ULTRASTRUCTURE IN BIOLOGICAL SYSTEMS

Edited by

Albert J. Dalton **Françoise Haguenau**

VOLUME 1 / Tumors Induced by Viruses: Ultrastructure Studies, 1962

VOLUME 2 / Ultrastructure of the Kidney, 1967

IN PREPARATION:

The Nucleus

The Membranes

ULTRASTRUCTURE OF THE KIDNEY

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PARIS, FRANCE

1967

ACADEMIC PRESS NEW YORK and LONDON

ACADEMIC PRESS INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS INC. (LONDON) LTD.
Berkeley Square House, London W.1

LIBRARY OF CONGRESS CATALOG CARD NUMBER : 66-16437

PRINTED BY THE ST. CATHERINE PRESS LTD., BRUGES, BELGIUM

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PREFACE

The monograph series on "Ultrastructure in Biological Systems" was begun not simply to start another group of reviews on ultrastructure but to fill what was felt to be a need for summary statements of the current status of particular areas of ultrastructural research in which significant and rapid advances are being made and in which considerable controversy and discussion are continuing. The ultrastructure of the mammalian kidney is certainly one of these areas.

One of the first applications of the electron microscope to problems in human pathology was to the kidney, and resulted in the demonstration of the hitherto undetected early thickening of the basement membrane of glomerular capillaries in glomerulonephritis. Yet many problems remain, particularly in relation to the correlation between function and the ultrastructure of components of the kidney—mesangium, glomerulus, juxtaglomerular apparatus, and the renal tubules.

It is only very recently that the mesangium has come to be accepted as real, and many questions remain as to the function of its cells. The existence of true membranes between foot processes of the epithelial cells of glomeruli is a newly established fact; but what this has to do with glomerular filtration is not known at present. Granules apparently secretory in nature have been identified in cells of the juxtaglomerular apparatus, but so far their presence has not been correlated with specific functional change. Artifacts introduced at fixation are now known to have considerable relevance in interpreting the ultrastructure of the normal nephron. Partly because evidence for this is quite recent, the correlation between fine structure and function of parts of the nephron is still in its infancy. Contributions of electron microscopy to an understanding pathological conditions have been several, but it would appear that the most valuable future contributions will come from the application of some of the more sophisticated techniques such as radioautography and ferritin-labeled antibody.

These are paraphrased views of the contributors to this monograph who, while acquainting the reader with the research being carried on in these areas, have also brought into focus the many problems still awaiting solution.

ALBERT J. DALTON
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November, 1966

THE FINE STRUCTURE OF RENAL TUBULES IN CORTEX AND MEDULLA[1]

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[1] The work from the authors' laboratories which is mentioned in this review has been supported in part by U.S. Public Health Service research grants A-06074, RG 5762, A 1702, and AM-07731.

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I. Introduction

The kidneys play a leading role in maintaining the internal environment of the body, the "milieu intérieur," in the optimal state for cellular function. In the course of evolution the kidney of the early chordates developed the ability to conserve sodium, enabling these chordates to leave the ocean and invade freshwater streams. Later the kidney became able to conserve water also and the chordates moved into a drier environment on land (H. W. Smith, 1951, 1959). Of course, many other substances have come to be selectively retained or excreted as necessary.

The localization of sites and the elucidation of mechanisms for handling each substance are challenges to investigators who must try to explain the chemical identification, analysis, and work performed by the kidney, as well as the alterations produced by disease—all in terms of the chemical composition and finer structures of the different renal cell types and their macromolecular components. Such an analysis will require clarification of the factors involved in filtration in the glomerulus, permeability, selective resorption, active and passive transport, and biochemical synthesis and secretion, whether into the bloodstream or into the urine. It is apparent that such an analysis will involve different fields including anatomy, histology, biochemistry, physiology, pharmacology, and pathology, as well as presenting challenges in the application and development of physical and chemical techniques at the macromolecular level. While renal processes cannot be fully understood until these intracellular processes are worked out, the high degree of structural and

functional differentiation in different parts of the nephron suggest that the kidney may well be the first organ in which will be clarified many of the secrets of cellular activity in transferring chemical energy of catabolism into the chemical work of active transport.

More electron microscopic work has been done on the glomerulus than on the tubules for two apparent reasons. First, the present electron microscopic techniques give better pictures of the glomerulus in health and disease because the glomerulus is less altered by present preparative techniques and by the trauma of needle biopsy, if one is performed. Second, most human renal disease seems to affect the glomerulus primarily. Although the etiological processes in the glomerulus are manifold and obscure, the functional alterations seem relatively simple. Passive glomerular filtration is affected either by changes in the permeability of the glomerular capillary wall or by changes in the capillary blood pressure. Although the tubules are secondarily affected in most renal diseases, they have much more complex physiological functions which accomplish most of the work of the kidney. Moreover, it is apparent that they are relatively labile structures, rapidly changing in response to interference with the blood supply and to many of the procedures necessary to study them. This biological sensitivity has made the interpretation of many electron microscopic studies quite difficult and demands stringent efforts to control conditions and discover the more sensitive reaction mechanisms of the cell. This is why studies of technical variations necessarily precede an analysis of any but the grossest physiological and pathological alterations. On the other hand, this sensitivity might well be expected of cells required to perform constantly so much chemical and osmotic work. Moreover, reactions to changes in the environment during study and fixation are clues to cellular changes under physiological and pathological conditions.

Certain elementary points are summarized in this review because some readers will be familiar with the kidney but not with electron microscopy, whereas others will be familiar with electron microscopy but not with the kidney. In order to keep within manageable proportions, the bibliography is not complete, but emphasizes reviews and recent references rather than the historical development of our knowledge.

II. Anatomy

The main anatomical features are given in histology and renal textbooks (von Möllendorff, 1930; Allen, 1962; Coupland, 1962). Only a few points will be mentioned here.

The different parts of the nephron are listed in Table I, which has been modified from von Möllendorff (1930), Sjöstrand (1944), and Rhodin (1958). The corresponding names in several languages are given in order to facilitate translation of foreign papers. The same part sometimes has different names in the same language; some of the equivalents are given in parentheses. In English, "part," "portion," and "segment" are used interchangeably. The division of the nephron given in Table I refers primarily

TABLE I

DIFFERENT PARTS OF THE NEPHRON AND CORRESPONDING NAMES IN FOREIGN LANGUAGES

English	German	Latin	French	Spanish
Glomerulus (renal corpuscle) (Malpighian corpuscle)	Nierenkörperchen	Corpusculum renis	Glomérule	Glomérulo (corpúsculo renal) (corpúsculo dc Malpighi)
Neck	Hals	—	Col	Cuello
Proximal tubule	Hauptstück	Portio principalis	Tube contourné proximal (I)	Tubo contorneado proximal
Convoluted (proximal) part	gewundener Teil	pars convoluta	la portion contournée	porción contorneada
Terminal part (straight part)	gestreckter Teil	pars recta (pars medullaris)	la portion rectiligne	porción recta
Thin segment (limb) of Henle's loop	Überleitungsstück (dünncr Teil der Henleschen Schleife)	Pars conducens	La branche grêle de l'anse de Henle (segment grêle)	Porción delgada del asa de Henle

Distal tubule	Mittelstück	Portio intermedia	Tube contourne distale (II) (segment intermediare)	Tubo contorneado distal
Ascending thick segment (limb) of Henle's loop	gestreckter Teil (aufsteigender Schenkel der Henleschen Schleife, breiter Teil)	pars recta	la branche large (ascendante) de l'anse de Henle (portion epaisse, anse large)	porción gruesa de la rama ascendente del asa de Henle
Medullary part	in Markstrahl	pars medullaris	—	—
Cortical part	in der Aussenzone	pars corticalis	—	—
Convoluted part	gewundener Teil	pars convoluta	segment contourne	porción contorneada
Macula densa	Zwischenstück	pars maculata	macula densa	mácula densa
Intercalated part	Schaltstück	pars intercalata	pièce intermediare	porción intercalar
Connecting part	Verbindungsstück	pars reuniens	canal d'union	—
Collecting tubule (excretory duct)	Sammelrohrsystem	Pars colligens	Tube collecteur (excréteur)	Tubo collector
Arched collecting tubule (proximal part, connecting tubule, cortical colting tubule)	proximaler Teil	—	—	tubo collector arqueado (porción cortical)
Straight collecting tubule	distaler Teil	—	tube droit	tubo collector recto
Part in cortex	—	—	—	porción cortical
Part in outer medulla	—	—	—	porción medular
Papillary duct of Bellini	—	ductus papillaris	canal papillaire (Bellini)	conductos de Bellini

to differentiated mammalian kidneys. However, the neck is not present in most mammalian kidneys although it is prominent in kidneys of reptiles, frogs, and fish. On the other hand Henle's loop is, with some exceptions, developed mainly in mammals, birds, and desert reptiles. Different species have long and short loops of Henle in different proportions. The ascending thick segments of the loop of Henle are the distinguishing feature of the outer zone of the medulla (Fig. 1). Until the past few years, the collecting tubule was not considered part of the nephron, but it is included now because more recent studies indicate that the collecting tubule participates in producing a hypertonic urine.

Two different conventions exist on the location of the junction of cortex with medulla. Some authors consider the cortex to include the glomeruli and convoluted tubules and place the descending, relatively straight portion of the proximal convoluted tubule in the medulla. Other authors include the straight portion in the cortex. It should be noted that the arcuate arteries do not mark the junction in all animals. In some animals the junction forms a relatively smooth plane, whereas in others it is quite folded.

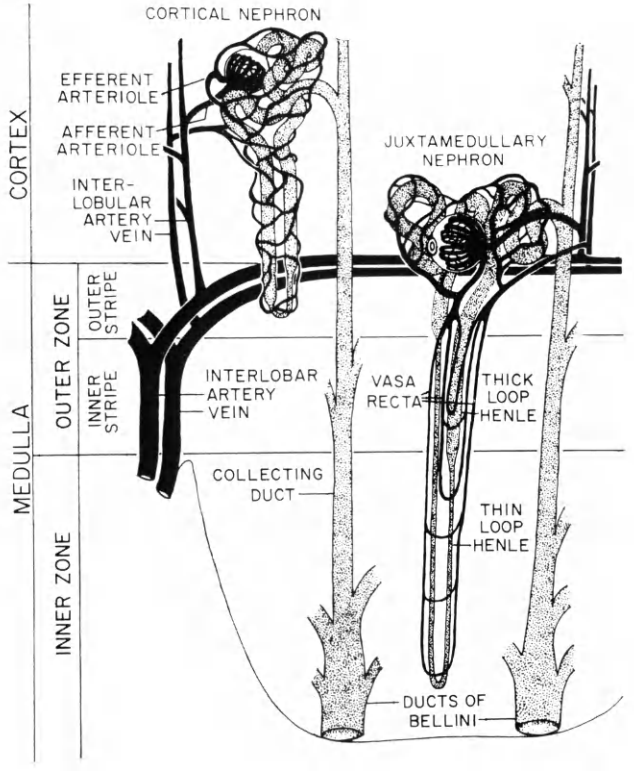


FIG. 1. Diagram of the different layers of the kidney, showing the structures found in each layer and their relationships. From Pitts (1963).

III. Renal Physiology

It is generally agreed (H. W. Smith, 1951) that the elaboration of urine involves three different processes: glomerular filtration, tubular resorption, and tubular secretion. The extensive physiological evidence for these processes has been the subject of recent reviews (Giebisch, 1962; Gottschalk, 1961; Berliner, 1963; Pitts, 1963; Ullrich and Marsh, 1963). Only a few highlights need to be presented here in order to indicate the main functions of the different portions of the nephron. Of course, many of these functions have not yet been correlated with cellular ultrastructure by electron microscopy.

A. Glomerular Filtration

Glomerular filtration has been established by micropuncture studies in amphibian and mammalian glomeruli. A relatively protein-free filtrate of plasma is forced through the walls of the glomerular capillaries by the hydrostatic pressure of the blood. This indicates that filtration is a passive process not requiring cellular work in the kidney. Glomerular filtration in the average normal man averages 180 liters per day (or 125 ml per minute) for a surface area of 1.73 square meters. Over 99 % is reabsorbed.

The glomerular capillary filter, on the basis of physiological studies (Pappenheimer, 1955; Pitts, 1963), seems to behave as if it were perforated by cylindrical pores approximately 75–100 Å in diameter. Direct electron microscopic evidence indicates that the filtration barrier is located in the central dense layer of the basement membrane, at least for some colloidal particles or molecules. Thorotrast particles, about 90–100 Å in diameter, pass out of the blood through the endothelial fenestrations or holes and penetrate the inner less dense layer of the basement membrane, but are normally stopped by the central dense layer (Latta *et al.*, 1960). Similar observations have been made with ferritin molecules about 100 Å in diameter (Farquhar *et al.*, 1961). Although little structure is usually revealed in the well-fixed normal glomerular basement membrane, some indication of thin fibrils arranged in the plane of the membrane has been seen (Farquhar, 1961; Kurtz, 1964). Indirect evidence also supports this concept of tangentially oriented long molecules forming a "brush heap" type of gel with the spaces between molecular chains acting as the filtration barrier (Latta, 1961).

B. Tubular Resorption

The large amounts of glomerular filtrate and the solutes dissolved in it necessitate resorption (reabsorption) of many substances if the resources of the body are to be conserved. The proximal tubule is primarily involved because it resorbs approximately 80 to 85 % of the glomerular filtrate.

1. *Active and Passive Resorption*

In active resorption a substance is transported across the tubular cells against a concentration or electrical gradient, and energy is expended in the process. The most prominent solute in the glomerular filtrate that is actively transported is sodium. For some other substances, the transport mechanism is clearly limited and any excess over the tubular maximal reabsorptive capacity (Tm) is excreted in the urine. Substances reabsorbed in this manner by the proximal tubule include glucose, phosphates, vitamin C, certain amino acids, lactate, etc.

In passive resorption, no energy is expended in the transport of a given substance. The chloride anion passively follows the sodium cation across the tubule cell. Water diffuses from the tubule following the osmotic gradient created by the resorption of sodium and chloride. A variable part of the urea diffuses back passively.

2. *Concentration and Dilution*

The ability of mammals and birds to form a hypertonic urine has long been correlated with the presence of a loop of Henle. However, clarification of the concentrating and diluting mechanism depended on the interesting hypothesis of countercurrent multiplication of concentration formulated by Kuhn and Ryffel (1942). Cryoscopic and micropuncture studies have confirmed this hypothesis and elaborated some of its details (Wirz *et al.*, 1951 ; Gottschalk, 1964).

Concentration of the urine depends on a hypertonic concentration being maintained in the medulla. The work involved appears to be performed by the thick ascending segment of Henle's loop, which pumps sodium out of the tubular fluid into the interstitial medullary tissue. It is necessary to assume that the ascending loop is relatively impermeable to water, while the descending limb is permeable. Only a small osmolar gradient need be established between the descending and ascending limbs at any level in order that concentrations can increase progressively toward the tip of the pyramid. The small gradient is then multiplied by countercurrent flow. The fluid that enters the distal convoluted tubule in the cortex is hypotonic and there is an osmotic gradient across the cells. If the kidney is forming a hypertonic urine (antidiuresis) the circulating level of antidiuretic hormone (ADH) of the posterior pituitary is high and makes the distal and collecting tubules more permeable to water. In the cortex, therefore, water passes out of the distal tubule until the contents become isotonic. When the fluid enters the collecting tubule in the medulla, water passes from the lumen to the hypertonic interstitium until the tubular fluid also becomes hypertonic. The loops of the vasa recta act as countercurrent exchangers and prevent the sodium in the hypertonic medulla from being washed away too rapidly.

In diuresis, when a dilute urine is being formed, a low ADH level lets the epithelium of the distal and collecting tubules become relatively impermeable to water, and the hypotonic fluid in the first part of the distal tubule remains hypotonic as it passes through the rest of the distal tubule and the medulla.

C. Tubular Secretion and Synthesis

The proximal tubule in the mammalian kidney can secrete two groups of organic compounds. The first group consists mainly of nonphysiological organic acids, such as *p*-aminohyppurate (PAH), phenol red, diodrast, etc. Creatine is also excreted by man and the rat, but not by the dog, sheep, or seal. The second group secreted consists of organic bases and includes guanidine, choline, histamine, hexamethonium, etc.

The distal tubule and, in part, the collecting tubule, secrete K^+, H^+, and NH_4^+ and stabilize the bicarbonate level of the body with the action of carbonic anhydrase. With these mechanisms the pH of the urine is controlled. Secretion of K^+ and H^+ may be linked by an ion-exchange mechanism to Na^+ reabsorption. The secretion of ammonium ions also involves their synthesis by the distal and collecting tubules.

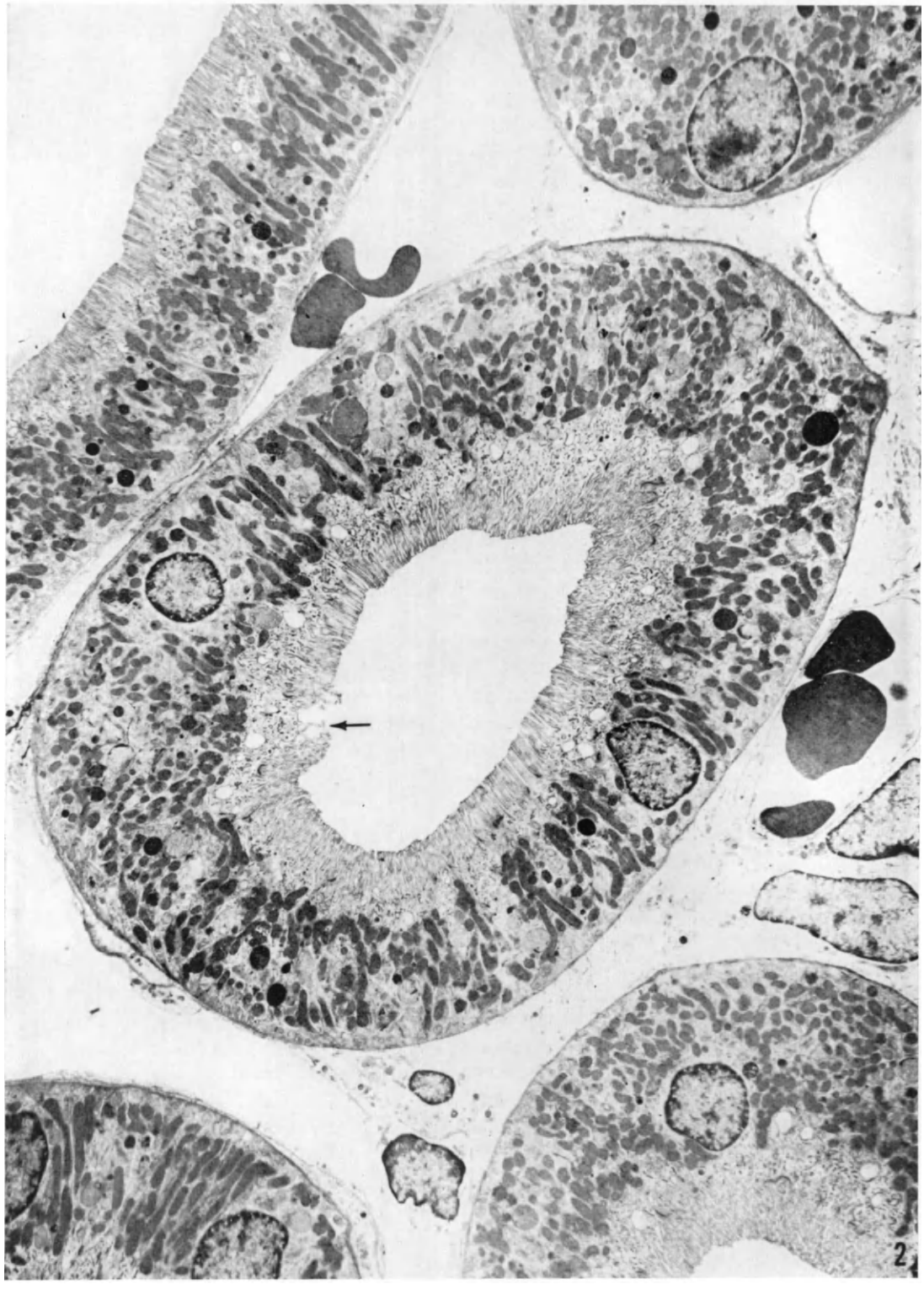
IV. Proximal Tubule

Some electron microscopic features of the proximal tubule were studied several years ago by Pease and Baker (1950) and Dalton *et al.* (1950, 1951). The ultrastructure at high resolution of the proximal tubule cells was revealed by the studies of Sjöstrand and Rhodin (1953) and Rhodin (1954). Some of the results by these and other authors (such as Pease, 1955a, b, c) have been reviewed more recently by Rhodin (1958, 1962a, b, 1963b), Kurtz (1964), and Caulfield (1964).

In this review the proximal tubule cells will be discussed with special attention to features that have been revealed with improvements in fixation and embedding procedures which have been developed during the last few years.

A. Fixation

The fixation technique greatly affects the structure of proximal tubule cells. Most apparent are alterations in the diameter of the lumens and in the size and shape of the cells. In the living animal micropuncture studies have demonstrated open lumens (Walker and Oliver, 1941; Gottschalk and Mylle, 1956). By using quick freezing *in vivo* Hanssen (1960) has shown open lumens by light microscopy and demonstrated that disappearance of the lumen usually occurs within 10 to 20 seconds after killing the animal. Therefore, the closed lumens shown by most electron microscopic studies must be artifactual. In electron microscopy open lumens were first demonstrated by Pease who dripped the fixative on the surface of the kidney in the living animal (Pease, 1955b). This work was later confirmed (Stone *et al.*, 1961) and extended (Maunsbach *et al.*, 1962a). The loss of lumens seems to be due to a rapid movement of fluid from the tubule lumen into the adjacent cells, producing considerable swelling. Fixation by dripping appears to give the best preservation of all cell details in tubules close to the surface of the kidney (Maunsbach *et al.*, 1962a) (Fig. 2). Fixation by perfusion with aldehydes (or osmium tetroxide under certain



conditions) also gives good results, preserving tubules throughout the cortex with open lumens and intact fine structure (Maunsbach, 1964d, 1966b, c).

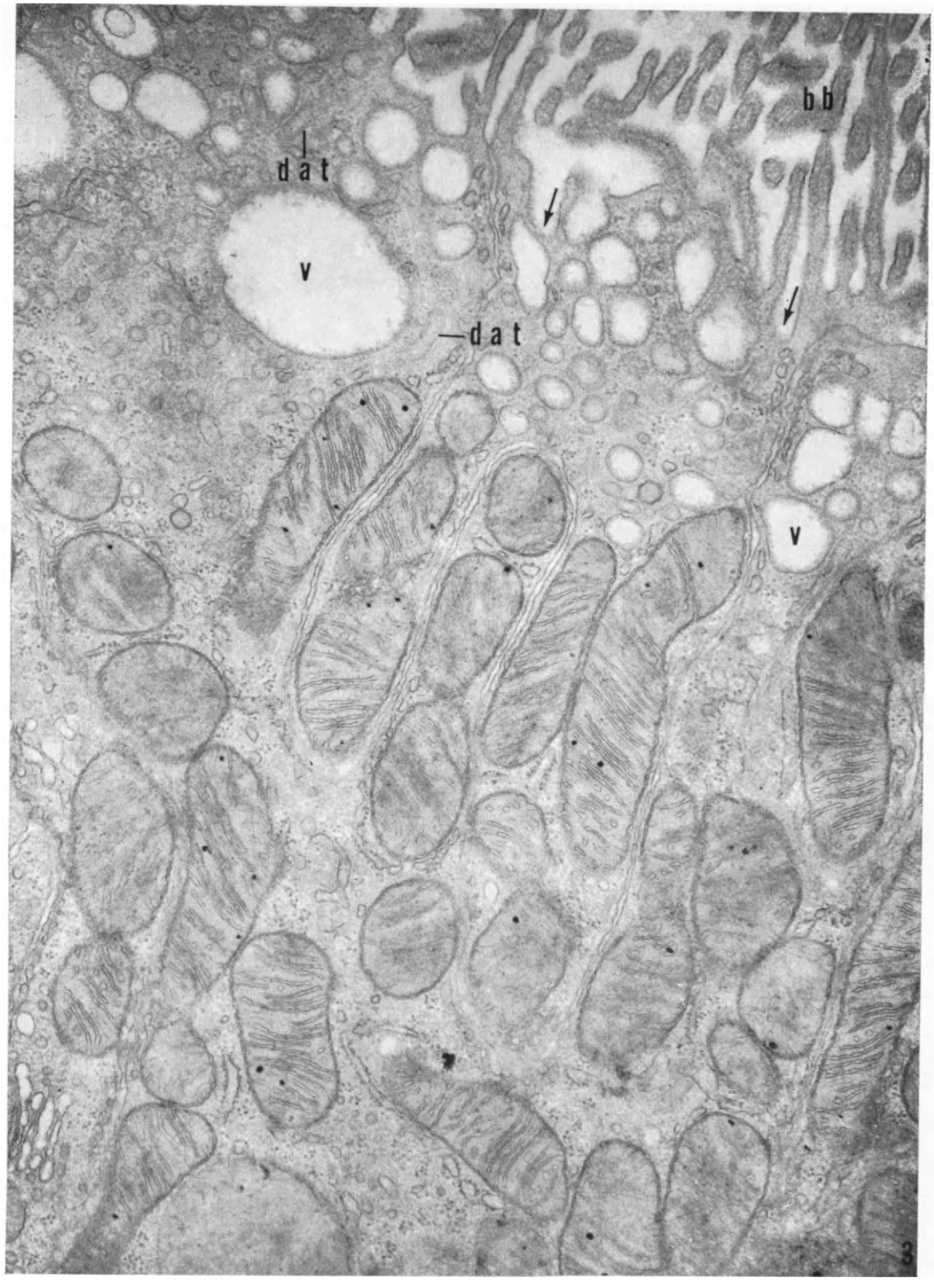
B. Plasma Membrane

The plasma membrane of the proximal tubule cell is triple-layered as are most cellular membranes (Robertson, 1957; Sjöstrand, 1960) and after staining the section with heavy metals it measures about 90 Å in thickness. An increasing amount of evidence indicates that the molecular structure for plasma membranes in general is a bimolecular layer of lipids sandwiched between layers containing proteins and perhaps other substances (Danielli and Davson, 1934; Sjöstrand and Rhodin, 1953; Sjöstrand, 1956, 1960; Robertson, 1957; Stoeckenius, 1963). The plasma membrane is thicker than many intracellular membranes (Sjöstrand, 1956, 1965), which shows that all membranes are not identical. The term "unit membrane" (Robertson, 1957, 1959) implies the same basic unit structure for all cellular membranes, a concept which is not supported by recent findings of variations in dimensions, staining properties, and the appearance of globular structures. The triple-layered plasma membrane of the proximal tubule cell is geometrically asymmetric after certain section-staining procedures (Sjöstrand, 1963a; Farquhar and Palade, 1963); the inner layer of the plasma membrane appears more intensely stained than the outer layer. This type of structural asymmetry was previously observed in other plasma membranes (Elfvin, 1961; Sjöstrand and Elfvin, 1962).

The basal membranes (infolded membranes) are part of the plasma membrane and cover the processes at the base of the cells (Sjöstrand and Rhodin, 1953) (Fig. 3). The infolded basal and lateral membranes divide the cytoplasm into processes which were seen early by light microscopists (see von Möllendorff, 1930) who found that they interdigitate extensively with processes from adjacent cells. These findings have been considerably substantiated by electron microscopic studies (Ruska *et al.*, 1957; Rhodin, 1958, 1962b, 1963b).

Pease (1955b) found separation of the membranes in areas of poor fixation and pointed out that this was a good indication that the membranes were osmotically sensitive and part of the plasma membrane of the cell. A similar separation has been noted by a number of investigators (Rollhäuser and Vogell, 1957; Ruska *et al.*, 1957; Rouiller and Modjtabai, 1958; Latta, 1960; Ruska, 1960), but it has been difficult to see common factors in the variety of conditions under which they have been described, suggesting that they may have a variety of causes including conditions of preparation or fixation (see Maunsbach *et al.*, 1962a). Further indication that the conditions of fixation are very important for the observed relationship between the infolded plasma membranes is the recent observation that adjacent triple-layered

FIG. 2. Survey picture of proximal tubules. These show open lumens and a rather even arrangement of the brush border. In one place the apical cytoplasm protrudes into the tubule lumen (arrow), but this may represent an artifact of fixation. Magnification: approx. ×3000.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membranes may appear fused, forming five-layered membrane complexes with complete absence of the extracellular space usually seen between adjacent cell membranes (Maunsbach, 1964d, 1966b). In a study of uranium poisoning in which fixation by dripping of the living kidney was carefully controlled, extracellular compartments or spaces were most pronounced at the start of the polyuric phase and were minimal or absent at other times and in the control animals (Latta, 1960; Stone *et al.*, 1961). A subsequent study of rats with neurohypophysectomy and unoperated control rats drinking large quantities of glucose solution showed that with polyuria large compartments would develop in proximal, distal, and collecting tubules (Latta *et al.*, 1962b). Compartments do not appear however in all conditions of massive polyuria: none were found in osmotic nephrosis produced by glucose, sucrose, or mannitol (Maunsbach *et al.*, 1962b).

Other evidence indicates that the basal membranes are functionally important. Studies by electron microscopic histochemistry have shown that phosphatase is associated with these membranes (Mölbert *et al.*, 1960a, b). Adenosinetriphosphatase (ATPase) activity has been found at the base of proximal tubule cells (Spater *et al.*, 1958; Goldfischer *et al.*, 1964; Wachstein and Besen, 1964b). This suggests that these membranes may be a major site of utilization of the energy stored in adenosine triphosphate (ATP), and hence that they may be sites of active transport, presumably of sodium which appears to be the main substance actively transported by the proximal tubule cells.

C. Brush Border and Cilia

The brush border (Fig. 3) seems to have an amorphous layer of material covering the triple-layered plasma membrane of the microvilli. This layer does not stain with phosphotungstic acid like that covering visceral glomerular epithelial cells (Latta, 1962). The microvilli contain alkaline phosphatase, which can be demonstrated by electron microscopy (Mölbert *et al.*, 1960b). There are some suggestions of structure within the microvilli. Vacuoles or tubules have been found (Sampaio *et al.*, 1958), and a tubular center has been seen under special conditions of fixation (Hanssen and Herman, 1962; Maunsbach, 1964e; Osvaldo and Latta, 1964). Whether these structures exist *in vivo* or represent preparation artifacts is uncertain at present.

Cilia have been found in proximal, distal, and collecting tubule cells (Latta *et al.*, 1961a). Usually no more than one to a cell is seen, but they seem even less frequent than that in normal cells. If they have a specific function it may be to mix the tubular contents and thereby facilitate resorption. More cilia have been found in abnormal circumstances. Many were seen in a hamster kidney tumor (Mannweiler and Bernhard,

FIG. 3. Apical portion of proximal tubule cells. Between the brush border projections (bb) are seen invaginations of the plasma membrane (arrows). In the apical cytoplasm are vacuoles (v) of different sizes and dense apical tubules (dat). Magnification: ×28,000.

1957). Several groups were found in the distal tubule of a patient who had disseminated lupus erythematosus (Latta, 1964). Here they had a definite 9 + 2 pattern.

D. Attachment Zones

Attachment zones are formed at the plasma membranes of adjacent proximal tubule cells. Similar attachment zones are present between cells in different parts of the body. Sjöstrand and Elfvin (1962) have described them as consisting of two types in mouse pancreas. The first type has a five- or seven-layered structure. The five layers are formed by the two triple layers of the plasma membranes of adjacent cells coming into contact so that the outer layers appear to fuse into a single layer. In the seven-layered structure the two triple layers are separated by a light interspace. The cytoplasm in the attachment zone has an increased density. The second type of attachment zone has a widened space between the plasma membranes, which is filled with an opaque material. The adjacent cytoplasm has an increased density which is quite marked just beneath the plasma membrane. This type of attachment is found on the basal side of the first type. It appears to be confined to a small area, in contrast to the first type which extends over long stretches. Farquhar and Palade (1963) have found similar structures joining epithelial cells. They use the names of tight junction or *zonula occludens* for the five-layered structure, intermediary junction (*zonula adhaerens*) for the seven-layered structure, and desmosome (*macula adhaerens*) for the localized or disclike zone of attachment (the second type of Sjöstrand and Elfvin). They believe that the term "terminal bar" used in earlier studies has been applied to what they now recognize as these three types of attachments and recommend that it should now be dropped in order to avoid confusion. If the term "terminal bar" continues to be used it should probably be applied to the first type of attachment zone of Sjöstrand and Elfvin (1962) or the tight and intermediary junctions of Farquhar and Palade (1963).

In the proximal tubule the tight junction is small and the intermediary junction is prominent (Farquhar and Palade 1963), whereas the situation is almost reversed in the distal and collecting tubules—the tight junction is prominent and the intermediary junction may be absent or discontinuous. In proximal tubules of the rat, desmosomes are small and infrequent, but in proximal tubules of other species, such as *Necturus*, they are quite prominent (Maunsbach, 1964e). Evidence that the attachment zones actually hold renal epithelial cells together and act as a barrier to passage of water or solutes is provided by several electron microscopic studies. The development of large extracellular compartments separating cells of the proximal, distal, and cortical collecting tubules up to their attachment zones in uranium poisoning (Latta *et al.*, 1961b; Stone *et al.*, 1961), in polyuria (Latta *et al.*, 1962b), and with hypertonic fixation (Maunsbach *et al.*, 1962a) indicates resistance to the movement of extracellular fluid back into the tubule lumen. Therefore, the region beneath the attachment zones in the kidney tubules forms a potential continuous space which seems to be concerned with fluid movement. Prevention of the passage

of larger molecules from the tubular lumen is demonstrated in hemoglobinuria (Miller, 1960; Farquhar and Palade, 1963) and ferritin microperfusion (Maunsbach, 1963).

E. Plasma Membrane Invaginations and Apical Vacuoles

Frequent plasma membrane *invaginations* into the cell are found between the microvilli of the brush border (Fig. 3). These apical invaginations are structurally specialized with a dense membranous component on the cytoplasmic side of the triple-layered plasma membrane and, in addition, have on their luminal side an amorphous coating similar to that covering the microvilli (Rhodin, 1962b; Farquhar and Palade, 1963; Maunsbach, 1963). Similar specialized sites are present in various cell types capable of pinocytosis (Roth and Porter, 1962).

Several distinct types of vacuoles and elongated tubules lie within the apical cytoplasm of the proximal tubule cells (Fig. 3). Most prominent are the *large apical vacuoles*, sometimes more than 1 μ in diameter. Between the large apical vacuoles and the lumen are profiles of plasma membrane invaginations, which may be mistaken for vacuoles unless serial sections are studied. Also seen between the large apical vacuoles and the invaginations are *apical tubules*, 600–700 Å in diameter. These apical tubules occasionally connect with the large apical vacuoles and often have electron-dense contents. In the same region are also elongated clear apical tubules of similar dimensions but usually more irregular in shape. Some of these clear tubules have occasional ribosomes on their surface indicating that the membranes are α-cyto-membranes (rough-surfaced endoplasmic reticulum).

Apical tubules and vacuoles have been related to the uptake of large molecules into the tubule cells. During massive hemoglobinuria, a dense material, presumably hemoglobin, fills the lumen of the tubules, the apical invaginations, and many vacuoles of different sizes (Miller, 1960; Ericsson and Dallner, 1962; Farquhar and Palade, 1963; Ericsson, 1964). Injection of ferritin into individual proximal tubules (Maunsbach, 1963) enables the uptake of individual molecules to be followed in stages. Ferritin molecules appear first in apical invaginations, then in small vacuoles and some dense apical tubules, and finally within 3 to 5 minutes in large apical vacuoles.

During osmotic nephrosis produced by injected glucose, sucrose, dextran, or mannitol (Maunsbach *et al.*, 1962b) the apical vacuoles increased in size and number, indicating that they may be involved in resorption of these substances from the tubular lumen. A small amount of inulin, which is a carbohydrate, can actually be absorbed from the proximal tubule lumen, as recently demonstrated in micropuncture studies (Scott *et al.*, 1964). The relative amounts taken up by pinocytosis or absorbed through the plasma membrane are, however, not known. In a study of inulin nephrosis an increase in the number of cytoplasmic bodies in proximal tubule cells was found (Simon *et al.*, 1964), but whether or not this indicates increased pinocytotic activity is uncertain at present.

The subsequent fate of resorbed substances is discussed later under the heading "Cytoplasmic Bodies" (Section IV, G).

F. Cytomembranes (Endoplasmic Reticulum or Ergastoplasmic Membranes)

A variety of membranes are present in the cytoplasm of proximal tubule cells besides those of mitochondria and the different types of cytoplasmic bodies. These membranes have been referred to with general terms such as cytomembranes, endoplasmic reticulum, or ergastoplasmic membranes.

The cytomembranes may appear as vesicles, elongated tubules, or flattened sacs. Some investigators have regarded all the different types of cytomembranes as part of a continuous or potentially continuous system (Porter, 1957, 1961; Robertson, 1959). Sjöstrand (1956, 1965) has characterized structural differences between the various groups of cytoplasmic membranes and believes that the different groups of membranes should not be regarded as parts of a single system. There has been considerable discussion of this point (see Porter, 1957, 1961; Haguenau, 1958; Sjöstrand, 1965). With new embedding media and staining techniques it has become possible to find structural differences between the membranes themselves. The cytomembranes may vary with respect to their thickness (Yamamoto, 1963; Sjöstrand, 1963a). Furthermore, while at moderate magnifications most membranes appear triple-layered, at high magnifications some, but not all, show a globular substructure (Sjöstrand, 1963a, b).

The cytomembranes in the proximal tubule cells can be divided into several different groups.

1. *α-Cytomembranes (rough-surfaced cytomembranes, rough-surfaced endoplasmic reticulum, ergastoplasmic membranes).* These membranes form tubular or vesicular profiles which are covered on their outer surface with small dense particles which apparently contain ribonucleic acid. The particles have been referred to as ribonucleoprotein (RNP) granules or particles, or as ribosomes. The α-cytomembranes have a thickness of approximately 55 Å and high-resolution electron microscopy reveals that they have a globular substructure (Sjöstrand, 1963a, b). They are undoubtedly very important in cell function. There is considerable evidence to indicate that rough-surfaced cytomembranes serve as a site of synthesis of protein (Palade and Siekevitz, 1956; Palade, 1961; Caro and Palade, 1964).

The ribosomes are also often seen in the cytoplasm of proximal tubule cells without apparent association with cytomembranes. When in groups they are often called polyribosomes (or polysomes) and are believed to be in the process of synthesizing cytoplasmic protein constituents.

2. *γ-Cytomembranes (smooth-surfaced cytomembranes, smooth-surfaced endoplasmic reticulum).* In addition to the rough-surfaced type of membranes there are considerable amounts of smooth-surfaced cytomembranes in proximal tubule cells. Although some authors regard these as part of the same system, others think that

variations in thickness, arrangement, or relationship to other cellular structures may be significant. Therefore we distinguish the following types of smooth-surfaced cytomembranes in proximal tubule cells.

a. Smooth-surfaced cytomembranes adjacent to microbodies. Most microbodies in the proximal tubule cells are surrounded by tubular and vesicular profiles of cytomembranes. Often these smooth-surfaced cytomembranes are connected with rough-surfaced cytomembranes. Their functional significance is not known.

b. Smooth-surfaced cytomembranes adjacent to plasma membranes. In some proximal tubule cells a large number of tubular or vesicular profiles of smooth-surfaced cytomembranes are present very close to the lateral plasma membranes of the tubule cells. This system, although observable in published pictures in several investigations has not been commented on until very recently (Bulger, 1964; Ericsson *et al.*, 1963). The function of these membranes is not known at present.

c. Smooth-surfaced cytomembranes without apparent association with other cellular structures. In the cytoplasm of proximal tubule cells are found tubular or vesicular profiles of smooth-surfaced cytomembranes which appear to have no direct relationship to other cellular structures, although they may occasionally connect with rough-surfaced cytomembranes. Although not very prominent in mammalian proximal tubule cells, they are quite abundant in proximal tubule cells of amphibians. In such cells they are often associated with dense granules assumed to represent glycogen. In proximal tubule cells of the dog, similar smooth-surfaced membranes were observed to be closely associated with lipid bodies which were experimentally induced during mobilization of free fatty acids from adipose tissue (Maunsbach and Wirsén, 1966).

The total thickness of most of the smooth-surfaced membranes listed under subsections *a*, *b*, and *c*, as well as the rough-surfaced cytomembranes referred to under subsection 1 above, is distinctly smaller than that of the plasma membrane, being of the order of 50 to 55 Å.

d. Golgi membranes. The Golgi cytomembranes compose the so-called Golgi apparatus (complex, system, substance, component) which is described as having three major components: flattened sacs (cisternae), large vacuoles, and clusters of small vesicles (Sjöstrand and Hanzon, 1954; Dalton and Felix, 1954, 1956; Dalton, 1961). In proximal tubule cells the Golgi apparatus is often quite prominent and it is usually located close to the nucleus. There is evidence from studies with electron microscopic histochemistry (Novikoff, 1963) that the Golgi apparatus in proximal tubule cells contains acid phosphatase. The acid phosphatase activity is usually present only in part of the Golgi apparatus, usually in some Golgi vesicles or one of the sacs. The Golgi apparatus shows a close relationship to some of the cytoplasmic bodies discussed below, in particular, those with acid phosphatase activity. Novikoff (1963) has proposed that these cytoplasmic bodies obtain their enzymes, at least in part, from the Golgi apparatus.

3. *Apical vacuoles and dense apical tubules.* These structures which occur mainly in the most apical part of the proximal tubule cells have been discussed above in

connection with plasma membrane invagination (see Section IV). They differ from the smooth-surfaced cytomembranes discussed under subsection 2 above with respect to their greater thickness, which is in the order of 90 Å (Maunsbach, 1964e).

G. Cytoplasmic Bodies

The cytoplasm of most proximal tubule cells contains a variety of cytoplasmic bodies or granules in addition to the vacuoles noted above. It is difficult to make a morphological distinction between such bodies and vacuoles, especially when many transitional forms may be found. Structures with clear, supposedly fluid contents are usually called vacuoles in electron microscopic papers, although it is possible that considerable material may have been extracted during the preparative procedures. Bodies or granules have denser, supposedly more solid contents.

Light microscopists have long recognized in normal renal proximal tubules the presence of cytoplasmic granules which can be differentiated from mitochondria (von Möllendorff, 1930; Oliver, 1948). Staining procedures reveal a variety of types in the normal kidney (Bencosme *et al.*, 1960). Fluorescence microscopy has revealed the presence of granules which have a typical autofluorescence different from that of the mitochondria (Sjöstrand, 1944). Some of these granules have been referred to as cytoplasmic granules in normal states and as colloid or hyaline droplets in abnormal states (Allen, 1962). Studies of proteinurias using labeled and unlabeled proteins (Smetana and Johnson, 1942; Smetana, 1947; Oliver, 1948; Latta *et al.*, 1951; Oliver and MacDowell, 1958; and others) indicate that some seem to be resorption droplets containing protein that has appeared in the glomerular filtrate. Many of the droplets take a periodic acid–Schiff stain, indicating that they contain insoluble or bound carbohydrates. Light microscopic studies have also shown that other materials seem to be resorbed by the proximal tubule cells. Such materials include lipids or lipoprotein (Smetana and Johnson, 1942), hemoglobin (Rather, 1948), polyvinylpyrrolidone or Kollidon (Heinlein and Hübner, 1958), dextrans (Mowry *et al.*, 1952), etc.

Changes in the frequency and distribution of cytoplasmic bodies in proximal tubule cells have been observed under a variety of experimental conditions. Owing to the large number of such studies only a few can be discussed here. Most studies have been concerned with the tubular handling of protein. At the light microscopic level very extensive studies have been carried out by Straus (1959, 1961, 1962, 1964a, b) and Novikoff (1960, 1961) who have used the plant protein, horseradish peroxidase. Peroxidase, which can be demonstrated by histochemical methods, rapidly appears in the glomerular filtrate and is then taken up by the proximal tubule cells in apically located vacuoles or bodies referred to by Straus as phagosomes. At later time intervals peroxidase is found in discrete bodies in the interior of the cell. By elegantly combining the histochemical staining methods for peroxidase and acid phosphatase Straus (1964a, b) and Novikoff (1960) showed that these peroxidase-positive bodies also contain acid phosphatase within 1 hour after the start of peroxidase absorption. This together with previous observations (Straus, 1958), indicates that cytoplasmic bodies

containing lysosomal enzymes directly participate in the cellular handling of absorbed peroxidase.

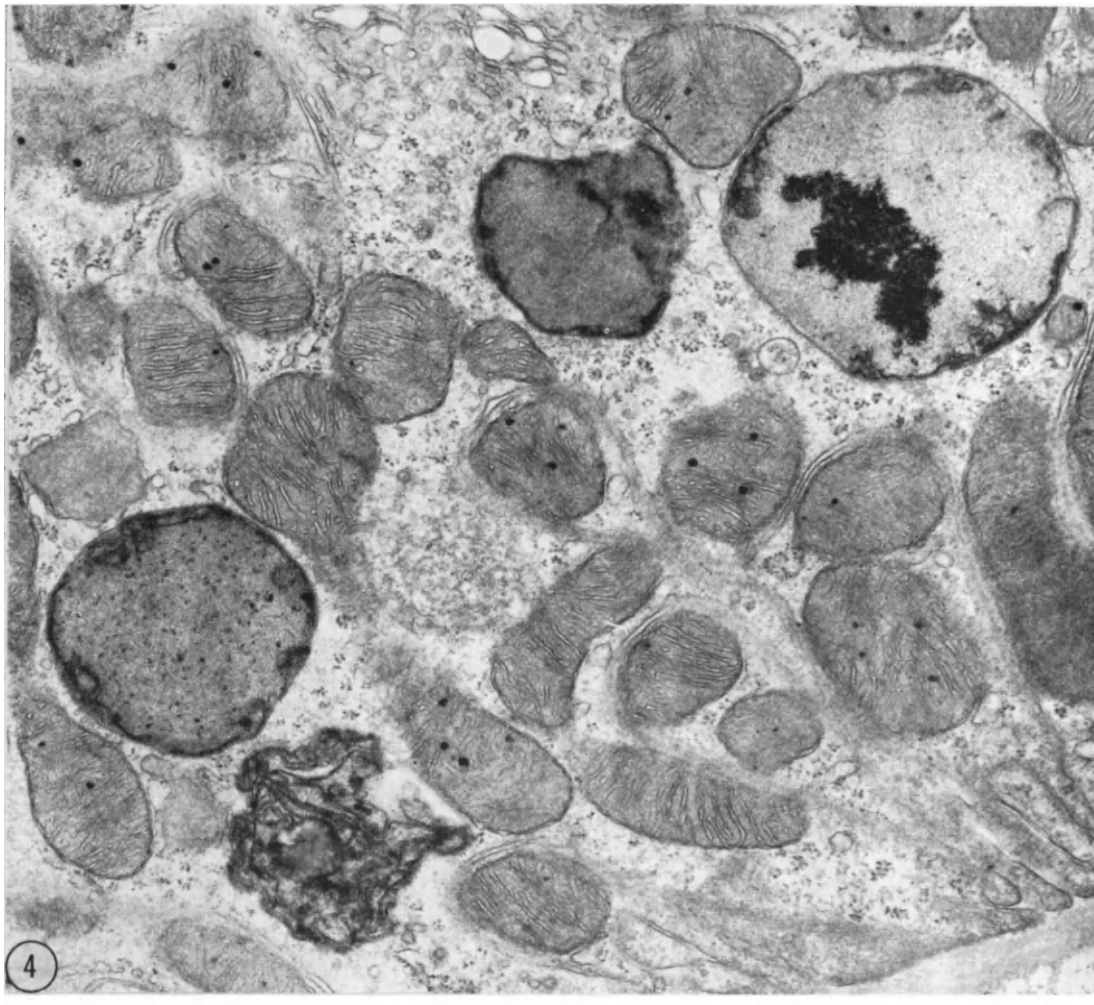
With the advent of electron microscopy a variety of structures about the same order of size as mitochondria were noted by many investigators in renal proximal tubules (i.e. Sjöstrand and Rhodin, 1953; Rhodin, 1954; Pease 1955b; Clark, 1957; Gansler and Rouiller, 1956; Stone *et al.*, 1961; Rouiller, 1961). Some bodies have been associated in electron microscopy with resorbed protein (Rhodin, 1954; Rouiller and Modjtabai, 1958; Stone *et al.*, 1961; Ashworth and James 1961; Kurtz and Feldman, 1962). Other materials which seem to produce cytoplasmic bodies observed electron microscopically are iron dextran or iron saccharide (Richter, 1957), Trypan blue (Trump, 1961), polyvinylpyrrolidone (Hübner, 1962), glucose, sucrose, mannitol, or dextran (Maunsbach *et al.*, 1962b) and sucrose (Trump and Janigan, 1962). At the electron microscopic level detailed studies have been made on the uptake and reabsorption of hemoglobin (Miller, 1960, 1962; Ericsson and Dallner, 1962; Ericsson, 1964; Miller and Palade, 1964). These combined structural and histochemical studies clearly demonstrate that acid phosphatase-containing bodies become involved during the hemoglobin absorption, although perhaps in slightly different ways in mouse (Miller, 1962; Miller and Palade, 1964) and rat (Ericsson, 1964). Details regarding the early stages of the uptake of materials from the lumen by apical invaginations and vacuoles has been mentioned above (Section IV, E).

It is possible to recognize some structurally distinct forms of cytoplasmic bodies, and there seems to be general agreement in the recent literature about the nomenclature of two of these cytoplasmic bodies, namely microbodies and multivesicular bodies.

The *microbody* is membrane-limited and often has an irregular shape. It has a homogeneous content except for a central density and is usually closely surrounded by cytomembranes. Cytoplasmic bodies of this type were first observed in mouse proximal tubule cells by Rhodin (1954) and referred to as microbodies because of their small size (0.2–0.3 μ). Structurally very similar bodies, although often somewhat larger, have been depicted also in rat proximal tubules (cf., Gansler and Rouiller, 1956; Engfeldt *et al.*, 1958; Maunsbach *et al.*, 1962b; Ericsson, 1964) and appear present in other species as well. Microbodies are said to contain uricase, D-amino acid oxidase, and catalase (Novikoff, 1961). Histochemically they do not stain for acid phosphatase.

The *multivesicular body* is surrounded by a membrane, has light contents, and contains several small vesicles. The multivesicular bodies do not form a distinct and separate group because they are sometimes found as part of a larger cytoplasmic body.

The remaining cytoplasmic bodies in many species constitute the majority of the bodies in the proximal tubule cells. They are very pleomorphic even in a single tubule cell and may vary considerably in ultrastructure in different species. They may contain dense homogeneous or granular material, light flocculent material, membranes (Thoenes, 1962b), crystalline material (Maunsbach, 1966a), dense pigment, or ferritin (Fig. 4).



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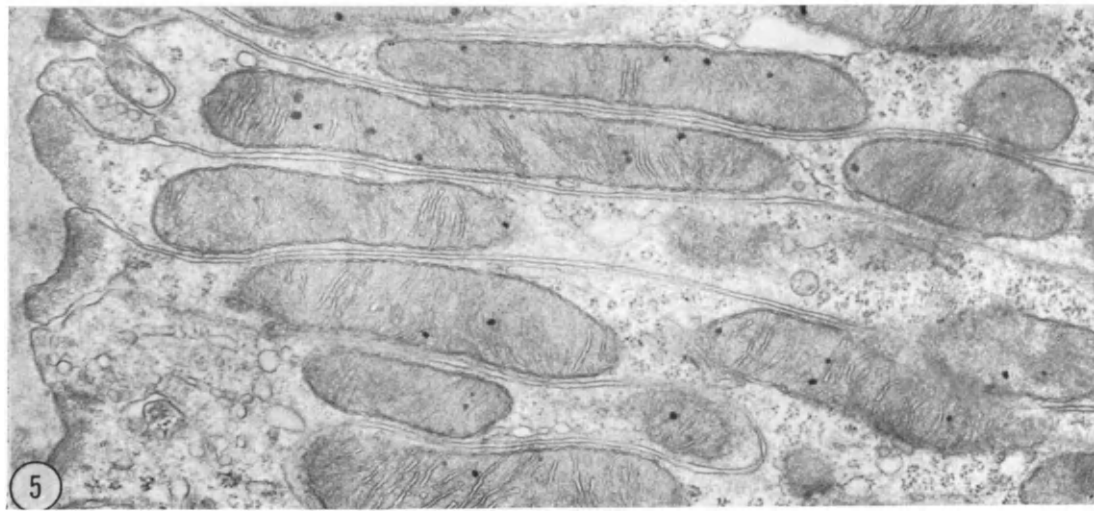

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There is at present considerable confusion and contradiction with respect to the nomenclature for these different bodies. Among the names that have been used by electron microscopists for these bodies (other than microbodies or multivesicular bodies) are cytoplasmic body, cytoplasmic granule, big granule, dense granule, hyaline granule, cytosome, metasome, droplet, absorption granule, absorption droplet, ovoid body, siderosome, lysosome, cytolysome, and cytosegresome. It seems desirable to the present authors to use names in electron microscopic descriptions which do not imply a functional concept or a certain origin (i.e., absorption granule or metasome) unless this has been established. Nor does it appear meaningful to use indiscriminately names indicating specific histochemical, biochemical, or chemical characteristics (such as lysosome) except in the cases when these specific properties have actually been demonstrated. In view of the pleomorphism of the cytoplasmic bodies in the proximal tubule cells the present authors prefer to use a general name such as cytoplasmic body or granule in morphological descriptions. If various morphological types need to be mentioned descriptive words can be used, such as cytoplasmic body with membranes or crystalline inclusions, etc. Among other names proposed for cytoplasmic bodies is the term "cytosome" (Linder, 1957). Unfortunately this term has already been used in cytology to refer to the cell body exclusive of the nucleus (Jones *et al.*, 1949). Some authors have used cytosome as a general term, whereas others have tried to apply it in a specific way to a single group of cytoplasmic bodies distinct from microbodies, multivesicular bodies, or apical vacuoles. However, the remaining bodies are heterogeneous morphologically with regard to contents and do not form a single group. A new way to characterize the cytoplasmic bodies in proximal tubule cells has been suggested by Maunsbach (1966a). On the basis of the structural properties of their limiting membrane or boundary, four different types of bodies were recognized in tissue fixed with osmium tetroxide or with glutaraldehyde followed by osmium tetroxide: Type I. Limited by a single membrane about 65 Å thick. Type II. Limited by a single membrane about 90 Å thick. Type III. Not limited by a distinct membrane. Type IV. Usually limited by two thin membranes.

Classification of the bodies on the basis of their limiting boundary represents an objective way to characterize these polymorphic cytoplasmic components. It may be used in electron microscopic investigations until more is known about the functional or biochemical properties of the cytoplasmic bodies. Of the different types of bodies, types II and IV contain acid phosphatase activity as demonstrated by electron microscopic histochemistry (Maunsbach, 1966a, e) and may correspond to lysosomes, which are discussed below. In view of the morphological heterogeneity of cytoplasmic

FIG. 4. Cytoplasmic bodies in basal part of proximal tubule cell from a female rat. This group exemplifies the polymorphism of these bodies which is often encountered in proximal tubule cells from normal rats. Magnification: ×28,000.

FIG. 5. Basal parts of interdigitating proximal tubule cells. These contain elongated mitochondria oriented perpendicular to the basement membrane at the left. Magnification: ×26,000.

bodies it is desirable to have other means of characterizing them. Considerable work has been done with two techniques, biochemical analysis and electron microscopic histochemistry. These have been useful in separating out certain cytoplasmic bodies which have some properties in common with lysosomes.

The term *lysosome* was applied to isolated subcellular particles which showed biochemically high activities of acid phosphatase, cathepsin, acid ribonuclease, β-glucuronidase, and other acid hydrolases (de Duve *et al.*, 1955; de Duve, 1959). Electron microscopically these liver fractions contained, besides mitochondria and unidentified structures, many membrane-limited dense granules, often containing electron-opaque grains resembling ferritin (Novikoff *et al.*, 1956). This identification has later been verified on more purified fractions (Baudhuin and Beaufay, 1963).

Straus (1954, 1956) isolated "droplet" fractions from homogenates of rat kidney cortex which resembled very much in biochemical properties those isolated by de Duve *et al.* (1955) from the liver. These fractions showed high concentrations of acid phosphatase, β-glucuronidase, cathepsin, acid DNase, and RNase which are all enzymes now considered characteristic of lysosomes. Another fraction, also containing high specific acid phosphatase activity, was isolated from homogenates of rat kidney cortex (Maunsbach, 1964c) and shown by electron microscopy to contain round, membrane-limited, dense bodies very similar to some of the cytoplasmic bodies found in sections of proximal tubule cells. Recently these bodies have been further purified and isolated in almost pure fractions (Maunsbach, 1966d).

Electron microscopic histochemistry shows that many cytoplasmic bodies in proximal tubule cells contain acid phosphatase (Novikoff, 1959, 1963; Miller, 1962; Ericsson and Trump, 1964). This indicates that many morphologically dissimilar bodies may resemble lysosomes, as suggested by the demonstration of a common enzyme.

The electron microscopic localization of acid phosphatase to certain membrane-limited cytoplasmic bodies raises the question whether such bodies should be referred to as lysosomes. De Duve (1963) wishes to retain the original biochemical definition of lysosomes and believes that this term should be used only provisionally by electron microscopists until several acid hydrolases are demonstrated in these bodies. Because not all cytoplasmic bodies contain acid phosphatase and because those that do have a variety of appearances, it does not seem appropriate for electron microscopists to call cytoplasmic bodies lysosomes unless they have at least done acid phosphatase stains on the particular tissue they are studying with the electron microscope.

For all these reasons, unless the specific nature of a body has been determined in each case, it seems preferable for electron microscopists to use in morphological descriptions a general term such as cytoplasmic body.

H. Mitochondria

The mitochondria of proximal tubule cells were first described in high-resolution studies by Sjöstrand and Rhodin (1953) and Rhodin (1954). They are characteristically quite large and elongated and oriented with their long axis perpendicular to the

basement membrane (Fig. 5). With respect to the localization of the mitochondria within the cell it should also be noted that they are especially abundant between the infolded basal and lateral cell membranes, as pointed out in many previous investigations. As also discussed above, these membranes are probably involved in tubular transport processes and the mitochondria are thus strategically located to provide energy for such processes. Their interior usually contains an appreciable number of inner membranes (or cristae), a matrix, and some intramitochondrial granules. Although the individual membranes [membrane elements according to Sjöstrand (1965)] in one inner membrane appear triple-layered at moderately high magnifications, recent high-resolution electron microscopic studies have shown that these membranes or membrane elements show a globular substructure (Sjöstrand, 1963c, 1965).

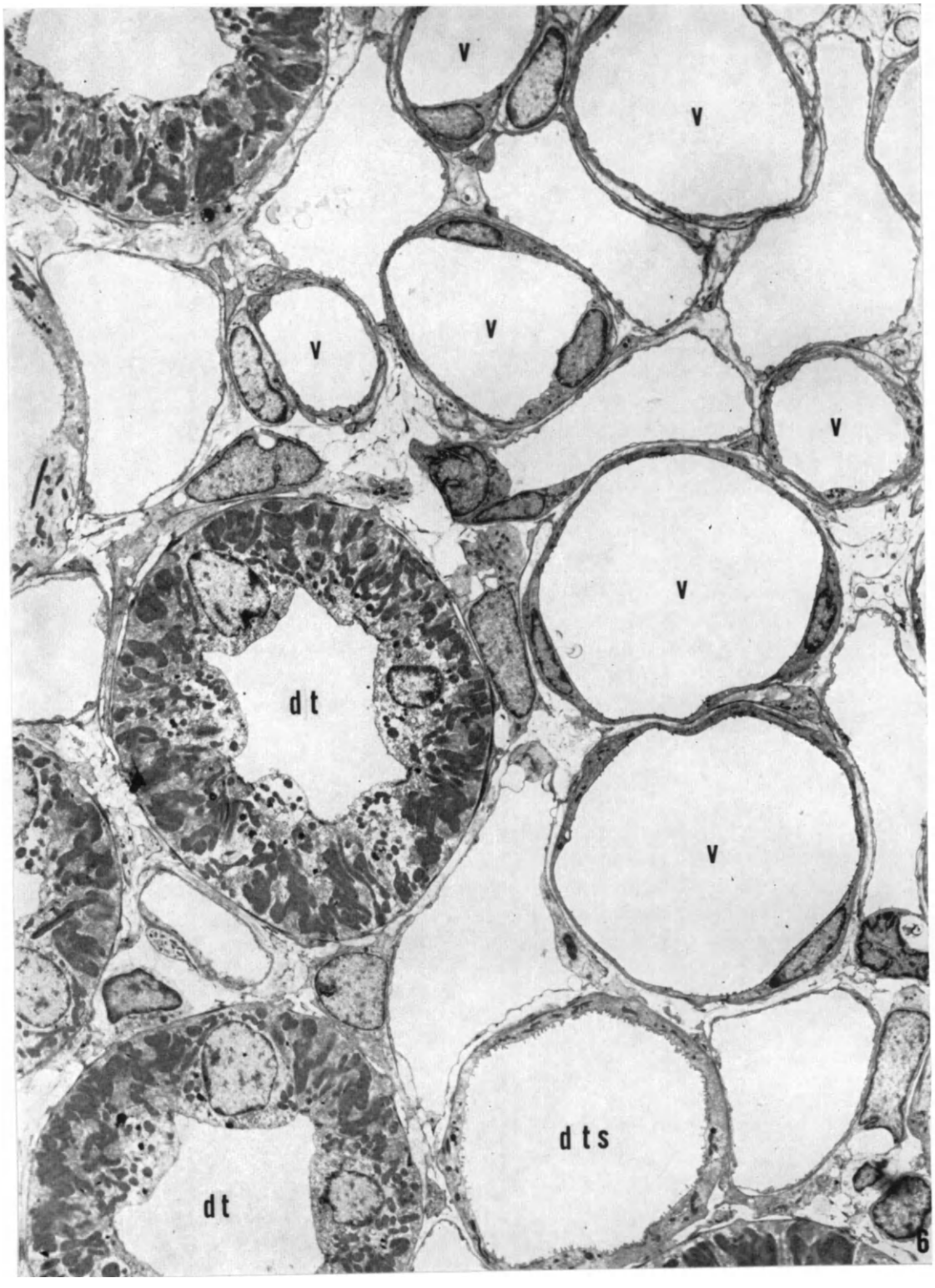
On the basis of biochemical evidence Green (1959) postulated the presence of specific units of the enzymes of the electron transport chain in the mitochondrial membranes. Recently Fernandez-Moran (1962, 1964) observed mushroomlike particles in isolated mitochondrial fragments by negative staining and referred to these particles as elementary particles. It was assumed that these elementary particles corresponded to the units of the electron transport chain previously described by Green (Fernandez-Moran *et al.*, 1964). However, this has recently been questioned (Chance *et al.*, 1964; Stasny and Crane, 1964; Sjöstrand *et al.*, 1964). In the latter study made partly on kidney mitochondria, Sjöstrand *et al.* proposed that the often tubelike fragments with attached elementary particles which are supposedly derived from disclike mitochondrial membranes may represent an artifact formed during the preparation and which may be more related to the myelin figures than to intact mitochondrial membranes.

I. Nuclei

The nuclei of proximal tubule cells have a fairly even distribution of chromatin granules, with small accumulations beneath the nuclear envelope. Nucleoli may be fairly prominent. Inclusions of unknown significance are occasionally seen. The nuclear envelope is composed of two triple-layered membranes which are usually parallel and enclose a light space. Ribosomes may be seen on the outer membrane. Nuclear pores are present and may offer a pathway between nucleus and cytoplasm.

J. Segmentation of the Proximal Tubule

Descriptions of the segmentation of the mammalian proximal tubule have been presented by T. Suzuki (1912) using light microscopy and in considerable detail by Sjöstrand (1944) using light and fluorescence microscopy. The proximal tubule of several mammalian species could be divided into at least three segments and in some species a fourth segment could be recognized. The first two segments in most species composed the convoluted part of the proximal tubule. The third (and fourth, if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present) were confined to the descending part of the proximal tubule, which in some species is rather straight but in others, such as the rat, is comparatively winding. Many other light microscopic studies have also demonstrated that the terminal part is different from the convoluted part (Foote and Grafflin, 1942; Longley and Fisher, 1954; Sternberg *et al.*, 1956; Straus, 1964b).

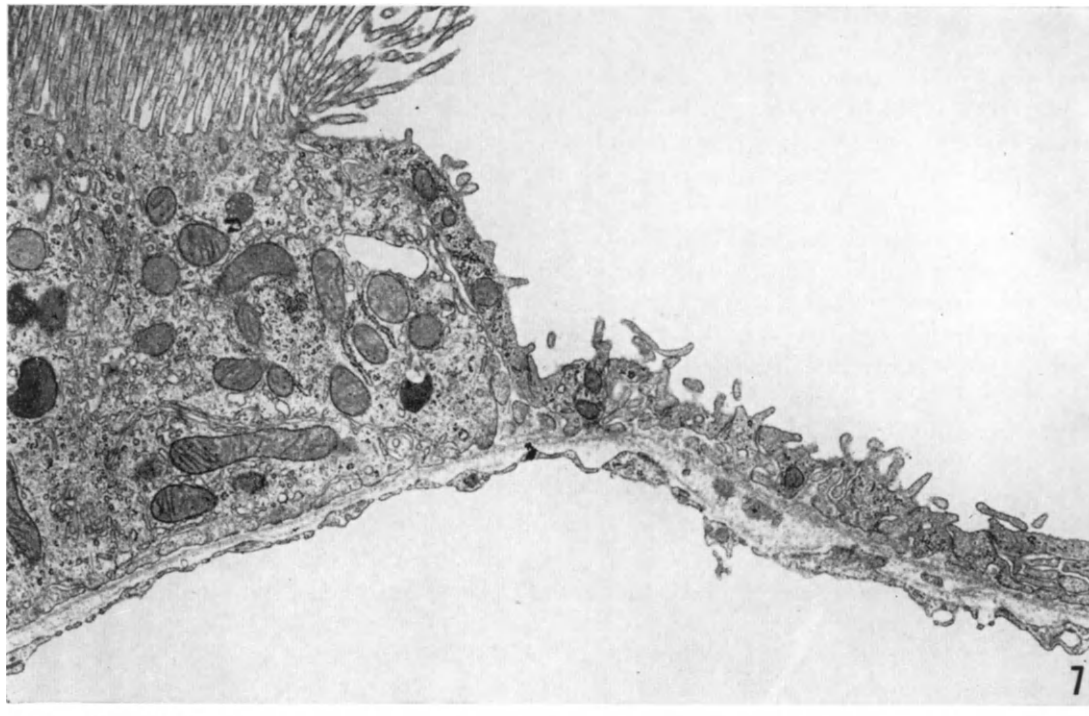
By electron microscopy segmental differences within the proximal tubule were noted by Rhodin (1954) who described differences between convoluted and straight portions of the proximal tubule in the mouse. Differences in the distal portion which include sparse development of basal infoldings and fewer and smaller mitochondria and apical vacuoles have subsequently been well established in other species (cf. Sakaguchi and Suzuki, 1958; Thoenes, 1961a; Caulfield and Trump, 1962).

Less attention has been paid to the ultrastructural differences that are found within the convoluted part of the proximal tubule, although in many mammalian species these are quite pronounced. Rhodin, however, has pointed out such differences between at least three parts of the proximal tubule in the mouse (Rhodin, 1958, 1962b). In particular Rhodin noted that the frequency and size of the mitochondria gradually decreased toward the end of the tubule and that the basal and lateral infoldings of the plasma membranes while being scanty in the last part of the tubule, were very prominent in the first part of the proximal tubule. More recently Maunsbach (1964b) has reported similar differences between three main segments within the rat proximal tubule and also observed a very characteristic segmental distribution of some of the cytoplasmic bodies in the tubule cells. The majority of these cytoplasmic bodies are acid phosphatase-positive as observed by electron microscopic histochemistry (Maunsbach, 1966e) and appear to correspond in size and localization to the autofluorescent granules observed by Sjöstrand (1944).

V. Thin Segment of Loop of Henle

Some animals, such as the beaver, have only a short loop of Henle which includes a very short thin descending segment that empties into the thick ascending segment. When the loops of Henle are situated entirely within the cortex, such nephrons have been referred to as cortical nephrons (Sperber, 1944), with the assumption that the collecting tubule is not functioning in resorption. Some animals have only long loops of Henle. These animals, largely desert rodents, also have long medullary papillae which contain greatly lengthened thin segments having long descending and ascending

FIG. 6. Inner stripe of outer zone of medulla. This shows the vascular bundle of the vasa recta (V) from the juxtamedullary glomeruli. The endothelial lining is continuous and without perforations. In contrast, the adjacent capillaries are fenestrated and have a more irregular shape. Ascending segments of distal tubules (dt) are seen at the left and the descending portion of a thin segment (dts) of Henle's loop lies at the bottom. The interstitial cells have different characteristics than those in the inner zone of the medulla (Figs. 12–14). Magnification: approx. ×3000.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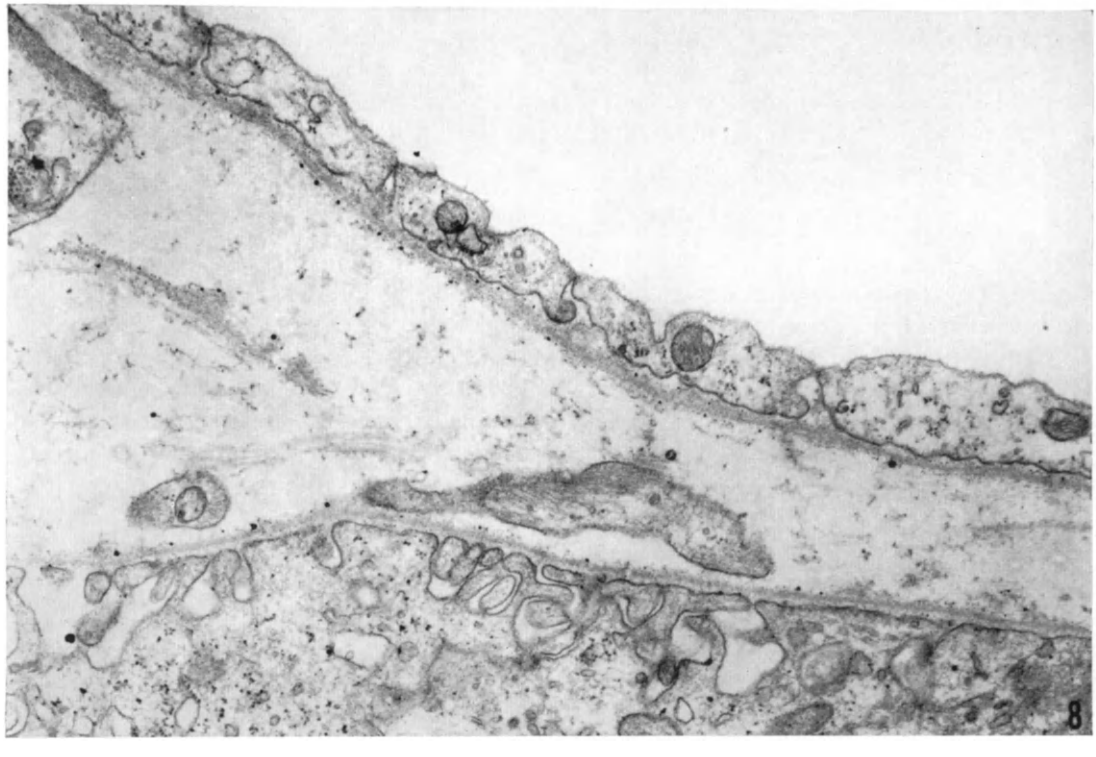
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portions. Most mammals have both long and short loops of Henle. The short ones arise from the outer cortical glomeruli while the long ones arise from the juxtamedullary glomeruli and may form about one-eigth of the nephrons in human kidneys. Physiological studies indicate that the long (or medullary) loops of Henle act as countercurrent multipliers of sodium. The function of the short loops with little or no thin segment is not clear. The simplicity of the cytoplasm in the thin segment and the relatively few mitochondria suggest that it may not require much energy or perform much work.

Early electron microscopic studies of the medulla did not distinguish two types of thin segments in inner and outer portions (Pease, 1955b, c; Takaki *et al.*, 1956; Sakaguchi and Suzuki, 1958; Rhodin, 1958; Novikoff, 1960). Differences were suggested by Thoenes (1961b). Lapp and Nolte (1962) pointed out that the thin segments in the outer medulla should be descending. Rhodin (1962b, 1963a, b) also recognized two types of thin segments but stated that in the papilla the ascending and descending portions cannot be differentiated. The point of transition between the two histological types of thin segments has not been established previously.

Fixation of the medulla by immersion results in rather poor preservation of cellular detail. Fixation by perfusion with glutaraldehyde and postfixation with osmium tetroxide have given good preliminary results which will be reported here (Osvaldo and Latta, 1965a, b, 1966a, b; Maunsbach, 1964d, 1966b) (Fig. 6).

A. Thin Segments with Elaborate Basal Processes (Descending Type)

In the rat there is an abrupt transition between the straight portion of the proximal tubules and the thin descending segment (Fig. 7). At this level, which constitutes the outer stripe of the outer medulla (Fig. 1), sections show the efferent arterioles of the juxtamedullary glomeruli (beginning to break up into vasa recta), the thick ascending limb of Henle's loop, and collecting tubules. The change at the luminal surface is remarkable: while the proximal tubules show a short but well-defined brush border, the thin segment presents only scattered microvilli in moderate numbers. Large numbers of basilar cytoplasmic extensions appear to interdigitate with similar extensions of neighboring cells. These extensions resemble the microvilli at the luminal border (Rhodin, 1962b, 1963b; Lapp and Nolte, 1962). The cytoplasm is somewhat dark, and moderate numbers of ribosomes and mitochondria may be found in any part of the cell. Attachment zones or terminal bars are found between the cells.

Fig. 7. Descending portion of thin segment of Henle's loop, in outer stripe of outer zone of medulla. This shows the junction of the thin segment with the proximal tubule and identifies the characteristics of the descending type of thin segment: microvilli at the luminal surface and interdigitating projections at the lateral and basilar surfaces. From Osvaldo and Latta (1966a). Magnification: × 11,000.

Fig. 8. Ascending type of thin segment of Henle's loop, in inner zone of medulla. This shows the simpler structure of the ascending type of thin segment. Attachment zones are apparent between the cell processes here, as well as in the descending portion. Magnification: × 22,000.

Bundles of fibrils may be found in the basal cytoplasm. Although these are predominant in the outer zone, some are seen in the inner zone.

B. Thin Segments without Elaborate Basal Processes (Ascending Type)

In the inner zone of the medulla most of the thin segments are characterized by a single layer of cell processes (Figs. 8, 12) (Pease, 1955c; Takaki *et al.*, 1956; Sakaguchi and Suzuki, 1958) which are said to represent interdigitations of adjacent cells (Rhodin, 1958). Attachment zones occur at the luminal junctions. Very few short microvilli are present. Mitochondria are infrequent in a light cytoplasm that contains very few ribosomes. The cell structure in these segments is much simpler than in the segments with the elaborate basal processes described above.

