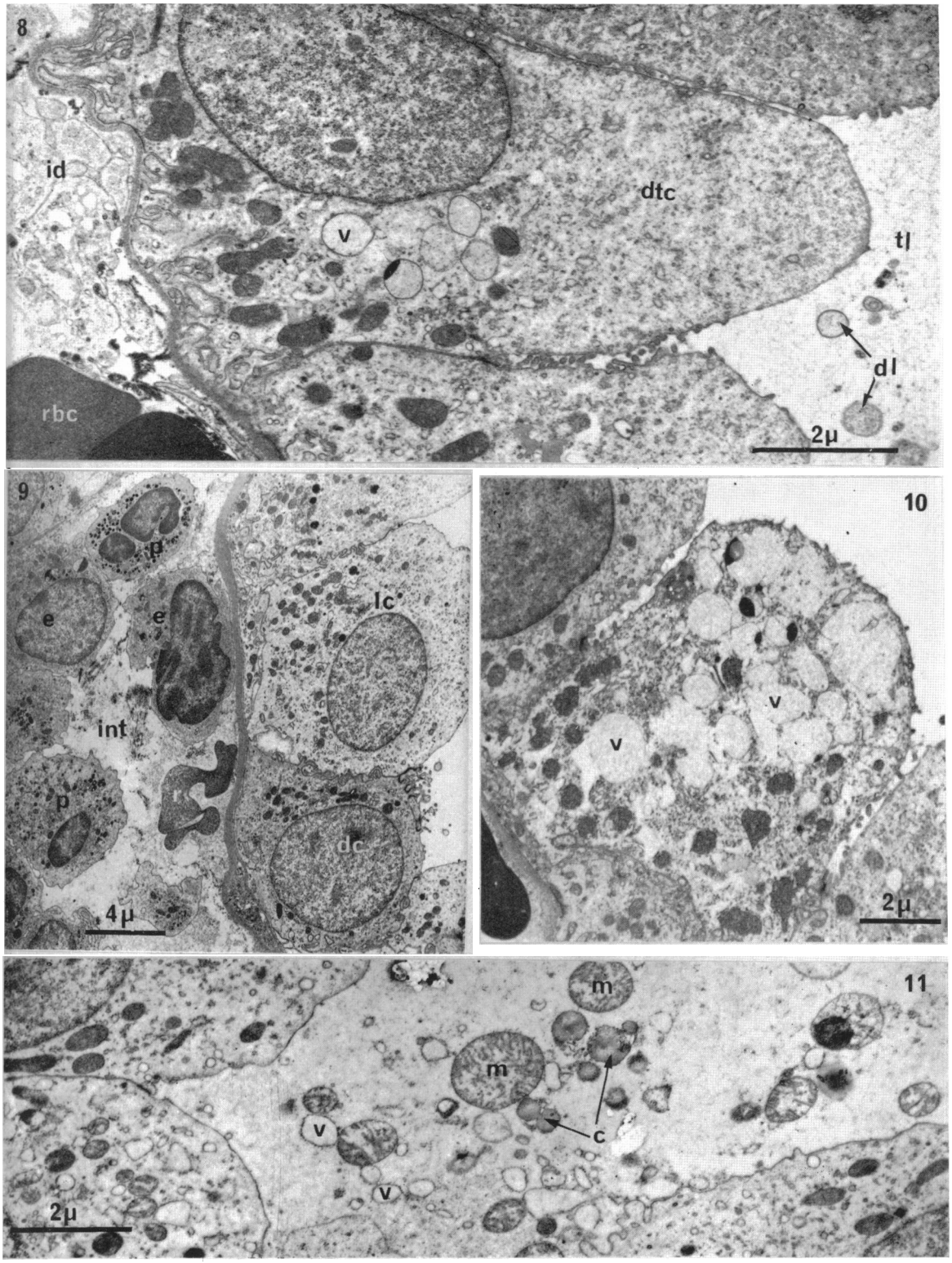
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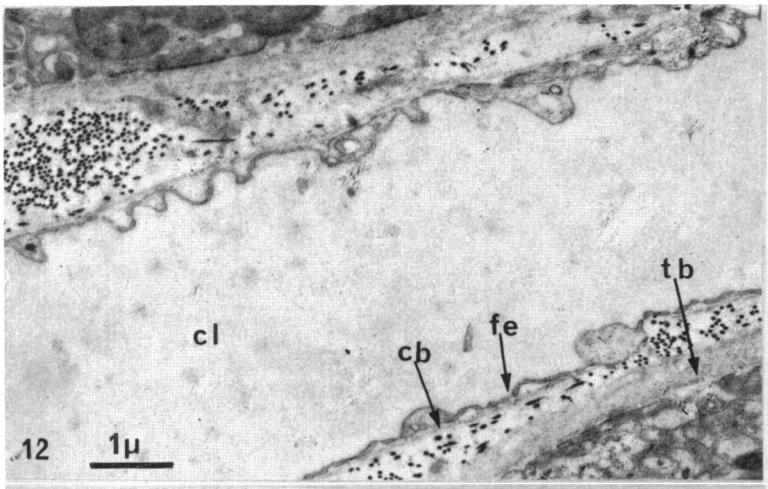
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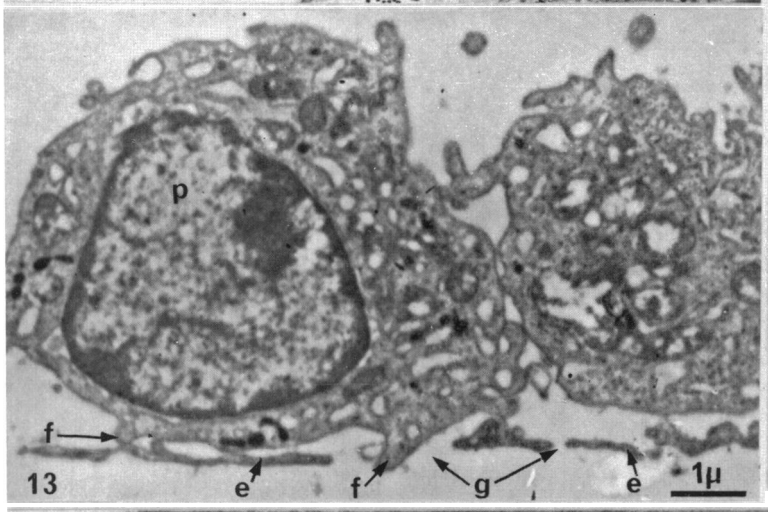
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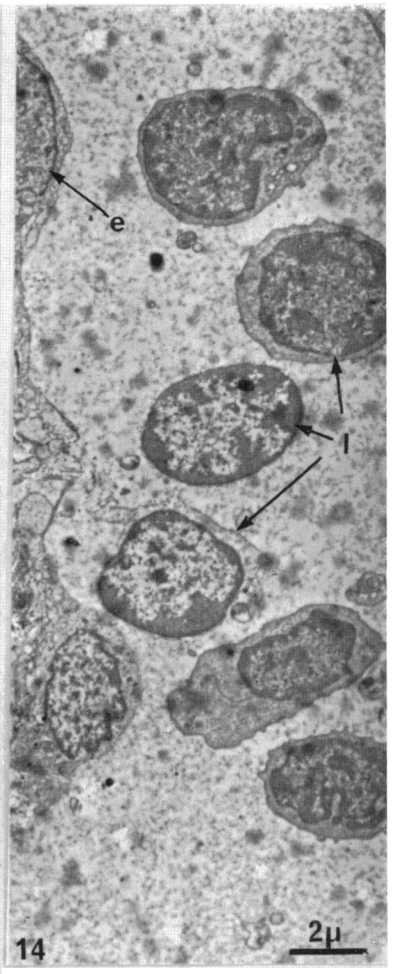
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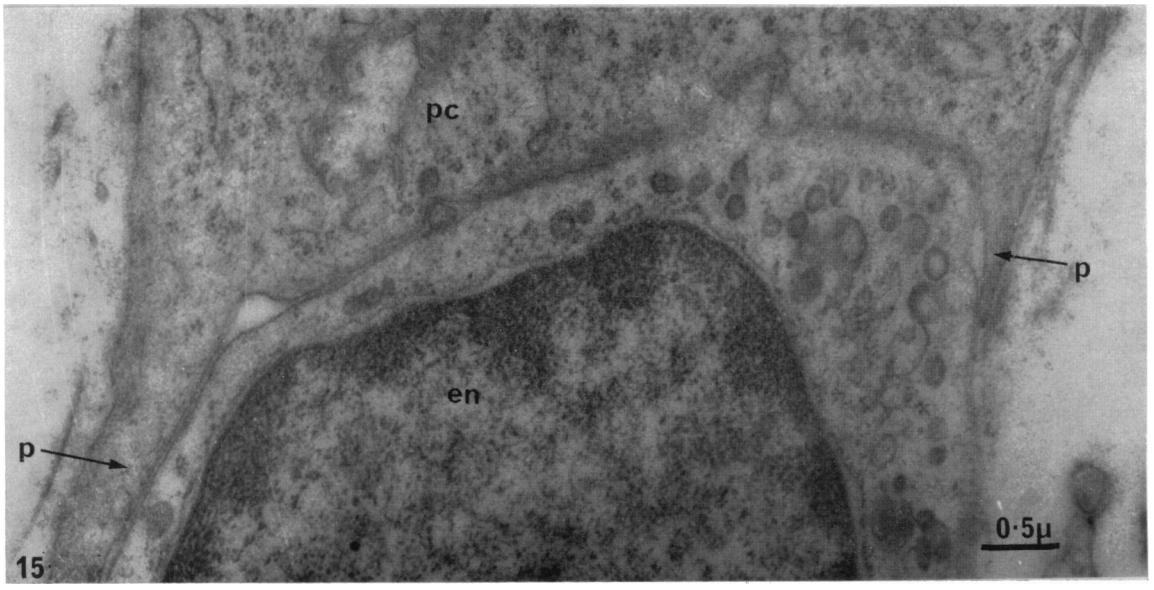
