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The Relation between Connective Tissue Cells and Intercellular Substances, including Basement Membranes [and Discussion]

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The relation between connective tissue cells and intercellular substances, including basement membranes

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[Plates 14 and 15]

Basement membranes are distributed widely in the body forming an extracellular matrix for epithelial and endothelial cells. The collagenous and glycoprotein constituents of basement membranes are synthesized by these two cell types. Disturbance of the interactions between basement membranes and their associated epithelial and endothelial cells can lead to the pathological changes seen in diseases involving basement membranes. These changes are illustrated here by reference to glomerulonephritis induced by the deposition of immune complexes in the glomerulus of the kidney, and chronic inflammatory changes occurring in the lung after inhalation of asbestos.

In these diseases basement membrane changes can occur in several ways. Hydrolytic enzymes released from inflammatory cells degrade basement membranes while other factors released from these cells may stimulate synthesis of basement membrane constituents by epithelial and endothelial cells. Alternatively the physical separation of epithelial and endothelial cells from their basement membranes by space-occupying substances such as immune complexes can interfere with feedback mechanisms leading to synthesis of basement membrane constituents and cell proliferation. Studies of these pathological changes at a cellular level should shed new light on the ways in which cells interact with their pericellular environment.

No man is an island and still less is each cell of which he is composed. Cells have complicated social relations with one another; for example, collaboration of at least three cell types – macrophages, T and B lymphocytes are required for effective immune responses. Cells also interact with their immediate environment. Among the connective tissue cells, this is most obvious in the case of cartilage, where the chondrocytes are enclosed in a firm matrix. In normal adult cartilage, relatively little matrix is synthesized, but if the matrix is digested away by proteases to release the chondrocytes, each one is turned on to synthesize matrix rapidly. When the cartilage is reformed synthesis again subsides. These observations suggest that the cell can in some way sense when its immediate environment has been disturbed and react by synthesizing more extracellular material. When the environment is restored to normal, synthesis is repressed again. The constituents of connective tissues which effect this type of feedback control have not been identified, but some preliminary results are interesting (Wiebkin & Muir 1973).

Interactions of connective tissue and epithelial cells in embryological differentiation are well known (Grobstein 1964). The role of enzymes secreted by epithelial and mesenchymal cells in connective tissue reorganization occurring during metamorphosis (Eisen & Gross 1965) and regeneration of functional limbs (Grillo, Lapiere, Dresden & Gross 1968) is also well established.

Disturbances of the systems which control synthesis of extracellular constituents are of great importance in pathology – for example, fibrogenesis, cirrhosis of the liver, osteoarthritis and diabetic nephropathy.

Basement membranes form an important part of the pericellular environment of both epithelial and endothelial cells. Contact of basement membranes with these cells almost certainly regulates their metabolism including the synthesis of basement membrane constituents themselves. Changes in the immediate environment of epithelial and endothelial cells cause both quantitative and qualitative alterations in the basement membrane components that they synthesize. Substances released by other cells, such as leucocytes can damage basement membranes and this disturbs the pericellular environment, thereby bringing about changes in the synthetic activity of the cells. The deposition of macromolecular aggregates such as immune complexes in basement membranes can also change the pericellular environment leading to activation of associated cellular elements. After describing briefly the structure and composition of basement membranes, we will discuss how disturbances of the pericellular environment can lead to changes in the structure and function of basement membranes. This will be illustrated by reference to diseases affecting the basement membranes in the glomeruli of the kidney and alveoli of the lung.

The structure and composition of basement membranes

Basement membranes are distributed throughout the body, forming an extensive extracellular matrix which supports almost all endothelial and epithelial cells. Ultrastructural studies have shown basement membranes to contain partially orientated networks of fibrils approximately 3–4 nm in diameter embedded in an otherwise amorphous material (Farquhar 1960; Palade & Bruns 1964; Jakus 1964). The strong reactivity of basement membranes with periodic acid-Schiff reagent indicated the presence of carbohydrate (McManus 1948; Leblond, Glegg & Eidinger 1957). This was confirmed chemically by Goodman, Greenspon & Krakower (1955) who demonstrated the present of hexose, fucose and hexosamine in glomerular basement membranes. The presence of collagenous protein in basement membranes was also suggested by these authors who detected significant amounts of hydroxyproline in preparations of basement membranes. The complete amino acid and carbohydrate composition of basement membranes from various sources (Kefalides 1973), has shown them to be rich in hydroxyproline, glycine and hydroxylysine which is consistent with the presence of collagen. However, in addition to glucose and galactose – the only two hexoses identified in mammalian collagens – basement membranes contain fucose, mannose, hexosamines and sialic acid.

Basement membrane components are synthesized by either epithelial or endothelial cells. Collagen is synthesized as a precursor which is cleaved by an endopeptidase before release from the cell (Lapiere, Lenaers & Kohn 1971; Bornstein, Ehrlich & Wyke 1972).

The total proline and hydroxyproline content of various basement membranes ranges from 14 to 18 %, considerably less than the value of 22 % given for mammalian collagens (Kefalides 1973). On the other hand basement membranes contain 3–5 times as much hydroxylysine as collagen. Basement membranes are rich in carbohydrates, varying from 11 % in the glomerulus to 14 % in the anterior lens capsule. Neutral hexose units in basement membrane collagen are the disaccharide glucosylgalactose and the monosaccharide galactose linked by an *O*-glycosidic bond to hydroxylysine. The mechanism by which these carbohydrate residues become attached to hydroxylysine has been outlined by Spiro (1973) who has identified various glycosyl

transferases in cells which synthesize basement membrane constituents (Spiro & Spiro 1971; Spiro 1974). The α-chain of basement membrane collagen is longer than that of interstitial-type collagen and Kefalides (1973) has suggested that it represents procollagen released into the extracellular space without being cleaved by the tissue endopeptidase described by Lapiere *et al.* (1971) and Bornstein *et al.* (1972).

In addition to collagen, basement membranes contain soluble glycoproteins which lack hydroxyproline, hydroxylysine and glucose (Kefalides 1973). These glycoproteins contain galactose, mannose, hexosamines, fucose and sialic acid. The sequence of these carbohydrates and the nature of their linkage to the protein is not known. Immunochemical studies have shown that at least two determinants in addition to collagen are present in basement membranes. These are thought to be representative of a low and a high molecular mass glycoprotein described by Kefalides (1972). The nature of the interaction between these glycoproteins and collagen is not known but molecular models have been proposed by Kefalides (1971, 1973).

Function of basement membranes

Two major functions of basement membranes are apparent. First they form an extensive extracellular matrix which supports epithelial and endothelial cells. Secondly they provide a continuous barrier preventing certain macromolecules from crossing capillary walls. The filtration capacity of basement membranes is determined by the structural organization of their constituent molecules. In the glomerulus proteins having the dimensions of ferritin accumulate in the subepithelial region of the basement membrane (Farquhar 1960) while horseradish peroxidase of molecular mass 40000 traverses the membrane to reach the urinary space (Graham & Karnovsky 1966).