The segments with a simpler structure probably represent at least a portion of the ascending limbs, not only because they differ from the segments identified as descending in the outer zone, but also because the transition with the thick portion of the ascending limb has been identified. The contrast is striking between the simple structure of the thin segment and the elaborate basal infoldings and numerous mitochondria of the thick portion. The simpler type of thin segment is more frequent in the papilla. It seems, therefore, that the transition between the two types probably occurs in the descending limb, but it is difficult to establish how far from the bend of the loop it takes place.

VI. Distal Tubule

The distal tubule is provisionally considered to begin where the ascending loop of Henle changes from its thin to its thick part and to end where two nephrons join to form the collecting tubule. This distinction may serve as a landmark until functional or morphological features become known which will clearly indicate the point of connection between the distal and collecting tubule. Possibly this transition may be gradual.

The first part of the distal tubule up to the association with its juxtaglomerular apparatus is referred to as the ascending or thick segment of Henle's loop and has a medullary and a cortical portion. The convoluted part of the distal tubule begins with the macula densa, continues with the intercalated part, and changes into the connecting part.

A. Ascending Part of Distal Tubule

The thick ascending segment of the distal tubule (thick segment of loop of Henle) is located in the inner stripe of the outer medulla (Fig. 1), together with the thin descending portion of the loop of Henle, collecting tubules, and the vascular bundles formed by division of the juxtamedullary efferent arterioles. The thick ascending segment has a characteristic structure which is distinguished by plasma membranes

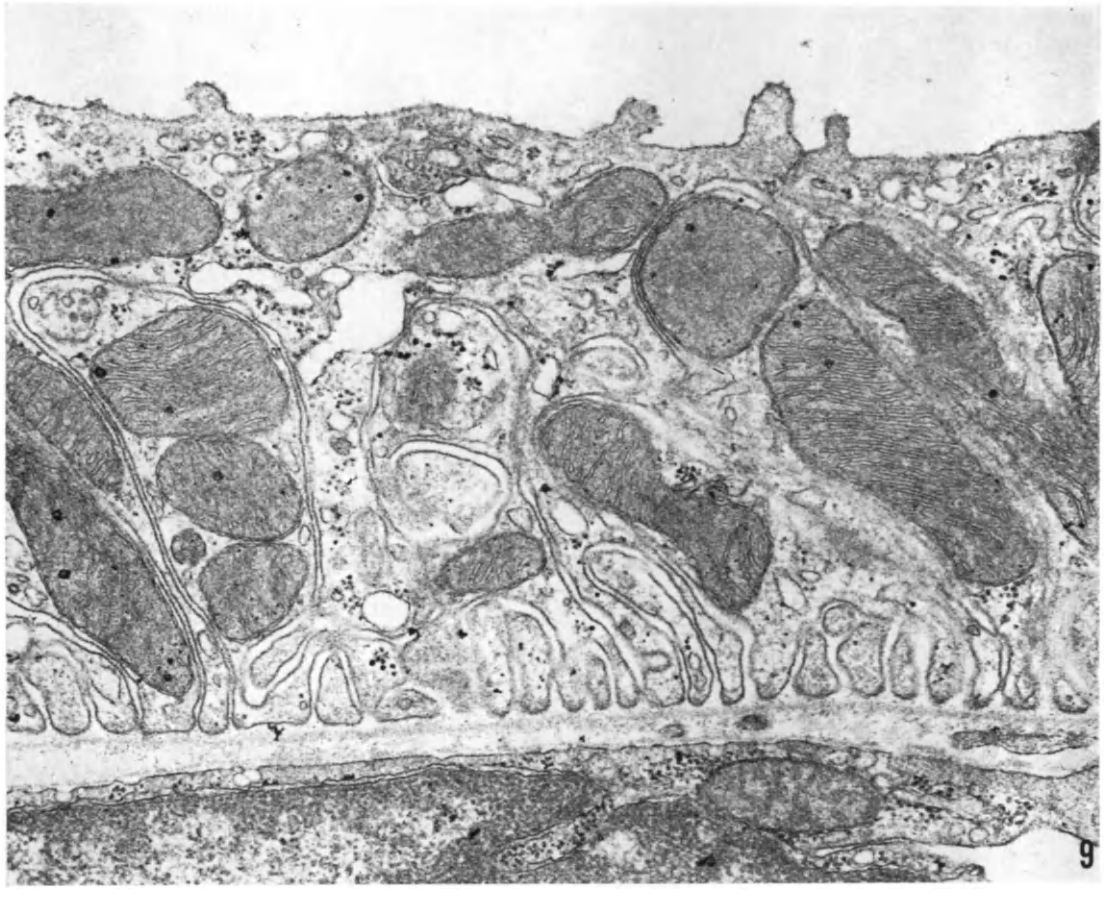


FIG. 9. Thick part of Henle's loop (straight ascending part of distal tubule) in outer zone of medulla. When compared with the convoluted portion of the distal tubule in the cortex, these cells are lower, the mitochondria are more irregular in shape and orientation, and the basal membranes are less developed. Magnification: × 13,000.

extending in from the base of the cell, sometimes to a level close to the lumen (Fig. 9) (Rhodin, 1958; Sakaguchi and Suzuki, 1958; Thoenes, 1961a). These membranes are of two types: either the membrane is folded back on itself with the adjacent plasma membranes belonging to the same cell (β-cytomembranes) or the membrane pairs are formed by large basal processes interdigitating with processes of adjacent cells. In the latter case, the adjacent membranes belong to two different cells. The mitochondria are elongated and lie close to the membranes, but do not line up as well as those in the cortical part of the distal tubule. The plasma membranes of these basilar infoldings are symmetrical, i.e., the osmiophilic inner and outer portions of the triple-layered structure are equal in thickness in contrast to the asymmetric membrane of the

proximal tubules, which has a thinner outer layer. Other details of the distal tubule have been noted, such as the absence of long microvilli, a small amount of endoplasmic reticulum, small vesicles found near the lumen, and small dark cytoplasmic bodies (Figs. 6, 9).

The close relationship of the many mitochondria to the infolded basal membranes suggests that these membranes may be the site of active sodium concentration in the medulla. ATPase activity has been found to be associated with the basal membranes by light microscopy (Spater *et al.*, 1958) and electron microscopy (Goldfischer *et al.*, 1964; Wachstein and Besen, 1964b). Such concentration of sodium could be accomplished only if the cells are relatively impermeable to water.

B. Convoluted Part of Distal Tubule

1. *Macula Densa*

The fine structure and the probable function of the macula densa as a feedback mechanism is discussed quite well in more detail elsewhere (see chapter by Hatt in this volume). The structure of the distal tubule becomes considerably altered in the macula densa of the juxtaglomerular apparatus at the hilus of the glomerulus where it comes in contact with the efferent and afferent arterioles (Latta and Maunsbach, 1962; Barajas and Latta, 1963a, b). The cells and their nuclei are packed closer together and the cells are usually higher than in the adjacent portions of the distal tubule. The mitochondria are fewer, shorter, and randomly arranged. The Golgi system is frequently subnuclear. There is much less basal infolding and interdigitation, but extracellular compartments are more frequent than in the rest of the distal tubule. The plasma membrane of the luminal portions of the cells, especially over the microvilli, seems to have an additional layer of material which stains with phosphotungstic acid (Latta, 1962).

2. *Intercalated Part of Distal Tubule*

The intercalated portion makes up the bulk of the distal convoluted tubule in the cortex. Its convolutions lie beneath the capsule and furnish the portion available for micropuncture studies. It has deep infolded plasma membranes and adjacent long mitochondria, giving it an appearance somewhat similar to that of the ascending thick portion of Henle's loop (Dalton *et al.*, 1951; Pease, 1955b, c; Rhodin, 1958; Thoenes, 1961b). The development of extracellular compartments like those in proximal tubules, under certain conditions of extensive polyuria, is morphological evidence that the distal tubule is also concerned with fluid and salt control (Stone *et al.*, 1961; Latta *et al.*, 1962a, b).

3. *Connecting Part of Distal Tubule*

The connecting part of the distal tubule is said to have lower cells with fewer and shorter mitochondria (Rhodin, 1958). The details of the transition between the distal and collecting tubules have not been worked out.

VII. Collecting Tubule

A. Cortical Collecting Tubule

The cortical collecting tubule is characterized by cells which have fewer mitochondria, fewer basal membranes, and a less prominent internal structure than the distal tubule (Pease, 1955b, c; Rhodin, 1958). They have β-cytomembranes and some basal membrane infolding (Pease, 1955b, c; Rhodin, 1958; Thoenes, 1961b) and develop extracellular compartments (Latta *et al.*, 1962a), furnishing some evidence of an additional site of regulation of fluid resorption.

1. *Light Cells*

Most light cells have a scalloped border with few microvilli. A single cilium can sometimes be found at the apex (Latta *et al.*, 1961a). Basal infoldings and interdigitations are fairly numerous, but are much shorter than those of the distal tubule. The mitochondria are relatively few and short. Cytoplasmic bodies sometimes show a layered or paracrystalline structure (Miller, 1961; Rhodin, 1962b). Some light cells are lower and have fewer microvilli, mitochondria, and basal infoldings.

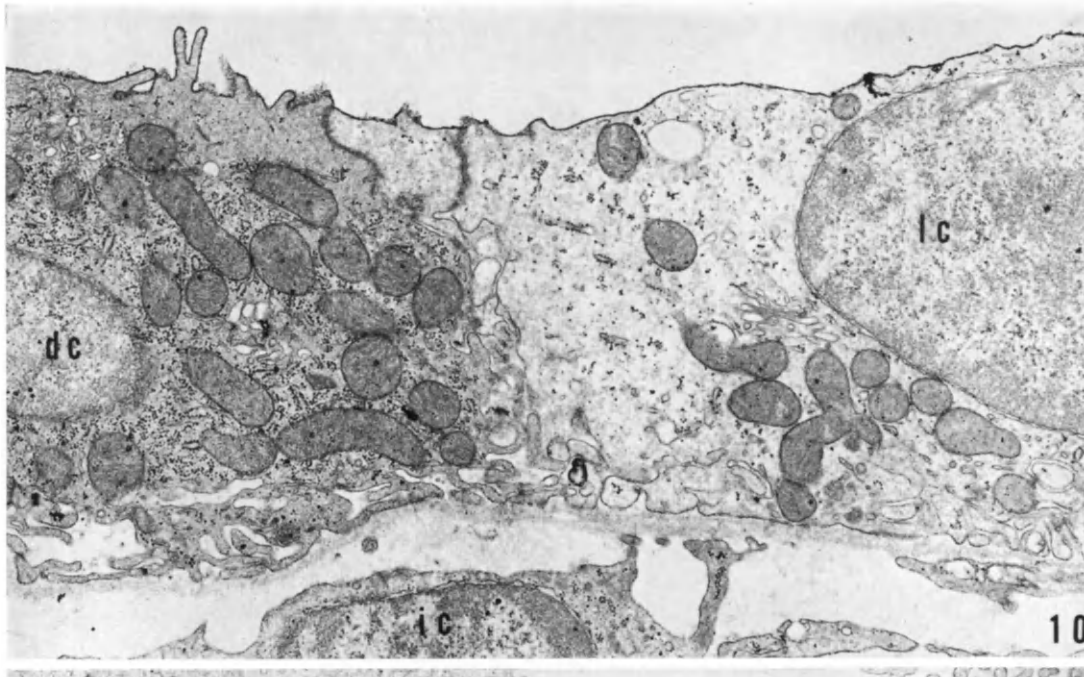
2. *Dark Cells*

The fine structure of dark cells was described by Rhodin (1958). These cells are much fewer in number than the light cells and generally occur singly, appearing as a darker cell between adjacent light cells. The darkness seems to be due to a denser, more osmiophilic cytoplasm.

The cells usually contain a number of prominent small vesicles located in the upper portion beneath moderate numbers of irregular short microvilli. Rounded or elongated mitochondria are more numerous than in light cells and show no particular orientation in the cytoplasm. Basal infoldings are more prominent than in adjacent light cells, and in material fixed by perfusion there is usually separation of the membranes. These cells seem to be increased in numbers in potassium depletion (Oliver *et al.*, 1957). The dark cells also seem to develop more prominent extracellular compartments with polyuria than adjacent light cells (Latta *et al.*, 1962b).

B. Collecting Tubule in Medulla

The medullary collecting tubules seem to contribute to the final step in the production of a hypertonic urine. Under the action of antidiuretic hormones, the cells permit water to pass from the lumen into the hypertonic medulla. This is a passive process and would seem to require little cellular work. However, adenosinetriphosphatase has been associated with the basal membranes (Wachstein and Besen, 1964b). By light microscopy Glimstedt *et al.* (1952, 1954) have observed functional changes in the collecting tubules. Among the changes were increases in nuclear volume after hydration under different hormonal states. Young and Wissig (1964)



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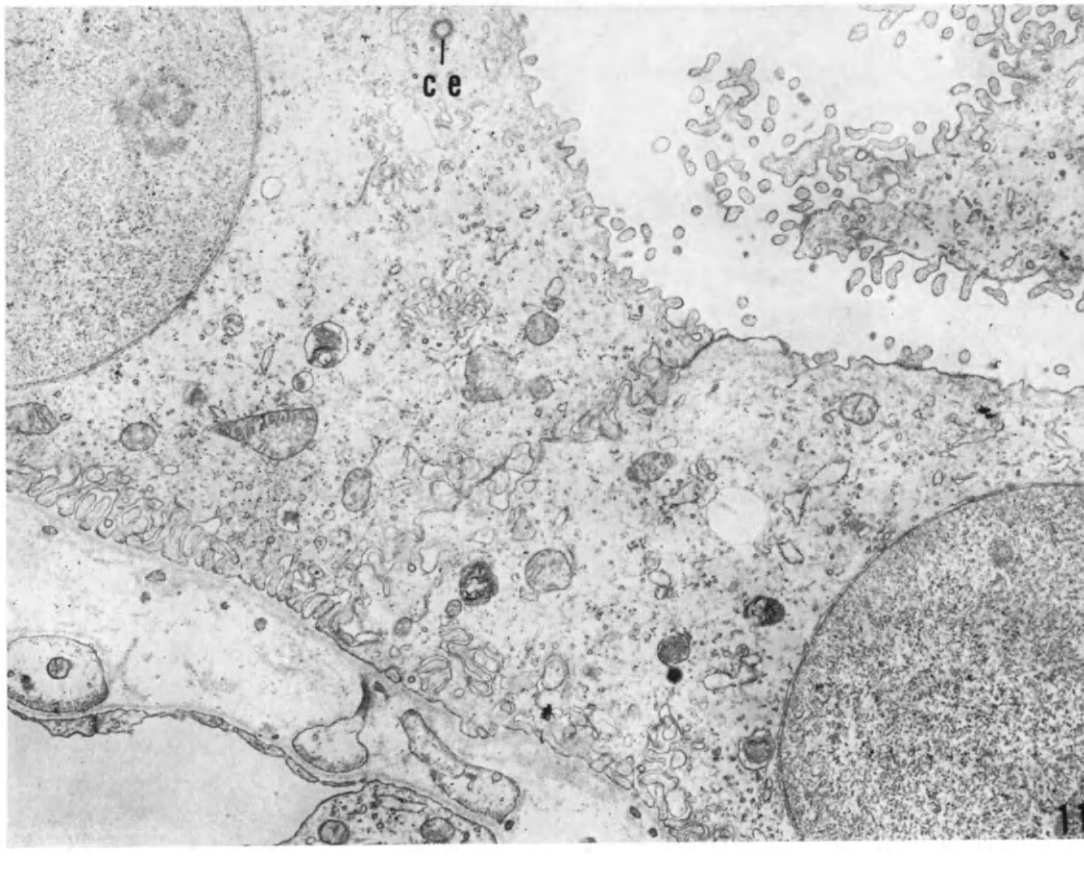

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divided the collecting tubule into four segments on the basis of cellular characteristics. They also observed concentration of plasma in the rete mirabile at the tip of the papilla and crenated red cells in the papilla.

By electron microscopy the medullary collecting tubule cells increase in height toward the tip of the pyramid. They contain fewer mitochondria and basal infoldings than the cortical collecting tubules (Figs. 10, 11). The cytoplasm of the light cells shows fewer organelles. A small number of apical vesicles may be found near the lumen. Lateral interdigitations are prominent beneath the attachment zones. The dark cells become less frequent in this portion of the tubule (Fig. 10). Numerous "cytosomes" (cytoplasmic bodies) have been noted (Lapp, 1960).

C. Papillary Duct

The papillary duct consists almost entirely of light cells which are larger but otherwise similar to those in the tubule above it. The base of the cell in the mouse kidney may contain lipid droplets (Rhodin, 1962b) and in the rat, small fibrillar bundles (Osvaldo and Latta, 1964). Some dark cells have been found in the papillary ducts of the rat (Osvaldo and Latta, 1964).

VIII. Other Structures in Cortex and Medulla

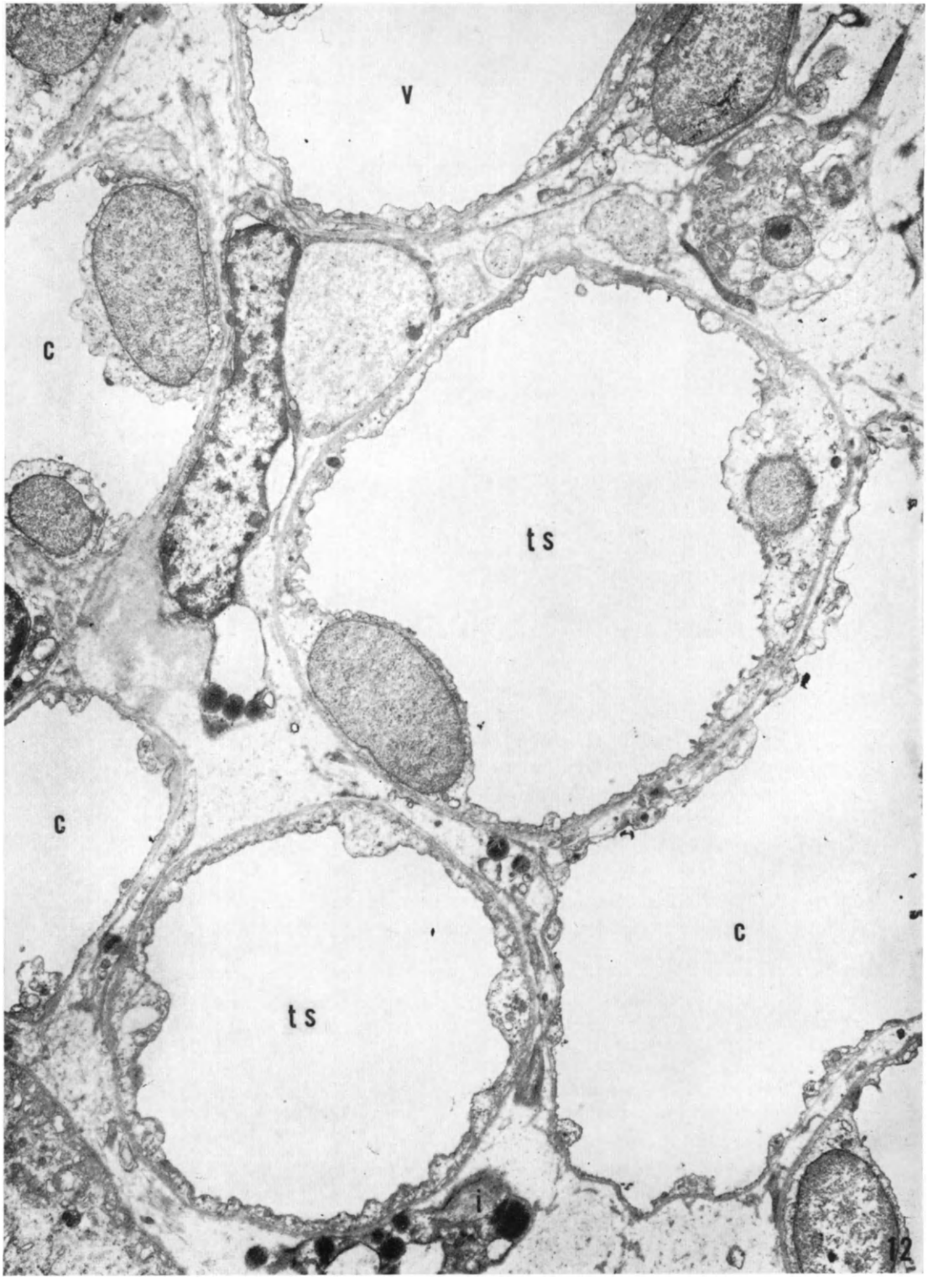
A. Vessels

Some of the prominent patterns of vascularization of the kidney have been the object of new studies using light microscopy and perfusions with contrasting media (Moffact and Fourman, 1963; Ljungqvist and Lagergren, 1962). Only a few early studies with the electron microscope have been presented on the structure of the vascular bundles of the outer stripe of the medulla and the medullary capillaries (Takaki *et al.*, 1956; Novikoff, 1960; Longley *et al.*, 1960; Lapp and Nolte, 1962).

The afferent arterioles are similar to those elsewhere in the body, except that the smooth muscle cells may contain granules, especially in the region of the

FIG. 10. Collecting tubule in outer zone of medulla. A light cell (lc) lies on the right and a dark cell (dc) lies on the left. The dark cells appear darker because of the denser cytoplasmic matrix, the number of mitochondria, and the ribosomes distributed through much of the cytoplasm. They usually show more separation of the basal processes and longer and more prominent microvilli than the light cells. Two cytoplasmic processes of an interstitial cell (ic) are in contact with the basement membrane of the collecting tubule. Magnification: × 37,000.

FIG. 11. Collecting tubule in inner zone of medulla. The cells have short microvilli and show lateral and basal infoldings. They are larger than the collecting tubule cells in the outer zone and have fewer mitochondria and more numerous cytoplasmic bodies. A centriole (ce) lies near the lumen. Magnification: × 11,000.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juxtaglomerular apparatus (see the chapter by Hatt in this volume). The efferent arteriole may have a thick wall on leaving the glomerulus, but this rapidly thins becoming a sinusoidal channel which quickly empties into the tubular capillaries (Barajas and Latta, 1963a).

Most capillaries in the cortex and medulla show large endothelial perforations or fenestrations (Pease, 1955a; Siadt-Pour, 1959). Rhodin (1962a) describes a diaphragm across capillary endothelial fenestrations in the renal cortex and medulla. The effectiveness of this as a semipermeable barrier in capillaries in the glomerulus and between cortical tubules may be questioned, because under the conditions of thorotrast injection, large particles are able to pass through the pores and basement membrane and are found beneath the endothelium and in the interstitial spaces (Latta *et al.*, 1960; Maunsbach, 1964e; Osvaldo and Latta, 1964). The capillaries near the juxtaglomerular apparatus seem to have few fenestrations and seem less permeable than elsewhere in the cortex (Latta and Maunsbach, 1962). In the medulla, on the other hand, the diaphragm across the fenestrations of the capillaries (Figs. 7, 14) appears to present a fairly effective barrier to the passage of thorotrast particles out of the bloodstream (Osvaldo and Latta, 1964).

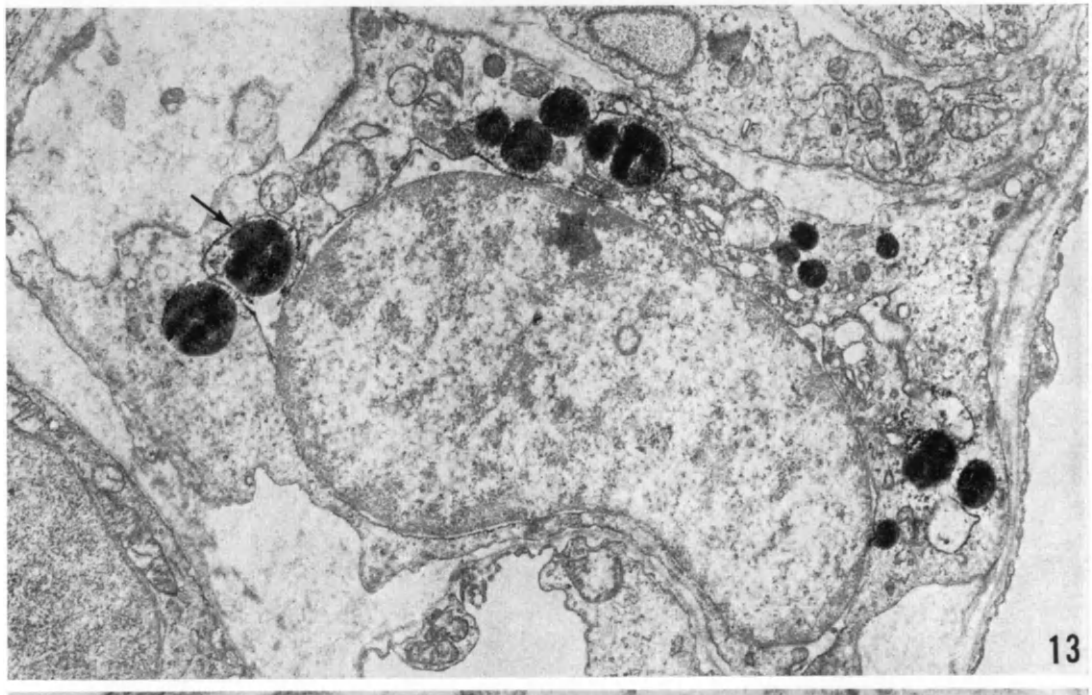
In the medulla are found large vessels having a thick endothelium without perforations (Fig. 6). They may be surrounded by cells, probably smooth muscle cells or pericytes. There are vessels of different caliber at each level in the medulla, and these vary in diameter as well as in structure. Structural and histochemical differences between vessels in the outer zone of the medulla have been reported (Longley *et al.*, 1960). At deeper levels in the medulla of the rat, most of the vessels seem to have fenestrations and it seems very difficult to identify definitely which vessel is ascending and which is descending. The endothelial cells frequently send small pointed prolongations through the basement membrane.

The renal lymphatics are said to be associated mainly with the blood vessels and their presence has been questioned in the parenchyma of the cortex or medulla. Although physiological studies of renal lymph have been performed (LeBrie and Mayerson, 1960), the lymphatics have not been demonstrated in the kidney by electron microscopy.

B. Nerves

Light microscopic studies have associated the nerves in the kidney mainly with the vessels. An electron microscopic study of the nerves in the juxtaglomerular apparatus has shown characteristic nerve endings of two types associated with smooth muscle cells and granular cells in the juxtaglomerular apparatus (Barajas, 1964).

FIG. 12. Inner zone of medulla. This shows the relations of thin segments (ts), capillaries (c), one of the vasa recta (v), and interstitial cells (i). The latter are different from the interstitial cells in the outer zone. Magnification: × 4400.



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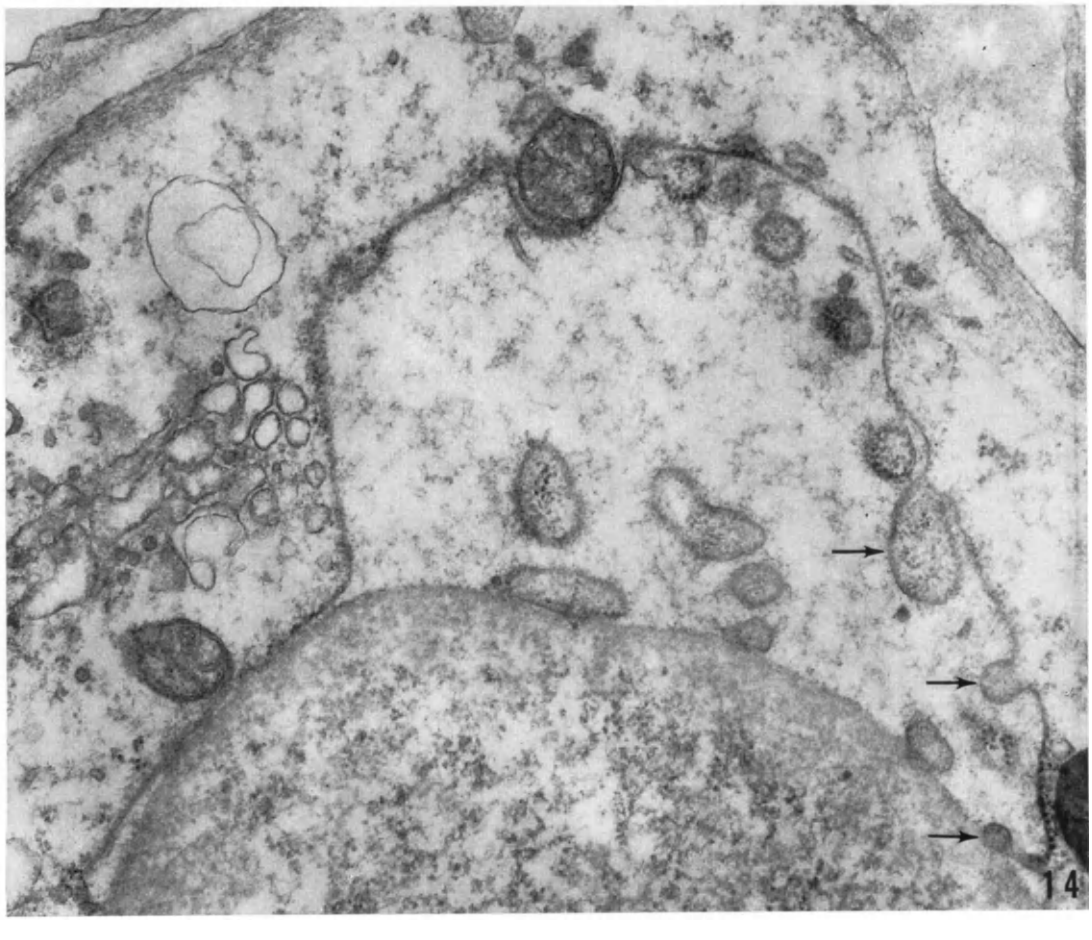

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C. Interstitial Cells

In the cortex, several types of cells are seen in the triangular spaces between tubules. Besides endothelial cells, these seem to represent fibroblasts, macrophages with various cytoplasmic bodies, and possibly other cell types. Ciliated cells have been found in the region of the juxtaglomerular apparatus (Barajas and Latta, 1963b), but the significance of this observation is not clear because cilia have been found in other mesenchymal cells such as fibroblasts and smooth muscle cells. Cells with many of the characteristics of plasma cells have been found repeatedly at the hilus of the glomerulus (Barajas and Latta, 1963b).

The presence of interstitial cells of an unusual type in the renal medulla has been noticed in early light microscopic studies (Renaut and Dubreuil, 1907) and also by electron microscopy (Novikoff, 1960; Leeson, 1961; Lapp and Nolte, 1962; Rhodin, 1962b; Rosen and Muehrcke, 1964). In the medulla of the rat, interstitial cells of two general types are found. In the outer zone, most of the interstitial cells resemble fibroblasts (Fig. 6), while the cells in the inner zone of the medulla have a peculiar appearance and contain numerous osmiophilic droplets (Figs. 12-14). A layer of moderately dense material covers a variable portion of the cell surface. The cytoplasm appears swollen in many cells and the numerous mitochondria may show focal swelling. Golgi membranes are prominent. Centrioles, multivesicular bodies, and various other cytoplasmic bodies may be found. A number of microtubules are scattered in the cytoplasm. Bundles of filaments also lie in the cytoplasm, usually near the plasma membrane. These bundles are particularly prominent in the smaller projections of the cytoplasm. Round dark bodies resembling lipid droplets are numerous and prominent in the cytoplasm. The nuclear envelope usually shows an irregular separation of the outer membrane. Often there is a marked enlargement of the perinuclear space with the outer nuclear membrane sending a large irregular projection for some distance into the cytoplasm. These projections are often covered with RNP granules and frequently contain vesicles of varying size, sometimes appearing as invaginations of adjacent cytoplasm. A few dark osmiophilic droplets have also been seen in the perinuclear space. In the nucleus, the chromatin material is concentrated at the periphery next to the nuclear envelope. A single dense layer of closely packed chromatin granules lies on the inner nuclear membrane.

FIG. 13. Interstitial cell in inner zone of medulla. This contains a number of lipidlike droplets, one of which appears to be in the perinuclear space at the left (arrow). Magnification: × 15,000.

FIG. 14. Interstitial cell in inner zone of medulla. The perinuclear space is frequently irregularly enlarged as shown here. The apparent inclusions in it seem to arise from invaginations of the cytoplasm as shown at the right (arrows). Bundles of fibrils lie just beneath the plasma membrane at the upper left and right. Magnification: × 35,000.

IX. Embryology

Renal structure does not seem to be completely developed at birth in the rat, mouse, hamster, rabbit, or in man (Clark, 1957; Leeson, 1957). In the rat, new nephrons are added in the outer nephrogenic zone during the first few weeks after birth. In man, formation of new glomeruli ceases before the fetus attains a weight of 2500 gm (Potter, 1961), but the human kidney at birth is not fully developed structurally or functionally.

Electron microscopic studies of the development of the kidney have usually emphasized the glomerulus, but some studies of tubular differentiation have been made. The early development of the rat metanephron has been traced with electron microscopy up to the initiation of glomerulogenesis and presented together with a literature review (Jokelainen, 1963). A primitive metanephrogenic blastemal cell cap at the blind end of the collecting tubule forms an independent renal vesicle surrounded by a basement membrane. The upper portion of the late vesicle represents the primitive tubular portion of the future nephron. The lumens become continuous after the vesicle fuses with the collecting tubule. In the lower portion of the vesicle the glomerulus begins as a cleft between epithelial cells in the middle of the outer vesicle wall.

Later stages of differentiation of tubule cells were presented by Clark (1957) and Y. Suzuki (1958). Clark (1957) found that the mouse nephrons continued differentiating for about 2 weeks after birth. At birth, the proximal tubule epithelium is low and the microvilli irregular. The membranes seem to develop by interlocking of adjacent cells. The mitochondria, sparse at birth, increase in number and size and dense granules appear in them. Many large round bodies which are seen in the cytoplasm disappear during the first week. These were thought to represent protein which passed through temporarily abnormally permeable newborn glomeruli and which was resorbed by the proximal tubules. The collecting tubules developed "pleating" of the basal cell membranes and dark cells containing small vacuoles appeared.

Y. Suzuki (1958) reported similar observations in mice, with the main exception of the basal intussusceptions which he thought developed by fusion of rows of vesicles. An alternate explanation is that the "vesicles" might represent cross sections of fingerlike projections from the basal membranes. If the present concept of interdigitation of basal processes from adjacent cells is correct, this interdigitation would have to develop subsequent to the stages he studied. Cytoplasmic granules were most numerous in the 12-hour-old rat. Leeson (1959) found that the developing mesonephrons and metanephrons in the rabbit were similar. He (1961) described an intertubular cell type in the postnatal hamster kidney cortex which decreased in number with age, few remaining in the adult kidney. He thought this cell was degenerate and either disappeared or differentiated into a fibroblast.

X. Renal Phylogenetic Differences

The discussion of tubule cell fine structure in this paper is primarily based upon observations made on rat and mouse kidneys which so far have been most extensively used in electron microscopic studies. However, it should be pointed out that considerable species variation exists between homologous cells of the nephron. These variations are obvious when comparing kidney tubule cells from different species of mammals and are very pronounced when comparing mammalian and amphibian tubule cells.

The variations that are present between mammalian proximal tubule cells of different species are mainly concerned with size, frequency, or sometimes structural modifications of certain cell organelles. Among examples of such differences are the larger size of microbodies in rat proximal tubule cells than in those of the mouse. Also, cytoplasmic bodies with dense contents are larger and more frequent in proximal tubule cells of the rat than of other mammals. Glycogen has been demonstrated in normal human proximal tubule cells (Biava, 1963), but appears to be absent in several other species.

The ultrastructure of homologous tubule cells in nonmammalian species varies considerably. Moreover, several segments of the nephron may be lacking in lower animals. Relatively few nonmammalian kidneys have so far been studied in detail by electron microscopy and therefore only a few examples can be given. A study of the kidney of a reptile, the "horned toad," demonstrated a number of differences from mammalian kidneys (Anderson, 1960). The proximal tubule of the amphibian mesonephric nephron has been studied in most detail (Bargmann *et al.*, 1955; Himmelhoch and Karnovsky, 1961; Karnovsky, 1963; Maunsbach, 1963; Christensen, 1963), whereas only one study seems to have been carried out of the amphibian pronephros (Christensen, 1964). The proximal tubule cells in the amphibian nephron are larger than those in mammalian kidneys; they usually have a less abundant brush border; and very characteristically they have no, or very few, basal plasma membrane infoldings. In addition, the cytoplasm, especially in *Necturus*, contains a very large amount of smooth-surfaced cytomembranes which form tubular or vesicular profiles and which are often surrounded by glycogen particles. It has been demonstrated by histochemistry (Himmelhoch and Karnovsky, 1961) that the *Necturus* proximal tubular cells appear to lack cytochrome oxidase and succinic dehydrogenase activity which are essential for aerobic metabolism. It has also been observed by electron microscopy and by measurements of intracellular potential differences that *Necturus* proximal tubule cells are little affected by cyanide, but are quite sensitive to iodoacetic acid (Maunsbach, 1964a), indicating that these proximal tubule cells may have a predominantly anaerobic metabolism. Furthermore, it may be noted that most amphibian proximal tubules are supplied primarily by venous blood

through the renal portal veins. The distal tubule of the amphibian kidney, which is supplied mainly by arterial blood, has principally the same ultrastructure as the mammalian distal tubule (Himmelhoch and Karnovsky, 1961), although in *Necturus* the cells may contain a large amount of glycogen (Maunsbach, 1964e).

XI. Other Tissues Transporting Salts or Fluids

Several other epithelial cells which have the capability of salt or fluid transport have structural features in common with kidney tubule cells. Most striking in this respect is the presence of plasma membrane infoldings as pointed out by Pease (1956). Among cells which show such infoldings from the basal surface are cells of the choroid plexus (Pease, 1956), the epithelial cells lining the excretory ducts of certain glands such as the salivary glands (Pease, 1956; Rutberg, 1961), and certain cells in the excretory ducts of insects (D. S. Smith and Littau, 1960; Copeland, 1964). The choroid plexus has infoldings of the "basal" surface adjacent to the aqueous humor (Pease, 1956; Holmberg, 1957).

Of special interest are the extrarenal glands for salt secretion which have developed especially in marine animals. The nasal salt glands of marine birds have deep plasma membrane infoldings at the basal and lateral surfaces (Doyle, 1960; Komnick, 1963). In the rectal salt glands of elasmobranchs, the infoldings involve primarily the lateral cell membranes (Doyle, 1962; Bulger, 1963). The lacrimal glands of sea turtles also have a lateral proliferation of membranes, but in the form of abundant microvilli (Ellis and Abel, 1964).

XII. Physiological and Pathological Reactions

Some reactions of the tubular epithelium with functional significance for cellular ultrastructure have been mentioned above. Other reactions will be considered more systematically here.

A. Fixation of the Kidney as a Whole

The changes occurring during fixation may be considered very sensitive alterations in response to adverse conditions that can occur under physiological or pathological stress. As stated in Section IV, A, dripping the fixative on the surface of the kidney in the living animal has been found to produce the best results (Pease, 1955a, b, c; Maunsbach *et al.*, 1962a). With this technique, the proximal tubule lumens are open and the tubules remain separated so that their basement membranes are not in contact. The capillaries contain uncompressed red blood cells. Extracellular compartments may occur in proximal, distal, and collecting tubules and seem to be produced by mechanical trauma and hypertonic solutions, but they may result under

other physiological and pathological conditions. Fixation by immersion favors apical swelling and loss of compartments in proximal tubules. The distal convoluted tubules, collecting tubules, and glomeruli are relatively resistant to these factors. However, the dripping technique is not effective in preserving well the deeper cortical or medullary tissues. Perfusion with solutions of osmium has been unsuccessful with medulla but perfusion with glutaraldehyde has given promising results in the cortex (Section IV) and in the medulla (see Sections VI–VIII).

B. Autolysis, Ischemia, and Shock

Studies of autolysis demonstrate the reactions of cells to more prolonged adverse conditions than are found during fixation and may be considered to indicate some of the reactions to be expected in the body during ischemia, infarction, shock, and perhaps other conditions. A light microscopic study of autolysis in the rat kidney has shown eventual swelling of the distal and collecting tubule cells in addition to the previously recognized early swelling of proximal tubule epithelium (Osvaldo *et al.*, 1965). Sequences in the development of pyknosis, karyorrhexis, and karyolysis in the different types of renal cells have been studied. Comparison with tissue prepared for electron microscopy showed that considerable shrinkage is produced by Helly's solution or Zenker-type fixation and paraffin embedding. Electron microscopic studies have shown the development of cytoplasmic bodies (possibly including lysosomes) and myelinlike figures associated with the brush border and basilar plasma membranes (Ito, 1962; Latta *et al.*, 1965). The persistence (Takaki *et al.*, 1955) and even extension (Ito, 1962; Osvaldo *et al.*, 1965) of the membrane systems of cells after many hours of ischemia is a surprising demonstration of their structural stability and dynamic behavior.

After clamping the renal artery for 1 hour, reactions of the tubules to ischemia were demonstrated (Thoenes, 1962a, 1964). The major changes were in the proximal tubules in which the cells underwent marked swelling and eventually ruptured into the lumen. The debris distended distal tubules. The thin segments of Henle and the collecting tubules showed only slight changes. Degenerative changes including multilocular inclusions have been described in human kidney biopsies in acute anuria (Dalgaard and Pedersen, 1961). Partial constriction of the renal artery produces changes which are described by Hatt elsewhere in this volume.

C. Poisoning

In acute uranium poisoning changes in the cortical tubules seem to be associated with three processes: necrosis, resorption of protein, and polyuria (Stone *et al.*, 1961). In pyknotic necrosis the chromatin of the nuclei becomes marginated at the nuclear envelope. With cellular swelling and rupture, swollen mitochondria and other fragments are released into the tubular lumen. Resorption of plasma proteins and necrotic material from the lumen probably accounts for the appearance of cytoplasmic

bodies with quite variable contents. The onset of polyuria was correlated with the development of extracellular compartments (Stone *et al.*, 1961; Latta *et al.*, 1962b).

Mercuric chloride after injection was found in the mitochondria of the pars recta of proximal convoluted tubules (Bergstrand *et al.*, 1959). Electron microscopic examination showed enlargement of mitochondria and destruction of lamellae. After cell fractionation mercury was found in mitochondria and microsome fractions. In contrast to this localization of inorganic mercury, organic mercury was distributed throughout the cortex. After poisoning rats with mercuric chloride (Mölbert *et al.*, 1964), spherical bodies and changes in the microvilli were noted in proximal tubules within the first few hours. Later, "cytosomes" developed.

In patients with chronic lead poisoning Richet *et al.* (1964) have found characteristic electron microscopic lesions in the proximal tubules consisting of intranuclear deposits, intracytoplasmic deposits thought to be siderosomes, and mitochondrial alterations.

Silica gel injected intraperitoneally into rats apparently produces deposits in several sites, including mitochondria of proximal tubules (Policard *et al.*, 1960a).

In acute diethylene glycol poisoning, swelling of the proximal tubule cells and the mitochondria were seen (Aizawa, 1962). Later the basal intussusceptions dilated and large vacuoles developed. Similar changes were noted in distal tubules.

The *d*-isomer of serine can cause necrosis of terminal portions of proximal convoluted tubules (Wachstein and Besen, 1964a). Two types of changes were observed. One type included marked vesiculation of the cytoplasm and an increase in size and number of "cytosomes" and multivesicular bodies. The other type of lesion was a coagulative necrosis characterized by increased density of mitochondria and all other subcellular structures.

Phlorizin, which is known to block sugar transport across proximal tubules, was reported to cause swelling of isolated kidney mitochondria, whereas ATP seemed to produce shrinkage (Burgos *et al.*, 1964).

Focal cytoplasmic degeneration with the appearance of cytoplasmic inclusions (or bodies) has been observed as a common response of cells, including renal tubule cells, to various injuries or even physiological states (Hruban *et al.*, 1963).

D. Ions, Hormones, and Vitamins

In a study of experimental potassium deficiency, MacDonald *et al.* (1962) observed splitting and thickening of the basement membrane of the thin portions of the loop of Henle. In kaliopenic nephropathy Biava *et al.* (1963) observed large extracellular compartments in proximal and distal tubules. Muehrcke and Rosen (1964) found mitochondrial abnormalities and an increase in intracellular osmiophilic bodies in proximal tubules of biopsies of six patients with kaliopenic nephropathy. In rats, they found that acid phosphatase-rich osmiophilic bodies were formed in collecting ducts, interstitial cells, Henle's loops, and capillaries of the papillary tip. Proliferation of the cells of collecting ducts in the inner medullary zone occurred together with a fourfold increase in lactic dehydrogenase activity. Potassium-deficient rats (Morrison

and Panner, 1964) developed granules in the cytoplasm of papillary collecting tubule cells. Similar granules were also seen in interstitial cells. Acid phosphatase was identified in the granules.

Excess DCA can produce dilatations of the intermembranous spaces (extracellular compartments) in proximal tubules (Hatt *et al.*, 1963). The distal tubule, on the other hand, shows other changes including an increase in the basal membranes.

After parathyroid hormone injections, Engfelt *et al.* (1958) found increased numbers of microbodies and large round bodies in proximal tubules. After calcium gluconate was injected intraperitoneally (Policard *et al.*, 1960b), needlelike crystals were observed in proximal tubule cells. Other deposits with concentric layers were found outside the base of the cells. Two types of renal calcification have been studied by Caulfield and Schrag (1964). Following injections of parathyroid hormone, needle-shaped crystals with the electron diffraction pattern of apatite appeared first in mitochondria and vacuoles and later in large areas of cytoplasm of the straight portions of proximal tubules. With recovery, calcified debris appeared in the lumens. After calcium gluconate injections, confluent granular deposits without a diffraction pattern (and thought to be calcium carbonate) appeared in the basement membranes of the convoluted portions of proximal and distal tubules. Extracellular compartments also occurred. Renal calcification has also been produced with large doses of vitamin D (Giacomelli *et al.*, 1964). Elongated crystals appeared in cytoplasmic vacuoles in proximal tubule cells. During the second week of the experiment smaller crystals were present extracellularly at the base of tubule cells. In some cases these extended between the cells toward the lumen and in other cases they extended along the basement membrane.

E. Renal Homotransplantation

Galle and de Montera (1962) during an electron microscopic study of the interstitial cellular infiltrate in a human renal homotransplant found that the greatest portion of the cells were mononuclear cells, consisting of lymphocytes, plasmocytes, and histocytes, and unidentified cells thought to be related to these. Although many pyroninophilic or ribosome-rich cells have not been identified, others have been related to mature and immature plasmocytes, fibroblasts (Galle and de Montera, 1962), and lymphoid or histocytic cells (Binet and Mathe, 1962). Many cells in graft-versus-host reactions were rich in ribosomes but contained little endoplasmic reticulum (Binet and Mathe, 1962), suggesting that the absence of endoplasmic reticulum or ergastoplasm may be connected with the absence of humoral antibodies (in contrast to the plasma cell which has been associated with the production of humoral antibodies). Kountz *et al.* (1963) concluded that the basic mechanism of rejection of homotransplanted kidneys in dogs was an immunological attack on the endothelium of the intertubular capillaries, caused by immature plasma cells arriving via the bloodstream. These cells became closely applied to the endothelial cells and it even seemed possible that cytoplasmic continuity might have been established at scattered

points. As endothelial and vascular destruction progressed, necrosis became common, especially in proximal tubules. No glomerular changes were observed. This work was subsequently published with considerably more detail (Williams *et al.*, 1964). Another detailed study with similar results was performed by Porter *et al.* (1964). They confirmed earlier studies in identifying the pyronine-positive cells infiltrating the homotransplant as resembling plasma cells, lymphocytes, or histiocytes (monocytes). Hamburger *et al.* (1965) have recently summarized their observations on transplanted human kidneys.

F. Various Disease Processes

In experimental hydronephrosis in rats (Novikoff, 1959) changes in acid phosphatase droplets or lysosomes of the proximal tubules have been noted. Within 6 hours the tubules dilate and extrude portions of the apical cytoplasm into the lumen, and the lysosomes contain myelin figures and mitochondria. Four to 7 weeks after removal of a ligature from the ureter of such a kidney, isolated areas of degenerating cells contain large irregular masses with acid phosphatase activity.

Tubular changes in human renal disease have been difficult to evaluate because of the sensitivity of the tubules to needle trauma. However, changes were marked enough to be considered significant in five cases of the Debre-de Toni-Fanconi syndrome (Jackson *et al.*, 1962). Early changes in the proximal and distal convoluted tubules suggested that the basic cause of the renal lesions lay in a metabolic deficiency because no deposits of crystals were identified in tubular cells. Cystine crystals were localized in interstitial cells and identified by electron diffraction. (The difficulties of obtaining an identifiable electron diffraction pattern from such a single organic crystal in a tissue section involve first, finding a crystal sectioned in a plane normal to the electron beam so that a symmetrical diffraction pattern can be obtained, and second, photographing the pattern rapidly with low-beam intensity because the diffraction pattern may disappear after a few seconds.)

Maleic acid produces changes in renal function of rats similar to those of the Debre-de Toni-Fanconi syndrome. Electron microscopic studies of early changes showed dehydration and shrinkage of the tubular cells and an increase in size of apical vacuoles (Worthen, 1963).

In two cases of renal diabetes (Monasterio *et al.*, 1964), the proximal tubule cells showed focal abnormalities, among which pleomorphic alterations in the mitochondria were prominent. In idiopathic renal glycosuria, needle biopsies of five patients revealed no abnormalities in fine structure (Freeman and Roberts, 1963).

The hyaline arteriolosclerotic deposits and the fine structure of renal arterioles in hypertensive patients have been studied with electron microscopy (McGee and Ashworth, 1963; Biava *et al.*, 1964).

The resorption of protein and other materials has been discussed under the heading "Cytoplasmic Bodies" (Section IV, G). The development of cytoplasmic bodies and ferritin has been observed in human kidneys in hemoglobinuria (Reger *et al.*, 1961).

G. Renal Tumors

A spontaneous renal adenocarcinoma of the frog contained, in a third of the cases, viral-like particles in the cytoplasm, nucleus, and intercellular spaces (Fawcett, 1956).

A renal tumor produced in male but not female hamsters by diethylstilbestrol showed infolded basal membranes and the surprising development of groups of typical cilia (Mannweiler and Bernhard, 1957). Although cilia are highly differentiated structures, such a development may indicate reversion to an earlier phylogenetic stage.

Human clear-cell carcinomas of the kidney (hypernephromas, von Grawitz tumors) have been studied and found to contain lipid droplets, much glycogen, and many swollen mitochondria (Oberling *et al.*, 1959). The finding of basal infolded plasma membranes and apical microvilli demonstrated the origin of these tumors from proximal convoluted tubule cells.

Polyoma virus, injected into newborn hamsters, produces renal sarcomas rapidly within 2 weeks (Howatson and Almeida, 1960). At later times, the virus particles seem to disappear from the cells (Dourmashkin, 1962).

A nephroblastoma (Wilm's tumor) has been produced in chickens by the myeloblastosis virus (Heine *et al.*, 1962). The tumor seemed to arise by differentiation of primitive nephrogenic cells residual in the postembryonic kidney. All cell types were involved in viral elaboration by budding from the plasma membrane.

XIII. Conclusion

Electron microscopy has revealed many complex details in cells of the different parts of the nephron. Although much information has been obtained, much work remains. Histological details and anatomical relationships are far from complete. Histochemical identification and localization of enzymes and tissue substances has only begun. Correlations between fine structure and function are largely speculative at present. Many interesting features have been studied and described in pathological reactions and disease states, but the information obtained has not yet reached the stage of being necessary in the diagnosis or treatment of disease, except in isolated cases. However, the field of electron microscopy of the kidney is expanding rapidly. The kidney promises to be one of the first organs in which the physiological and clinical significance of changes in cellular fine structure will be established.

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THE STRUCTURE AND FUNCTION OF THE GLOMERULAR MESANGIUM

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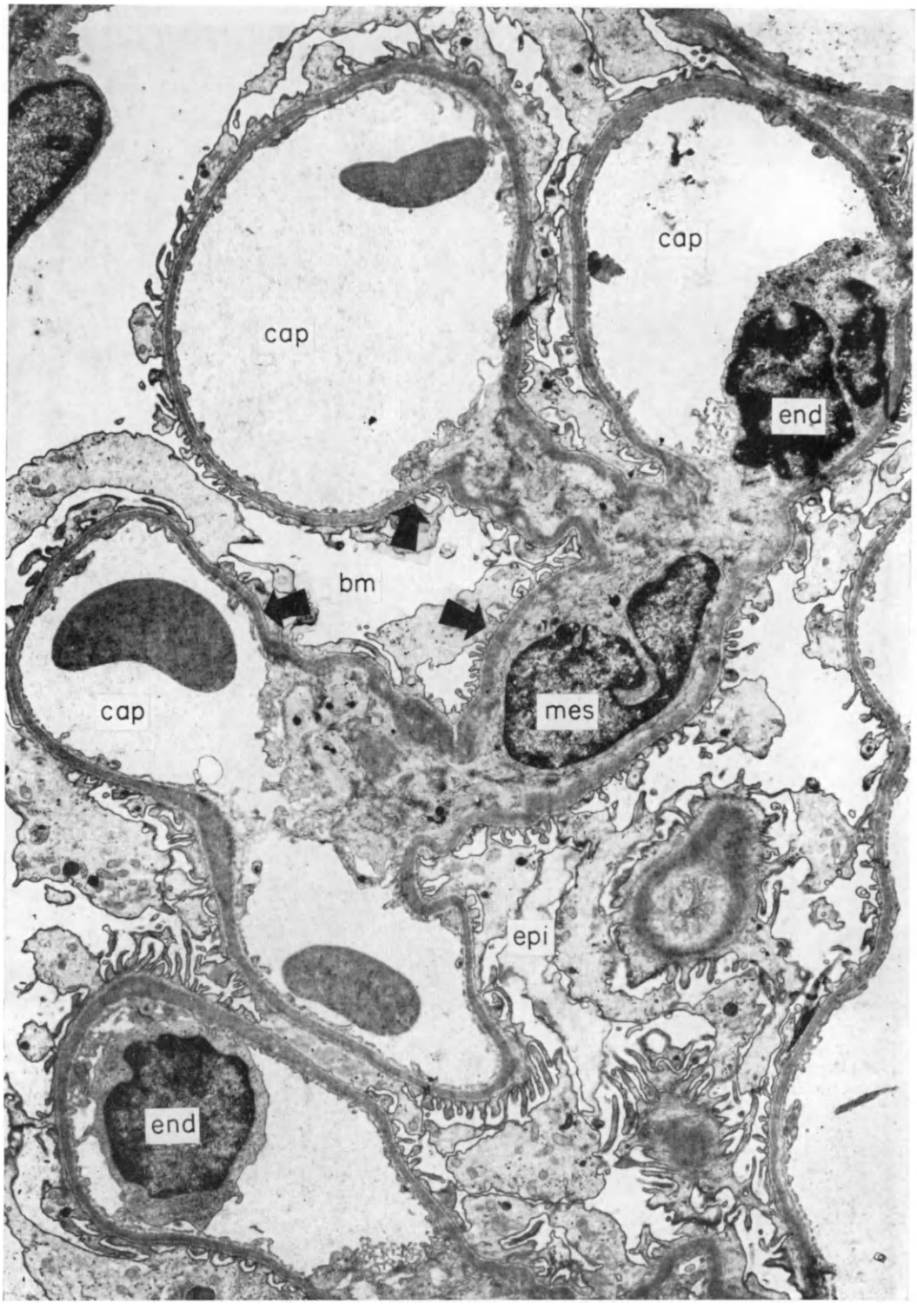
I. Introduction

The problem of the general structure of the mammalian glomerulus has been of interest for a good many years. Despite extensive studies by means of light microscopy two problems remained unsolved however:

(1) the fine structure of the capillary wall and especially the structure of the basement membrane separating the endothelium from the epithelium;

(2) the existence in the glomerulus of a third cell type—the mesangial cell.

The introduction of the electron microscope provided a new technique of particular importance for the study of these problems. The early investigators were primarily concerned with the problem of the fine structure of the capillary wall, and the problem of the existence of a third cell type was not investigated systematically. On the basis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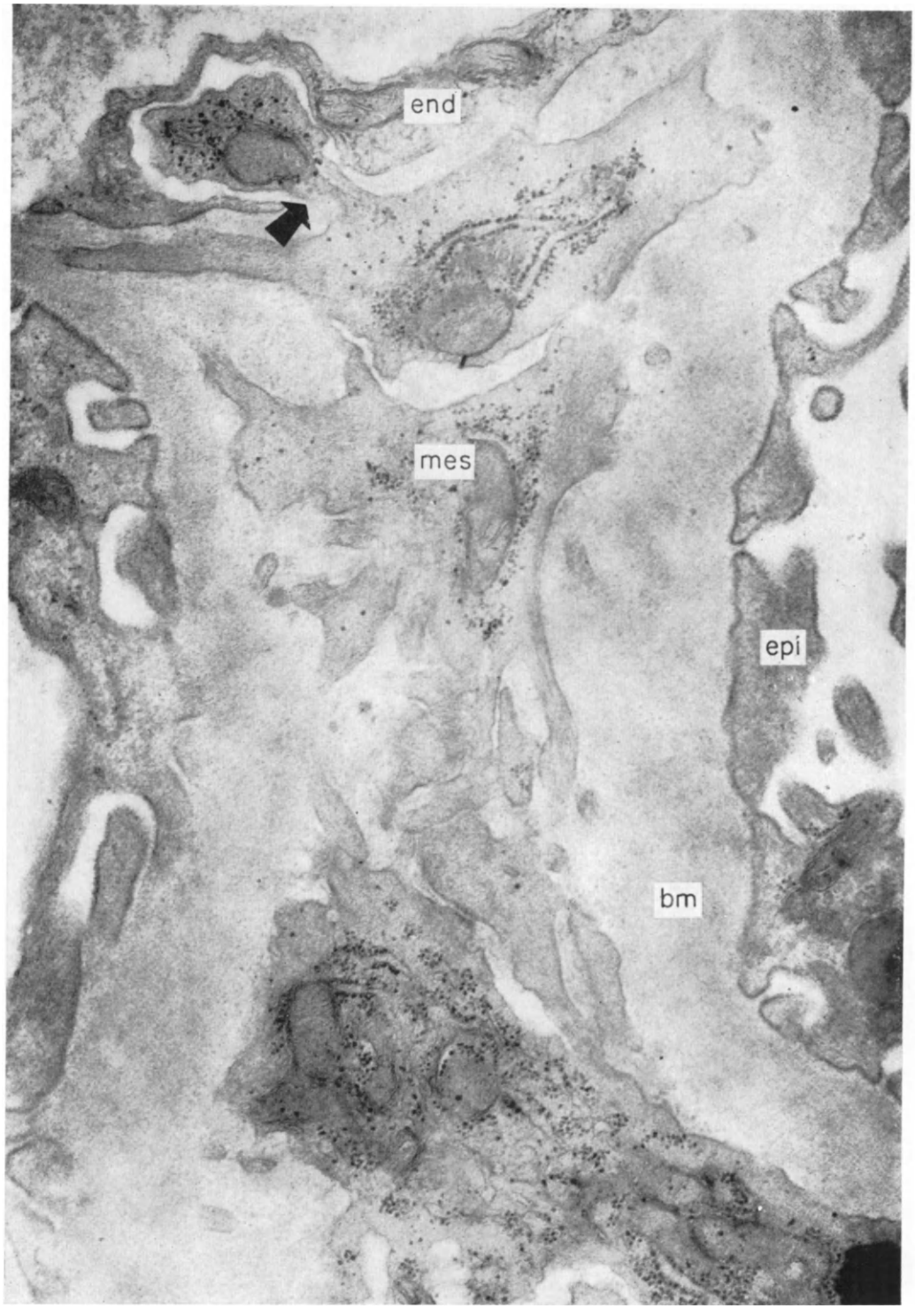
of a rather superficial examination it was generally admitted that there was no third cell type in the glomerulus and the mesangial cells were considered as endothelial cells. Two facts supported this view. First, electron microscopic studies of connective tissue did show a characteristic periodicity in the collagen fibrils surrounding the fibroblasts. Since no such fibrils could be found in the glomerulus it was concluded that the mesangial cells were not fibroblasts as originally described by Zimmermann (1933). Second, the original conception on which the mesangium hypothesis of Zimmermann was built was proved to be false. Indeed, Zimmermann's original description of a mesangium, consisting of fibroblasts supporting the glomerular capillary loops, was based on the hypothesis that the glomerulus is formed by the invagination of isolated capillary loops in the closed end of a tubule. The resulting topographical relationship between each loop and the invaginated basement membrane was then identical to the relationship existing between the bowel and its supporting mesenterium. Each capillary loop was thus supposed to be supported by a mesangium made up of a double basement membrane sheet containing fibroblasts. In analogy with the term mesenterium, Zimmermann coined the word mesangium. Although the general topography of the basement membrane is compatible with the invagination hypothesis, further embryological studies (Hall and Roth, 1957; Kurtz, 1958) established that the glomerulus is not formed by invagination but by the progressive differentiation *in situ* of a group of cells of mesenchymal origin. Also it turned out from microdissections and serial sections that each lobule of a glomerulus does not consist of a single isolated capillary loop, but that in each lobule numerous anastomoses exist (Hall, 1954; Boyer, 1955; Bohle and Herfarth, 1958; Bonhomme *et al.*, 1961). The similarity between the capillary loops of the glomerulus and the intestine was thus only apparent. On this basis the hypothesis of Zimmermann was rejected and most investigators admitted there was no third cell type in the glomerulus. All the cells located on the luminal side of the basement membrane were then assumed to be endothelial cells.

Recently there has been a complete reversal of this trend, and most of the authors, especially those using epoxy resins as embedding medium, consider the mesangial cells to be different from the endothelial cells and agree on the existence of two different cell types on the luminal side of the basement membrane. Considerable controversy persists however about the nature of these cells. Several extensive reviews on this subject have been published recently (Farquhar and Palade, 1962; Michielsen, 1962; Jones *et al.*, 1962; Suzuki *et al.*, 1963). Therefore it seems desirable to limit the present review to the actual knowledge on the topographical localization of the

FIG. 1. This figure shows a section through the periphery of a glomerulus. The arrows indicate how the basement membrane (bm) covers the intercapillary space. Consequently the basement membrane does not entirely surround the capillary lumens (cap). The mesangial cell (mes) is limited by the basement membrane and by the endothelial cells (end). The mesangial cell is interposed between the capillary lumens and is directly exposed to the intracapillary pressure, without the protection of a basement membrane. Approximate magnification: ×3000.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mesangial cells, their ultrastructure, nature, and function. No space will be devoted to the historical evolution of the ideas about the mesangial cells.

II. The Topographical Localization of the Mesangial Cell

At the vascular pole of the glomerulus the capillary basement membrane is in direct continuity with the basement membrane of Bowman's capsule. This capillary basement membrane covers the anastomosing capillary loops grouped in different lobules. It separates the glomerulus into two distinct parts: the capsular space covered with visceral and parietal epithelial cells and the luminal space. Inside this luminal space we find the capillary lumens with their endothelial lining and the mesangial cells in the intercapillary space. These mesangial cells are thus limited by the endothelial cells and by the basement membrane. As a rule they do not come into direct contact with the capillary lumens, but they fill the space between adjacent capillary lumens. It is obvious that under such circumstances, the basement membrane does not entirely surround the capillary lumens (Fig. 1).

In sections of a glomerulus the mesangial cells will show different topographical relations:

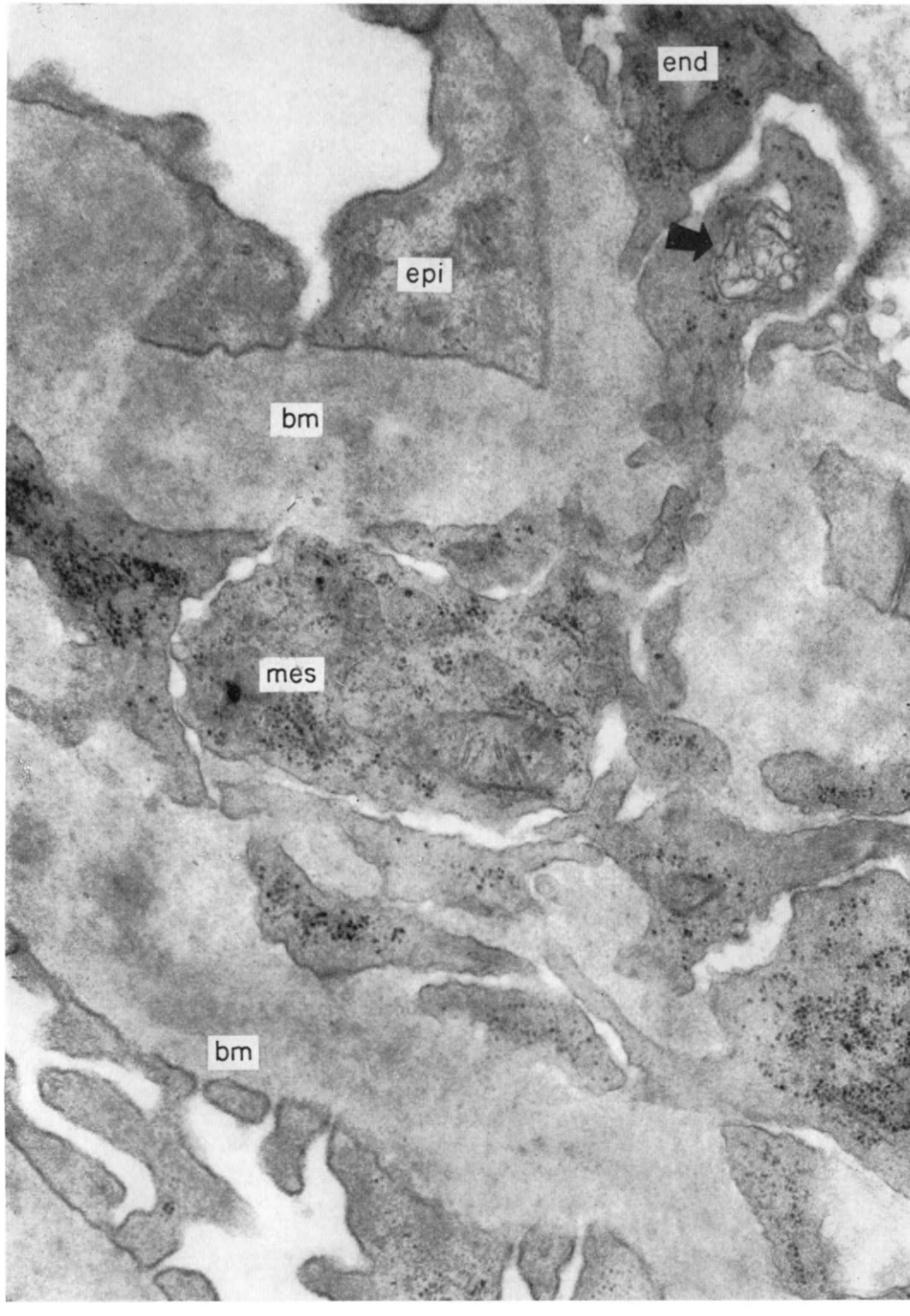
(1) A mesangial cell can be situated between two isolated capillary loops. This corresponds to a tangential section through the periphery of the capillary tuft. Since the mesangial cell is much smaller than the adjacent capillary loops, the basement membrane penetrates for some distance between the capillary loops and is fixed on the mesangial cell.

(2) Other mesangial cells are located in the center of several capillary loops (Fig. 1). One mesangial cell can thus come in contact with several capillary loops. In this case, the capillary loops are located around the central mesangial cells as trefoil leaflets.

(3) In other sections, the mesangial cells are alternating regularly with capillary loops in series of four or five, like knots of a chain.

(4) In some sections, the mesangial cells are found in an intracapillary position. They appear fixed on the basement membrane and covered by endothelial cells. Farquhar and Palade (1962) consider this intracapillary position as the usual one. The intercapillary position seen in many places is interpreted as resulting from tangential sections through intracapillary cells fixed on the wall of a capillary loop. The explanation for these different topographical relations cannot however be given by the study of single sections. Serial sections (Bohle and Herfarth, 1958) clearly demonstrate the relationships between the capillary loops, the basement membrane, and the intercapillary cells. From these reconstructions it appears clearly that the mesangial cells are located between different capillary loops; their position is thus intercapillary and not intracapillary.

FIG. 2. This figure illustrates the irregular spinous processes of the mesangial cell. The matrix surrounding these processes has an irregular density. The section goes through the neck of a diverticulum (arrow) of the mesangial cell (mes). Approximate magnification: ×31,000.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In optical as well as in electron microscopy the relationship between the macula densa and the vessels of the vascular pole has been extensively studied. Between the glomerular vessels and the macula densa there exists a small group of cells that have been described by several names: pohlkissen (Zimmermann, 1933), pseudo-Meissnerian cells (Goormaghtigh, 1936), and lacis cells (Oberling and Hatt, 1960; Latta and Maunsbach, 1962a). These cells penetrate into the glomerulus in the space between the vas afferens and vas efferens and the basement membrane of Bowman's capsule folding over these vessels (Bohle and Herfarth, 1958). Where these vessels divide into numerous capillaries, the lacis cells divide into various branches and spread as mesangial cells between the capillary loops and their anastomoses (Oberling and Hatt, 1960; Latta and Maunsbach, 1962a).

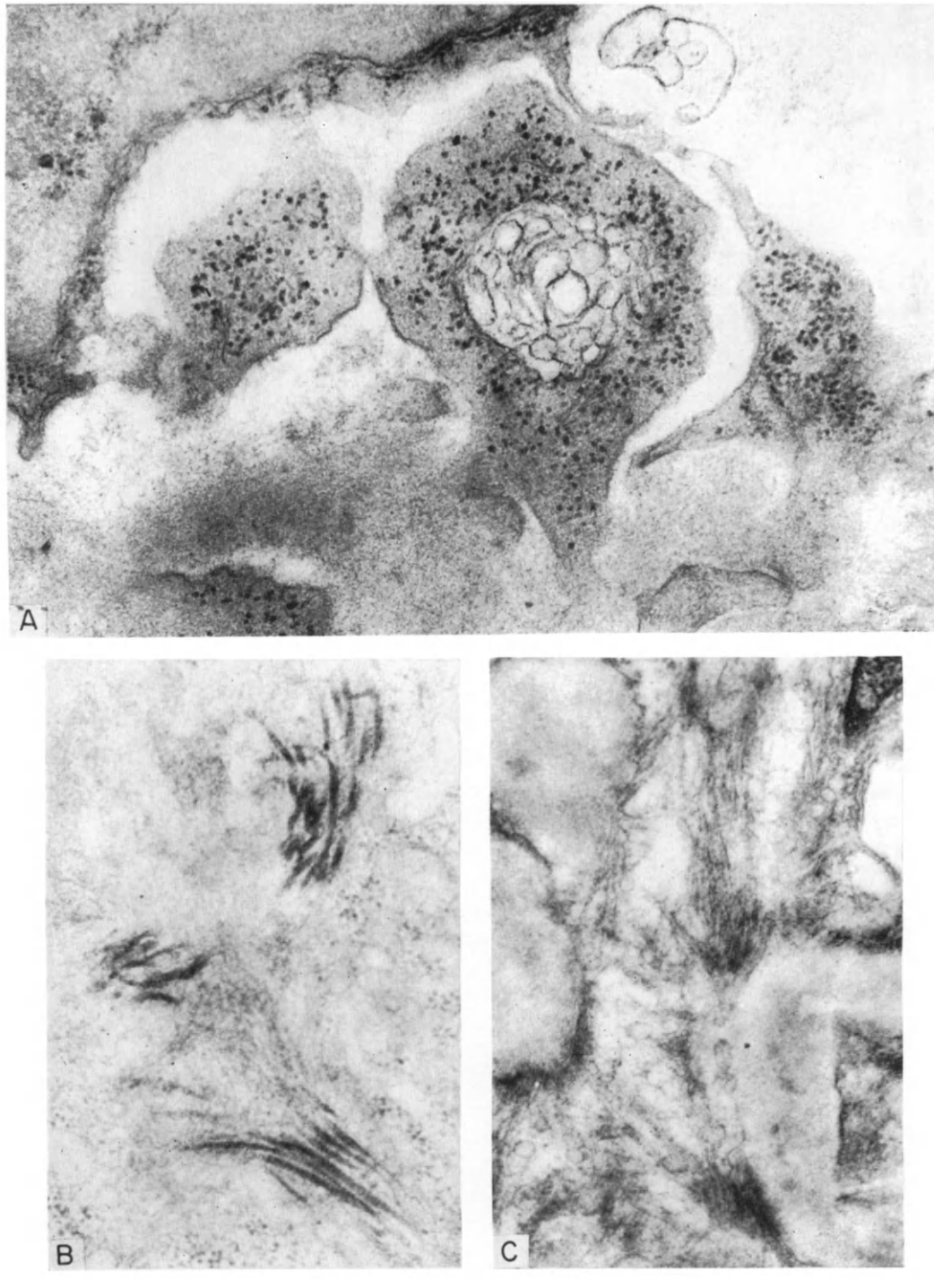
Thus the mesangial cells constitute a complex tissue filling the spaces between the capillary loops with numerous branches in continuity with the lacis cells which penetrate the glomerulus between the glomerular vessels.

III. The Fine Structure of the Mesangial Cell

A. The Mesangial Matrix

The mesangial cells are surrounded by an amorphous substance, the mesangial matrix. Different names have been proposed for this amorphous substance: basement membranelike material (Farquhar *et al.*, 1957), spongy material (Farquhar and Palade, 1962), mesangial matrix (Suzuki, 1959), spongelike tissue (Jones, 1963), and intercellular matrix (Latta, 1961). At first sight a similarity between this substance and the basement membrane could be suggested. There are in fact numerous structural and functional differences. The lamina densa, i.e., the central dense part of the normal basement membrane is surrounded on both sides by a less dense space, lamina rara interna and lamina rara externa. The lamina rara externa is clearly limited by the filtration slit membrane joining the foot processes of the epithelial cells. The lamina rara interna is less well limited. Where the basement membrane covers the intercapillary space, it is evident (Fig. 1) that the lamina densa can be seen as a single line covering the intercapillary space. There seems however to exist a continuity between the lamina rara interna and the amorphous substance surrounding the mesangial cells. Farquhar and Palade (1962) named it the inner loose layer of the basement membrane and considered that there is a relationship between this loose layer and the mesangial matrix. They state however that owing to the lack of information on the chemical nature of these substances their interpretation is only tentative. If the relationship between the mesangial matrix and the lamina rara interna is

FIG. 3. This figure illustrates the irregular density of the mesangial matrix surrounding the mesangial cell. In some places real channels seem to exist between the spinous processes. At the top of the figure (arrow), a diverticulum of the mesangial cell contains a cluster of membranes and vacuoles. Approximate magnification: ×31,000.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hypothetical, it is however beyond doubt that this matrix is distinct from the lamina densa which is the principal part of the basement membrane. The essential features of this substance are its variable density and irregular deposition around the mesangial cells (Figs. 2 and 3). In some places, it penetrates deeply between the irregular cytoplasmic processes of the mesangial cell; in others there are deep invaginations of the cell membrane containing some of this matrix. In still other places the matrix is scanty and the adjacent cell membranes come into close contact. The density of this matrix is so variable that in some places it is as dense as the lamina densa of the basement membrane, whereas in other places it is so loose that open spaces remain between the processes of the mesangial cell (Fig. 3).

