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THE STRUCTURE OF THE KIDNEY IN RELATION TO AGE AND DIET IN WHITE RATS DURING THE WEANING PERIOD

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Young mammals at birth are commonly found to be unable to secrete a urine as concentrated as that which adults of the same species can form (McCance, 1948; Falk, 1955). Although the neurohypophyses of some young mammals are poor in antidiuretic hormone (Heller & Lederis, 1959), the failure to produce a concentrated urine during dehydration is due to the immaturity of the kidney (Heller, 1961), exogenous antidiuretic hormone having, for example, only a slight action in babies a week old (Heller, 1944). Similarly, the normal concentrating response of adults to antidiuretic hormone can first be obtained in rats only between 23 and 33 days of age (Heller, 1952; Falk, 1955; Dlouhá, Křeček & Křečková, 1958: Křeček, Dlouhá, Jelínek, Křečková & Vacek, 1958; Křeček, Dlouhá & Křečková, 1958).

The evidence of sampling by micropuncture from individual nephrons (Wirz, 1957; Gottschalk & Mylle, 1959), together with the observation that there is through all tissues of the renal medulla a gradient of hypertonicity, rising towards the pelvis (Ullrich, Drenkhahn & Jarausch, 1955), has led to the general conclusion that renal concentration depends on medullary structures. More particularly, the experimental findings have found their most satisfactory explanation so far in the hypothesis that the production of a hypertonic urine results from flow in a hairpin countercurrent concentrating system (Wirz, 1957). The loop of Henle is an essential part of such a system and therefore, according to the hypothesis, essential to the action of antidiuretic hormone in the formation of an hypertonic urine.

For this reason it was felt desirable to investigate the structural development of the kidney in relation to the known sequence of events in functional maturation. If the countercurrent hypothesis is correct, absence or, possibly, defect of Henle's loops should be found only in a kidney not yet capable of a full adult concentrating response to antidiuretic hormone. Peter (1927) and Gruenwald & Popper (1940) did in fact find that the nephrons of the human kidney are not all structurally mature at birth, and Sperber (1941) found post-natal elongation of Henle's loops in kittens. The studies to be reported here, however, were carried out with rats, in view of the large body of experimental findings available on the formation of urine in the young of those animals. Baxter & Yoffey (1948) described histological changes in the kidneys of rats in the period immediately after birth, but they gave no information on the medulla. In our investigation histological changes in both cortex and medulla have been sought during the first 5 weeks of post-natal life, and an attempt made to affect these changes by variations in diet. Some of the findings have already been published in summary (Boss, Dlouhá, Kraus & Křeček, 1962).

METHODS

Animals and diet. White rats of both sexes, of the Konarovice strain, were reared on four different dietary regimes in Prague, and the kidneys, after fixation and embedding in paraffin wax, were brought to Bristol for histological investigation.

The rats were fed thus: (1) One group was left with the mothers and with free access to water and Larsen rat diet (Fábry, 1959). Animals so reared were killed on the 1st, 11th, 23rd or 33rd day of life. These rats began to be weaned when about 2 weeks old; weaning is generally complete in similar animals at about 4 weeks of age. (2) A second group was treated similarly, but was removed from the mothers at 14 days of age. Rats of this group were killed on the 23rd or 33rd day. (3) A third group was fed in the same way as the second, but had access to 3 % NaCl solution as well as to water. They took NaCl solution and fresh water in such proportions as to obtain an average of 0·7 % NaCl in their water, irrespective of the absolute amount drunk. Rats were killed on the 23rd or 33rd day. (4) A fourth group differed from the second in that the Larsen diet was replaced by the high-fat diet, referred to by Fábry (1959) as II/c. Dividing the dietary groups according to the age at killing, there were ten sub-groups altogether; each comprised 4 rats, making a total of 40 animals for the entire investigation.

Histological methods. Rats, suddenly stunned, were then killed by cutting their neck vessels; the smaller animals were completely beheaded. The kidneys were immediately removed and placed in fixative. Kidneys from rats other than those 1 day old were cut coronally before fixation.

In every group of four animals one kidney from each of two rats was fixed in Susa and one in neutral buffered isotonic 4 % formaldehyde. From each of the other two rats one kidney was fixed in a 5 % solution of glacial acetic acid in absolute ethanol at 4° C, and one in a mixture of 9 parts of saturated aqueous $HgCl_2$ and 1 part of commercial formalin. The kidneys fixed in buffered formaldehyde, and those fixed in a mixture of acetic acid and alcohol, were not used in the studies reported here; thus the results to be presented depend on one kidney from each rat. All material was embedded in paraffin wax, and most sections were cut coronally at 6 μ (Susa) or 4 μ (formalin-sublimate) so as to include all zones of the kidney in each.