Changes in basement membranes during disease

Structural and functional alterations in basement membranes have been observed in a number of diseases including several types of glomerulonephritis, systemic lupus erythematosus, renal vein thrombosis and diabetes mellitus. The most striking change in many of these diseases is a greatly increased thickness of the glomerular basement membrane, suggesting increased synthesis or decreased degradation of the basement membranes' constituents. Little information is available on changes in composition of basement membranes in many of these conditions; only diabetes mellitus has been studied extensively, from this point of view. In this disease thickening of the glomerular basement membrane is observed together with an increase in mesangial matrix. These changes are often related to the duration of the disease and are associated with increased glomerular permeability and significant proteinuria. Beisswenger & Spiro (1973) have isolated basement membranes from normal and diabetic human glomeruli and performed a detailed analysis of the glycoprotein material. Diabetic basement membranes contain significantly more hydroxylysine and glycosylgalactose disaccharides linked to this residue. There is a decrease in lysine content so that the total amount of lysine and hydroxylysine is the same as in normal basement membranes. Lesser changes in the levels of hydroxyproline, glycine, valine and tyrosine occur; the number of heteropolysaccharide units remain constant. These observations showed that there is an increase in the hydroxylation of lysine residues in the basement membranes of diabetics which leads to the overproduction of glycosylgalactose residues. This alteration in subunit production may in turn lead to the defective packing of the peptide chains of basement membranes resulting in the glomerular permeability which is

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observed in diabetics. Recent studies in alloxan-diabetic rats (Spiro & Spiro 1971) have shown an increased activity of the glucosyl transferase involved in the synthesis of the hydrolysine-linked disaccharide while the activity of the galactosyltransferase required for the assembly of heteropolysaccharide units is unaltered. Treatment of the diabetic animals with insulin restores the glycosyl transferase activity to normal with a consequent slowing down of basement membrane synthesis. Beisswenger & Spiro (1973) have suggested that certain substances, the levels of which are controlled by insulin, can play a role in regulating the post-ribosomal steps such as hydroxylation of lysine and carbohydrate attachment involved in the assembly and cellular export of basement membrane components. Diabetes may therefore be an example of a disease where a general metabolic defect causes an increased synthesis of abnormal basement membrane subunits which are functionally deficient.

Recently, Westberg & Michael (1973) and Kefalides (1974) have failed to confirm the findings of Beisswenger & Spiro (1973). No increases in hydroxylysine or glucosylgalactose content of basement membranes isolated from kidneys of diabetic patients was found. No explanation for this discrepancy can be offered and further studies are required. It is possible that the different methods used for the preparation of basement membranes may yield products of differing compositions. Simultaneous analysis of basement membranes prepared from the same starting materials by the two methods should resolve this possibility.

The deposition of immune complexes in basement membranes in the kidney

The deposition of complexes results in increased synthesis and abnormal function of glomerular basement membranes. Small soluble immune complexes may pass through the basement membrane to accumulate at the epithelial side or alternatively larger, insoluble complexes are deposited in a subendothelial position (Germuth & Rodriguez 1973). The site of deposition of immune complexes has a decisive influence on the changes seen in the glomerulus. Complexes which traverse the basement membrane induce prominent changes in epithelial cells while those that are deposited in a subendothelial position induce changes in endothelial and mesangial cells.

The deposition of immune complexes in the glomerulus can change the pericellular environment in at least three ways. First, they can recruit inflammatory cells by generating pharmacological mediators of acute inflammation. This occurs through fixation of complement by immune complexes with the consequent formation of chemotactic fragments which attract polymorphonuclear leucocytes to the site of deposition. Polymorphonuclear leucocytes contain enzymes which degrade basement membrane proteins (Cochrane & Aikin 1966; Janoff 1972) and interaction with immune complexes results in the release of lysosomal enzymes (Henson 1972) from these cells. Certain types of glomerulonephritis illustrate this type of change. Acute glomerular injury induced by injection of anti-basement membrane serum results in the accumulation of large numbers of polymorphonuclear leucocytes in the glomerulus with the rapid development of proteinuria (Cochrane, Unanue & Dixon 1965). Analysis of the urine shows the presence of glomerular basement membrane material and acid proteinase, a lysosomal enzyme able to degrade this material (Hawkins & Cochrane 1968). Similar changes in the glomerulus are also seen in experimental autoimmune glomerulonephritis induced by immunization with heterologous glomerular basement membrane antigens (Steblay 1962). In both these types of glomerulonephritis immune complexes are formed *in situ* by the interaction of circulating antibodies with basement membrane antigens. If the antibodies are of the

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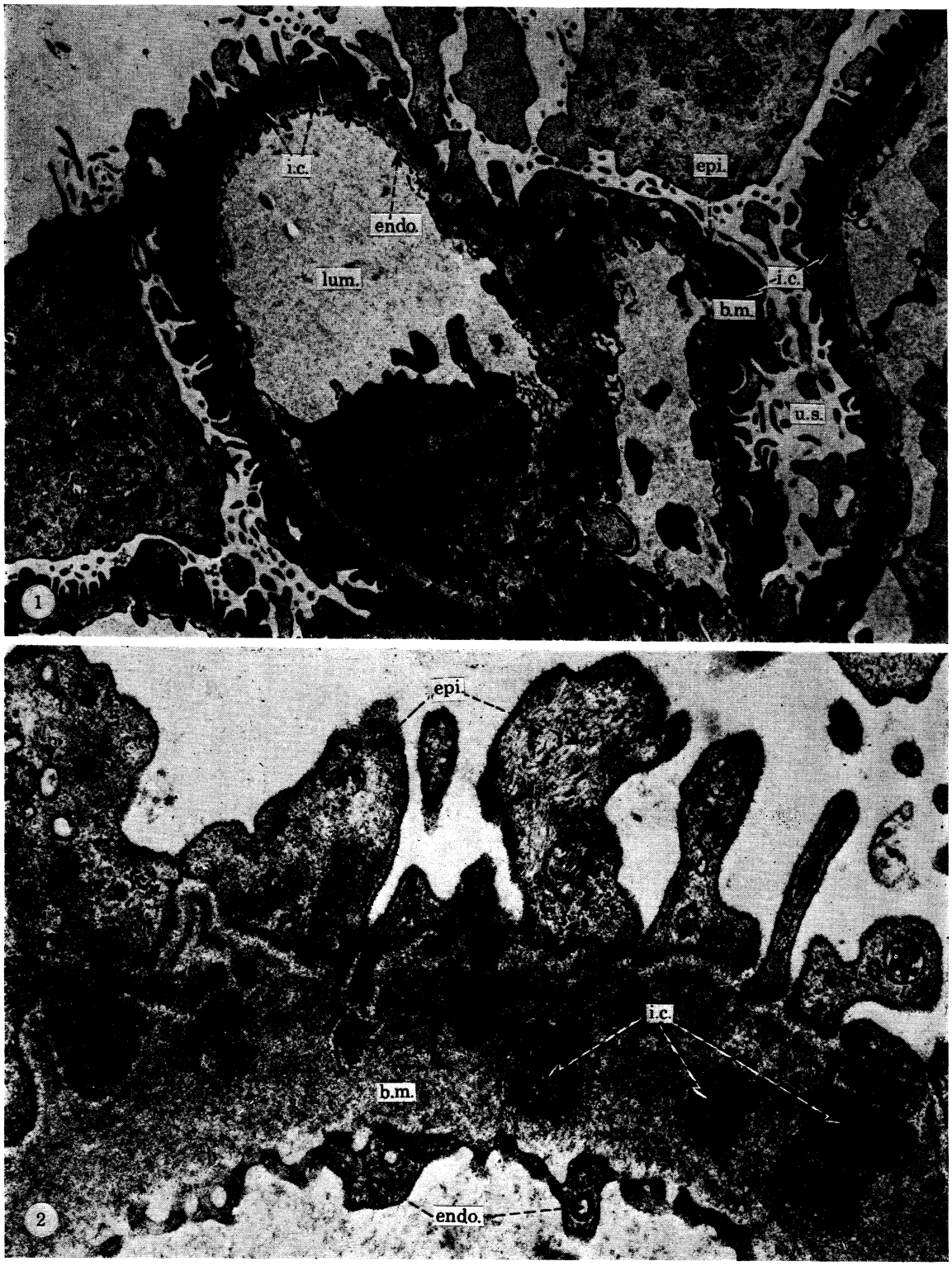

FIGURE 1. Electron micrograph of a renal biopsy from a Nigerian child with nephrotic syndrome showing the deposition of immune complexes (i.c.) in the basement membrane (b.m.). Note slight thickening of the basement membrane and abnormality of epithelial cell (epi.) morphology. The basement membrane separates the lumen (lum.) of the glomerular capillary lined by a layer of endothelial cells (endo.) from the urinary space (u.s.). (Magn. × 6500.)