Although most of the earlier workers denied the existence of periodic fibrils in the mesangial matrix, there is a growing evidence that such fibrils can be found. Spiro (1959) reported the existence of collagen fibrils in a case of amyloidosis. The significance of this finding could not however be established because it could be interpreted as a secondary invasion of a glomerulus by fibroblasts coming from periglomerular connective tissue. In 1959 Bencosme *et al.* demonstrated the presence of collagen fibrils in the intercapillary region of the rat glomerulus after uranium poisoning. In 1961 Hinglais-Guillaud and Galle described collagen fibrils in the mesangial matrix of proliferating mesangial cells in human cases of lobular glomerulonephritis and amyloidosis. Recently Kimmelstiel *et al.* (1962) found collagen fibrils in the noduli from a case of diabetic glomerulosclerosis. The presence of periodic fibrils is however not restricted to pathological material. In normal rats such collagen fibrils were regularly found after staining with phosphotungstic acid (Latta, 1961; Michielsen, 1962). They occur in small groups of ten to fifteen fibrils (Fig. 4b). We found them most often in the mesangial matrix between the intercapillary cells and the basement membrane. Although Latta was able to demonstrate in several places the existence of a major periodicity of 500–700 Å, we could only find a periodicity of less than 100 Å. These groups of collagen fibrils were scanty, but we found them constantly in normal rats near the vascular pole as well as near the urinary pole of the glomerulus. Recently Suzuki *et al.* (1963) reported similar findings in the avian glomerulus. Although they could not find collagen fibrils in the normal rat, they found them around the pathological intercapillary cells in cases of Masugi nephritis.

The mesangial cells are thus surrounded by a matrix of irregular density in which there are, at least in some species, periodic fibrils occurring in small bundles. These fibrils are shorter than the usual collagen fibrils and it is in some cases only that a major periodicity, similar to that of collagen, can be seen.

FIG. 4. (A) Detail of a mesangial cell showing a cluster of membranes and vacuoles in the center of a diverticulum. Approximate magnification: ×52,000. (B) After staining with phosphotungstic acid small groups of collagen fibrils can be found in the mesangial matrix. Approximate magnification: ×60,000. (C) In the cytoplasm of the mesangial cell small groups of parallel running fibrils are fixed on osmiophilic densifications of the cell wall. Approximate magnification: ×43,000.

B. The Mesangial Cell

The limits of the mesangial cells are extremely irregular with numerous spinous processes extending into the surrounding matrix and invaginations of the cell membrane penetrating deeply into the cytoplasm (Figs. 2, 3). In some places corresponding to tangential sections of the cell wall it is difficult to differentiate clearly the cell membrane from the surrounding matrix (Fig. 2). In other places there are limited osmiophilic densifications on the cell membrane. In methacrylate-embedded material the cell membrane has often an indentated irregular appearance, but after embedding in epoxy resins the cell limits are smooth. One of the main characteristics of the mesangial cells is the presence of small intracellular fibrils dispersed in small bundles at the periphery of the cell and fixed on the osmiophilic densifications of the cell wall (Fig. 4c). This structure is similar to the attachment bodies of the muscle cells in the arterial wall (Pease and Molinari, 1960). The central part of the cell is usually free of these fibrils.

In addition to these intracellular fibrils the mesangial cell contains the usual cytoplasmic organelles: endoplasmic reticulum, mitochondria, a Golgi apparatus, centrioles, and an indented nucleus. Finally the mesangial cells contain small vesicles, some multivesicular bodies, and dense bodies surrounded by a single membrane.

In his original publication (1933), Zimmermann described small processes of mesangial cells extending into the capillary lumens (Intrakapillarhöckerchen). This was confirmed by Mac Manus (1948) and Goormaghtigh (1951). These structures were studied by electron microscopy and described as diverticula of the intercapillary cell by Yamada (1955), Michielsen (1961, 1962), and Farquhar and Palade (1962). It is clear that the exact structure of these diverticula can only be seen in sections going through the neck of the diverticulum (Fig. 3). In transverse sections they will appear as double-walled cysts, the outer wall corresponding to the cell membrane of the endothelial cell. These structures were described as double-walled cysts by Bergstrand and Bucht (1958). Although they are true diverticula of the mesangial cytoplasm, these structures are usually described as devoid of cell organelles. In normal animals we often found in the central part of these diverticula a cluster of concentrically arranged membranes and vacuoles (Figs. 3, 4a). The surrounding cytoplasm is especially rich in ribonucleic acid (RNA) granules. As far as we know this structure has not been described before.

IV. The Nature of the Mesangial Cell

A. The Fibroblast Hypothesis

Numerous hypotheses have been proposed concerning the nature of the mesangial cell. In the original description by Zimmermann the mesangial cells were considered to be fibroblasts. In electron microscopy, however, it is obvious that there are important differences between mesangial cells and fibroblasts. The mesangial matrix is

less developed than the ground substance of the fibroblasts and in normal as well as in pathological reactions the collagen fibrils are so scarce that for several years all investigators agreed that there was no collagen in the glomerulus. The cytoplasm of the mesangial cell is also completely different from the cytoplasm of the fibroblast. The extreme development of the endoplasmic reticulum is lacking in the mesangial cells. The general structure is also different as the fibroblast does not present numerous spinous processes containing bundles of small fibrils fixed on attachment bodies.

B. The Endothelial Cell Hypothesis

The fibroblast hypothesis of Zimmermann has never been generally accepted even in optical microscopy, and many pathologists (Allen, 1951; Bell, 1950; Mac Manus, 1950) admitted that all the cells located on the luminal side of the basement membrane were endothelial cells. The endothelial cell hypothesis received considerable support from earlier work in electron microscopy. No collagen fibrils were found in the mesangial matrix and it was admitted that there was no difference between the fine structure of the mesangial cells and the endothelial cells. The identity between endothelial and mesangial cells was accepted by many authors with considerable variation. Some described the capillary lumens as anastomosing channels in a syncytial mass of endothelial cells (Elias, 1957). Others (Mueller *et al.*, 1955) described a cellular stalk of syncytial endothelial cells supporting the capillary lumens. Other authors described two different types of endothelial cells: the cells lining the capillary lumens, with loose cytoplasmic structure and poor in particles; the cells between the capillary loops, interluminal cell aggregates (Kurtz and Mac Manus, 1959) or deep cells (Farquhar and Palade, 1962), with a denser cytoplasm. Many authors actually share this point of view and believe that the mesangial cells are endothelial or less differentiated mesenchymal cells (Suzuki, 1959).

C. The Smooth Muscle Cell Hypothesis

The similarity between mesangial cells and the cells of the vascular wall of the arterioles is stressed by other authors. Farquhar and Palade (1962) mention the similarity with the fixed pericytes. There is actually a growing evidence that the mesangial cells are in fact more or less modified smooth muscle cells. This hypothesis was first proposed by Goormaghtigh (1942) on the basis of the study of the pathological reactions of the mesangial cells. Embryological studies of De Winiwarter (1943) supported this view. In electron microscopy Yamada (1955) confirmed the hypothesis of Goormaghtigh and described the occurrence of intracellular fibrils and intracapillary colliculi. This hypothesis was further developed by Michielsen (1961, 1962) on a topographical and cytological basis. The arguments in favor of the fundamental identity between the mesangial cells and the smooth muscle cells are the following: (1) There is a continuity between the mesangial cells and the cells of the juxtaglomerular apparatus (Oberling and Hatt, 1960). (2) The irregular cell borders, the presence of a mesangial matrix containing some collagen fibrils, the

intracellular fibrils fixed on the attachment bodies of the cell wall, all these fundamental characteristics of the mesangial cells are similar to those of the smooth muscle cells. (3) The smooth muscle cells present a great variability of structure. This is well known since the work of Ruyter (1925) showing the disappearance of the typical fibrillar structure of the smooth muscle cells and their transformation into secretory cells. A similar secretory activity has occasionally been seen in mesangial cells in optical (Okkels, 1929; Goormaghtigh, 1951) and electron microscopy (Michielsen, 1962). An additional argument for the fundamental identity of the mesangial and smooth muscle cells is given by the studies of Dunihue and Boldosser (1963). They showed that the response of the mesangial cells to mineralocorticoid deficiency was similar to the response of the juxtaglomerular cells and of the media cells of renal arterioles.

Whatever final opinion one may have about the nature of the mesangial cells, one can only agree with Farquhar that "the cell differentiation is more diverse and more finely graded than currently assumed as exemplified by our difficulty in classifying the third cell type found in the glomerulus."

V. The Function of the Mesangial Cell

The general structure of the glomerulus suggests that one of the main functions of the mesangial cells could be to support the capillaries and their numerous anastomoses. As the basement membrane does not entirely surround the capillary lumens it must be firmly fixed to the mesangial matrix and the interlocking spinous processes of the mesangial cells in order to resist the intracapillary pressure. This supporting function is experimentally demonstrated: elective destruction of the mesangial cells results in a ballooning of the capillary lumens and a fusion of the lumens into a big vacuole (Kawaji and Oyama, 1960).

When tracer particles are injected into the circulation it has been shown that these particles (ferritin, Farquhar and Palade, 1962; Thorotrast, Latta *et al.*, 1961; colloid ferric hydroxide dextran complexes, Kawamura, 1961) are mostly retained by the basement membrane acting as a filter. Very soon after injection (Farquhar and Palade, 1962) ferritin particles are present not only in the mesangial matrix, but also in small vacuoles, multivesicular bodies, and larger dense bodies in the mesangial cells. Later, the amount present in the mesangial matrix decreases and the ferritin content of the dense bodies increases. After 4 days the content of the dense bodies becomes heterogeneous suggesting a digestive process. This demonstration of a phagocytic activity of the mesangial cells led Farquhar to the hypothesis that the function of the mesangial cells would be to remove the residues of filtration accumulating against the glomerular basement membrane.

Within 5 to 15 minutes after injection, an important accumulation of the injected tracer is however found in the mesangial matrix (Farquhar and Palade, 1962) or in the less dense areas of this matrix (Latta *et al.*, 1961). This observation is most

unexpected as the classic renal physiology considers only a glomerular filtration through the periphery of the capillary wall with subsequent modification of the filtrate by tubular activity. The demonstration of a high degree of permeability of the mesangial matrix raises the question of the existence of another pathway for the glomerular filtrate. Indeed, the mesangial matrix is in direct continuity with the matrix surrounding the lacis cells, the ground substance surrounding the smooth muscle cells of vas afferens and vas efferens and the basement membrane of the macula densa. Such a transport had already been proposed by Oberling and Hatt (1960) who suggested that urine is picked up by the macula densa cells and circulates in the network of basement membranes surrounding the juxtaglomerular apparatus and the mesangial cells. It seems however highly improbable that urine could circulate against the pressure gradient existing between the capillary lumens and the distal tubular lumen. A flow in the opposite direction, from the capillary lumens toward the macula densa, would be more likely. At this moment there are however very few experimental data to support this view. Latta and Maunsbach, (1962b) mention: "within five to ten minutes after injection of thorotrast, when particles are distributed through the circulation and have passed into the channels between intercapillary cells in the centrolobular regions of glomeruli, particles can rarely be found between the cells of the lacis. At the hilus intercapillary matrix substance fills most of the space between cells and prevents passage of large amounts of particles. Later a few particles can rarely be found in cytoplasmic bodies of granular cells." This work of Latta demonstrates experimentally the possibility of a flow between the capillary lumens and the juxtaglomerular apparatus. Only limited amounts of the injected tracer material follow this way, but this does not exclude the possibility that particles smaller than the injected tracer or a "filtrate" of blood plasma could reach the juxtaglomerular apparatus in significant amounts. Latta believes that the greatest part of the tracer material circulating in the mesangial matrix must be carried back into the bloodstream. As a possible site for this, he indicates the region of the central part of the glomerulus where the capillaries join the efferent arteriole. The absence of a sufficient pressure gradient between the capillary lumens and vas efferens is however an argument against this hypothesis. The existent pressure relations would rather be in favor of a filtration toward the macula densa, which is known to be directly connected with the basement membrane network of the lacis cells.

Finally it must be stressed that no function has hitherto been described for the diverticula of the mesangial cells bulging into the capillary lumens. Their intracapillary situation suggests a possible role as pressoreceptors. Directly interposed between the capillary lumens, without the protection of a basement membrane, the mesangial cells are directly exposed to changes in the intracapillary pressure. With the special topographical relations between the mesangial cells and the juxtaglomerular apparatus in mind, the question can be raised whether the mesangial cells are not sensitive pressoreceptors, controlling the secretions of renin by the juxtaglomerular cells. There is actually no proof for this hypothesis which rests only on the anatomical relations, but fits the known physiological data.

It can be concluded that two functions of the mesangial cells are well established: the supporting of the capillary loops and the phagocytic activity of the cells. The permeability of the mesangial matrix for tracer material is well established, but the significance of this phenomenon is still obscure.

VI. Conclusion

Recent electron microscopic studies have definitively resolved the problem of the existence of a third cell type in the renal glomerulus. Although there is actually complete agreement on the main basic facts about the structure of these cells, their exact nature is still a subject of controversy. In our opinion the most likely hypothesis is that these cells are modified smooth muscle cells. The glomerular capillaries must then be considered as modified arterioles, a condition entirely logical for vessels interposed between two arterioles.

The mesangial cells support the capillary loops and have a phagocytic activity. The demonstration of the permeability of the mesangial matrix to tracer substances is actually difficult to understand in the light of the data on renal function. More correlation studies between function and structure are needed to elucidate this problem.

ACKNOWLEDGMENT

The authors are indebted to Professor De Duve for the use of his electron microscopy facilities and to Dr. Baudouin for his helpful advice.

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SOME MORPHOLOGICAL CONSIDERATIONS OF TRANSPORT IN THE GLOMERULUS[1]

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I. Introduction

Transportation of material from capillary lumens to an extracapillary position may be considered from various viewpoints. One approach has been to consider the capillary wall as a functionally homogeneous unit in which the passage of particles is regulated in a mechanical sense, i.e., the size and charge of particles passed is dependent on the size and charge of pores present in the capillary wall. The movement is thus contingent upon a constant hydrostatic pressure gradient (Pappenheimer, 1953; Bott and Richards, 1941). Another variant of the mechanical barrier philosophy

[1] This work was partially supported by an award from the United States Public Health Service A-1393.

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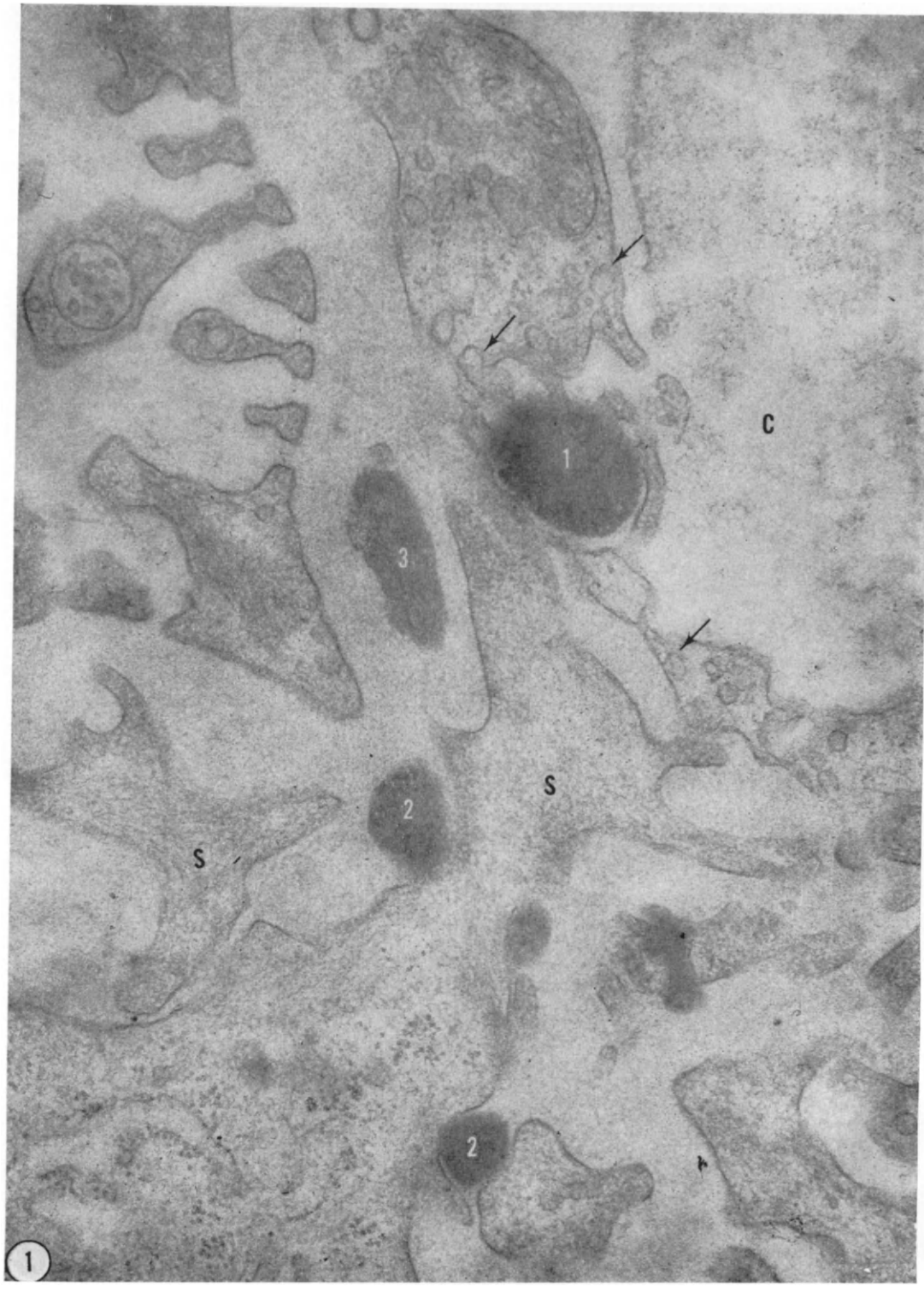

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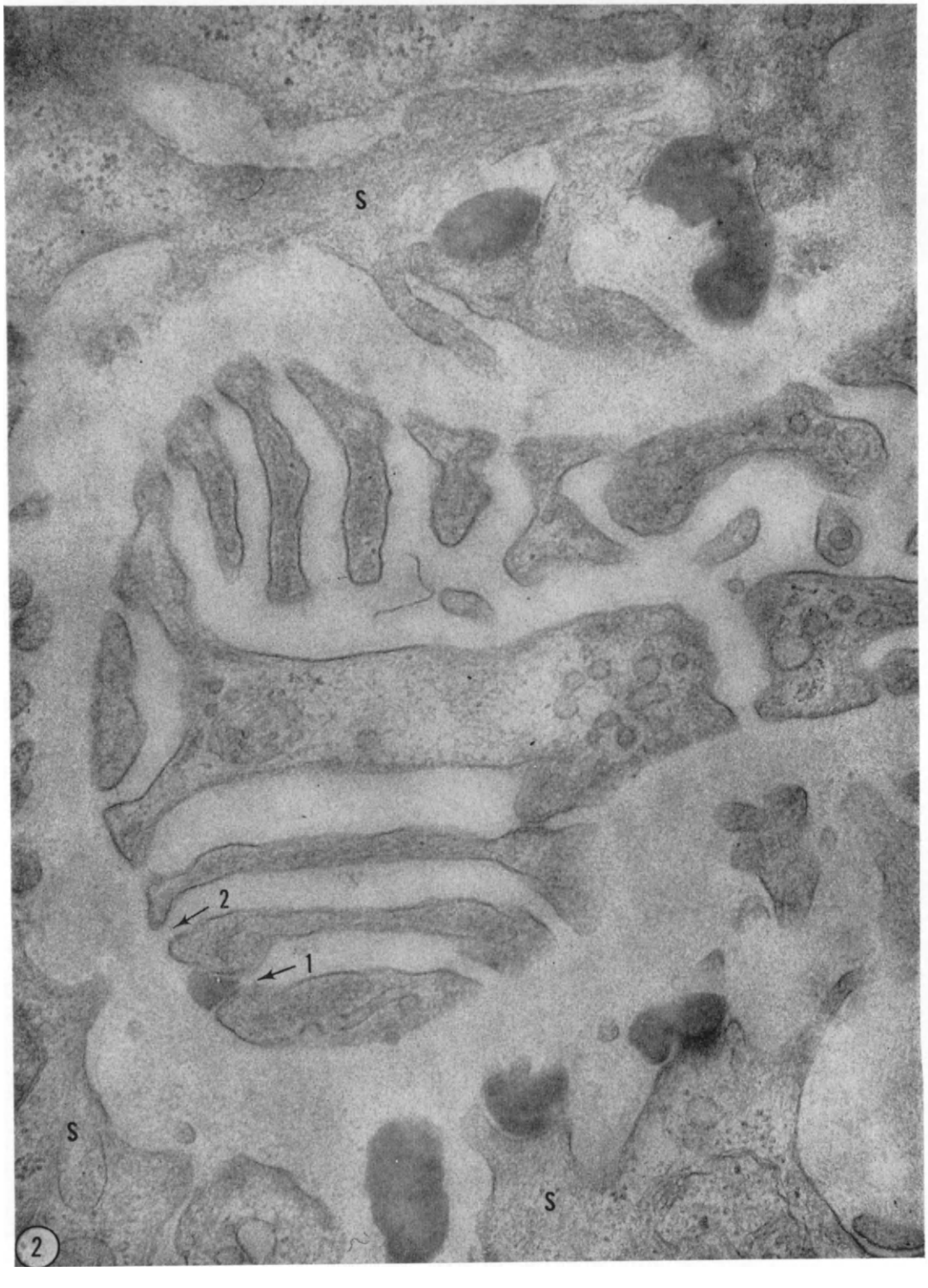
is the consideration of transport as a process of diffusion, dependent upon concentration gradients across the capillary wall, which in this case is thought of as a semipermeable membrane (Chinard *et al.*, 1955). The mechanical and mathematical concepts mentioned above are based upon concentration measurements of test substances on both the inside and outside of capillaries within the glomerulus or elsewhere, and the resulting theories are proposed to explain the findings with little attempt to ascertain the actual pathway taken by the transported material.

Another approach to the transport problem has made use of the electron microscope to study the glomerular wall in various conditions, but without the use of visible tracers. The structures seen under these conditions have given rise to speculations on transport, but they suffer from a deficiency which is the reverse of the mathematical-mechanical models. Whereas in the theories derived only from concentration measurements there is no possibility of ascertaining how the material moved from one side of the capillary to the other, in the observations of structure there is no possibility of recognizing what material is being transported by the structure being examined if the test material is not specifically identifiable. Hall (1957) developed a theory of filtration based on the apposition of epithelial cell foot processes to each other, thereby forming long, interconnecting "slit-pores" which were thought to regulate the size and quantity of filtered material. Sitte (1959) produced nephritis in rats and found a concomitant swelling of the basement membrane. Based on his observations he postulated that the filter of the kidney is the basement membrane and that it changes pore size by swelling or shrinking, thus changing the distance between the interlacing fibrils which make up its substance. Information obtained from more recent techniques suggests that both of these speculations have serious defects.

Since it would seem desirable to see material in various stages of transport through layers of the capillary, studies have been made utilizing the electron microscope and electron-dense tracers which could be directly visualized. These materials have included ferritin (Farquhar, 1960; Farquhar *et al.*, 1961), dextran (James and Ashworth, 1961), Imferon (Kawamura, 1961), Thorotrast (Latta and Maunsbach, 1962), and globin [see Section III, C (Menefee *et al.*, 1964a)]. From these observations using different materials, it appears that there are several possible paths for transport through the capillary wall and that each kind of particle may have its own preferred transit route. These variations will be discussed in more detail in a later section of this chapter.

FIG. 1. Twenty-five minutes after globin injection. A globin aggregate (1) has been taken up in a region containing many pinocytotic vesicles (arrows) which interconnect in several places suggesting a juxtacollicular region. Globin (2) is within sponge fibers, and globin (3) is also part way through the basement membrane. Some processes of stalk cells (S) demonstrate their fibrillar cytoplasm. The capillary lumen is at "C." Foot processes are seen along the basement membrane in the upper left. Magnification: ×45,600.



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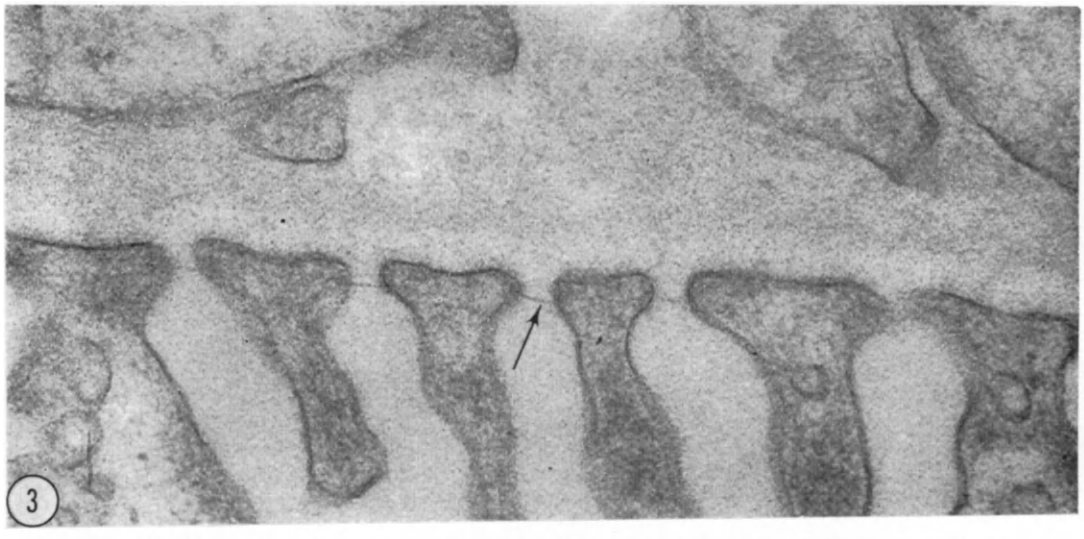
II. Structural Considerations

A. Basement Membrane

Glomerular endothelium is separated at all places from glomerular epithelial cells by a single basement membrane (Mueller, 1958). This positioning of the basement membrane so that material moving from the capillary lumen to Bowman's space must traverse it has led to the notion that the basement membrane is the primary filtering device of the glomerulus (Sitte, 1959; Farquhar, 1960; Farquhar *et al.*, 1961). Some support has been added to this opinion by the fact that fibrils are seen to be embedded in the membrane (Rhodin, 1955; Yamade, 1955; Sitte, 1959; Kurtz and Mc Manus, 1960; Farquhar, 1960) and an analogy has been drawn between this fibrillar arrangement and that of a filter. The organization of observed fibrils has variously been described as a meshwork, feltlike, or simply as fibrils embedded in an amorphous matrix. Kurtz and Mc Manus (1960) applied uranyl acetate to their material and obtained selective staining of the fibrils which they measured as having a diameter of 30–40 Å. The fibrils of fixed tissue are also visible without special stains (Figs. 1–3). We consider it probable that these fibrils are not permanent components of the basement membrane but rather that they represent short-range order interactions between the component protein molecules. The more general structure of the basement membrane is probably a thixotropic gel in which the transient dynamic interaction between several protein molecules can occur; thus the fibrils are only the fixed representatives of a continuously changing interrelationship (Section III, C).

The question of the embryonic origin of the glomerular basement membrane has been answered by Kurtz and Mc Manus (1959) who demonstrate that in the developing embryo there is no invagination of a pre-existing cup by a growing capillary tuft. Rather, the epithelial cells and prospective endothelial cells are first seen as solid groups which hollow out their respective lumens *in situ*. When the epithelial cells first begin to develop foot processes, basement membrane material is seen to appear between the developing endothelial cells and the adjacent epithelial cell processes. At the hilus the basement membrane of the glomerulus fuses with the basement membrane of the capsule (Mueller, 1958). Which cells are responsible for elaborating basement membrane material is not so clearly established. Kurtz and Feldman (1962) found that if young rats were given silver nitrate the basement membrane material was labeled with silver and could be identified in the electron microscope. These young rats were then killed at intervals and the deposition of new basement membrane

FIG. 2. Twenty-five minutes after globin injection. Globin aggregates are seen in several places within sponge fibers near stalk cells (S). The stalk cells are seen to have prominent fibrils in their cytoplasm. Slit membranes are seen between the foot processes; number "1" is displaced by a globin aggregate, whereas number "2" is in its normal position near the basement membrane. Magnification: ×45,600.



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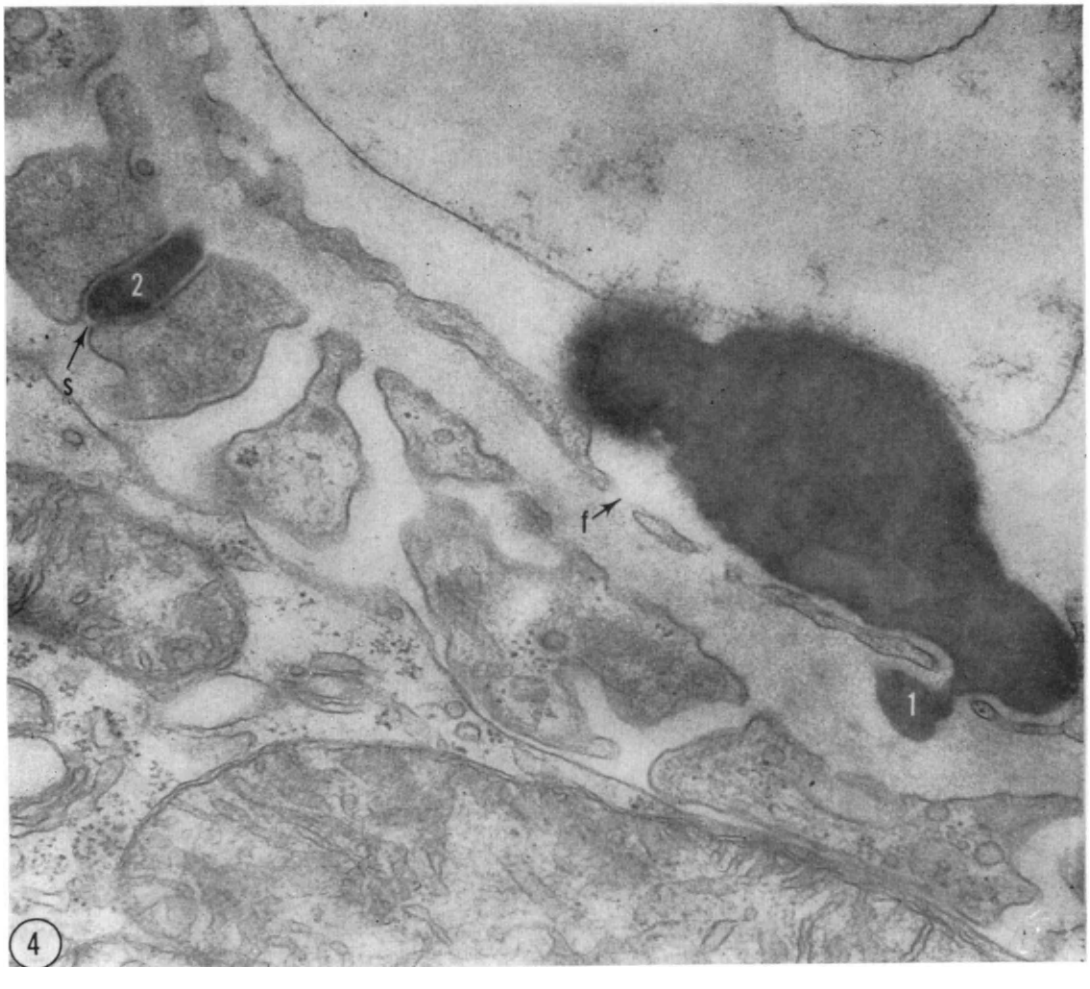

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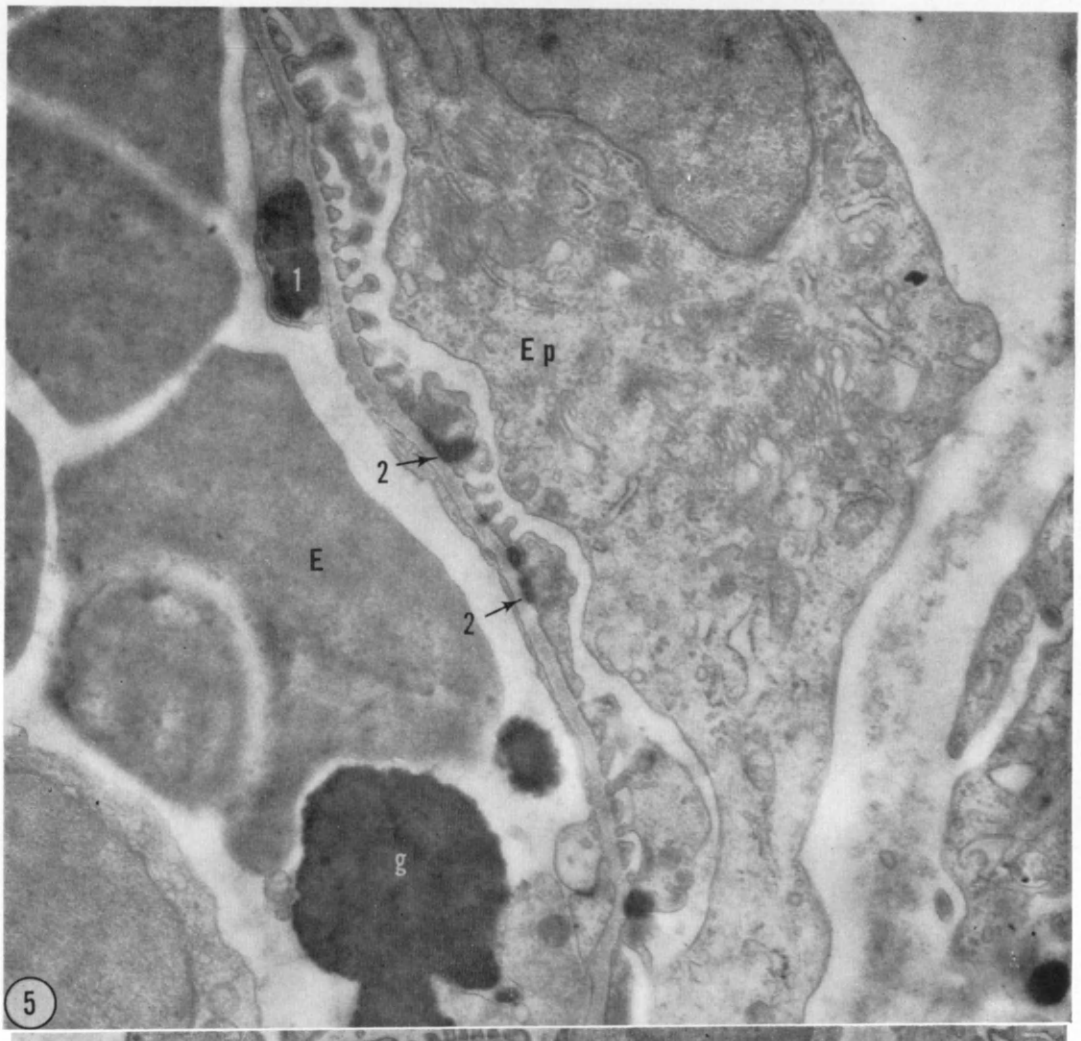
material observed. They found that all of the new material was deposited on the epithelial side. Furthermore, if rats having labeled basement membranes were made nephrotic, the new material was all deposited on the epithelial side. Other workers (Farquhar *et al.*, 1959; Jones, 1963; Dachs *et al.*, 1964; Movat *et al.*, 1961) have observed that in both diabetic nephropathy and nephritis the new basement membrane-like material is deposited mostly on the endothelial side and predominantly in the stalk region. In the normal aging of rats the central dense zone of basement membrane becomes thicker and the less dense zones on each side become thinner (Ashworth *et al.*, 1960). From these diverse reports it seems that epithelial cells, stalk cells, and endothelial cells may all be involved in the elaboration of basement membrane material and that different conditions may result in a selective increase of activity in one of the possible types.

B. Epithelium

The epithelial cells of the glomerulus are first arranged in the embryo as a solid aggregate surrounding the presumptive mass of endothelial cells (Kurtz and Mc Manus, 1959). As the endothelial cells begin to form hollow tubes, the epithelial cells are separated by clefts which eventually become Bowman's space. Very early in development the epithelial cells manifest processes on the side nearest the endothelium. These processes further alter in the adult to become the familiar foot processes which interdigitate with each other and cover all parts of the basement membrane of the glomerulus. The space between foot processes is fairly constant and is closed by a membrane (Fig. 3) which has been called "filtration slit membrane" (Yamada, 1955). This membrane has been said by some (Farquhar *et al.*, 1961) to represent a modified desmosome, but the resemblance is not very striking (see Section III, C). The free surface of the epithelial cells has a thickened membrane (Figs. 3 and 4) which may have some effect on water permeability (Latta and Cook, 1962). The epithelial cell contains in quantity the elements usually associated with a high rate of metabolism, i.e., mitochondria, ribosomes, Golgi material, and ergastoplasm. The foot processes are frequently seen to have small vesicles which are taken to represent pinocytotic activity (Yamada, 1955). The capsular and glomerular epithelial cells are derived

FIG. 3. Normal epithelial foot processes connected by slit membranes (arrow) and resting on basement membrane. The unit membrane on the free surface of the foot process is somewhat thicker than that of the endothelial cells (top of micrograph). Fibrils are seen within the basement membrane. Magnification: ×49,000.

FIG. 4. Glomerular wall 30 minutes after globin injection. An aggregate of globin within the capillary lumen extends through an opening in the endothelium (possibly a fenestration) and forms an expansion (1) within the basement membrane. Another fenestration with a diaphragm across it is seen at "f." Another globin aggregate (2) is seen between two foot processes whose slit membrane (s) is displaced. Unstained fibrils are visible within the basement membrane. These fibrils are thought to represent fixed micells. Magnification: ×48,700.



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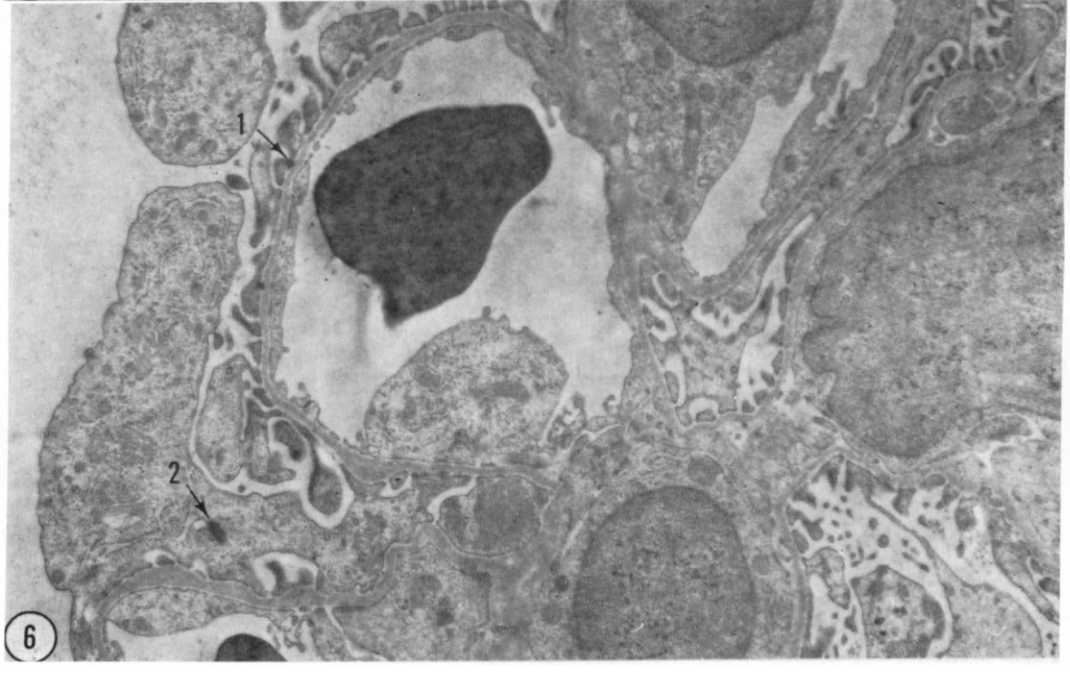

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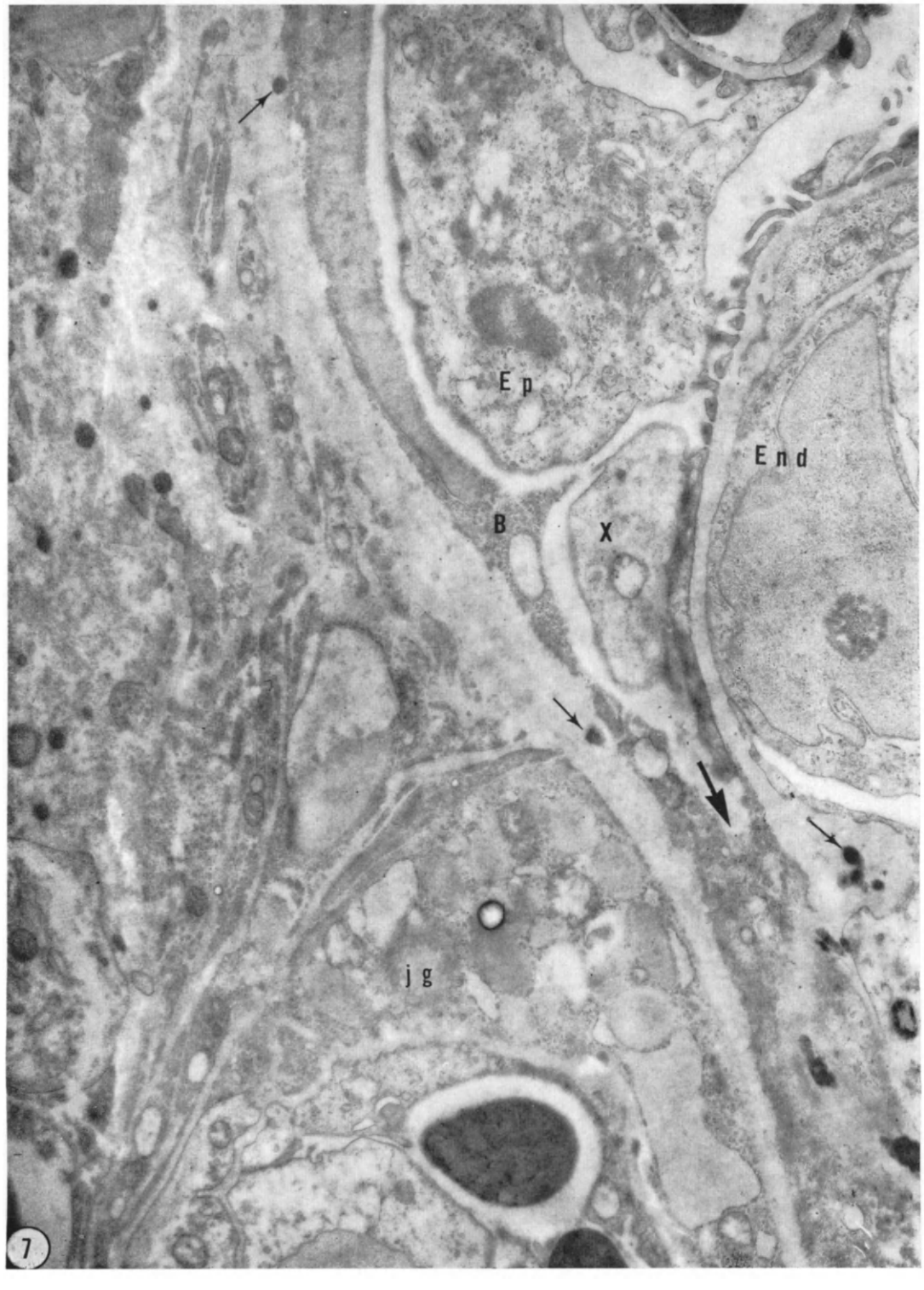
from the same embryonic cell mass (Kurtz and Mc Manus, 1959) and in the adult they are continuous with each other at the hilus (Mueller, 1958). However, their structure is considerably different (Figs. 5–7).

C. Endothelium

The endothelial cells are first seen in the embryo as a solid mass which subsequently develops channels within it and thus forms the capillaries of the glomerulus (Hall and Roth, 1957; Kurtz and Mc Manus, 1959). The original thick-walled endothelial cells become much thinner, with age (Ashworth *et al.*, 1960) and eventually in the adult are greatly attenuated cells. The nucleus and a relatively small amount of surrounding cytoplasm is usually located at the side of the capillary nearest the stalk region (see Section II, D) and the cytoplasm is extended as a thin sheet to surround the lumen (Figs. 4, 8, and 9). The attenuated portion of the cell is further thinned at fairly regular intervals to form what appears as round depressions when seen in surface view (Fig. 9) or as thin membranes in transverse section (Fig. 4). These thinned out areas have been called fenestrations and are closed by a membrane or "diaphragm" (Rhodin, 1962). Fenestrae have previously been considered to be openings in the cytoplasm by some workers and have been implicated in fluid transport. If the fenestrations were not closed they would offer no impedence whatsoever to flow, because they are of the order of 1000 Å diameter and are numerous. More recently (Luft, 1964) the diaphragm has been thought to consist of the external components of two unit membranes. The inner protein and lipid layers reflect back at the margin of the fenestration with the result that the membrane across the fenestration is composed of two protein layers with their attached mucoprotein or mucopolysaccharide furry layer (Ito, 1964). It has been postulated by Rhodin (1962) that the molecular structure of the endothelial diaphragm provides the filtering action of the kidney. Thus small molecules could pass through the protein meshwork, while large molecules could be held back. This model would account for the known filtering effect of the glomerulus (Pappenheimer, 1953), while still allowing for active transport of certain materials through the cytoplasmic portion of the endothelium, and it is in general agreement with our own experimental results (Section III, C).

FIG. 5. Two hours following globin injection. Globin aggregate (g) in the capillary lumen may be compared to the erythrocytes (E) for density of staining and general contour. A particle of globin (1) has been transferred through the endothelium by modified phagocytosis. Other globin (2) has traversed the basement membrane and come to lie beneath foot processes and one of the aggregates appears to be migrating into a slit between foot processes. The high content of ribosomes, Golgi material, ergastoplasm, vesicles, and mitochondria typical of epithelial cells (Ep) is also demonstrated. Magnification: ×11,400.

FIG. 6. Six hours after globin injection. Most of the globin has left the vascular system by this time. One small aggregate is between foot processes (1). Arrow "2" indicates a rare dense aggregate which may be globin within an epithelial cell. Magnification: ×6800.

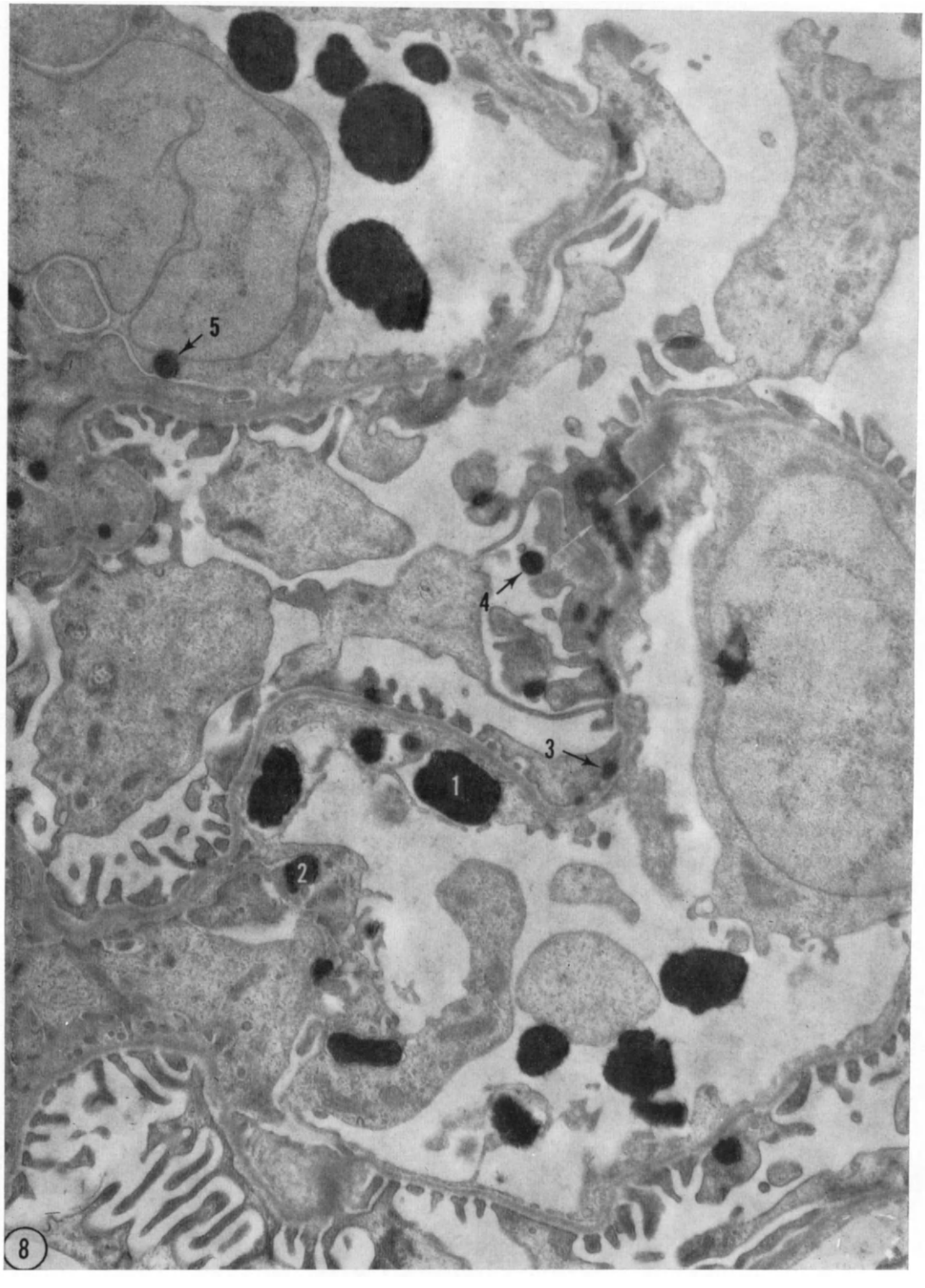

Ep
End
B
X
jg
7

The attenuated portion of the endothelial cytoplasm contains little metabolic equipment although it is frequently seen to have small vesicles (Figs. 1, 10, and 11) just as do other endothelial cells (Palade, 1953). These vesicles have been thought to be pinocytotic, and Palade (1953) postulated that fluid might be transported in them. The cytoplasm near the stalk contains a few ordinary cell constituents and frequently is seen to contain a complex system of channels or interconnecting vesicles (Figs. 1, 11, and 12). The endothelial cells are perforated in some regions near the stalk by processes from the stalk cells (Yamada, 1955; Jones *et al.*, 1962). The interconnecting vesicles of the endothelial cell are most frequently seen at this region of perforation.

D. Stalk Region

In addition to the endothelial cells lining the lumen of capillaries in the glomerulus and the epithelial cells whose foot processes cover over the external surface of the glomerular basement membrane, a third type of cell occupies a region inside the continuous basement membrane and between the endothelial cells. The fine structure of this cell type was first described by Yamada (1955). His observations have been confirmed by the majority of later workers (Bencosme *et al.*, 1959; Latta *et al.*, 1960; Movat and Steiner, 1961; Farquhar and Palade, 1962; Jones *et al.*, 1962; Latta and Maunsbach, 1962). An assortment of names has been given to the components of this region which is bounded by endothelium and basement membrane, but we shall use the terminology provided by Jones *et al.* (1962). The *glomerular stalk* consists of specialized cells which are irregular in contour and form close interdigitations with basement membrane-like material. The fibers which are always continuous with the basement membrane, as shown by three-dimensional reconstruction, are called *sponge fibers* and their intertwining with the stalk cells is thought to provide the primary support of the glomerulus. Sponge fibers appear to have a structure similar to basement membrane although they may vary somewhat in density (Latta and Maunsbach, 1962). The cell which produces the sponge fibers is in some question since Kurtz and Feldman (1962) make the point that no new material is deposited in the stalk region in their experimental nephrosis, although many others find new material deposited in the stalk in a variety of diseases and experimental conditions (Bencosme *et al.*, 1959; Farquhar *et al.*, 1959; Movat *et al.*, 1961; Jones, 1963; Dachs *et al.*, 1964).

FIG. 7. Portion of a juxtaglomerular cell (jg) near the hilus of a glomerulus. Glomerular epithelial cells (Ep) may be compared to the epithelial cells of Bowman's capsule (B). An apparent intermediate cell (X) is in contact with the glomerular basement membrane in one portion and its cytoplasm is reflected away (large arrow) to become continuous with Bowman's capsule epithelium. A glomerular endothelial cell (End) is also present. Globin (small arrows) is present within various portions of the glomerular basement membrane, Bowman's capsule basement membrane, and juxtaglomerular basement membrane. Magnification: ×9100.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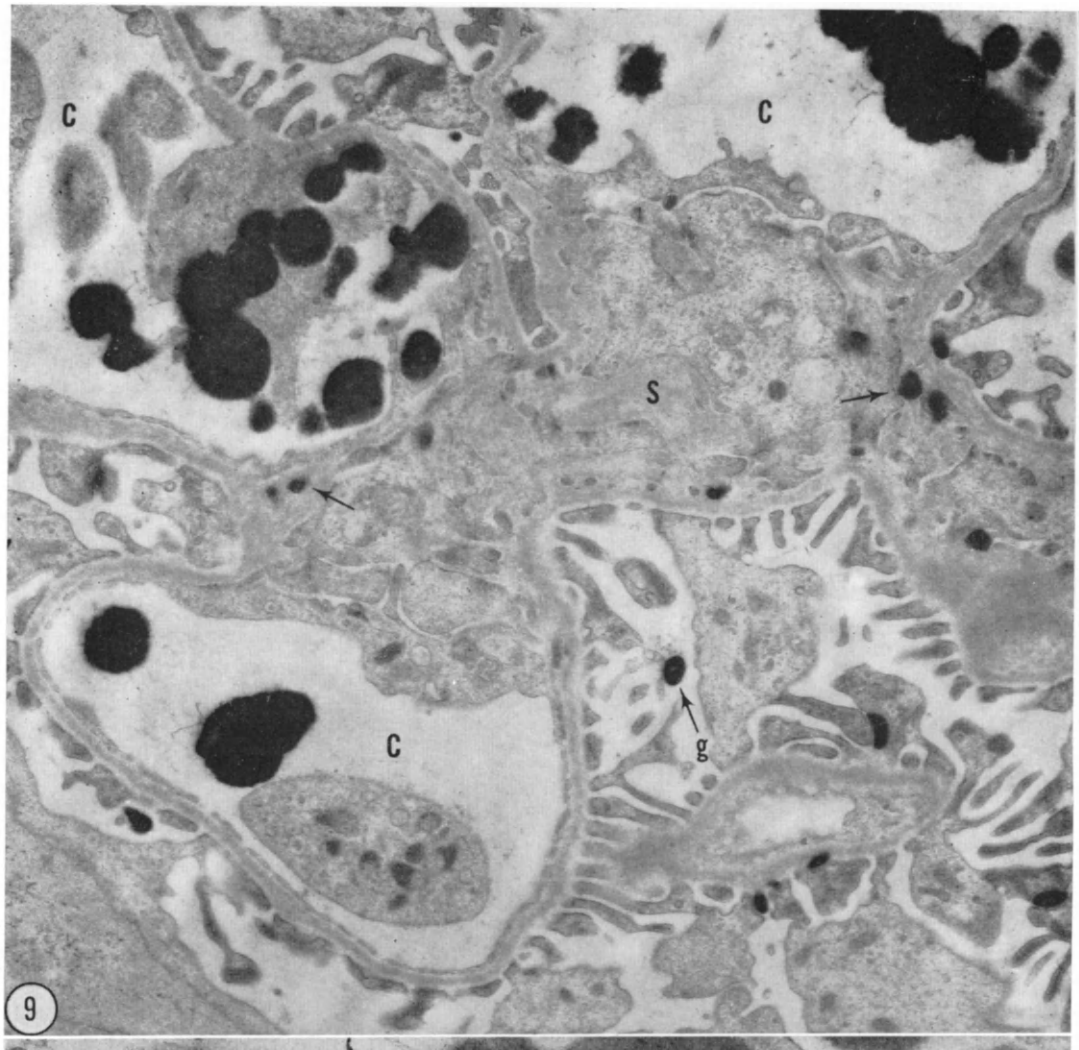
In addition to the irregularly projecting cytoplasm, the stalk cell is characterized by cytoplasmic fibrils (Figs. 1 and 2) and a regular-shaped nucleus. Some of the cytoplasmic processes project through the meshwork of sponge fibers to contact the endothelial cells or to actually penetrate them and enter the lumen (Yamada, 1955; Jones *et al.*, 1962). These special projections have been called *cytoplasmic probes* (Fig. 12). There are many similarities between the stalk cells and those of the juxtaglomerular apparatus both in the cell shape as related to the sponge fibers and in cytoplasmic content. Both cells have cytoplasmic fibrils and sometimes stalk cells have granules like those of the juxtaglomerular cells. Continuity between stalk cells and juxtaglomerular cells is seen at the hilus and material is found to pass from the sponge fibers of the stalk to the intercellular substance of the juxtaglomerular apparatus so that a functional as well as physical relationship probably exists (Latta and Maunsbach, 1962). Stalk cells are phagocytic, at least under some conditions (Yamada, 1955; Kawamura, 1961; Farquhar and Palade, 1962; Latta and Maunsbach, 1962).

III. Pathway of Transported Material

A. Small Ions and Water

No method has yet been found which will permit direct visualization of water, small ions, or smaller carbohydrates during their passage through the capillary wall. Observations based on the concentrations of test substances on both sides of the wall demonstrate that material can indeed move from the lumen of the capillary to Bowman's space. By ignoring the structure of the capillary wall and considering it as a uniform barrier, two somewhat different mathematical models have been derived. Pappenheimer (1953) considers the passage of material through the glomerulus as a filtration process with the hydrostatic pressure of the blood as the filtration force. He then estimates that the capillary wall contains a population of "pores" with a radius of 30–40 Å and a population density of 2×10^9 per cm^2. Chinard *et al.* (1955) consider the capillary wall as a semipermeable membrane and think of the migration of material as a diffusion process with different rates for different substances based on their diffusion coefficients and concentrations. Despite experimental evidence and computations which are impressive, it is difficult to conceive of the endothelial and epithelial cytoplasm as a passive barrier, although the basement membrane could

FIG. 8. Rat glomerulus 1 hour after globin injection. Several globin aggregates are within the capillary lumen. Number "1" indicates an aggregate partially engulfed by endothelial cytoplasm. Aggregate "2" is on the distal side of the endothelial cell and is lodged in a sponge fiber. Number "3" is globin lying beneath a foot process on the epithelial side of the basement membrane. Another globin aggregate (4) has passed into Bowman's space. Number "5" is a globin particle partially extruded into a sponge fiber from endothelial cytoplasm on the distal side of the nucleus. The unlabeled white arrow indicates a tangential cut of basement membrane with globin extending from the endothelial side to the epithelial foot process on the distal side. Magnification: ×10,900.



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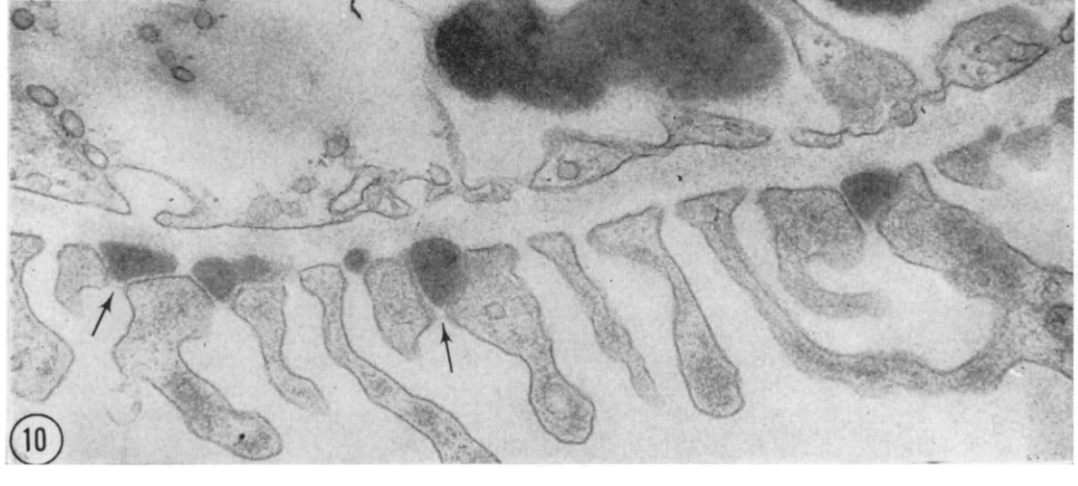

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act as one. Palade (1953) suggested that fluid might be transported across endothelial cells within pinocytotic vesicles and discharged on the distal side. Since evidence of pinocytotic activity is seen in both endothelial and epithelial cells of the glomerulus it may be that some transport through the cellular components is active, whereas movement through the basement membrane is passive (Rinehart, 1955). With the present knowledge of endothelial diaphragm structure (Luft, 1964; Rhodin, 1962), it is possible to postulate that a filter effect could be produced by the diaphragm which would act on small ions and molecules without the impedence to flow that would be offered by the full thickness, lipid-containing unit membrane. Whatever the mechanism, water and small molecules cross the glomerular membrane and are resorbed by the tubular epithelium, even when the ureter is occluded (Omachi and Macey, 1959).

B. Small Protein and Similar Size Molecules

1. *Light Microscopy of Hemoglobin and Other Material*

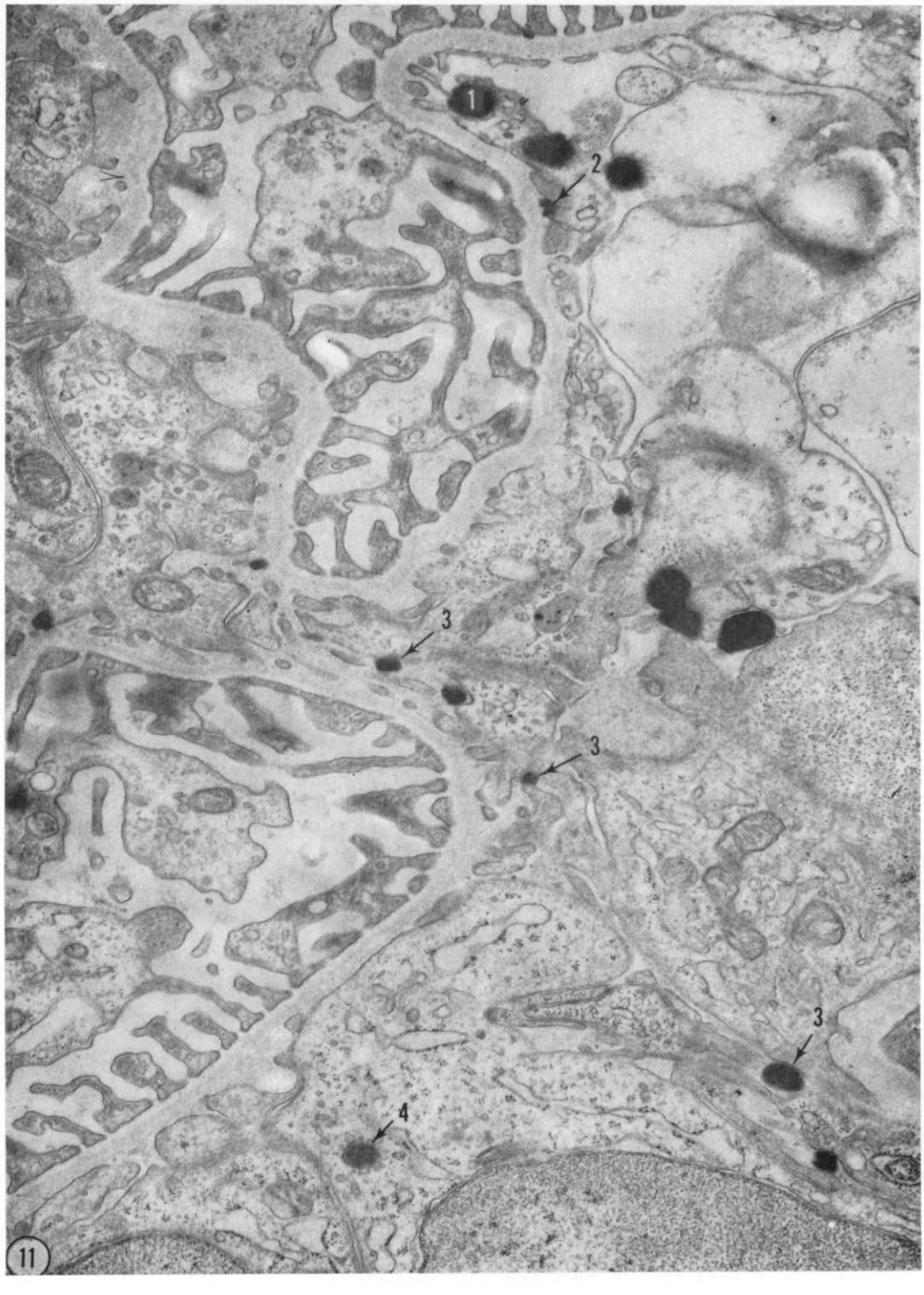
For many years it has been known that protein molecules at least as large as hemoglobin could cross the glomerulus and enter the urine (Yorke and Nauss, 1911). This has been confirmed repeatedly since then (Baker and Dodds, 1925; De Gowin *et al.*, 1937; Bywaters *et al.*, 1941; Bywaters and Stead, 1944; Yuile *et al.*, 1945; Corcoran and Page, 1947). By the use of histochemistry at the light microscope level, it has been shown that a wide variety of amino acids and proteins at least as large as 35,000 molecular weight could be passed by the intact glomerulus (Bott and Richards, 1941; Oliver *et al.*, 1954a, b; Lee, 1954; Oliver and Mac Dowell, 1958).

2. *Dextran*

When dextran particles of approximately 150 Å diameter are injected into the peritoneal cavity, they are eventually found inside the epithelial cells of the glomerulus (James and Ashworth, 1961) where they are visible in the electron microscope. Although published micrographs do not demonstrate the fact with certainty, it appears that the dextran is aggregated in clumps within the cytoplasm without any surrounding membrane. It was not ascertained whether the particles were taken up directly or taken up within vesicles whose walls later disappeared.

FIG. 9. A sponge fiber (S) is indicated within a stalk surrounded by three capillaries (lumen of each indicated by "C"). The arrows indicate typical globin aggregates within sponge fibers, several of which are seen to be continuous with the basement membrane. A particle of globin (g) is free in Bowman's space. One hour after globin injection. Magnification: ×11,800.

FIG. 10. Thirty minutes after globin injection. Globin is seen within the capillary lumen (at top), and several aggregates are seen between epithelial foot processes. The slit membranes (arrows) are displaced by the globin aggregates, and the foot processes are pushed apart. Magnification: ×45,700.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3. *Thorotrast*

The fate of Thorotrast injected intravenously is somewhat different from that of dextran. A short time after injection, Thorotrast can be seen within the sponge fibers of the stalk region (Latta *et al.*, 1960), and a few hours after its appearance in the stalk it is found within the basement membrane-like intercellular material of the juxtaglomerular apparatus (Latta and Maunsbach, 1962). It is rarely found within either the endothelial or epithelial cells, but is sometimes seen inside vesicles of the stalk cells.

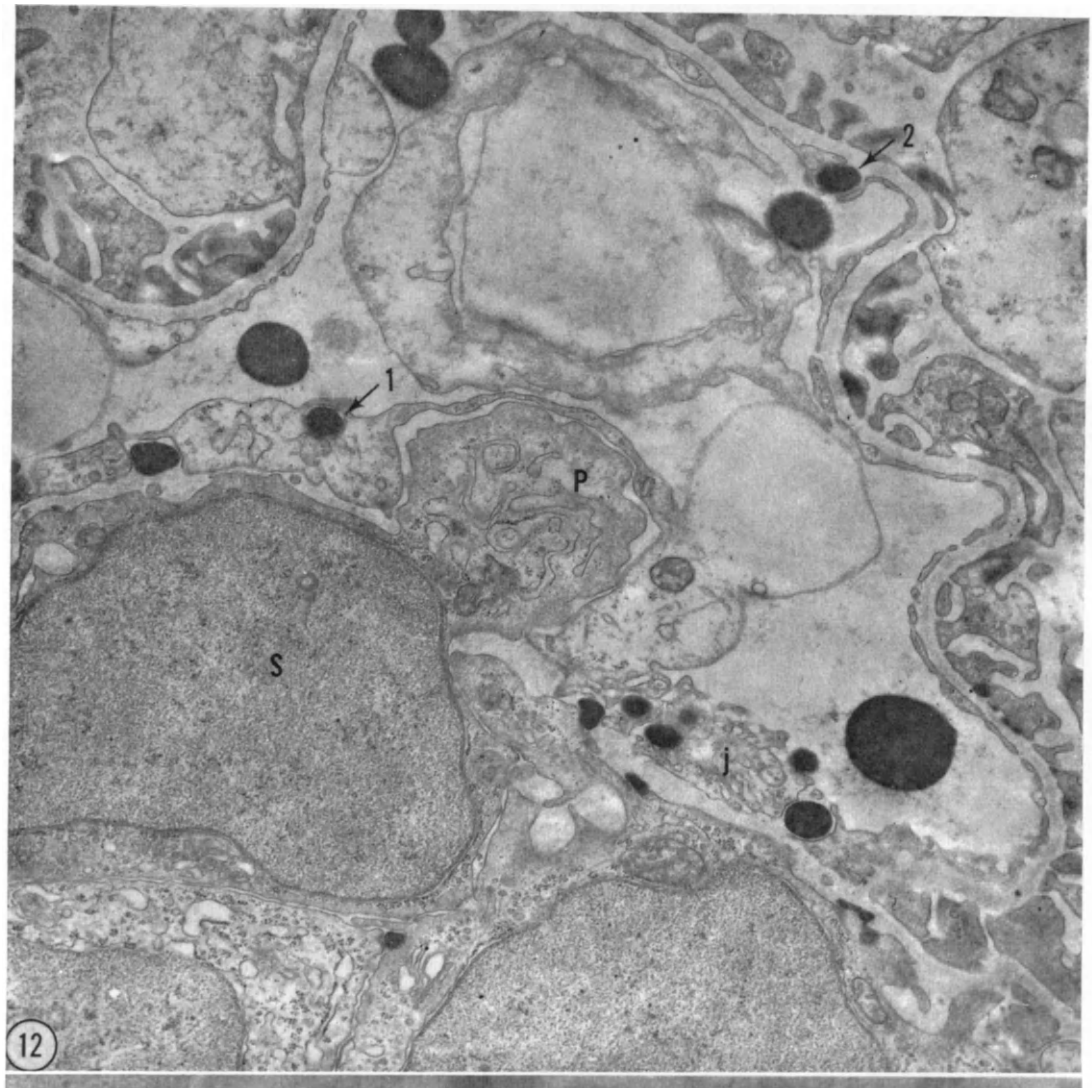
4. *Ferritin*

The pathway taken by intracapillary ferritin as it crosses the glomerular wall has been described as follows (Farquhar, 1960; Farquhar *et al.*, 1961). The particles are found in the capillary lumen and on the endothelial side of the basement membrane a few minutes after intravenous injection. The fenestrae were considered to be "freely permeable to ferritin," although the published photographs do not demonstrate an increased concentration of ferritin in the region of fenestrae either on the lumen side or the basement membrane side. If there had been a selective migration of particles through the fenestrae, those particles would be expected to accumulate within the basement membrane in the vicinity of their entrance. There is, however, a series of observations at different time intervals which demonstrate that particles accumulate on the endothelial side of the basement membrane without any special distribution in relation to the fenestrae. Transport of ferritin thus appears to us to be through all parts of the endothelium. After entering the capillary side of the basement membrane, the ferritin slowly migrates across to the epithelial side where it is taken up by pinocytosis in the foot processes. The small vesicles containing ferritin aggregate to form larger vacuoles which become condensed in the Golgi region of epithelial cells (Farquhar and Palade, 1960). The ferritin is later extruded into Bowman's space. Little evidence was found for ferritin transport through the slits between foot processes. The stalk region contained ferritin both in the sponge fibers and within vacuoles in the stalk cell cytoplasm.

5. *Imferon*

Kawamura (1961) traced the pathway of Imferon with the electron microscope as it traversed the glomerulus. He found the characteristic particles in the cytoplasm of endothelial, epithelial, and stalk cells; some particles were free in the cytoplasm of all cells and some were within vesicles bounded by membranes. He felt that the bulk of particles traversed the endothelial cells by way of the "pores" although the same

FIG. 11. Portion of a capillary and stalk region 10 minutes after globin injection; globin within a fairly typical juxtacollicular region is at "1." Number "2" indicates globin on the distal side of the endothelial cell cytoplasm. Several globin particles are also seen within sponge fibers (3). One globin aggregate appears within a vacuole in a stalk cell (4). Magnification: ×15,200.



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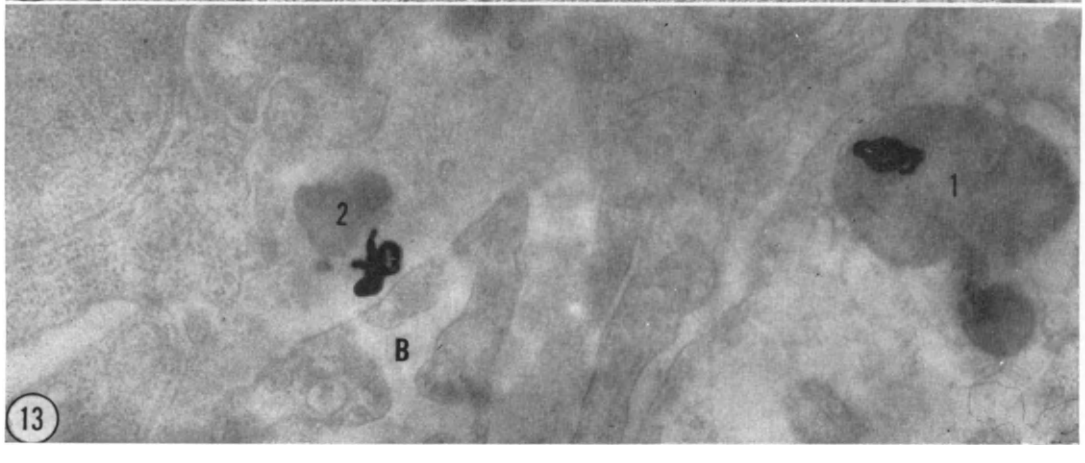

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objection applies to his evidence as to the evidence that ferritin goes through the fenestrae (Section III, B, 4). He also found particles within the sponge fibers of the stalk.