Sections from material fixed in Susa were stained with Ehrlich's haematoxylin and eosin, by Heidenhain's Azan, or by the Feulgen reaction with light green as a counterstain: all three methods were used for each block. Sections of kidneys fixed in the formalin-sublimate mixture were stained for mitochondria by the method of Bharadwaj & Love (1959), as this technique had already been found useful in distinguishing cells of different types in the renal tubules (Boss, 1961). (The removal of nucleic acids, recommended by the authors, was omitted, as it did not affect the result and sometimes caused sections to lift off the slides).

All the stained sections from each block were examined for the presence, structure and spatial relations of all segments of the nephron.

RESULTS

Effects of diet

Within each age group kidneys were compared with respect to the features, discussed below, which were found to vary between rats of different ages. Neither these nor any other features were found to differ with diet.

Differences with age

Nephrogenic zone. In the first post-natal day the outer half of the cortex consisted of a nephrogenic zone. In this region the types of tubules were not readily distinguished, and Malphighian bodies were represented by spheres of cuboidal or low columnar epithelium invaginated from one side, as in the description of the nephrogenic zone of the human foetus by Potter & Thierstein (1943). At 11 days the division of the cortex into two zones was less obvious, but the outer region was still markedly poorer in capillaries and richer in mitosis than the inner. At 23 days all trace of the nephrogenic zone had been lost.

Malpighian bodies. Even within the more differentiated zone of the cortex the Malpighian bodies, although having capillaries and a capsular space, were immature at birth (Pl. 1*A*). The epithelium of Bowman's capsule was not as thin as in the adult but varied from thick squamous to cuboidal, being generally taller on the visceral than on the parietal side of the cavity, the appearance being that depicted by Klein, Burdon-Sanderson, Foster & Brunton (1873) for the human foetus and described by Gruenwald & Popper (1940) for the new-born baby. At 11 days and afterwards the epithelium of Bowman's capsule was thin, as in the adult (Pl. 2*A*). There appeared to be an increase in the number of capillaries in the glomerular tuft between the 1st and 11th days, but no counts were made. In general, the parietal basement membrane of Bowman's capsule appeared, in the 1-day kidneys, to be thickest in Malpighian bodies with the lowest epithelium.

Proximal convoluted tubules (cortical segments). The proximal convoluted tubule has two segments. In the rat the second, medullary part is long and contorted, and will be discussed separately. It is the first, cortical segment which corresponds to classical descriptions of the proximal tubule (Boss, 1961). The following description does not apply to the primordial proximal tubules, without brush borders, found only in the nephrogenic zone.

In the day-old rats the epithelium of the cortical parts of the proximal tubules showed intracellular spaces, such as might be seen after the removal of droplets by the lipid solvents used in embedding. These were similar to the spaces corresponding to lipid droplets in the kidneys of carnivores. These spaces do not occur in similar sections prepared from adult rats (Boss, 1961) and were, in the studies described here, absent at 11, 23 and 33 days. Previous work on adult rats (Boss, 1961) showed that the cells of the proximal tubules contained lipid, which was not dissolved out during embedding to leave clear spaces, whether after Susa, formalin-sublimate or formalin-chromic acid fixation; this lipid was probably present as lipoprotein. Lipid droplets such as these were absent in all the rats used in the present studies. It thus appears that lipid of one kind was present at 1 day old, while neither this nor lipid of the other, adult type occurred in the proximal tubules at 11, 23 or 33 days of age.

The mitochondria were crowded to the sides of each cell in the proximal tubules at 1 day of age, by the central space which may have represented the site of dissolved lipid. At 11 days the mitochondria were not thus restricted in position, but their palisaded arrangement was fully developed only by 23 days. It appeared that an increase in number forced them into a pattern of parallel packing. At 23 and 33 days, therefore, the arrangement of mitochondria in the proximal tubules was similar to that found in the adult.

At 1 day old the capillary density among the proximal convoluted tubules appeared similar to that in the adult, but the capillaries were less densely arranged at 11 days; possibly the formation of new tubules had outrun the development of capillaries. At 23 and 33 days the number of proximal tubules and contiguous capillaries appeared again to be approximately in the adult proportion. No counts were made on any sections.