FIGURE 2. Electron micrograph of a renal biopsy from a Nigerian child with nephrotic syndrome. Detail of immune complex deposition in the basement membrane. (Magn. × 36 500.)

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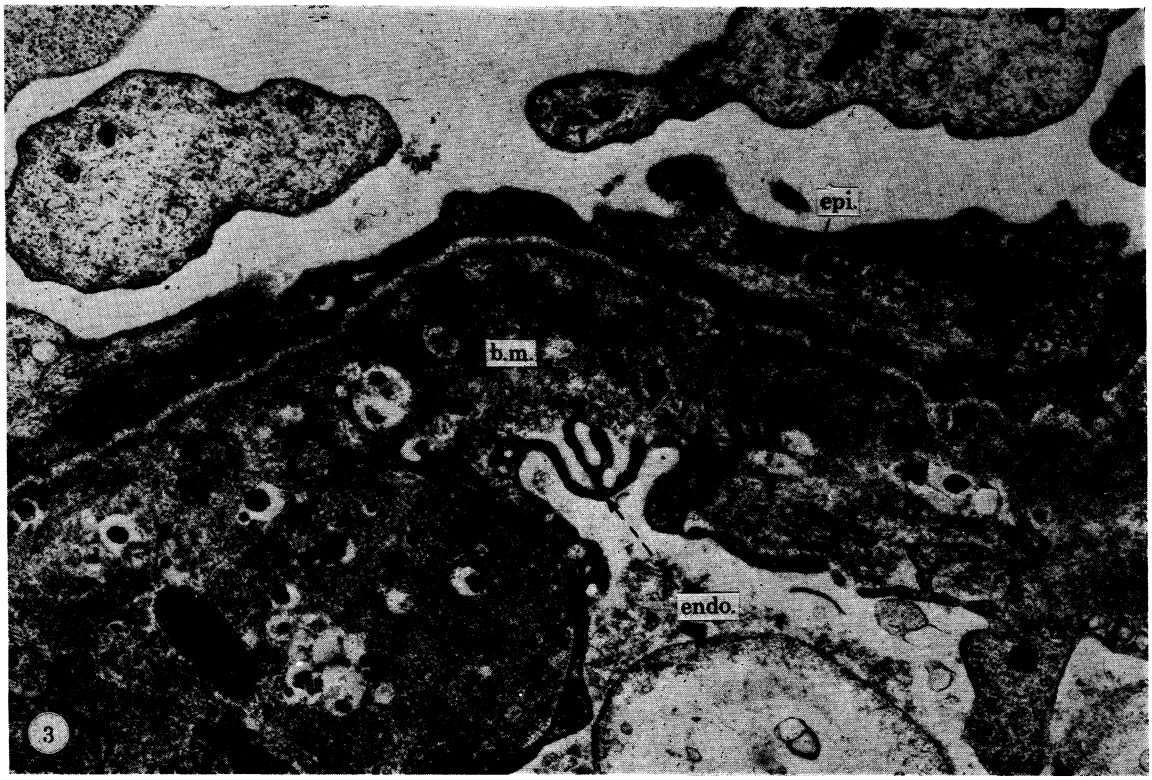

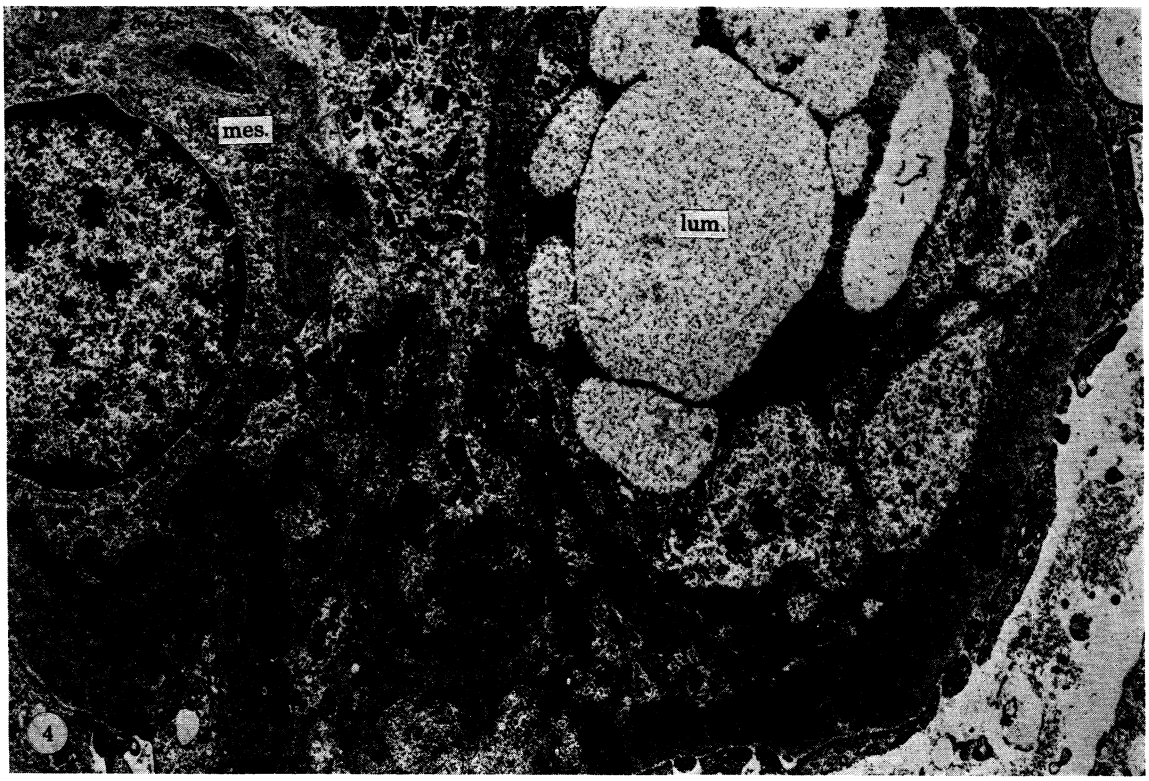

FIGURE 3. Electron micrograph of a renal biopsy from a Nigerian child with nephrotic syndrome. Note thickening of basement membrane and abnormalities in endothelial cell structure. (Magn. ×18000.)

FIGURE 4. Electron micrograph of a renal biopsy from a Nigerian child with nephrotic syndrome showing advanced disease with marked proliferation of mesangial cells (mes.) partially occluding the capillary lumen. (Magn. ×10000.)

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complement fixing type generation of pharmacological mediators with recruitment of polymorphonuclear leucocytes can occur.

In other types of glomerulonephritis immune complexes form at sites distant to the glomerulus so that complement fixation does not occur in the kidney. In these cases infiltration of polymorphonuclear leucocytes would not be expected. Studies in human glomerulonephritis of various unknown etiologies support this possibility (Allison *et al.* 1969; Houba *et al.* 1970; Germuth & Rodriguez 1973). In addition recent studies of experimental autoimmune glomerulonephritis show that not all the antibodies deposited in the kidney are complement-fixing (Couser, Stiltman & Lewis 1973).