6. *Other Indirect Observations*

Pathological studies of a patient with paroxysmal nocturnal hemoglobinuria showed aggregates of granules which were structurally indistinguishable from ferritin within both the visceral epithelial cells and Bowman's capsule (Reger *et al.*, 1961), so hemoglobin may take a course across the capillary wall similar to that of ferritin.

Protein-losing nephropathies of various kinds lead to several changes in the different cells, but if any alternation is found, it is usually in the epithelial cell and is frequently associated with vacuoles which contain protein (Farquhar *et al.*, 1957a, b; Sitte, 1959; Movat *et al.*, 1961; Lannigan and McQueen, 1962).

C. Globin as a Large Colloid

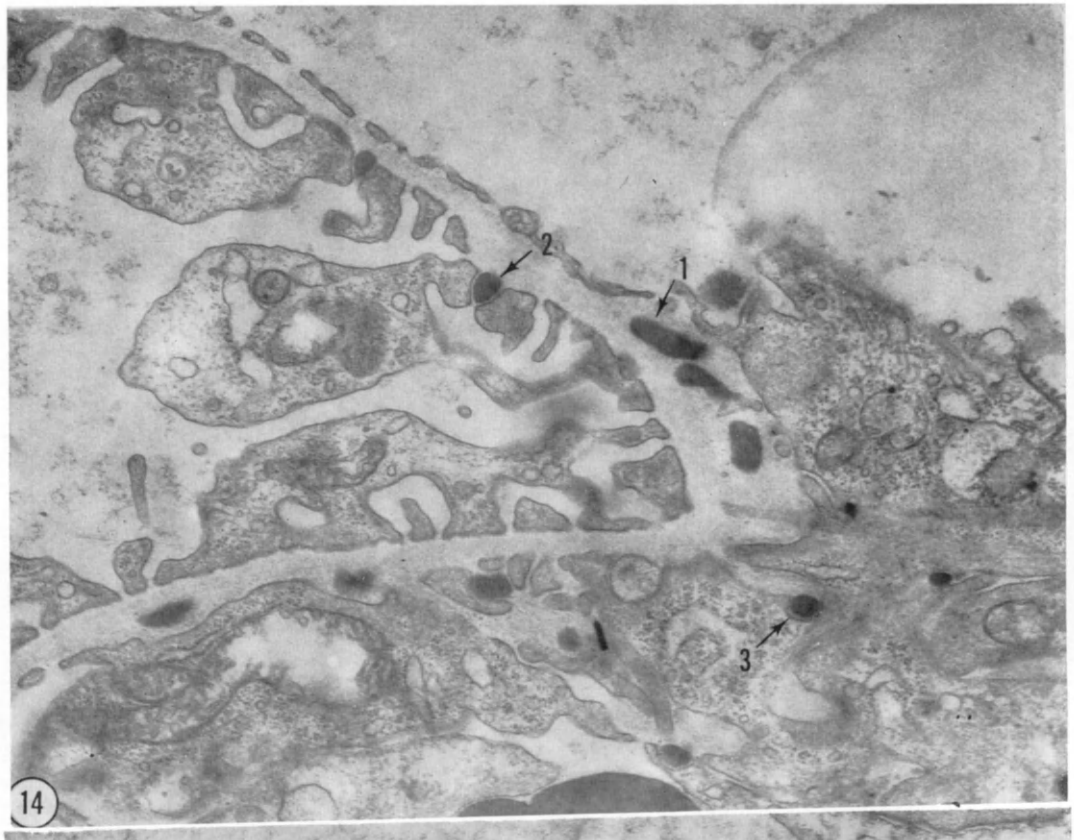
1. *Experimental Design*

Female Sprague-Dawley rats were given intravenous injections of 0.75 gm purified human globin (Menefee *et al.*, 1964a) per kg of body weight. Kidney tissue was taken at several time intervals from 1 minute to 6 hours after injection. In addition to the regular globin injections, two animals were given globin which had been labeled with tritium and their kidneys were taken at 1 and 2 hours; these served to confirm the identification and localization of unlabeled globin which might possibly have been mistaken for some other substance even though it has a characteristic appearance in the electron microscope.

The animals were anesthetized with ether and the kidneys were perfused with a fixative made as follows: (percentages are weight/volume) 2% osmium tetroxide, 2 parts; 7.3% polyvinylpyrollidone, 1 part; 4% sodium nitrite, 1 part. To this mixture was added just sufficient phenol red indicator to give a color (yellow) and the solution was titrated with 0.01 *N* sodium hydroxide to a brick red color (pH 7.3). The pH remained constant for several days at room temperature. If the fixative

FIG. 12. Portion of a capillary and glomerular stalk 10 minutes after globin injection. Several globin aggregates are within the capillary lumen. Arrow "1" indicates globin contained within a vacuole within an endothelial cell. Arrow "2" indicates globin in contact with the basement membrane and still partly surrounded by a vacuole membrane. The juxtacollicular region (j) has several globin particles within the interconnecting vesicles. A stalk cell (S is within the nucleus) has a cytoplasmic probe (P) projecting toward the capillary lumen. Magnification: ×11,400.

FIG. 13. Autoradiograph from the glomerulus of a rat that had been injected 2 hours previously with radioactive globin. The characteristic exposed grains are over two globin particles. Number "1" is a particle within the capillary lumen and number "2" is a particle within a sponge fiber, just entering the basement membrane. "B" indicates Bowman's space. Magnification: ×26,500.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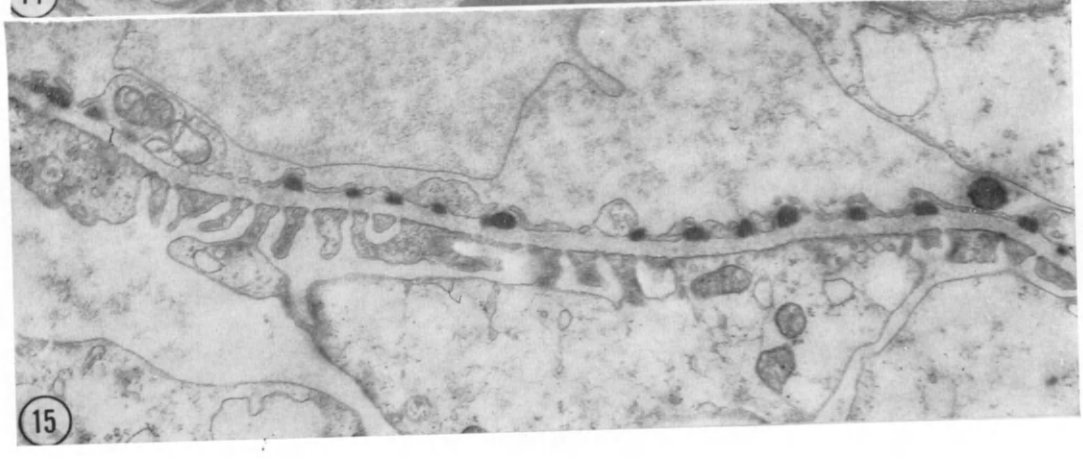
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turned yellow during fixation it was an indication of excess acid and it was replaced with fresh fixative. In this way the changes characteristic of acid fixation were eliminated. After arterial perfusion the kidneys were removed, sliced into small segments, and placed in an excess of fixative for an additional 30 minutes. The tissue was then washed in isotonic saline, dehydrated in graded ethanol solutions, and embedded in epoxy resin. Sections were cut on the LKB Ultrotome, stained with lead (Dalton and Zeigel, 1960), and photographs made with an RCA EMU 3F or 3G electron microscope.

Electron microscope autoradiographs were made by applying a gelled film of Ilford L-4 emulsion directly from a loop to sections mounted on grids according to the method of Caro and van Tubergen (1962). Optimum activity was reached at about 6 weeks.

2. *Observations on Glomeruli during Globin Transport*

The purified globin as it is used in these experiments aggregates within the vascular system to form small, usually submicroscopic particles. This aggregation is apparently similar to that occurring *in vitro* when a diluted solution of globin is warmed to 37° C (Menefee *et al.*, 1964a). When fixed with an osmium tetroxide fixative, the particles in the blood are homogeneous and are somewhat more electron-dense than erythrocytes cut to the same section thickness (Fig. 5). Another identifying feature is the irregular surface profile which usually differs from the smooth membrane-limited surface profile of erythrocytes. When radioactive globin is injected, similar aggregates are found which give positive autoradiographs (Fig. 13).

Within 1 minute or less following intraveneous injection of globin it is identifiable in the proximal tubule (Menefee *et al.*, 1964b). It reaches that location by going through the endothelial cells, basement membrane, and between foot processes. The slit membranes are displaced and apparently disrupted during this transit. Globin also migrates into the stalk region from which it moves through the basement membrane and between foot processes.

a. *Endothelium.* With one exception, the type of transport of globin through endothelial cells may be considered as phagocytosis of the aggregate on the lumen side followed by migration of the resulting membrane-bound particle and extrusion on the basement membrane side. One method of engulfment, which appears similar

FIG. 14. Thirty minutes after globin injection. Arrow "1" indicates globin within the basement membrane. Arrow "2" is a globin aggregate between two foot processes with a slit membrane displaced away from the basement membrane. Arrow "3" is a globin aggregate within a sponge fiber. Magnification: ×22,100.

FIG. 15. Several aggregates of globin are lined up on the basement membrane side of the endothelium 4 minutes after injection. Many of the globin droplets are larger than the thickness of endothelial cytoplasm and have been carried across by a modified phagocytosis (see text). Magnification: ×12,900.

to erythrophagocytosis (Essner, 1960), consists of the extension of cytoplasmic processes outward from the cell to surround the particle followed by fusion of the extended processes around the particle; this sequence results in a cytoplasmic vacuole bounded by a single membrane and containing an aggregate of globin which completely fills it (several stages of this are seen in Figs. 8, 9, and 12). Extrusion on the distal side may be similar to the extrusion of secretory granules (Palade, 1959; Parks, 1962) in which the vacuolar membrane fuses with the cell membrane, followed by breakdown of the fused membranes at one point so that the continuity of surface membrane is maintained while the contents are exposed to the basement membrane (Figs. 5, 8, and 14).

A more frequently seen mechanism of movement from capillary lumen to basement membrane side of the endothelial cell can be described as a modified phagocytosis. This latter method is seen in the attenuated part of the cell and appears to be due to the fact that the thickness of the cytoplasm in those regions is considerably less than the diameter of the globin aggregate which it attempts to engulf. The result is that the globin is extruded on the distal side by the time that the cytoplasm has fused on the lumen side (Figs. 5 and 15). A possible third variation of phagocytosis occurs in the juxtacollicular region. Many pinocytotic vesicles are found which interconnect to form irregular channels at least part way through the cell. When globin is seen in this region, it usually does not completely fill the irregular vesicles in which it is found (Figs. 1 and 12). This morphological relationship suggests that globin may be transported in the juxtacollicular region as an incidental passenger in a region engaged in active transport under many different conditions. An unusual observation is illustrated in Fig. 4 which demonstrates an aggregate of globin passing through a fenestration. The fenestrae do not often manifest transport of globin and even in the most attenuated part of the endothelial cells where modified phagocytosis occurs the fenestrae usually do not have globin passing through them but rather are carried to one side or the other of the engulfed particle (Fig. 15).

b. *Basement Membrane.* After traversing the endothelium, globin is extruded into either the stalk region or directly into the basement membrane. The droplet then crosses the basement membrane and either passes directly between epithelial foot processes or comes to lie beneath a foot process (Figs. 5 and 8). The basement membrane does not appear deformed in any way by the globin present within it (Figs. 1, 2, 4, 10, 12, and 13). If the basement membranes were composed of a stable meshwork of protein filaments or were in any other way unyielding, the migration of particles as large as the globin aggregates should produce a piling up of material in the direction of flow and possibly a rarefaction behind. If the membranes were rigid enough to maintain pore sizes of fixed diameters, the globin aggregate could not pass through at all.

Small protein or colloidal particles have been observed to cross the basement membrane and to migrate through the basement membrane-like substance of the sponge fibers (Farquhar, 1960; Latta *et al.*, 1960; Farquhar *et al.*, 1961; Kawamura,

1961; Latta and Maunsbach, 1962), and no visible deformation of the basement membrane accompanies the passage of these small particles. Ferritin accumulates on the endothelial side of glomerular basement membranes; this has been taken as evidence of the filtering effect of the basement membrane (Farquhar *et al.*, 1961). Since ferritin eventually passes through the membrane to the epithelial side, the concept of a "filter" might better be changed to diffusion barrier. The diffusion coefficient of a particle within the substance of the basement membrane would then become the limiting factor in its movement there without having any effect on transport through other components of the capillary wall.

Farquhar *et al.* (1961) thought of the basement membrane as "... a gel-like structure with two fine fibrillar components embedded in an amorphous matrix." We would like to qualify that description to specify that the characteristics are those of a *thixotropic* gel and that the fibrils which are present in fixed tissue probably represent only a temporary relationship in an ongoing interaction between protein molecules making up the gel. Such a continuing flux of combination and recombination between protein molecules is characteristic of gels, and the regions of well-ordered aggregation are called *micells.* A micell is made up of portions of several different molecules and each molecule entering into its formation may enter into other micells in other parts of its length at the same time. The rate of breakdown and reformation of the components into new micells is dependent on many factors such as the state of hydration of the gel, the ionic concentration, kinds of intermolecular bonding, temperature, and pressure. The kind of regional interaction which occurs between molecules making up a micell has been called "short-range order" and is known to be augmented by fixation and dehydration [Frey-Wyssling (1948) presents a thorough discussion of gels in biological systems]. It is most likely these fixed micells which we see in the electron microscope. Passage through the basement membrane of particles of all sizes, including the large globin aggregates, without obvious deformation can most easily be explained if the basement membrane is a thixotropic gel which liquefies in any local region where a critical pressure is exceeded. The pressure could be provided by active extrusion of the particle from an endothelial cell into the membrane or by the transmission of vascular pressure to a particle already present within the membrane. The concept of local liquefaction is obviously not necessary when considering the passage of diffusible molecules; so in this latter case the basement membrane may act as a diffusion barrier in a manner analogous to a chromatographic column.

c. *Epithelial Cells.* When globin reaches the distal side of the basement membrane it may accumulate temporarily beneath a foot process and may even indent the cell membrane (Figs. 3 and 8), but it is not taken up by the epithelial cell. Those globin aggregates, which go first to the area beneath the foot process, appear to migrate along the cell surface until they reach a slit and then pass between the foot processes (Figs. 5 and 8). Other aggregates apparently reach the distal side of the basement membrane directly in line with a slit and go through it. The aggregates of globin are

not completely fluid as demonstrated by the fact that they push the foot processes apart and displace the slit membrane during their passage (Figs. 2, 4, 5, 10, and 14). When resistance is encountered by the aggregates, such as that offered by the foot processes to lateral displacement, they tend to be flattened in the direction of greatest force (Fig. 4). The two foregoing features indicate that globin aggregates are semifluid particles.

The fact that the slit membrane is moved out of its normal position near the basement membrane by globin when it passes between foot processes is an important indicator of slit membrane structure. The connection between foot processes has been described as a "desmosome-like structure" (Farquhar *et al.*, 1961) but it must be noted that a desmosome (Farquhar and Palade, 1963) occupies two dimensions on the surface of each of the two cells joined by it, whereas the slit membrane is very elongate in one dimension and very thin in cross section. Rarely two foot processes are seen to be separated by a larger than normal space unoccupied by globin and with a slit membrane attached to one foot process only, as though it has been broken (Menefee *et al.*, 1964a). It may be assumed that the migrating globin disrupts the slit membrane but that normal relations are resumed very quickly after its passage because very few such displacements are found in the absence of a globin particle. After passing between foot processes, the globin becomes free in Bowman's space (Fig. 8) and passes on down the nephron where some of it is taken up by tubule cells and some excreted in the urine (Menefee *et al.*, 1964b).

d. *Stalk Region.* Globin taken up by endothelial cells is discharged into sponge fibers of the stalk as well as into basement membrane. Most of the globin remains within sponge fibers until its eventual discharge through the basement membrane; only a small amount is taken up by stalk cells (Figs. 1, 2, 8, 9, 11–14). Globin appearing in the basement membrane of the juxtaglomerular apparatus (Fig. 7) may arrive there by a route similar to that of Thorotrast (Latta and Maunsbach, 1962), i.e., by way of sponge fibers which are continuous with it. Globin remains in the stalk region longer than in other parts of the glomerulus, but by 6 hours after injection most of it has been discharged from the stalk also (Fig. 6). Since globin is found at random within sponge fibers and since there is no deformation of the sponge fiber material, it may be assumed that there is little impedence to its movement there. This suggests a thixotropic gel characteristic for sponge fibers just as it does for basement membrane.

IV. Summary

Each component of the glomerulus has its own relationship to material being transported through it, and the mode of transport within any one glomerular structure may vary with the kind of material being transported. No evidence is presently available with respect to the route taken by water or small ions, but a considerable

number of electron microscopic observations have been reported on small proteins, small colloidal particles, and larger globin aggregates. Ferritin and Imferon have been thought to pass primarily through endothelial fenestrae, although the recent observation of diaphragms across these specialized regions raises a reasonable question about the validity of previous interpretations of transport through them. Imferon has also been noted to pass through endothelial cells. The presence of pinocytotic vesicles within the endothelial cytoplasm has led to the postulation that this is a method of moving material across the cellular part of the wall. Phagocytosis of relatively large particles by endothelial cells has been reported here and may actually be only a large scale extension of pinocytotic activity. The diaphragms across fenestrae may act as the filter for small molecules so that bulk flow and filtration could be the means of moving some materials, whereas others are carried by active transport.

The basement membrane has been postulated to act as a filter by those who considered the endothelial fenestrae to be actual openings which would allow free passage of material. The fibrillar appearance of fixed basement membranes was taken as support of their filterlike function. The observed interaction between glomerular basement membranes and both small and large particles leads us to conclude that the basement membrane is a thixotropic gel which may allow the passage of large particles, even including blood cells, by localized liquefaction followed by almost immediate reconstitution afterward. The same gel structure could act as a diffusion barrier for smaller ions and water.

Reaction of stalk cells to identifiable test materials which have passed across the endothelium varies with the material. Some substances such as ferritin, Imferon, and Thorotrast are taken up to some extent by stalk cells, and the material may be either contained within vesicles or free in the cytoplasm. Globin is not taken up to nearly as great an extent by stalk cells but rather tends to exist freely within the sponge fibers between stalk cells, thus suggesting a gel structure similar to that of the basement membrane.

Epithelial cells manifest a selective absorption of materials, e.g., they do not take up globin but are quite active in the uptake of certain other proteins. Globin passes between epithelial foot processes on its way from the basement membrane to Bowman's space. The foot processes are displaced laterally and the slit membrane is moved from its normal position by the passage of globin. Behavior of the slit membrane when force is exerted on it by passing globin leads to the conclusion that the slit membrane is a specialized structure.

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THE JUXTAGLOMERULAR APPARATUS

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I. Introduction

The complex structure known as the juxtaglomerular apparatus is found in the region of the hilus of mammalian renal glomeruli wedged in a triangular pocket formed by the afferent and efferent vessels and macula densa of the distal convoluted tubule. It seems ideally situated to play a primary role in the regulation of cortical blood flow in the kidney.

We shall see that, although its morphology is now well known, its exact physiology is not; the different hypotheses postulated concerning this subject have been partially refuted or not definitely confirmed.

II. Historical Background

It was Ruyter (1925) who, studying mitochondrial stains on sections of kidney from the white mouse, described for the first time the differentiation of the wall of the renal arteriole before it penetrated into the glomerulus. He showed that the common smooth muscle was replaced by cells whose abundant cytoplasm contained granules which took up the mitochondrial stains. He called these cells "epithelioid" and compared them with similar elements described by Schumacher (1907) in the arteriovenous anastomosis of the coccygian glomus, believing that, like the latter, these cells must play a role in the local regulation of blood flow.

The development of these epithelioid cells after the first extrauterine week through differentiation of smooth muscle cells and the persistence of myofibrils in some of them seemed in Ruyter's eye to justify the concept that such elements were derived from smooth muscle.

He further noted in these cells the disappearance of the internal elastic membrane and an abundance of filaments stained by the Bielchowsky technique. He interpreted this as evidence of a rich neural plexus.

Ruyter found these cells in mice and rats but not in man, monkeys, or other higher vertebrates.

Oberling (1927), studying human kidney sections, was struck by the epithelioid structure of cells in the tunica media of the preglomerular arteriole which made these cells very comparable to the myoepithelial elements described by Pierre Masson (1924) in digital glomi and tumors derived therefrom.

Oberling showed that their cytoplasm contained fuchsinophilic granulations as revealed by the Masson trichrome stain. In addition, these cells extended into the adventitia of the vessel and appeared to be richly innervated, from which came the expression "housse neuro-musculaire" of the glomerular artery in man.

Okkels (1929) showed the presence of analogous cells in the vascular hilus of the glomerulus in the frog. These cells could be seen inside the glomerulus itself in the transitional zones between the arteriole and capillary loops and they contained granules which stained with Regaud's hematoxylin.

Okkels and Peterfi (1929) by mechanically stimulating the different vascular segments with a micromanipulator, demonstrated that the glomerular capillaries did not react, whereas the neuromyoarterial apparatus responded with a violent contraction to the slightest excitation. From this observation they concluded that this apparatus plays a primary role in the local regulation of blood flow.

In 1932 Goormaghtigh redescribed this preglomerular structure in man and the cat and confirmed its similarity to the arteriovenous anastomoses in the skin. In addition to the epithelioid cells, he described a group of morphologically different cells piled up in columns that he compared to the tactile corpuscules of the skin and to the schwannian elements which gave rise to the term "pseudo-Meissnerian cells" used with respect to these structures.

In the same year, Zimmermann, in his fundamental study of the glomerulus structure, was unable to confirm Ruyter's description. He showed that just before the glomerulus the arteriole wall showed a nodular swelling composed of from five to fifteen flattened cells sometimes made up of clear agranular cytoplasm which on one side pushed in the arterial endothelium and on the other rested against the distal segment of the convoluted tubule.

He gave the name "Polkissen" (polar pad) to this nodular swelling. In delineating the characters of the distal tube (already noticed by Peter in 1907), he called the epithelium at this point the "macula densa" because of the pile of cells that constituted it and the accumulation of nuclei found there.

Not having found the granular cells described by Ruyter and Oberling (however, they appear in Fig. 5 in his article) and being ignorant of Goormaghtigh's all-too-recent description of pseudo-Meissnerian cells, he introduced two new ideas—that of the Polkissen and of its intimate relation to the macula densa.

Goormaghtigh, in describing the "myoneuroarterial juxtaglomerular" ensemble later, viewed it as composed of two different structures which had been grouped together by Zimmermann under the name of "Polkissen": the epithelioid cells described by Ruyter and Oberling and the palissadelike pseudo-Meissnerian cells which he himself had described.

He stated that the epithelioid elements constitute the wall of the afferent arteriole, while the pseudo-Meissnerian cells were lodged in the angle between the afferent and efferent vessels, the ensemble being in close contact with the macula densa of Zimmermann. This macula densa seemed to him to be a highly differentiated segment of tubular epithelium to which he accorded a sensory plate function which, via the palissadelike cells, could inform the preglomerular arteriole as to modifications occurring in the terminal portion of the corresponding nephron. This description underwent no notable modification until the advent of electron microscopy.

Montserrat (1934), Kaufman (1942), and Schloss (1946) confirmed Oberling's description of the epithelioïd cells in the human kidney.

In birds and amphibians, Edwards (1940) found the macula densa, which he called "epithelial plate," and described its close relationship to the vascular hilus of the glomerulus. According to him, fish have the same structure but without epithelioid differentiation of the afferent arteriole.

McManus (1942), in describing this structure, insisted upon the abundance of reticular fibers throughout the region.

Oberling (1944), using the silver impregnation techniques of Gros-Schultz, and de Muylder (1945, 1948) obtained pictures which they interpreted as being those of a highly developed, perivascular neural plexus.

The physiological properties of the juxtaglomerular apparatus have been discussed in a succession of numerous contradictory papers.

Following Goormaghtigh (1936, 1942; Goormaghtigh and Grimson, 1939) and his pupil Elaut (1934) hypotheses first proposed that this structure played a role in blood pressure equilibrium.

At the time, Goldblatt *et al.* (1934) had demonstrated the role of the ischemic kidney in causing hypertension and had put forth the foundations of his humoral theory of hypertension of renal origin: the ischemic kidney liberates a large dose of renin which is responsible for a rise in blood pressure.

Goormaghtigh and Grimson (1939), having shown the proliferation of epithelioid cells in the ischemic kidney, postulated that they could be the site of renin elaboration.

Finding this phenomenon under numerous pathological conditions (severe scarlatina, eclampsia, acute glomerulonephritis, crush syndrome) in which he observed a similar hypertrophy of the epithelioid cells, Goormaghtigh (1942) considered the endocrine function of the renal arterioles as one of the essential factors in the regulation of arterial blood pressure.

This hypothesis advanced the juxtaglomerular apparatus from the modest position of local circulatory regulator to one more ambitious—it could be made responsible for hypertensive diseases. This gave rise to numerous articles for and against such a hypothesis.

In its favor, three facts are presented: (1) The frequent, if not constant, coexistence of hyperactivity of the juxtaglomerular structure with arterial hypertension in man (Weiss and Parker, 1939; Graef, 1940; Kaufman, 1942; de Muylder, 1945, 1948; Des Prez, 1948; Mayer, 1952; Turgeon and Sommers, 1961; Demopoulos *et al.*, 1961; Crocker *et al.*, 1962; Itskovitz *et al.*, 1963; Boughton and Sommers, 1963), and in experimental pathology (Elaut, 1934; Dunihue and Candon, 1940; Dunihue, 1941; McManus, 1942; P. M. Hartroft, 1957; Tobian *et al.*, 1958; Masson *et al.*, 1964; Rapp, 1964; Verniory and Potvliege, 1964). (2) the sensitivity of these structures to modification of intrarenal arterial pressure which render it, in some cases, essentially a barosensitive area; and (3) favorable proof of renin synthesis at this site.

Against the latter, the following discordant facts are marshalled: (1) In human arterial hypertension, the juxtaglomerular apparatus appears more often altered than hyperactive (Oberling, 1944; Bohle, 1959; Pitcock and Hartroft, 1958). (2) The site of renin elaboration is far from ascertained. (Kaplan and Friedman, 1942). (3) Hartroft's (1953) perfection of a reliable technique for the semiquantitative evaluation of the juxtaglomerular activity, by measuring the average amount of granulation in any given kidney, has permitted a better understanding of its behavior under different experimental conditions. She concluded that, far from always being linked to modifications in arterial tension, the apparatus activity appears to depend primarily on the balance of electrolytes. On that point, it reacts in a manner parallel to the glomerular zone of the adrenal cortex in that it seems to be a relay.

These studies take on significance after the demonstration of relationships existing between the kidney and the adrenal cortex, between the action of renin and of angiotensin on aldosterone secretion, which, as we know from Davis (Davis *et al.*, 1961, 1962), makes the juxtaglomerular apparatus one of the regulatory centers of glomerular activity.

Bartter's (Bartter *et al.*, 1962) clinical observations concerning cases of hyperaldosteronism unaccompanied by hypertension, plus his discovery upon autopsy of

diffuse hyperplasia of the juxtaglomerular apparatus, gave to these recent concepts the support of human pathology.

None of the preceding facts can be disproved. They must all be integrated into a single doctrine. Can such a doctrine conciliate all the roles thus far proposed for the juxtaglomerular apparatus: barosensitivity and chemosensitivity, glomerulotropic activity, and blood pressure regulation?

As will be seen, this question still lacks a definite answer despite the progress made by electron microscopy in this field.

III. The Juxtaglomerular Apparatus of the Rat as Seen in the Electron Microscope

In the rat, mouse, rabbit, and cat, under normal conditions (Bohle, 1959; Oberling and Hatt, 1960a, b; P. M. Hartroft and Newmark, 1961; Thoenes, 1961; Bucher and Reale, 1961a, b, 1962a, b; Reale *et al.*, 1963; Latta and Maunsbach 1962a, b) in experimentally induced hypertension (Hatt, 1961, 1963; Hatt *et al.*, 1962, 1963, 1964), and after adrenalectomy (Barajas and Latta, 1963b; Dunihue and Boldosser, 1963) the juxtaglomerular apparatus appears essentially as it has been described by Ruyter, Oberling, Zimmermann, and Goormaghtigh.

A. Technical Problems

After trying several methods, we finally chose *in vivo* fixation of a fragment of renal cortex by injecting $\frac{1}{4}$ to 1 ml 2% osmic acid at pH 7.4 (Palade, 1952), 1 mm under the renal capsule.

After several minutes, the kidney was removed and cut into 1- to 2-mm slices. The pieces to be studied were selected from among the blackest fragments, then recut into fine sections $\frac{1}{2}$ mm thick, 2 to 3 mm wide, and finally submerged in the fixative for 1 hour.

The tissue was embedded in Epon according to the Luft technique (1961) with the fragments placed flat on the bottom of the gelatin capsules.

After examining a thick section in the phase-contrast microscope, the block was trimmed to expose one glomerulus, chosen for its perfect fixation. Thus prepared, the glomerulus was cut into ultrathin sections which were collected on formvar-coated grids with a thick section (1 μ) being made after each grid. Five to six grids were thus prepared, each one corresponding to a thick section which could easily be observed in the phase contrast microscope. With this method one can examine only those grids which seem to contain a juxtaglomerular apparatus.

Orientation of the tissue is of little importance. Sections parallel to the renal cortex give a longitudinal view, and sagittal sections a transversal view of the afferent arteriole.

B. Topographical Anatomy

Three morphologically different structures can be distinguished in the juxtaglomerular apparatus (cf. Diagram I, Plates I–VI).

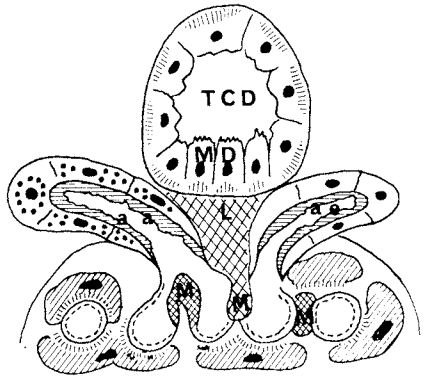


Diagram 1. Diagram of the juxtaglomerular apparatus. The afferent arteriole (aa) with its epithelioid cells enters the glomerulus. Exit of the efferent arteriole (ae). The distal convoluted tubule (TCD), differentiated in macula densa (MD) passes by the vascular hilus of the glomerulus. One sees the lacis (L) which is continuous with the wall of the intraglomerular vessels where its constitutes the mesangium (M). [Reprinted by permission of the Expansion scientifique française (Hatt *et al.*, 1964).]

1. *The Afferent and Efferent Vessels*

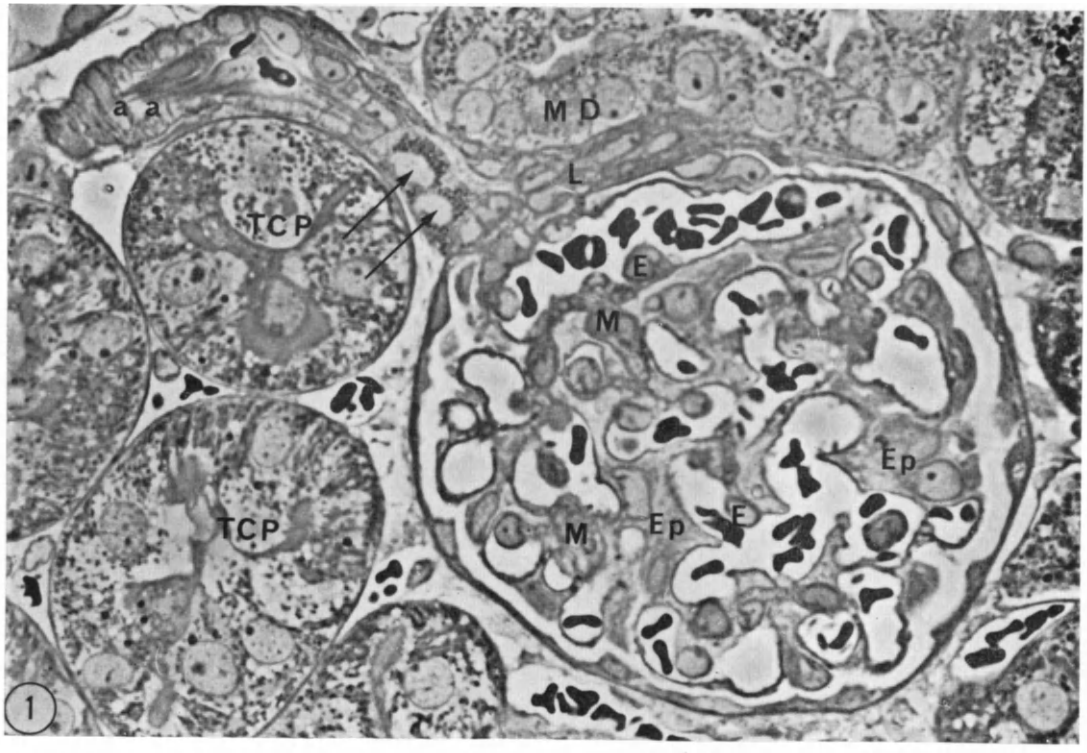
The afferent arteriole with its epithelioid cells rich in endocrine structures and the efferent arteriole devoid of these structures [this opinion is not unanimously held, Barajas and Latta (1963a) having observed granular cells in the efferent arteriole] make up the vascular hilus of the glomerulus.

2. *The Lacis*

The blood vessels form an obtuse angle in which is found that portion of the Polkissen of Zimmermann represented by the pseudo-Meissnerian cells of

Plate I. The juxtaglomerular apparatus in the light microscope. Kidneys from rats fixed in osmic acid, sections 1 μ thick, photographed in the phase-contrast microscope. One sees the afferent arteriole (aa), its epithelioid cells (arrow), the lacis (L), the macula densa (MD), the glomerulus with its endothelium (E), its epithelium (Ep), and its mesangium (M).

Fig. 1. Mercurial anuria. Rat sacrified 16 hours after an injection of $HgCl_2$ (12 mg/kg). The epitheloid cells are the site of the beginning of hypergranulation. The proximal convoluted tubules (TCP) are clearly altered. Magnification: ×800. *Fig.* 2. Overdosage of salt and Doca. Rat sacrificed 3 months after unilateral nephrectomy, implantation of 100 mg Syncortyl and addition of 1% NaCl to the drinking water, obvious hypertension. The epithelioid cells are degranulated and swollen, beginning glomerulosclerosis. Magnification: ×800.


a a
MD
L
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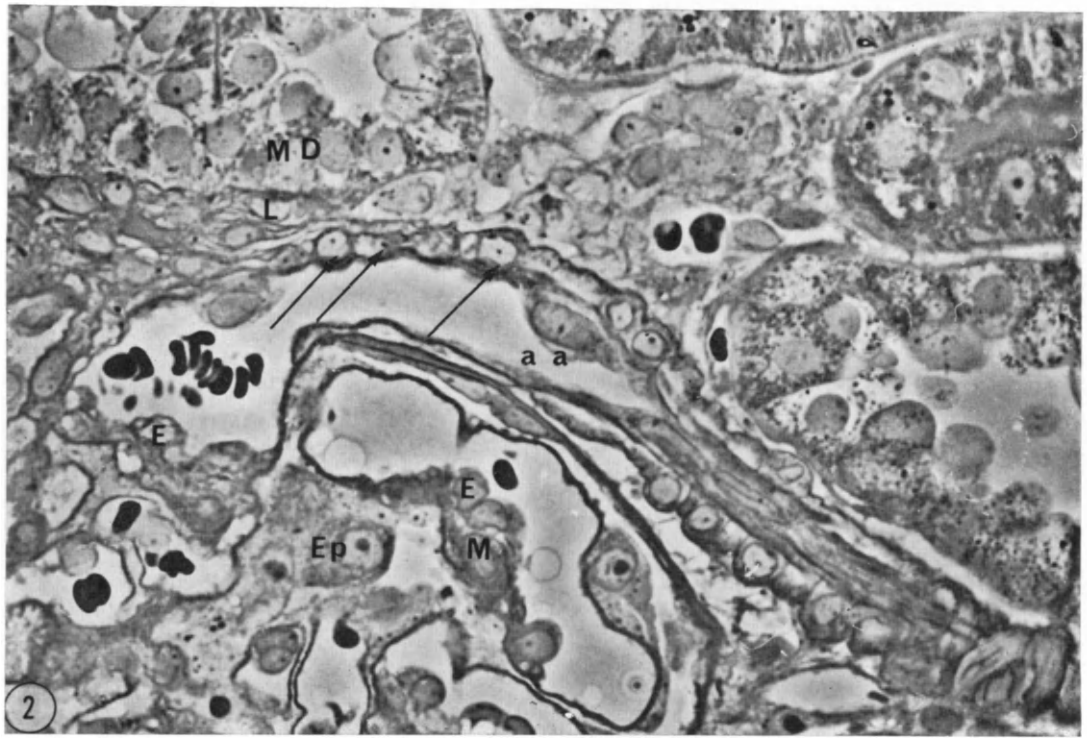
1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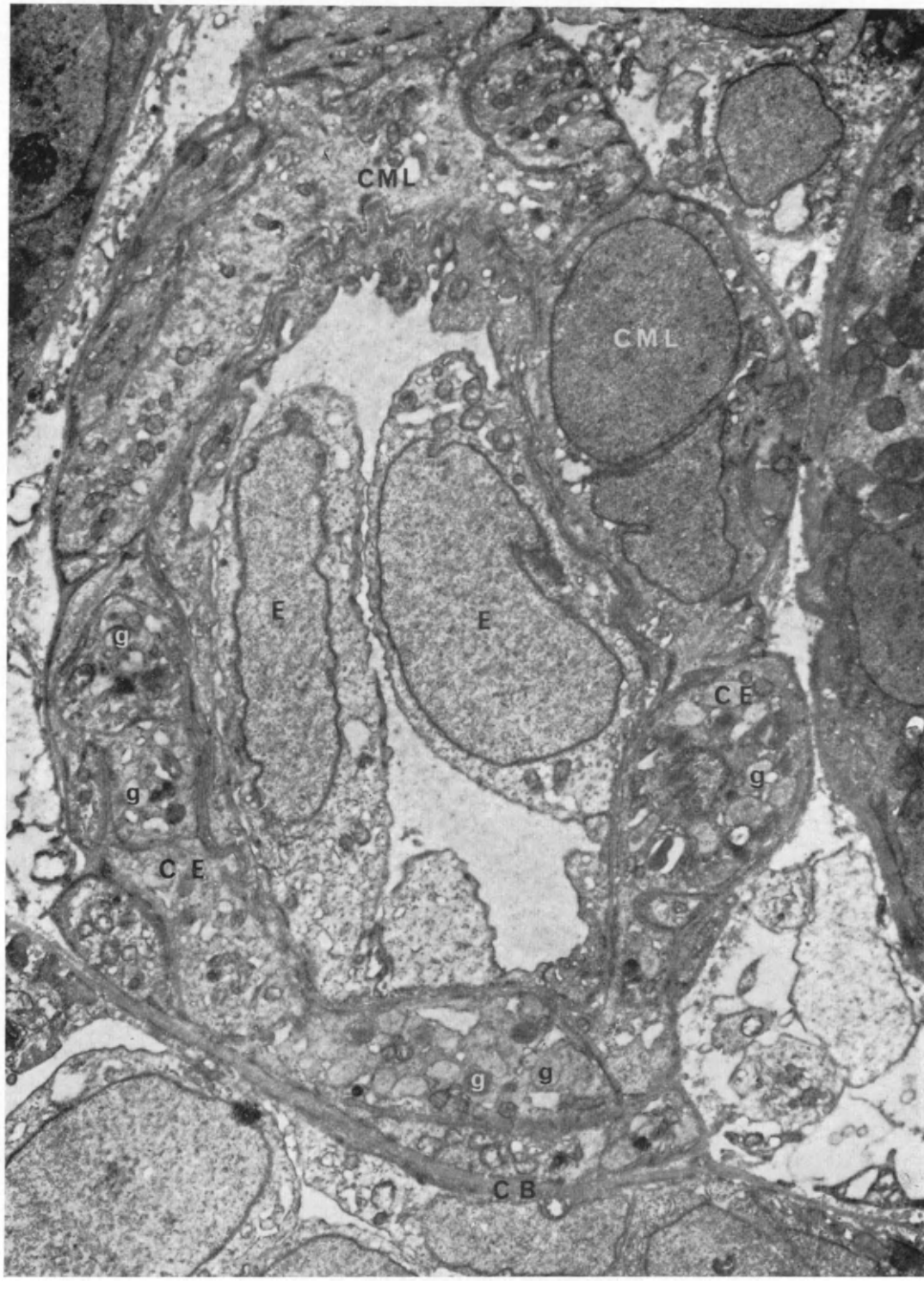
CML
CML
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CE
CE
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g
CB

Goormaghtigh. The latter is a group of relatively undifferentiated cells which appear fusiform in certain sections and which are embedded in a dense mesh of basement membranes, hence the name "lacis" (Oberling and Hatt, 1960a, b).

3. *The Macula Densa*

The distal convoluted tubule ascending from the medulla nestles into the vascular hilus of the glomerulus where its basement epithelium rests against the afferent arteriole, the lacis, and the efferent arteriole.

At this point, the tubular epithelium takes on a rather particular appearance owing to the stacking up of its cells; this was perfectly described by Zimmermann when he called this the "macula densa."

4. *Interelationships of the Various Structures*

a. *Upon Entering the Glomerulus, Do the Vessels Perforate Bowman's Capsule or Is the Capsule Reflected back around Them?* This question, a source of classic discussion, has been settled by electron microscopy. The connective tissue web of the Bowman's capsule joins, with no break in continuity, the basement membrane system which surrounds all cells in this region. This occurs at the level of the afferent and efferent vessels, as well as in the lacis and the first segments of the stalk.

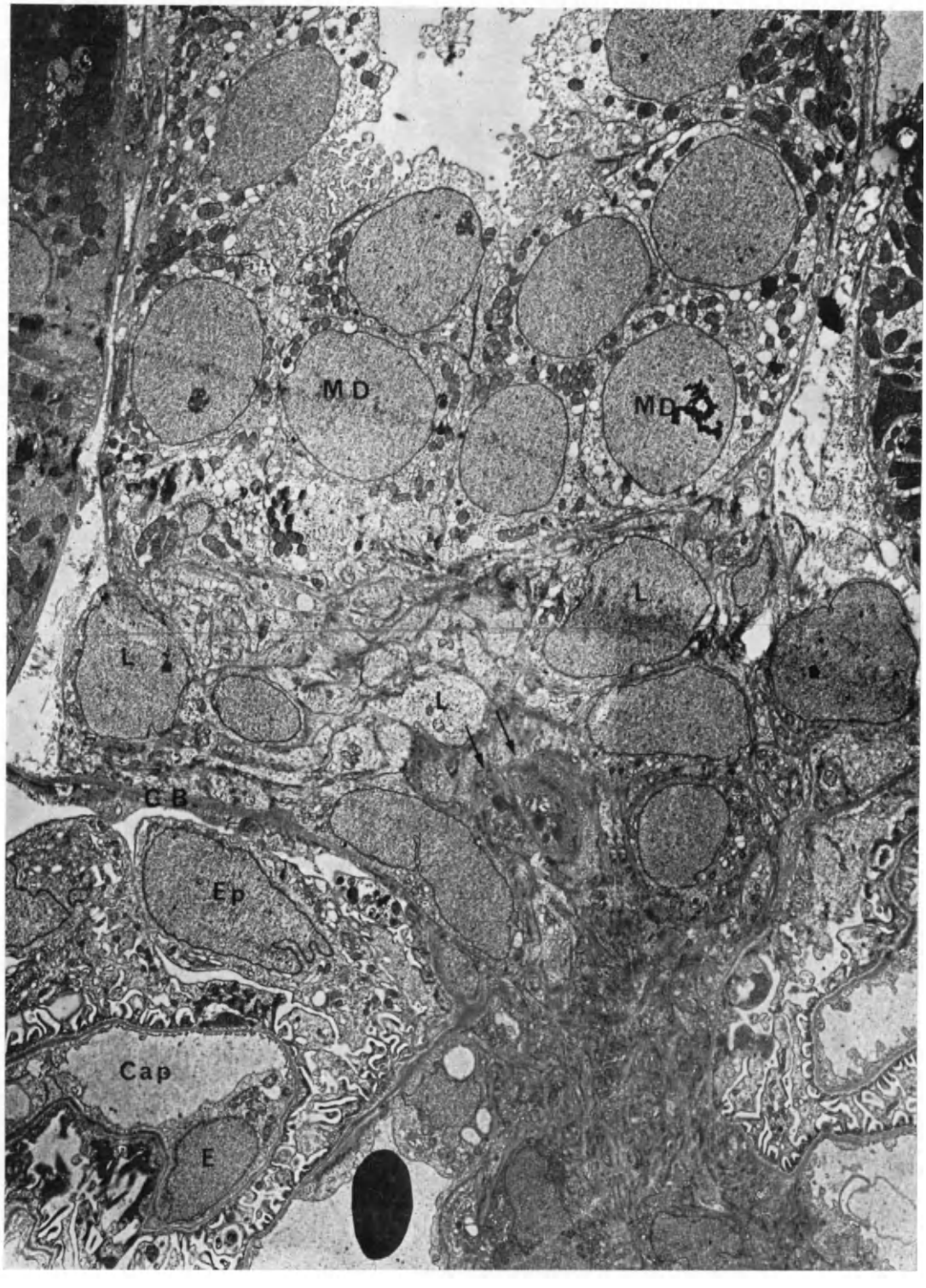
As for the parietal epithelium which lines the internal face of Bowman's capsule, it is reflected back around the first branchings which are visible just after the penetration of the vessels into the glomerulus. These are covered by the epithelium which, starting from the first capillary loop, constitutes the podocytic epithelium.

b. *Relationship between the Arteriolar Tunica Media and the Mesangium.* At first sight, it seems that the tunica media of the afferent and efferent vessels, by their prolongation inside the stalk, constitute the intercapillary tissue of the glomerulus. Dunihue and Boldosser (1963) showed that in adrenalectomized cats the mesangium had the same appearance as the epithelioid cells in the tunica media of the afferent arteriole.

In fact, the mesangium appears to be an intraglomerular extension of the lacis, as will be seen later.

c. *Relation to the Lacis.* The lacis fills up the triangle delineated by the afferent and efferent vessels on the sides and the macula densa at the base. It is wedged in between the two arterioles, from where it penetrates into the glomerulus. At this point sections show that it is prolonged into the mesangium.

PLATE II. Afferent arteriole. Oblique section of the vessel before it enters the glomerulus in which is seen the Bowman's capsule (CB). The epithelioid cells (CE) with their granules (g) are a continuation of the common smooth muscle cells (CML) at the point where the latter enters the glomerulus. The endothelium (E) is pushed into the lumen of the vessel, control rat. Magnification: ×7500.


MD
MD
L
L
L
GB
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Cap
E

Opposite the hilus the lacis comes into close contact with the epithelium of the distal tubule, being separated from it only by the thin basement membrane of the tubule.

d. *Relation to the Macula Densa.* As we have seen, the macula densa lies in intimate contact with the afferent arteriole, the lacis and the efferent arteriole, in that order.

The three-dimensional models constructed by Barajas and Latta (1963a) clearly demonstrate that the macula densa is in contact with the efferent arteriole over a greater length than with the afferent vessel.

C. Ultrastructure of Constituents

1. *Epithelioid Cells.*

The epithelioid cells occupy the media of the afferent arteriole where they replace the common smooth muscle cells of the undifferentiated arteriole.

a. *Normal* (Plates II and VII, Fig. 1). Under normal conditions of circulation and electrolyte balance, there are four to six cells which are grouped just in front of the place where the arteriole penetrates the glomerulus. Sometimes a second group of cells is visible above the first. This group is located a certain distance from the glomerulus in the wall of an arteriolar branch slightly after the latter separates from the interlobular artery. Barajas and Latta found a third group in the efferent arteriole.

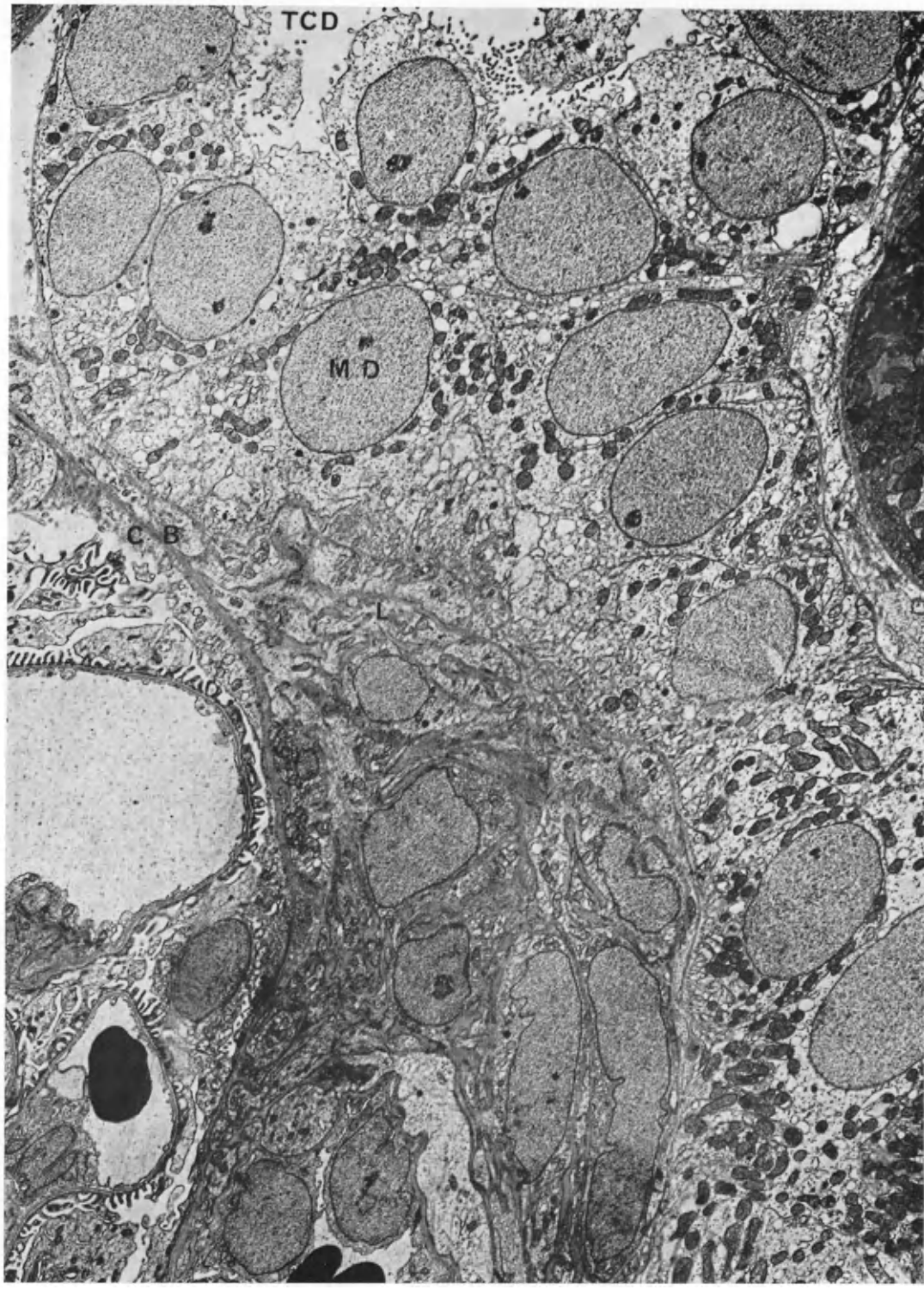
The epithelioid cells are separated from the arteriolar endothelium by a simple basement membrane devoid of elastic fibers. This membrane is continuous, working its way between the epithelioid cells and mixing with the neighboring basement membranes of the lacis and the capsule of Bowman.

The cell membrane contains only a few pinocytic microvesicles; the nucleus is no different from those of the adjacent smooth muscle cells. The endoplasmic reticulum is composed of sacs whose membranes are sparsely peppered with ribosomes and inside which there is sometimes a homogeneous, slightly osmiophilic substance which appears as incipient secretion granules.

The paranuclear Golgi apparatus, clearly more developed than in the nearby smooth muscle cells, has the usual membranovesicular appearance and surrounds a rarely seen centriole.

The oval mitochondria have almost rectilinear and parallel cristae. The ribosomes are more or less evenly distributed throughout the cytoplasm. Some seem to be attached to the membranes of the endoplasmic reticulum although not often enough to constitute an authentic ergastoplasm.

Plate III. The macula densa and the lacis. Oblique, tangential section of the afferent arteriole shortly after its entrance into the glomerulus where one sees the Bowman's capsule (CB), the capillaries (Cap), the endothelium (E), and the epithelium (Ep). Also present are the cells of the macula densa (MD), piled up on one another, and the lacis in the middle of which are two epithelioïd cells (arrow) containing secretion granules, control rat. Magnification: ×4900.


TCD
M D
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L

Secretion granules are either rounded or oval, homogeneous, and usually osmiophilic. They are limited by a single, sometimes split membrane, whose appearance is similar to that of the endoplasmic reticulum.

Multivesicular bodies are present. Certain appear osmiophilic and are crammed with secretion product.

Myofibrils exist in some places, particularly at the periphery of the cell, which substantiates the link between these cells and those of the smooth muscle.

This mixture of structures indicates a double differentiation (muscular and endocrine) and justifies the designation "myoepithelioid" which has been given to these cells.

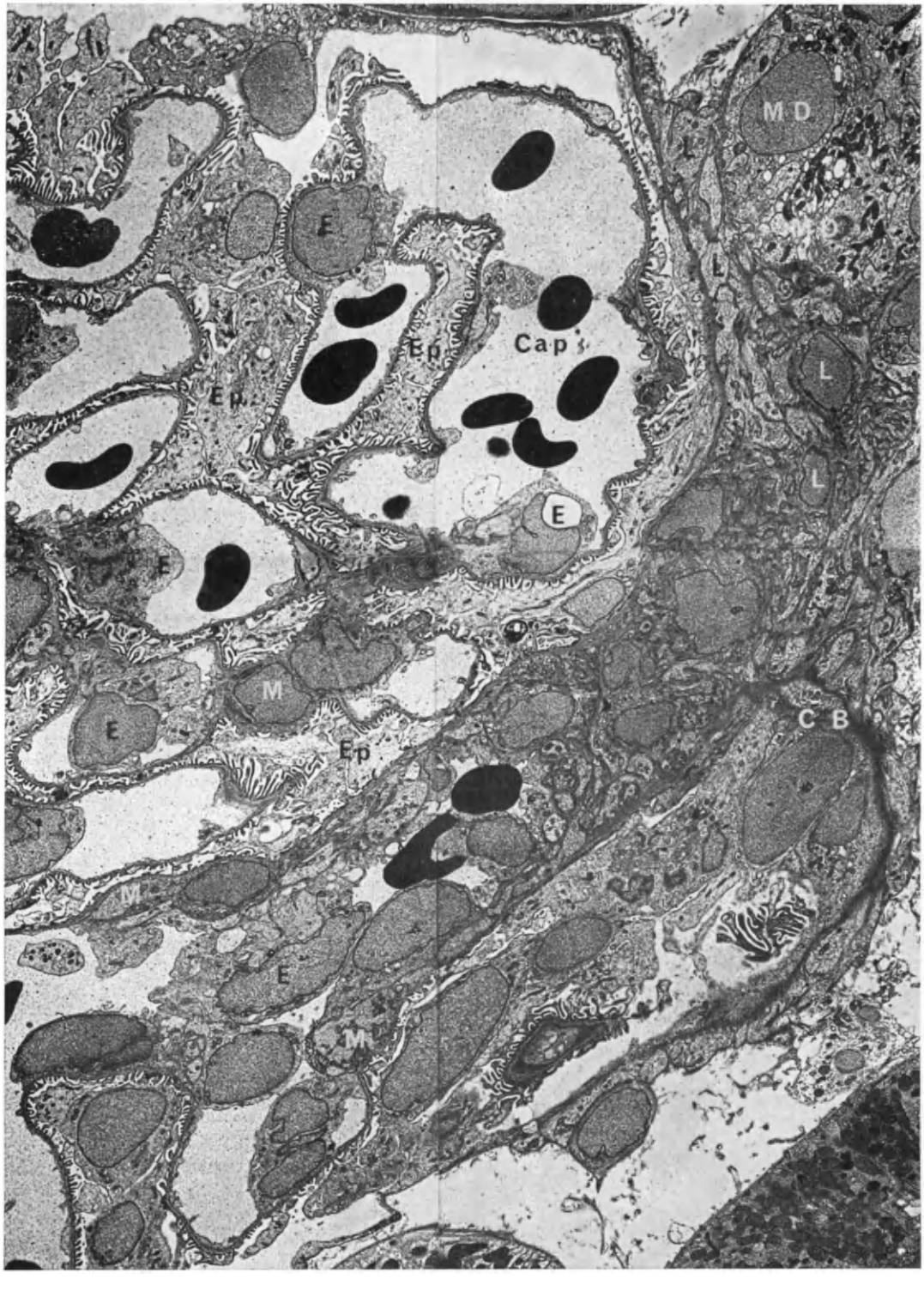
b. *Hyperactivity.* Under conditions of hyperactivity, the changes in appearance of epithelioid cells confirm their endocrine nature. The conditions under which hyperactivity occurs are: (1) renal ischemia created by partial constriction of the renal artery. Characteristic pictures of hyperactivity only appear when both kidneys are intact. Examination must be made within the first 3 weeks following clamping, for beyond this time the percentage of hyperactive juxtaglomerular complexes diminishes. (2) Sodium-free diets, adrenalectomy, and mercurial anuria. Within 24 hours injection of $HgCl_2$ (12 mg/kg) into the rat brings about an intense, proximal tubulonephritis and also elicits hyperactivity of all juxtaglomerular apparatus, thereby facilitating study of the different stages of secretion granule synthesis.

In these cases, two kinds of modifications occur (Plate VII, Fig. 2; Plate VIII, Figs. 1, 2; Plate IX) as follows.

Hyperactivity of the epithelioid cells, properly speaking, manifests itself as a swelling of the endoplasmic reticulum mesh, inside which appears an osmiophilic substance destined to become the secretion granule. This substance appears homogeneous, unstructured, and having equal density from one lacuna to another. The number of secretion granules increases. They can rapidly occupy all or part of the cytoplasm, leaving relatively little space for other structures. The matrix of the granules remains homogeneous, although the granules appear often less dense than normal. The population of ribosomes increases, becoming concentrated in the narrow cytoplasmic spaces which remain. The mitochondria similarly appear more numerous and smaller.

The neighboring smooth muscle cells and certain lacis cells become the site of an endocrine activity not found under normal conditions. The endoplasmic reticulum dilates, accompanied by the appearance of secretion granules and by an increase in ribosomes. Thus the number of epithelioid cells grows at variable but important rates, going, for example, from four to twenty cells in a few days (mercurial anuria) or a few weeks (renal ischemia or sodium-free diet).

PLATE IV. The macula densa, the lacis, and the efferent arteriole. The distal convoluted tubule (TCD) passes by the vascular hilus of the glomerulus at the point of exit of the efferent arteriole. The arteriole is separated from the macula densa (MD) by the lacis (L). Bowman's capsule (CB) is continuous with the basement membrane system of the lacis, control rat. Magnification: ×4900.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c. *Hypoactivity* (Plate VII, Fig. 3; Plate VIII, Figs. 3, 4). Hypoactivity is seen under the following conditions: a sodium-rich diet with an overdosage of Doca (deoxycorticosterone acetate), unilateral renal ischemia when the nonischemic kidney is examined, and perinephritis of one kidney.

The epithelioid cells become notably impoverished in cellular constituents. The secretion granules disappear from the cell leaving in their stead a few voided sacs composed of a single membrane. The mitochondria, as well as the ribosomes, diminish in number. In certain cases (overdosage of Doca or in the kidney paired with an ischemic organ), the juxtaglomerular cell is generally swollen and clear as if it were the seat of an edema.

It is often difficult under these conditions to differentiate between these cells and those of the nearby smooth muscle which have undergone similar changes, as have the lacis cells. The total number of granulated cells falls way below par.

d. *Conclusions.* These changes in appearance confirm the endocrine nature of the myoepithelial cells and the real significance of their "degree of granulation." An increase in granulation coexists with all the cytological indications of hypersecretion, whereas a reduction is indicative of hyposecretion.

But such evidence does not supply clear answers to questions posed by the cytologists. Does the substance of the secretion granules represent the hormone itself, a precursor of it, or its storage form? What are the functions of the Golgi, the mitochondria, the endoplasmic reticulum, and ribosomes in the elaboration, excretion, and storage of the hormone?

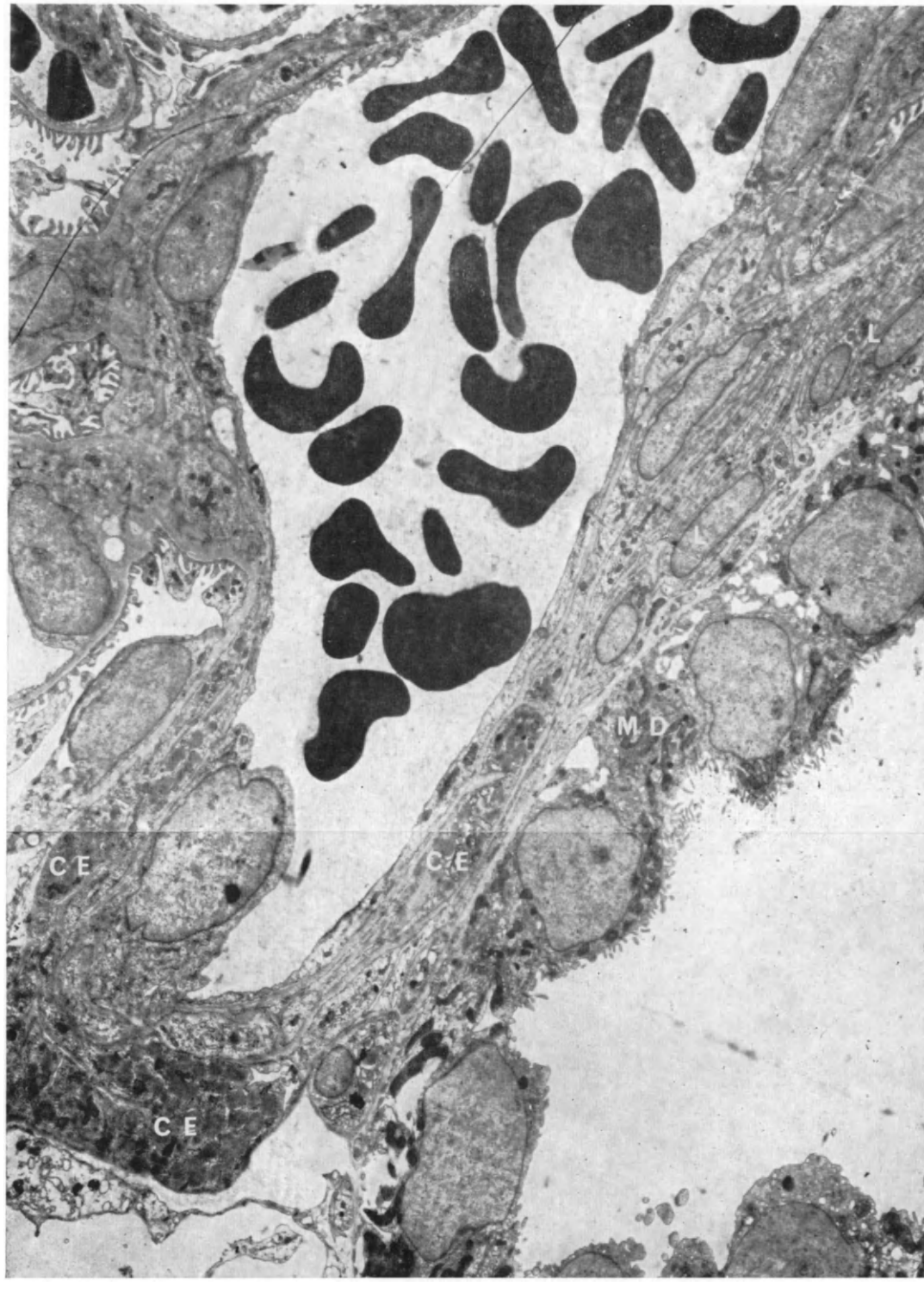
At this time, opinions on this subject can be no more than mere conjecture.

2. *The Lacis*

The lacis (Plates III–VI) fills the triangular space limited by the two glomerular vessels and the macula densa and is composed of cells piled up in a dense network of basement membranes.

a. *Under Normal Conditions.* The cells of the lacis are rarely totally visible, any one section usually showing only a narrow portion of each cell. On the few longitudinal sections seen, they appear elongated and tortuous with a prominent perinuclear zone containing an average-size Golgi apparatus. The cells have long tapered processes of vesicular cytoplasm (an appearance due to its rich endoplasmic reticulum) and small, ovoid mitochondria with rectilinear cristae. The cell membrane is not particularly rich in microvilli. Here and there, an intracytoplasmic fibrillar structure is discernible.

PLATE V. Relationship of the lacis to the mesangium. Longitudinal section of one of the first intraglomerular branchings of the afferent arteriole. The lacis (L) unites the base of the macula densa (MD) with the glomerulus; one sees the capsule of Bowman (CB), the capillaries (Cap), the endothelium (E), and the epithelium (Ep). The lacis is continuous with the wall of the intraglomerular vessels where it constitutes the mesangium (M), control rat. Magnification: ×3700.


L
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C E
C E

Generally speaking, these lacis cells have no clearly defined differentiation nor are they comparable to nerve cells, but they are clearly different from their epithelioid and muscular neighbors.

Their morphological resemblance to the intercapillary cells of the glomerulus is, however, evident. In the occasional longitudinal section showing both the lacis and one of the primary intraglomerular capillaries, it is easy to see that the former, with no break in continuity, nor modification of structure, projects into the wall of the primary capillary of the stalk where it becomes the mesangium.

Just as in the latter instance, the cells of the vascular pole of the lacis are closely related to one another by a tortuous system of overlapping basement membranes which are in close contact with the other nearby basement membranes.

b. *Under Certain Experimental Conditions.* The changes in appearance of the lacis add little supplementary knowledge to the preceding observations.

Latta and Maunsbach (1962a), who noted, as we have, the structural link between the lacis and the mesangium, tried without success to discover their phagocytic properties. Thorotrast, injected into the animal, while concentrated in the mesangium cells, also appeared in the lacis but to a lesser degree.

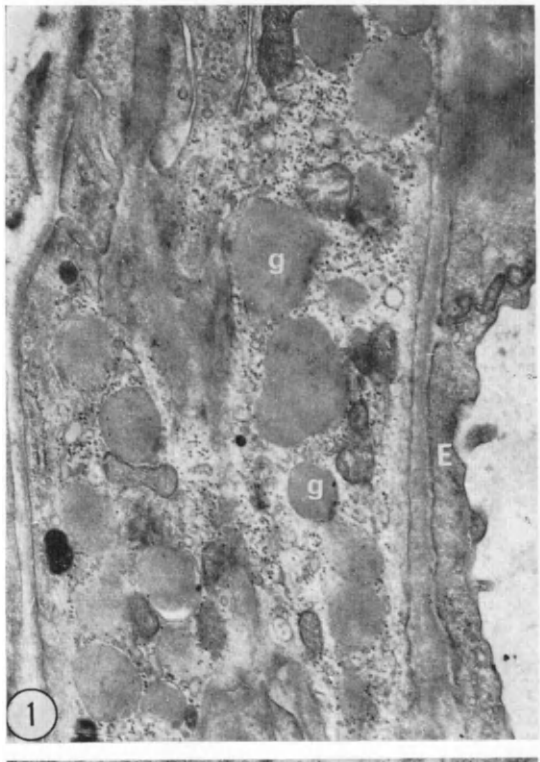
In studying modifications occurring in different experimentally produced nephropathies we observed that the response of the stalk cells was almost exactly like that of the tunica media of the afferent and efferent vessels on the one hand and of the mesangium on the other.

In renal ischemia and sodium-deficient diets, the lacis participates to a certain extent in the endocrine activity; some of its cells as well as the epithelioid cells fill up with secretory product. In acute renal ischemia accompanied by tubular degeneration with relative preservation of the vascular structures, the macula densa disappears and the lacis cells are no longer visible. It is, therefore, impossible to say whether the cells have actually disappeared or whether they have been transformed into epithelioid cells, the extraordinary proliferation of the latter being an indirect argument in favor of the latter hypothesis.

In the kidney paired with an ischemic kidney and in animals receiving overdoses of salt and Doca (Plate X), the lacis can undergo alterations identical with those occurring in the tunica media of the afferent arteriole and the mesangium: swelling and clarification of the cells, thickening of the intercellular basement membranes, resulting in a true hyaline infiltration of the afferent and efferent vessels, the lacis, and the stalk.

c. *Conclusions.* In conclusion, one must ask about the nature of the lacis. Is it an adventitious girdle composed of slightly differentiated, ubiquitous cells, that can

PLATE VI. Over-all view of the juxtaglomerular apparatus. Longitudinal section of the afferent arteriole at its point of entry into the glomerulus. One sees the epithelioid cells (C) with their secretion granules, the macula densa (MD), and the lacis (L). Rat after 1 month on a sodium-free diet. Magnification: ×4400.



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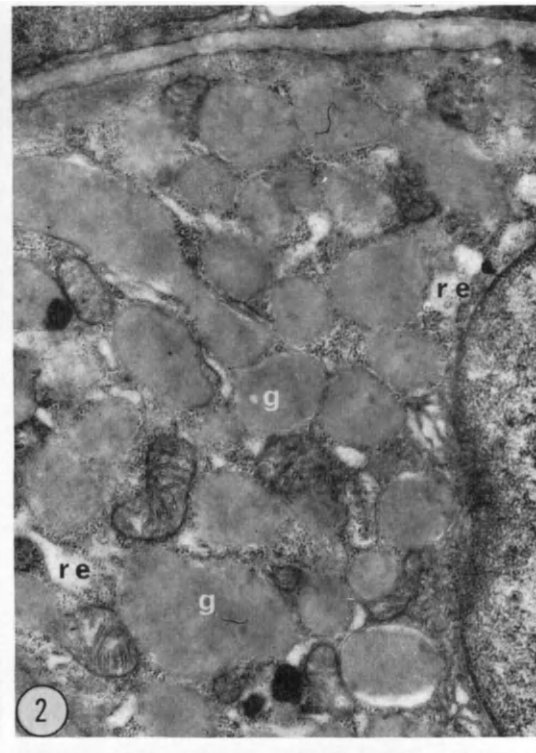

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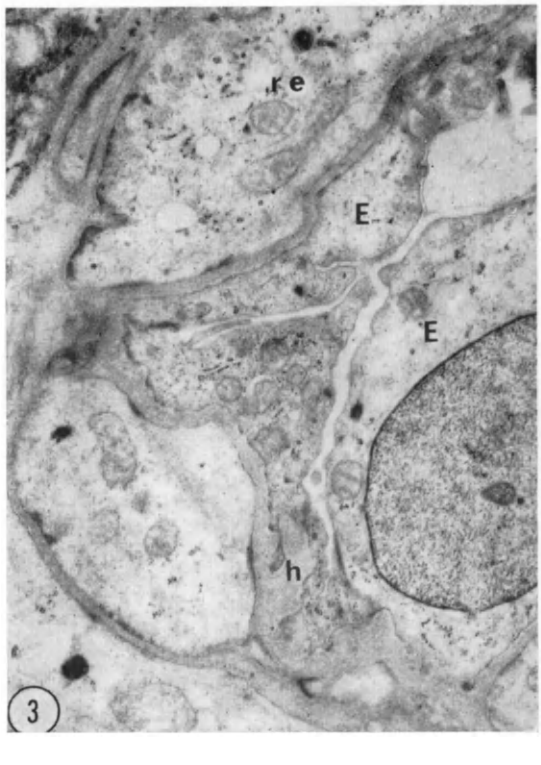

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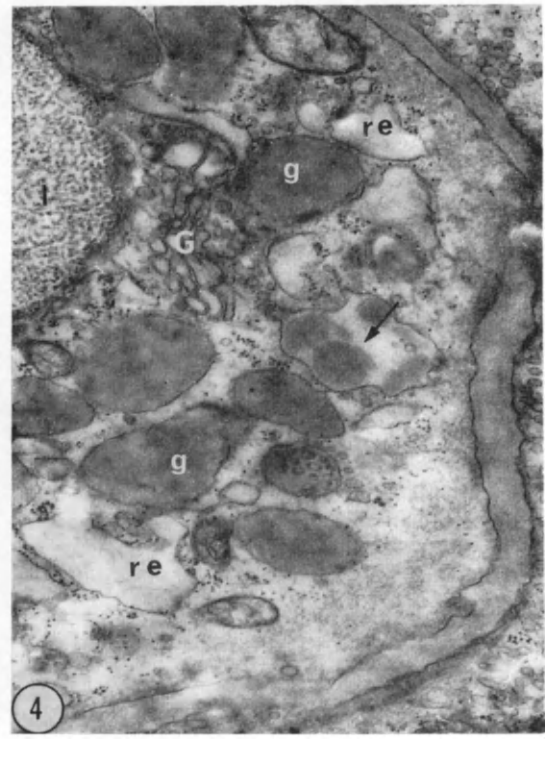

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transform into macrophages in the same manner as the mesangial cells? Or is it simply a sustaining tissue with no particular physiological properties? Finally, is this structure a transmitter of osmotic information? It is still impossible to be definitive on this subject.

In any case, the electron microscope has eliminated the first hypothesis concerning the neurological role of the lacis and proved that, if silver impregnation reveals a highly developed plexus, it is not a neural plexus, but rather a richly anastomosed network of inert basement membranes.

3. *The Macula Densa*

The macula densa (Plates III, IV, VI, XI) consists of epithelial cells of the distal convoluted tubule and arises at the point where the tubule touches the vascular hilus of the glomerulus. It comes in contact with the afferent arteriole and its epithelioïd cells, with the lacis, and with the efferent arteriole.

The cells of the macula densa possess all the characteristics of the common distal tubular epithelium, being comprised of a basal pole with its membraneous intracytoplasmic projections and an apical pole whose surface erupts into microvilli. The Golgi takes a low, paranuclear position around the nucleus, which is at the base of the cell. These cells are distinguished by the following characteristics.

a. *Normal Aspects.* The close proximity of the cells is due to the relative scantiness of their cytoplasm and gives the impression in the light microscope that the nuclei are piled up, as mentioned by Peter and Zimmermann.

The mitochondria are scattered throughout the cell rather than gathered in its base as in the rest of the distal tubule.

The basement membranes are not rectilinear nor do they show the order or parallelism characteristic of the distal tubule. They are few, oriented in all directions, and not regularly associated with the mitochondria. The plasma membrane at the apex of the cell appears thicker than at the base.

