AMINONUCLEOSIDE NEPHROSIS

I. Electron Microscopic Study of the Renal Lesion in Rats*,‡

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Plates 13 to 17

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The renal glomeruli of many children with lipoid nephrosis have shown no abnormalities when examined with the light microscope (1). However, electron microscopic studies of renal biopsies from children suffering from the nephrotic syndrome have revealed consistent changes in structure of the glomerular epithelial cells (2, 3) which appear to be characteristic of the nephrotic syndrome in man (4). In each case studied, coalescence of the foot processes of the epithelial cell, associated with an apparent "smudging" of the cytoplasm along the outer aspect of the glomerular basement membrane, has been observed. Furthermore, swelling and vacuolization of the glomerular epithelial cells have been prominent in most of the biopsies. These observations suggest that the pathogenesis of nephrosis may involve an insult to the glomerular epithelial cells.

Recently an experimental disease resembling human nephrosis has been produced in rats by the subcutaneous injection of an aminonucleoside, 6-dimethylamino purine, 3-amino-*d*-ribose (5, 6). The disease which occurs in rats is so strikingly similar to the nephrotic syndrome in man that it was considered worth while to study kidney tissue from animals with aminonucleoside nephrosis and to compare the lesion occurring in animals with the drug induced nephrosis to the lesions observed in the human disease. In the present study, kidney tissues from animals sacrificed serially during the development of aminonucleoside nephrosis have been examined by light and electron microscopy. The purpose of this report is to describe serial ultramicroscopic changes in the glo-

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merular epithelial cell produced by aminonucleoside, to compare the abnormalities observed to those occurring in human nephrosis, and to correlate the development of proteinuria with the development of the ultramicroscopic lesion in the experimental animals. The findings permit the conclusion that aminonucleoside nephrosis in rats resembles nephrosis in man in its ultramicroscopic manifestations.

Methods

Immature, male Sprague-Dawley rats weighing 85 to 220 gm. were used throughout the course of the study. Experimental animals received daily subcutaneous injections of 1.5 mg. per 100 gm. body weight of aminonucleoside[1] as a 0.5 per cent solution. Control animals received daily subcutaneous injections of an equal volume of distilled water.

Three groups of animals were studied as follows:

Group I consisted of five control and five experimental animals studied to evaluate the completely developed disease. Daily injections of aminonucleoside were given to the experimental animals until they died or were sacrificed when death seemed imminent. All animals were sacrificed by the 18th day after injections were started and autopsies were performed immediately after death or at the time of sacrifice.

Group II consisted of 14 control and 42 experimental animals studied to evaluate the progressive ultramicroscopic changes in the kidney and to obtain data on blood chemistry and urine protein excretion. Daily injections of the drug were given to the animals in the experimental group for 10 to 12 days. Several animals were sacrificed each day beginning on the 4th day after initiation of injections.

Group III consisted of 12 experimental animals given aminonucleoside as above. In these animals, the daily urinary protein output was determined in order to obtain additional data for correlation of proteinuria with the development of the ultramicroscopic renal lesion.

The rats were fed a Purina fox chow diet *ad libitum*, had unlimited water, and were housed by pairs in separate cages. 12 to 24 hour urine specimens were collected from animals housed separately in metabolic cages having access only to water.

Physiological Observations—Body weights were recorded periodically and animals were examined daily for any signs of edema. Blood was drawn from the abdominal aorta prior to sacrifice and the following determinations were carried out:—

(*a*) Total serum protein by the Weichselbaum biuret method (7).
(*b*) Serum cholesterol by the method of Abell *et al.* (8).
(*c*) Blood urea nitrogen by the technique of Gentzkow (9).
(*d*) Paper electrophoresis of serum proteins using a veronal buffer at pH 8.6 (ionic strength of 0.1) and a Spinco electrophoresis apparatus. Electrophoresis was continued for 16 hours at a potential of 70 volts and a current of 5 milliamperes.

Urinary protein output was measured by two methods. In Group I, determinations were carried out with the standard semiquantitative precipitation technique using heat and acetic acid–NaCl solution. In Groups II and III, quantitative urinary protein excretion was determined by initial trichloroacetic acid precipitation in the cold and subsequent analysis by a modification of the method developed by Hiller *et al.* (10) utilizing an alkaline copper sulfate reagent.

Preparation and Examination of Tissues for Microscopy—Kidney tissues from 25 experimental and 9 control animals were prepared for electron microscopy. At the time of sac-

[1] Kindly supplied by Dr. Stanton M. Hardy, Lederle Medical Research Department, American Cyanamid Co., Pearl River, New York.

rifice, animals were anesthetized with ether and the kidneys exposed. One cubic millimeter blocks were cut immediately from the kidney cortex and immersed in 1 per cent buffered osmic acid at 4°C. for 2 hours. The blocks were then rinsed twice in buffer, dehydrated in serially increasing concentrations of alcohol, and embedded in 90 per cent butyl–10 per cent methyl methacrylate. Details for the method used to prepare the tissues for study by electron microscopy are available elsewhere (11).

Sections approximately 200 to 500 A units in thickness were cut with a Servall Porter-Blum microtome (12), mounted on formvar-coated copper specimen grids, and examined in an RCA EMU-3 electron microscope. Sections 1 micron thick were obtained from the same blocks and stained with either Wright-Giemsa (13) or gallocyanin-phloxine stain (14). In this manner, light and electron microscopic study of the same glomerulus was possible.

Complete autopsies were performed on all animals. Sections of spleen, lung, liver, heart, kidney, and adrenal were stained with hematoxylin and eosin for study by light microscopy and sections of kidney were also stained with periodic acid–Schiff's reagent.