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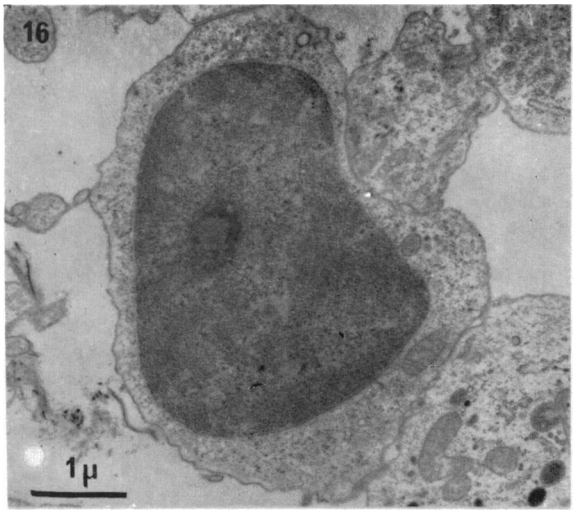
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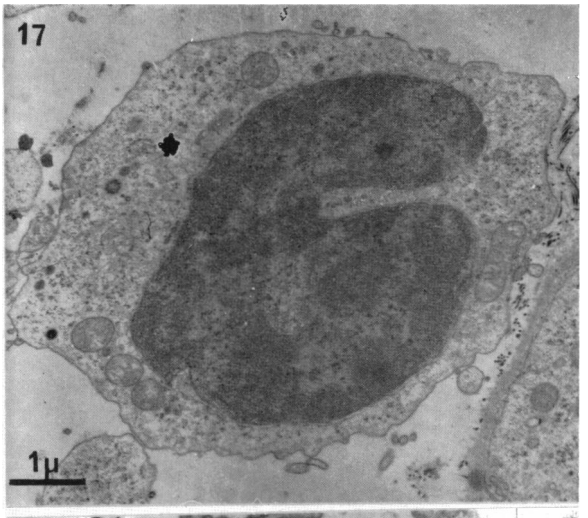
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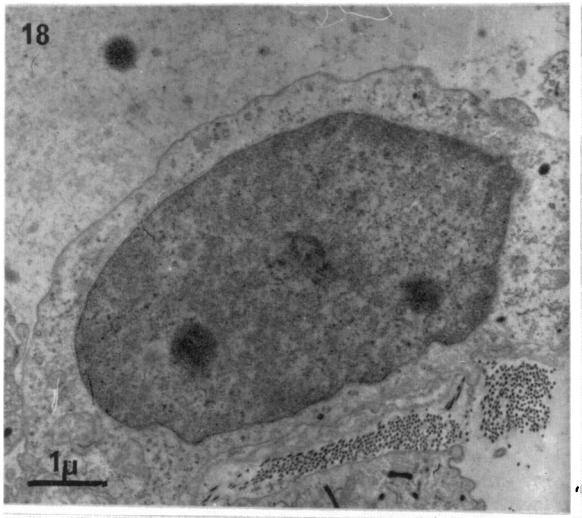
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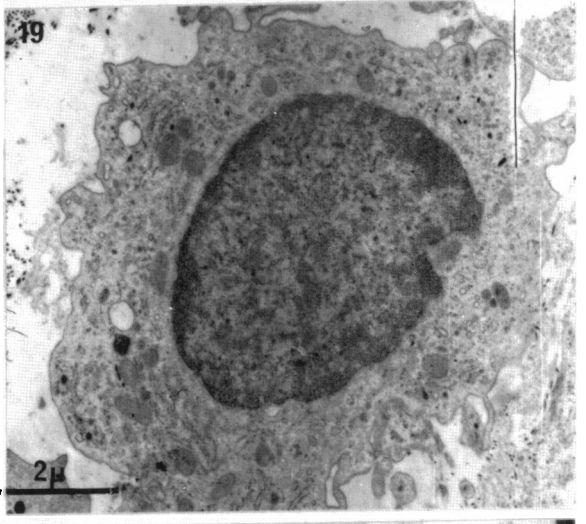
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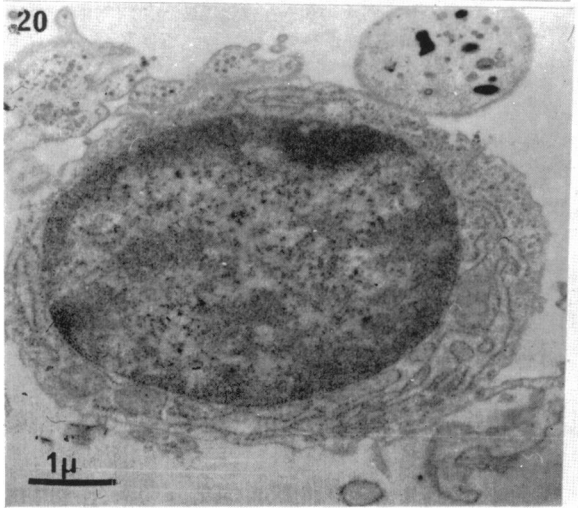