The intercellular spaces, often opening down into the base of the cells, bring the tubular lumen close to the basal pole; consequently, the urine circulating there comes

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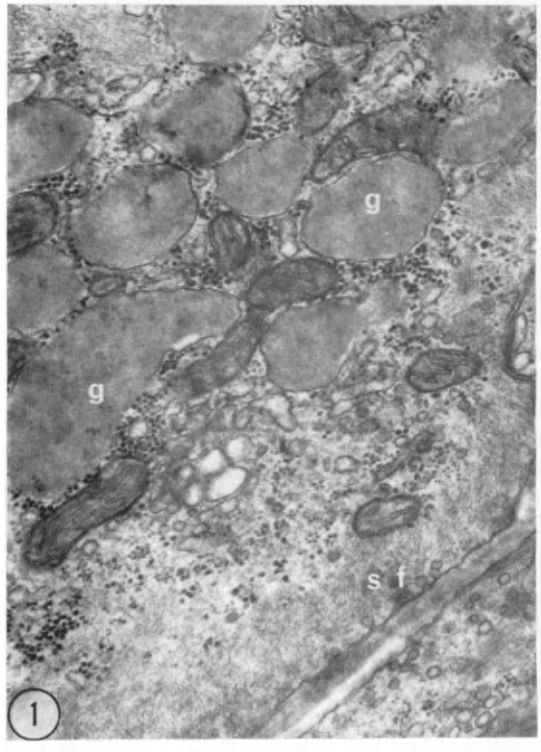
PLATE VII. Epithelioid cells under normal conditions and in an ischemic kidney. Various states of the epithelioid cells with their secretion granules (g), endoplasmic reticulum (re), and ribosomes.

Fig. 1. Normal control rat. Magnification: ×17,000. *Fig.* 2. Rat with unilateral renal ischemia of 3-months duration, ischemic side. Note the density of secretion granules and of the ribosomes. Magnification: ×17,000. *Fig.* 3. Rat with unilateral renal ischemia of 3-months duration, non-ischemic side. Degranulation and edema of the endothelium (E) and epithelioid cells, hyaline infiltration under the endothelium (h). Magnification: ×12,500. *Fig.* 4. Metaischemic arterial hypertension in a rat after 3 months of unilateral ischemia. The ischemic kidney was removed and the hypertension persisted. The remaining kidney was examined. Note the Golgi apparatus (G) and the appearance of grains in the heart of the dilated openings of the endoplasmic reticulum (arrow). Magnification: ×26,000.



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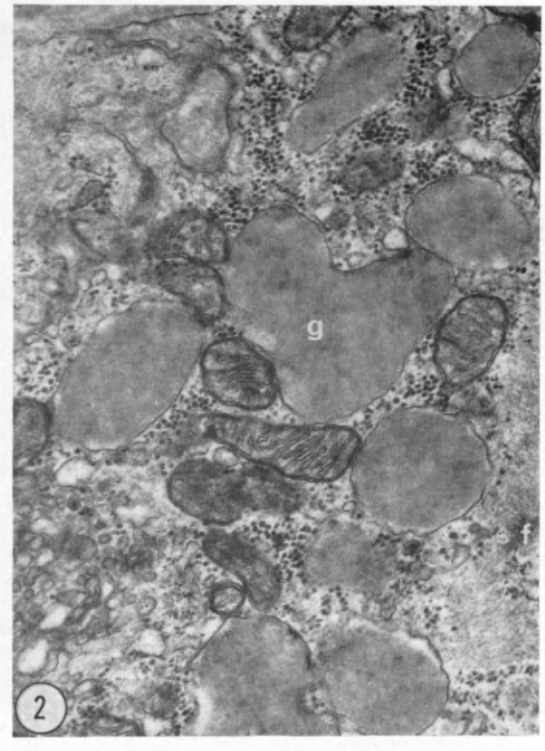

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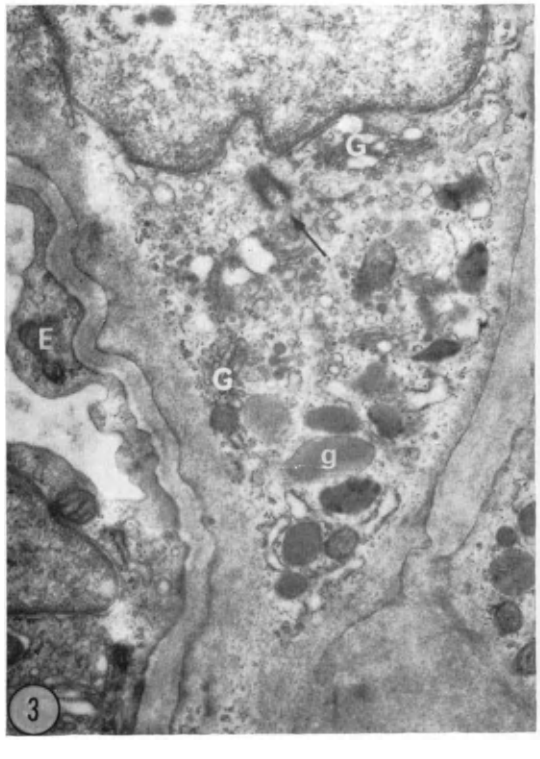

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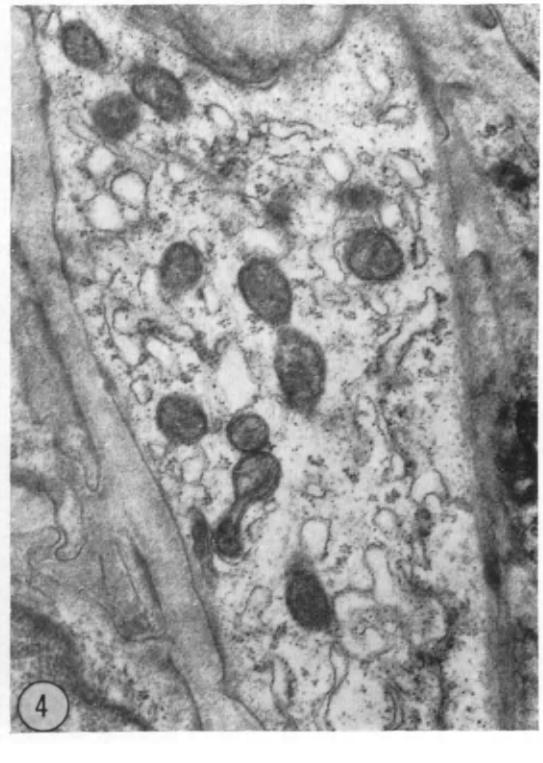

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in direct contact with the basement membrane. A cilia, implanted in the apical pole and whose extremity bathes in urine, was observed once.

b. *Under Conditions Which Modify the Activity of the Epithelioid Cells.* The macula densa is concomitantly modified. With sodium-deficient diets and with minor renal ischemia, the appearance of the macula densa does not differ from its normal appearance. We have been unable to detect the site of glucose-6-phosphate dehydrogenase activity which, under these conditions, is increased (Hess and Pearse, 1958). In such cases, the ribosomes and the Golgi apparatus seem more apparent and the basement membranes more organized, but these are not prominent features.

In major renal ischemia, accompanied by tubule degeneration and relative conservation of vascular structures, the macula densa atrophies to nothing, while beside it, lie extraordinarily hyperplastic and hyperactive epithelioid cells. This would seem to prove that the presence of the former is in no way necessary for the activity of the latter.

In the kidney opposite the ischemia and under overdosage of salt and Doca, the cells of the macula densa become clear, swollen, and their fine structure is reduced with respect to the mitochondria, Golgi, and the ribosomes.

c. *Conclusion.* All these structures do not indicate an evident role for the macula densa. They signify, however, that (1) this is a banal epithelium, devoid of any differentiation indicative of a neural plaque, as Goormaghtigh implied; (2) the reabsorption activity with which it has been attributed appears, under normal conditions, to be quite small.

D. Summary

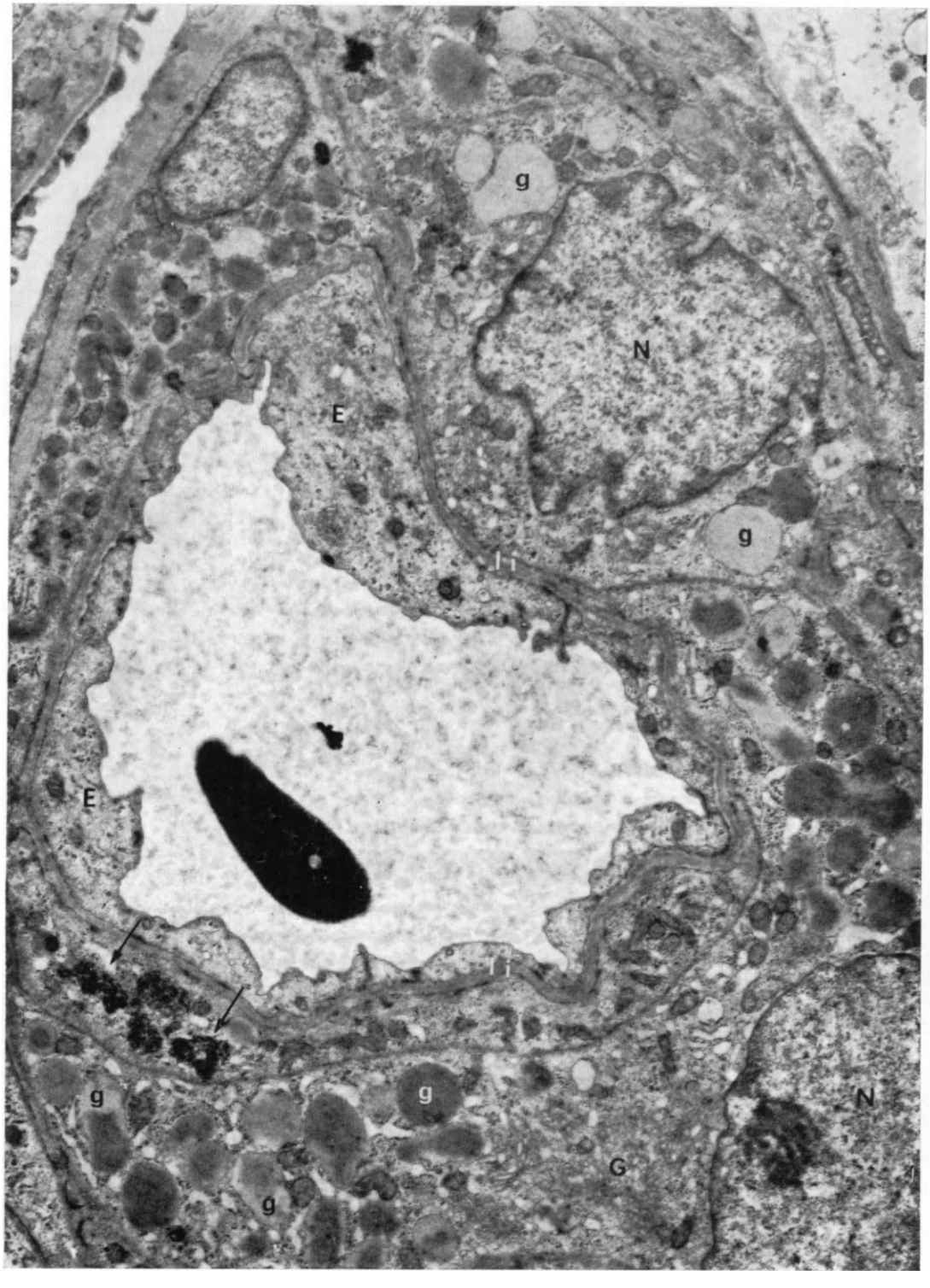
The electron microscope affords the following confirmations and corrections of preceding descriptions of the juxtaglomerular complex.

It confirms existing topographical observations about the region between the hilus and the macula densa.

Regarding the three elements therein, electron microscopy corroborates the myoepithelial appearance of the media cells of the afferent arteriole, as described by Ruyter and Oberling, and confirms beyond any doubt the endocrine nature of their structure, for it reveals changes in their activity under various experimental conditions.

Plate VIII. Epithelioid cells in various functional states.

Figs. 1 *and* 2. Rat injected with $HgCl_2$ (12 mg/kg) 40 hours before sacrifice. Complete anuria. An intense endocrine activity is apparent in the epithelioid cells and lapses over into the neighboring smooth muscle cells. Fibrillar structures (sf) of the smooth muscle are present. Note the density of the secretion granules (g) and ribosomes. Magnification: ×25,000. *Fig.* 3. Rat subjected to 21 days of renal ischemia, nonischemic side. Degranulation of the epithelioid cells (E). The centriole (arrow) and Golgi apparatus (G) are discernible. Magnification: ×16,000. *Fig.* 4. Same case as in Fig. 3. Total degranulation of an epithelioid cell which is no longer distinguishable from a lacis cell. Magnification: ×23,000.


g
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Further, electron microscopic evidence weakens hypotheses concerning the presence of neural elements not only in Goormaghtigh's pseudo-Meissnerian cells which are relatively undifferentiated and identical to intercapillary mesangium cells, but also in the dense argentophil network of basement membranes surrounding them. Both the membranes and the cells continue into the heart of the glomerulus where they constitute the mesangium.

As for the macula densa of Zimmermann, it is a segment of distal convoluted tubule whose particular appearance could only be accounted for as indicative of a reabsortive activity and which, by the loose character of the intercellular bonds, facilitates direct contact between the tubular contents and the "lacis." This latter structure, judging from its abundance of basement membranes, allows a rapid transfer of electrolytes and therefore cannot be eliminated as a possible means of exchange at this site.

If the endocrine function of the epithelioid cells is well established, the exact roles and natures of the lacis and macula densa are not. Modifications appearing in this region under different experimental conditions seem to indicate that they participate along with the epithelioid cells in certain functions, which ones, however, are far from clear.

This gap in our knowledge represents one of the principal obstacles to the understanding of the physiological role of the juxtaglomerular complex.

IV. Physiological Significance

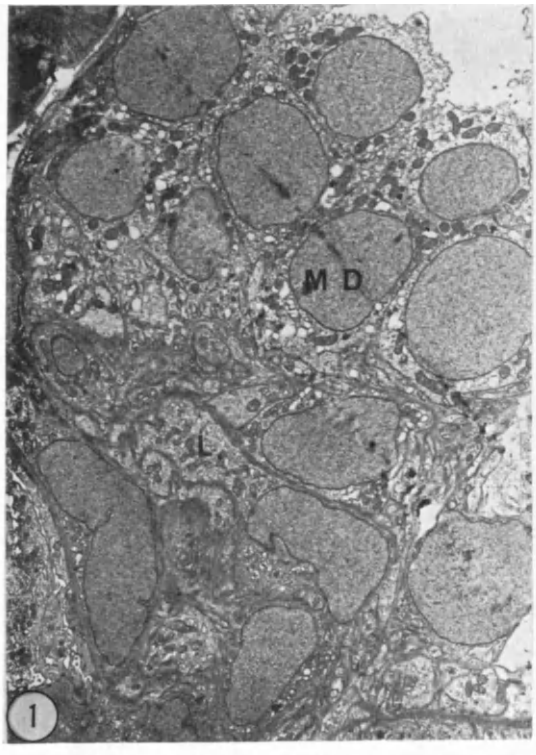
While the electron microscope confirms the endocrine nature of the juxtaglomerular complex and assigns its place precisely to the epithelioid cells, it has not yet permitted a far reaching study of its functions.

The exact site of renin elaboration in the juxtaglomerular apparatus, the barosensitive or chemosensitive nature of its elements, its role in the regulation of blood pressure, electrolyte balance, and adrenal cortex activity are current topics of discussion rather than settled questions.

A. Secretory Activity

This hypothesis, formulated from the first light microscope investigations, is clearly ascertained by the electron microscope, which shows that the epithelioid cells possess all the structures necessary for active secretion.

Plate IX. Hyperactivity of epithelioid cells seen in an early stage. Rat injected subcutaneously with $HgCl_2$ (12 mg/kg) 16 hours before sacrifice. Beginning of anuria. Transverse section of the afferent arteriole showing its endothelium (E), its internal limiting membrane (li) devoid of elastin, its epithelioid cells with their nuclei (N), Golgi apparatus (G), and secretion granules (g). The granules are at different stages of maturation. Abnormal inclusions (arrow) in a cell are perhaps mercury. Magnification: ×8700.



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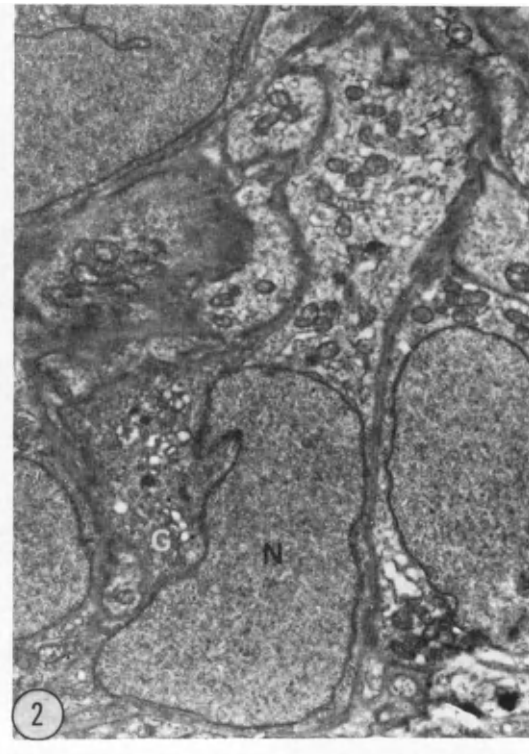

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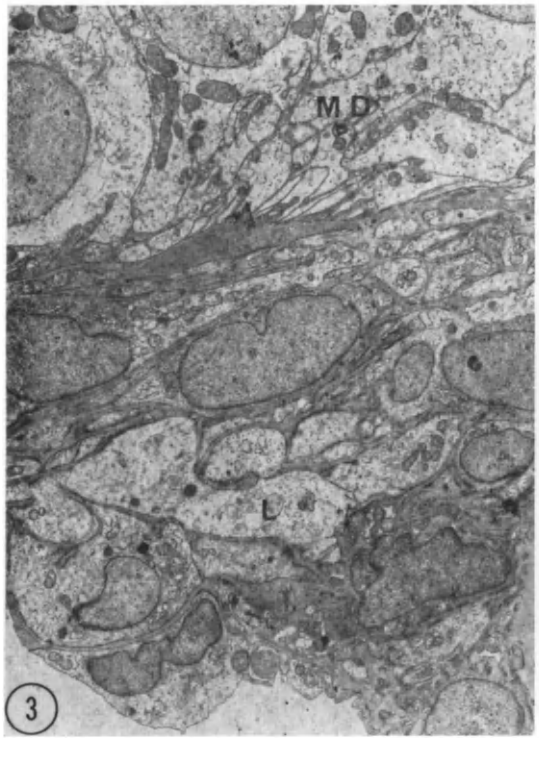

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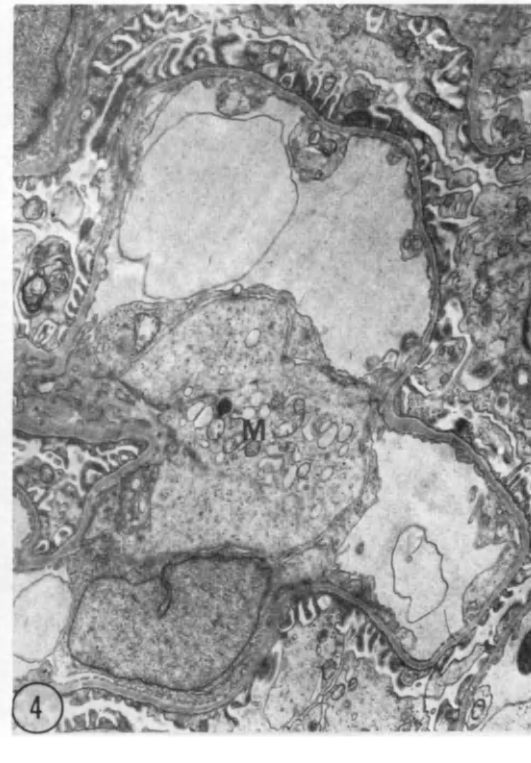

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1. *Review of the Ultrastructure*

It has been shown above that under normal conditions these structures are limited to four or five preglomerular cells. In hyperactivity, they can be seen in the neighboring smooth muscle cells and certain lacis cells. In hypoactivity, the number of epithelioid cells diminishes; in each cell the endocrine organelles rarify to the point of disappearing.

These changes are not accompanied by modifications of the macula densa and lacis regularly enough to be able to say that the other constituents of the juxtaglomerular complex are involved in the secretory process.

This opens the discussion as to the validity of the two cytological tests previously proposed for studying the activity of the juxtaglomerular apparatus: the Hartroft technique and the glucose-6-phosphate dehydrogenase test.

2. *Validity of the Hartroft Technique*

This semiquantitative measurement of the degree of granulation of the juxtaglomerular complex was proposed by P. M. Hartroft and Hartroft (1953) for appraising the average activity of this region in any given kidney.

We have personally employed this technique in all our experiments and have found it in agreement with electron microscopic observations. This is not surprising considering that it measures the degree of granulation in the epithelioid cells and that the electron microscope confirms that this degree of granulation is, of all the cytological characteristics indicative of secretory activity, the most evident (and the only one available on the light microscope level).

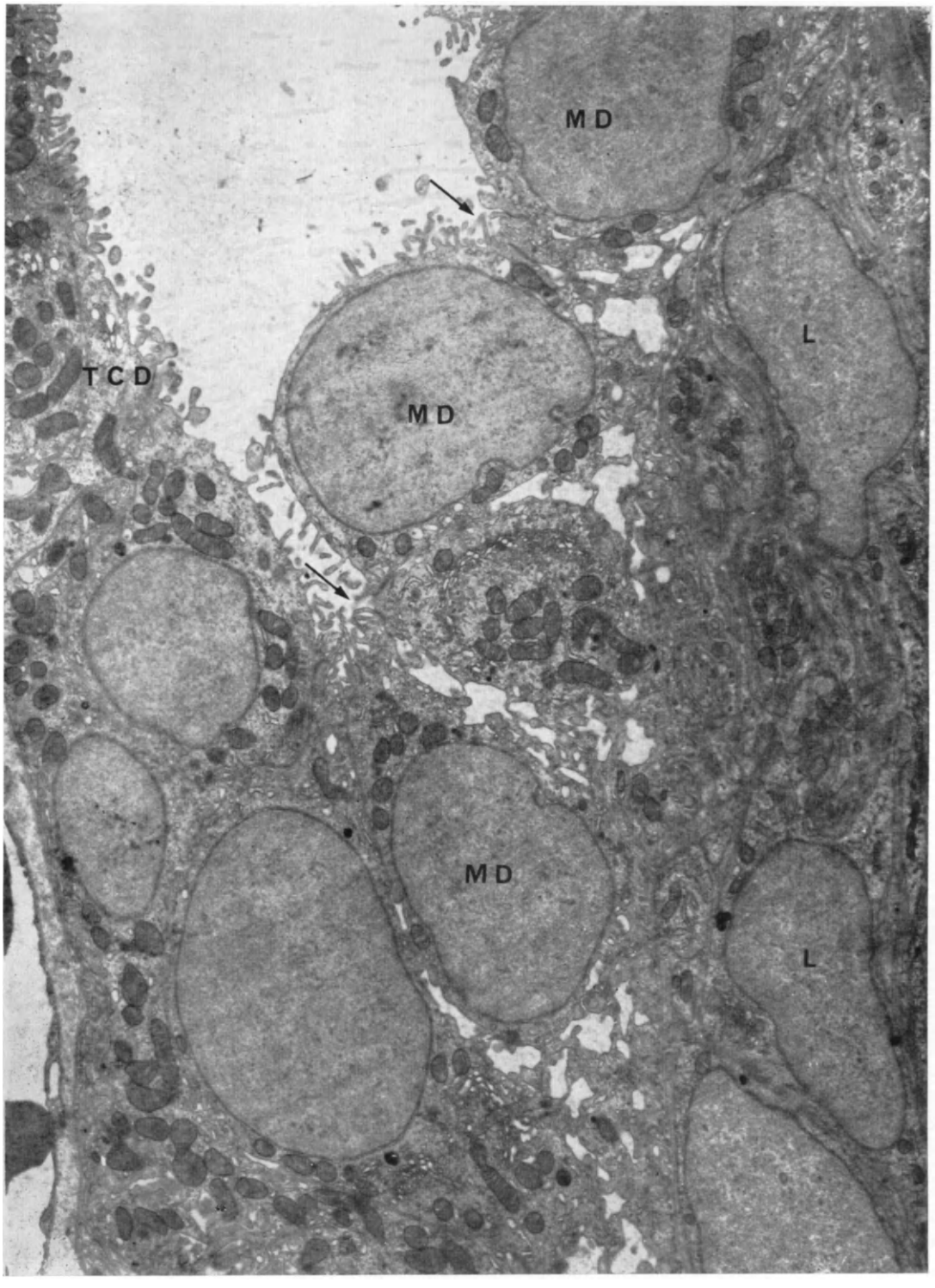
Hypergranulation coincides with hyperactivity, whereas hypoactivity or degranulation is linked with inhibition of epithelioid cell activity.

Again, certain conditions of validity must be underlined.

It is necessary, as P. M. Hartroft and Hartroft prescribed, that a minimum of 100 glomeruli are counted (experience showing that not all juxtaglomerular apparati in any one kidney, notably in renal ischemia, have the same activity at the same time); to refere to a statiscally significant number of control rats, kept under the same conditions, particularly as regards their electrolytes; and to take into account the delay necessary for the maturation of the secretory granules, particularly in experimental pathology, the length of this delay and the chronology of Hartroft's calculations being little known and probably varying with the experimental design.

PLATE X. The lacis and the mesangium.

Fig. 1. Normal control rat. The macula densa (MD) and lacis (L) are seen. Magnification: ×3200. *Fig.* 2. Same case as in Fig. 1. Detail of a lacis cell with its nucleus (N), Golgi apparatus (G), and its tapering cytoplasmic projections. Magnification: × 7800. *Fig.* 3. Rat which received overdoses of salt and Doca following unilateral nephrectomy. Swelling and clarification of the lacis cells. Magnification: ×4800. *Fig.* 4. Same case as in Fig. 3. A mesangial cell is swollen and clear, as are the lacis cells. Magnification: ×7000.


MD
TCD
MD
L
MD
L

3. *Glucose-6-phosphate Dehydrogenase Activity*

Hess and Pearse (1958), Hess and Gross (1959), and Gross and Hess (1960) have shown that, in the epithelium of the distal convoluted tubule, particularly at the level of the macula densa, the histochemically detectable activity of glucose-6-phosphate dehydrogenase increased under conditions of hyperactivity of the juxtaglomerular complex (renal ischemia, sodium-deficient diets, after adrenalectomy) and diminished in inverse circumstances (kidney opposite to ischemia, overdoses of Doca and sodium).

Under these conditions, electron microscopy does not reveal any clearly defined structural modifications except for certain expansion of ordinary structures—Golgi, ribosomes, and mitochondria.

In certain cases of acute renal ischemia, the macula densa appears degenerated in contrast to the hyperactive epithelioid cells, which would seem to show that the integrity of the former structure is not necessary for the functioning of the latter.

The role attributed to glucose-6-phosphate dehydrogenase in the elaboration of renin is possibly correct but, as we shall see, the site of synthesis of renin itself has not yet been pinpointed. In any case, conclusions about this would be premature at this time.

B. Conditions Which Modify the Secretory Activity

Conditions which modify secretory activity can be classified under three headings according to whether the hormonal, circulatory, or electrolyte balance is the disturbed system.

1. *Circulatory Disturbances*

Arterial hypotension, whether general (repeated hemorrhages) or localized in the kidney (renal ischemia), induces hyperactivity of the juxtaglomerular apparatus, whereas hypertension induces inhibition of juxtaglomerular functioning.

This notion, drawn from Goormaghtigh's work (1936, 1942; Goormaghtigh and Grimson, 1939), has since been confirmed (P. M. Hartroft, 1957; Tobian *et al.*, 1958) and is based, above all, on the appearance of the juxtaglomerular complex during unilateral renal ischemia. In a kidney subject to ischemia such that the blood supply is diminished without causing atrophy, there is a hyperplasia and hypergranulation of the epithelioid cells which can, in these instances, move high enough in the interlobular artery so as to create a veritable endocrine metaplasia in segments of the normally undifferentiated arterial wall. In the controlateral kidney, on the contrary, the epithelioid cells become void of secretion grains.

Interpretation of these facts is usually made as follows: as a barosensitive zone,

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Plate XI. The macula densa. Longitudinal section of the distal convoluted tubule (TCD) through the macula densa (MD). The epithelium of the common distal tubule can be distinguished from that of the macula densa, the latter resting on the lacis (L) cells; rat on a sodium-rich diet. Magnification: ×6500.

the juxtaglomerular apparatus responds to a fall in blood pressure in the ischemic kidney by a hyperactivity, whereas an increase in blood pressure in the opposite kidney results in inhibition.

Our studies carried out on the rabbit and rat (Hatt, 1961, 1963; Hatt *et al.*, 1962, 1964) paint a more complex picture.

In the majority of cases of unilateral renal ischemia (the opposite kidney being intact), there is a hypergranulation of the ischemic organ and a degranulation in the opposite kidney. However, this hypergranulation is inconstant and subject to quite large variations depending on the degree and duration of the ischemia, on the one hand, and level of hypertension, on the other.

In acute ischemia, hypergranulation only occurs if the cortical vascular structures are preserved and results in a curious sort of cortical atrophy, where the vessels remain unscathed and apparently still perfused with blood, among degenerated and clearly nonfunctional tubular epithelium. In these cases, the most excellent pictures of hypergranulation of the epithelioid cells are to be seen.

In certain cases of segmental ischemia, hypergranulation occurs in some areas of the parenchyma, other juxtaglomerular complexes appearing normal or even showing degranulation. It is as if foci of ischemia coexisted with zones of vicariance.

In minor renal ischemia with no tubular degeneration, hypergranulation, seemingly constant in the first weeks of ischemia, can give way to a normal level of granulation after 2 months, particularly if the arterial hypertension rises high.

There does not seem to be a close parallel between the degree of granulation and blood pressure level—certain cases of marked hypergranulation not being accompanied by hypertension and acute hypertension coexisting with a strictly normal amount of granulation.

In the controlateral kidney, degranulation occurs regularly, whether or not there is arterial hypertension.

After removal of the ischemic kidney, the amount of granulation in the remaining organ tends to rise, reaching a point close to normal, whether hypertension disappears or not. Regranulation of the juxtaglomerular apparatus in metaischemic hypertension is a frequent, if not regular, occurrence.

In ischemia of a solitary kidney (after nephrectomy of the opposite organ) the level of granulation is normal or slightly decreased. It can be reduced to zero in certain cases of acute ischemia of a lone kidney, that is rapidly complicated by renal insufficiency.

The sum of these results (not counting reactivation of the juxtaglomerular complex in metaischemic hypertension which has not been studied from this point of view) is in agreement with recent results concerning the amount of renin extractable under comparable conditions (Pitcock *et al.*, 1959; Fisher, 1961; W. S. Hartroft and Hartroft, 1961; Bing, 1962a; Tobian, 1962; Regoli *et al.*, 1962a, b).

In unilateral ischemia where the opposite kidney is intact, figures corresponding to the amounts of renin in the ischemic kidney vary; some figures are about normal, others higher, having no relation to blood pressure level.

In ischemia of a solitary kidney, the amounts of renin are normal or below normal.

Under these conditions, it is no longer possible to sustain the simple diagram previously proposed. While, in classic renal ischemia, hyperactivity of the juxtaglomerular apparatus is a usual and quasi-regular phenomenon during the acute phase, it is not so beyond the third week of the experiment. Hyperactivity exists commonly in an ischemic kidney not causing hypertension and is lacking in lone ischemic kidney. Finally, in the kidney opposite the ischemia, inactivity of the juxtaglomerular complex is the rule, regardless of the arterial blood pressure. The activity returns to normal following removal of the ischemic kidney, whether or not this operation effects a fall in pressure.

As we shall see, these facts cast doubt on the barosensitivity of the juxtaglomerular apparatus and limit its responsibility *vis à vis* hypertension of renal origin.

2. *Electrolyte Disorders*

Sodium deficiency activates and excess of sodium inhibits the juxtaglomerular apparatus.

These facts, established for the first time by P. M. Hartroft and Hartroft (1953), have been well confirmed since then (P. M. Hartroft, 1963). They point toward the juxtaglomerular region as a chemoreceptive rather than a barosensitive zone.

Under the above conditions, this region reacts parallel to the glomerular zone of the adrenal cortex (Deane *et al.*, 1948; P. M. Hartroft and Hartroft, 1955), from which comes the hypothesis concerning the relationships existing between the kidney and the adrenal cortex, relationships that have been largely confirmed and detailed in recent years.

On the other hand, overdosage of potassium or deficiency in potassium does not seem to have the same effect. Excess of potassium, in particular, activates the zona glomerulosa of the adrenal cortex without significantly modifying the juxtaglomerular region (P. M. Hartroft, 1963). The latter zone, according to our experiments (administration of KCl, 25% in drinking water, for 2 months) even appears to be inhibited despite a marked stimulation of the zona glomerulosa.

In man and animals, various pathological conditions with edema—cardiac insufficiency (P. M. Hartroft, 1963) and cirrhosis of the liver in man (Reeves *et al.*, 1962), ligation of the inferior vena cava (Davis *et al.*, 1962) and aminonucleoside-induced nephrosis (Tobian *et al.*, 1962) in animals—are accompanied by a hypergranulation of the juxtaglomerular apparatus which one tends to attribute to dilution hyponatremia.

Whatever the exact mechanism of this chemosensitivity (to which we shall refer again), it seems, at present, limited to modifications in the sodium level that act simultaneously on the juxtaglomerular apparatus and the adrenal cortex. To a certain extent this distinguishes sodium disorders from other dyselectrolytemias which, like overdosage of potassium, act on the adrenal cortex without affecting the juxtaglomerular complex.

3. *Hormonal Control and Adrenal Cortex Relationships*

The role of the anterior hypophysis is a subject of much controversy. The stimulating action of ACTH (adrenocorticotropin) injections has been pleaded (Marks *et al.*, 1960) and then denied. Hypophysectomy has no effect either on the degree of granulation or on the stimulating effect of sodium deficiencies (P. M. Hartroft, 1963).

On the other hand, the links uniting the juxtaglomerular apparatus with the zona glomerulosa of the adrenal cortex are at present based on a group of perfectly coherent facts which point to the former as a regulating center of the activity of the latter, i.e., of aldosterone secretion.

Let us briefly recall that the causes of hypergranulation of the juxtaglomerular apparatus, in particular, sodium-free diets, renal ischemia, incomplete constriction of the abdominal aorta or of the inferior vena cava, are accompanied by a cytologically demonstrable hyperactivity of the zona glomerulosa and an increasing aldosterone secretion. Sodium-rich diets, on the contrary, reduce the activity of both structures (P. M. Hartroft and Hartroft, 1955).

Adrenalectomy in animals (Dunihue, 1949; Dunihue *et al.*, 1963; Kohlhardt and Voth, 1962) like Addison's disease in man (McManus, 1950), brings about a hyperactivity of the juxtaglomerular complex. Overdoses of Doca (P. M. Hartroft and Hartroft, 1953) or aldosterone (G. M. C. Masson, 1962) reduce the degree of granulation.

Davis *et al.* (1961, 1962) proved that certain experimental conditions bring about hyperaldosteronism on the condition that both kidneys are intact, bilateral nephrectomy hindering the appearance of the same.

The full significance of these observations was brought about by the demonstration of the glomerulotropic power of renin and angiotensin (Laragh *et al.*, 1960; Davis *et al.*, 1961, 1962; Carpenter *et al.*, 1961; Biron *et al.*, 1961, 1962). Everything happens as if there existed between the juxtaglomerular apparatus and the zona glomerulosa the close relationships linking any two glands (Katz *et al.*, 1962). Juxtaglomerular apparatus activates or represses adrenal cortex in direct proportion to the amount of renin produced. The latter, in return, acts on the former in relation to the amount of aldosterone secreted.

C. The Different Hypotheses Concerning the Physiological Role of the Juxtaglomerular Apparatus

1. *Does the Juxtaglomerular Apparatus Produce Renin?*

This hypothesis, postulated for the first time by Goormaghtigh, has been the object of passionate debates which are still not over.

Marshalled against this hypothesis is the evidence that renin is present in the mesonephros and metanephros of the pig embryo before the appearance of the juxtaglomerular complex (Kaplan and Friedman, 1942) and absent in the kidneys of certain fish, which possess epithelioid cells (Bohle and Walvig, 1964).

In support of such a notion are numerous experimental facts indicating that a parallelism usually exists between the amount of granulation in the juxtaglomerular apparatus and the quantity of renin that can be extracted from the kidney. The causes of hypergranulation are accompanied by an increase in the amount of renin, whereas degranulation is paired with a fall in the level of renin.

One of the most demonstrative arguments in favor of Goormaghtigh's thesis was furnished by Edelman and Hartroft (1961) who found that fluorescent anti-renin antibodies injected into the rabbit and dog were localized selectively in the cells of the preglomerular arteriole.

Cook and Pickering (1959), by magnetically isolating glomeruli impregnated *in vivo* with iron oxide and then separating out their vascular pole, showed that renin is selectively found in the latter.

Bing and Kazimierczak (1962, 1963), using microdissection, arrived at the same conclusion remarking, however, that, if renin seemed to be localized in the vicinity of the juxtaglomerular complex, it was found not in the afferent arteriole wall but in the epithelium of the distal convoluted tubule at the level of the macula densa.

Chandra, Skelton, and Bernardis (1964), using differential centrifugation of kidney homogenates, separated out a structure rich in renin and thought it to be the protein secretion granules of the epithelioid cells.

Whatever the uncertitudes on this subject, one can in any case be certain that the degree of granulation in the juxtaglomerular apparatus of a given kidney is always parallel to the amount of renin therein.

2. *Is the Juxtaglomerular Apparatus Barosensitive or Chemosensitive?*

The notion of barosensitivity stems from the behavior of the juxtaglomerular complex in hypertensive renal ischemia, the ischemic organ being hyperactive and the opposite kidney displaying hypoactivity.

Perfusions of isolated kidneys (Tobian *et al.*, 1959) seem to confirm this concept according to which the blood pressure existing in the afferent arteriole regulates the activity of the epithelioid cells.

Opposed to this hypothesis are several experimental facts which show that the juxtaglomerular complex activity is modified without apparent change in the renal perfusion pressure. As we have seen, hypergranulation occurs following sodium-deficient diets or adrenalectomy. Hypogranulation or degranulation appears during sodium-rich diets, overdosage of Doca, and in the kidney opposite an ischemia, even in the absence of arterial hypertension. Regranulation in a clipped kidney occurs during metaischemic arterial hypertension (Hatt *et al.*, 1964).

Therefore the notion of chemoreceptivity, maintained by P. M. Hartroft in 1963, whereby it is the level of sodium which regulates the juxtaglomerular complex, implies that an excess of sodium has an inhibiting effect, whereas a deficiency has a stimulating influence.

Here again, it is necessary to agree on the significance of these modifications in sodium content. How are these modifications to be transmitted?

We have seen that, by their location, the epithelioid cells can receive information from both sides, from the blood circulating in the afferent arteriole, on the one hand, and from the urine flowing in the distal convoluted tubule across the macula densa and the lacis, on the other.

If the theory of chemosensitivity is accepted, it should be admitted that the epithelioid cells are extremely sensitive with respect to changes in the blood plasma, for electrolyte variations in the blood are almost nil under several conditions during which important modifications in the activity of the juxtaglomerular complex are observed.

The epithelioid cells, however, could be sensitive to electrolyte changes occurring in the urine of the distal tubule, whose composition, unlike that of the plasma, should change, according to the theory of chemoreceptivity (cf. Ullrich *et al.*, 1963). An excess of circulating sodium increases sodium excretion and a deficiency decreases it.

It can even be wondered whether changes in renal blood pressure do not act indirectly on the juxtaglomerular apparatus via the repercussions such changes have on the composition of urine in the distal tubule. Renal ischemia diminishes sodium excretion and intravenal hypertension increases it.

Undoubtedly, we must wait to increase, correct, and extend our knowledge concerning the composition of urine in the distal tubule under different experimental conditions.

Many questions remain, which cannot be answered without a thorough structural and histochemical (Gomba *et al.*, 1962, 1963) understanding of this apparatus. These facts, however, lead us to believe that the juxtaglomerular apparatus plays an important role in local regulation of the electrolytes.

3. *Its Role in Arterial Hypertension*

The work of Goormaghtigh (1932, 1936, 1942; Goormaghtigh and Grimson, 1939) and Elaut (1934) has given a histological foundation to the humoral theory of arterial hypertension elaborated by Goldblatt *et al.* (1934) by showing the juxtaglomerular apparatus as the site of renin elaboration and its hyperactivity as the cause of hypertension, owing to the large quantities of renin put in to the circulatory system.

Without considering in detail the contradictory opinions on this subject (cf. Oberling and Hatt, 1960b) and in order to limit discussion to the histological behavior of the juxtaglomerular apparatus, the actual status of the question can be summed up as below.

a. *Experimental Hypertension.* In experimental hypertension (cf. Hatt *et al.*, 1964), only renal ischemia (Goldblatt type) is accompanied by hyperactivity of the juxtaglomerular apparatus with the following reservations. (1) In the ischemic kidney, it exists regularly only in the acute period of unilateral ischemia; (2) there is no direct relationship between its extent and the level of arterial blood pressure; certain ischemias, accompanied by a marked hypergranulation, do not result in hypertension;

(3) hyperactivity is lacking in a number of cases of hypertensive chronic renal ischemia; (4) hyperactivity does not exist in ischemia of a lone kidney.

Among other experimentally induced hypertensive nephropathies, perinephritis (Page type) is the source of contradictory observations. According to Dunihue (1941), it is accompanied by a temporary hyperactivity of the juxtaglomerular apparatus, whereas our observations reveal a precocious degranulation (Hatt, 1963).

With overdosage of salt and Doca, as in hypertension due to adrenal regeneration, it is unanimously agreed that the juxtaglomerular apparatus exhibits a total degranulation (P. M. Hartroft and Hartroft, 1953; Tobian *et al.*, 1958; Hatt *et al.*, 1963, 1964; Rapp, 1964; Verniory and Potvliege, 1964).

The sum of these facts, coupled with data concerning levels of renin (Gross, 1960, 1964; Bing, 1962a; Regoli *et al.*, 1962a, b; Gross *et al.*, 1964), shows that neither the juxtaglomerular apparatus nor the renin-angiotensin combination can necessarily be considered part of the mechanism of hypertension.

One of Bing's (1962b) recent experiments makes this fact perfectly evident by showing that rats given overdosage of salt and Doca have a very low level of renin in their kidneys. Some are moderately hypertensive, others are normal. In these rats, clipping of the renal artery elicits a hypertension whose level and evolution can be superimposed on that of the hypertension appearing after renal ischemia in control animals who had not been previously treated with salt and Doca. Whatever level of renin there is in the kidney, ischemia evokes the same hypertension.

b. *Hypertension in Man.* Concerning man, the most discordant observations have been published. For some (Weiss and Parker, 1939; Graef, 1940; Kaufman, 1942; de Muylder, 1945, 1948; Des Prez, 1948; Mayer, 1952; Turgeon and Sommers, 1961; Crocker *et al.*, 1962; Itskovitz *et al.*, 1963) hyperactivity of the juxtaglomerular apparatus is the result of numerous hypertensive nephropathies running the gamut from ischemia (Goldblatt type) to pyelonephritis and toxemia of pregnancy. For others, hypergranulation of the juxtaglomerular apparatus serves as a prognostic test in the surgery of unilateral hypertensive nephropathies. The removal of a kidney containing such hypergranulation effects a cure of the hypertension more often than does the removal of a kidney devoid of hypergranulation. (Crocker *et al.*, 1962; Itskovitz *et al.*, 1963). For still others, on the contrary (Oberling, 1944; Bohle, 1954; Pitcock and Hartroft, 1958), the juxtaglomerular apparatus is more often impaired than hyperactive.

For the moment, therefore, it is difficult to be definitive. It nevertheless seems impossible to reconcile such discordant facts within the simple framework proposed by Goormaghtigh. If the juxtaglomerular apparatus does play a role in hypertension via the secretion of renin, this mechanism can be considered only in very special cases of renal ischemia. This role is neither exclusive nor mandatory, for numerous cases of arterial hypertension, whether they be of renal origin or not, seem to be independent of it.

Finally, one cannot exclude the fact that, even in cases of hypertensive renal

ischemia where the juxtaglomerular apparatus is hyperactive, it plays only an accessory role in the hypertension and is essentially dependent on intrarenal commands made by the electrolyte balance.

4. *Its Role in Electrolyte Balance*

The juxtaglomerular apparatus can exert its power in at least two ways: by the intermediary of the control that it has on aldosterone secretion through the adrenal cortex and by direct action on the kidney.

a. *The Links between the Juxtaglomerular Apparatus and Adrenal Cortex.* Within the framework of sodium exchange, the function of the juxtaglomerular apparatus is clear enough. A deficiency in sodium stimulates renin secretion, which increases the secretion of aldosterone from the adrenal cortex, which, in turn, has an inhibitory effect on the excretion of sodium. Inversely, an excess of sodium inhibits renin secretion, which results in hypoaldosteronism and an increase in sodium excretion.

If the coming into play of this system can be considered physiological and useful, since the electrolyte disturbance is both primary and solely responsible, it can also be considered, in certain cases, to enter into vicious pathological circles.

It is a result of hyperaldosteronism that one encounters edematous syndromes of cardiac deficiency and cirrhosis in which the sodium deficiency is linked to a hyponatremia by dilution and where the adrenal demand tends to worsen the water-sodium retention.

This is also the case in hyperaldosteronism secondary to renal ischemia whose aggravating character is indicative of the pathological nature of the disease.

All this happens as if, adapted to the peculiar conditions which effect electrolyte disturbances by excess or deficiency of sodium, the functioning role of the juxtaglomerular apparatus no longer occurs when commanded by an unexpected pathway.

Under these circumstances, it seems that the ties existing between the juxtaglomerular apparatus and the adrenal cortex function in only one direction, i.e., it is the hyperactivity of the former which evokes a similar reaction in the latter.

Inversely, there are conditions where hyperaldosteronism, when linked to a direct stimulation of the zona glomerulosa, can act as a break on the juxtaglomerular complex. This is notably the case in potassium-rich diets and, undoubtedly, also in the Conn type of primary hyperaldosteronism, like experimental overdosage of Doca and aldosterone.

b. *Direct Renal Action.* This action is poorly understood. The only facts worth mentioning are as follows.

(1) Action of renin and angiotensin on renal circulation: Both these hormones are vasoconstrictors and reduce the renal blood flow without our knowing exactly to what extent or whether they modify the relative distribution of cortical and medullary blood flow (cf. Page and Bumpus, 1962; Gross, 1963).

(a) Action of renin on the tubular reabsorption: Whether it acts directly on the tubule or indirectly by causing a constriction of the efferent arteriole, renin seems to

reduce reabsorption in the proximal as well as in the distal convoluted tubule (Peters, 1962; Vander, 1963).

Here again there are many question marks: the meaning of the different behaviors of the hypertensive and normotensive subjects regarding renal excretion of sodium when undergoing angiotensin perfusion (Biron *et al.*, 1962; Brown and Peart, 1962); the connections between the activity of the juxtaglomerular complex, the level of renin in the kidney, and sodium excretion (Brunner *et al.*, 1962); the relationships which exist between glomerular filtration, proximal reabsorption, and the activity of the juxtaglomerular apparatus (Leyssac, 1963a, b, 1964a, b).

5. *Conclusion*

At present, the place of the juxtaglomerular apparatus in the sodium balance can be defined as an intermediary between the kidney and the adrenal cortex; the major role in the maintenance of sodium balance is finally assigned to aldosterone.

Does the juxtaglomerular apparatus function in other kinds of electrolyte balance? Does it intervene directly in the mechanisms of excretion and reabsorption by the intermediary action of renin on renal circulation? It is perhaps in the latter direction rather than in that of the hypertensive role that we should seek justification for the vasoconstrictive properties of the renin-angiotensin system.

V. Summary

After briefly considering our knowledge of the structure and function of the juxtaglomerular apparatus as it appeared after light microscope study, we have reviewed the further precisions and corrections made possible by the electron microscope.

The intimate relationships existing between the hilus of the glomerulus and the macula densa have been confirmed.

The myoepithelioid cells of Ruyter and Oberling are indeed endowed with all the attributes of an active secretory system. The conditions for and ultrastructural appearance of this activity have been studied.

The macula densa of Zimmermann appears to be a banal epithelium, devoid of differentiation which would allow us to consider it in the role of a nervous plate. It seems to have a mild reabsorptive activity and has a notable particularity—the spacing of its cells which allows the urine to come in direct contact with the glomerular hilus.

The lacis consists of that portion of the Polkissen of Zimmermann described by Goormaghtigh under the name of "pseudo-Meissnerian cells." It is a mass of relatively undifferentiated cells with no structural resemblance to nerve cells, which are entangled in a dense network of richly anastomizing basement membranes. These cells are identical to the mesangium cells which appear to be an intraglomerular extension of the lacis.

The physiological significance of the juxtaglomerular apparatus has been discussed

with particular attention to its role in renin production, its barosensitive or chemosensitive nature, and its function in the mechanism of arterial hypertension of renal origin and in electrolyte balance.

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ULTRASTRUCTURAL PATHOLOGY OF THE GLOMERULUS

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I. Introduction

The widespread use of percutaneous renal biopsy and the feasibility of repeated sampling have been of immense value to the detailed study of the fine structure and morphogenesis of glomerular lesions. Biopsies have been most frequently performed

in conditions associated with diffuse glomerular disease where the relatively small sample can be considered representative of the underlying condition. The fresh material obtained by biopsy is more suitable for electron microscopic studies than tissue secured several hours post mortem although, with the newer techniques of fixation and embedding, considerably more information can now be obtained from autopsy material than was heretofore possible. It should be noted, however, that most of the ultrastructural studies of renal pathology reported in the literature have been done on biopsy material.

In the organization of this chapter, considerable emphasis has been given to the group of clinical disorders which can be most conveniently considered under the term chronic Bright's disease. Owing to the large amount of information on this subject, both the clinical and the experimental aspects of all these disorders have been considered and discussed in the same section to facilitate correlation. Owing to the heterogeneous nature of the other glomerular lesions discussed in subsequent sections and the fact that much of the experimental work pertaining to them has been directed toward the elucidation of specific problems, it was found more convenient in considering each entity to discuss the human and experimental data together.

The last section has been devoted to the vascular changes in hypertension even though these changes are often not considered part of glomerular pathology. The glomerular capillaries, however, constitute an arteriolar portal system and can advantageously be considered in conjunction with the pathology of the renal arterioles. Furthermore, it has recently been shown that granular changes in the juxtaglomerular and mesangial cells may be very similar, and the suggestion has been made that the latter constitute a special variety of smooth muscle cell.

In this review, the pathology of the mesangium has been selected for special comment on account of its controversial nature and relatively recent acceptance as a distinct glomerular entity. Prior to 1962, most investigators studying the ultrastructure of normal and diseased glomeruli agreed that the classical concept of the mesangium was erroneous and that the cells seen in the core of the glomerulus were, in fact, endothelial in nature. As more studies of human and experimental material became available and as techniques improved, it became evident that the functional pathology of the glomerulus could be best explained by considering the mesangium as a special structural and functional unit within the glomerulus. Furthermore, since many physiopathological data are essential for an understanding of the ultrastructural pathology of the glomerulus, and to avoid repetition we shall precede our detailed discussion of glomerular lesions by a brief introduction on the physiopathology of this structure with particular emphasis on the role of the mesangium.

The selection of photographs and diagrams has been made to illustrate points discussed in the text. This selection of illustrations was only possible as a result of the generosity and cooperation of several authors, to all of whom we are extremely grateful.

II. Physiopathology and Ultrastructure of the Diseased Glomerulus

A. Physiopathology

1. *Normal Filtration*

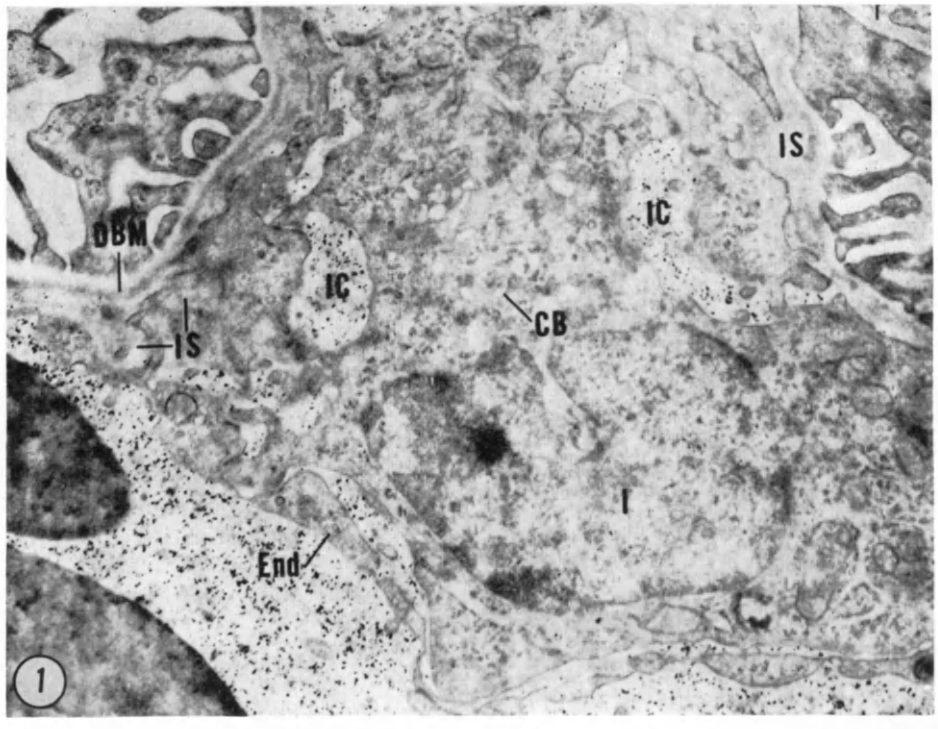
Although the normal structure and function of the glomerulus has been discussed in another chapter in this volume those points which are particularly pertinent to a clear understanding of glomerular ultrastructural pathology will be briefly reviewed.

Our knowledge of the filtration process has been clarified by the use of particles possessing high electron-scattering capacity (Figs. 1–3). As a result of these studies (Farquhar and Palade, 1961, 1962; Farquhar *et al.*, 1961; Farquhar, 1964; Latta *et al.*, 1960; Kurtz and Feldman, 1962b; Kawamura, 1961; Vernier and Birch-Andersen, 1963), specific functions have been ascribed to the four components of the glomerular tuft.

The *endothelium* acts as a coarse mesh filter, permitting and perhaps regulating the access of plasma to the basement membrane by varying the number and size of its fenestrae. This particular function has been little studied under normal or pathological conditions. Occasional references to changes in the size of these fenestrae have been made (Feldman *et al.*, 1963; Brown *et al.*, 1963), but no systematic study of this problem has been reported.

The *lamina densa* of the basement membrane is the main barrier to the passage of medium-sized molecules. Farquhar *et al.* (1961) have shown that in rats ferritin molecules pass readily through the endothelial pores and accumulate in the subendothelial space and mesangial matrix (Fig. 2). Latta *et al.* (1960) found that thorotrast particles less than 90 Å pass through the basement membrane, whereas larger ones behaved like ferritin. In normal rats, Farquhar *et al.* (1961) showed that a few ferritin molecules also accumulate in the subepithelial space, thus indicating the imperfect nature of the filtration barrier. In animals rendered nephrotic by aminonucleoside, these particles readily pass through the lamina densa and are incorporated into the epithelial cells (Fig. 3). These findings are interpreted as evidence that the lamina densa is the main filtration barrier, albeit an imperfect one, for medium-sized molecules. It is possible that there may be several different mechanisms by which particles pass through the glomerular filter. Small molecules, such as electrolytes, glucose, and urea may readily pass through the molecular lattice, whereas slightly larger molecules, such as ferritin, may be retained by the lamina densa because they are too large to pass readily between the intermolecular spaces of the basement membrane. Still larger particles, such as globin, may as a consequence of greater molecular pressure or area of contact, produce liquefaction of the membrane at the site of contact if, as Menefee *et al.* (1964) suggest, the membrane behaves as a thixotropic gel.

The *epithelial cells* may absorb and metabolize molecules passing through the lamina densa. The reactivity of the epithelial cells and the conditions in which large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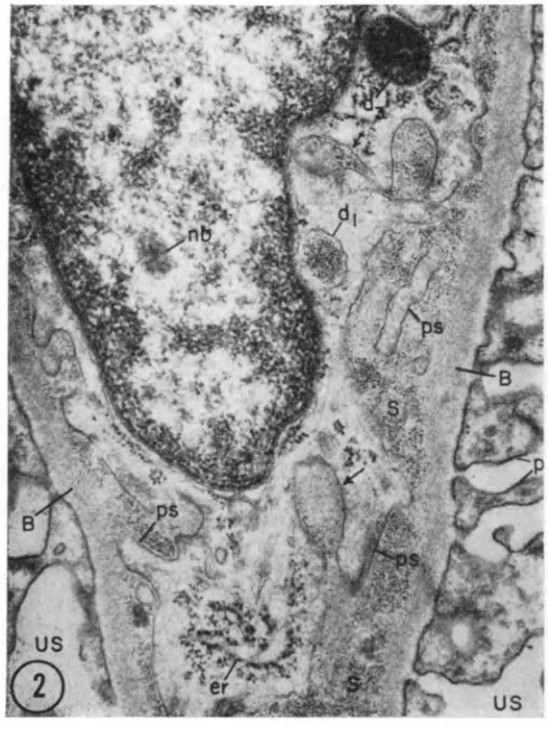
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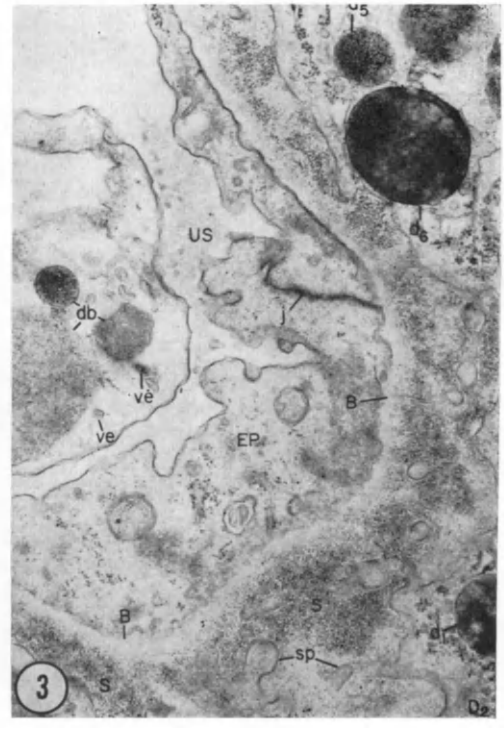
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molecules pass through the basement membrane have led Farquhar *et al.* (1961) and Farquhar and Palade (1961) to postulate that these cells normally have a regulative function and that they behave as monitors to the basement membrane. In their view, enlargement of the foot processes, conversion of desmosomes into tight junctions, and increased pinocytic activity may act to check excessive loss of protein and perhaps other plasma constituents through the defective basement membrane.

The *mesangium* removes filtration residues and thus prevents clogging of the basement membrane. The function of the mesangium in filtration has been greatly clarified by studies in which tracer particles were injected into the circulation. Latta *et al.* (1960) have shown that thorotrast particles, several hundred Angstroms in

FIG. 1. Centrolobular region after Thorotrast injection. Intercapillary channels (IC) are filled with Thorotrast particles within 10 minutes after injection. Little intercellular substance (IS) is seen in this section, in contrast to larger amounts seen in other sections. Few particles penetrate the denser parts of intercellular substance. Embedded in Epon. Magnification: ×14,000. From Latta *et al.* (1960).

FIG. 2. Deep cell in a normal rat 1 day after ferritin administration. Ferritin molecules literally fill the spongy areas between the basement membrane and the deep cell, whereas their concentration falls off sharply in the basement membrane proper where they occur at relatively low concentration. The contours of the deep cell appear ruffled owing to the presence of numerous processes (ps) of varied form. The spongy material packed with ferritin fills the space between the processes; some (arrows) deeply indent the cytoplasm and are connected to the cell surface by a narrow channel. Within the deep cell cytoplasm a whole spectrum of dense bodies can be seen: from some (d_1), in which the ferritin appears at the same concentrations as in the extracellular deposits, to others in which the tracer is markedly concentrated (d_3), suggesting that the ferritin residues incorporated by the deep cells undergo progressive condensation within these bodies. The fact that some of the spongy masses deeply indenting the deep cell cytoplasm are partially free of ferritin suggests that the spongy material is incorporated in a manner similar to the ferritin residues. Note that in many places a zone of 100 Å along the surface of the cell membrane or along the membrane bounding the phagocytic pockets is free of ferritin. The bodies marked (nb), frequently encountered in the nuclei of deep cells, are distinct from nucleoli; their significance is unknown. Magnification: ×30,000. From Farquhar and Palade (1962).

FIG. 3. Axial region of a glomerulus in a nephrotic rat 12 hours after ferritin administration, showing massive deposits of the tracer in the spongy areas of an axial region and segregated within numerous large dense bodies of heterogeneous composition (d_1, d_5, d_6) in the cytoplasm of a deep cell. Two deep cells showing numerous complicated cytoplasmic processes are seen in the upper and right corners. A continuous epithelial layer interrupted only by a single cell junction (j) is seen to the left covering the outer surface of the basement membrane. Note that while the ferritin seems to penetrate freely the relatively loose spongy areas, its concentration falls off sharply at the level of the basement membrane proper (B). Several small vesicles (ve) and dense bodies (d) containing ferritin are also present in the epithelial cytoplasm.

Note also the heterogeneity of the dense bodies in the deep cell as well as those in the epithelium. The tracer concentration varies not only from one dense body to another but also within the same dense body (e.g., d_1, d_6). Moreover, in many of these there are masses of material partially or completely free of ferritin. The findings suggest that such composite or heterogeneous bodies arise as a result of fusion of absorption droplets of different ages, some of them antedating the incorporation of the tracer. Magnification: ×18,500. From Farquhar and Palade (1962).

diameter, have ready access to mesangial cells through intercapillary channels (Fig. 1). On this basis, they postulated that mesangial cells would be capable of reacting quickly to changes in plasma content and perhaps regulating capillary flow. Farquhar and Palade (1962) (Figs. 2 and 3) concluded from their work that the mesangial cells were responsible for removing filtration residues and degraded basement membrane, thus continuously unclogging and reconditioning the main barrier to filtration. The studies of Menefee *et al.* (1964) (see also chapter by Menefee and Mueller in this volume), using large globin molecules, did not suggest an active role of the mesangial cell although some accumulation of particles was noted in the "sponge matrix" and "stalk" cells (mesangium).

2. *Proteinuria*

Proteinuria has long been regarded as the most consistent abnormality in impaired glomerular function. Consequently, many investigators have studied the ultrastructural defects associated with this condition in both human and experimentally produced glomerular disease in an attempt to establish the morphological basis of proteinuria (Ellis, 1958, 1959; Post, 1960; Ashworth and James, 1961; Anderson and Recant, 1962). The structural alterations in the epithelial cells accompanying proteinuria were found to be similar regardless of the etiology and to resemble closely the changes seen in human disease characterized by the nephrotic syndrome. Fusion of the foot processes of the epithelial cells with the formation of clear vacuoles and hyaline droplets in these cells has been the earliest and most consistent abnormality noted. Figure 3 and its legend sum up the changes seen in the glomerular capillaries in cases with severe proteinuria.

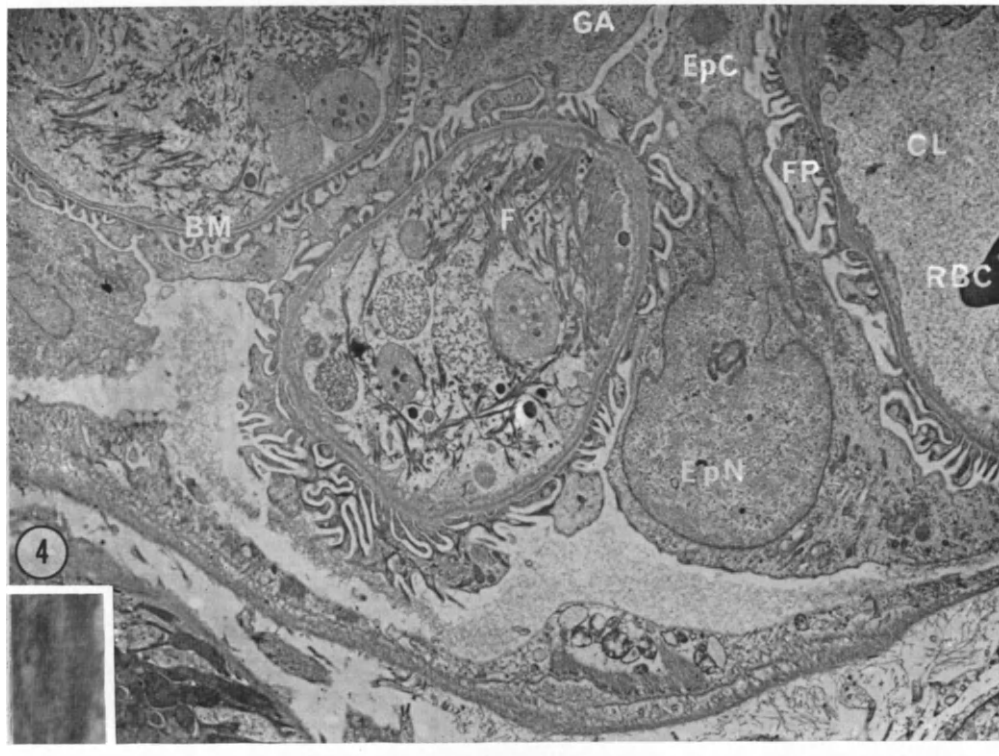
Until Vernier (1961a, b) demonstrated fusion of the foot processes in dogs following rapid infusion of canine serum albumin, it was not clear whether the epithelial cell changes were the cause or result of the proteinuria. Subsequent workers (Ashworth and James, 1961; Anderson and Recant, 1962; Fisher and Hellstrom, 1962; Fisher and Klein, 1963; Fisher *et al.*, 1964) have directly or indirectly confirmed Vernier's concept that proteinuria per se in the absence of renal disease may result in fusion of foot processes. It is now generally agreed that the changes in the epithelial cells result from an increase in the amount of protein passing through the basement membrane and reflect increased absorption and degradation of such protein.

At the present time, there is no unanimity of agreement among investigators attempting to explain the morphological changes in the basement membrane responsible for increased permeability. This is not surprising when we consider the difficulties encountered by electron microscopists in their attempts to elucidate the fine structure of the basement membrane (Yamada, 1955; Bergstrand and Bucht, 1958; Sitte, 1959; Kurtz and McManus, 1960; Farquhar *et al.*, 1961; Farquhar, 1964; Menefee *et al.*, 1964; Hall, 1965). Holes up to 100 Å in diameter have been described in the basement membrane (Spiro, 1959, 1960; Spiro *et al.*, 1961) and were believed to represent the basic defect responsible for proteinuria. These observations have not been confirmed by subsequent workers (Farquhar and Palade, 1961; Movat,

1960a). In both experimental and clinical conditions characterized by acute proteinuria, Movat *et al.* (1961a, b) noted swelling and loosening of the basement membrane (quellung) with imbibition of the plasma constituents. Their findings and interpretations are in accord with Sitte's (1959) postulated mechanisms of abnormal filtration whereby the passage of large molecules through the basement membrane requires a loosening of its lamellar structure. It is not inconceivable that such a mechanism might operate through a basement membrane which had the characteristics of a thixotropic gel as postulated by Menefee *et al.* (1964).

The relation of hyperproteinemia to glomerular changes was emphasized by Fisher and Hellstrom (1962). These investigators showed that rats infused with subthreshold and threshold quantities of homologous and heterologous protein developed fusion of foot processes and other changes characteristic of proteinuria in the absence of proteinuria. Similar changes were described in patients with multiple myeloma (Fisher *et al.*, 1964) in whom no proteinuria was demonstrated. In both reports the morphological changes were attributed to the hyperproteinemia present. The authors were unable to identify any specific defect in the basement membrane despite the fact that it was twice the normal thickness in multiple myeloma and that increased glomerular permeability without detectable changes in the basement membrane was present in the rats infused with globulin and bovine serum albumin (BSA) but not in those receiving homologous albumin. The increased thickness of the basement membrane in multiple myeloma as opposed to the normal appearance in the experimental animals was attributed by Fisher *et al.* (1964) to the prolonged course of the clinical disease in contrast to the acute nature of the experimental condition. It is of interest that Fisher and Klein (1963) were unable to detect structural alterations in the basement membranes of aminonucleoside-treated rats in which the nephrotic syndrome was markedly reduced by the concomitant administration of adenine even though significant proteinuria persisted. Ferritin clearance studies in these animals resulted in significantly less extraluminal deposition of the marker in the aminonucleoside plus adenine-treated rats. This observation, together with previous knowledge of glomerular epithelial changes in proteinuric states, is consonant with the contention that aminonucleoside exerts its toxic effect on the lamina densa of the basement membrane despite the failure to discern significant ultrastructural alteration in the lamina densa.

The experiments of Fisher and Hellstrom (1962) introduce another important facet in glomerular physiopathology which has not yet been adequately explored, i.e., the specific effect of changes in the concentration and biochemical nature of the circulating proteins on the glomerular morphology and function. These authors found that rats infused with 0.8 gm of homologous globulin exhibited intracapillary fibrin deposition (Fig. 4) and gross hematuria. Similarly, Arhelger and Langford (1965) have induced lesions resembling nephrotoxic serum nephritis or the generalized Shwartzman reaction in rabbits infused for several hours with angiotensin. The authors felt that these lesions resembled those reported by Vassalli *et al.* (1963b) following liquoid administration (Fig. 5).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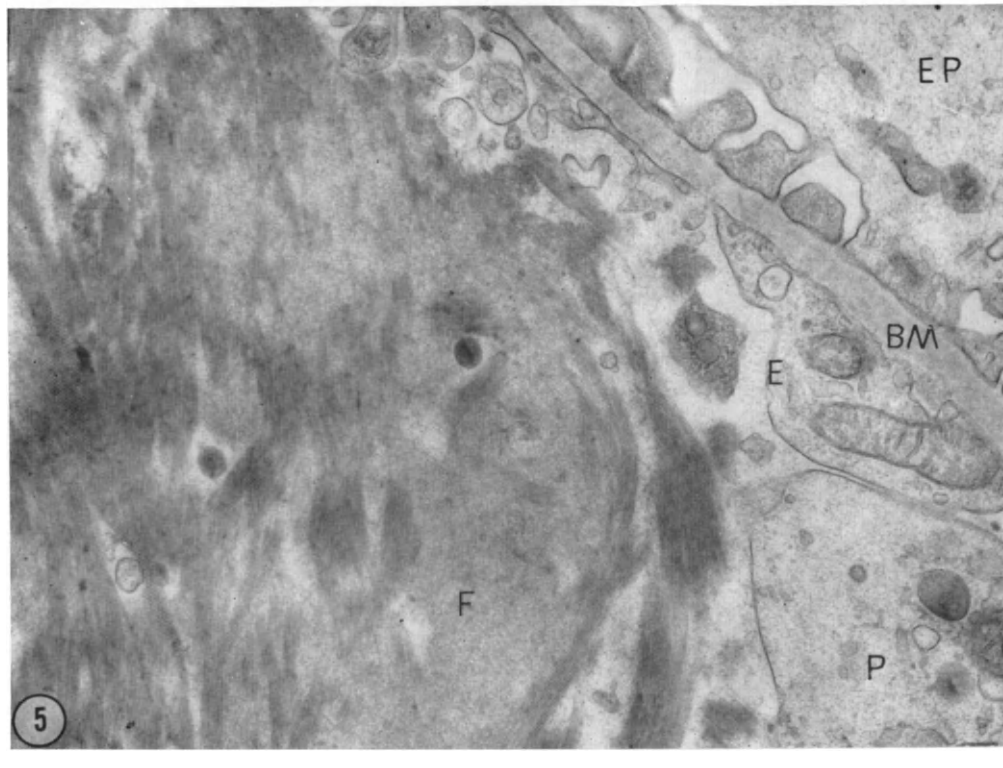
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In contrast with previous observations, Kurtz and Feldman (1962b) have induced massive proteinuria with only minimal changes in the epithelial cells in rats given intraperitoneal injections of BSA. Similar results were reported by Karl *et al.* (1964) in rats receiving intraperitoneal injections of homologous albumin. In addition, these latter investigators, on the basis of extensive enzymatic studies in rats, concluded that protein loss through the kidney rather than a specific biochemical lesion in the nephrotic state is responsible for decreased alkaline phosphatase activity and increased glucose-6-phosphate dehydrogenase observed in nephrosis.

In a recent paper, Miller and Palade (1964) have contributed a detailed electron microscopic, cytochemical study of the changes associated with the lytic activities of the lysosomal complex in the glomerulus during the passage of normal and excessive quantities of marked proteins across the glomerular capillary wall. These authors found lysosomal enzymatic activity in the same membrane-bounded structure which incorporated the exogenous proteins. They also remarked that the mesangial cells contained the largest number of lysosomal bodies. In addition, they made the interesting observation that in nephrotic rats, the ferritin molecules appeared free in the cytoplasmic matrix of mesangial cells within 30 minutes after injection.

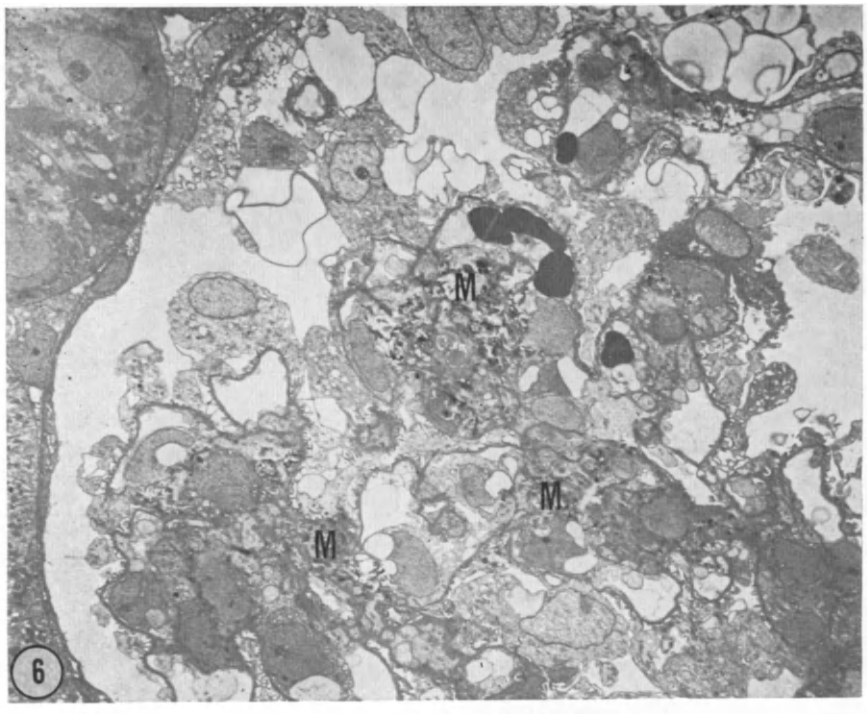
From this brief review of the literature, it is apparent that there are large gaps in our knowledge of the mechanisms of proteinuria and its relationship to ultrastructural changes. The work of Miller and Palade (1964) indicates the value of combining electron microscopy with cytochemistry, and it is to be hoped that in the future other workers will pursue the study of biochemical and physiological changes at the ultrastructural level using the sophisticated techniques currently available.