Experimental Results

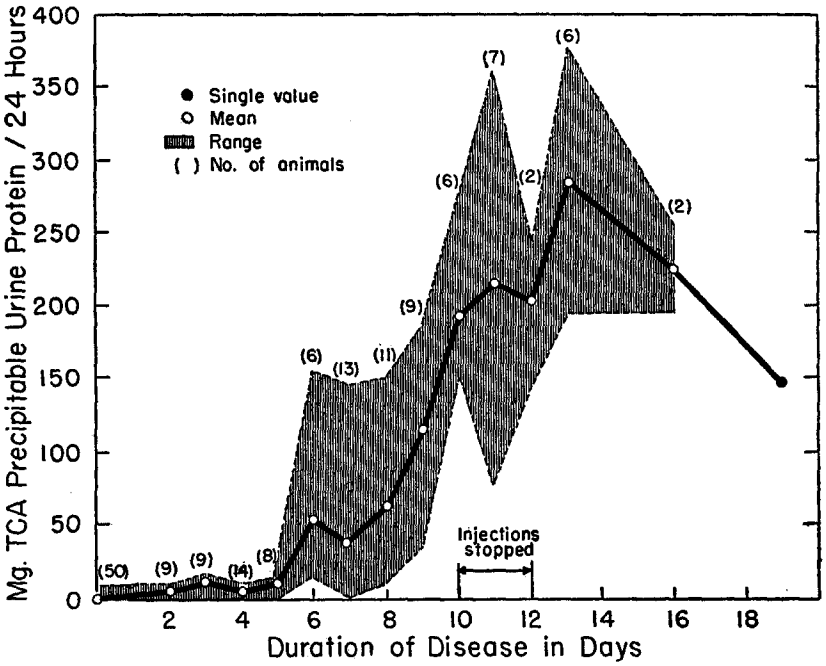
Protein was absent from the urine of normal young animals (90 to 120 gm.) and present in very small amounts in older rats (140 gm. and above). Among 50 normal animals a mean quantity of 0.4 mg. of protein (range 0 to 13 mg.) was excreted per 24 hours. Increased urinary protein excretion was noted in the experimental animals by the 6th day after initiation of the aminonucleoside injections and the degree of proteinuria increased progressively, reaching a peak by the 13th day of the study (Text-fig. 1). Serum albumin levels were lower than normal in the experimental groups by the 8th day of the experiment and serum cholesterol and blood urea nitrogen values rose progressively after the 11th day. Comparison of electrophoretic patterns of serums from nephrotic and normal animals indicated that the principal decrease in serum protein occurred in the albumin fraction.

Few animals survived more than 13 daily injections of aminonucleoside. However, if injections were discontinued between the 10th and 12th day, some of the animals ultimately recovered completely. Four animals were sacrificed on the 19th to 28th day of the experiment (9 to 18 days after injections of aminonucleoside were discontinued), when recovery was incomplete. The urinary protein excretion in this group decreased, but the blood urea nitrogen and serum cholesterol values remained significantly elevated in all animals at this stage of recovery.

Microscopic Findings.—Examination of microscopic sections of the spleen, liver, lungs, heart, and adrenals of both the experimental and control animals revealed no specific abnormalities, and the kidneys of all control animals appeared normal. Prominent pathologic changes were observed in the kidneys of the experimental animals with the fully developed disease and consisted of numerous eosinophilic tubular casts, dilatation of the proximal, distal, and collecting tubules, and flattening of the epithelium of the distal tubules. Although no abnormalities of the renal glomeruli from experimental animals were appar-

ent in sections stained by the hematoxylin and eosin (H and E) method, periodic acid-Schiff (PAS) stains revealed definite thickening of the glomerular membranes from rats sacrificed late in the course of the disease (Figs. 1 to 3).

Thickening of the glomerular membranes of experimental animals sacrificed after the 12th day of the study was also apparent by microscopic study of stained thin sections of osmium-fixed, methacrylate-embedded kidney tissue.



TEXT-FIG. 1. Quantitative urine protein excretion of rats receiving aminonucleoside (Groups II and III).

These thin sections also revealed abnormal masses of homogeneous, basement membrane-like material which appeared to be connected to the glomerular membrane (Fig. 4).

Ultramicroscopic Findings

Glomerular Morphology in Control Animals.—The glomerular capillary wall has three components: the endothelium, the basement membrane, and the epithelium. The endothelial cell cytoplasm becomes attenuated peripheral to the nucleus and forms a very thin fenestrated membrane which lines the capillary internally. Distinct interruptions or "pores" (15) are noted in cross-sections of this layer. The basement membrane is a homogeneous, amorphous layer, 800 to 1200 A in thickness. External to the basement membrane, the

epithelial cells lie between the capillary loops. Organization of the epithelial cell cytoplasm is characterized by a complex system of primary processes which further divide into secondary or "foot processes" (16). The foot processes rest upon the basement membrane of the glomerular capillary, and spaces which connect with Bowman's space occur at regular intervals between the adjacent foot processes. That portion of the foot process nearest the basement membrane is usually much more dense than is the remaining cytoplasm of the epithelial cell.

Glomerular Morphology in Aminonucleoside Nephrosis.—The glomerular morphology of kidney specimens from rats given daily injections of aminonucleoside was correlated with the duration of treatment in each animal prior to sacrifice, and the serial pathologic changes will be described in the sequence in which the changes appeared to develop.

Fourth through Sixth Day.—The glomeruli from rats sacrificed after four to six daily injections of aminonucleoside did not differ significantly from control animals and were considered to be normal. The mitochondria of endothelial and epithelial cells were carefully studied in an attempt to detect minimal morphologic alterations in these structures which might precede the more obvious changes to be described, but no abnormalities were noted (Figs. 5 and 6).

Seventh through Tenth Day.—The earliest glomerular change noted was swelling of the primary and secondary "foot processes" of the epithelial cells. This was consistently observed in rats given more than seven daily injections of aminonucleoside. The foot processes were broader than normal and appeared to fuse to one another along the basement membrane, resulting in loss of spaces between adjacent foot processes (Fig. 7). Distortion of the foot processes of the epithelial cells from animals studied after 7 to 8 days of administration of aminonucleoside was variable from capillary to capillary and from glomerulus to glomerulus. However, fusion of the epithelial cell foot processes was a uniform finding in those animals treated 9 or more days.