Disturbances of the pericellular environment by deposited immune complexes

These observations suggest that another mechanism, independent of inflammatory mediators and polymorphonuclear leucocytes may be acting to account for the changes found in the glomerulus in the immune complex nephropathy. The accumulation of space occupying substances such as immune complexes, would lead to a physical separation of either epithelial cells or endothelial from the basement membrane. This separation may stimulate these cells to synthesize connective tissue constituents. Thus immune complexes which penetrate the basement membrane would be expected to stimulate epithelial cells while large complexes which fail to penetrate the basement membrane would stimulate endothelial cells. Physiological and ultrastructural observations suggest that the penetration of macromolecules into the glomerular basement membrane depends mainly on their size (Oliver & Essner 1972). Substances with molecular masses varying from 80000 to 250000 traverse the basement membrane and accumulate in a subepithelial situation while larger molecules remain in a subendothelial position.

Renal biopsies from West African children with nephrotic syndrome associated with malaria have shown that there is localized or diffuse thickening of glomerular capillary walls, an increase in periodic acid-Schiff positive material in the basement membrane and an increase in the number of mesangial cells. Immune complexes and complement have been detected in the glomeruli by immunofluorescence techniques (Allison *et al.* 1969; Ward & Kibukamusoke 1969; Houba *et al.* 1970). They are deposited in one of two patterns or a combination of both. In one the deposits are in the form of coarse granules which contain C3, IgM and IgG_3. The other deposits contain IgG but no IgM or complement. It is notable that clinical prognosis was less favourable in those patients in which no complement was demonstrable in the glomeruli (Allison & Houba 1974). The absence of complement, a factor chemotactic for polymorphonuclear leukocytes, indicates a mechanism of tissue alteration independent of granulocytes. This suggests that the immune complexes exert their effects within the glomerulus by means other than the generation of pharmacological mediators.

The ultrastructure of glomeruli from Africans with nephrotic syndrome has been described in detail elsewhere (Allison *et al.* 1969; Houba *et al.* 1970; Hendrickse *et al.* 1972). Immune complexes are deposited within the basement membrane (figure 1, plate 14). Deposition of immune complexes in a subepithelial position (figure 2, plate 14) results in marked changes in the morphology of epithelial cells. Prominent among these is the fusion of podocytes which normally show regularly interrupted contact with the basement membrane. When immune complexes are deposited in a subendothelial position there is a focal thickening of the basement membrane (figure 3, plate 15) and an increase in the amount of cytoplasm, polyribosomes and other organelles within endothelial cells. It would be of great interest to obtain information on

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the capacity of these cells to synthesize basement membrane constituents. The cellular changes described above may reflect increased synthesis of basement membrane constituents due to the disturbances of the pericellular environment by the deposition of immune complexes. It is possible that constituents of basement membrane may inhibit the synthetic activity of the endothelial cells in a manner similar to that by which hyaluronic acid reduces the incorporation of sulphate into proteoglycan by chondrocytes (Wiebkin & Muir 1973). As a first approximation it may be supposed that the cells continue to synthesize basement membrane until a layer of sufficient thickness separates the endothelial and epithelial cells. Then a feedback inhibition system comes into play, preventing the further synthesis while normal contact of the plasma membrane and basement membrane is maintained. However, if immune complexes or other extraneous materials are inserted between the plasma membrane and basement membrane, the feedback ceases to operate and the cells are again stimulated to synthesize basement membrane.

The interaction of immune complexes with mesangial cells

The existence of a third cell type in the glomerulus was established by the classical study of Farquhar & Palade (1962). Mesangial cells are found on the luminal side of the glomerular basement membrane, but not usually in contact with the capillary lumen. They have long cytoplasmic processes which extend along the inner surface of the basement membrane. The cells are embedded in a spongy material which can be distinguished from basement membrane by its looser texture, lower density and the presence of small bundles of fine fibres. Farquhar & Palade (1962) found that mesangial cells endocytose high molecular mass tracers such as ferritin, colloidal gold and thorotrast which localized in the subendothelial region of the glomerular basement membrane after intravenous injection. The tracers accumulate in membrane bounded vesicles which undergo progressive condensation to form dense bodies. The accumulation of the high molecular mass tracers by the mesangial cells is accompanied by the formation of an increased number of free and membrane attached ribosomes suggesting an overall increase in cellular activity. Farquhar & Palade (1962) concluded that one function of the mesangial cell is to ingest and dispose of high molecular mass substances, including immune complexes which are deposited on the glomerular basement membrane. This function is aided by their long cytoplasmic processes which 'sweep' along the basement membrane, recognize and endocytose deposited macromolecules.

It is therefore clear that mesangial cells share some of the functional properties of the cells of the reticulo-endothelial system. Mesangial proliferation occurs where high molecular mass immune complexes are deposited in a subendothelial position. When these deposits induce severe changes, the mesangial cells show marked cellular activation with an increase in mesangial matrix and an ability to penetrate damaged capillaries (figure 4, plate 15).

Recent studies by Mauer, Fish, Blau & Michael (1972) and Mauer, Fish, Day & Michael (1974) have provided further evidence for the uptake and degradation of macromolecules by mesangial cells. The administration of nephrotoxic serum to rats causes a marked increase in the uptake of ^{125}I-labelled aggregated human immunoglobulin G. This increase is apparent at low dosages of the nephrotoxic serum which do not cause glomerular damage as indicated by the absence of proteinuria or significant light-microscopic changes.

The uptake of immune complexes by the cells of the reticulo-endothelial system has been shown both *in vivo* (Benaceraff, Sebastyen & Cooper 1959) and *in vitro* (Steinman & Cohn 1972). One of the consequences of the phagocytosis of immune complexes by mononuclear

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phagocytic cells *in vitro* is the selective release of lysosomal acid hydrolases (Cardella, Davies & Allison 1974). This phenomenon has been observed by exposing mouse peritoneal mononuclear phagocytes to various types of immune complexes formed at equivalence (Cardella *et al.* 1974). The release of enzyme is dependent on time (figure 5) and also the concentration of immune complexes (figure 6). Enzyme release is seen within 1 h of exposure to immune complexes and continues for at least 24 h. Selective release of acid hydrolases is seen with immune complexes at concentrations of 5 μg/ml of antigen and above (figure 5). The release of lysosomal enzymes was shown to be selective in nature by measuring the distribution of a cytoplasmic marker enzyme, lactate dehydrogenase between cells and culture medium. In no instance was the level of lactate dehydrogenase in the medium of the cultures exposed to immune complexes increased above the control values. Also, cellular levels were not decreased in treated cultures, indeed cultures exposed for 24 h show significant increases in enzyme activity at certain concentrations of immune complexes (figure 7).

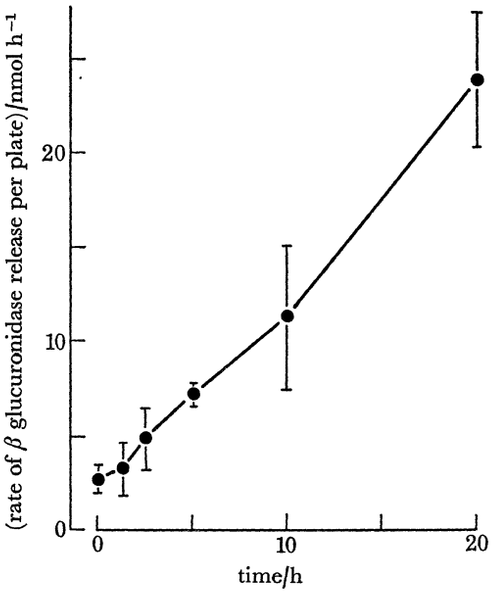

FIGURE 5. The time dependence of mononuclear phagocyte lysosomal enzyme release induced by immune complexes. (Reproduced with permission from *Nature, Lond.* **247**, 46–48 (1974).)