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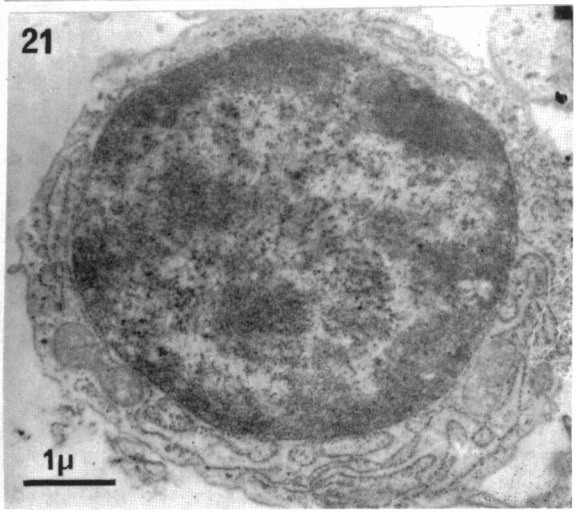
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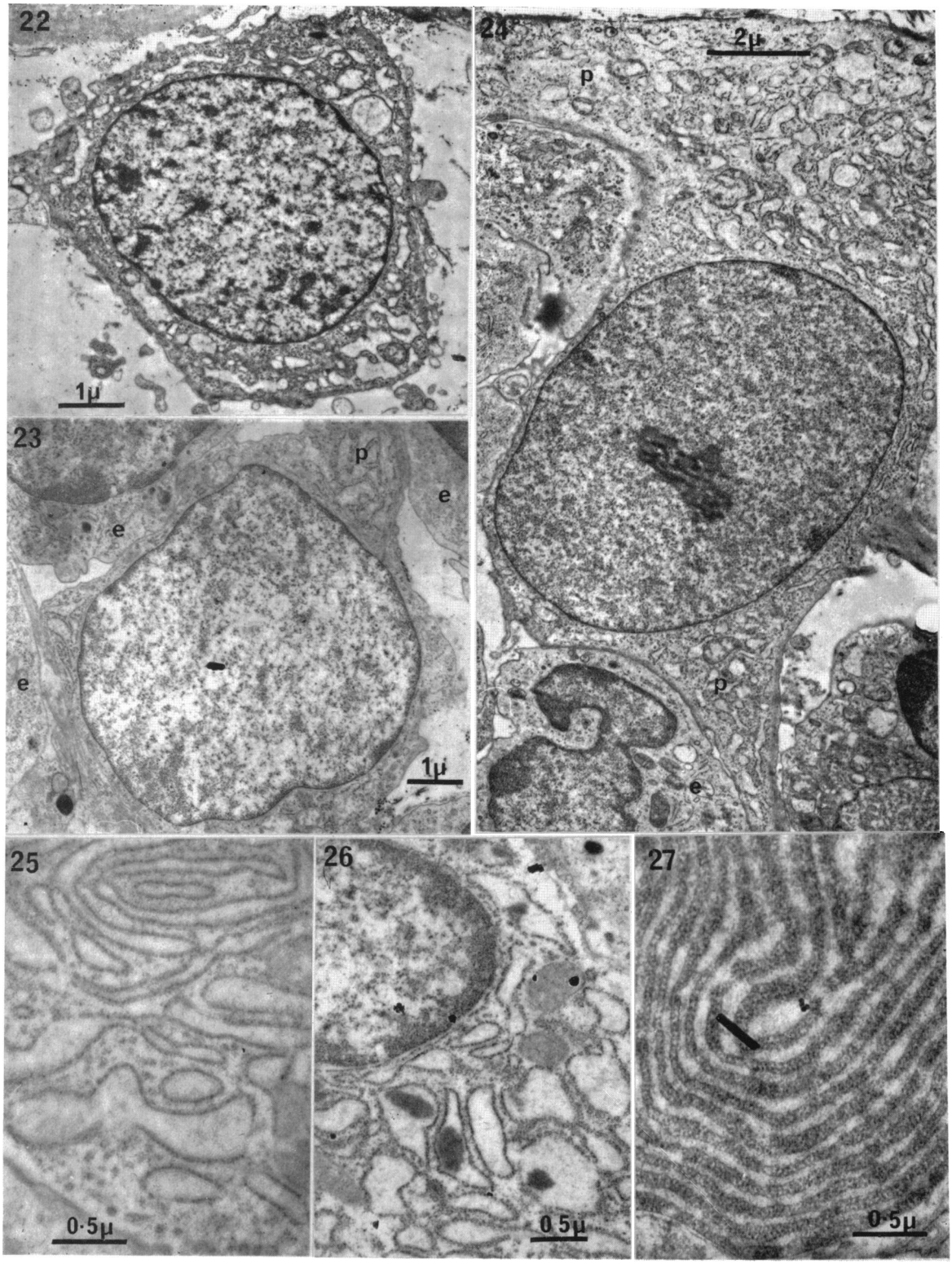
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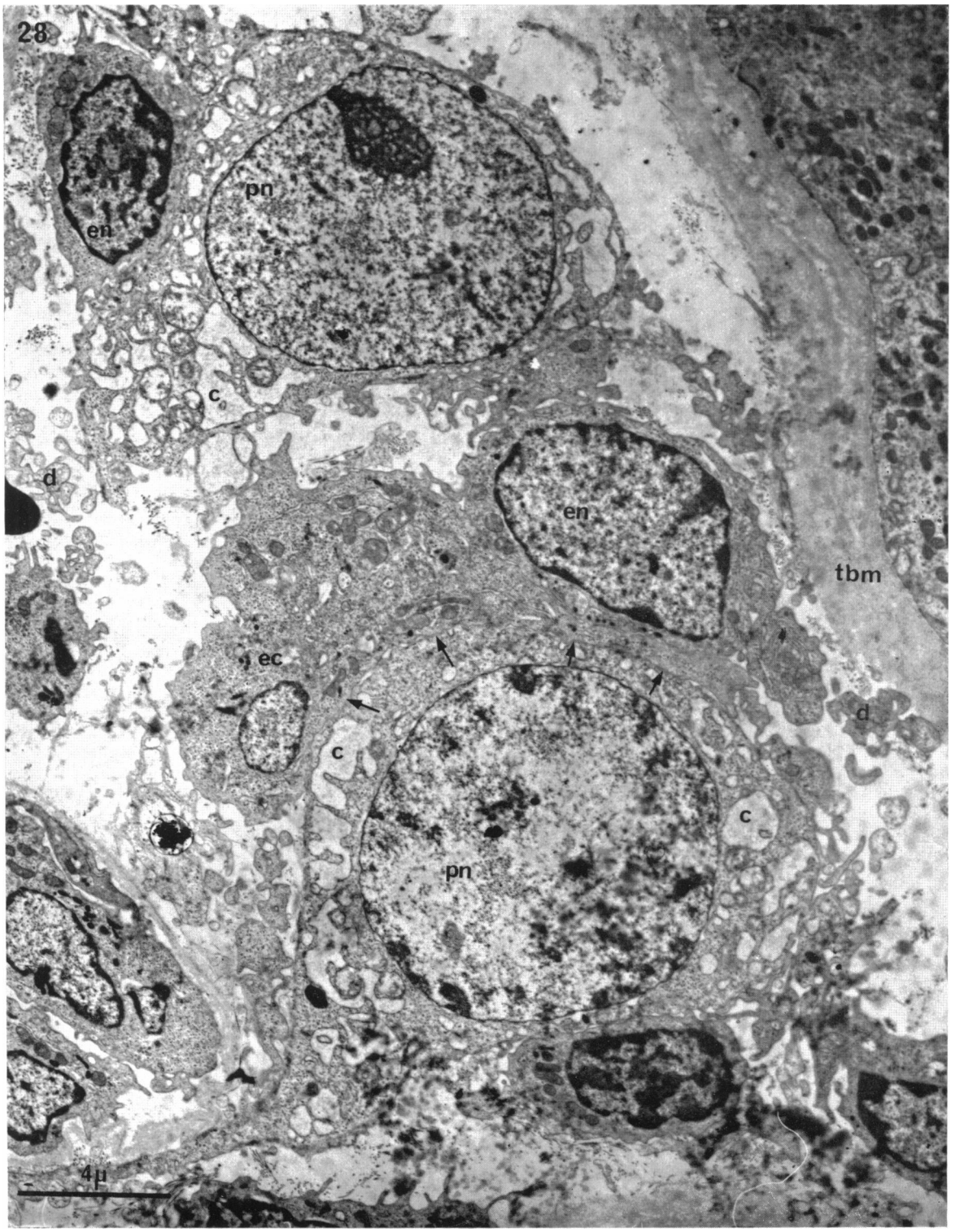
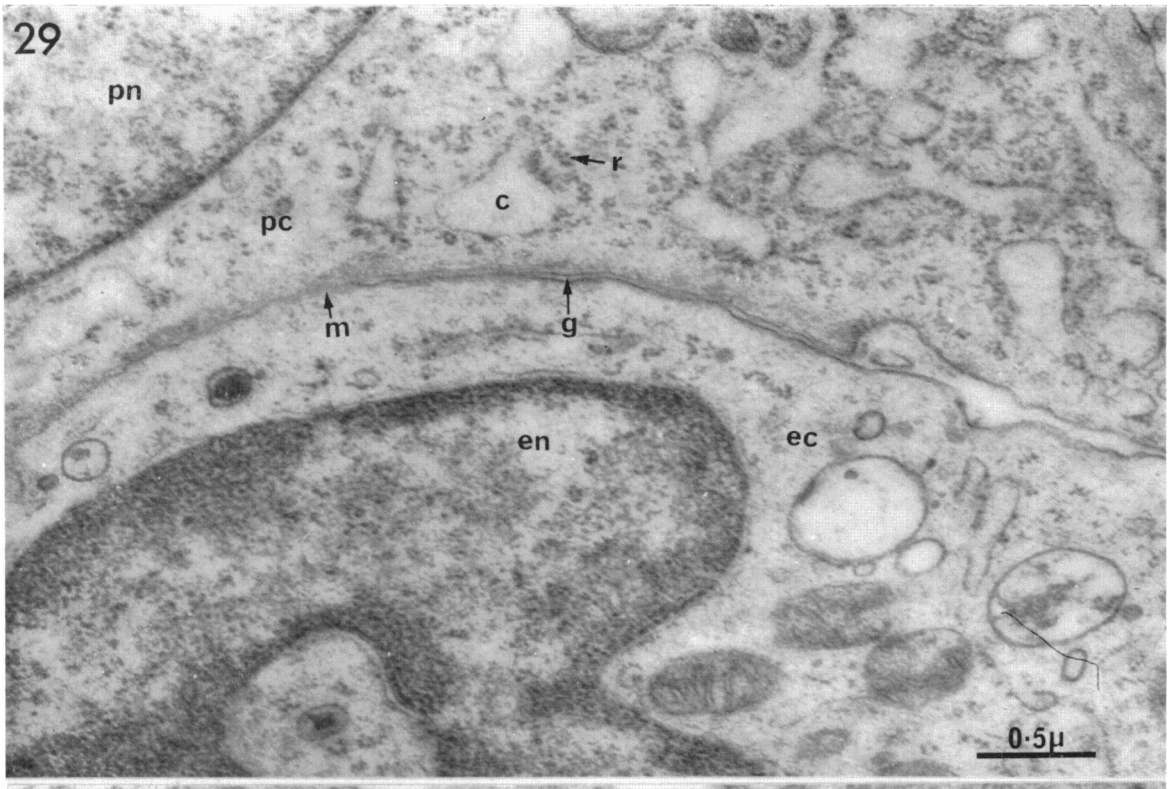


P. L. WILLIAMS AND OTHERS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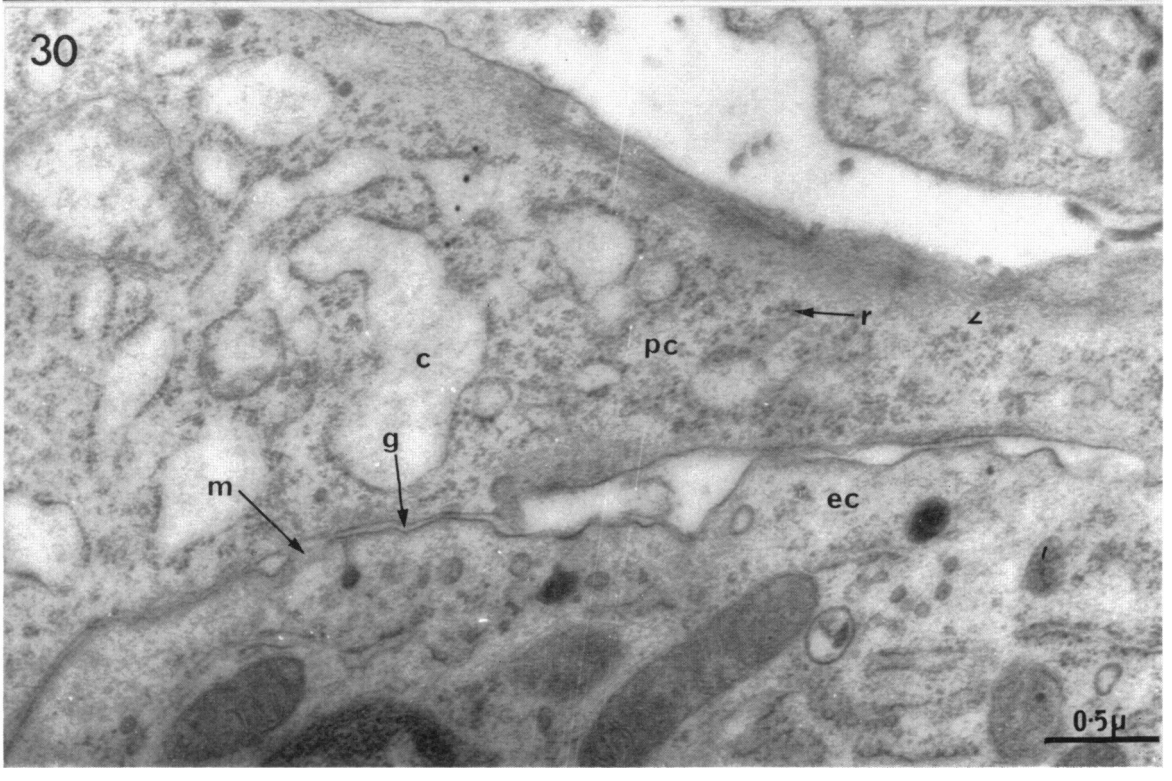