The glomerular epithelial cells of animals sacrificed after 7 or more days of treatment contained larger and more numerous cytoplasmic vesicles and greater amounts of granular material than did the epithelial cells of normal animals. Other cytoplasmic detail within the epithelial cell, including fine mitochondrial structure, did not appear abnormal.

Eleventh through Eighteenth Day.—The changes in the epithelial cell, described above, were more prominent in animals which had received eleven to sixteen daily injections of aminonucleoside. Dense accumulations of epithelial cytoplasm which extended along the basement membrane with only infrequent interruptions replaced the epithelial foot processes in all glomeruli. Vacuole formation and increase in granular material within the cytoplasm of the epithelial cell were more extensive than that observed earlier in the course of the serial studies (Fig. 8).

Hypercellularity of glomeruli, consisting of an increase in endothelial cells, was occasionally observed by the 11th day but was consistently present by the 14th day of the experiment. The increase in number of endothelial cells was associated with an accumulation of dense homogeneous material which resembled basement membrane morphologically. This basement membrane-like material occurred as amorphous bands and irregular masses between the endothelial cells and appeared to encroach upon the capillary luminal spaces. Occasional glomeruli, in which this process of capillary obliteration was more extensive, showed open capillary lumina only at the periphery of the lobule (Fig. 9). These changes were correlated with the glomerular membrane thickening observed by light microscopic study of kidney sections stained with periodic acid-Schiff reagent.

Renal Tubular Structure in Control Animals.—The morphology of the normal proximal (17) and distal (18) tubule from various animal species have been described. Although some variation in the organization of the tubular cell at different levels of the nephron is recognized, the following brief review of typical tubule cells is presented for reference to the pathologic changes observed.

Proximal tubule cells are limited on the luminal border by ultramicroscopic villi which arise from a complex infolding of the plasma membrane forming the brush border. This layer in longitudinal sections appears as multiple folds of double membranes at the apex of the cell. The limiting plasma membrane at the base of the cell folds apically, dividing the cytoplasm of the cell into a number of compartments of variable size and shape. An amorphous homogeneous layer 800 A in thickness forms the basement membrane which is continuous from cell to cell around the basal circumference of the tubule. Numerous oval and cylindrical mitochondria are seen within the cytoplasm of the cell, the greatest concentration being in the basal region of the cell, and double walled intramitochondrial membranes or cristae are visible in mitochondria properly oriented during sectioning. Minute vesicles, granules, and membranes of the endoplasmic reticulum contribute to the complex organization of the cytoplasm of the proximal tubular cell. The nucleus is oval, has a double walled membrane, and contains a homogeneous, finely granular nucleoplasm.

A typical cell of the distal tubule is characterized by an elaborate system of infoldings of the basal plasma membrane which divide the basal cytoplasm into a number of rather uniform compartments oriented perpendicularly to the basement membrane. This organization represents the striated border of the distal tubule as it is recognized in the light microscope. The basement membrane is similar to that of the proximal tubule. A plasma membrane, which folds to form occasional finger-like projections, limits the lumen of the distal tubule cell. Mitochondria in the distal tubule tend to be more cylindrical than those of the proximal tubule, but the morphology is otherwise similar (Fig. 10).

Tubular Morphology in Aminonucleoside Nephrosis.—Rats given one to six

daily injections of aminonucleoside showed no abnormalities of tubular morphology. The earliest lesion consisted of apparent vacuole formation within the mitochondria of the distal tubules of rats treated for 7 days. Careful study of the mitochondria revealed that the vacuoles were due to focal swelling between the intramitochondrial membranes (cristae) resulting in an approximately twofold increase in the normal distance between the cristae (Fig. 11).

This abnormality was present in the proximal tubules somewhat later in the course of the disease. Apparent progression of this abnormality resulted in severe swelling of mitochondria and fragmentation of cristae in some renal tubules of animals sacrificed on the 12th to 16th day of the experiment. Animals sacrificed after ten daily injections of aminonucleoside frequently showed additional changes in the cytoplasm of both the proximal and distal tubular cells. The density of the cytoplasm of some tubules was greatly decreased, suggesting swelling and a probable increase in water content of the cytoplasm of the cells (hydropic change). Large, rounded, dense masses of osmophilic material, thought to represent reabsorbed protein or fat, were also observed within the tubular cells. These bodies probably represent the hyalin droplets, previously observed in nephrosis by light microscopy and previously described in electron microscopic studies of the tubular cells in lipoid nephrosis in man (19).

Glomerular Morphology in Animals Which Had Partially Recovered.—Four rats which were studied 9 to 18 days after the last injection of aminonucleoside showed decreasing proteinuria and disappearance of ascites. Electron microscopic examination of glomeruli from these animals revealed numerous areas of relatively normal epithelial cell foot process organization, while other portions of glomerular capillaries were covered by an uninterrupted layer of epithelial cell cytoplasm, similar to the abnormality described above. Distribution of normal and abnormal epithelial cells was variable from glomerulus to glomerulus and from capillary to capillary within a glomerulus.

DISCUSSION

Electron microscopic examination of the glomeruli from rats treated with aminonucleoside revealed definite abnormalities of the epithelial cells. Serial studies of the development of this lesion indicated that the first change was a swelling and coalescence of the foot processes beginning on the 7th day after injections of aminonucleoside were begun. This was followed by fusion and disappearance of most of the primary cytoplasmic processes, and finally by an increase in density of the cytoplasmic layer immediately adjacent to the basement membrane proper. In addition, large vacuoles containing granular material, similar in appearance to precipitated protein, were observed within the cytoplasm of epithelial cells of the nephrotic animals. These abnormalities of the epithelial cells of rats treated with aminonucleoside are identical with the morphologic changes which we have described in a variety of forms of nephrosis in

man (4), and indicate that these abnormalities may represent a unifying lesion linking together nephrotic syndromes produced by diverse etiologic agents.