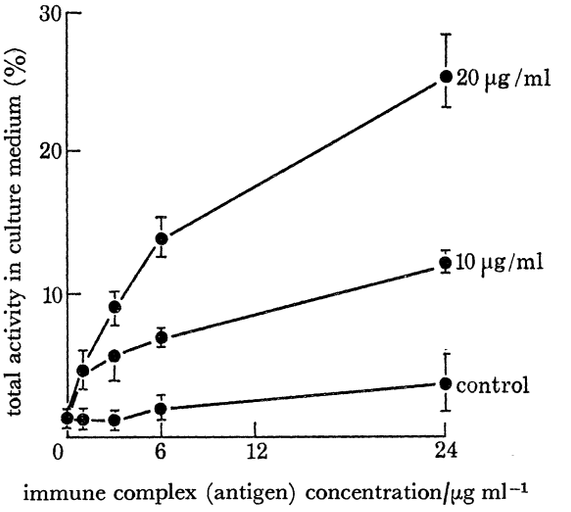

FIGURE 6. The release of a lysosomal enzyme (β-glucuronidase) from mononuclear phagocytes exposed to increasing concentrations of immune complexes. (Reproduced with permission from *Nature, Lond.* **247**, 46–48 (1974).)

The addition of antigen or antibody alone does not cause selective release of acid hydrolases at any of the concentrations used. It is also clear that other biologically inert substances such as latex or anatase do not cause lysosomal enzyme release from mononuclear phagocytes (Davies, Page & Allison 1974). However, several substances known to induce chronic inflammation such as Group A streptococcal cell walls (Davies, Page & Allison 1974; Page, Davies & Allison 1974), dental plaque (Page, Davies & Allison 1973) and carrageenan (Davies, Allison, Dym & Cardella 1974) bring about selective release of lysosomal enzymes in a manner similar to immune complexes. Since several of the lysosomal enzymes present in mononuclear phagocytes are known to degrade connective tissue constituents (table 1), we have suggested that their selective release may account for certain aspects of the tissue damage that is seen in chronic

inflammation (Allison & Davies 1974). Because mesangial cells are involved in the clearance of immune complexes in the glomerulus, release of their acid hydrolases could also lead to degradation of basement membrane constituents. Such a release from mesangial cells could occur during the endocytosis of immune complexes into their pseudopodia which are in continuous contact with the glomerular basement membrane. Such a possibility is supported by the findings of Mauer *et al.* (1973), that the formation of immune complexes within the glomerular mesangium by sequential injection of antigen and antibody into experimental animals leads to a glomerulonephritis involving the mesangium and endothelium.

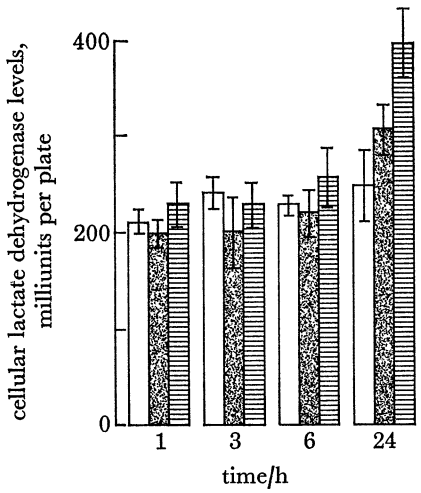

FIGURE 7. The levels of lactate dehydrogenase in mononuclear phagocytes exposed to immune complexes. (Reproduced with permission from *Nature, Lond.* **247**, 46–48 (1974).) □, control; ▦, immune complexes, 20 μg/ml; ▤, immune complexes, 10 μg/ml.

TABLE 1. TISSUE-DEGRADING ACID HYDROLASES IN MACROPHAGES

tissue component	enzyme	reference
collagen	collagenase	Wahl, Wahl, Martin & Mergenhagen (1973)
	cathepsin B1	Davies, Finlay & Allison (1974)
protein polysaccharides	hyaluronidase	Goggins, Lazarus & Fullmer (1968)
	cathepsin B1	Davies, Finlay & Allison (1974)
	acid proteinase	Cohn & Wiener (1963)
elastin	elastase	Janoff (1972)
basement membranes	acid proteinase	Cohn & Wiener (1963)
C5	neutral proteinase	Snyderman, Shin & Dannenberg (1972)
hormones	cathepsin C	Davies, Finlay & Allison (1974)
alveolar surfactant	phospholipases	Franson & Waite (1973)

Not all the products released by mononuclear phagocyte cells cause tissue damage and degradation, and a similar situation with mesangial cells may explain the proliferative changes seen in certain kinds of glomerulonephritis. Indeed, in the experimental model described by Mauer *et al.* (1973) endothelial cell proliferation is one of the consequences of the formation of immune complexes within the mesangial cells. Factors that stimulate cell proliferation may be lysosomal or non-lysosomal in origin. Polymorphonuclear leucocyte lysosomes are known to stimulate synovial proliferation when injected intra-articularly (Weissmann, Spilberg & Krakauer 1969) while the addition of cell fractions rich in lysosomal enzymes to cells cultured

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in vitro results in accelerated growth (Ryan & Cardin 1967). It has been claimed that mononuclear phagocytes exposed to toxic particles such as silica, release fibrogenic materials (Heppleston & Styles 1967; Kilroe-Smith, Webster, van Drimmelen & Marasas 1973; Burrel & Anderson 1973). It is also clear that humoral factors from mononuclear phagocytes promote the growth and division of multipotential stem cells in bone marrow (Golde, Finley & Cline 1972; Chervenik & Lo Buglio 1972). The various ways in which mesangial cells can influence the cells of the glomerulus and extracellular environment is summarized in figure 8. It has not yet been possible to isolate glomerular mesangial cells so that their reactivity to macromolecular substances can be analysed.

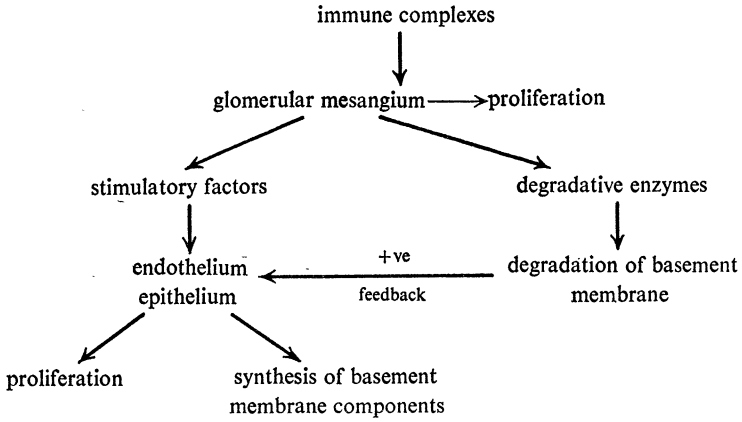

FIGURE 8. Schematic representation of the role of the mesangial cell in disturbances of the pericellular environment of the glomerulus induced by immune complexes.