3. *The Pathology of the Mesangium*

Prior to 1952, the mesangium was a site of great interest to pathologists although the exact function of this tissue in health and disease was not known. Following the advent of early electron microscopic papers denying its existence, pathologists tended to ignore the mesangium and rather concentrated on lesions whose location was undisputable, such as those seen in the epithelial or endothelial cells and in the basement membrane. Over the past 5 years, a large amount of information has gradually been accumulated on the fine structure and functional pathology of the mesangium. In 1959, collagen was induced selectively and in large amounts in this region by

FIG. 4. Portion of glomerulus from rat sacrificed immediately after infusion with 0.8 gm of homologous globulins, revealing intracapillary fibrin deposition (F). Epithelial cytoplasm (EpC) is increased and there is focal blunting and swelling of foot processes (FP). Epithelial nucleus (EpN) is more bizarre than observed in saline control. CL, Capillary lumen; RBC, red blood cell; BM, basement membrane or lamina densa. Magnification: ×6600. From Fisher and Hellstrom (1962).

FIG. 5. Portion of glomerular capillary loop from a rabbit infused with angiotensin for 13 hours prior to biopsy. The lumen is filled with dense material composed of granular fibrinoid (F) and fibrin with its characteristic periodicity. P, platelet; E, endothelium; BM, basement membrane; EP, epithelium. Magnification: ×19,000. Courtesy of Dr. R. B. Arhelger and Dr. H. G. Langford.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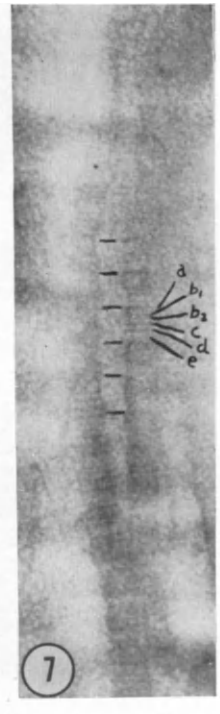
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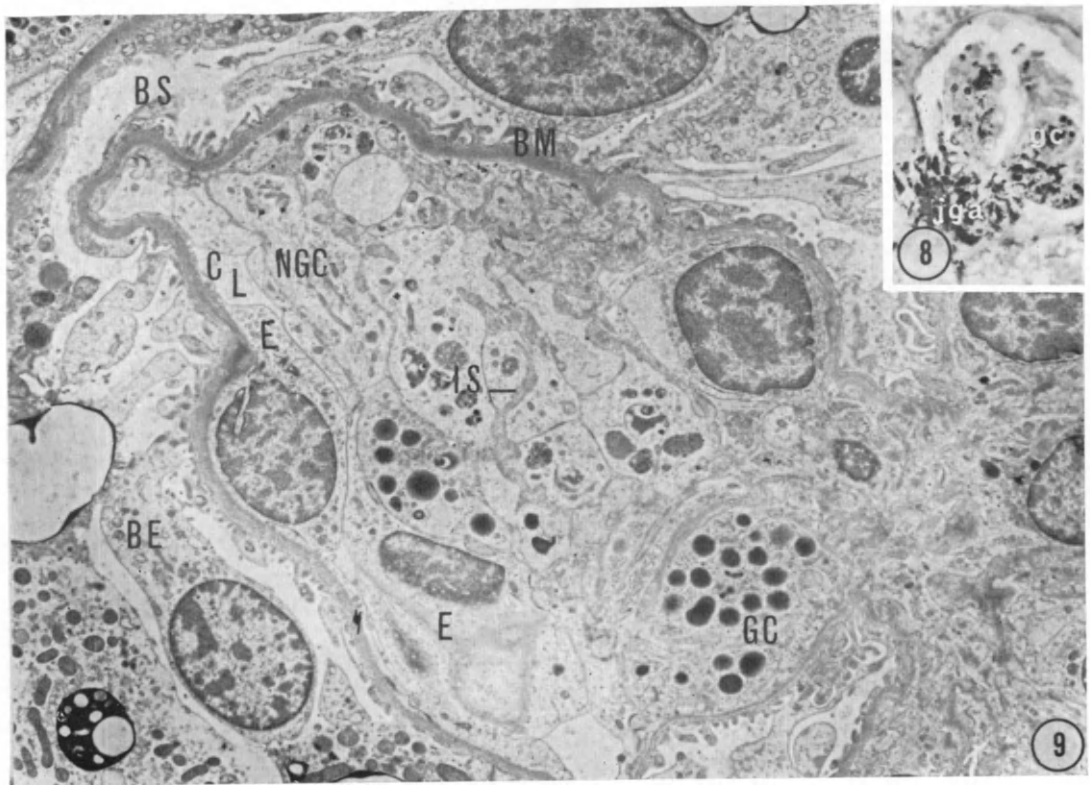
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Bencosme *et al.* (1959) in rats receiving a single injection of uranium nitrate (Figs. 6 and 7). The presence of collagen in the mesangium was subsequently described in the normal frog (Yamada, 1960), in the rat (Latta, 1961), and in diseased glomeruli (Habib *et al.*, 1961; Suzuki *et al.*, 1963; Dachs *et al.*, 1964). The presence of collagen fibers in the mesangial matrix in normal glomeruli and the increased amounts found in this area in disease clearly indicated that the mesangium was anatomically and functionally different from the rest of the glomerular structures. This contention was further supported by the ability of Habu snake venom to selectively lyse mesangial cells as reported by Sakaguchi and Kawamura (1963) and Suzuki *et al.* (1963) who described the electron microscopic changes of this lesion. Two hours after injection of the venom, the mesangial matrix was edematous and mesangial cells were still present. After 8 hours, mesangial cells were usually completely degenerated and the mesangial matrix dissolved. Within 10 hours there was dissolution of the mesangium and conversion of the glomerulus into a blood-filled cavity surrounded by basement membrane and epithelial cells. This experiment emphasizes not only the topographical and structural differences between the mesangium and other glomerular structures but also their different reactivity to injurious agents. Sakaguchi and Kawamura (1963) believed that these experiments also indicated the important supporting function of the mesangium.

Based on purely anatomical considerations, several authors (Yamada, 1955; Latta *et al.*, 1960; Huhn *et al.*, 1962; Michielsen, 1961) believe the mesangial cells to be modified smooth muscle cells. In addition to a supporting role, they have attributed a contractile function to this structure which would exert some degree of control over glomerular blood flow. The works of Hatt *et al.* (1963) and Dunihue and Boldosser (1963) further support the smooth muscle nature of mesangial cells. Hatt and collaborators showed that in rats in which deoxycorticosterone hypertension had been induced, the mesangial cells underwent alterations similar to those occurring at the same time in the smooth muscle cells of preglomerular and afferent arterioles. In

FIG. 6. Mesangial lesion, uranium poisoning. Section from a rat killed 6 days after subcutaneous injection of uranyl nitrate. The mesangial lesion is characterized by dark irregular deposits of variable density (M). Under higher magnification these deposits appear to be rich in collagen fibers (see Fig. 7).

FIG. 7. Collagen fibers in a centrolobular lesion. Five and sometimes six subperiods can be identified. Six days after injection of uranium. Magnification: ×120,000. From Bencosme *et al.* (1959).

FIG. 8. Photomicrograph of glomerulus from mineralocorticoid-deficient cat showing morphological continuity of juxtaglomerular apparatus granular cells with similar cells within the glomerulus. jga, Juxtaglomerular apparatus; gc, granular cell. Bowie's stain. Magnification: ×480. From Dunihue and Boldosser (1963).

FIG. 9. Electron micrograph of glomerular capillary loop from a cat deficient in mineralocorticoid for 9 months. The capillary lumen (CL) is almost occluded by granular (GC) and nongranular (NGC) mesangial cells. BS, Bowman's space; BM, basement membrane; E, endothelium; IS, intercellular substance. Lead hydroxide. Magnification: ×4500. From Dunihue and Boldosser (1963).

these animals, both the smooth muscle cells and the mesangial cells became edematous, and the basement membrane-like material surrounding these cells showed hyaline change.

Dunihue and Boldosser (1963) made the interesting observation that mesangial, medial, and juxtaglomerular cells all responded to mineralocorticoid deficiency (adrenalectomized cats) by hypertrophy, hyperplasia, and development of cytoplasmic granules. These granules were similar in staining reaction and ultrastructure to those of the granular cells of the juxtaglomerular apparatus (Figs. 8 and 9). The authors suggested that certain dense bodies seen in mesangial cells (Fig. 9) probably represent different phases in the life cycle of these granules. In a recent report on lipofuscin-like granules in vacuolar smooth muscle and juxtaglomerular cells of human kidneys, Biava and West (1965) have discussed in detail the structure, distribution and pathological variation of these granules. From their work, one wonders whether the "dense bodies" seen by Dunihue and Boldosser (1963) in the mesangial cells of adrenalectomized cats are not similar to the lipofuscin-like granules described by Biava and West (1965). For more information on this subject, see Section II, M. Dunihue and Boldosser (1963) commented on the multiplicity of functions attributed to the mesangial cells and suggested the possibility that the mesangium may be constituted by a heterogeneous cell population. It is to be hoped that in future work, immunohistochemical studies with anti-renin (Hartroft, 1963) will be used to clarify the role of these cells in renin secretion.

One aspect of mesangial cytopathology which has not been much explored and is poorly understood is the function of the pseudopods which the mesangial cells send through the endothelial cytoplasm into the capillary lumen. Although extension of mesangial cytoplasm into the glomerular capillary lumen was well recognized by Zimmermann (1933) and has subsequently been confirmed by electron microscopy (Yamada, 1955; Farquhar and Palade, 1962; Huhn *et al.*, 1962), its significance both in normal and pathological conditions is still unknown. The fact that the pseudopods increase in many pathological conditions indicates that these structures probably have an important role in the pathological physiology of the glomerulus. It is of interest with respect to the possible muscular origin of the mesangial cells that Hatt *et al.* (1962), working with Goldblatt hypertensive kidneys, have described swollen cytoplasmic projections of the smooth muscle cells in the interlobular arteries which also extended into the lumen of the vessel and closely resembled the mesangial pseudopods.

With present day knowledge of the physiological action of the mesangial cells in "unclogging" the glomerular filter, it seems that most mesangial lesions are the result of "clogging" of the filter mechanism by an abnormal amount of deposited material, either because the material itself cannot be properly phagocytized and digested or because the amount coming to it exceeds the handling capacity of the cell. This situation provides pathogenetic significance to deposits found in the mesangial area.

It seems possible that certain proteins or mixtures of proteins with other substances may be circulating in the bloodstream and eventually gain access to the subendothelial

region of the basement membrane through the interaction of a number of variables which are still poorly understood. From here, they may go to the mesangium if they have not reached it directly through the interconnecting channels. In this location, depending on the nature of these substances, they may be taken care of by the mesangial enzymatic machinery of digestion or if this is not possible, some type of mesangial reaction may be established which results in a variety of changes, some cellular and some involving the matrix. In the early stages, these changes will probably be cellular, whereas when the cell has been overcome, mesangial deposits might be expected to appear. It might be anticipated that the possibility of finding deposits would increase with the molecular size of its constituents, but this need not necessarily follow in all circumstances as some substances could be noxious and damage mesangial cells directly, irrespective of their size.

It follows that, in conditions associated with the deposition of extraneous material in the mesangium, it may be worthwhile to investigate the biochemistry of the deposit to determine whether its constituents are present in normal or increased amounts in the blood or whether they are present in a precursor state in the bloodstream and finally deposited in the mesangial area as a result of interaction between mesangial enzymes and these precursors. For instance, as suggested by Shimamura and Sorenson (1965), it is possible that amyloid is synthesized at some other site but becomes polymerized first in contact with the mesangium because of special local environmental conditions. The same could occur in hepatic glomerulosclerosis (see Section II, K), diabetic glomerulosclerosis (see Section II, G), and in states leading to fibrin formation (see Section II, C, 1). One even wonders when collagen forms rapidly in the mesangium in large amounts like in uranium nephropathy (Bencosme *et al.*, 1959) whether it was not preformed elsewhere.

It is also possible that inhibiting substances may be found in some patients and that these could interfere with the "unclogging" mechanism. The presence of a specific mesangial toxin in the venom of the Habu snake (Sakaguchi and Kawamura, 1963; Suzuki *et al.*, 1963) suggests the possibility that other less toxic but equally specific substances could exist and come in contact with mesangial cells, thus depressing their activity. Farquhar (1964) believes that depression of normal mesangial function may be responsible for the lesions of the basement membrane and the mesangial matrix in diabetic glomerulosclerosis.

Review of the literature suggests that evaluation of the phagocytic activity of the mesangium by electron microscopy may be of value in observing the role of this structure in the pathogenesis of a given glomerular disease. It will not be surprising if a primary disease of the mesangium or a group of diseases primarily due to defective mesangial cells are described in the future. In this respect, the recent suggestion by Hamburger and associates that the nodular glomerulonephritis is one such condition (see Section II, B, 4) is of great interest. Moreover, the work of Galle (1964) who described fibrinoid deposits exclusively localized to the interluminar space (mesangium) in a group of young patients with mild proteinuria and microscopic hematuria may represent another variety of mesangial disease.

B. Bright's Disease

1. *Acute and Subacute Glomerulonephritis*

a. *Acute Glomerulonephritis.* The diagnosis of acute glomerulonephritis is usually made on the basis of clinical and urinary findings, and biopsies are seldom taken. Short reports on the ultrastructural changes have been contributed by earlier investigators (Vernier *et al.*, 1958; Farquhar, 1959; Spiro, 1960; Churg *et al.*, 1962; Kimmelstiel *et al.*, 1962b; Trump and Benditt, 1962). Although electron microscopy has clarified many of the confusing findings of light microscopy in acute glomerulonephritis, perhaps the most significant observation which was completely unrecognized before the advent of electron microscopy was the discovery of deposits of undetermined nature located on either side of and within the lamina densa and the mesangium. These deposits were first described by Movat *et al.* (1962), followed shortly by Kimmelstiel *et al.* (1962b). There was good evidence from the literature that they were probably composed of precipitated antibody products with some blood protein added. That this is indeed the case has recently been shown by Seegal *et al.* (1965) employing the immunoferritin technique. The work of these authors is discussed in the section on the immunological aspects of this condition. A recent report by Strunk *et al.* (1964) is of particular interest because these authors were able to obtain sequential biopsies in a severe case of acute poststreptococcal glomerulonephritis and to correlate the ultrastructural changes in the kidney with the clinical findings from the stage of complete anuria to resolution. Because of its special interest this case will be discussed in some detail.

The patient was a $5\frac{1}{2}$-year-old white boy who manifested symptoms of his disease 30 days after a sore throat which occurred during a streptococcal infection associated with scarlet fever. The patient's brothers and sisters both developed scarlet fever, whereas the patient had only a sore throat. The initial findings are summarized in Table 1 and are characteristic of severe, acute glomerulonephritis. Anuria lasted from day 2 to day 17 and, without dialysis, this boy would probably not have survived. From day 17 to day 560 his renal function steadily improved. He was discharged on the 100th day of his disease and remained asymptomatic after leaving hospital.

The first biopsy was taken 9 days after the onset of the disease while the patient was anuric. This biopsy showed marked hypercellularity of the glomeruli due to mesangial and endothelial cell proliferation and to infiltration by numerous polymorphonuclear leukocytes and macrophages (Fig. 10). Hypercellularity resulted in a severe reduction of glomerular capillary lumens. Because of the obliteration of capillaries and general distortion of normal structures, the authors found it frequently impossible to determine whether a given mononuclear cell was endothelial, mesangial, or blood monocytic in nature. The mesangial matrix was considerably distorted and contained dense deposits similar to those found in the basement membrane and described below. In some areas, the matrix was greatly attenuated and broken, whereas in others it was swollen and mottled. It is of interest that the "edema" seen

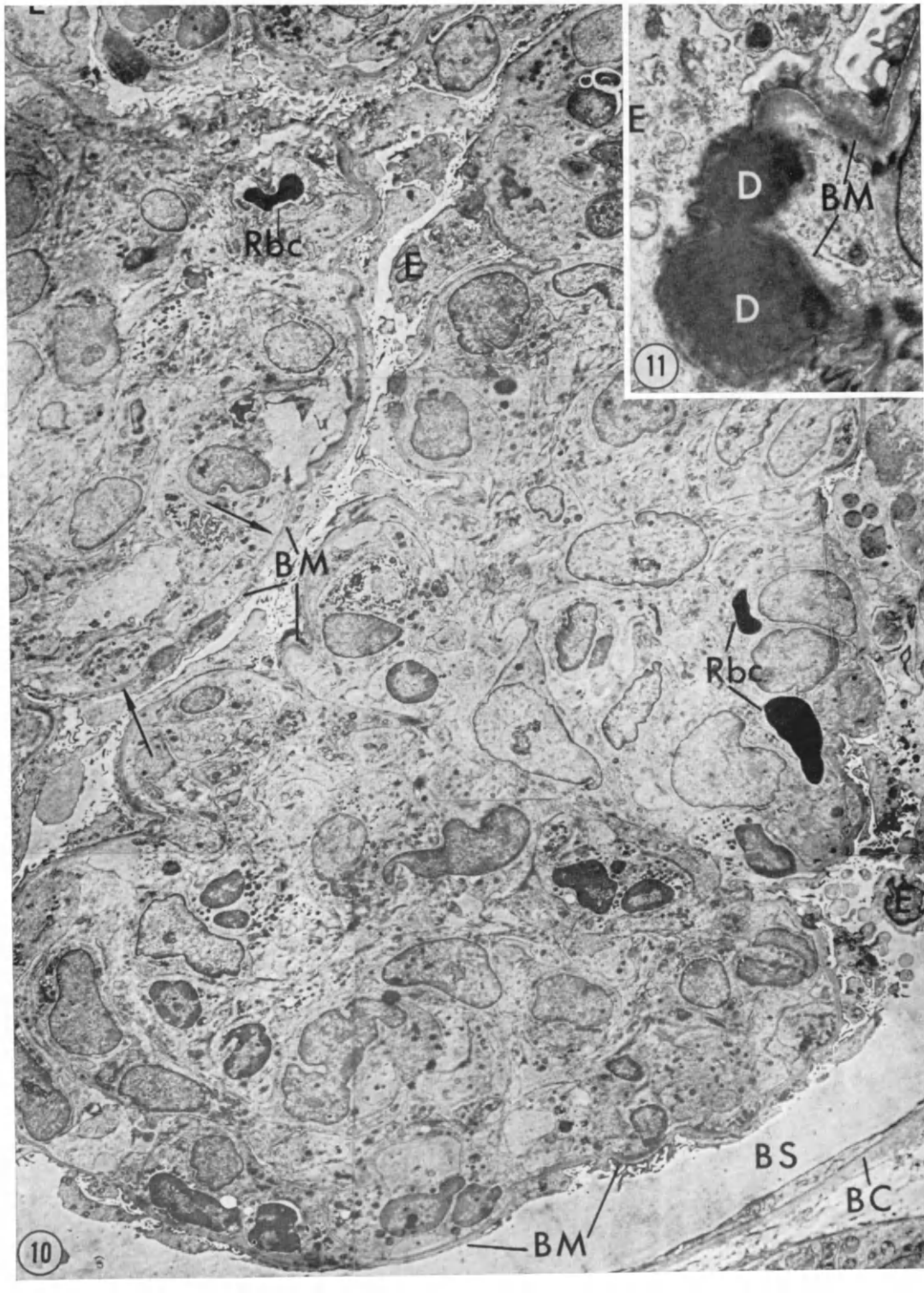
TABLE I

CLINICAL SUMMARY[a, b]

Day	Event	Urine Output	SPGR	Protein	Microscopic	Blood				Other data
						BUN	HCT	WBC	ASO	
−30	Scarlet fever in family, patient had strep throat, strep epidemic in community	—	—	—	—	—	—	—	—	—
0	Slight edema, nausea, vomiting	—	—	3+	2–3 RBC	—	—	—	—	—
1	Marked edema, nausea, vomiting	—	—	—	—	—	—	—	—	—
2	—	15 ml	—	—	—	—	—	—	—	BP, 105/65
3	—	15 ml	—	—	—	—	—	—	—	—
4	—	4 ml	—	—	RBC casts, 15–20 RBC	203	34	10,150	—	—
5	University of Washington Hospital hemodialysis	—	—	300 mg %	RBC granular and ghost casts	201	29	11,000	—	BP, 150/90
9	Biopsy	—	—	—	—	114	25	—	—	—
12	Hemodialysis	—	—	—	—	150	—	—	1250	—
14	—	—	—	—	—	—	—	—	—	10 mg prednisolone 4 times a day
17	First urine output	200	—	—	RBC casts	123	—	—	—	—
19	Hemodialysis	353	—	—	—	157	—	—	—	—
29	Biopsy	700	1010	100 mg %	50 RBC, RBC casts, 10 WBC	99	20	20,000	—	—
72	Biopsy	900	1017	100 mg %	Many RBC	41	21	17,000	333	BP, 124/70
100	Discharge	715	1014	3+	10–20 RBC, no casts	28	26	11,700	—	10 mg prednisolone every day; BP, 138/70
560	Biopsy	Normal	—	0	0–1 RBC	19	43	—	100	BP, 115/70

[a] The abbreviations used in this table are: SPGR, specific gravity; BUN, blood urea nitrogen; HCT, hematocrit; WBC, white blood cells; RBC, red blood cells; ASO, antistreptolysin titer.

[b] From Strunk *et al.* (1964).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in the mesangium by light microscopy in acute glomerulonephritis has been shown by Strunk *et al.* (1964) to be an agglomeration of cell cytoplasm.

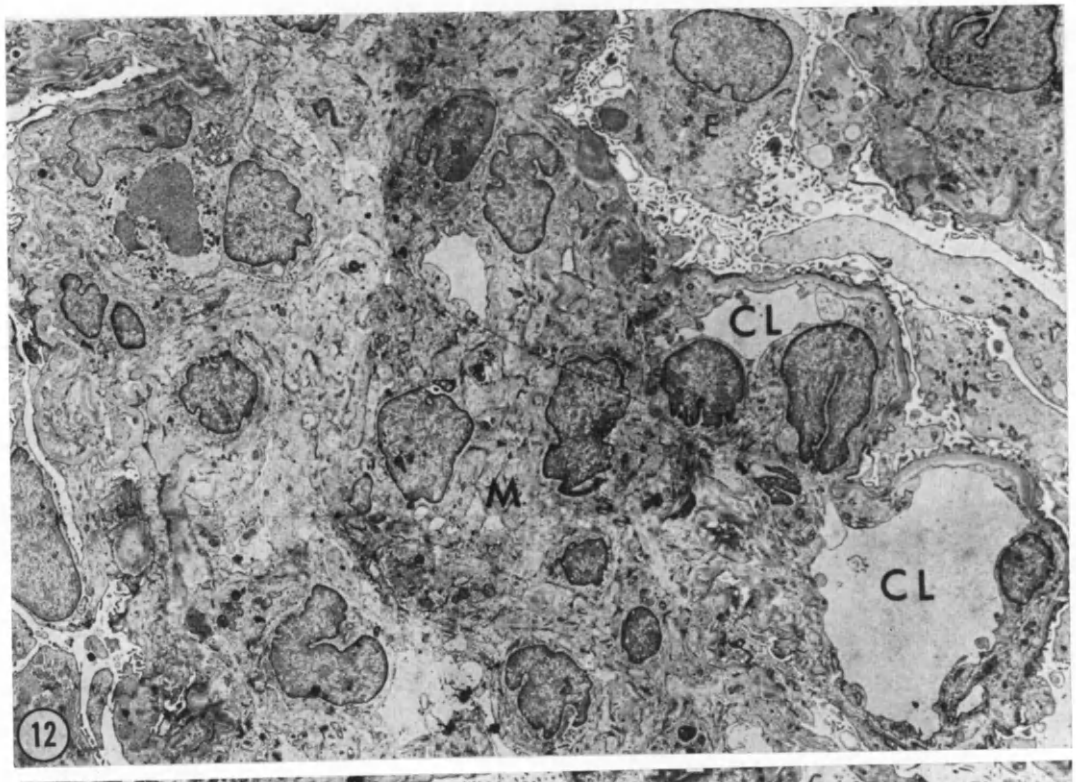
Frequent deposits of extracellular material with a greater electron density than the basement membrane were present between the epithelial cells and the basement membrane and occasionally within and beneath this membrane as well (Figs. 10 and 11). Some parts of the glomerular basement membrane were thicker than normal, whereas others were attenuated. The lamina densa and the lamina rara externa were little changed except where deposits were present. In contrast, the lamina rara interna was absent in many areas. The endothelial cells were greatly swollen, obliterating the endothelial pores. The epithelial cells showed extensive fusion of foot processes and a slight increase in the number of microvilli.

The second biopsy, taken on day 29 after the onset of the disease when the 24-hour urine output had been increasing for 11 days from 200 to 800 ml, showed very early changes indicating a return toward normality. There was a reduction in the number of cells in the glomerular lobules and occasional capillary lumens were patent (Fig. 12). At this stage it was easier to distinguish the nature of the individual cells. The mesangial cells had decreased in number and size, but vacuoles and masses of moderately dense granular material, unbounded by membrane, were present within them. The mesangial matrix was increased in amount and in many areas appeared with a prominent fibrillar pattern, but no periodicity was evident in these fibers. The dense deposits seen in the matrix in the earlier biopsy were considerably diminished.

The appearance of the basement membrane had changed significantly. The deposits in the membrane which were found in the previous biopsy were greatly reduced. In many foci the lamina rara interna and externa appeared widened, whereas the lamina densa exhibited a moth-eaten appearance (Fig. 15). Small masses of material resembling the lamina densa were frequently seen isolated in the lamina rara externa (Fig. 16). Tongues of epithelial cytoplasm extended into these regions. The changes in the basement membrane were interpreted by Strunk *et al.* (1964) as indicating that some material, such as the dense deposits previously present, had been removed from the membrane by the phagocytic activity of the epithelial cells (Figs. 15 and 16). Wisps of dense material similar to lamina densa were also seen in the lamina rara interna in juxtaposition to the endothelium (Fig. 17). The endothelial cells, although

FIG. 10. Acute glomerulonephritis. Nine-day biopsy specimen, showing a montage incorporating approximately one-sixth to one-eighth of the glomerular tuft and one complete lobule and portions of two others. Epithelial cells (E) are not prominent. The basement membrane (BM) externally bounds the enlarged hypercellular lobule. In some areas (arrows) it is markedly thinned. Three red cells (Rbc) are entrapped within the lobule. The hypercellularity is due to a confusing array of mononuclear cells and leukocytes. Bowman's space (BS) and capsule (BC) are present in the lower portion of the micrograph. Magnification: ×1500. From Strunk *et al.* (1964).

FIG. 11. Acute glomerulonephritis. A 29-day biopsy specimen showing deposits (D) between epithelium (E) and basement membrane (BM). In this instance they extend into the substance of the membrane. The epithelium (E) is hypertrophic. Magnification: ×9200. From Strunk *et al.* (1964).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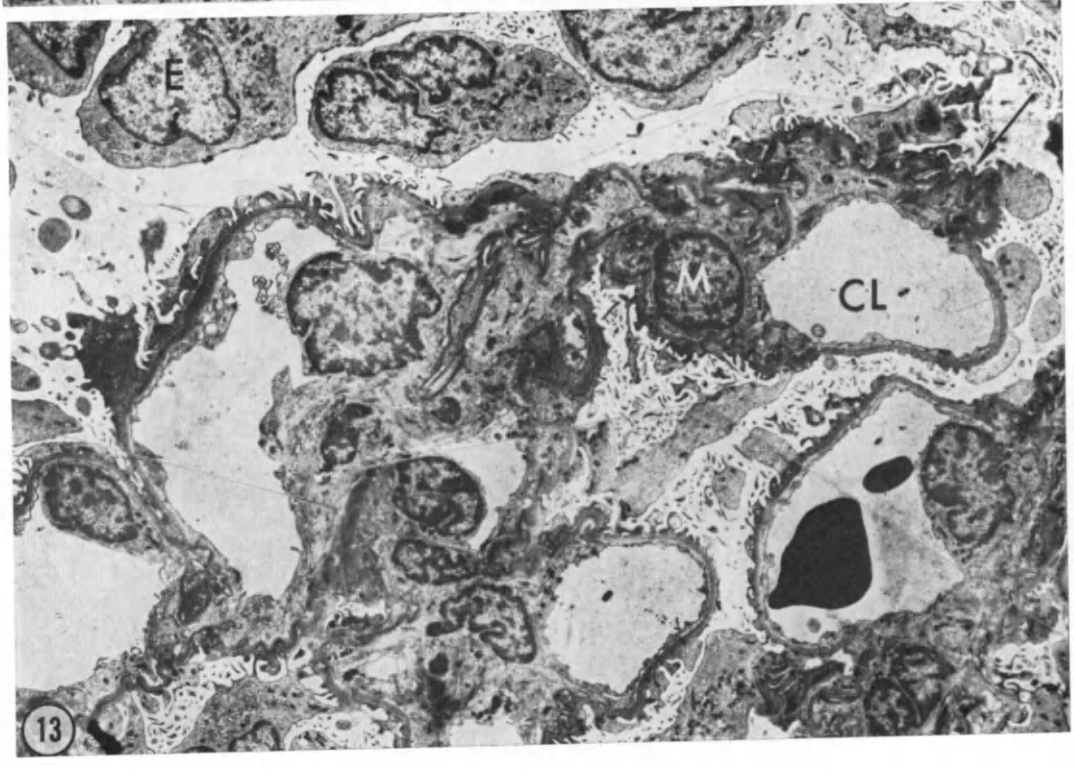
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less swollen, were still larger than normal and only a few typical fenestrated membranes were seen. In contrast with the previous biopsy, the epithelial cells showed considerable activity (Fig. 12). They were hyperplastic and contained numerous vacuoles and deposits of different size and density and a very prominent endoplasmic reticulum which frequently contained dilated cisternae. Microvilli were also abundant. Extensive fusion of the foot processes was still present.

The third biopsy was taken on day 12 after the onset of the disease when the urine output was normal. This biopsy showed many glomeruli which appeared almost normal by light microscopy although several still showed expansion of the mesangium in the center of the glomerular lobule. Electron microscopy confirmed the presence of the expanded mesangial areas and, in addition, revealed that the process of resolution of the lesions in the basement membrane described in the second biopsy (Figs. 15–17) was still very incomplete. Many capillary lumens were still small and the endothelial cells lining them were still considerably enlarged. The enlarged mesangium seen by light microscopy was shown by electron microscopy to correspond to unopened capillary loops containing swollen endothelial cells and a collapsed and wrinkled basement membrane (Fig. 13).

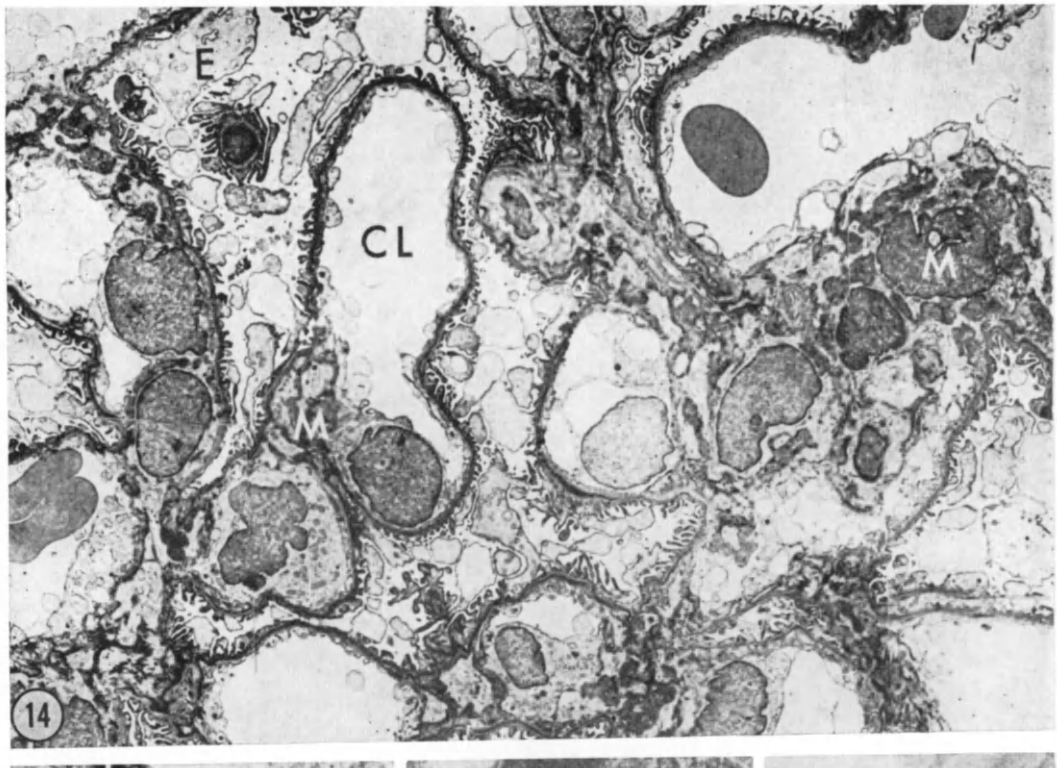
In many areas the basement membrane appeared normal, but the focal changes described in the preceding biopsy (Figs. 16 and 17) could still be found. Most of the epithelial cells had reverted to normal except for the presence of extensive fusion of the foot processes which was, however, considerably less than in the preceding biopsy.

The fourth biopsy was taken 560 days after the onset of the disease when there were no clinical abnormalities. It showed that many glomeruli had returned to normal when seen by both light and electron microscopy (Fig. 14). In some glomeruli, however, study of the ultrastructure showed that the mesangium was still thickened owing mainly to the persistence of some of the unopened capillary loops previously noted in the preceding biopsy.

The basement membrane appeared normal in most areas although a few focal changes similar to those described at 72 days still persisted but were less marked. Irregularities of the epithelial surface had almost disappeared (Figs. 15 and 16). The most frequent abnormality seen at this time was the presence of a thin layer of lamina densa in juxtaposition to the endothelium (Fig. 17). The epithelial and endothelial

FIG. 12. Acute glomerulonephritis. A 29-day biopsy specimen, showing portions of glomerular lobules and of an arteriole. One lobule shows capillary loops (CL) with varying degrees of patency at the periphery of the still expanded mesangium (M). Capillary waists are widened. Some peripheral capillary wall abnormalities are evident. Epithelial cells (E) are enlarged. Magnification: ×1500. From Strunk *et al.* (1964).

FIG. 13. Acute glomerulonephritis. A 72-day biopsy specimen; a montage of a portion of a glomerular tuft showing patency of many capillary lumina (CL). The mesangium (M) remains expanded to varying degrees. Note the decrease in size of epithelial cells (E) and the occasional capillary loop irregularities (arrows). Magnification: ×1500. From Strunk *et al.* (1964).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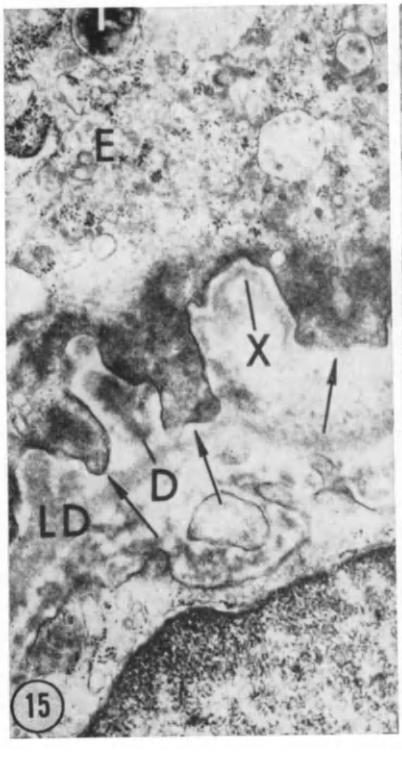
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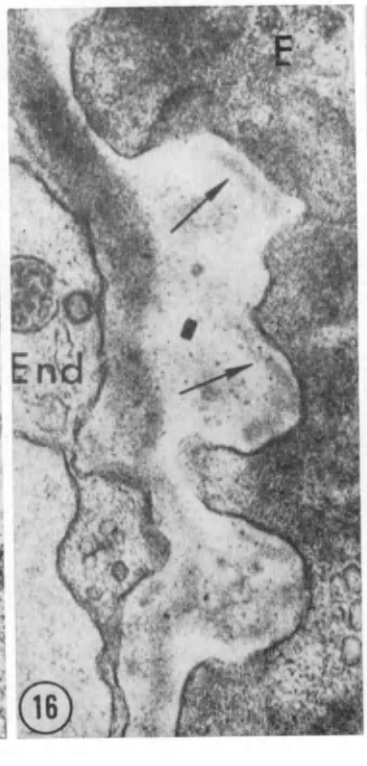
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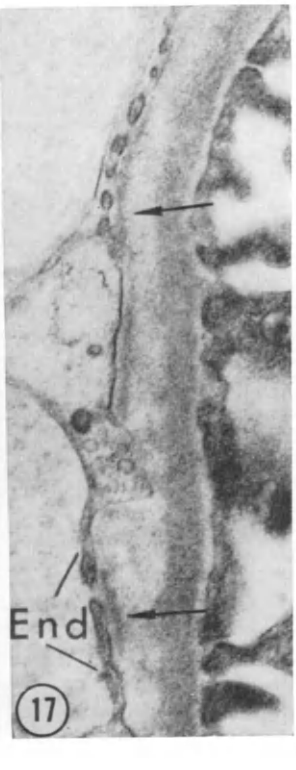
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cells usually appeared normal although occasionally some fusion of epithelial foot processes was seen. A few glomeruli were small, collapsed, and nonfunctional. These obsolete glomeruli showed no evidence of inflammatory scarring and resembled those produced by ischemia.

Although the presence of deposits in the basement membrane in acute glomerulonephritis has been previously well described by Movat *et al.* (1962) and Kimmelstiel *et al.* (1962b), the report by Strunk *et al.* (1964) was the first description of the sequential changes leading to the removal of the deposits and repair of the basement membrane. These authors suggest that epithelial as well as mesangial cells are concerned with the removal of the dense deposits and the replacement or regeneration of new basement membrane and mesangial matrix. They also postulate that the endothelial cells may contribute to the repair of the basement membrane following removal of the deposits. This postulate is in agreement with the work of Pierce *et al.* (1963) which indicated that the endothelial cell participates in the production of the basement membrane independently from the associated epithelial cells.

If, as suggested by Strunk *et al.* (1964), both endothelial and epithelial cells contribute to the formation of the basement membrane, it is possible that the "splitting" of the lamina densa so frequently observed in glomerular diseases may indicate regeneration of new lamina densa from either endothelial or epithelial cells to replace the original basement membrane which may have been destroyed by the disease process.

In addition to describing the evolution of the deposits and regeneration of the basement membrane, the work of Strunk *et al.* (1964) has greatly clarified the ultrastructural changes of the mesangium in acute glomerulonephritis. It had previously been thought, on the basis of light microscopy, that this area became edematous

FIG. 14. Acute glomerulonephritis. A 560-day biopsy specimen; a montage incorporating portion of a glomerular tuft showing striking return to normal from the previous time periods. A few foci of persistent mesangial (M) thickening are seen. Most mesangial regions are normal as are the capillary loops (CL). Epithelial cells (E) are not prominent. Magnification: ×1500. From Strunk *et al.* (1964).

FIG. 15. Acute glomerulonephritis. A 29-day biopsy specimen showing an altered area of basement membrane. Note the partial disruption of lamina densa (LD), the irregular lucent areas, and the extension of epithelial cytoplasm (arrows) into the defects in the basement membrane. The epithelium (E) contains inclusions (I) of moderately dense material. Two dense areas (D) resembling residual deposits are present. A thin line of lamina densa is closely applied to the epithelium (X). Magnification: ×12,000. From Strunk *et al.* (1964).

FIGS. 16 AND 17. Acute glomerulonephritis. Two micrographs, one (Fig. 16) showing wisps of basement membrane (arrows) applied to the undersurface of the epithelium (E) with a relatively intact endothelial (End) surface of the basement membrane, while in Fig. 17 the endothelial surface (End) is irregular and contains fine wisps of basement membrane material juxtaposed to the endothelium (arrows). Configurations as depicted by Fig. 16 are most common at 29 days and are seen with about the same frequency as those in Fig. 17 at 72 days. The configurations represented by Fig. 17 are most common at 560 days. Figure 16, magnification: ×35,000. Figure 17, magnification: ×21,000. From Strunk *et al.* (1964).

in the acute phase, whereas the electron microscopic studies in this report clearly showed that what appeared to be edema by light microscopy was, in fact, infiltration by cellular elements, some of which were derived from proliferation of endothelial and mesangial cells, whereas others were of hematogenous origin. This report also showed that the persistence of lobular stalk thickening, which is the most common residual change in acute glomerulonephritis, was due to continuing hypercellularity of the mesangium, an excess of extracellular material in the mesangium, and persistence of collapsed capillary loops. Failure of resolution of these changes leads to the picture of subacute glomerulonephritis.

b. *Subacute Glomerulonephritis.* This condition has not been extensively studied. Kinoshita and Fujisaki (1963) described changes in subacute glomerulonephritis characterized by marked proliferation and swelling of glomerular capillary cells, development of epithelial crescents, and deposition of osmiophilic substance. The mesangial areas were greatly widened as a consequence of hypercellularity, but the origin of the cells was difficult to decide. The epithelial cells were edematous, and the foot processes were extensively fused and smudged. The mitochondria were swollen, and the endoplasmic reticulum was prominent within the epithelial cells and microvilli were seen. The lamina densa varied in thickness and was usually thinner than normal. There were variable but extensive deposits of osmiophilic substance around the mesangial cells and between the lamina densa and the endothelial cells. The epithelial cells of Bowman's capsule were increased in number. The epithelial crescent contained many mitochondria and endoplasmic reticulum and merged with the glomerular epithelial cells. The basement membrane of Bowman's capsule was thickened and split into fine fibrils. Most of these changes are nonspecific and could well be considered on morphological grounds to represent incomplete resolution of the acute changes described above.

c. *Immunological Aspects.* Recently Seegal *et al.* (1965) have applied the immunoferritin technique to human biopsy material from subjects with glomerulonephritis. The tissue was stained with ferritin-labeled antisera to 7S gammaglobulin, 1c and type 12 streptococcal antigens. In acute glomerulonephritis, electron microscopy revealed deposition of the antisera in the electron-dense deposits present among proliferating endothelial and mesangial cells, in the basement membranes, in the deposits on the endothelial side of the basement membrane, and in some focal subepithelial deposits. Binding of ferritin-labeled antibodies was also demonstrated on the electron-dense precipitates occurring in the capillary lumens and in the Bowman spaces of the most severely diseased patients. Similar localization of antibodies was also seen in the dense deposits present in patients with subacute glomerulonephritis.

Their studies included 19 patients in whom the first biopsies were taken 6 months or more after the onset of the disease. Antibodies to human proteins were present in the glomeruli in slightly over 50% of the biopsies and in the views of the authors support the hypothesis that antigen-antibody complexes continue to play a role in the progression of the disease at this stage. Preliminary studies of the biopsies from

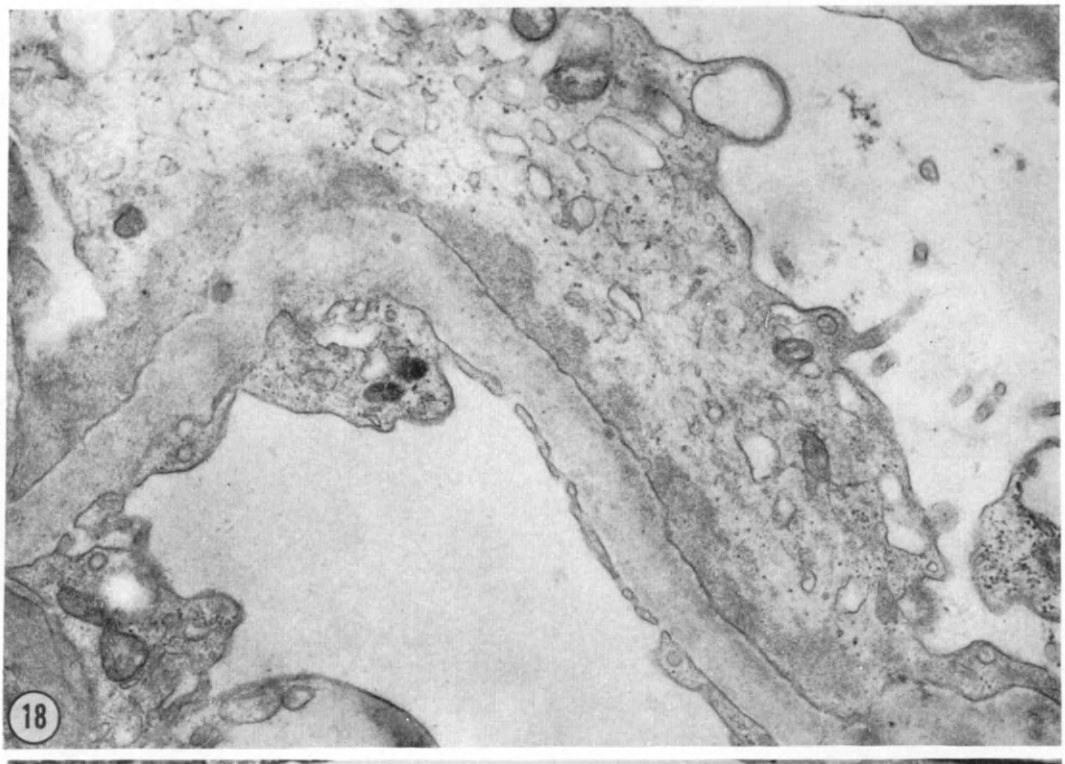
these patients with subacute glomerulonephritis indicated the finding of type 12 streptococcal antigens in four out of five cases. The exact significance of these findings is not yet clear.

On the basis of this work, Seegal and associates hypothesized that in acute glomerulonephritis antigen-antibody complexes may circulate in the blood, aggregate in the glomerular capillaries, penetrate among proliferating cells, and form deposits beneath the basement membrane in a process which also involves complement. In these complexes, type 12 streptococcal products appeared to be a source of antigen in at least six of the ten cases studied. In subacute nephritis, the presence of gamma globulin and complement in glomeruli was felt to indicate that antigen-antibody complexes might continue to play a role in the progression of the disease. Preliminary studies on the binding of streptococcal antigens in the same area suggested that these antigens play a part in the complex. It is not clear whether the antigen is bound immunologically or whether fibrinogen may be present in this area and capable of binding M protein. At the present time the exact immunological role of streptococcal antigens remains to be elucidated.

2. *Lipoid Nephrosis of Children*

Lipoid nephrosis of children has been largely responsible for stimulating investigators to apply electron microscopy to the study of renal pathology, because by light microscopy the glomeruli in this condition usually appear normal despite severe proteinuria. Farquhar and associates (Farquhar *et al.*, 1957a, b; Vernier *et al.*, 1956, 1958) were the first to emphasize the potential of electron microscopy in the study of renal pathology in this condition. These investigators found that the most characteristic lesion was a marked decrease in the number and frequently a complete absence of the foot processes of the epithelial cells (Fig. 18). This observation has been confirmed by subsequent workers (Folli *et al.*, 1958; Latta, 1960; Sorel *et al.*, 1960; Movat, 1960a, 1961; Steiner *et al.*, 1961; Movat *et al.*, 1961b). It cannot, however, be considered pathognomonic as it is seen in many other conditions, particularly those associated with significant proteinuria.

The cytoplasm of the epithelial cells facing the basement membrane frequently contains a dark and homogeneous substance which is characteristic of lipoid nephrosis (Fig. 18) but may also appear in other conditions where marked alterations of the epithelial side of the basement membrane occur. It is of interest that Rhodin (1964) has demonstrated a similar dark material in the epithelial side of the basement membrane of human embryos before the foot processes are formed. It is possible that this dark material is in some way related to the substances making up the filtration slit membrane found between the foot processes in mature kidneys. Other changes found in the epithelium in lipoid nephrosis of children include marked vacuolization, swollen mitochondria, and lipid and protein droplets. Microvilli have frequently been described on the epithelial cell surface facing the urinary space (Latta, 1960; Movat *et al.*, 1961b). They have sometimes been referred to under the misleading term "osmiophilic bodies" (Folli *et al.*, 1958; Steiner *et al.*, 1961).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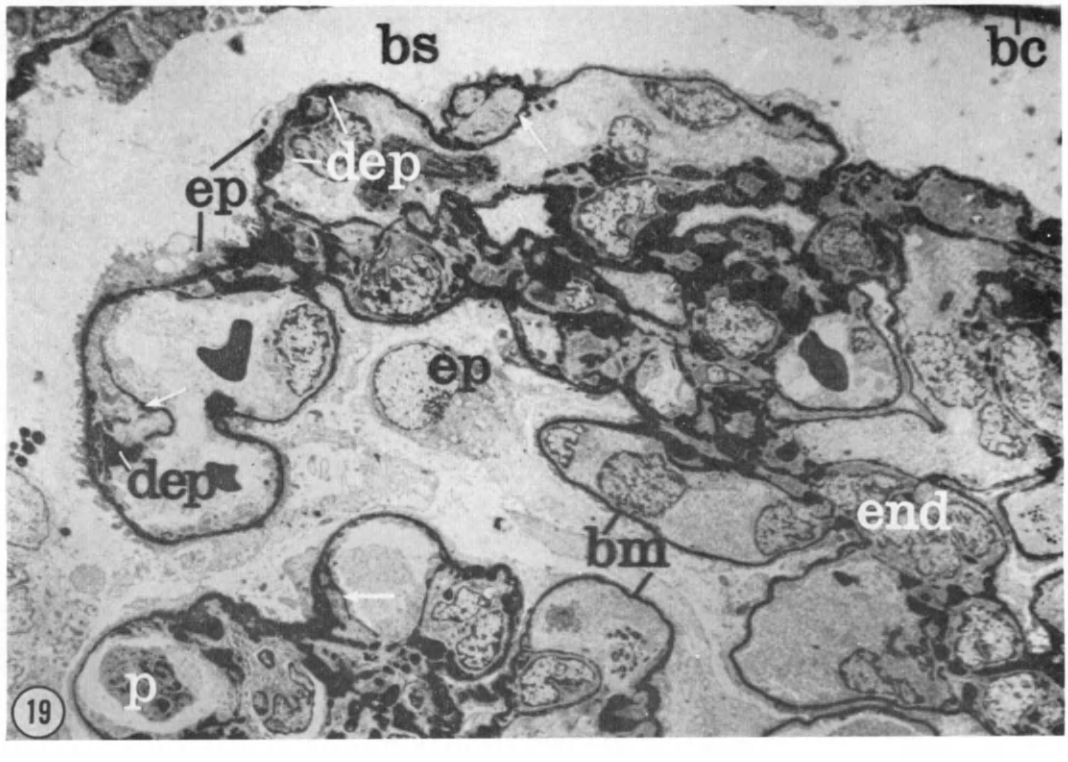
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The endothelium is usually unchanged, although occasionally microvilli similar to those seen in epithelial cells may be seen in the endothelium (Steiner *et al.*, 1961).

Recent studies by Movat (1960a, 1961) and Steiner *et al.* (1961) have provided additional information on the structural alterations which occur in the basement membrane in this condition. Using the periodic acid–silver methenamine technique, these workers reported diffuse swelling of the glomerular basement membrane with areas of imbibition and irregular rarefaction. In cases of long-standing lipoid nephrosis, these investigators noted focal deposits between the endothelium and the lamina densa as well as in the mesangium (Fig. 19). Although some of these changes in the basement membrane have been illustrated by others (Vernier *et al.*, 1956, 1958; Farquhar *et al.*, 1957a, b; Folli *et al.*, 1958; Spiro, 1959, 1960; Latta, 1960; Sorel *et al.*, 1960), Movat and Steiner were the first to insist upon the importance of these findings and suggested that the changes observed were compatible with the deposition of an extraneous or exogenous material in the lamina rara interna, such as antigen-antibody complexes. Such material might possibly induce disruption of the polymerized molecular structure of the basement membrane and lead to swelling and increased capillary permeability.

Kimmelstiel *et al.* (1962b) reported two cases of lipoid nephrosis in which it was felt that the most conspicuous change was a deposition of an electron-dense band in the outer portion of the basement membrane. In addition, these authors also found nodular deposits on both sides of the basement membrane similar to those seen in adult glomerulonephritis. It is unfortunate that no silver methenamine stains were done in these cases as it is difficult to correlate and interpret the results of these investigators in relation to the findings described by others.

In 1964, Rosen *et al.*, on the basis of studies on lipoid nephrosis in children and idiopathic membranous glomerulonephritis in adults, reported neither basement membrane change nor osmiophilic deposits in the subjects with lipoid nephrosis although there was complete fusion of the foot processes. In contrast to the patients with membranous glomerulonephritis, the lesions in these subjects showed much less tendency to progression. The authors concluded that membranous glomerulonephritis and lipoid nephrosis were distinct clinical and pathological entities. It is

FIG. 18. Portion of a glomerular capillary loop from an 11-year-old child with "pure" untreated nephrosis 3 weeks after the onset of symptoms. Micrograph shows complete loss of the epithelial foot process organization and increased density of the cytoplasm in relation to the basement membrane. Small and medium-size vacuoles and microvilli are common in the epithelial cell. Magnification: ×22,000. Courtesy of Dr. C. G. Biava.

FIG. 19. Portion of glomerulus of child with history of long-standing lipoid nephrosis. In this electron micrograph one can see signs of early chronic glomerulonephritis. These consist of intracapillary (subendothelial) deposits (dep) of a dense argentophilic material and slight proliferation of endothelial (mesangial?) cells (end). Note that in some areas (arrows) the deposits are attenuated to form structures which in the past have been referred to as "splitting" or "reduplications" of the basement membrane (bm). Periodic acid-silver methenamine. Magnification: ×1240. From Movat (1961).

evident from the discrepancies between the work of Rosen *et al.* (1964), Kimmelstiel *et al.* (1962b), Movat (1960a, 1961), and Steiner *et al.* (1961) that further work is indicated to clarify the pathological changes in this condition, and it is hoped that future workers will standardize their terminology, diagnostic criteria, and experimental techniques.

3. *Membranous Glomerulonephritis (Idiopathic)*

This condition which is characterized by a nephrotic syndrome and diffuse thickening of the walls of glomerular capillaries has been extensively studied because of its clinical and anatomical similarity by light microscopy to lipoid nephrosis in children. By means of electron microscopy, the two conditions are readily distinguished.

There is general agreement that by electron microscopy the most striking, if not pathognomonic lesion, in membranous glomerulonephritis is an extensive accumulation of material which is usually denser than the basement membrane, between the lamina densa and the fused foot processes of the epithelial cells (Fig. 20). The relationship of this material to the epithelial cells is such that even by light microscopy a characteristic "alternating spike and dome" pattern can be recognized if very thin sections are stained with periodic acid-silver methenamine (PASM). In 1959, Movat and McGregor applied this technique to electron microscopy and described deposits between the endothelium and the lamina densa which were very similar to those seen in lipoid nephrosis of children (Steiner *et al.*, 1961; Movat *et al.*, 1961b). They also described a dense precipitate not only within the basement membrane but also between the mesangial cells. Their findings are in agreement with those of Galle *et al.* (1962). Moreover, in contrast with previous reports (Movat and McGregor, 1959), Movat *et al.* (1961b) noted that the basement membrane was frequently swollen in this condition. Rosen *et al.* (1964) found that membranous glomerulonephritis was characterized by diffuse thickening or splitting of the glomerular basement membrane associated with subepithelial silver methenamine-positive projections. However, these authors did not observe the subendothelial deposits described in this condition by Movat and McGregor (1959) and Movat *et al.* (1961b). Faith and Trump (1964) studied a number of patients with different nephropathies and concluded that basement membrane thickening alone or subepithelial deposition alone could occur in diseases in which proteinuria predominated. In contrast, subendothelial or intramembranous deposits were found in cases where the endothelial cells were most affected. The chemical nature and mode of formation of these deposits is still under investigation. There is increasing evidence that the deposits are probably composed of a mixture of blood proteins with antigen-antibody complexes (Movat and McGregor, 1959; Spargo and Arnold, 1960; Dixon *et al.*, 1961). Movat *et al.* (1961b) have speculated that the adult epithelial cell may be less well equipped metabolically than that of the child to handle large quantities of protein passing through the outer basement membrane, with the result that protein deposits may occur between the epithelial cells and the basement membrane. Others, however, believed that the

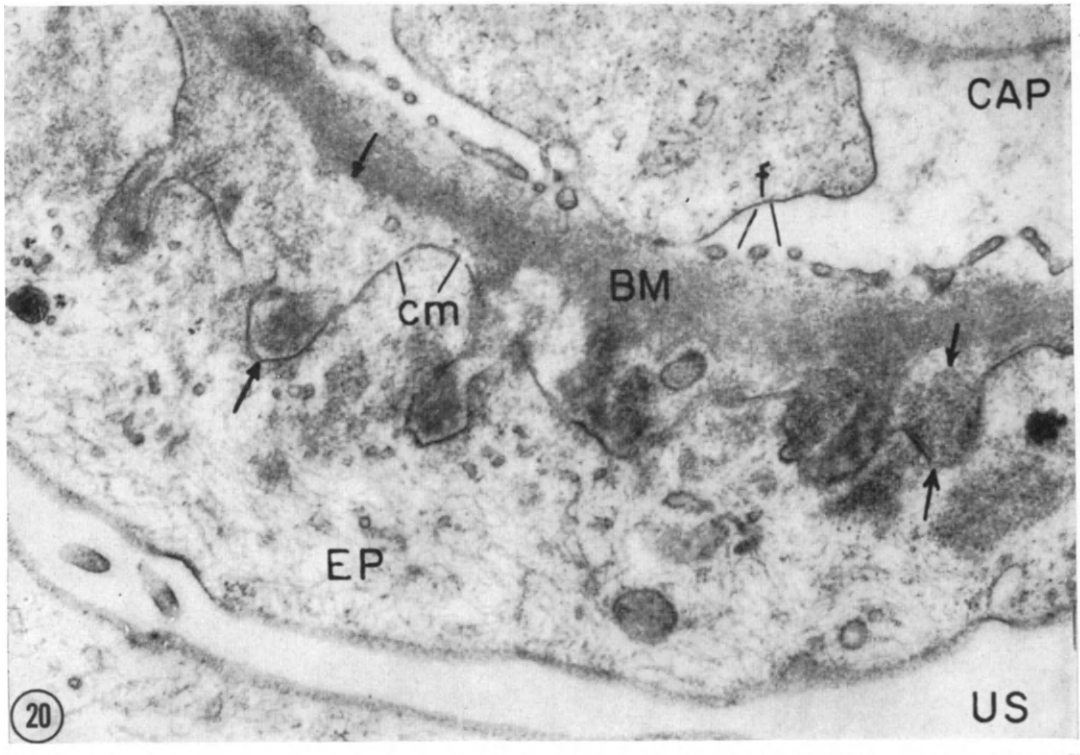
deposits consisted, at least in part, of material synthesized locally by the visceral epithelium (Trump and Benditt, 1962). This last suggestion provides an alternative hypothesis to the commonly held view that the deposits are derived from material circulating in the blood.

4. *Lobular Glomerulonephritis*

This type of chronic glomerulonephritis was isolated as a morphological entity by Allen in 1951. It is characterized by hyaline nodules in the mesangial region and is clinically associated with a nephrotic syndrome. The latter association may be more apparent than real as the bulk of renal biopsy material is derived from patients with the nephrotic syndrome. One of the most exhaustive studies of this condition has been carried out by Hamburger and his associates while studying 108 cases of nephrosis of unknown origin (Habib *et al.*, 1961; Galle *et al.*, 1962). They described fifteen cases which were characterized clinically by the unusual finding of gross hematuria and an age distribution predominantly in the second and third decade. According to these authors it appears that lobular glomerulonephritis characteristically begins as a cellular proliferation of the mesangium which is later followed by a progressive accumulation of mesangial matrix. In time, there is a considerable increase in the number of fibers in the matrix. These fibers exhibit a periodicity of 100 Å which is believed by the authors to represent the collagen subperiods of recently formed collagen fibers (Habib *et al.*, 1961). The fibers are found in the midst of hyaline masses in the mesangial matrix and also between cells. During the development of the mesangial lesion, the capillaries, including the epithelial cells and basement membrane, are little changed and the epithelial foot processes may remain intact over wide areas. In some cases, the capillary wall may be thickened by subendothelial and even subepithelial deposits. Galle *et al.* (1962) concluded that, if the mesangium is an autonomous tissue, then lobular glomerulonephritis can be considered a specific pathological condition of this tissue. The morphological differences between membranous and lobular glomerulonephritis are strongly against the possibility that the lobular lesion is an evolution of the membranous as had been thought from studies based on light microscopy. The abundance of collagen fibers in the mesangial region in lobular glomerulonephritis is only equalled experimentally by uranium nephropathy in the rat (Bencosme *et al.*, 1959).

The etiology of lobular glomerulonephritis is still unsettled. Movat, discussing the paper of Habib *et al.* (1961), reported that he had found the lobular picture in three different clinical cases: (1) following acute glomerulonephritis, (2) in a patient with pure nephrosis, and (3) in a patient who had so-called latent nephritis characterized solely by hematuria and slight proteinuria. It is of interest that hematuria was considered the most constant clinical finding associated with lobular glomerulonephritis by Habib *et al.* (1961).

In a recent study of renal biopsies performed in young, adult patients, with chronic nephropathies characterized by moderate proteinuria and microscopic hematuria, Galle (1964) found extensive deposits in the mesangial area. By light microscopy,



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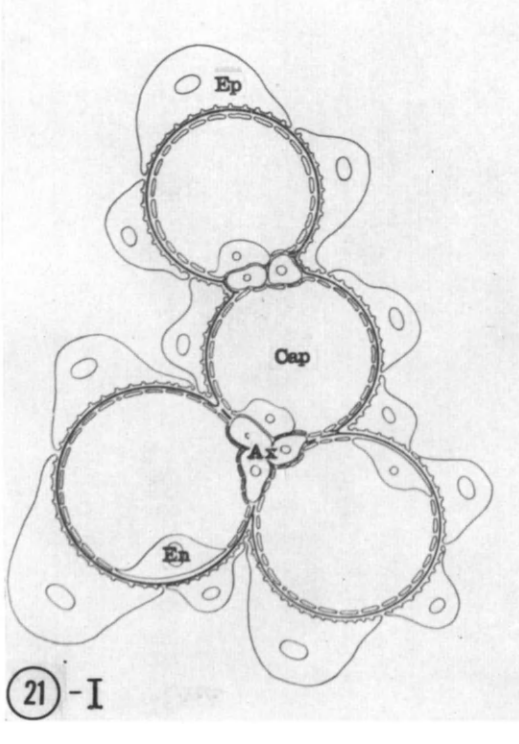

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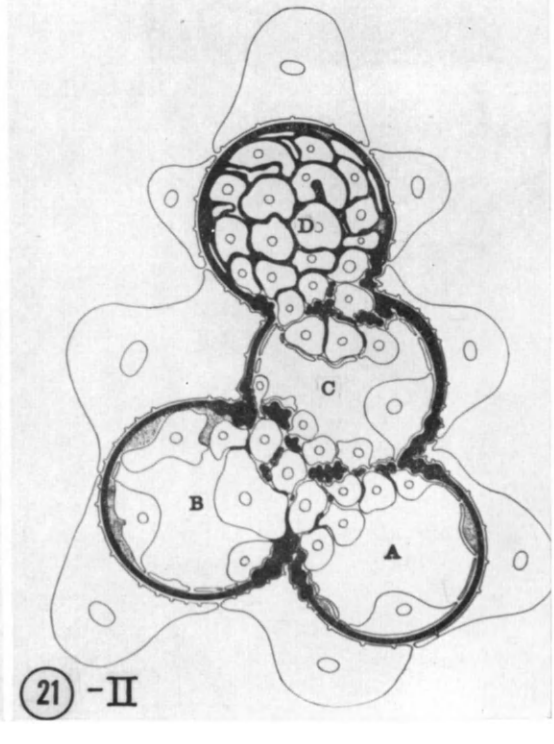

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these could not be distinguished from fibrin thrombi in the capillary lumens. Electron microscopy, however, revealed that these large deposits were not intraluminal but were confined to the mesangial area and structurally were composed of fine granular material resembling fibrinoid. The possibility that these deposits represent an early stage in the evolution of lobular glomerulonephritis cannot be excluded.

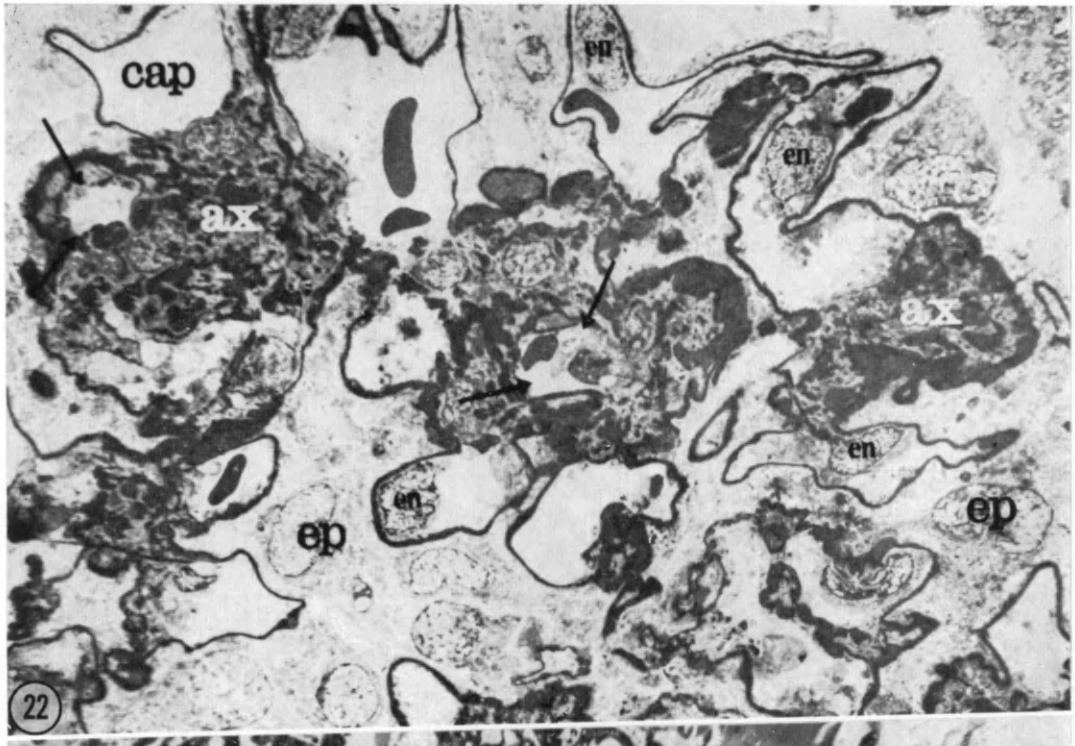
5. *Chronic Glomerulonephritis*

Prior to 1962 (Steiner *et al.*, 1962a, b), no detailed electron microscopic study of glomerular changes in chronic glomerulonephritis had been reported, although some features had been described in reports on the nephrotic syndrome (Farquhar *et al.*, 1957a; Farquhar, 1959; Spiro, 1959, 1960; Spargo and Arnold (1960); Habib *et al.*, 1961). In general, chronic glomerulonephritis is characterized by progressive occlusion of glomerular capillaries. Ultimately, this leads to the classic obsolete glomeruli of the light microscopists. Steiner *et al.* (1962a, b) have suggested that the process varies depending on whether a capillary loop is peripherally or centrally situated. The sequence of events is schematically illustrated in Fig. 21. In both central and peripheral capillary loops, proliferation of the mesangium, increase of the mesangial matrix, proliferation of endothelial cells, thickening of the lamina densa, and formation of deposits between the endothelium and lamina densa appear to be the main factors responsible for the final obliteration of the capillary lumen (Figs. 22 and 23). Alterations of the epithelial cell foot processes are common but not specific, and complete fusion can occur over a given capillary loop. Such changes are equally common over totally obsolete loops or over patent ones. Similar findings were reported by Kinoshita and Fujisaki (1963). The nature of the intracapillary material is undetermined. It may represent antigen-antibody complexes alone or an aggregate of macromolecules or platelet material resulting from transformation of thrombocytes in capillary lumens (Steiner *et al.*, 1962a). The material responsible for the general

FIG. 20. A portion of the glomerular loop from a case of long-standing membranous glomerulonephritis. The basement membrane (BM) is clearly seen. Heterogeneous material, some of it electron-dense, is deposited on the epithelial side of the basement membrane and extends between foot processes. The subepithelial space is markedly widened (arrows). The epithelial foot processes are broad and flattened. Magnification: ×10,000. Courtesy of Dr. M. G. Farquhar.

FIG. 21. Diagrams illustrating the development of individual capillary obsolescence. Diagram I represents normal capillary loops. The peripherally located loops are in contact with one axial (mesangial) region (Ax) each, and the central one is in contact with two at opposite poles.

Diagram II analyzes the processes leading to obsolescence. Loop A shows early concentric obliteration caused by thickening of the lamina densa and formation of subendothelial deposits. Loop B shows an advance of these processes. Concentric narrowing is increased by endothelial hypertrophy and hyperplasia, and some eccentric axial encroachment is developing. Loop C shows the concentric axial encroachment of a centrally located capillary. Loop D is obsolete. The lumen is filled with cells and basement membrane-like material. The cells at this stage are given the prefix "intracapillary" since it is impossible to say whether they are axial (mesangial) or endothelial in origin. From Steiner *et al.* (1962b).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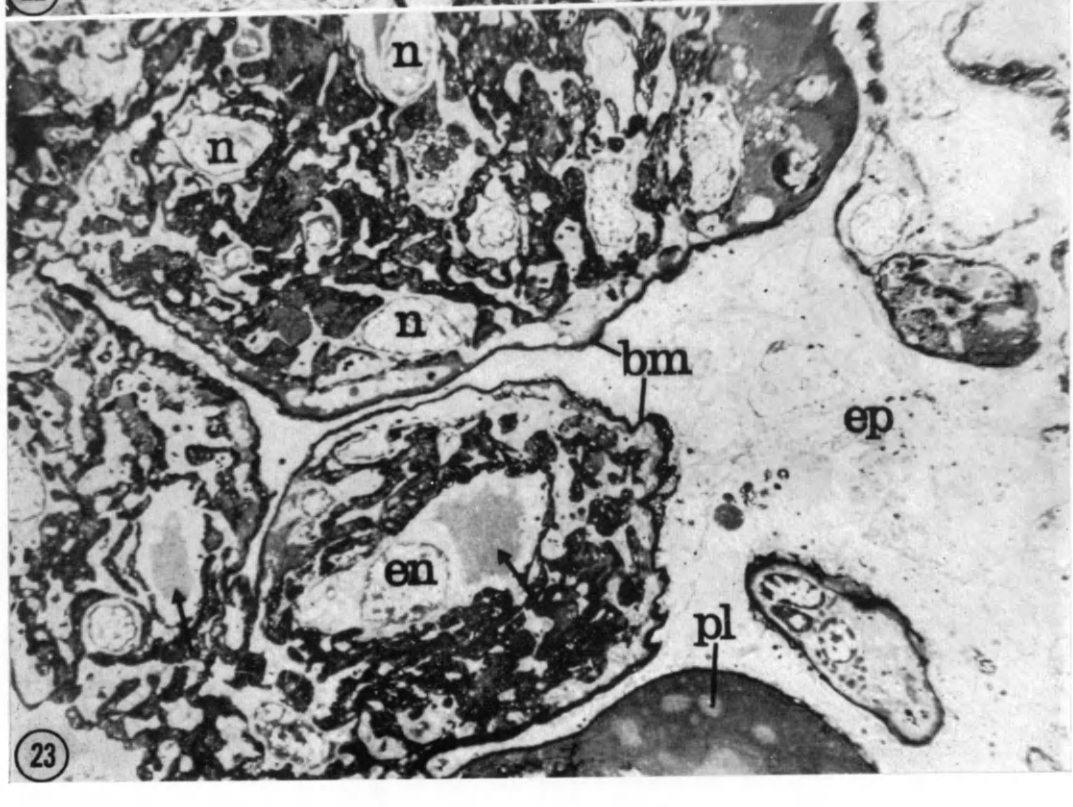
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swelling of the basement membrane is also unknown. At the present time the nature of the stimulus leading to mesangial proliferation and to the increased deposition of mesangial matrix is the subject of intensive investigation (see Section II, A, 3). The recent studies of Seegal *et al.* (1965) showing the presence of 7S gamma globulin, Beta 1C and antigens of type 12 streptococci in the glomeruli of patients with chronic glomerulonephritis are particularly relevant to this problem.

Although considerable "scarring" is present in chronic glomerulonephritis, the participation of collagen in this process is poorly understood. Steiner *et al.* (1962a) found no collagen in the mesangial matrix in well-established chronic glomerulonephritis although fibers of undetermined nature were present in this location. Spiro (1959) reported the occurrence of collagen in advanced cases of membranous glomerulonephritis but only where the glomeruli were completely hyalinized. Habib *et al.* (1961) found occasional collagen fibers in the hyaline nodules of lobular glomerulonephritis, and similar observations have been made by Hinglais (1961).

Movat *et al.* (1961b) have pointed out the similarities between acute, chronic, and membranous glomerulonephritis, and the long-standing lipoid nephrosis of children, all of which are characterized by swelling and imbibition of the basement membrane and an accumulation of deposits on the luminal side and in the mesangium. These authors believed that the deposits are ultimately converted into basement membranelike material. Regardless of whether the clinical history is that of lipoid nephrosis, chronic nephritis, or membranous glomerulonephritis, Movat *et al.* (1961b) concluded that changes in the mesangium and on the luminal side of the capillary membrane may be identical. The remarkable similarity between the intracapillary lesions in these three conditions does not extend to the extracapillary deposits (exudate).

Movat *et al.* (1961b) and Steiner *et al.* (1961) have suggested that the lack of exudate in lipoid nephrosis of children in contrast to the large diffuse deposits in membranous glomerulonephritis (lipoid nephrosis of adults) is due to the greater capacity of the epithelial cells in children to handle this exudate. The occurrence of focal subepithelial deposits in acute glomerulonephritis in children is considered by these authors to be due to a local breakdown or saturation of the epithelial cell

FIG. 22. Chronic glomerulonephritis. Electron micrograph from a somewhat advanced axial (mesangial) reaction with moderate expansion of the axis. Both axial (mesangial) cell hyperplasia and new PAS-positive material in axial regions lead to an increased prominence of these areas and trapping of some capillaries (arrows). Peripherally placed capillaries are still patent. Periodic acid-silver methenamine. Magnification: ×1128. From Steiner *et al.* (1962b).