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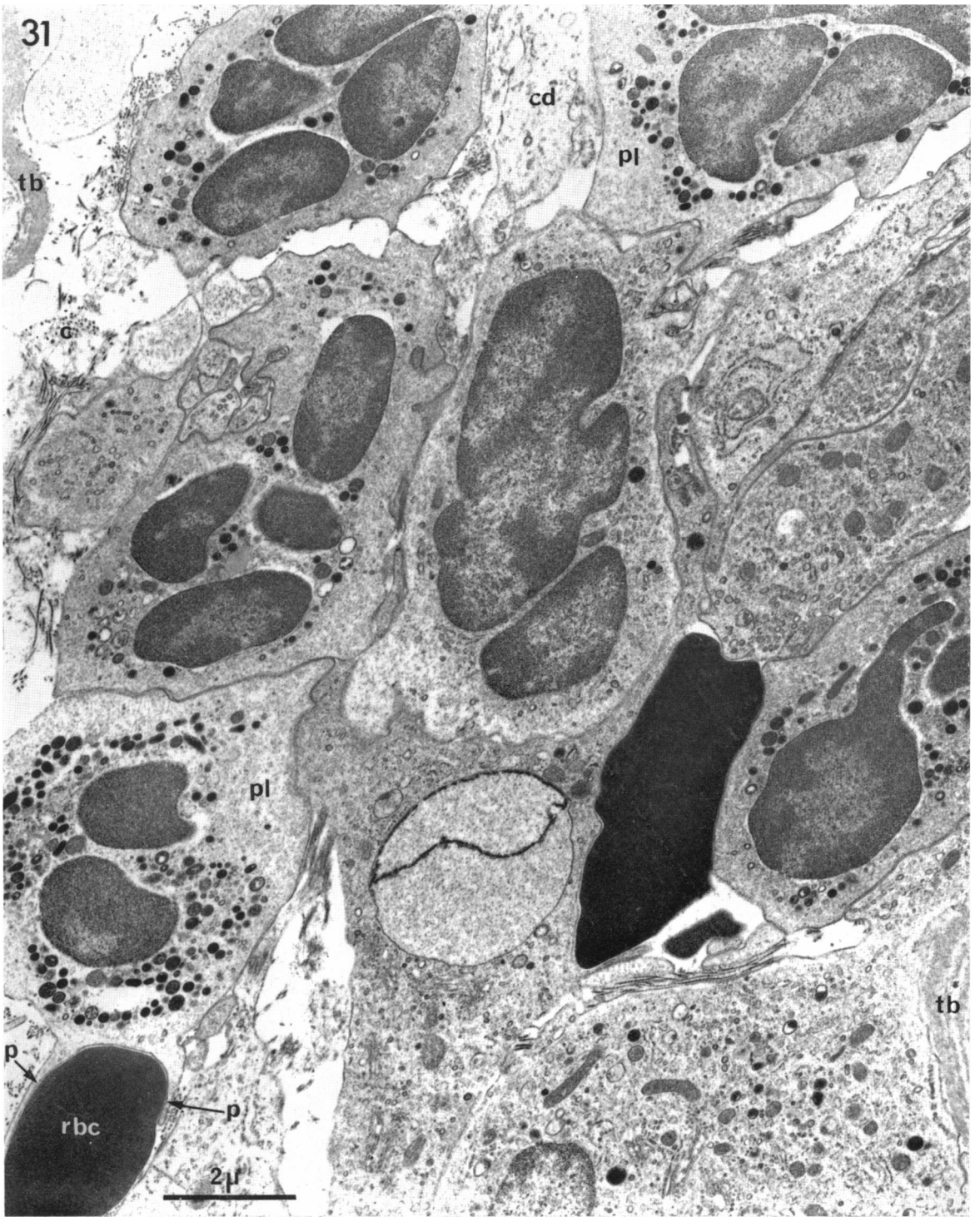
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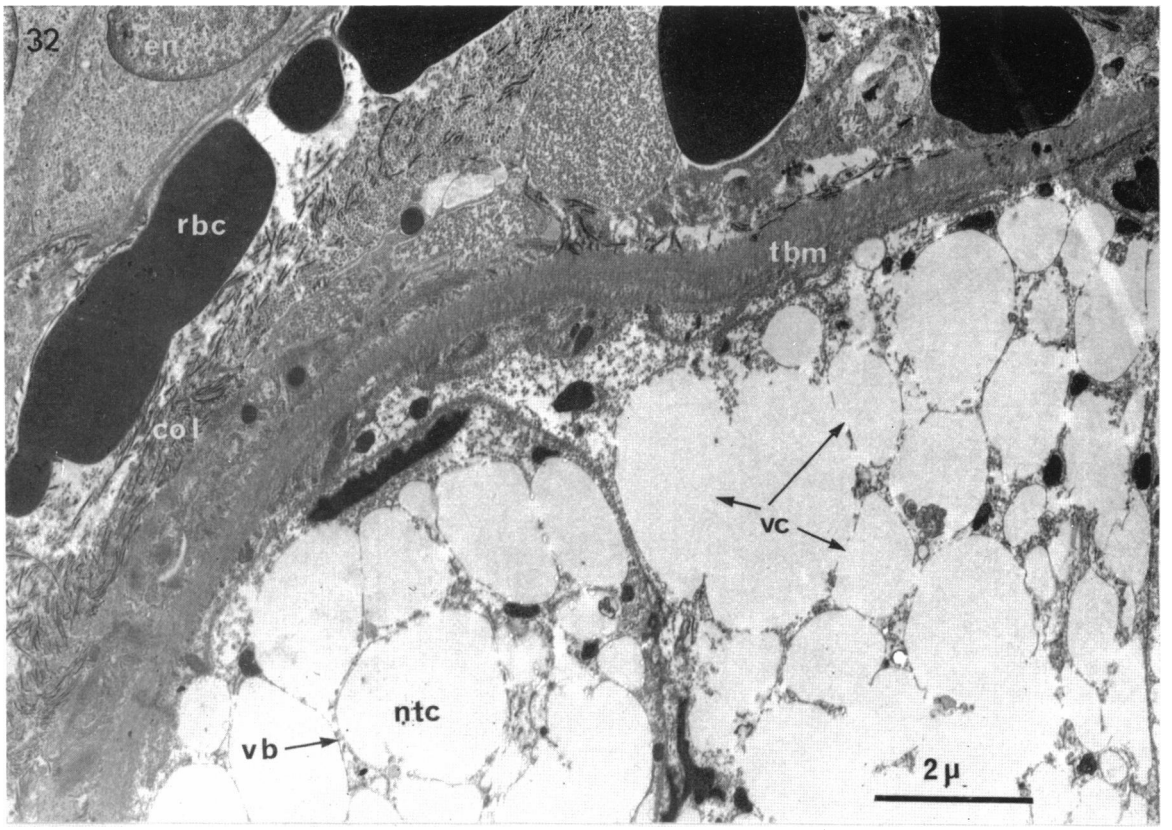
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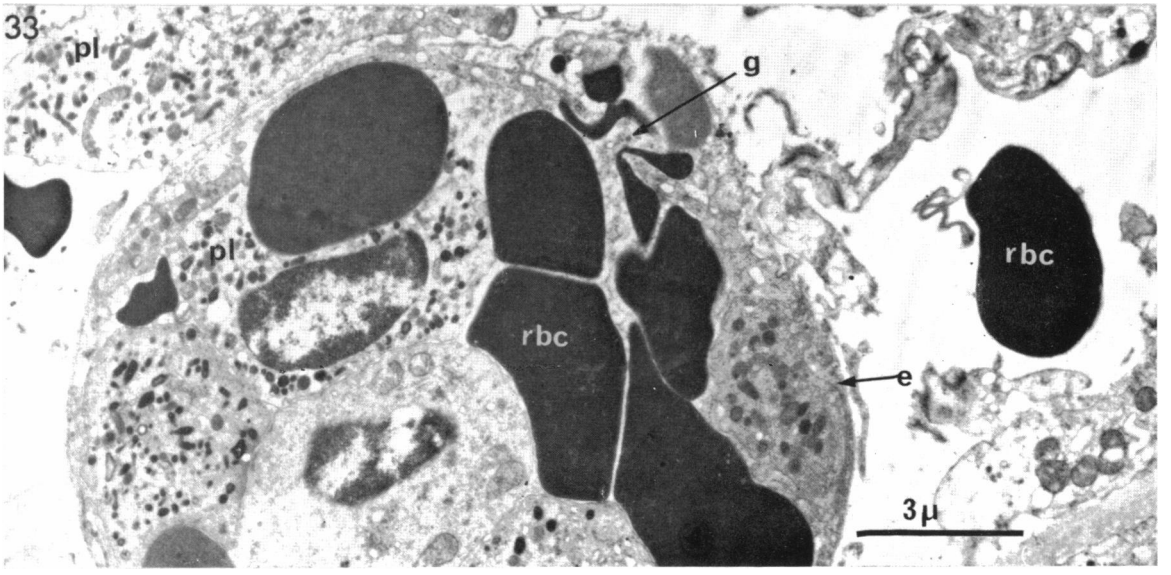
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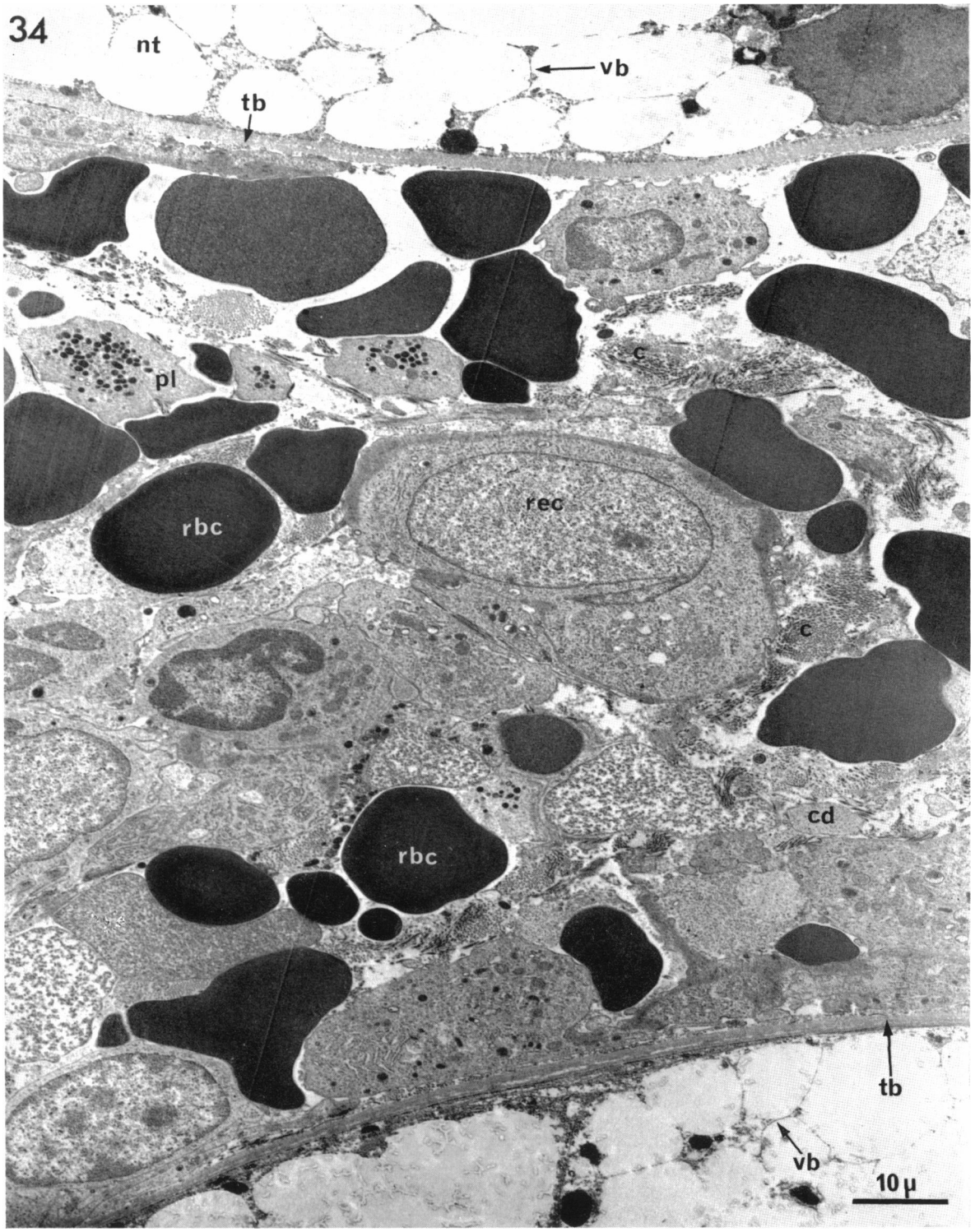
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PLATE 7