Proximal convoluted tubules (*medullary segment*). These parts of the nephrons are sufficiently long and contorted in the rat to form a broad zone, the outer medullary stripe (Longley & Fisher, 1954). At 1 day old they were either very short or very few, and there was no outer stripe to the medulla. At 11 days some medullary segments of proximal convoluted tubules could be seen partly differentiated and appeared to arise by the modification of cells without brush borders at the upper ends of Henle's loops. (If the latter were in fact present, they almost all must have consisted entirely of cuboidal cells; see below.) At 11 days there were some fully differentiated medullary segments of proximal tubules, and these appeared similar to the corresponding structures in the adult. At 23 and

33 days not only were the individual medullary segments adult in appearance, but they formed a continuous outer medullary stripe.

Thin segments of loops of Henle. At 1 day old these were difficult to find; an occasional one was seen. It was only a little easier to find them at 11 days (Pl. 1*B* and 2*B*). At 23 and 33 days, however, the thin segments of Henle's loops appeared as frequently in each field of the microscope as would be expected in a section of adult rat kidney.

Thick segments of loops of Henle. In the kidneys of the day-old rats the only medullary tubules, excepting an exceedingly rare thin segment, were of cuboidal epithelium and similar to each other. Although these tubules were rather different at 11 days (Pl. 1*B* and 2*B*), there was the same lack of variety within the age group. It is not possible to say whether these tubules at 1 and 11 days were collecting ducts and thick segments of Henle's loops, or collecting ducts only. At 1 day of age they all appeared, structurally, to be collecting ducts but at 11 days some were ambiguous in appearance. At 23 and 33 days thick segments were adult in appearance, readily distinguishable and abundant. (If there were few thick segments at 11 days, and therefore, in the presence of only a few thin segments, few loops, the differentiation of the medullary parts of the proximal tubules must have arisen largely at junctions with distal tubules, and not with loops.)

Medullary connective tissue. In day-old kidneys the tubules in the medulla were not packed closely together but were separated by a loose connective tissue resembling embryonic mesenchyme. There were few capillaries. At 11 days this loose connective tissue was still present but at 23 and 33 days the medullary connective tissue consisted mainly of basement membranes, as in the adult animal. There was some increase in the number of capillaries at 11 days, and in the older kidneys they appeared to be as abundant as in the adult organ.

Distal convoluted tubules. These are present at 11, 23 and 33 days. They may also have been present in the day-old rats but, if so, they were not typical in appearance. What may have been distal tubules in the 1-day kidneys were smaller than at later stages, with cells taller in proportion to their width and without rod-like mitochondria. The possibility of confusion with radicles of the collecting duct system therefore arose. At 11, 23 and 33 days the appearance of distal tubules was as in the adult, with palisaded mitochondria in the deeper part of the cell but not in the more superficial cytoplasm. The tubules were distinguishable from thick segments of Henle's loops, which had similar mitochondria, but which were without the superficial cytoplasmic layer.

Collecting ducts. There were at all ages collecting ducts similar in appearance to those found in the adult. The smallest collecting ducts present at 11 days were smaller than any found at 1 day, presumably because of the outgrowth of ducts of further orders of subdivision during the intervening period (Pl. 1*B* and 2*B*).

Mitosis. Mitosis was strikingly abundant in the day-old kidneys, especially in the nephrogenic zone, where there were 5–10 figures in each low-power field, about 1 mm in diameter. Cell division, although less abundant, was still easily found at 11 days, but rare at 23 days and not present in every section at 33 days. The primordia of Malpighian bodies in the nephrogenic zone contained dividing cells, but no mitosis was present in these structures at more mature stages. Fully differentiated cells of both parts of proximal convoluted tubules were found very rarely in mitosis at any age, but dividing cells were more easily found in less differentiated cells adjoining the distal ends of proximal convoluted tubules. Still more division figures were seen in thick segments and distal tubules, but none were ever present in either the papillary parts of collecting ducts or in thin segments of Henle's loops. Connective tissue both cortical and medullary, contained abundant mitotic figures at 1 day, but at later stages cell division was found in only some of the slides from each block.

DISCUSSION

The disappearance of the nephrogenic zone at some time between the 11th and 23rd days corresponds with the finding of Baxter & Yoffey (1948) that storage of intra-vitally administered dye by proximal convoluted tubules extends to the outermost cortex at 12 days; a tubule was able to segregate the dye when its brush border had developed. Potter & Thierstein (1943), defining the nephrogenic zone not by the state of the tubules but by the presence of immature glomeruli, found that the zone persists in the human foetus or infant up to a body weight of 2·0–2·5 kg. In the studies reported here we found that the zone of immature tubules was also the zone of immature glomeruli, if maturity is defined by the presence of the brush border and glomerular capillary tuft respectively. Nevertheless, we found that, in day-old kidneys, even the more mature Malpighian bodies, not in the nephrogenic zone, had cuboidal epithelium lining Bowman's capsule. Similarly, otherwise mature proximal tubules did not exhibit the adult palisaded arrangement of mitochondria at 1 or 11 days of age.