Changes in the pericellular environment affecting basement membranes in the lung

As in the kidney, changes in the pericellular environment in the lung can lead to the degradation or synthesis of basement membrane components. Three main cell types are present in the alveoli. The type I epithelial cell is difficult to visualize except by electron microscopy because of its attenuated, well-spread cytoplasm. Its flattened nuclei are similar to those of the endothelial cells, usually being situated at the junction between the alveolus and its capillary. The type II epithelial cell is more rounded and contains numerous mitochondria and lysosomes. This cell is thought to produce lung surfactant. Alveolar mononuclear phagocytes are derived from peripheral blood monocytes or from precursors resident in the lung. These cells ingest and dispose of a variety of foreign particles which enter the lung. In some instances, failure to carry out this function results in damage to the alveolus with either degradation or thickening of the basement membrane (Spencer 1968).

The inhalation of a wide variety of toxic particles results in an inflammatory response leading to structural changes in the alveolus. In recent years there has been much interest in the lung lesions induced by asbestos since this particle constitutes an all too frequent environmental hazard which can produce respiratory disease, bronchogenic carcinoma and mesotheliomas of the pleural cavity (Whipple 1965). The pathology of the asbestosis is characterized by a mononuclear cell granuloma with giant cell formation, fibrogensis, an increase in the size of epithelial cells and thickening of the alveolar basement membrane.

It is thought that the initial event after the arrival of asbestos particles in the lung is their attachment to and phagocytosis by alveolar mononuclear phagocytes (Allison 1968; Suzuki, Churg & Ono, 1972). The toxic effects of asbestos on mononuclear phagocytes has been

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described by Allison (1968) and Miller & Harington (1972). Recently we have found that small amounts of asbestos release large quantities of acid hydrolases from mononuclear phagocytes without any detectable loss of cell viability. Mouse peritoneal mononuclear phagocytes exposed to various concentrations of asbestos for 24 h show a concentration-dependent increase in lysosomal enzyme activity in the culture medium, a drop in the cellular level and no overall change in the total amount (figure 9). This release of enzyme is selective since there is no release of lactate dehydrogenase in cultures exposed to any of the concentrations of asbestos used. In addition there are significant increases in cellular levels of lactate dehydrogenase in cultures exposed to concentrations of asbestos greater than 2 μg/ml. After an initial lag period of 3 h lysosomal enzyme release occurs in a rapid time-dependent manner (table 2) so that by 24 h 62.8 ± 1.7 % of total enzyme activity is in the culture medium compared to 22.9 ± 3.5 % in control cultures. The initial delay in enzyme release is probably due to the protective effect of serum proteins which coat the asbestos particles. This coat is digested within secondary lysosomes exposing the reactive groups of asbestos which are then free to interact with lysosomal membranes.

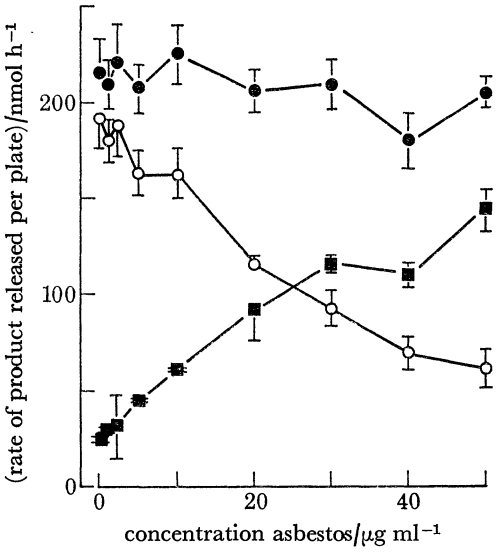

FIGURE 9. The redistribution of a lysosomal enzyme (β-galactosidase) in mononuclear phagocytes exposed to increasing concentrations of chrysotile asbestos (U.I.C.C. Chrysotile A) for 24 h. ●, total; ○, cells; ■, culture medium.

Similar changes in the alveolus in the presence of inhaled asbestos would result in the release of basement membrane-damaging enzymes from alveolar mononuclear phagocytes. Depletion of basement membrane components in the pericellular environment of epithelial cells might stimulate their proliferation and synthesis of extracellular constituents. Alternatively products released from mononuclear phagocytes could stimulate directly the synthesis of basement membrane constituents by epithelial cells.

The ability of proteolytic enzymes released from phagocytic cells to damage lung tissue is shown by the high incidence of chronic obstructive pulmonary disease in patients with an inherited deficiency of α1-trypsin inhibitor (Eriksson 1965). The absence of the inhibitor allows released enzymes to act upon the lung tissue for prolonged time periods to cause cumulative damage which leads to chronic disease. Similar changes can be induced in experimental

animals by intratrachael administration of pancreatic elastase in serum deficient in α1-trypsin inhibitor (Kaplan, Cuhn & Pierce 1973). On the other hand no damage is seen when elastase is administered in normal serum.

The increased production of interstitial collagen by fibroblasts could also result from the effect of soluble substances released from mononuclear phagocytes exposed to asbestos (Heppleston & Styles 1967; Kilroe-Smith *et al.* 1973; Burrell & Anderson 1973). The extreme resistance of asbestos to degradation and clearance from the lung accounts for the chronicity of the lesion that it induces. Recently, Suzuki *et al.* (1972) have shown the transformation of alveolar epithelial cells into phagocytic cells during asbestosis. That the transformed cells are derived from epithelial cells is shown by their attachment to basement membrane and to neighbouring epithelial cells by characteristic tight junctions. This transformation is associated with thickening of the basement membrane (Suzuki *et al.* 1972). These changes may result from the direct interaction of asbestos particles with epithelial cells or from a primary interaction with alveolar mononuclear phagocytes which may secrete mediators that induce the phagocytic function of epithelial cells.

TABLE 2. THE TIME-DEPENDENCE OF THE SELECTIVE RELEASE OF LYSOSOMAL HYDROLASES FROM MONONUCLEAR PHAGOCYTES BY ASBESTOS (50 μg/ml)

time h	*N*-acetyl-β-D-glucosaminidase activity in the culture medium (%)	
	control	asbestos, 50 μg/ml
0	3.9 ± 1.3	4.1 ± 1.8
1	3.9 ± 0.6	3.9 ± 0.8
2	4.5 ± 0.6	6.5 ± 1.8
3	3.4 ± 1.0	9.7 ± 1.8*
4.5	4.2 ± 0.8	16.9 ± 1.0*
6	4.5 ± 1.4	27.5 ± 1.7*
9	7.5 ± 1.7	42.6 ± 1.9*
14	9.8 ± 0.4	56.3 ± 1.9*
24	22.9 ± 3.5	62.8 ± 1.7*

* $P < 0.01$.

Pulmonary surfactant is thought to play an essential role in maintaining the stability of the alveolar structure ensuring efficient gaseous exchange. This phospholipid material is synthesized by type II alveolar epithelial cells (Adamson & Bowden 1973) and is released presumably by exocytosis. Besides albumin the other major components of the alveolar fluid are two glycoproteins with a composition similar to those of basement membrane glycoproteins (Lynn, Bhattacharyya, Passero & Tye 1973). These two substances have molecular masses of 36000 and 62000 and are of similar composition containing 8 % carbohydrate, 1 % hydroxylysine and 13–15 % glycine. They accumulate in excessive amounts during alveolar proteinosis and in rats infected with *Pneumocystis carina*. Their function is not known but their insolubility in water and high content of hydrophobic residues suggest that they may interact with the phospholipid surfactant to form part of the alveolar film. The release of lysosomal enzymes from alveolar mononuclear phagocytes would be expected to have an adverse effect on the levels of surfactant since these cells contain phospholipases which can degrade surfactant (Franson & Waite 1973). The subsequent decrease in the levels of surfactant could stimulate a feedback mechanism which

would increase surfactant production. The increased amount of surfactant seen in certain lung diseases may result from its overproduction by type II epithelial cells in an attempt to compensate for its increased breakdown by phospholipases.