Fig. 28. Functioning homotransplant (72 hr.) showing the cellular contents, and relationships, of the intertubular spaces. tbm, Tubular basement membrane; pn, nucleus of immature plasma cell; c, cisternae in cytoplasm of immature plasma cell; en, endothelial cell nucleus; ec, endothelial cytoplasm; d, cytoplasmic débris. Arrows indicate one of the numerous lines of conjunction between endothelial and plasma cell membranes.

PLATE 8

Figs. 29 and 30. Functioning homotransplant (72 hr.) showing close apposition of endothelial cell and immature plasma cell. pn, Plasma cell nucleus; en, endothelial cell nucleus; pc, plasma cell cytoplasm; ec, endothelial cytoplasm; c, cisternae; r, free ribosome rosette; g, 200 Å. gap between adjacent unit membranes; m, ? continuity of cytoplasm.

PLATE 9

Fig. 31. Anuric homotransplant—an intertubular field. tb, Tubular basement membrane; cd, cytoplasmic débris; pl, polymorphonuclear leucocyte; p, pseudopodia; rbc, red blood corpuscles; c, collagen.

PLATE 10

Fig. 32. Anuric homotransplant. tbm, Basement membrane of proximal convoluted tubule; ntc, necrotic tubular cell; vb, vacuolar boundaries; vc, areas of vacuolar coalescence; rbc, extravascular erythrocyte; en, rounded, isolated endothelial cell; coll., collagen.

Fig. 33. Oliguric homotransplant—showing complete blockage of an intertubular vessel. e, Endothelium; rbc, erythrocytes; pl, polymorphonuclear leucocyte; g, endothelial gap with escaping erythrocytes.

PLATE 11

Fig. 34. Anuric homotransplant showing two neighbouring, necrotic proximal convoluted tubules and the contents of the intertubular space. nt, Necrotic tubule; vb, vacuolar boundaries; tb, tubular basement membrane; rbc, erythrocytes; rec, rounded endothelial cell; pl, polymorphonuclear leucocytes; c, bundles of collagen fibres.