Further evidence of a relationship of severe proteinuria which occurs in nephrosis to the epithelial cell abnormalities described above is provided by the observation of partial restoration of normal foot process structure in partially recovered animals in this study and in children during remissions of nephrosis, whether the latter have occurred spontaneously or have been induced by treatment with adrenal steroids (1).

In view of these findings, the role of the epithelial cell in glomerular filtration and in the pathologic physiology of proteinuria deserves reconsideration. The parallel development of proteinuria and the obliteration of normal epithelial cell foot process structure, together with the appearance of large vacuoles within the cytoplasm of these cells, might be interpreted as indirect support for the suggestion of Rinehart (20) that filtration takes place through the epithelial cell rather than between the foot processes. According to this view, the ultrafiltrate passes through three membranes: the endothelium, the basement membrane (lamina densa), and the epithelium, before reaching Bowman's space. In accord with this hypothesis, the granular material observed within the epithelial cytoplasmic vacuoles might represent protein-rich filtrate traversing the cell. Thus it is possible that the morphologic changes observed in the epithelial cells in nephrosis reflect an increase in the permeability of the membrane to serum proteins due to changes in these cells and that the primary site of the pathology of the disease is the epithelial cell.

An alternative possibility is that the lamina densa is the rate-limiting membrane for protein filtration, in which case the observed abnormality of the epithelial cell might be considered as a secondary or concomitant alteration of the glomerular capillary in nephrosis. Coalescence of the epithelial cell foot processes could then be considered as an adjustment of the cell to prevent protein losses across an abnormal basement membrane. Although no consistent abnormalities of the basement membrane have been detected in either human or experimental nephrosis, this concept cannot be excluded on morphologic evidence alone.

Finally, it is conceivable that the proteinuria of nephrosis could be the result of either active secretion of protein or decreased reabsorption of protein by the renal tubule. Alterations of the fine structure of mitochondria in the distal tubule were observed at the time of onset of proteinuria in rats given aminonucleoside for 7 days. As the proteinuria became more severe, similar abnormalities in mitochondria of proximal tubules and an over-all decrease in tubular cytoplasmic density (hydropic changes) developed, and the tubular cells contained irregular masses of dense material thought to be protein. Whether these abnormalities reflect a primary defect in either tubular secretion or reabsorption, or whether they represent secondary responses of the tubule to the presence of a protein-rich filtrate, could not be determined by the present study.

The morphologic changes in the kidney in human nephrosis (3, 5, 21) are correlated with the duration and the severity of the disease. Thus, early in the course of nephrosis, light microscopic studies revealed no abnormalities of the glomerulus, while later in the disease (mixed nephrosis-nephritis or the nephrotic syndrome with chronic glomerulonephritis) scarring of the glomerulus was evident. However, the epithelial cell abnormality which we have described was present by electron microscopy in all cases of human nephrosis which we have studied regardless of their severity or duration. Similarly, the glomeruli from animals with aminonucleoside nephrosis showed no definite pathological alterations by light microscopv until late in the course of the disease. Endothelial cell proliferation and accumulation of dense, homogeneous material similar in appearance to the lamina densa was evident only late in the course of the disease, when glomerular membrane thickening was readily detectable by light microscopy.

It has previously been pointed out that the basement membrane, as seen in the light microscope, represents all three layers of the glomerular capillary membrane (3). Swelling of the epithelial cell and fusion of the foot processes probably represent the earliest manifestation of the glomerular basement membrane thickening (22) noted in human nephrosis by light microscopy. The more severe basement membrane thickening demonstrated by special stains late in the course of human nephrosis or experimental aminonucleoside nephrosis in rats is doubtless related to the accumulation of the basement membrane-like material mentioned above.

Previous reports of aminonucleoside nephrosis (5, 6) have described uremia as a characteristic finding in rats studied late in the course of the disease, yet no satisfactory explanation for uremia has been presented. In the present experiments, endothelial cell proliferation and accumulation of dense basement membrane-like material in the glomeruli of rats studied after the 11th day of the experiment was associated with the development of uremia in those animals, suggesting that the uremia might be due to a decrease in capillary luminal area with a concomitant decrease in renal blood flow. Since the number of glomeruli examined in each animal was small, we cannot be certain that the observed decrease in capillary luminal area satisfactorily explains the uremia. Further studies of the cytotoxic effects of aminonucleoside on other tissues of the rat and discrete physiologic studies of kidney function at this stage may elucidate a possible prerenal source or define the renal basis of the uremia.

SUMMARY

Experimental renal disease was produced in young rats by daily subcutaneous injections of 6-dimethylamino purine, 3-amino-*d*-ribose (aminonucleoside). The physiologic, biochemical, and light microscopic changes were similar to those observed in human nephrosis.

Electron microscopy of the glomeruli from animals which received seven or

more daily injections of aminonucleoside revealed characteristic abnormalities of the epithelial cells of the glomerular capillaries. These changes consisted of swelling, coalescence, and eventual obliteration of the epithelial cell foot processes and an increase in the number and the size of epithelial cytoplasmic vacuoles. The serial development of the ultramicroscopic pathologic changes in the epithelial cells, as observed by study of animals through the course of the disease, indicated that the smudging of the foot processes occurred at the time of onset of severe proteinuria.

Further changes, consisting of hypercellularity due primarily to an increase in the number of endothelial cells and an increase in the amount of basement membrane-like material, were regularly observed by the 12th day after injections were begun. These abnormalities were correlated with the development of uremia in the later stages of the disease process.

The distal and proximal renal tubules were abnormal by the 7th day after injections were begun, and showed localized swelling between the cristae of the mitochondria and a decrease in over-all cytoplasmic density, described as hydropic change.

Electron microscopy of glomeruli from animals which had partially recovered from aminonucleoside nephrosis revealed areas of normal epithelial cell morphology. This observation was interpreted as evidence of partial reversal of the ultramicroscopic changes in the recovery phase of the disease.