Concluding remarks

It is apparent that certain pathological changes in diseases involving the kidney and the lung result from the disturbance of a delicate balance that exists normally between cells and their pericellular environment. These changes may involve the degradation of connective tissue components by mediators of acute and chronic inflammation released from inflammatory cells. On the other hand proliferation of connective tissue cells and an increase in their extracellular matrix can occur. The latter may result from a direct stimulus released into the extracellular environment by inflammatory or reticuloendothelial cells or as a consequence of a positive feedback response to depletion of the pericellular environment. A similar response is elicited by the separation of connective tissue cells from their pericellular environment by macromolecules such as immune complexes. It is clear that pathological processes represent both degradative and proliferative changes of cells and their connective tissue products. Host defence mechanisms represent an ultimately favourable balance of these changes brought about to resolve adverse changes in normal cell to cell and cell to pericellular environment relationships. Study of pathological changes at a cellular level can therefore be expected to shed new light on the ways in which cells interact as well as their functional dependence on their pericellular environment.

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Discussion

Professor J. B. Lloyd (*Biochemistry Research Unit, Keele University, Staffordshire*). Dr Davies, you have shown how phagocytic uptake of several materials that cause inflammation *in vivo* is accompanied by extrusion of lysosomal enzymes. Is there a correlation between ability to induce inflammation and ability to induce enzyme release, or is enzyme release a universal concomitant of the phagocytosis of particles?

P. Davies. Release of lysosomal enzymes does not always accompany phagocytosis by mononuclear phagocytes (Davies, Allison & Page 1974). This phenomenon seems to be restricted to substances such as group A streptococcal cell walls (Page *et al.* 1974); immune complexes (Cardella *et al.* 1974); carrageenan (Davies, Allison, Dym & Cardella 1974) and asbestos which can induce chronic inflammatory lesions when administered *in vivo*.

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A. H. S. Rahi (*Institute of Ophthalmology, Judd Street, London W.C.*1). What explanations have you got for the two different morphological pictures in immune complex nephrites. Whereas in experimental models there is marked polymorpho-nuclear response, in a clinical situation (e.g. s.l.e.) no such inflammatory reaction is observed. If it is accepted that deposition of immune complexes in vessels is followed by complement activation and accumulation of leucocytes, one should be able to see a similar morphological change in human immune-complex nephritis.

P. Davies. There are at least two alternative explanations. First, immune complex formation and complement fixation may occur at sites remote to the kidney. This would mean that pharmacological mediators, including substances chemotactic for polymorphonuclear leucocytes, formed by activation of the complement system would not be concentrated in the kidney. The accumulation of polymorphonuclear leucocytes observed in certain types of experimental nephritis where complexes are formed *in the kidney* would not therefore be expected. Secondly, certain types of immune complexes do not fix complement. In this situation chemotactic factors derived from cleaved components of complement would not be formed so that polymorphonuclear leucocytes would not be expected to accumulate preferentially at sites of immune complex formation.

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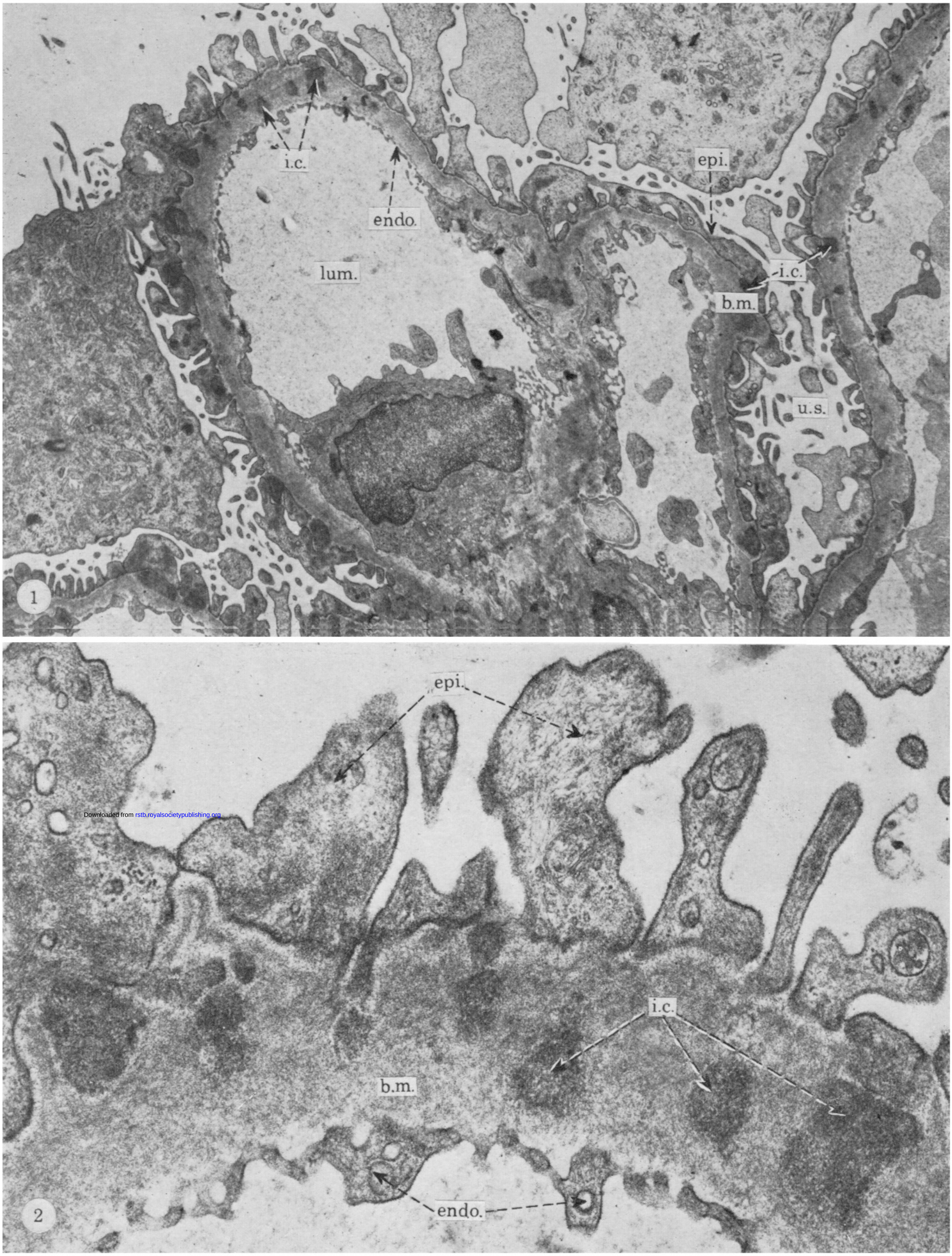
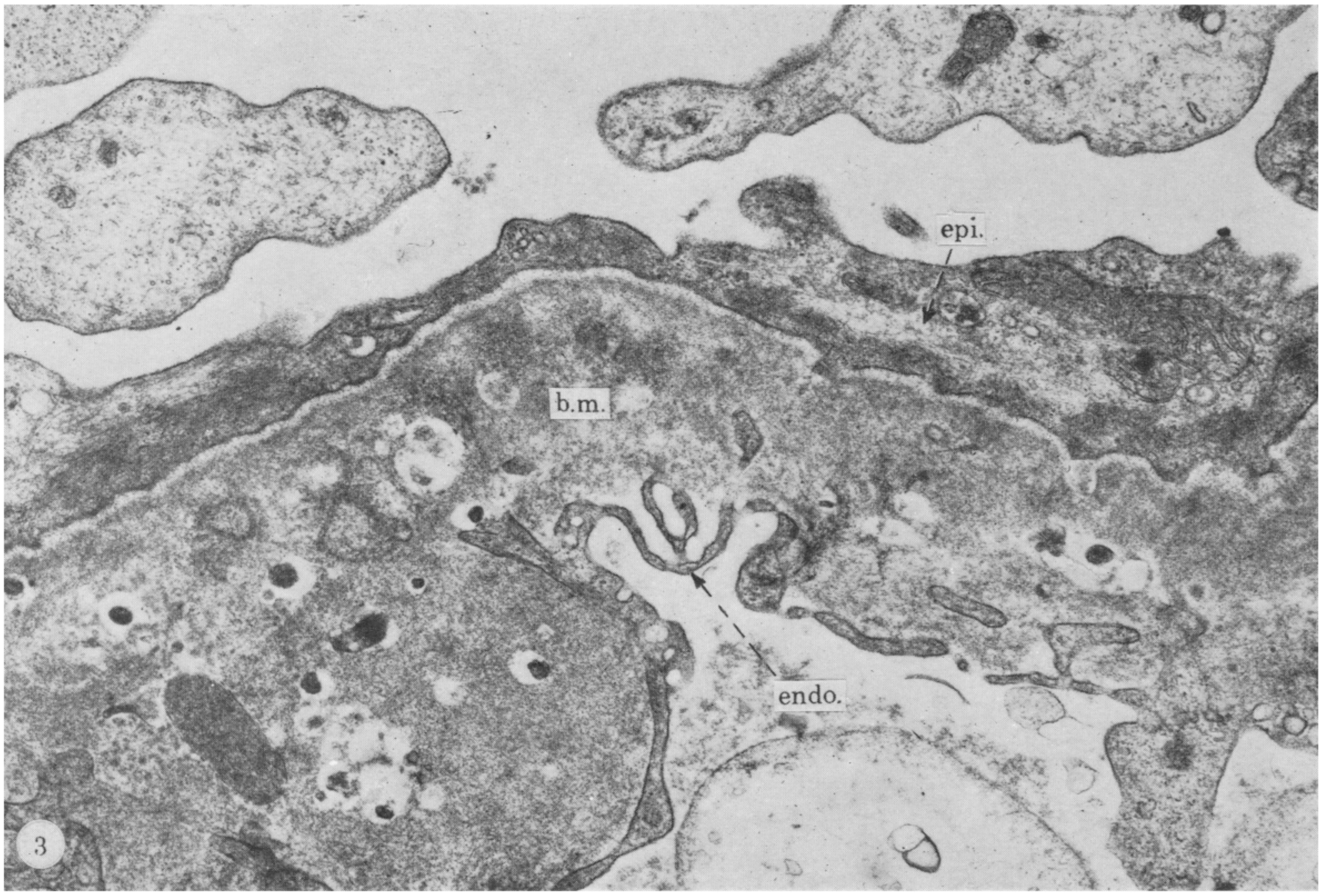