FIG. 23. Terminal phase of the axial reaction. Obsolescent glomerular loops. Small capillary lumina (arrows) still remain. The irregularly thickened basement membrane forms the outer boundary of loops. On its luminal side lies a mixture of PAS-positive basement membrane-like material and some paler material of lesser electron density. The nuclei of proliferated cells can be seen between this material particularly in the loop at the upper border of the picture. These cells can only be designated as intracapillary, since they may originate from either axial or endothelial locations. Periodic acid-silver methenamine. Magnification: ×3530. From Steiner *et al.* (1962b).

clearing mechanism. The role of the epithelial cell in cases of increased permeability of the basement membrane has been discussed elsewhere (Section II, A, 2).

C. Experimental Nephritis and Nephrosis

1. *Nephrotoxic (Anti-kidney Serum) Nephritis*

Nephrotoxic (anti-kidney serum) nephritis has been the most extensively studied form of experimental glomerulonephritis. It is outside the scope of this paper to discuss in detail the clinical and immunological aspects of this condition. The readers are referred to the excellent reviews by Peters and Freedman (1959a, b, c) for further information on this subject. We shall, however, emphasize those morphological findings which resemble or help to understand some of the changes occurring in human glomerulonephritis. Although the exact functional changes and the sequence in which they occur vary with the type of experiment, it is generally agreed that the condition is biphasic. The early reaction occurs within 24 hours after injection of the nephrotoxic serum and is characterized by oliguria, hematuria, and mild proteinuria. After 5 to 10 days, the proteinuria becomes progressively more marked and may become irreversible.

It is difficult to compare the results described in nephrotoxic serum nephritis by various workers (Piel *et al.*, 1955; Simer, 1955; Reid, 1956; Miller and Bohle, 1957b; Sakaguchi *et al.*, 1957; Churg *et al.*, 1960; Movat *et al.*, 1961a; Arhelger *et al.*, 1961a; Suzuki *et al.*, 1963; Feldman *et al.*, 1963) because of the variations in response to the nephrotoxic serum according to species and also because of the differences in techniques employed. There is, however, general agreement that all the components of the capillary wall are involved to a certain extent shortly after the injection of the serum. The interpretation of the results is still an open question under active discussion, although the significance of the phenomena involved is becoming more clearly understood as indicated in a recent short review by Dixon (1965).

a. *Early Changes (12 to 72 Hours).* The earliest changes seen are swelling of the basement membrane with occasional splitting of the lamina densa. This finding has been described in mice (Simer, 1955; Reid, 1956), rabbits (Sakaguchi *et al.*, 1957), rats (Piel *et al.*, 1955; Churg *et al.*, 1960; Feldman *et al.*, 1963), and dogs (Movat *et al.*, 1961a). The last authors believed that swelling of the basement membrane resulted from the direct action of the nephrotoxic serum and was the morphological basis for the immediate proteinuria and considered the lesion analogous to that seen in lipoid nephrosis of children. Feldman *et al.* (1963) found that in the rat the thickening of the basement membrane was due to wispy deposits of a material denser than the membrane situated between the endothelium and the basement membrane (Fig. 24). Although this lesion usually occupied the full peripheral length of the capillary wall, there were some loops which were spared. On the basis of fluorescence microscopy performed in this material by Hammer and Dixon (1963), Feldman *et al.* (1963)

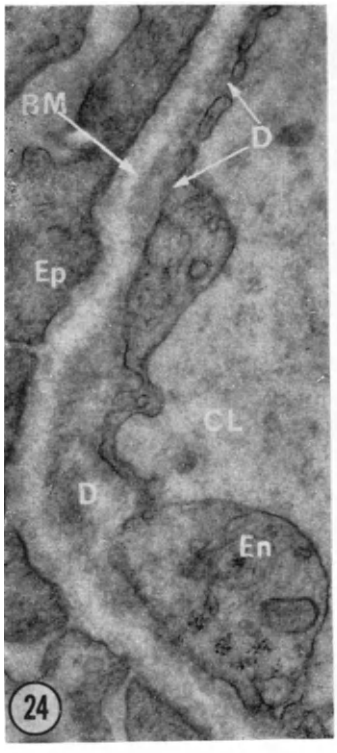
believed that the deposits seen with the electron microscope were partly or wholly composed of complexes of antigen (basement membrane), antibody (rabbit nephrotoxic serum), and host complement. These views are supported by the work of Andres *et al.* (1962) who were able, by means of ferritin-conjugated antibody techniques, to identify the specific glomerular sites at which nephrotoxic globulins localized in the basement membrane in acute nephrotoxic serum nephritis. These authors found that 1 week after the injection of the anti-kidney serum, the nephrotoxic globulins appeared most concentrated in the glomerular basement membrane and in the membrane-like material contained in distended cisternae of the endoplasmic reticulum. With a similar but modified immunochemical technique, Arhelger *et al.* (1963) showed that 12 hours after the injection of chicken anti-rabbit nephrotoxic serum into rabbits, the greatest amount of nephrotoxic agents were found fixed to the less dense zones of the basement membrane on either side of the lamina densa (Fig. 25).

The early cellular reaction in rats has been described by Piel *et al.* (1955) and Churg *et al.* (1960) as consisting mainly of swelling and vacuolization of endothelial cells. Such changes were considered to be responsible for the initial oliguria and azotemia seen in the animals (Churg *et al.*, 1960). Feldman *et al.* (1963), also working with rats, found the lesions in the cellular components of the glomeruli somewhat less marked and more comparable to those described by Movat *et al.* (1961a) in dogs. These latter authors found changes in the cellular components of the glomerulus which varied from minimal to moderate and consisted of fusion of foot processes of epithelial cells and marked prominence of the endothelial and mesangial cells owing to an increase in the number of cellular components, such as the Golgi apparatus and the endoplasmic reticulum.

Microvilli on the surface of the epithelial cells were described by Sakaguchi *et al.* (1957). In the initial stages of Masugi nephritis in the rabbit, these microvilli appear as rod-shaped projections about 50–100 mμ wide and 1 mμ in length. When cut transversally, they appear as short rods or small granules. Similar microvilli were noted in nephrotoxic nephritis in dogs (Movat *et al.*, 1961a) and rats (Churg *et al.*, 1960). The significance of these structures is not clear although they have been found in conditions associated with severe proteinuria induced by many different mechanisms, such as protein nephritis (Robertson and More, 1961), uranium nephropathy (Bencosme *et al.*, 1959), aminonucleoside nephrosis (Feldman and Fisher, 1961), and have also been found in patients with lipoid nephrosis (Steiner *et al.*, 1961).

b. *Late Changes.* The late changes affect all parts of the glomerulus and appear after the first or second week depending on the animal and the experimental procedure employed. Thereafter, they progress for months and eventually glomerular architecture may be markedly distorted or completely destroyed.

The most characteristic change is in the basement membrane which is increased in thickness mainly due to an extraneous deposit which occurs in the luminal side of the basement membrane and is only rarely found in the subepithelial position (Movat *et al.*, 1961a). This deposit is particularly well seen in electron micrographs from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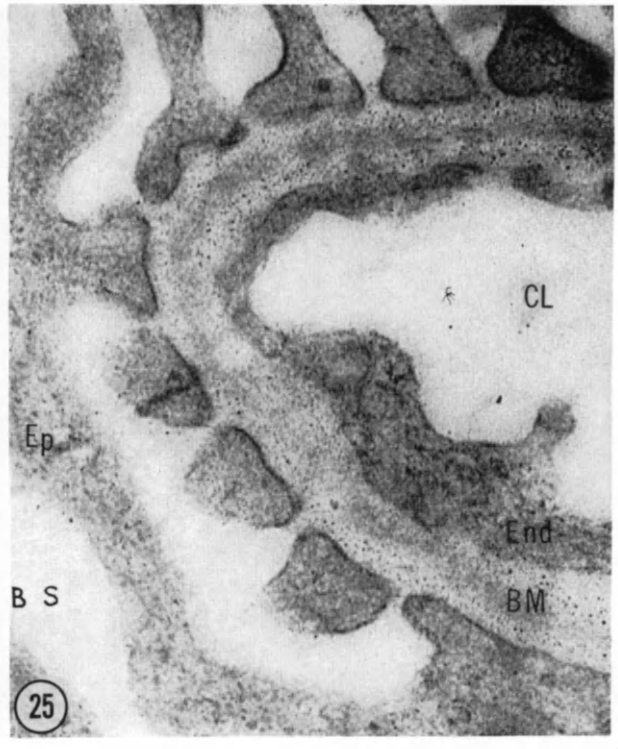
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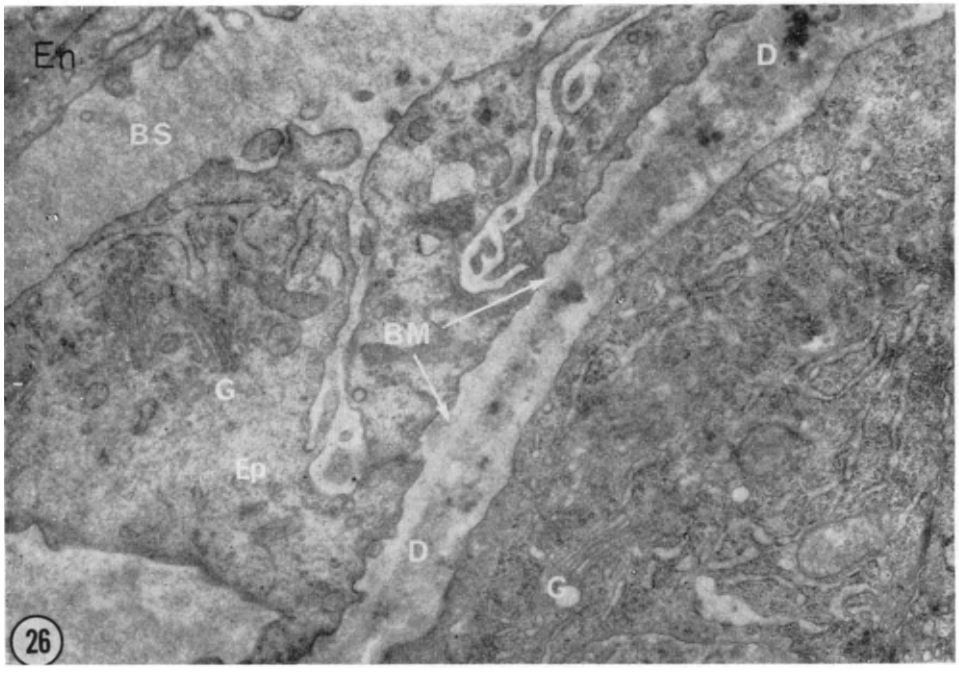
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periodic acid–silver methenamine-stained sections (Movat *et al.*, 1961a). In the rat, the most prominent change was considered by Feldman *et al.* (1963) to be related to the presence of "an inhomogeneous, dense subendothelial deposit that lifted off the endothelial cytoplasm from its basement membrane" (Fig. 26). In animals with nephrotoxic nephritis of more than 3-months duration, Movat *et al.* (1962) found focal argentophilic deposits on both sides of the basement membrane (Fig. 27). The subepithelial deposits resembled those found in membranous glomerulonephritis, although in the dog they were considerably more localized. Changes similar to those in membranous glomerulonephritis were also reported in nephrotoxic serum nephritis in rats by Churg *et al.* (1960), and lesions indistinguishable from this condition were seen in rats rendered nephrotic by injections of homologous kidney extract and *Hemophilus pertussis* vaccine (Blozis *et al.*, 1962).

Arhelger *et al.* (1961a) found they could increase the severity and frequency of chronic renal lesions if the animals received a gram-negative endotoxin in addition to the anti-kidney serum and noted that a finely granular fibrinoid material frequently appeared in the subendothelial position and in the basement membrane. This fibrinoid deposit contributed to the thickening of the basement membrane and was also found in the swollen endothelial cells. There was a striking morphological similarity between these electron microscopic changes and those found in lupus nephritis. Similar subendothelial fibrinoid deposits can be induced in rabbits by combination of nephrotoxic serum and low doses of abdominal radiation (Arhelger *et al.*, 1961b); severe radiation, however, prevents the development of glomerular damage and the demonstration of antibodies in the basement membrane by immunohistochemical techniques.

The cellular changes in the later stages of nephrotoxic nephritis are rather nonspecific (Movat *et al.*, 1961a; Feldman *et al.*, 1963). Endothelial cells show minor swelling, whereas epithelial cells show marked changes associated with proteinuria, such as fusion of foot processes, hyaline droplets, etc. Both endothelial and epithelial

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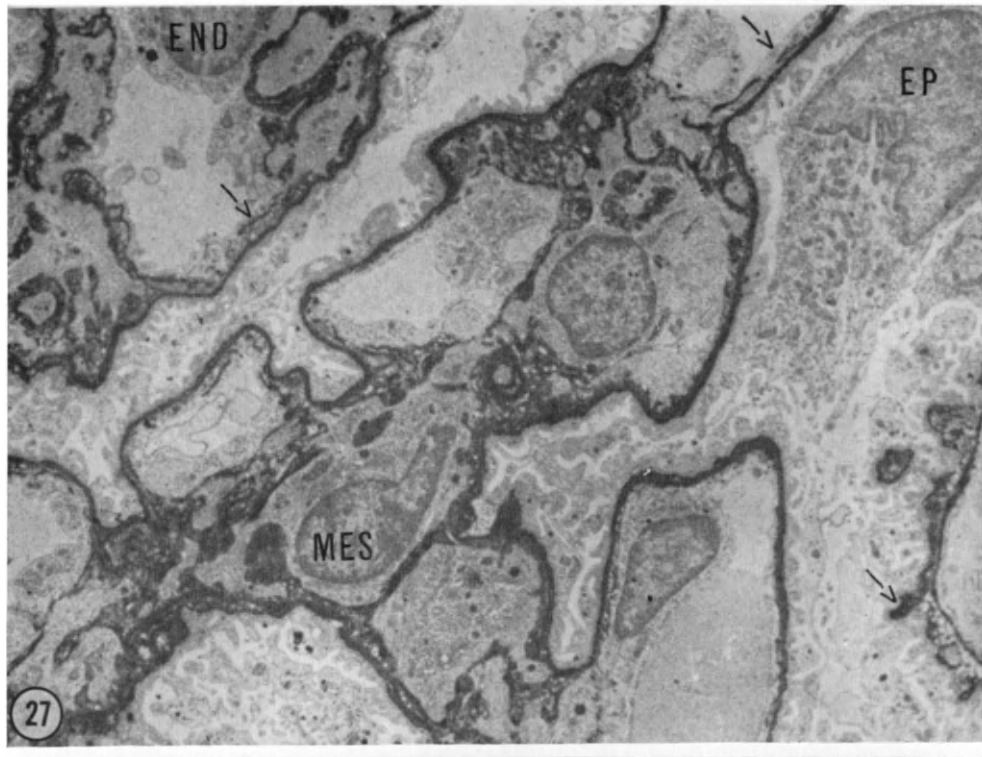
FIG. 24. Segment of capillary wall from a tolerant rat 1 day after injection of rabbit nephrotoxic serum (RNTS). The basement membrane (BM) is thickened by deposits (D) of dense material. Foot processes (Ep) are somewhat broadened. Endothelial cytoplasm (En) is lifted away from its basement membrane. Magnification: ×37,500. From Feldman *et al.* (1963).

FIG. 25. Glomerular capillary wall from a rabbit given nephrotoxic serum. The section was treated with ferritin-conjugated rabbit (anti-chicken globulin) gamma globulin (RAC gamma globulin). Note the dense concentration of ferritin along the basement membrane (BM). Ferritin is present also to a lesser extent in the endothelium (End) and epithelium (Ep). Occasional ferritin granules are seen in the capillary lumen (CL) and Bowman's space (BS). Magnification: ×60,000. From Arhelger *et al.* (1963).

FIG. 26. A segment of basement membrane (BM) from a normal rat 8 days after injection of rabbit nephrotoxic serum (RNTS). An inhomogeneous granular deposit (D) is visible next to or within the luminal side of the basement membrane. A part of the endothelial cytoplasm (En) displays an extensive, rough-surfaced endoplasmic reticulum and numerous free ribonucleoprotein particles. In the epithelial cell (Ep) are seen a Golgi zone (G) and several sacs of granular ergastoplasm near Bowman's space (BS). Magnification: ×18,000. From Feldman *et al.* (1963).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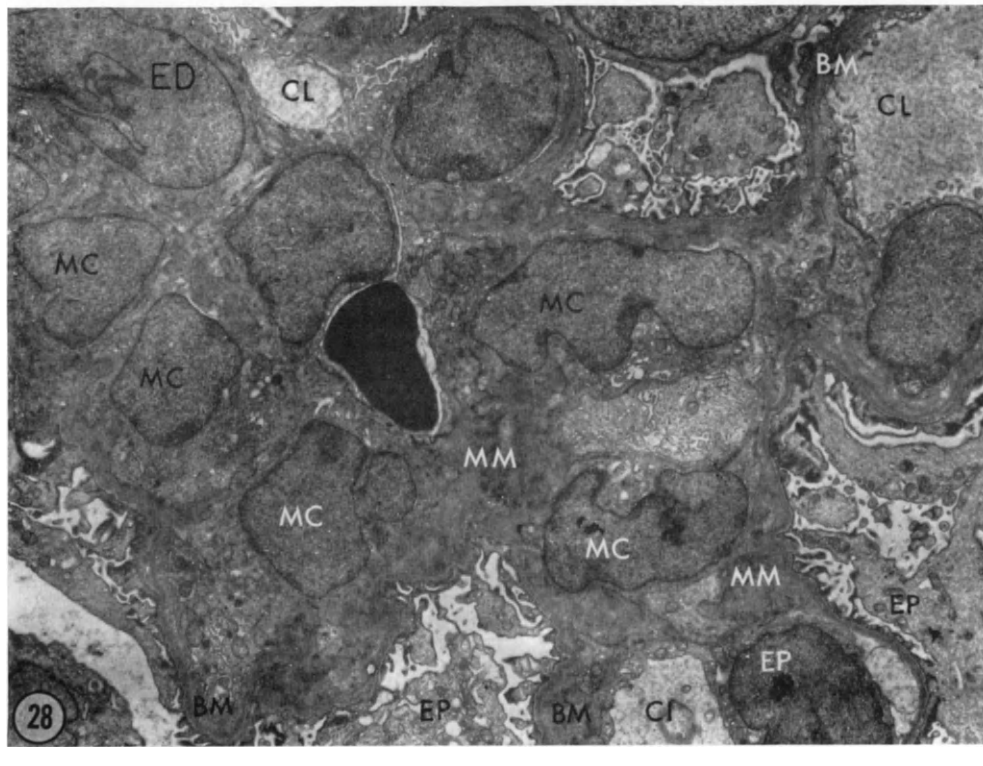
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cells also show an increase in the number of mitochondria, ergastoplasmic membrane, Golgi apparatus, and other cytoplasmic structures known to be associated with increased enzymatic activity and protein synthesis. These cellular changes are probably related to increased permeability of the basement membrane and to attempts by the cells to maintain the integrity of the membrane and perhaps to remove the extraneous deposits from it (Kurtz and Feldman, 1962a, b; Trump and Benditt, 1962; Strunk *et al.*, 1964; Feldman *et al.*, 1963).

Although most authors, notably Sakaguchi *et al.* (1957) and Suzuki *et al.* (1963), who have studied nephrotoxic serum nephritis have referred to the mesangium, the latter workers made a special study of the reactions in this tissue in rats injected with nephrotoxic serum. Three weeks after the administration of the serum these authors found edema of the mesangial matrix and proliferation of the mesangial cells (Fig. 28). "Unusual blood spaces" in the mesangium lined by mesangial cells were also observed by Suzuki *et al.* (1963) (Fig. 28). These may represent residuals of former lumens or may represent "canalization." In addition, mesangial fibrils became more prominent (Figs. 28 and 29), and as the lesion progressed, a small number of collagen fibers began to appear in the mesangial matrix (Figs. 29 and 30). Concurrently, small deposits of a dark granular material, presumably protein in nature, appeared in the mesangial matrix and occasionally within the mesangial cytoplasm. With the progression of time, the changes evolved so that by the third month there was a marked increase in the number of mesangial cells, mesangial matrix and collagen fibers (Fig. 30).

The interpretation of the late changes seen in nephrotoxic serum nephritis is still inconclusive, although Feldman *et al.* (1963) have suggested, on the basis of work employing normal and tolerant animals studied by immunofluorescent and electron microscopy, that in the secondary phase of nephrotoxic nephritis the host antibody reacts with a heterologous nephrotoxic antibody which, when bound to the glomerulus acts as an antigen to the host. This reaction appears to be responsible for the progressive and irreversible glomerular damage in contrast with the first phase reaction which is reversible and self-limited.

The ultrastructural changes, particularly those which appeared from the eighth to twelfth week, such as synechia, collapse of glomerular loops, elaboration of collagen with focal fibrosis, etc., were felt to be nonspecific in that they were not the direct

FIG. 27. Canine nephrotoxic serum nephritis of 3-months duration showing argentophilic deposits (arrows) on the luminal and to a lesser extent on the epithelial side of the basement membrane. There is mesangial cell (MES) proliferation and increased prominence of the mesangial matrix. Foot processes of epithelial cells are preserved in many areas. Periodic acid-silver methenamine. Magnification: ×4400. Courtesy of Dr. H. Z. Movat.

FIG. 28. Acute (Masugi) nephritis. There is marked cellularity of the mesangium. In the center of the lobule is a lumen filled by a red cell. This may be a residual of a former lumen or may represent "canalization." BM, Basement membrane; CL, capillary lumen; ED, endothelial cell; EP, epithelial cell (including foot process); MC, mesangial cell; MM, mesangial matrix. Magnification: ×3500. From Suzuki *et al.* (1963).

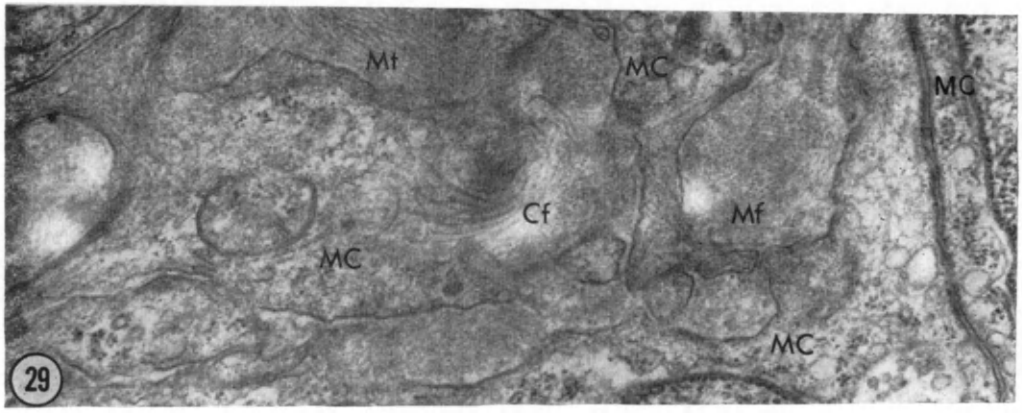

FIG. 29. Acute (Masugi) nephritis. Collagen fibers appear adjacent to a mesangial cell whose membrane has been cut tangentially. The mesangial matrix in the vicinity of the fibers contains numerous mesangial fibrils but is less dense in the area of fiber formation. Cf, Collagen fiber; MC, mesangial cell; Mf, mesangial fibrils. Magnification: ×28,000. From Suzuki *et al.* (1963).

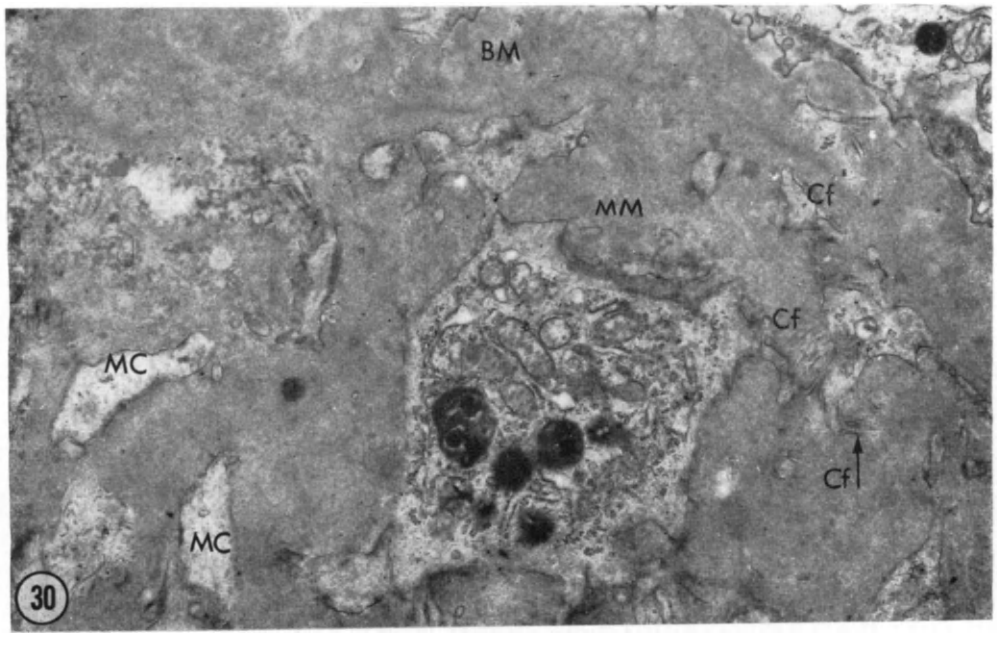

FIG. 30. Chronic (Masugi) nephritis. There is marked enlargement of mesangial matrix. Separation between the basement membrane and the mesangial matrix is not always clearly defined. Collagen fibers are found in the matrix adjacent to the mesangial cell membrane and occasionally in the basement membrane. The mesangial cell contains dense granules, some resembling lysosomes. BM, Basement membrane; Cf, collagen fiber; MC, mesangial cell; MM, mesangial matrix. Magnification: ×17,500. From Suzuki *et al.* (1963).

result of the immunological reaction but rather a general manifestation of glomerular injury. Similar nonspecific glomerular changes have been observed in a variety of diseases, such as aminonucleoside poisoning (Ericsson and Andres, 1961), uranium nephropathy (Bencosme *et al.*, 1959), diabetes (Farquhar *et al.*, 1959), and hydronephrosis (Pak Poy and Robertson, 1959).

The occurrence of fibrin formation in glomerular capillaries in nephrotoxic nephritis and in other glomerulopathies is well recognized (Churg *et al.*, 1960; Movat *et al.*, 1961a). However, no special pathogenetic significance was attributed to this finding until the recent work of Vassalli and co-workers (Vassalli *et al.*, 1963b; Vassalli and McCluskey, 1964; Simon and Chatelanat, 1963) suggesting that fibrin formation in glomerular capillaries is a most important factor in the development of the glomerular lesions seen in many forms of human and experimental nephritis. Vassalli *et al.* (1963b) induced intravascular fibrin formation in rabbits by injecting them with polyanetholsulfonate (Liquoid Hoffman La Roche), thromboplastin, or thrombin. The animals developed an acute lesion characterized by swelling and proliferation of endothelial and mesangial cells, leukocytic infiltration, which was predominantly neutrophilic and clumping of platelets. Deposits of fibrin and fibrinoid material (Figs. 31 and 32) were also seen usually between endothelium and basement membrane but also in the mesangium (Fig. 33). Phagocytosed fibrin was present in swollen endothelial and mesangial cells. Following the acute stage, these authors observed features indicating the development of progressive glomerular obliteration, such as mesangial proliferation with increased amounts of mesangial matrix, hyalinization, and collagen formation (Fig. 34). Various forms of basement membrane abnormality were also noted, such as abnormal subendothelial or subepithelial deposits and duplication or splitting of the lamina densa.

Vassalli and McCluskey (1964) showed that anticoagulation prevented the development of the fibrin and fibrinoid deposits and resulted in a marked diminution or suppression of intracapillary cell swelling and proliferation and complete prevention of crescent formation and glomerulosclerosis. Proteinuria, however, was not modified. On the basis of these findings, they concluded that the immune reaction within the glomerulus probably initiated the coagulation process leading to the formation of fibrin and fibrinogen derivatives in this condition. They suggested that phagocytosis of this material or its precursors by endothelial and mesangial cells led to swelling and proliferation of these cells and concluded that the coagulation process was an essential factor in the development of the glomerulosclerosis. These findings are of interest in view of the work of Kantor (1964) showing that mice and rats injected with type 12 streptococcal M protein, which contains a fibrinogen-precipitating factor, developed glomerular lesions characterized by deposition of eosinophilic, hyaline, material in the glomerular capillary bed. This material was identified as M-fibrinogen complexes by the immunofluorescent technique. Eight to 10 days after injection the animals developed secondary proteinuria at which time circulating anti-M antibodies were demonstrable. These findings suggest the possibility that similar alterations in the coagulation process may be responsible for


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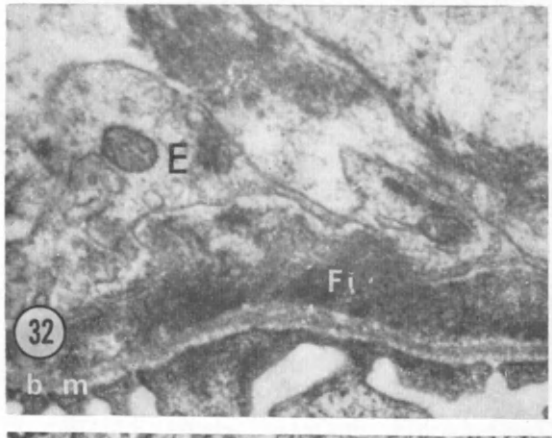
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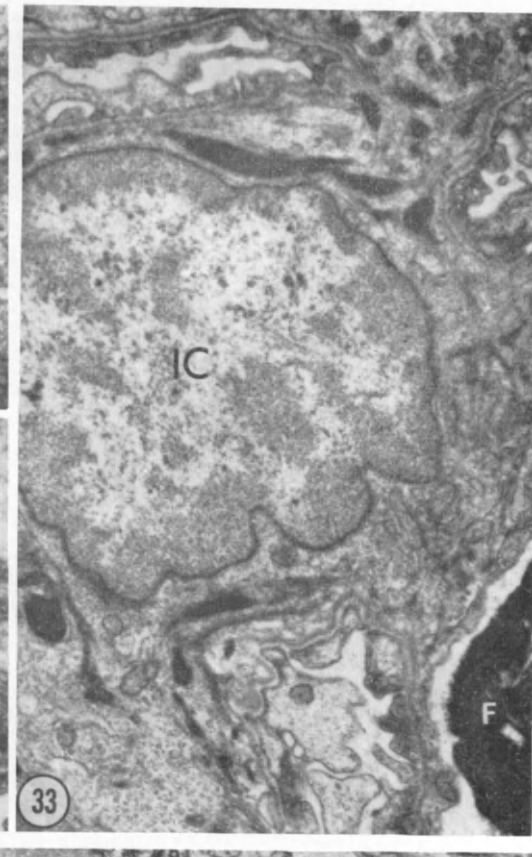
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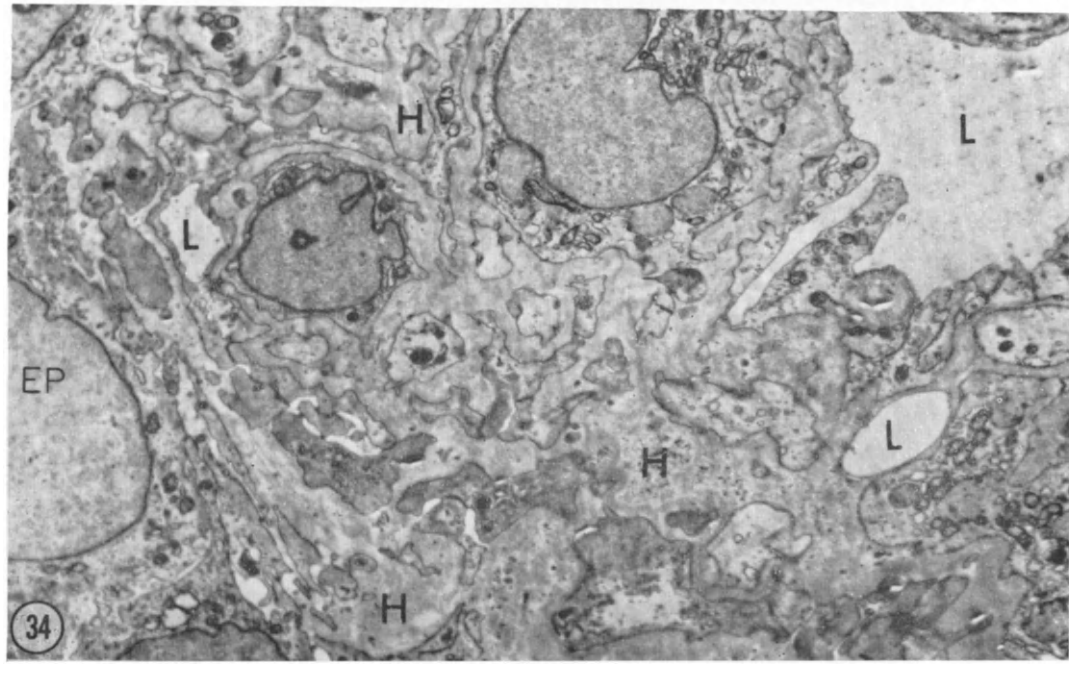
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the glomerular lesion induced by Group A streptococci in human subjects at least in some instances.

2. *Serum Sickness Nephritis (Foreign Protein Nephritis)*

Lesions resembling human glomerulonephritis have been produced experimentally by injecting one or two large doses of heterologous serum (antigen) or a purified derivative thereof into rabbits (Feldman, 1958, 1959; Sitte, 1959; Robertson and More, 1961). The lesions are self-limited and subsequently there is complete recovery.

In recent years repeated injections of moderate doses of heterologous serum or purified derivatives have been used with great success to produce a wide range of glomerular lesions resembling those found in human renal disease. If a state of antigen-antibody equivalence is maintained by daily measurement of antibody production and administration of an equivalent dose of antigen (Dixon *et al.*, 1961; Andres *et al.*, 1963), a certain percentage of the rabbits treated with bovine serum albumin will produce such large quantities of antibody that neutralization with exogenous antigen cannot be attained. Such animals usually developed an acute self-limited glomerulonephritis similar to that seen in animals receiving a single large dose of antigen. Animals which produced very little antibody did not develop any lesion. The most interesting animals, however, were those in which a state of antigen-antibody equivalence could be maintained for a prolonged period of time by daily injections of antigen. This group developed subacute and chronic glomerulonephritis similar to that seen in man.

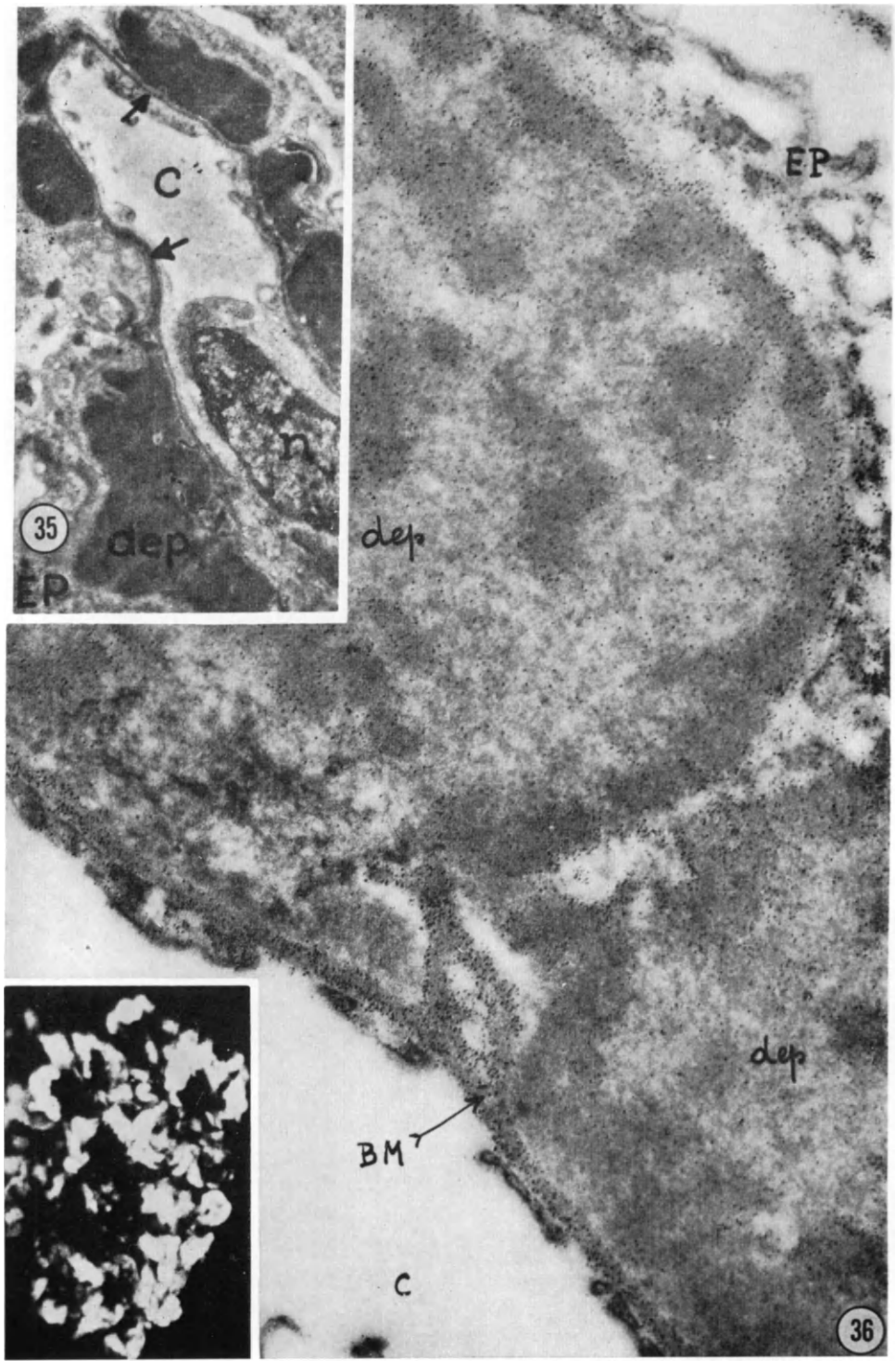
a. *Acute Glomerulonephritis.* Although a considerable amount of work has been done on the passage of heterologous protein across the glomerulus at the ultrastructural level, there are relatively few studies dealing with acute glomerulonephritis due to serum sickness. Feldman (1958, 1959) has described swelling and proliferation of glomerular endothelial cells as the most prominent change found in the glomeruli of animals with acute serum sickness disease. Although this author did not find thickening of the basement membrane, he noted a few focal deposits, usually in the

FIG. 31. Intravascular fibrin formation. Fibrillar fibrin (F) is deposited in the capillary lumen against somewhat swollen endothelial cells (E). Fibrin threads are in continuity with a granular substance (Fi) which has been deposited against the basement membrane (bm), apparently through a gap between two endothelial cells. Magnification: ×29,000. From Vassalli *et al.* (1963b).

FIG. 32. Intravascular fibrin formation. Granular fibrinoid (Fi) is deposited between the basement membrane (bm) and the endothelium (E). The capillary lumen contains similar material mixed with fibrin fibrils. Magnification: ×31,000. From Vassalli *et al.* (1963b).

FIG. 33. Intravascular fibrin formation. An intercapillary region contains fibrin (F), also present in the capillary lumen. IC, Intercapillary cell. Magnification: ×11,000. From Vassalli *et al.* (1963b).

FIG. 34. Intravascular fibrin formation. A severely damaged glomerulus. Marked deposition of hyaline material (H) distorts the normal glomerular structure. EP, Epithelium; L, capillary lumen. Lead staining. Magnification: ×5000. From Vassalli *et al.* (1963b).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luminal side of the membrane but sometimes found on the epithelial side as well. Feldman (1959), by correlating light and electron microscopy, concluded that the deposits seen in the luminal side of the basement membrane and in the mesangial matrix were fibrinoid in nature, whereas he was unable to determine the nature of those found subepithelially. The epithelial cells showed marked to moderate loss of foot processes in this condition.

Sitte (1959) studied changes in the basement membrane as early as 30 minutes after the injection of foreign protein into rats and concluded that there is a distinct thickening of the basement membrane even after this short interval, whereas the swelling of endothelial and epithelial cells is not significant at this stage. The basement membrane may be increased twofold, mainly as a result of thickening of the lamina densa. Sitte also remarked that there was a thickening of the mesangial matrix and an increased number of what he called endothelial vesicles. From the description and pictures, it is most likely that the latter correspond to the intraluminal processes of mesangial cells. From 1 to 3 days after the administration of foreign protein, this author noted persistent thickening of the basement membrane and progressive swelling and proliferation of endothelial cells, whereas 8 days after the injection of foreign protein, endothelial and epithelial cells appeared relatively normal. Sitte also concluded that the increased permeability to protein observed in these animals was the result of damage to the basement membrane by the foreign protein and that the rest of the changes were secondary to this increased permeability. The exact nature of the damage induced in the basement membrane is unknown.

A special study of the mesangial area was undertaken by Robertson and More (1961) who found in rabbits injected with bovine gamma globulin an increase in the number of mesangial cells and of the ergastoplasmic membranes. On the basis of their work, these authors insisted upon the ultrastructural differences between the mesangial

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FIG. 35. Serum sickness nephritis. A section of kidney tissue stained with silver methenamine. The capillary lumen (c), in the central part of the figure, is partially occupied by the nucleus of the endothelial cell (n). In the capillary wall two argentophilic components are visible; the basement membrane (arrows) and the deposits (dep) which are located between the basement membrane and the epithelial cytoplasm (EP). The epithelial foot processes are almost completely fused. Silver methenamine staining. Magnification: ×90,000. From Andres *et al.* (1963).

FIG. 36. Serum sickness nephritis. Section of a capillary wall from rabbit showing between the basement membrane (BM) and the epithelial cytoplasm (EP) large deposits (dep) of foreign material. The first component of the deposits is represented by aggregates of greater electron density which are tagged by ferritin-conjugated antibody to BSA. In the second lighter component only a few ferritin granules are localized. Ferritin localization is also evident in the glomerular basement membrane. Magnification: ×60,000.

In the insert there is a glomerulus from the same rabbit kidney, frozen in a carbon dioxide–butyl alcohol bath, sectioned in a cryostat at the thickness of 4 μ, "stained" with fluorescein-labeled rabbit anti-BSA, and photographed in ultraviolet light microscope. The fluorescent globulin is localized along the capillary walls with beaded appearance, which resembles the deposits seen by electron microscopy in Fig. 35. Magnification: ×350. From Andres *et al.* (1963).

and endothelial cells at a time when this distinction was not readily recognized by most investigators.

Acute glomerulonephritis was also produced by Dixon *et al.* (1961) in animals receiving repeated injections of heterologous protein which responded to this insult by the development of proteinuria and the production of a large amount of antibody. In general, the changes both by light and electron microscopy were similar to those described in the "one-shot serum sickness" (Feldman, 1958, 1959; Robertson and More, 1961). From correlated immunohistochemical and electron microscopic studies, Dixon and associates (1961) suggested that the antigen was localized in the subendothelial deposits.

b. *Chronic Glomerulonephritis.* The most constant and prominent alterations seen in chronic serum sickness glomerulonephritis consisted of extensive deposits of electron-dense material usually situated in the epithelial side of the basement membrane but frequently involving the lamina densa and the subendothelial space (Dixon *et al.*, 1961; Andres *et al.*, 1963). These deposits stained strongly with the silver methenamine technique (Andres *et al.*, 1963) and in the most severe cases imparted a beaded appearance to the basement membrane (Fig. 35). By immunohistochemical methods, including the use of ferritin-labeled antibody, Andres *et al.* (1963) demonstrated that large quantities of antigen were present in these deposits (Fig. 36). Ferritin granules were also seen to be localized in the glomerular basement membrane. In the deposits, some areas which were denser than others also reacted more intensely to the ferritin. The epithelial cells showed fusion of foot processes in relation to these deposits, whereas endothelial cells showed more moderate proliferation. The large number of subepithelial deposits and their general distribution closely resembled that seen in human cases of membranous glomerulonephritis. Some of the changes described disappeared with time, so that by the end of 6 months epithelial cells appeared normal, and the subepithelial deposits which were still present appeared less dense and their antigen content was remarkably diminished.

Results of studies with ferritin conjugated antibodies (Andres *et al.*, 1963) have shown that antigen and aggregates (probably antigen-antibody complexes) present in the blood cross the endothelium and basement membrane and accumulate in the form of dense deposits between the basement membrane and the epithelial cytoplasm. Andres *et al.* (1963) also made the interesting observation that contrary to what might be expected, it was difficult to visualize rabbit gamma globulin (presumably antibody to bovine serum albumin) in these deposits using the ferritin-conjugated antibody to rabbit globulin. They also demonstrated that rabbits, which were very active antibody producers and which died in anaphylactic shock following injection of bovine serum albumin, formed embolic deposits of antigen-antibody complexes in the lumen of glomerular capillaries.

While we have emphasized the immunological aspects of serum sickness nephritis, we should not omit mention of the interesting work of Vassalli and collaborators (Vassalli *et al.*, 1963b; Vassalli and McCluskey, 1964) on the glomerular lesions

resulting from intravascular fibrin formation already discussed in some detail in connection with serum nephritis (See Section II, C, 1). These authors were able to reproduce most of the lesions described in acute and chronic glomerulonephritis by manipulating fibrin formation in the glomerulus. Among other lesions, deposits on either side of the basement membrane were observed. These authors believed that fibrin may be present in association with immune complex since it is known that antigen-antibody complexes act on platelets to produce fibrin (Bettex-Galland *et al.*, 1963; Siqueira and Nelson, 1961) and that immune complexes can produce considerable deposition of fibrin in glomeruli *in vivo* (Lee, 1963).

3. *Aminonucleoside Nephrosis*

Since the discovery by Frenk *et al.* (1955) that aminonucleoside (6-dimethylamino-purine-3-amino-d-ribose) induced a nephrotic syndrome in rats, a tremendous amount of work has been done in an attempt to correlate the changes seen in these animals with those found in lipoid nephrosis of children. Many investigators have described the morphological changes at the electron microscopic level (Vernier *et al.*, 1959; Feldman and Fisher, 1959; Harkin and Recant, 1960; Ericsson and Andres, 1961; Lannigan *et al.*, 1962). Moreover, many experiments dealing with permeability have taken advantage of the marked increase in permeability to proteins which occurs in the glomerular capillaries of aminonucleoside nephrotic rats (see Section II, A, 2).

a. *Acute Stage.* During the acute phase following administration of aminonucleoside, animals show marked proteinuria, edema, and hyperlipemia. In these respects they are similar to human cases of lipoid nephrosis in children. At the ultrastructural level it is interesting that there is no change in the glomeruli of aminonucleoside-treated rats until immediately prior to the onset of proteinuria. The first alterations occur in the epithelial cells which show fusion of foot processes, numerous, rather large vacuoles, and dense bodies of different sizes and internal structures (Fig. 37). Pinocytic vesicles, in contact with the basement membrane, are increased in number. Similarly, microvilli which are usually rare in normal animals become quite abundant. Endothelial damage is characterized by swelling, proliferation, and partial obstruction of the capillary lumens (Ericsson and Andres, 1961). At a later stage, the mesangial region shows proliferated mesangial cells and increased amounts of basement membrane-like material (Vernier *et al.*, 1959). Movat (1962) has described the presence of a few collagen fibers in the basement membrane of rats 6 days following a single injection of aminonucleoside. Farquhar and Palade (1961), in the course of cytophysiological studies dealing with the permeability of glomerular capillaries, have made the important observation that desmosomal areas between foot processes of normal animals become narrower in aminonucleoside nephrosis and resemble tight junctions. These authors interpret this change as the formation of "water-tight" seals to prevent the loss of protein through the "filtration slit."

b. *Chronic Stage.* By various means, rats rendered nephrotic with aminonucleoside have been kept alive and studied for periods up to $1\frac{1}{2}$ years. Certain differences

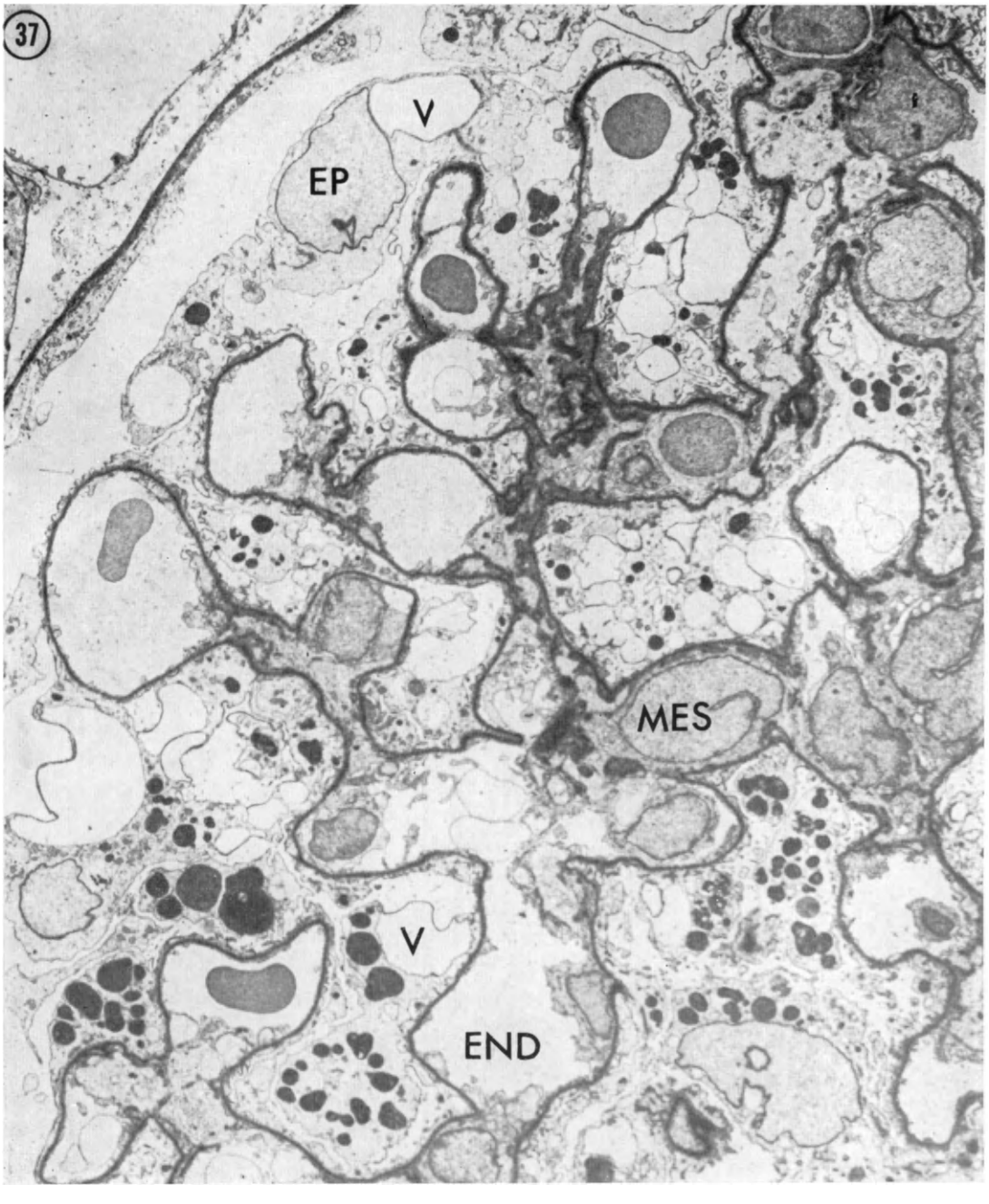


FIG. 37. Glomerulus from a rat with aminonucleoside nephrosis 6 days after a single injection. There is marked vacuolization (V) of epithelial cells (ep) and extensive loss of foot process. Large dark protein absorption "droplets" are abundant in epithelial cells. There is moderate proliferation of mesangial cells. Periodic acid-silver methenamine. Magnification: ×2200. Courtesy of Dr. H. Z. Movat.

exist between various reports which are probably due to the different manipulations of the animals. The results can be summarized by saying that after a period of prolonged proteinuria, those animals which have received the largest doses of aminonucleoside develop azotemia, hypertension, and renal failure (Ericsson and Andres, 1961). Proteinuria alone was present in those which received a single injection of aminonucleoside (Lannigan *et al.*, 1962).

The most obvious ultrastructural changes in the chronic stage of aminonucleoside nephrosis are thickening of the basement membrane and progressive accumulation of extraneous material of moderate density most commonly on the luminal side of the basement membrane but also on the epithelial side. The nature of these deposits is not clear, and it has been suggested that they may represent basement membrane-like material (Ericsson and Andres, 1961; Feldman and Fisher, 1961). The distribution of these deposits bears a striking resemblance to that seen in chronic cases of lipoid nephrosis of children by Movat *et al.* (1961b). No changes were observed in the basement membrane in animals which received only a single injection of aminonucleoside despite the fact that they had proteinuria for as long as 112 days (Lannigan *et al.*, 1962). These animals still showed cytological changes of the epithelial cells, such as loss of foot processes, osmiophilic droplets, and vacuolated cytoplasm after 112 days. Similar findings have been noted in all other cases of long-standing aminonucleoside nephrosis where, in addition, changes in the basement membrane were also seen.

Fusion between visceral and parietal epithelial cells was not infrequent at this stage. Endothelial cells showed varying degrees of swelling, and as the subendothelial deposits became larger, obliteration of the entire glomerulus occurred; and when seen under light microscopy, such glomeruli appeared completely hyalinized. Hyalinized glomeruli were seen in animals without hypertension, and the elevation of blood pressure occurring with azotemia and renal failure cannot be considered the cause of the glomerular changes (Ericsson and Andres, 1961).

Recently Fisher and Klein (1963) have treated animals simultaneously with aminonucleoside and adenine. The latter substance partially inhibits the action of the aminonucleoside so that the proteinuria is less severe. They were, however, unable to find the ultrastructural alterations in the glomerulus that could be responsible for the proteinuria. As a result of these studies, they concluded that aminonucleoside probably exerts its nephrotoxic effect on the lamina densa of the basement membrane rather than on the cellular elements of the glomerulus. Although their work suggests that the primary action of aminonucleoside is on the lamina densa, it does not rule out the possibility that the changes in this structure may be secondary to alterations either in the endothelial or epithelial cells. Whatever the explanation, once the basement membrane is damaged and more permeable to protein, proteinuria becomes established and the changes in the epithelial cells, such as loss of foot processes, hyaline droplets, vacuolization, increase in the endoplasmic reticulum and size of the Golgi complex, and development of microvilli are still considered to be secondary to the proteinuria. Finally, with the excessive passage of protein across the basement membrane, a certain amount is deposited subendothelially or subepithelially, as well

as in the basement membrane itself. As part of the same process of protein deposition in the basement membrane, the mesangium becomes overcharged with excess protein, and deposits also appear in the mesangial matrix. Eventually there is an increase in the mesangial matrix and in the total thickness of the glomerular basement membrane. This corresponds to what is normally referred to as hyalinization of the basement membrane and of the mesangial area.

D. Correlation between Bright's Disease and Experimental Nephritis and Nephrosis

The glomerular morphology of both Bright's disease and experimental nephritis and nephrosis can be considered in terms of the cellular reaction, the basement membrane changes, and the occurrence of deposits in the basement membrane and mesangial area. It must be recognized that the number of potential alterations which the glomerular structures can undergo in response to injury is limited. It is therefore not surprising that certain well-defined conditions such as membranous glomerulonephritis and lupus nephritis, while having a characteristic electron microscopic picture, may at times show ultrastructural changes common to both diseases. Despite this difficulty, the electron microscopic findings are often sufficiently characteristic to permit the recognition of distinct renal conditions which could not be diagnosed by light microscopy alone.

Over the past decade a variety of deposits occurring in the basement membrane and mesangial matrix in human and experimental renal disease have been extensively studied by electron microscopy. These studies have contributed greatly to our understanding of the morphology and pathogenesis of the deposits which are such a prominent feature of so many glomerular diseases.

For instance, the subendothelial deposits found in human cases of acute glomerulonephritis (Movat, 1960a; Movat *et al.*, 1962), progressive lipoid nephrosis (Movat, 1960a; Movat *et al.*, 1961b), and chronic glomerulonephritis (Steiner *et al.*, 1962a) resemble those described in nephrotoxic serum nephritis. The occasional focal subepithelial deposits seen in dogs with acute nephrotoxic serum nephritis are similar to those described in acute glomerulonephritis of children (Movat *et al.*, 1962). Dogs with chronic nephrotoxic nephritis develop subendothelial and subepithelial deposits which are morphologically analogous to those found by Steiner *et al.* (1962a) in human patients with chronic glomerulonephritis. The subepithelial deposits in nephrotoxic serum nephritis produced in rats by injection of kidney extract with adjuvant (Blozis *et al.*, 1962) closely resemble the changes seen in human cases of membranous glomerulonephritis. Repeated injection of foreign protein can produce comparable lesions in the rabbit (Dixon *et al.*, 1961).

Alterations in the basement membrane are usually associated with deposition of extraneous material; however, in some instances, changes have been described in the absence of deposits, and in other situations the basement membrane may appear morphologically normal despite significant functional derangement. In both amino-

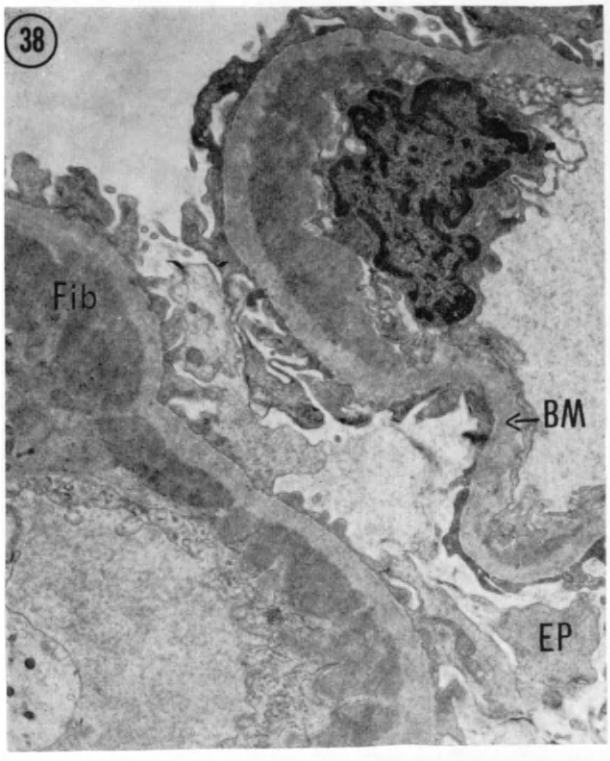
nucleoside nephrosis of the rat and idiopathic lipoid nephrosis of children, there is no evidence of basement membrane abnormality in the early stages, whereas in the chronic phase of both conditions, thickening of the basement membrane with deposition of basement membranelike material on the subendothelial surface can occur (Feldman and Fisher, 1961; Ericsson and Andres, 1961). Splitting of the basement membrane has been observed in acute glomerulonephritis in humans (Strunk *et al.*, 1964), nephrotoxic nephritis in the dog (Movat *et al.*, 1961a), and in experimental intravascular fibrin formation (Vassalli *et al.*, 1963b). Although there is no general agreement as to the pathogenetic significance of this finding, the suggestion by Strunk *et al.* (1964) that it may represent regeneration of the basement membrane from both endothelial and epithelial cells merits further investigation.

The cellular reaction in both clinical and experimental glomerular disease may be very similar. In conditions associated with proteinuria, the epithelial cells undergo characteristic, although nonspecific changes. In the early stages of acute glomerulonephritis, both experimental and human, there is proliferation and swelling of the mesangial and endothelial cells. These cells show large numbers of organelles, suggesting increased metabolic activity (Movat *et al.*, 1961a; Strunk *et al.*, 1964). The role of intravascular fibrin formation in the pathogenesis of Bright's disease and experimental nephritis has recently been emphasized by the work of Vassalli and co-workers (Vassalli *et al.*, 1963b; Simon and Chatelanat, 1963; Vassalli and McCluskey, 1964) who concluded that fibrin and its precursors are responsible for many of the cellular reactions of the endothelial and mesangial cells.

The role of immune mechanisms in both clinical and experimental nephritis is well recognized. The application of the immunoferritin technique to the study of the glomerular deposits has demonstrated the presence of immunological reactants in these lesions in nephrotoxic serum nephritis (Arhelger *et al.*, 1963), in serum sickness nephritis (Andres *et al.*, 1963), and in human patients with acute and subacute glomerulonephritis (Seegal *et al.*, 1965). The recent paper by Seegal *et al.* (1965) is of particular interest in that it revealed deposition of gamma globulin, complement, and type 12 streptococcal products in identical areas in an electron-dense material situated between proliferating endothelial and mesangial cells in capillary lumens on the endothelial side of the basement membrane and within basement membranes.

E. Disseminated Lupus Erythematosus

The glomerular lesion seen in systemic lupus erythematosus (SLE) may take several forms and also vary with the duration of the disease. In the very early stages there may be small focal areas of endothelial hypercellularity. Such lesions, referred to by Pirani *et al.* (1961) as lupus glomerulitis, are usually unaccompanied by abnormal urinary findings. In these very focal and minute lesions there is already some evidence of thickening of the basement membrane and fusion of foot processes. The picture, however, is not diagnostic of lupus although highly suggestive, since similar findings have also been seen in anaphylactoid purpura (Vernier *et al.*, 1961) and asymptomatic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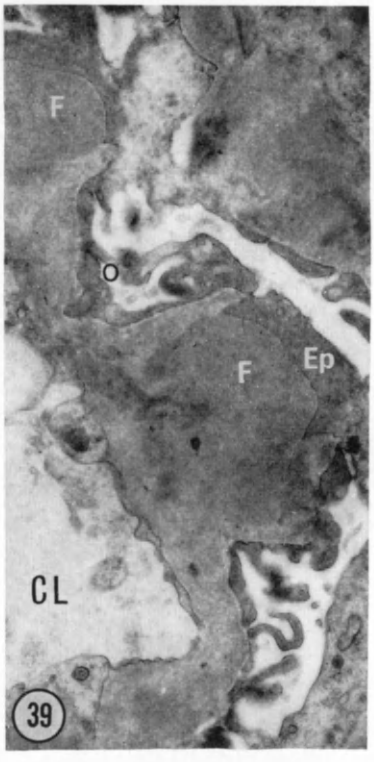
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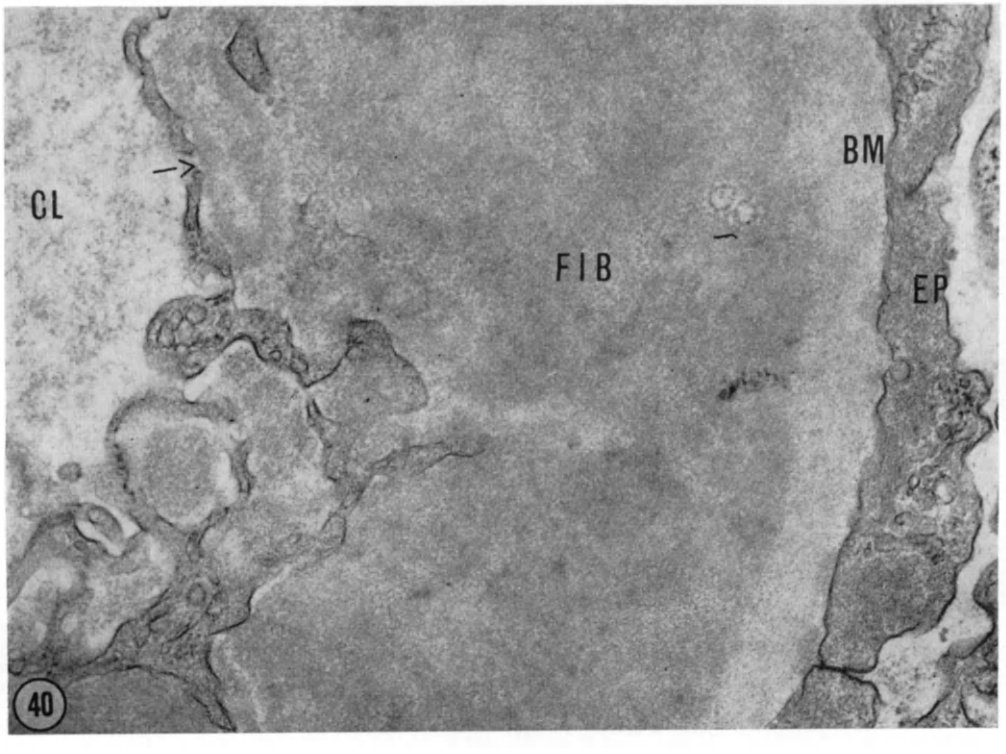
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persistent proteinuria (Pollak *et al.*, 1958). When severe renal lesions develop, they are characterized by focal glomerular necrosis, obliteration of capillary loops with fibrinoid changes, karyorrhexis, and formation of hematoxyphil bodies and have been referred to as lupus nephritis (Pirani *et al.*, 1961).

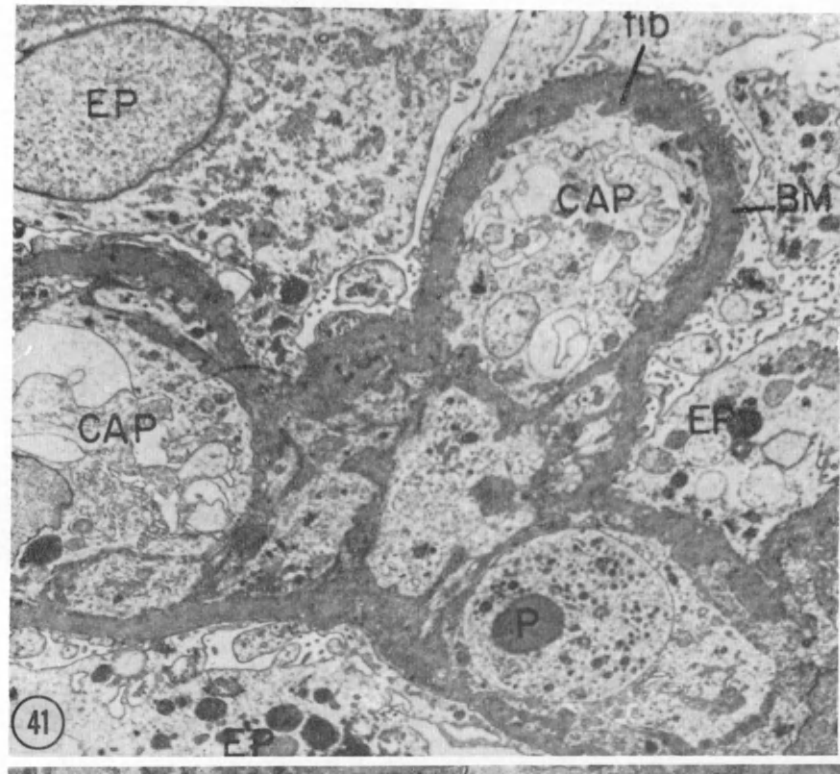
It is generally agreed that the most characteristic glomerular lesions seen by electron microscopy in lupus nephritis are marked endothelial cell proliferation, fibrinoid deposits, and thickening of the basement membrane, particularly in the peripheral glomerular loops (Farquhar *et al.*, 1957a; Vernier *et al.*, 1958; Farquhar, 1959; Spargo and Arnold, 1960; Spiro, 1960; Pirani *et al.*, 1961; Kimmelstiel *et al.*, 1962b; Brown *et al.*, 1963). The fibrinoid deposits are clearly recognized in electron micrographs by virtue of their great density and particulate nature (Farquhar, 1959). They are usually found between the basement membrane and the endothelium; however, a few deposits may be seen on the epithelial side and in the mesangium (Figs. 38–40). As a result of these fibrinoid deposits, the basement membrane appears considerably thickened by light microscopy, thus giving the so-called "wire loop" appearance (Fig. 38).

Changes in the epithelial cells are variable, although loss of foot processes is common in areas where there is extensive subendothelial fibrinoid deposition. Fibrinoid material is frequently seen in the subendothelial side of the basement membrane, and morphological evidence of phagocytosis of the fibrinoid material by the endothelial cells has been demonstrated by Brown *et al.* (1963). Arhelger *et al.* (1961a) have suggested that after pinocytosis by the endothelial cell, fibrinoid material is transported to the subepithelial region and eventually reaches the urinary space. Recently Vassalli *et al.* (1963b) have studied the glomerular lesions resulting from intravascular fibrin formation produced by different means and concluded that many of the ultrastructural changes observed were similar to those described in several human disorders, such as SLE. (The work of Vassalli and collaborators is discussed in more detail in Section II, C, 1.)

FIG. 38. Portions of two glomerular loops from a 13-year-old girl with typical disseminated lupus erythematosus for 7 months showing thickening of the basement membrane (BM) and extensive fibrinoid (fib) deposits. These are mostly subendothelial although some are also in the mesangial matrix. Epithelial cells (EP) show areas where foot processes are lost and the cytoplasm of these cells is very dense. Fibrinoid is recognized by its great density and finely particulate appearance. Magnification: ×8000. Courtesy of Dr. C. G. Biava.

FIG. 39. Adjacent glomerular capillary walls from a case of systemic lupus erythematosus (10-year-old female) showing extensive deposition of fibrinoid (F). The fibrinoid is recognized by its density and deposits are seen subendothelially, within the lamina densa (BM), and in a subepithelial location. In two areas broadened epithelial foot processes (Ep) adjacent to fibrinoid deposits are extremely dense, possibly indicating diffusion of the material into the cytoplasm. Magnification: ×9300. Courtesy of Dr. R. B. Arhelger.

FIG. 40. A higher magnification from the same biopsy as in Fig. 38 shows a large deposit of coarsely granular fibrinoid (FIB) on the luminal side of the basement membrane (BM) and also in the endothelial pores (arrow). Capillary lumen, CL; epithelial cell, EP. Magnification: ×34,000. Courtesy of Dr. C. G. Biava.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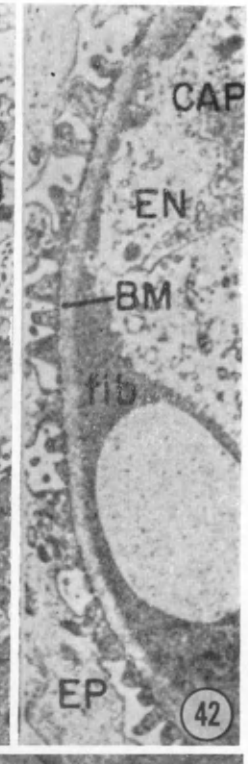
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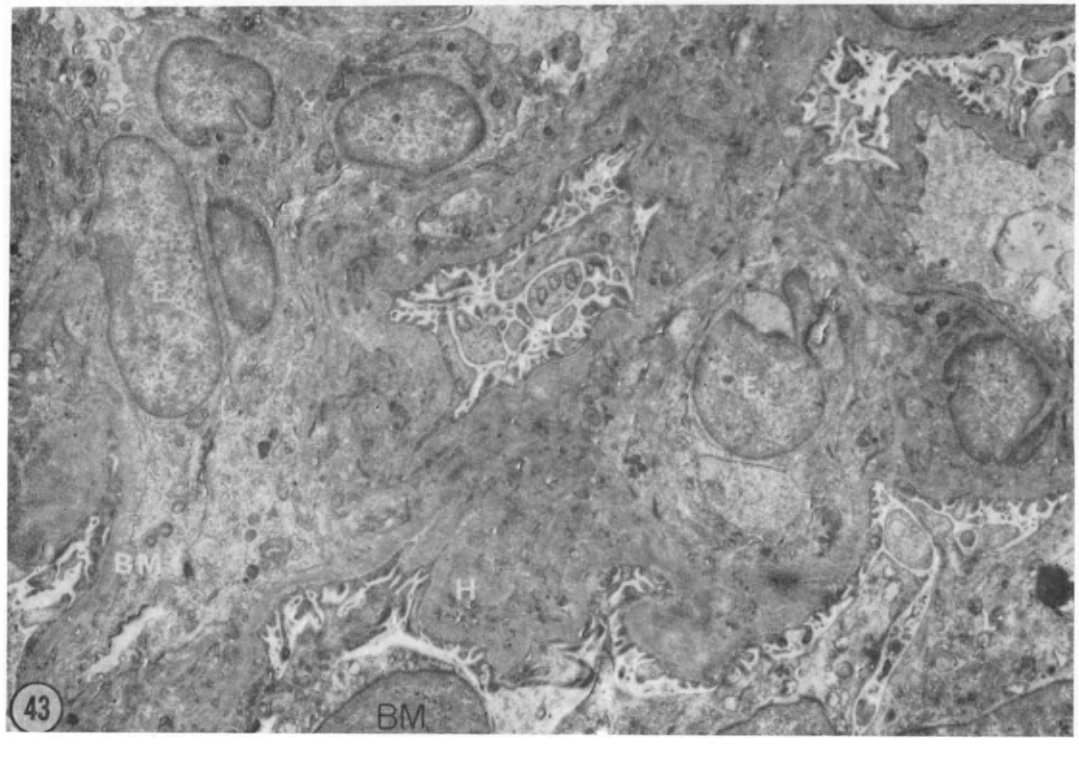
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Recent work by Wolfe *et al.*, (1963) has shown that it is possible to induce lesions similar to those found in lupus nephritis by infusing dogs with plasma from patients suffering from SLE. By the use of fluorescent antibody techniques, they concluded that an immunological mechanism was involved in the production of these lesions. Although electron microscopic studies were not made, their work suggests a model for further exploration of the interrelationships between the cytotoxic factor and the development of the renal lesions in SLE.

In an excellent discussion of the literature dealing with the relation between changes in ultrastructure and functional derangements in SLE, Harvey *et al.* (1963) suggested that the association of decreasing globulin clearance and clinical improvement observed in patients with SLE could be explained by two different pathogenetic mechanisms. In the first instance the improvement may simply reflect a diminution of the rate of deposition of gamma globulin within the kidney. Alternatively, the decrease in the globulin clearance may result from improvement in the integrity of the basement membrane, i.e., a decrease in permeability to large molecules (globulin). Since the available data is not adequate to distinguish between these two possibilities, it is hoped that further studies may elucidate the role played by the plasma proteins in the ultrastructural and clinical evolution of lupus nephritis.

F. Toxemia of Pregnancy

The glomerular lesions in pre-eclampsia and eclampsia have been the object of several electron microscopic studies in recent years (Spargo *et al.*, 1959; Farquhar, 1959; Altchek, 1961; Hopper *et al.*, 1961; Mautner *et al.*, 1962; Fiaschi and Naccarato, 1962; Guilhem *et al.*, 1962; Pirani *et al.*, 1963a; Meriel *et al.*, 1963). It is generally

FIG. 41. Portion of a glomerulus from a patient with pre-eclampsia and the nephrotic syndrome shown at relatively low magnification. Severe endothelial swelling has greatly restricted the capillary lumina (CAP). Moreover, the cytoplasm of many of the epithelial cells (EP) contains large numbers of vacuoles and dense bodies (i.e., hyaline or protein absorption droplets). A thin layer of fibrinoid (fib) is present between the endothelium and basement membrane (BM) in virtually every loop. At this magnification it is difficult to distinguish the fibrinoid deposits from the basement membrane. Magnification: ×5600. From Hopper *et al.* (1961).