These observations of the fine structure of pathologically altered glomeruli and tubules in aminonucleoside nephrosis are similar to our findings in human nephrosis as revealed by electron microscopy of serial renal biopsies.

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EXPLANATION OF PLATES

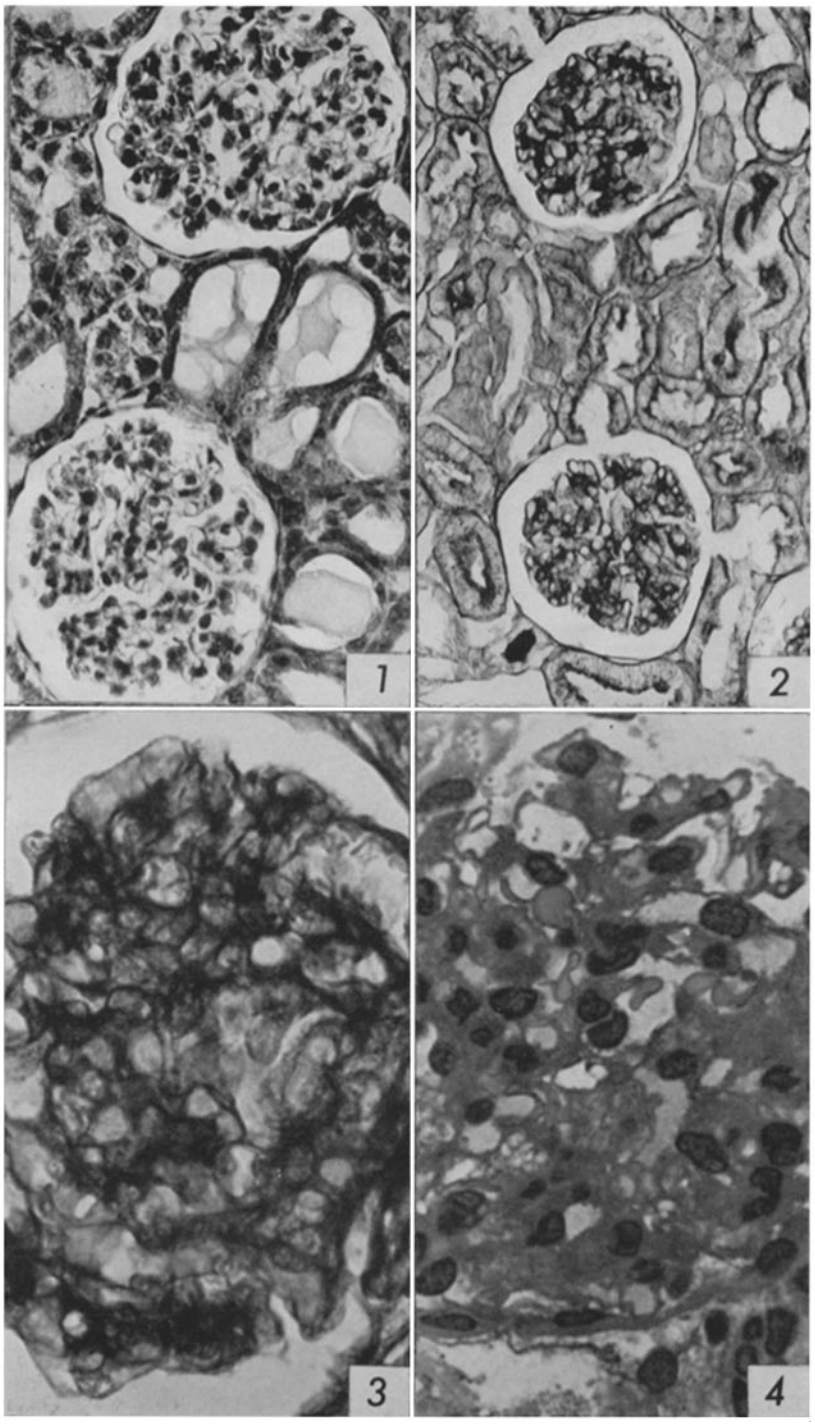
PLATE 13

FIG. 1. Hematoxylin-eosin stain of a section of kidney from a nephrotic rat sacrificed on the 16th day of the experiment. Note the protein casts and dilated tubules. The glomeruli appear normal. × 180.

FIG. 2. Periodic acid–Schiff (PAS) stain of a kidney section from a rat sacrificed on the 9th day of the experiment. The glomerular basement membranes show thickening in some areas. × 120.

FIG. 3. Periodic acid–Schiff stain of a kidney section from a rat sacrificed on the 16th day of the disease. The entire glomerular loop demonstrates diffuse basement membrane thickening. × 400.

FIG. 4. Gallocyanin-phloxine stain of a 1 micron section of kidney cut from a block which had been fixed with osmic acid and embedded in methacrylate. The rat was sacrificed on the 16th day of the experiment. The diffuse membrane thickening shown here may be compared with the changes seen in an electron micrograph (Fig. 9).



(Vernier *et al.*: Aminonucleoside nephrosis. I)