FIGURE 1. Electron micrograph of a renal biopsy from a Nigerian child with nephrotic syndrome showing the deposition of immune complexes (i.c.) in the basement membrane (b.m.). Note slight thickening of the basement membrane and abnormality of epithelial cell (epi.) morphology. The basement membrane separates the lumen (lum.) of the glomerular capillary lined by a layer of endothelial cells (endo.) from the urinary space (u.s.). (Magn. × 6500.)

FIGURE 2. Electron micrograph of a renal biopsy from a Nigerian child with nephrotic syndrome. Detail of immune complex deposition in the basement membrane. (Magn. × 36 500.)



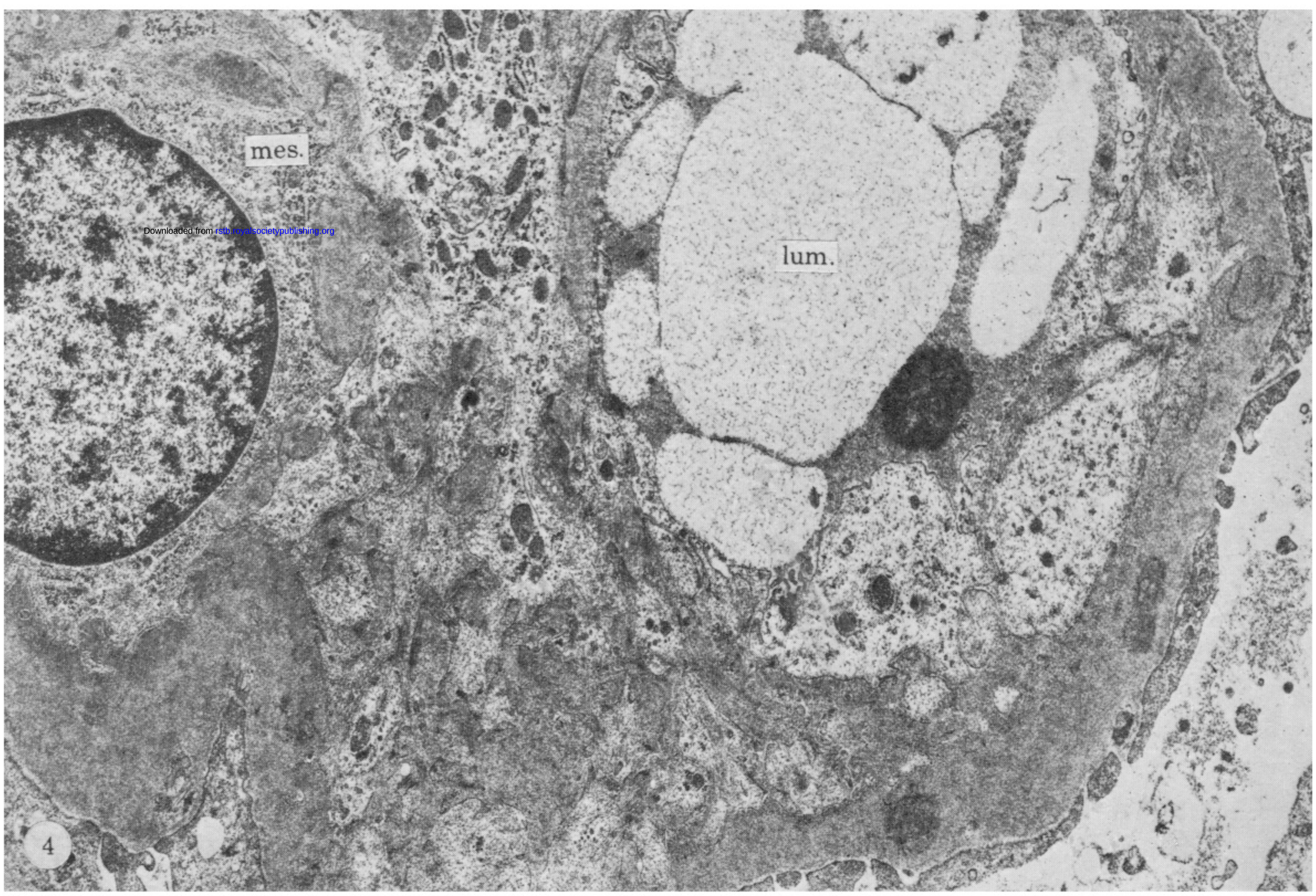


FIGURE 3. Electron micrograph of a renal biopsy from a Nigerian child with nephrotic syndrome. Note thickening of basement membrane and abnormalities in endothelial cell structure. (Magn. × 18000.)

FIGURE 4. Electron micrograph of a renal biopsy from a Nigerian child with nephrotic syndrome showing advanced disease with marked proliferation of mesangial cells (mes.) partially occluding the capillary lumen. (Magn. × 10000.)