FIG. 42. Glomerular loop from patient with pre-eclampsia. Pronounced swelling of endothelial cytoplasm (EN) has greatly restricted the lumen. Fibrinoid (fib) is present subendothelially between the endothelium and the basement membrane (BM) (subendothelial). Fibrinoid may be distinguished from basement membrane by virtue of its greater density and punctate texture. The epithelial foot processes and basement membrane appear essentially normal. Magnification: ×11,500. From Hopper *et al.* (1961).

FIG. 43. History: This is the case of a 21-year-old negro who developed pre-eclampsia during the third trimester of her first pregnancy. Biopsy at that time showed scattered epithelial foot process fusion, mild to moderate thickening of lamina densa, mild degree of endothelial swelling and proliferation. No fibrinoid deposition was seen in this case. The micrograph is from her second biopsy $1\frac{1}{2}$ years later. It shows extensive thickening of the basement membrane (BM), and mesangial matrix by hyaline material (H). The capillaries are nearly occluded by endothelial cells (E). Magnification: ×3900. Courtesy of Dr. R. B. Arhelger.

agreed that the changes in pre-eclampsia and eclampsia differ only in the severity of the lesion, being most dramatic in eclampsia.

The pathological picture is characterized by reduction of the lumen of the glomerular capillaries owing mainly to marked proliferation and swelling of both the endothelial and mesangial cells (Fig. 41). Endothelial cells show extensive vacuolization, bleb formation, and prominence of most cellular organelles, resulting in obliteration of most of the endothelial fenestra. Material resembling the lamina densa has also been described in endothelial cells (Pirani *et al.*, 1963a). Between endothelial cells and basement membrane, there is frequently a deposit of finely granular or amorphous material of varying electron density (Fig. 42). Some of the darker deposits have been considered to correspond to the fibrinoid seen by light microscopy (Harper *et al.*, 1961; Pirani *et al.*, 1963a; Meriel *et al.*, 1963). It is of interest that in fatal eclampsia, fibrin thrombi have been found in the glomeruli by some workers (Pirani *et al.*, 1963a).

When proteinuria was severe, similar deposits were also found in the mesangial area, including mesangial cells. In cases with clinical evidence of toxemia for a few weeks or more, the mesangial cells and the mesangial matrix were very prominent owing to swelling and proliferation of the mesangial cells, as well as to an increase in the deposits in the mesangial matrix (Mautner *et al.*, 1962; Meriel *et al.*, 1963).

Moderate and focal thickening of the basement membrane has been described by some (Pirani *et al.*, 1963a; Meriel *et al.*, 1963); whereas others considered the changes minimal or absent (Harper *et al.*, 1961; Mautner *et al.*, 1962).

The epithelial cell changes are very mild even in the presence of proteinuria and are mostly related to the formation of hyaline droplets, a few vacuoles, and moderate swelling. The foot processes in general appear normal. Focal fusion of foot processes may be found in some loci, particularly in relation to severely damaged glomerular loops, but in general the area involved accounts for only a very small proportion of the total loop.

Several investigators have been able to obtain biopsies before and after delivery, thus establishing that, in general, most of the changes observed during the toxemic stage may disappear with time (Harper *et al.*, 1961; Pirani *et al.*, 1963a; Mautner *et al.*, 1962; Meriel *et al.*, 1963). However, there is no general agreement as to the extent and speed at which these changes can regress. In fact, Mautner *et al.* (1962) found swelling of endothelial cells as late as 2 years post partum, and in these cases there was also some degree of enlargement of the mesangial area. Moreover, Meriel *et al.* (1963) found a group of cases "dysgravidie mineure" in which fibrinoid deposits were absent but in which the histological changes were suggestive of irreversible chronic glomerulonephritis. Arhelger (1965) is also of the opinion that in some patients with pre-eclampsia fibrinoid deposits are absent, but changes similar to those in early chronic glomerulonephritis are found. These patients do not go into remission but instead develop chronic renal disease progressing to eventual azotemia 1 or 2 years later. Morphologically, these individuals show changes consistent with chronic glomerulonephritis (Fig. 43).

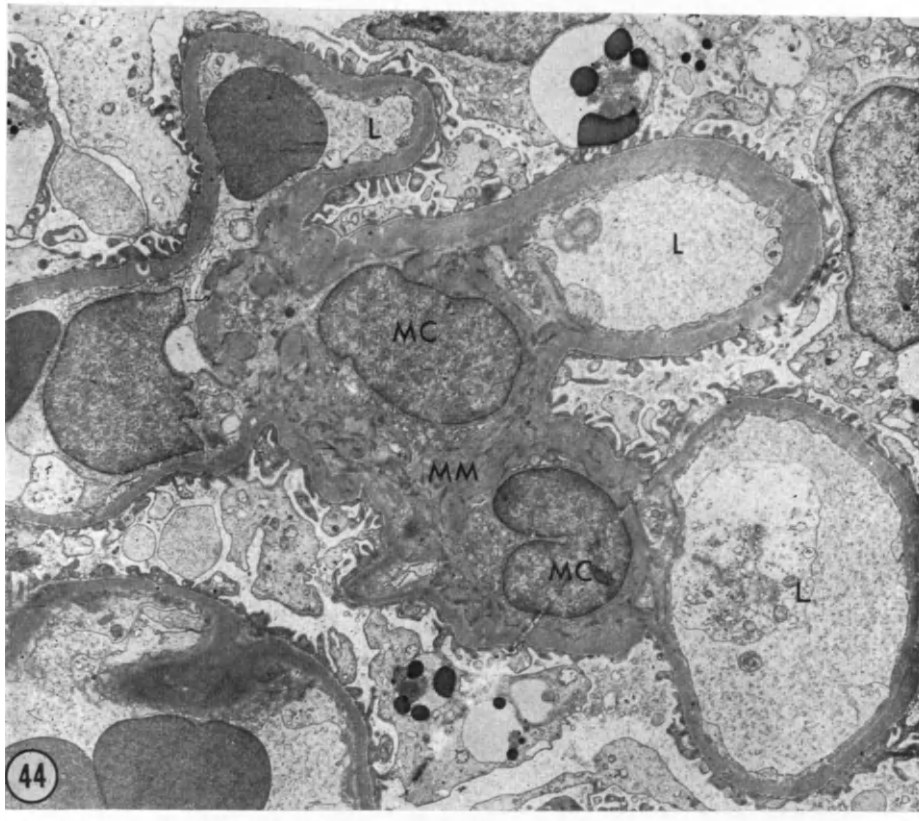
Harper *et al.* (1961) believe that endothelial and mesangial cells are probably responsible for the removal of the fibrinoid material from the glomerular capillaries. Certainly endothelial swelling seemed to be the most significant lesion and was always present when the patient had clinical evidence of toxemia at the time of biopsy. Similarly, the finding of deposits was directly related to the presence of proteinuria at the time of biopsy, whereas changes in the foot processes were absent despite the presence of proteinuria.

In recent years, Vassalli *et al.* (1963a) have studied the role of a prolonged intravascular clotting time on the accumulation of fibrin in glomeruli by immunofluorescence and have shown that fibrin is basic to the pathogenesis of glomerular damage, not only in pregnancy but in many other conditions. These authors found that fibrin was deposited along the basement membrane, whereas gamma globulin was only occasionally demonstrated, generally in the form of irregular deposits. Since fibrinoid material, as well as the other deposits may disappear post partum, one may question to what extent mesangial, endothelial, or epithelial cells contribute to the removal of this material in toxemia of pregnancy and also in other conditions where fibrinoid deposits occur, such as lupus and diabetic glomerulosclerosis. It is known from experimental studies in animals that the cellular components of the glomeruli play an important role in the removal of fibrin deposits, and it is probable that the same mechanism may be operative in toxemia of pregnancy in humans (Vassalli *et al.*, 1963b).

G. Diabetic Glomerulosclerosis

Among the human renal conditions investigated by electron microscopy, diabetic glomerulosclerosis has been one of the most extensively studied. This is probably due to the fact that although this condition can be suspected in a diabetic with proteinuria, an unequivocal diagnosis requires a renal biopsy. In addition, the pathogenesis of diabetic glomerulosclerosis has long been a source of controversy, particularly with respect to the existence of the mesangium. Moreover, the glomerulus is one of the most important sites of diabetic microangiopathy which has recently been the subject of numerous clinicopathological investigations. For further information regarding the nature and clinical course of this condition, the reader is referred to a recently published "Proceedings of a Conference on Small Blood Vessel Involvement in Diabetes Mellitus" (Siperstein *et al.*, 1964).

The electron microscopic picture is characterized by focal accumulations of hyaline, nodular deposits in the mesangial region, and diffuse deposition of hyaline material in the walls of glomerular capillaries (Farquhar *et al.*, 1959; Bergstrand and Bucht, 1959, 1964; Kimmelstiel *et al.*, 1962a; Farquhar, 1964; Azerad *et al.*, 1964; Ireland *et al.*, 1964; MacDonald and Ireland, 1964; Bloodworth and Engerman, 1964; Dachs *et al.*, 1964). It is generally believed that the earliest changes appear in the mesangium where the delicate strands of mesangial matrix become widened two- to threefold (Fig. 44) (Dachs *et al.*, 1964). Mesangial fibrils which are present in the



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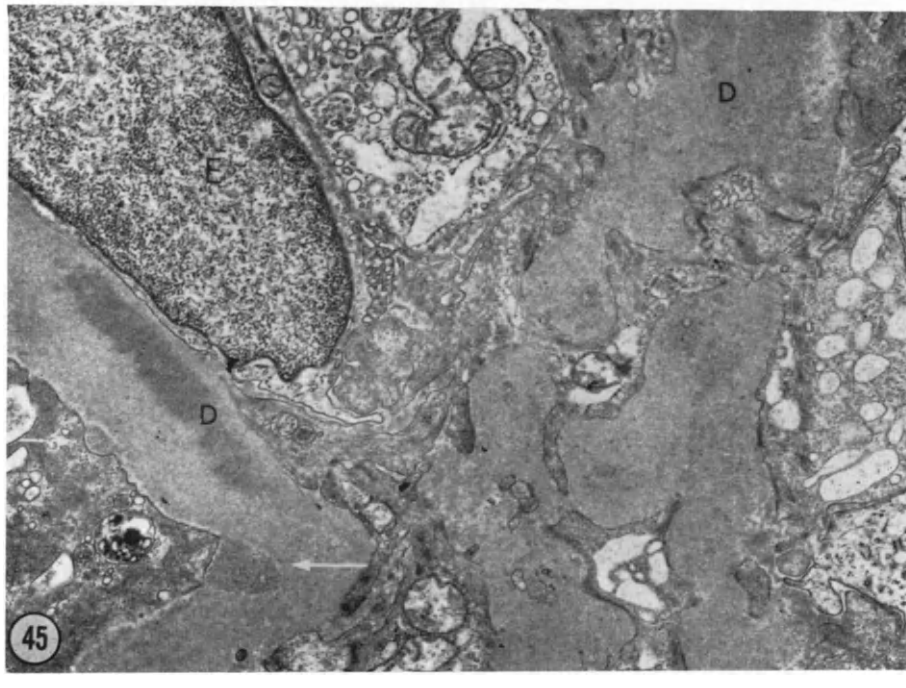

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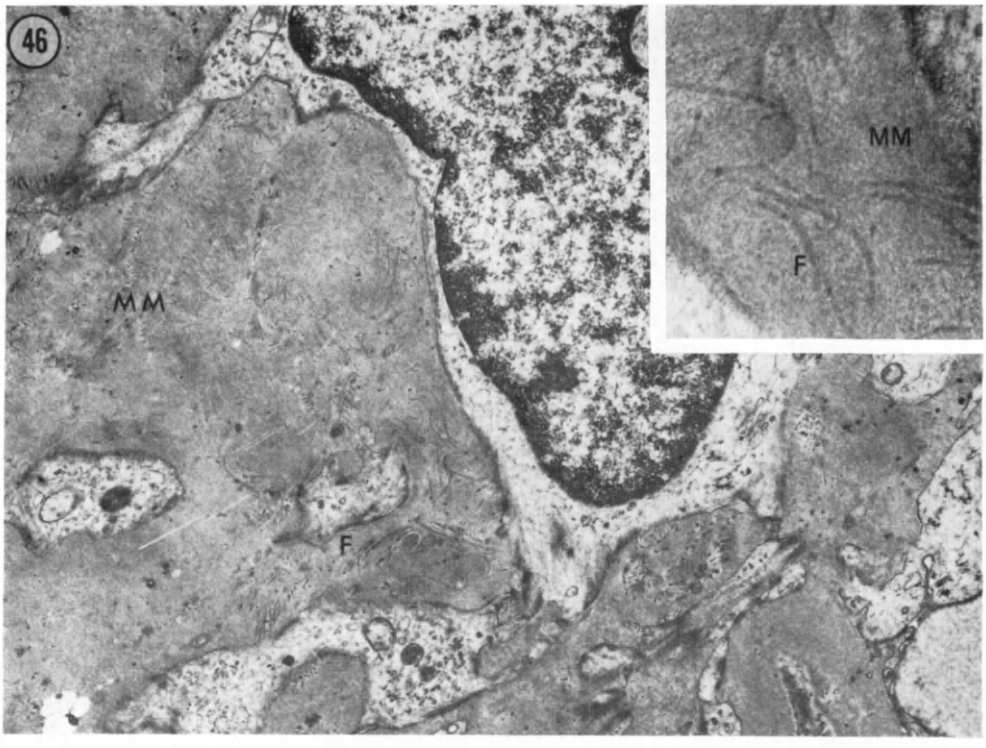
mesangial matrix appear normal at this stage (Suzuki *et al.*, 1963; Farquhar, 1964; Dachs *et al.*, 1964). Concurrently the electron opacity of the mesangial matrix increases so as to resemble and sometimes even exceed the density of the basement membrane proper (Fig. 45) (Suzuki *et al.*, 1963; Dachs *et al.*, 1964). Eventually the mesangial matrix increases in amount to form the typical nodular lesions of diabetic glomerulosclerosis (Fig. 46). These nodular lesions may contain in addition to mesangial matrix, heterogeneous deposits composed of osmiophilic droplets, amorphous accumulations of electron-dense granular material, and immature and mature collagen fibers (Fig. 46). Recent work by Farquhar (1964) has shown that a significant proportion of the "hyaline" material in diabetic glomerulosclerosis is composed of substances similar to the mesangial matrix including the presence of the 110 Å fibrils. The mesangial cells show little change, although according to Farquhar (1964) cytoplasmic organelles are even less developed than normally. Accumulation of granular, electron-dense material which is probably protein, as well as some small electron-dense droplets, presumably lipid, may be seen in mesangial cells (Suzuki *et al.*, 1963).

The most characteristic and earliest change in the capillary wall in diabetic glomerulosclerosis is a twofold or greater thickening of the basement membrane (Fig. 44). The normal fine fibrillarity of the basement membrane is exaggerated, and deposits of granular material similar to those found in the mesangial matrix can be seen on both epithelial and endothelial sides of the membrane (Fig. 45) (Dachs *et al.*, 1964). Extensive accumulations of a similar type of material under the endothelium has been referred to as the "exudative" lesion (Farquhar *et al.*, 1959; Dachs *et al.*, 1964). The fine structure of the so-called exudative lesion has been described by Farquhar *et al.* (1959) and distinguished from the fibrinoid deposits appearing in disseminated lupus erythematosus, eclampsia, and subacute and chronic glomerulonephritis, in which similar types of fibrinoid deposits may also occur. In diabetes, this fibrinoid deposit characteristically appears as a crescentlike homogeneous mass, the "fibrin" cap which may eventually occlude the capillary lumen. The significance of the exudative lesion in diabetes is unknown.

Endothelial and epithelial changes are not marked except when there is a severe nephrotic syndrome, in which case there may be swelling and vacuolization of both endothelial and epithelial cytoplasm. Dachs *et al.* (1964) have made the interesting anatomoclinical correlation that in patients with a nephrotic syndrome there was a membranous transformation of the glomerular basement membrane, although mild degrees of basement membrane thickening might be present without proteinuria.

FIG. 44. Early diabetic glomerulosclerosis. A glomerular lobule exhibits thickened capillary basement membranes and increased mesangial matrix ("basement membrane-like material") (MM). Two cells (MC) are seen in the mesangium. L, Capillary lumen. Magnification: ×3250. From Dachs *et al.* (1964).

FIG. 45. Diffuse diabetic glomerulosclerosis. Finely granular electron-dense deposits (D) appear at the lower left within the basement membrane and along the epithelial surface of the membrane (arrows). At the upper right, similar material lies within the mesangial matrix. E, Endothelial cell. Magnification: ×14,250. From Dachs *et al.* (1964).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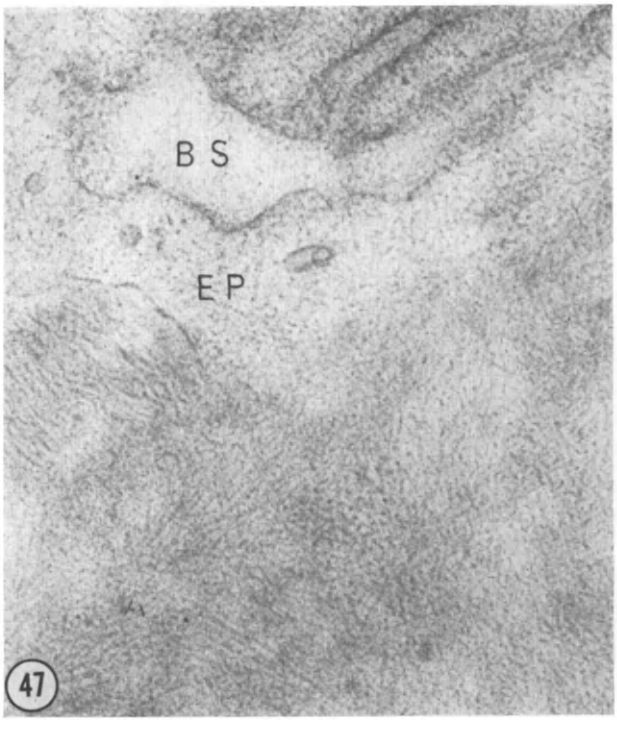
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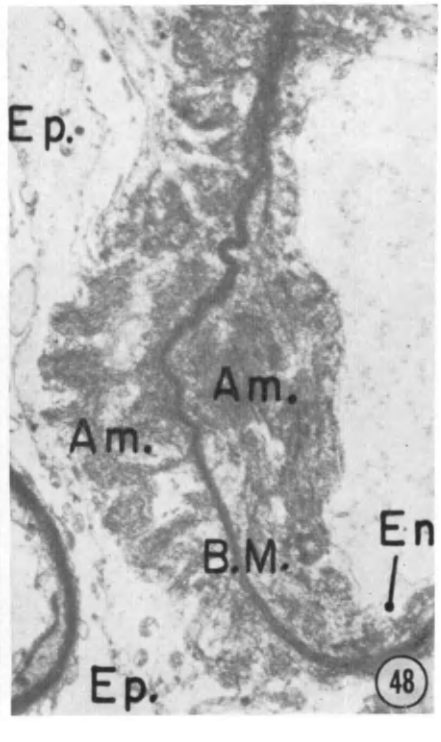
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Dachs *et al.* (1964) have suggested that under normal conditions precursors of the basement membrane and mesangial matrix are carried by the blood to the mesangial area where they are polymerized by the mesangial cells. The disturbance of carbohydrate metabolism in diabetes may somehow facilitate an excessive deposition of mesangial matrix. It is of interest that in diabetics, microangiopathies are found elsewhere in the skin, eye, and skeletal muscle. The outstanding lesion is also characterized by extensive thickening of capillary basement membranes. Fine collagen fibers, such as those found in diabetic glomerulosclerosis, were also common in the thickened capillary basement membrane of skeletal muscles in diabetic patients (Bencosme *et al.*, 1965).

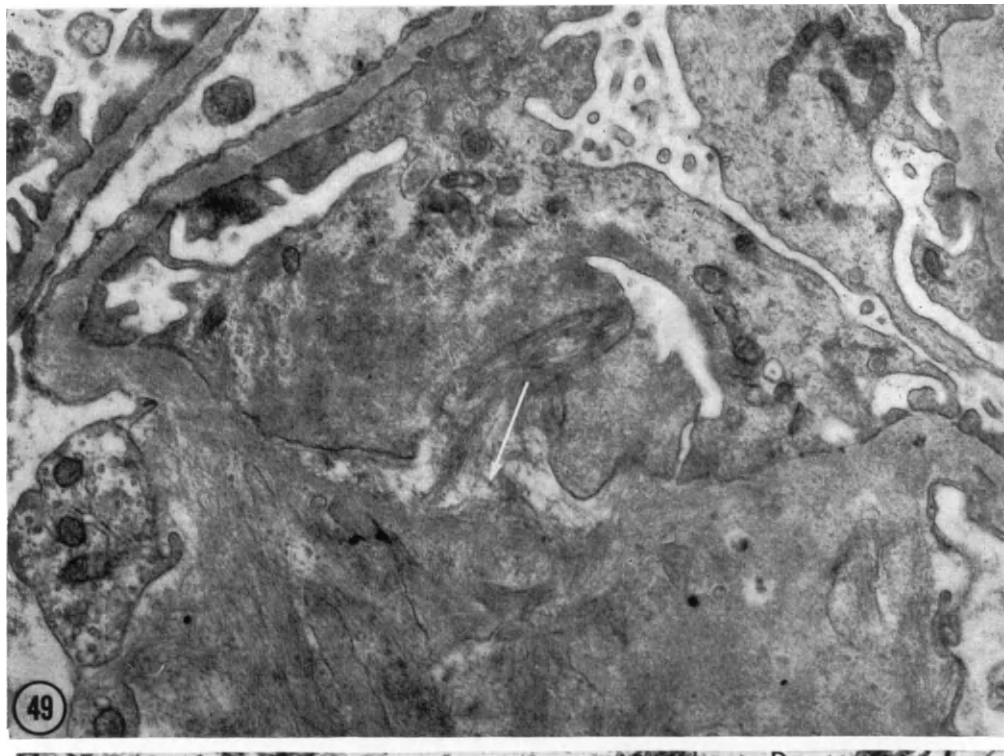
To explain the mechanism whereby mesangial matrix becomes hyalinized and the basement membrane increases in thickness, Farquhar (1964) suggested that there is probably a slowing down in the normal turnover process of basement membrane owing to a depression in the activity of the mesangial cells to incorporate, degrade, and finally remove basement membrane components. In support of this concept, she reported that mesangial cells are poorly developed in terms of cytoplasmic organelles, thus showing little evidence of phagocytic activity. In summary, the lesion of diabetic glomerulosclerosis would not be due to excessive production of basement membrane or mesangial matrix but rather to a diminution in the turnover of membrane components and mesangial material.

The development of glomerulosclerosis in experimental diabetes without the appearance of retinopathy (Gibbs *et al.*, 1964; Bloodworth and Engerman, 1964) has been taken to indicate that retinopathy should be considered a separate entity in contrast to the "diabetic microangiopathy" which is part of the diabetic syndrome (Bloodworth and Engerman, 1964). From the study of surgical diabetes (pancreatectomy), it is believed that the kidney lesions are similar regardless of whether they appear in people with primary or secondary diabetes (Ireland *et al.*, 1964; MacDonald and Ireland, 1964). Since the lesions may occur in patients with less than a 1-month history of diabetes, Azerad *et al.* (1964) believed that this angiopathy is an inherent part of the diabetic condition and not merely a complication of the latter. This view is shared by Camerini-Dávalos *et al.* (1964) who made a special study of vascular

FIG. 46. Nodular diabetic glomerulosclerosis. The mesangial matrix (MM) contains fibers (200–300 Å wide) of immature collagen. Magnification: ×10,750. Insert magnification: ×35,000. From Dachs *et al.* (1964).

FIG. 47. Renal amyloidosis. Small portion of glomerular capillary wall showing a deposit of amyloid fibrils (A). In cross section these fibrils have the appearance of tiny beads. EP, Epithelium; BS, Bowman's space. Magnification: ×50,000. Courtesy of Dr. R. B. Arhelger.

FIG. 48. Portion of two capillary loops from a patient having renal amyloidosis. Amyloid (Am) is seen on either side of the intact basement membrane, i.e., between endothelium (En) and basement membrane and between epithelium (Ep) and basement membrane (BM). The filamentous texture of amyloid can be appreciated. A small portion of a normal capillary loop is present in left lower corner. Osmium tetroxide, periodic acid-silver methenamine. Magnification: ×2200. From Movat (1960b).



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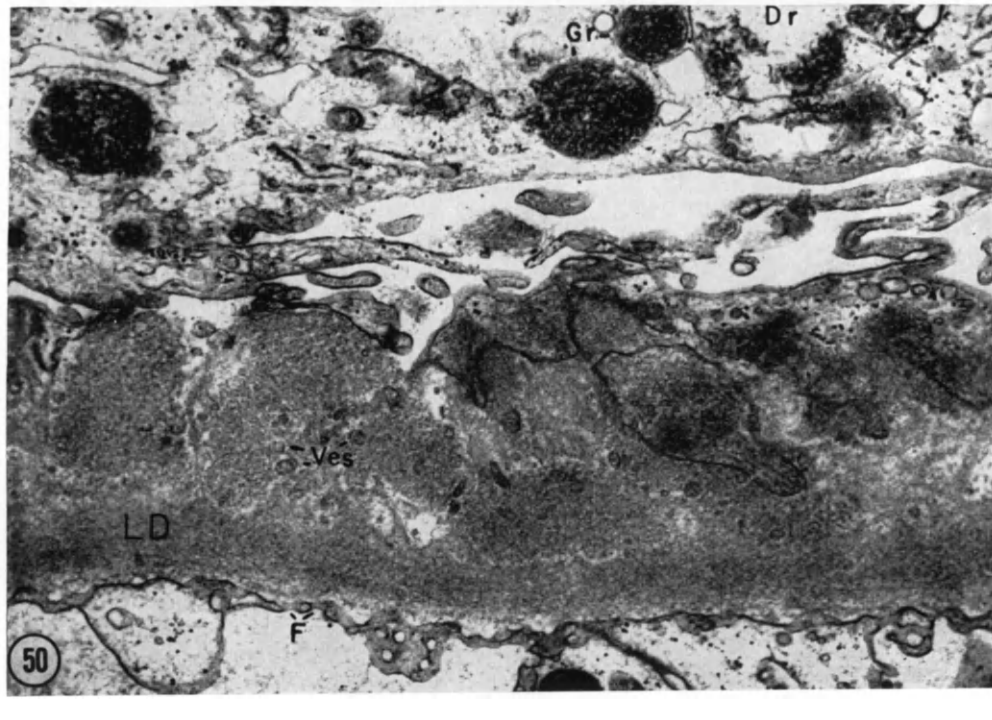

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changes in prediabetics and concluded that "the underlying metabolic defect may cause significant and identifiable renal pathology before the defect in glucose metabolism is recognized."

H. Amyloidosis

The diagnosis of renal amyloidosis, whether primary or secondary, is readily made by light microscopy. In the earlier stages, however, the electron microscope may be helpful, and by means of this instrument it has been possible to relate the deposition of amyloid to the various structures of the glomerulus, such as epithelium, basement membrane, mesangial cells, and endothelial cells. It has also provided information which may throw light on the pathogenesis of this condition.

The characteristic appearance of amyloid under the electron microscope is that of a fibrillar material (Fig. 47). The individual fibrils are of the same range of thickness, 50 to 100 Å, as mesangial fibrils, but are more sharply defined and more irregularly oriented (Suzuki *et al.*, 1963). These fibrils measure up to 0.5 μ in length, are arranged in a very loose, irregular meshwork and when studied under very high magnification, appear as a triple-layered structure and may show a rough beading or periodicity (Sorenson and Shimamura, 1964).

Regardless of whether amyloidosis of the renal glomerulus is primary or secondary (Geer *et al.*, 1958; Spiro, 1959; Movat, 1960b; Bergstrand and Bucht, 1961; Suzuki *et al.*, 1963; Hinglais and de Montera, 1964) or experimentally produced (Miller and Bohle, 1957a; Cohen and Calkins, 1960; Caesar, 1963; Sorenson and Shimamura, 1964), the first characteristic lesion is the deposition of amyloid in the mesangial matrix and between this region and endothelial cells (Fig. 49) (Hinglais and de Montera, 1964; Suzuki *et al.*, 1963; Sorenson and Shimamura, 1964). Later the deposits may extend into the peripheral part of the capillary loop and appear on both sides of the lamina densa but only rarely is the dense portion of the basement membrane involved (Hinglais and de Montera, 1964; Sorenson and Shimamura, 1964; Suzuki *et al.*, 1963). This late involvement of the lamina densa has been well illustrated in human subjects by Movat (1960b) using the silver methenamine technique to demonstrate the relative impunity of the lamina densa in the midst of severe

FIG. 49. Amyloid on the epithelial and mesangial sides of the basement membrane. The latter is infiltrated with amyloid fibrils and distorted so that it is barely recognizable (arrow). Magnification: ×14,000. From Sorenson and Shimamura (1964).

FIG. 50. Renal vein thrombosis. Portion of a glomerular capillary loop showing granules (Gr) and droplets (Dr) in the epithelial cytoplasm. Fenestrations (F) of the endothelium are seen at several points. The lamina densa (LD) is seen as an intact electron-dense layer, while the entire basement membrane is thickened. Vesicles (Ves) are seen mainly on the epithelial side of the lamina densa and occasionally within it. Individual dense granules of 150 to 200 Å can be seen in lumen (near F), in endothelial and epithelial cytoplasm, and in Bowman's space. The villuslike epithelial projections are visible. Magnification: ×30,000. From Panner (1963).

amyloid deposits on both sides of it (Fig. 48). This author, however, remarked that in early lesions, amyloid lay only on the inner side of the basement membrane.

Most authors agree that small amyloid deposits can be found between the endothelium and basement membrane. When large deposits are present in these sites, severe displacement of endothelial cells and narrowing of the capillary wall occurs. Alterations in epithelial cells are seen only when amyloid occupies the entire width of the basement membrane. These epithelial cell alterations consist of fusion of foot processes and increased cytoplasmic density. Sorenson and Shimamura (1964) did not find amyloid on the epithelial side except in those areas where deposits were also present on the endothelial side. It is of interest that these authors observed large amounts of amyloid within vascular spaces as well as tufts of amyloid fibrils in endothelial cytoplasmic invaginations. A recent paper by Shimamura and Sorenson (1965) has shown that in murine amyloidosis, the amyloid localizes initially in the mesangium from where it extends laterally beneath the endothelium. Eventually deposits of amyloid compress the capillaries and extend through and beyond the basement membrane into a subepithelial location.

The histogenesis of glomerular amyloidosis has been studied in early cases of amyloid deposition in humans (Hinglais and de Montera, 1964; Suzuki *et al.*, 1963) and in experimental animals (Sorenson and Shimamura, 1964; Hinglais *et al.*, 1964; Shimamura and Sorenson, 1965). The work of these investigators strongly suggests that mesangial cells play an important role in the pathogenesis of this condition. Sorenson and Shimamura (1964), who studied the function of the reticuloendothelial cells in splenic and hepatic amyloidosis (Heefner and Sorenson, 1962; Sorenson *et al.*, 1964), have suggested that mesangial cells, by virtue of their reticuloendothelial characteristics, may well play an important role in the pathogenesis of renal amyloidosis. Although the pathogenesis of amyloidosis is open to speculation, it has been proposed that immune mechanisms may be involved. However, studies of amyloid with ferritin-coupled anti-gamma globulin have led several investigators (Gueft and Ghidoni, 1963; Paul and Cohen, 1963) to conclude that it is unlikely that amyloid fibers are formed by an antigen-antibody reaction.

At the present time it is undecided whether amyloid arrives at the glomerulus already formed (Hinglais and de Montera, 1964) or whether it is directly synthesized *in situ*, perhaps by mesangial cells. The possibility also remains that amyloid is synthesized elsewhere and converted into a recognizable fibril in the glomerulus due to special local factors. Although the studies of Sorenson and Shimamura (1964) could be interpreted as indicating the passage of tufts of amyloid fibrils through the endothelial cells, their work does not exclude the other two possibilities.

The formation of amyloid in cell cytoplasm has been suggested on the basis of electron microscopic studies in renal amyloidosis (Hjort and Christensen, 1961) and also in solid carcinoma of the thyroid gland (Albores-Saavedra *et al.*, 1964). Working with amyloidosis of the spleen, Gueft and Ghidoni (1963) considered the amyloid to be a pathological scleroprotein formed at the periphery of histiocytes within cytoplasmic pockets.

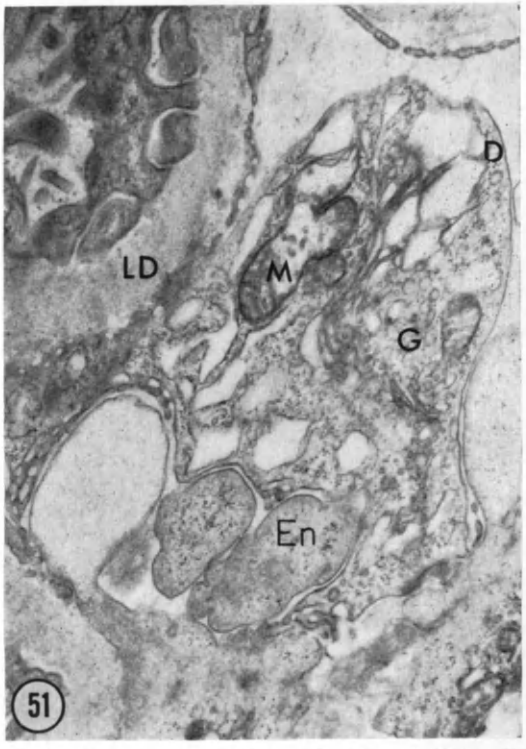
The studies of amyloidosis by electron microscopy in the past have served to delineate the anatomical relationships of the amyloid deposits within the glomerulus. It is hoped that in the future the use of cytochemical and radioautographic techniques, in conjunction with electron microscopy, will help to elucidate the histopathogenesis of this condition.

I. Renal Vein Thrombosis in the Adult—Glomerular Lesions

Renal vein thrombosis in the adult is a recognized cause of the nephrotic syndrome, and nephrosis can be produced experimentally in rats by subtotal ligation of renal veins. In reporting a case of renal vein thrombosis associated with the nephrotic syndrome, studied by light and electron microscopy, Panner (1963) reviewed in detail the relevant literature on the subject, from both the functional and morphological standpoints. This author demonstrated by electron microscopy that the lesions which occur in the glomerulus are similar to those seen in membranous glomerulonephritis and in some cases of idiopathic nephrotic syndrome in adults (see Section II, B, 3). In Panner's case, the changes were present in all glomeruli examined and showed approximately equal degrees of severity. The most characteristic finding was thickening of the basement membrane and the foot processes (Fig. 50). The lamina densa was moderately thickened up to 3000 Å. Vesicles up to 1000 Å in diameter were present in large numbers in the subepithelial deposits. The endothelial cells were normal, whereas the epithelial cells showed gross lesions. These included marked fusion of foot processes, development of microvilli, vacuolization, and numerous dense bodies.

Pirani *et al.* (1963b), in a short communication, reported six cases of the nephrotic syndrome due to renal vein thrombosis which was confirmed by autopsy in each instance. They described similar changes to those seen by Panner but felt that a spongy appearance of the outer portion of the basement membrane was a more common finding than the subepithelial deposits. While recognizing the diagnostic difficulties in such cases, they believed that the greater amount of interstitial edema and fibrosis present in their patients facilitated the distinction from membranous glomerulonephritis.

The origin of the albuminuria and the pathogenesis of the nephrotic syndrome which occurs in renal vein thrombosis are not completely understood despite the fact that a nephrotic syndrome can be easily produced experimentally by subtotal ligation of one renal vein (Omae *et al.*, 1958). It may be that the nephrotic syndrome associated with this condition is induced by increased renal venous pressure and is etiologically related to the nephrosis which is occasionally found in severe congestive heart failure and constrictive pericarditis. Whether an increase in venous pressure alone can result in the histological changes described is not at present clear. The problem should be readily amenable to investigation, and no doubt further work in this area will elucidate the pathogenesis of this condition and contribute to our understanding of the ultrastructural changes in the nephrotic syndrome.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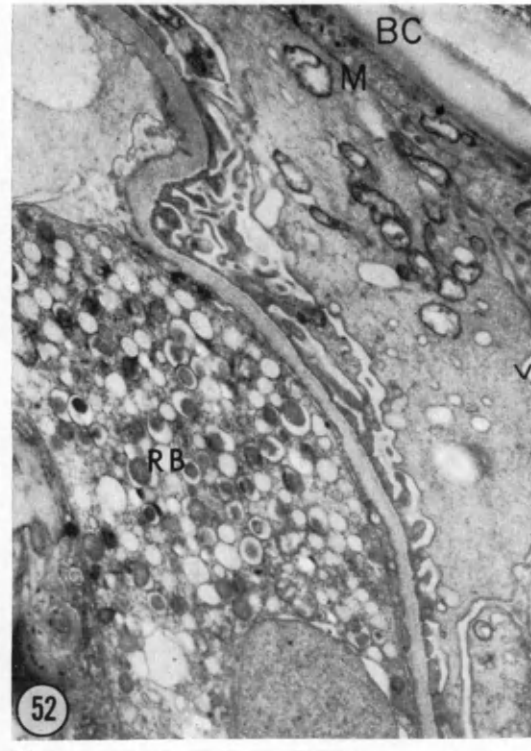
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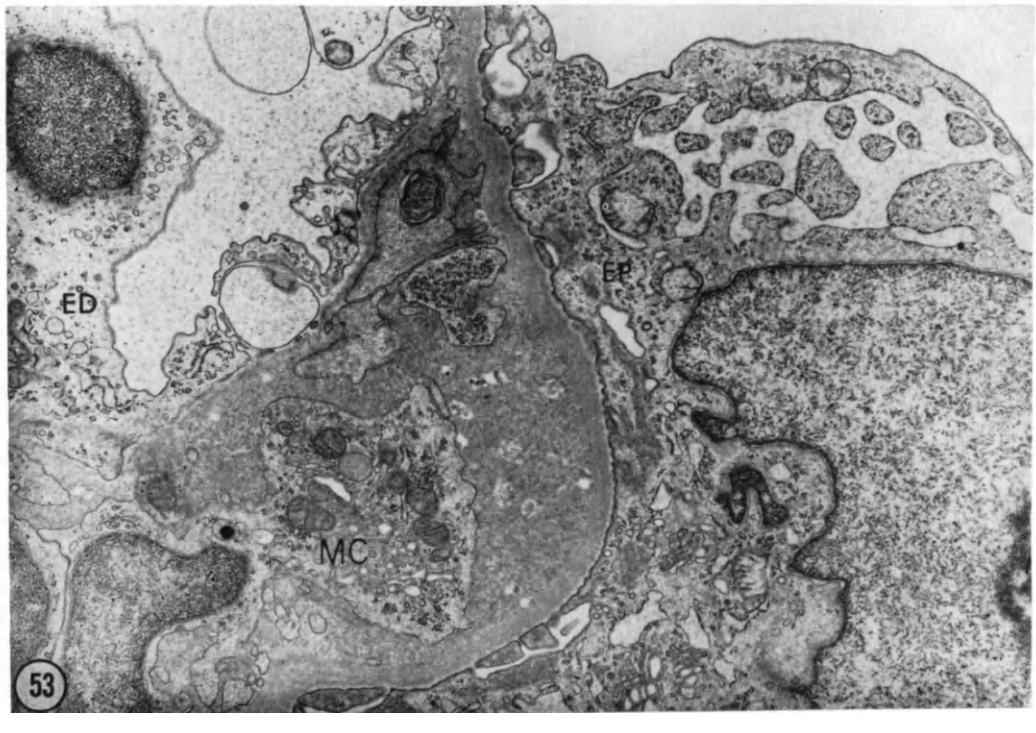
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J. Multiple Myeloma—Glomerular Lesions

It is well recognized that patients with multiple myeloma may develop renal damage, but the occurrence of glomerular lesions has only recently been recognized as a result of electron microscopic studies by Costanza and Smoller (1963) and particularly by Fisher *et al.* (1964). These latter authors, in a detailed clinicopathological study of seven cases, made the interesting observation that the glomerular lesions in multiple myeloma were characterized by marked thickening of the lamina densa up to twice normal, without any identifiable ultrastructural alteration (Fig. 51). It is unfortunate that no periodic acid–silver methenamine stain was done in these cases to rule out the possibility of uniform deposits or to provide some explanation for the marked thickening of the lamina densa. Epithelial, endothelial, and mesangial cells, however, showed changes similar to those found in other proteinuric states in man and experimental animals, so that fusion of the foot processes together with the presence of vacuoles and hyaline droplets in the epithelial cells were common. Endothelial cells showed moderate swelling. In two cases, these authors found cells attached to the endothelium which bore a strong resemblance to the plasma cell and contained Russell bodies (Fig. 52) as described by others (Bessis, 1961; Fisher and Dimling, 1964).

Fisher *et al.* (1964) concluded from their clinicopathological study that the appearance of glomerular lesions in this condition was correlated with the elevation of serum protein and not with the presence of abnormal proteins nor the occurrence of proteinuria. They interpreted their findings as indicating that the glomerular changes in multiple myeloma are the result rather than the cause of the proteinuria. This assumption is in agreement with the work of Fisher and Hellstrom (1962) who studied the different effects of infusion of homologous and heterologous protein on the structure and function of the glomerular capillary wall in rats.

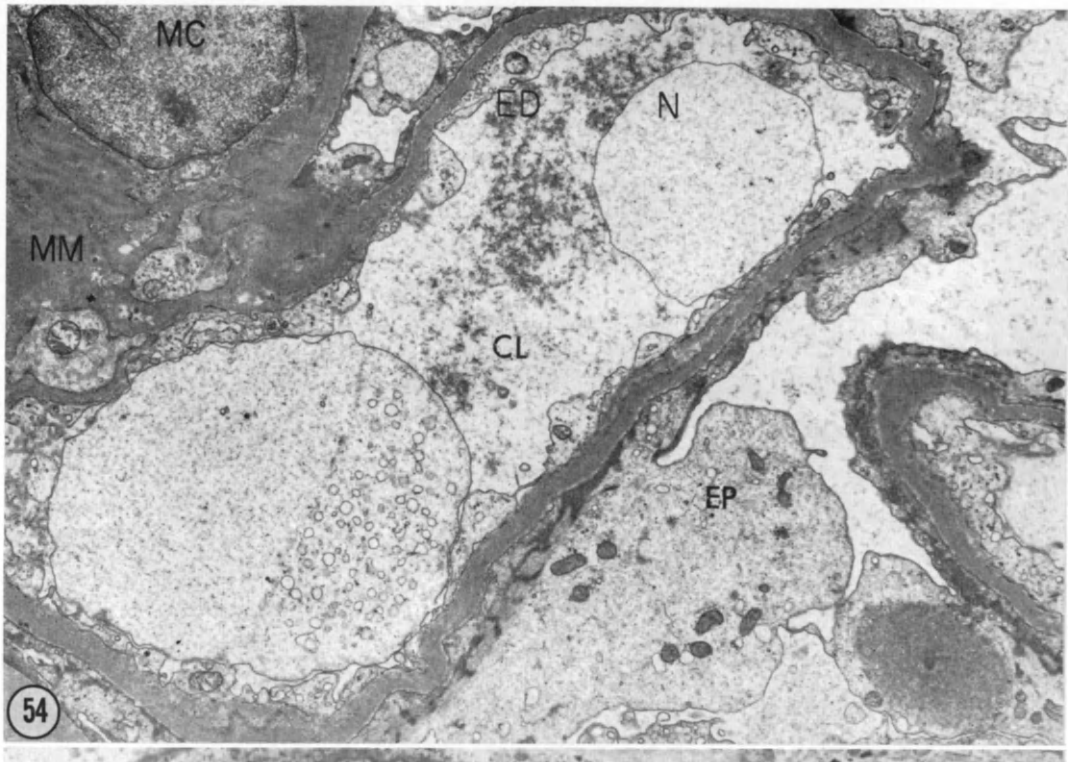
K. Hepatic Glomerulosclerosis

This term was introduced by Sakaguchi *et al.* (1964) to replace that of cirrhotic glomerulosclerosis, since these authors believed that the changes to be described are

Fig. 51. Portion of a glomerular capillary from a patient with multiple myeloma showing prominent Golgi structures (G) in endothelial cytoplasm (En). The lamina densa (LD) is homogeneous but thick. Magnification: ×15,000. From Fisher *et al.* (1964).

Fig. 52. Multiple myeloma. Portion of cell containing cytoplasmic bodies reminiscent of Russell bodies (RB) in endothelial position. The epithelial cell cytoplasm contains vacuoles (V) and prominent but distorted mitochondria (M). Portion of Bowman's capsule is also noted (BC). Magnification: ×7500. From Fisher *et al.* (1964).

Fig. 53. Hepatic glomerulosclerosis. Glomerulus after carbon tetrachloride administration for 4 months. Stage of deposition, grade 1. More conspicuous accumulation of proteinaceous material under the basement membrane and in the mesangial matrix. There are also coarser aggregates surrounded by a clear zone. ED, Endothelial cell; EP, epithelial cell; MC, mesangial cell. Magnification: ×10,000. From Sakaguchi *et al.* (1964).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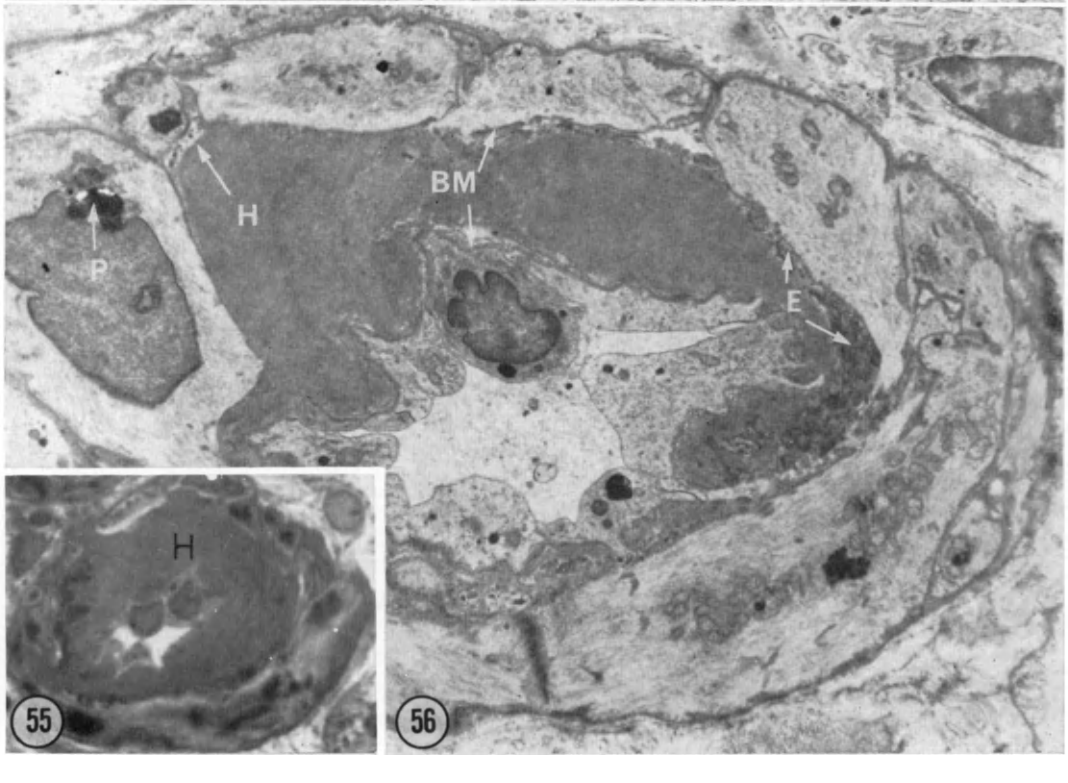
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related to liver derangement of different etiologies and are not necessarily limited to hepatic cirrhosis. A careful study of the electron microscopic changes taking place in the glomeruli of animals suffering from liver derangement induced by carbon tetrachloride or ethionine have been described by Sakaguchi *et al.* (1964). According to these authors, rats treated with carbon tetrachloride or ethionine develop progressive glomerular changes characterized by fusion of epithelial foot processes and deposits of an amorphous or granular material between the endothelium and the basement membrane and also in the mesangium (Fig. 53). In addition, the mesangial matrix becomes more abundant, and the basement membrane increases in thickness (Fig. 54). It is of interest that these changes are not necessarily associated with proteinuria, since little or no protein is found in the urine of these animals. The changes described in the rat take from 6 to 7 months to develop.

From the time sequence involved, Sakaguchi *et al.* (1964) concluded that the material deposited, which is of unknown origin, may represent protein from damaged liver cells or protein produced by a diseased liver in sufficient amounts to be trapped in the glomeruli, in the subendothelial space, and in the mesangium in the same way that other proteins are transported according to the general filtration and unclogging theory postulated by Farquhar and Palade (1962). One very exciting aspect of this condition is the lack of proteinuria despite the presence of lesions known to be usually associated with this condition, such as marked loss of foot processes, the presence of large subendothelial deposits, and the marked enlargement of the mesangial area. This experimental preparation, with further manipulation, may prove to be an excellent model for the study of the relationship between the anatomical changes in the glomerulus, membrane permeability, and proteinuria.

L. Fixed and Orthostatic Proteinuria

Robinson *et al.* (1961) conducted an extensive clinicopathological study on 56 asymptomatic patients with reproducible orthostatic proteinuria. By light microscopy

FIG. 54. Hepatic glomerulosclerosis. Glomerulus after carbon tetrachloride administration for 7 months. Stage of organization, grade 2. There is fusion of foot processes, thickening of the basement membrane, and increase in the amount of mesangial matrix (MM). CL, Capillary lumen; ED, endothelial cell; EP, epithelial cell; MC, mesangial cell. Magnification: ×7000. From Sakaguchi *et al.* (1964).

FIG. 55. A lobular arteriole showing intimal hyaline deposit (H). Elastic fibers appear as a discontinuous darker band (deep blue stain in section). Endothelium and smooth muscle cells are clearly distinguished. Osmium-fixed, Epon-embedded, toluidine blue stain. Magnification: ×350. From Biava *et al.* (1964).

FIG. 56. The same lobular arteriole shown in Fig. 55. Hyaline material (H) is deposited in the intimal space on the lumen side of elastic fibers (E) and is separate from endothelial and smooth muscle basement membrane (BM). Elastic fibers are displaced and compressed against smooth muscle cells. Endothelial and muscle cells are well preserved and contain dense pigment bodies (P). A large wedge-shaped deposit is infiltrating between smooth muscle cells (arrow). Magnification: ×3600. From Biava *et al.* (1964).

these authors found hypercellularity of the glomerular tuft in some cases, slight thickening of the capillary wall, and PAS-positive droplets in "close proximity to the epithelial periphery of the glomerular tuft." Five such cases were studied by electron microscopy. The findings confirmed the fact that many glomeruli appeared normal, whereas others showed mild focal alterations. In these, the most prominent abnormalities involved the epithelial cells where fusion of foot processes, hyaline droplets, and vacuolization of the epithelial cytoplasm developed and microvillation appeared. The basement membrane was found by Robinson and associates to be irregular, "split and frayed," particularly in the region of the lamina rara interna. These authors were unable to determine the significance of this finding, whereas the changes in the epithelial cells were consistent with increased protein filtration. If, as suggested by Strunk *et al.* (1964) and Rhodin (1964), basement membrane is formed by both endothelial and epithelial cells, it is tempting to speculate that the "fraying and splitting" of this structure observed by Robinson *et al.* (1961) may result from a more rapid turnover of an abnormally fragile membrane. Further studies with higher resolution and perhaps with specific stainings, such as silver methenamine, may clarify the pathogenesis of this interesting condition.

M. Radiation Nephritis

Rosen *et al.* (1963) have briefly reported a case from a patient who received more than 3000 R to each kidney over a period of 8 weeks during therapy for ovarian carcinoma. Five months later, the patient developed a nephrotic syndrome. A renal biopsy showed by electron microscopy that the endothelial cells were markedly swollen, vacuolated, and contained osmiophilic droplets. The epithelial aspect of the basement membrane was well maintained, but on the endothelial side there were large quantities of spongy, lamina densa-like material which often surrounded endothelial or mesangial cells. Epithelial cells were vacuolated and contained osmiophilic droplets as well as showing focal fusion of the foot processes. Mitochondrial and nuclear abnormalities were frequently seen in the glomerular cells. The authors concluded from retrospective studies of autopsy cases and a review of the literature that the glomerular component is more important than previously thought and represents a characteristic feature of this disease. Fusion of the foot processes of epithelial cells has been shown to follow X-radiation in mice subjected to a single dose by Pasinetti *et al.* (1962). The changes occurred as early as 24 hours following irradiation but had reverted to normal by day 21, indicating reversibility. Further studies in this field, and especially those in which clinicopathological correlations are performed, will be awaited with great interest.

N. Hydronephrosis

Few studies have been concerned with the glomerular changes in hydronephrosis (Pak Poy and Robertson, 1959; David, 1963). In mice and rabbits in which unilateral uretheral obstruction was induced for periods ranging from 1 to 29 days, David (1963)

found increased thickness of the basement membrane and deposits in the subepithelial region of the basement membrane. The most complete study on this subject to date is that of Pak Poy and Robertson (1959). These authors have given an excellent description of the progressive changes seen in the glomeruli of rats following complete obstruction of one ureter during a 20-week period. Lesions of glomeruli appeared as progressive collapse of glomerular capillaries. In the early stages of collapse, the peripheral segment of the capillary loop loses its approximately circular outline and becomes U-shaped. The next stage is the approximation of the limbs of the U, with complete or partial loss of the lumen in that region. If only small segments of the capillary wall are involved in the collapsing process, the basement membrane takes a heavy, undulating appearance. With time, the capillary basement membrane becomes thickened and there is loss of cellular elements.

The completely hyalinized glomeruli appeared as round, dense hyaline masses with a few red cells and some collagen fibers in the periphery together with some remnants of the Bowman's capsule. This finding frequently helped to identify the nature of the hyaline mass. A very interesting and probably fairly characteristic lesion in some glomeruli which appeared to be still functioning was the presence of ruptured Bowman's capsules. The gap was usually small, but it sometimes occupied as much as a quarter of the capsule at the plane of sectioning. Although focal loss of foot processes was seen by the authors, this finding was usually restricted to areas in the vicinity of the capsular defects. In a study of human cases of hydronephrosis, one of us (Bencosme and Ozen, 1965) has been able to confirm most of the observations of Pak Poy and Robertson (1959) in human material including rupture of the Bowman's capsule.

Pak Poy and Robertson (1959) believed that the presence of ruptured capsules in glomeruli which contained functioning capillaries supported the hypothesis of Hinman (1945) which suggested that urine secreted from high pressure glomeruli escapes into the interstitial space and from there into low pressure glomeruli where it is reabsorbed back into the bloodstream.

O. Hypertension—Lesions of Arteries and Glomeruli

It is well recognized that the kidney is important in the development of some types of hypertension, although there is no general agreement on which specific renal structure is responsible for the elevated blood pressure. It is beyond the scope of this presentation to discuss the vast literature on this subject, and the reader is referred to two recent national (Metcoff, 1963) and international symposia (Genest *et al.*, 1964) dealing with the kidney and the pathogenesis of hypertension. We will rather limit ourselves to those reports in which ultrastructural changes in the glomeruli, and renal arterioles have been discussed in relation to hypertension and kidney pathology.

Studies dealing with human material are very limited and have been directed primarily to the small arteries and arterioles (McGee and Ashworth, 1963; Biava *et al.*, 1964). Kinoshita and Fujisaki (1963) made a brief mention of nonspecific

changes in the glomeruli in five cases of essential benign hypertension. A few investigators have attempted to study the kidney by electron microscopy in animals with experimental hypertension. Some have concentrated on the glomerulus (Ashworth and Grollman, 1959; Geer *et al.*, 1961; Lynn, 1963; Anderson, 1963), whereas others have placed more emphasis on the arterioles and the juxtaglomerular apparatus (Hatt and Dontcheff, 1959; Hatt, 1961; Hatt *et al.*, 1962, 1963).

1. *Hyaline Arteriolarsclerosis*

Although hyaline arteriolarsclerosis may occur in normotensive, elderly individuals, it is characteristically found in hypertensive subjects. In the first study of human renal hyaline arteriolarsclerosis by electron microscopy, the hyaline was considered to be composed of thickened and confluent basement membranes of endothelial and smooth muscle cell origin (McGee and Ashworth, 1963). In older lesions these thickened basement membranes (hyaline material) were found to contain atrophic smooth muscle cells and some osmiophilic particles, probably lipid remnants from disappearing smooth muscle cells or filtered material from the blood (McGee and Ashworth, 1963). These findings were attributed to a combination of aging and increased arteriolar tension or vasoconstriction resulting in an excessive elaboration of basement membrane by both endothelial and smooth muscle cells. In contrast to the observations and interpretations of previous investigators, Biava *et al.* (1964), focusing on the morphological features of renal arteriolar hyalinosis, concluded that arteriolar hyaline was predominantly deposited within the intimal spaces in the walls of arterioles (Figs. 55 and 56). The arteriolar hyaline appeared morphologically distinct from collagen, elastic tissue, basement membrane material, fibrin, and amyloid. At high magnification, hyaline was found to be composed of an aggregation of moderately dense granules about 200 Å in diameter, apparently unrelated to the other cellular or intercellular constituents of the arterial wall (Fig. 57). In severe hypertension, arteriolar hyaline was seen to infiltrate and obliterate the basement membranes of the endothelial and smooth muscle cells as well as the elastic tissue. The authors postulated that the deposits were due to excessive passage of plasma protein into the arteriolar walls as a result of increased endothelial permeability. They also believed that the main plasma components which form the hyaline are globular proteins and lipoproteins rather than polymerized fibrillar proteins. The same authors found that in hypertensive patients the features described differed only in frequency and degree from those observed in normotensive individuals.

2. *Hyperplastic Elastic Arteriosclerosis*

The hyperplastic elastic arteriosclerosis of interlobular arteries and larger afferent arterioles in the kidneys of patients with chronic benign hypertension were investigated by McGee and Ashworth (1963). These authors found that the characteristic changes were thickening of endothelial and muscle cell basement membranes, formation of nonperiodic fibrils and of collagen fibrils in the intima, localization in the intima of minute lipid particles in the size range of chylomicrons, hypertrophy of

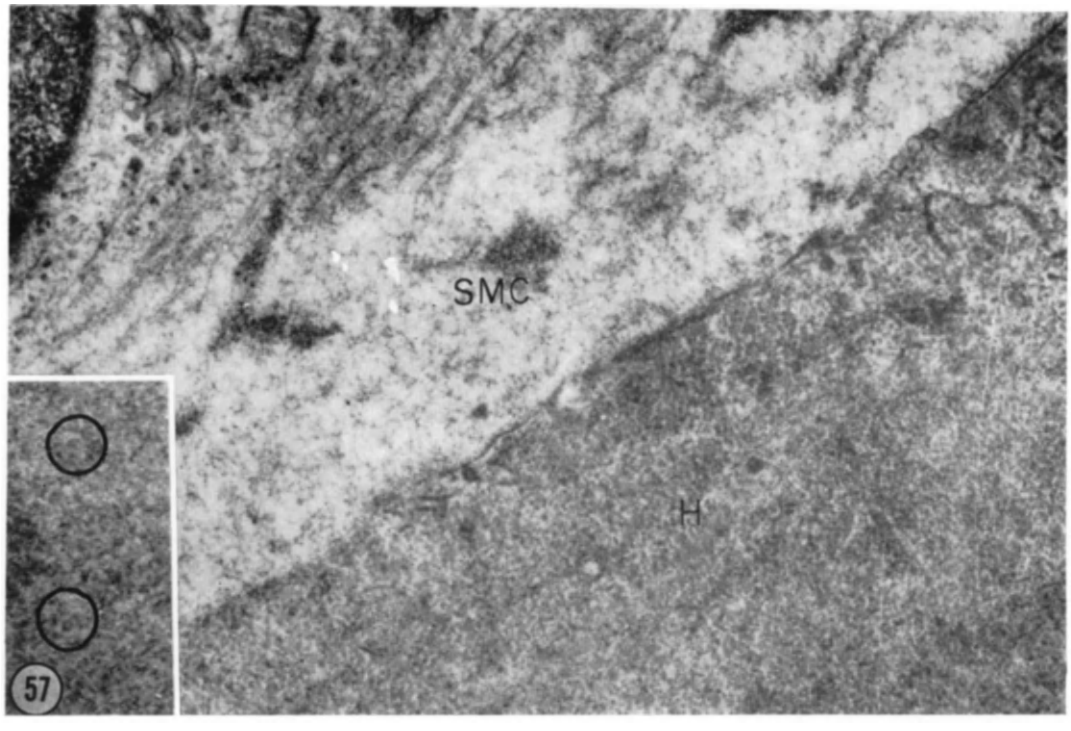


FIG. 57. Peripheral field of a large intimal hyaline deposit. Hyaline material (H) has completely replaced the muscular basement membrane and is immediately juxtaposed to the cell membrane of a medial smooth muscle cell (SMC). Magnification: ×26,000. *Insert*: High magnification of the hyaline showing granular elements about 200 Å in diameter (circle). Magnification: ×56,000. From Biava *et al.* (1964).

the elastic fibers, proliferation of smooth muscle cells and fibrocytes in the intima, and atrophy of medial smooth muscle cells. McGee and Ashworth are of the opinion that the hyperplastic elastic lesion of small arteries in the kidney in hypertension is basically similar to that seen in atherosclerosis of larger arteries and structurally similar to hyaline arteriolarsclerosis. Based on these similarities, they postulated a common pathogenetic mechanism for these lesions in contrast to the view of Biava *et al.* (1964) on the pathogenesis of hyaline arteriosclerosis.

3. *Experimental Hypertension*

Because of the small number of reports dealing with the electron microscopic picture of the kidney in experimental hypertension and the heterogeneous methods used, it is difficult to draw definite conclusions from the literature although some interesting information has become available in this field.

The work of Esterly and Glagov (1963) is of interest in demonstrating the relationship between adrenal cortical function, endothelial hyperpermeability, and renal

vascular lesions. These authors found an increased permeability of the renal artery in rats rendered hypertensive by uninephrectomy and administration of sodium chloride and deoxycorticosterone acetate (DOCA). In these animals the main renal artery showed vacuolization and degeneration of endothelial cells, adhesion of blood cells to the endothelium, widening of the subendothelial space, appearance of blood cells, such as macrophages and cell fragments and smooth muscle cells in the subendothelial space, vacuolization and degeneration of medial smooth muscle cells, and accumulation of extracellular osmiophilic material in the media. These findings were interpreted as indicating that hypertension or other hemodynamic factors in these experimental animals altered the permeability of the arterial wall. It is of interest that these changes, although less pronounced, have much in common with those found in hyperplastic elastic arteriosclerosis in humans by McGee and Ashworth (1963). Moreover, the material deposited in the subendothelial space of the arteries in these hypertensive animals had a similar distribution to that of hyaline arteriolarsclerosis as described in humans by Biava *et al.* (1964). Unfortunately, the magnification used by Esterly and Glagov (1963) does not permit a definite conclusion on this point. It would be of interest to use this model for longer periods of time and to study small arterioles with appropriate magnification to see if arteriolar hyaline as described by Biava *et al.* (1964) develops in these animals.

Hatt and associates (Hatt and Dontcheff, 1959; Hatt, 1961; Hatt *et al.*, 1962, 1963) have emphasized the importance of the arterioles and medium-sized arteries in the pathogenesis of experimental hypertension. These investigators, working with rabbits rendered hypertensive by renal arterial stenosis, concluded that hypertension is constant in bilateral ischemia of moderate or severe degrees. In cases with moderate ischemia, they described changes in the arterioles which they believed to be compatible with secretory hyperactivity of smooth muscle cells. These changes occurred a week after clamping the main renal arteries at the time hypertension began and included hypertrophy of the Golgi apparatus and of the endoplasmic reticulum, vacuolization of the cytoplasm, and an increase in the number of mitochondria, some of which became vacuolated. After the fourth week when hypertension was well established, the muscle cells in the media of the arterioles appeared somewhat different. The cells were swollen and much clearer, apparently as a result of the loss of myofibrils. At this stage there was no evidence of increased secretory activities, and the ergastoplasm, Golgi apparatus, and mitochondria appeared normal. This "clearing" of smooth muscle cells has been referred to by light microscopists as epithelial transformation. Hatt *et al.* (1962) found no consistent abnormalities in the juxtaglomerular apparatus except for increased vacuolization of the cells from the macula densa or of the lacis. No changes were found in the efferent arterioles or in the glomeruli at any stage during the entire experimental period which extended up to 120 days. The absence of glomerular changes in these rabbits is in contrast to the mesangial activity found by Lynn (1963) in the early stages of Goldblatt hypertension in dogs.

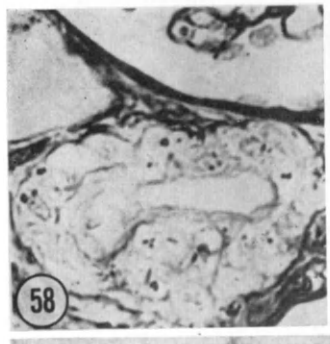
Hatt and collaborators hypothesize that if the ischemic kidney elaborates some hypertensive substance, it might be produced by the smooth muscle cells of the renal

arterioles. Other workers, however, feel that the changes in the smooth muscle cells are degenerative in nature (McGee and Ashworth, 1963; Esterly and Glagov, 1963; Geer *et al.*, 1961).

The few studies available of the fine structure of the glomerulus in experimental hypertension have not clarified the role of this organ in this condition, and on the basis of these reports it seems unlikely that the glomerulus is concerned with the development of hypertension in a cause and effect relationship. Moreover, the glomerular lesions seen in animals with induced hypertension may well be side effects of the experimental procedure. Ashworth and Grollman (1959) described thickening of the basement membrane and swelling of epithelial cells with fusion of the foot processes in rats with hypertension induced by choline deficiency. These authors attributed the basement membrane changes to choline deficiency and the epithelial changes to proteinuria. In adrenal regeneration hypertension of rats, Geer *et al.* (1961) showed alterations of epithelial cells which were related to proteinuria, such as fusion of foot processes and thickening of basement membrane. In addition, these authors demonstrated proliferation of mesangial cells and deposition of fibrinoid material in the mesangial matrix. Some of the mesangial cells showed ovoid bodies reminiscent of those described by Dunihue and Boldosser (1963) in adrenalectomized cats and believed by these authors to represent juxtaglomerular granulated cells. The fibrinoid changes in the basement membrane and in the mesangial matrix were considered by Geer *et al.* (1961) to be related to salt loading or to some changes in the electrolyte composition of the body fluids. Whether the salt or other electrolyte changes act directly upon the basement membranes or through the cells could not be established.

In rats with hypertension induced by unilateral nephrectomy, salt, and administration of DCA in the form of Percorten (CIBA), Anderson (1963) found the most outstanding change to be marked swelling of the endothelial and mesangial cell cytoplasm. Because of the extent of endothelial swelling, several capillary lumina were occluded. Epithelial cells showed evidence of protein leakage, fusion of foot processes, and hyaline droplets in epithelial cells. No lesions were seen in arteries or arterioles.

It is apparent that our knowledge of the morphology and pathological physiology of hypertension is still very incomplete. The increasing use of electron microscopy, however, is revealing new subcellular structures while leading to re-evaluation of previously recognized cell organelles. A recent paper by Biava and West (1965) is particularly pertinent to this point. These authors reported the light and electron microscopic features of a particular type of lipofuscin-like granule found in the cytoplasm of vascular smooth muscle cells (Figs. 58 and 59), granular myoepithelioid cells (Fig. 60), and in the cells of the lacis of the juxtaglomerular apparatus in the kidneys from 114 human subjects ranging in age from 2 to 70 years. Under the electron microscope, these granules appeared as dense bodies in which three main structural components were recognized: (1) fat droplets, (2) coarsely granular matrix composed of randomly scattered particles 55 to 60 Å in diameter, and (3) crystalloids composed



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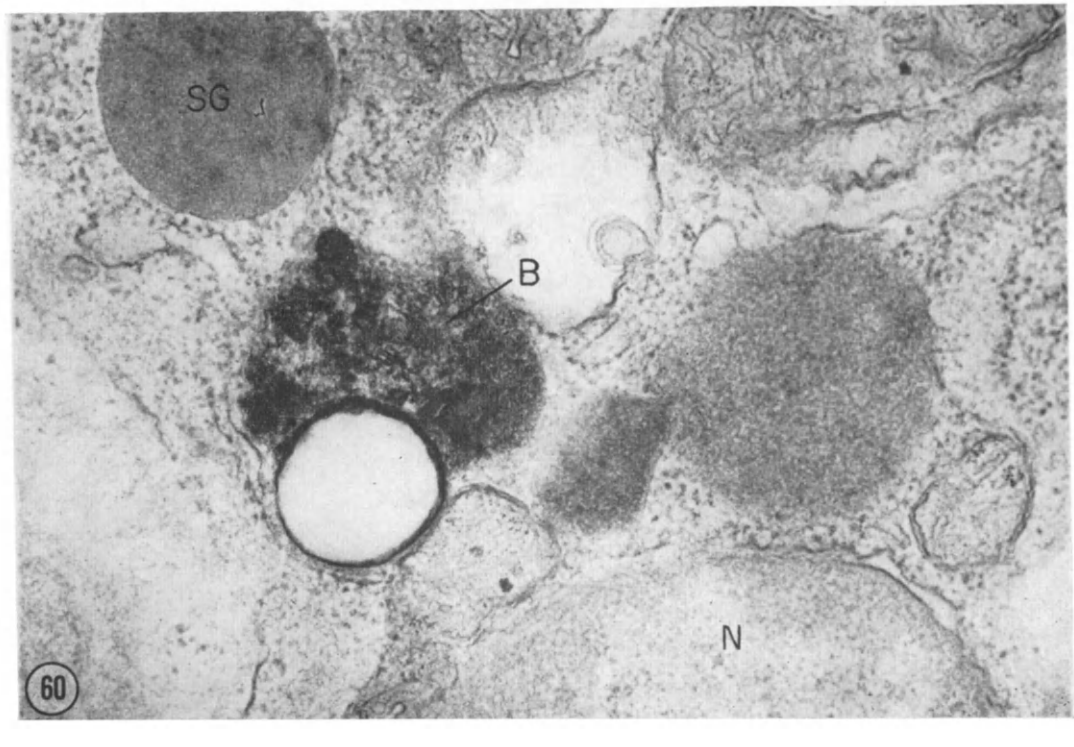

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of particles about 55 Å in diameter, arranged in regular hexagonal lattices. These structures closely resembled lipoprotein crystals and appeared to be derived from progressive crystallization of matrix particles. These bodies, which have been previously largely disregarded, were found to be present at all ages but were particularly prominent in hypertension, senility, and diabetes. The authors considered them to be of physiological significance although their exact function remains to be determined.

III. Concluding Remarks

At the present time, a fortunate combination of multiple approaches to renal physiopathology is permitting a better and more dynamic interpretation of the changes associated with abnormalities of glomerular function. Judicious application of this knowledge to the study of kidney disease is facilitating not only the precise morphological delineation of previously unrecognized glomerular lesions but also improved clinicopathological correlation of many disease entities. Moreover, several of these entities have been further subdivided on the basis of their clinical and ultrastructural characteristics. In this connection, it is possible that in some instances the coexistence of distinct and etiologically different lesions in the same clinical syndrome may be detected.

In the field of general pathology, the electron microscopic study of renal lesions has been progressing rapidly and has already contributed greatly to our understanding of many conditions. This has been particularly true of proteinuria which has been extensively studied owing to its intimate relationship to glomerular disease. It is now evident that changes may appear at the ultrastructural level prior to the development of significant proteinuria at a time when the glomeruli may appear completely normal by light microscopy. Although this observation has not been exploited in the diagnosis of early lesions, its potential contribution to clinical investigation should not be underestimated.

It is well recognized by those studying ultrastructure that further advances in our knowledge will require a greater use of the more sophisticated techniques, such as

FIG. 58. Renal arteriole in a normotensive 69-year-old patient showing several small smooth muscle cell granules. Silver methenamine stain. Magnification: ×1000. From Biava and West (1965).

FIG. 59. Cluster of dense bodies in an arteriolar smooth muscle cell of a normotensive 70-year-old patient. The bodies are predominantly composed of well-developed crystalloids (*insert*). Matrix substance is scanty. Lead hydroxide stain. Magnification: ×30,000. *Insert* magnification: ×92,000. From Biava and West (1965).

FIG. 60. Small dense body in a granular myoepithelial cell of juxtaglomerular apparatus in a normotensive 12-year-old patient. The body (B) is surrounded by an ill-defined single membrane. It is composed of a matrix substance and crystalloids similar to those in the dense bodies of arteriolar smooth muscle cells. Clusters of matrix particles show denser packing suggestive of early foci of crystallization. A specific secretory granule (SG) is seen adjacent to the dense body. N, Nucleus. Lead hydroxide stain. Magnification: ×40,000. From Biava and West (1965).

radioautography, immunochemistry, and cytochemistry at the ultrastructural level in conjunction with conventional electron microscopy. In the few instances where such techniques have been employed, they have greatly clarified our understanding and the pathogenetic significance of extraneous deposits in the basement membrane and mesangial matrix in human and experimental glomerulonephritis. It is to be hoped that in the future the use of these techniques will be extended and applied to other diseases.

The nature and molecular arrangement of the basement membrane and the mesangial matrix are of great importance. Recent developments in this field as a result of high-resolution electron microscopy and enzyme histochemistry indicate more and more clearly the close relationship of these structures to collagen. The available evidence suggests that mesangial matrix is more closely related to fibrous tissues than is the basement membrane. Differences in morphology and in reactivity of the basement membrane and mesangial matrix to the same stimuli, however, indicate important differences between them. No doubt further developments in our understanding of the physicochemical nature of these structures will greatly influence our future concepts of renal physiopathology.

There has been little mention in the literature of the fine structural changes which occur in the cell organelles in renal disease even though these changes are apparent in published electron micrographs. This omission is probably due to the fact that our knowledge of ultrastructural physiopathology is relatively limited and has been confined to a few specific conditions. Although high-resolution studies of renal lesions have been very restricted in the past, with general improvement in techniques and instrumentation, such studies are now being more widely performed. As a consequence, many investigators are studying the fine structural changes of cell organelles in greater depth, and it is anticipated that in the near future more valid generalizations will be possible regarding the functional significance of these changes.

The multiplicity of functions attributed to the mesangium constitute one of the most intriguing new fields of glomerular pathology. Although the best documented role of this structure pertains to its phagocytic activity and its relationship to filtration and proteinuria, the other functions are briefly discussed in the section dealing with the physiopathology of the mesangium and are now sufficiently well documented to merit further study. Despite the fact that the mesangium has only recently been accepted by electron microscopists, a firm foundation to which further knowledge can be added has already been established. It can be anticipated that the recognition and study of the mesangial changes in a variety of glomerulopathies, which in the past have been largely overlooked, will greatly clarify our understanding of glomerular diseases.

ACKNOWLEDGMENTS

This work has been supported by grants from the Medical Research Council, MA-1017 (Bencosme) and MT-1428 (Morrin) and an Ontario Heart Foundation Grant, 2-12 (Bencosme).

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Printed in Belgium