PLATE 14

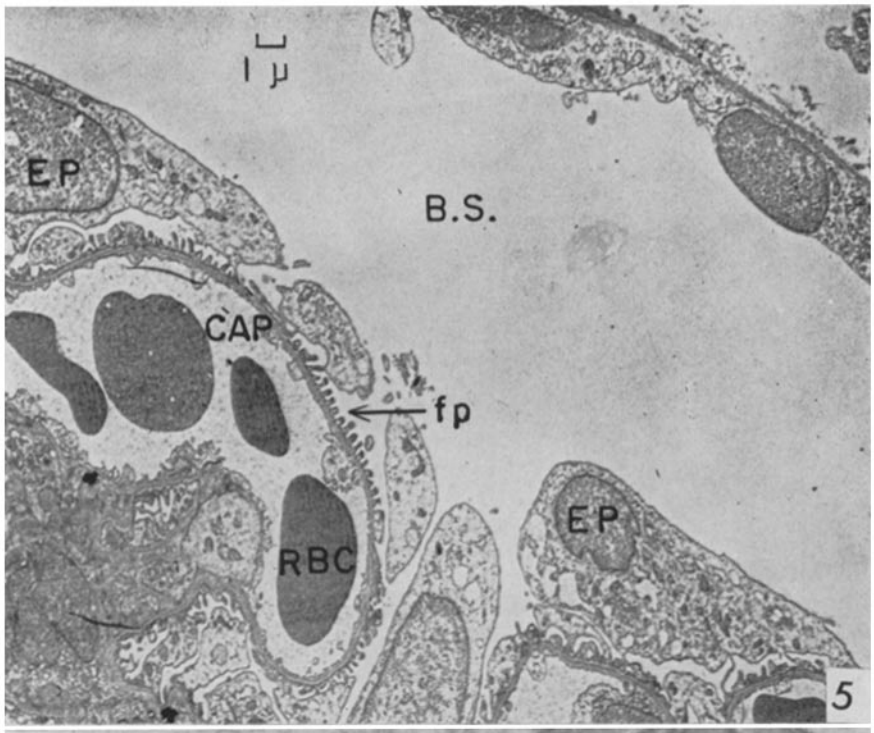
FIG. 5. Low power electron micrograph of a section of kidney from a rat sacrificed on the 4th day of the experiment. The glomerular morphology is not significantly different from that of the untreated rats.

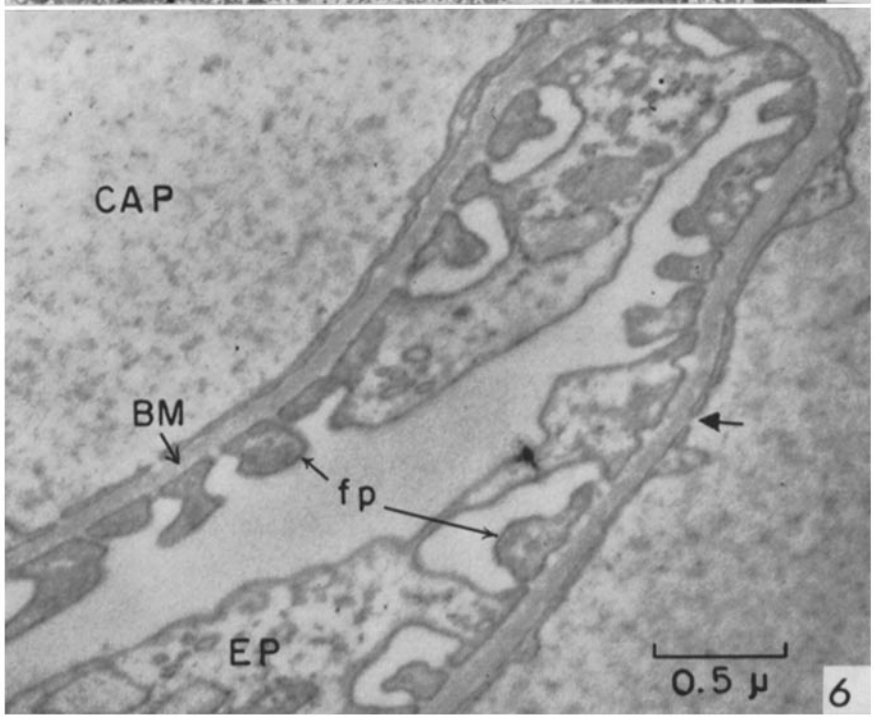
FIG. 6. Higher magnification electron micrograph of a section of kidney from a rat which had received four daily injections of aminonucleoside. The epithelial foot processes are normal. The arrow on the extreme right indicates an endothelial pore.

Explanation of Abbreviations Figs. 5 through 11

END, endothelium	*B.S.*, Bowman's space
BM, basement membrane	*B.C.*, Bowman's capsule
EP, epithelium	*fp*, foot process
RBC, red blood cell	*m*, mitochondria
CAP, capillary	

Linear markers indicate the relative magnification of each illustration.

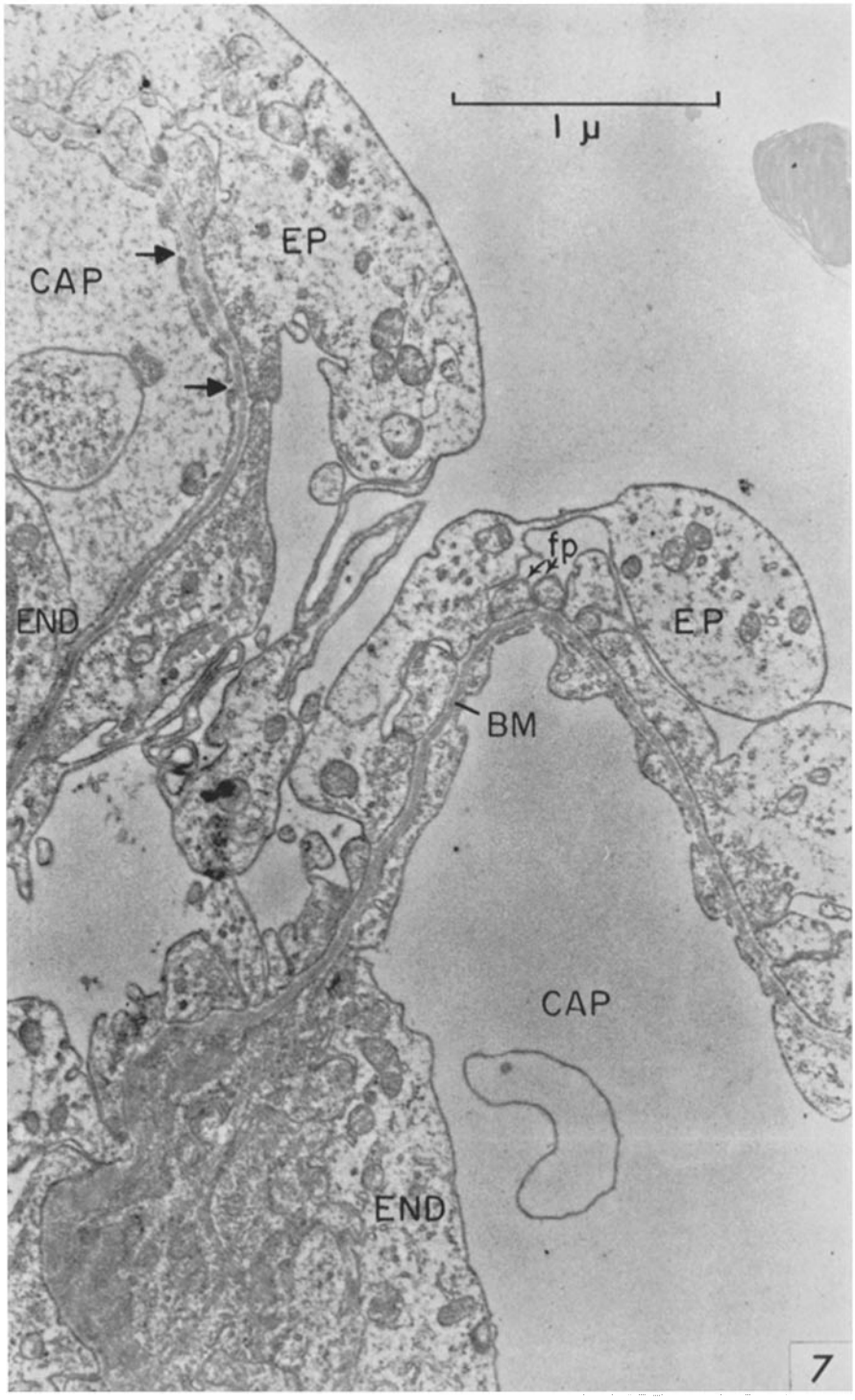




(Vernier *et al.*: Aminonucleoside nephrosis. I)

PLATE 15

FIG. 7. Electron micrograph of a section of kidney from a rat which was given ten daily injections of aminonucleoside. Swelling and fusion of the epithelial cell foot processes around two glomerular capillaries are shown. The endothelium and basement membranes are normal. Endothelial pores are indicated by arrows.

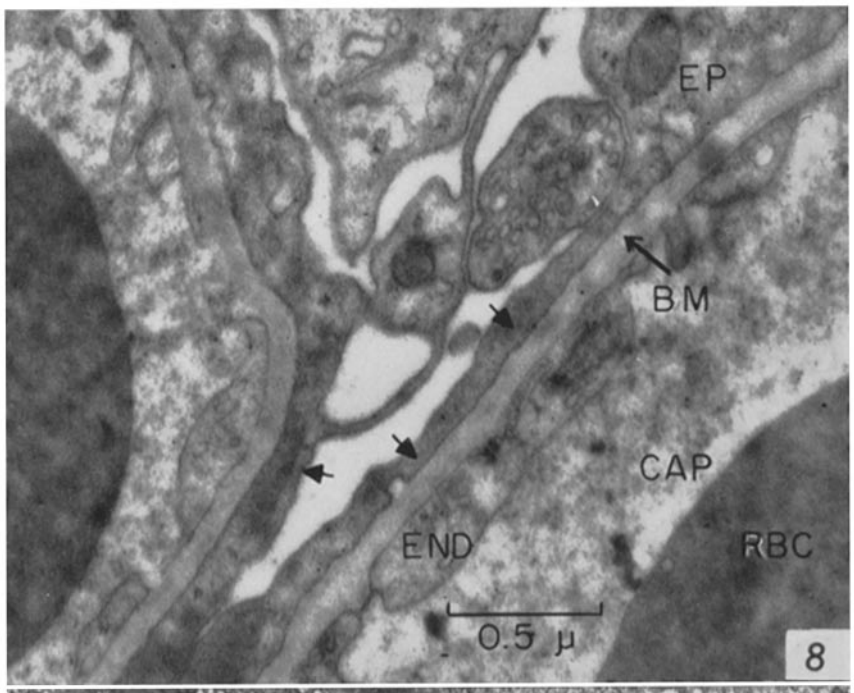


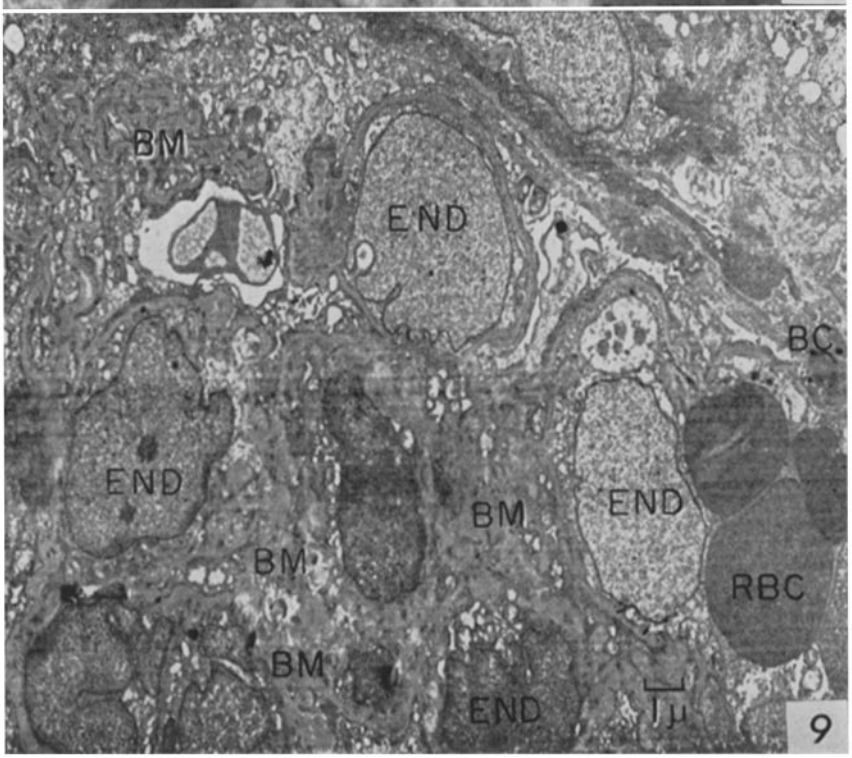
(Vernier *et al.*: Aminonucleoside nephrosis. I)

PLATE 16

FIG. 8. Higher magnification electron micrograph of a section of kidney from a rat which was sacrificed on the 16th day of the experiment. The layer of fused epithelial cell foot processes is indicated by arrows. The endothelium and basement membrane are normal.

FIG. 9. Low magnification electron micrograph of a section of kidney from a rat which was sacrificed on the 16th day of the experiment. This is the same glomerulus illustrated in Fig. 4. Except for the capillary on the extreme right, the circulation has been occluded by an increase in the number of endothelial cells and by an increased amount of basement membrane-like material. The epithelial cell foot processes are seen to be fused into a continuous layer, similar to the change observed in Fig. 8.



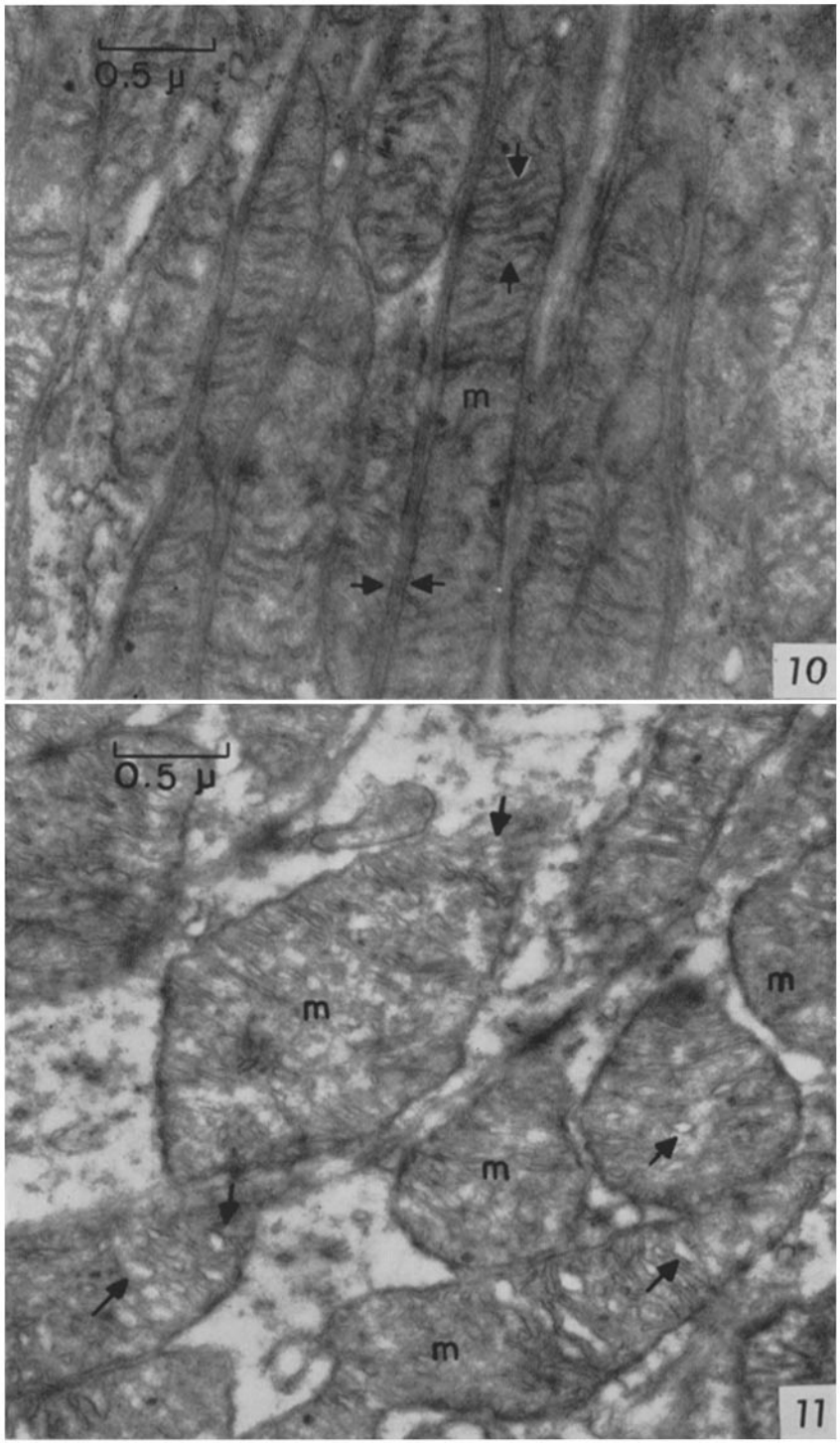


(Vernier *et al.*: Aminonucleoside nephrosis. I)

PLATE 17

FIG. 10. High magnification electron micrograph of mitochondria (longitudinal section) in a distal tubule cell from a normal rat. Note the mitochondrial tubular membranes (cristae) indicated by vertical arrows. The horizontal arrows point out the parallel double membranes of two adjacent mitochondria and the intervening infolded plasma membrane.

FIG. 11. High magnification electron micrograph of mitochondria (cross-section) in a distal tubule from a rat which was sacrificed on the 8th day of the experiment. Note the separation of the membranes of the cristae and the formation of microvacuoles (arrows).



(Vernier *et al.*: Aminonucleoside nephrosis. I)