Between 11 and 23 days of age three changes were observed in the medulla; thin segments of Henle's loops increased from sparseness to adult density, spaces between tubules were more fully vascularized and reduced to the thickness of basement membranes, and the medullary parts of proximal convoluted tubules increased in number to form an outer stripe to the medulla.

Whether the absence of thin segments indicates an entire absence of Henle's loops, or whether thick segments may be present but unrecognized, it appears that loops of mature form are abundant only after some date between the 11th and 23rd days. This is the period when, as pointed out at the beginning of this paper, the rat first responds in an adult fashion to antidiuretic hormone. This correspondence between functional and structural observations is consonant with the hairpin countercurrent theory, in which a loop of Henle with specified functions is necessary for the production of a hypertonic urine under the influence of antidiuretic hormone. Owing to the difficulties in the early cytological recognition of the thick segment, the development of the loops of Henle in the rat is now being checked by observation on lengths of tubule in digested kidneys.

Short diffusion distances between the limbs of loops would make the countercurrent system more effective, so that the close packing of loops, becoming complete between 11 and 23 days, may also be related to the maturation of the concentrating mechanism. The development of medullary capillaries should also play a part in the initiation of the concentrating mechanism, since the vasa recta should make the countercurrent system more efficient (Gottschalk, 1960).

Nothing is known of the functions of the medullary parts of proximal convoluted tubules, which have so far been beyond the reach of micropuncture (Ullrich, 1960). They vary greatly in form between species; some small mammals such as the rat have a broad outer medullary stripe of contorted tubules (Longley & Fisher, 1954), while the rabbit, for example, has in this position only straight segments of proximal tubules (Huber, 1932). On this part of the nephron, Ullrich (1960) has commented: 'The second part of the proximal tubule, the *pars recta*, is already partly included in the countercurrent system of the renal medulla'.

Even if the adult reaction to antidiuretic hormone depends upon a structurally adult medulla, some concentrating mechanism is present even at 10 days in the rat. Čapek & Heller (1962) have found a medullary sodium gradient rising from the cortex towards the pelvis, even at this age, when the gradient can also be made steeper by allowing the animals to become dehydrated.

These changes in the kidney occur at the onset of the weaning period, but the changes of diet used in this experiment were without histological effect. Indeed, it seems reasonable, not that weaning causes structural changes in the kidney, but the converse. The maturation of renal structure is an aspect of the same development which is manifested in other workers' experiments by the onset of the adult concentrating reaction to antidiuretic hormone. This in turn may liberate the young rat from the need for drinking as continuously as hitherto, so that it can more freely wander from its mother and explore other sources of food. This would suggest that the kidney of the new-born guinea-pig, which takes a mixed diet from birth, should have a more mature renal medulla than has the new-born rat. This is so (Boss, unpublished observation).

Since the human infant of more than 2·0–2·5 kg has no nephrogenic zone, the kidney may be considered as generally more mature than that of the new-born rat. Nevertheless, Peter (1927) found not only that the proximal convoluted tubules lengthened during the first 2 years of postnatal life, but also that, at birth, the loops of Henle were not fully elongated in the youngest nephrons. Since the new-born human infant has only a slight response to antidiuretic hormone (Heller, 1944), there is an opportunity here, as in the rat, to attempt to correlate histological development with the onset of the adult reaction to the hormone. By such correlations, in both species, light may be thrown on the functions of the various segments of the nephron.

SUMMARY

1. The kidneys of rats 1, 11, 23 and 33 days old were examined histologically.

2. Between 11 and 23 days of age, i.e. in the period when weaning begins and when the adult response to antidiuretic hormone is first found, the loops of Henle first became mature in structure, closely packed, and accompanied by capillaries at the approximate density found in the adult.

3. The outer stripe of the medulla, consisting largely of the second segments of proximal convoluted tubules, formed in the same period.

4. The cortex at birth had a nephrogenic zone, but some immature features of Malpighian bodies and proximal tubules were evident even in the more mature zone of the cortex.

5. Forced weaning at 14 days, whether on to a standard diet, a diet rich in fat, or a diet with access to extra salt, did not perceptibly affect the development of the kidney.

6. The results are in agreement with the countercurrent theory of renal concentration, and it is suggested that weaning in rats may be initiated by renal maturation.

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EXPLANATION OF PLATES

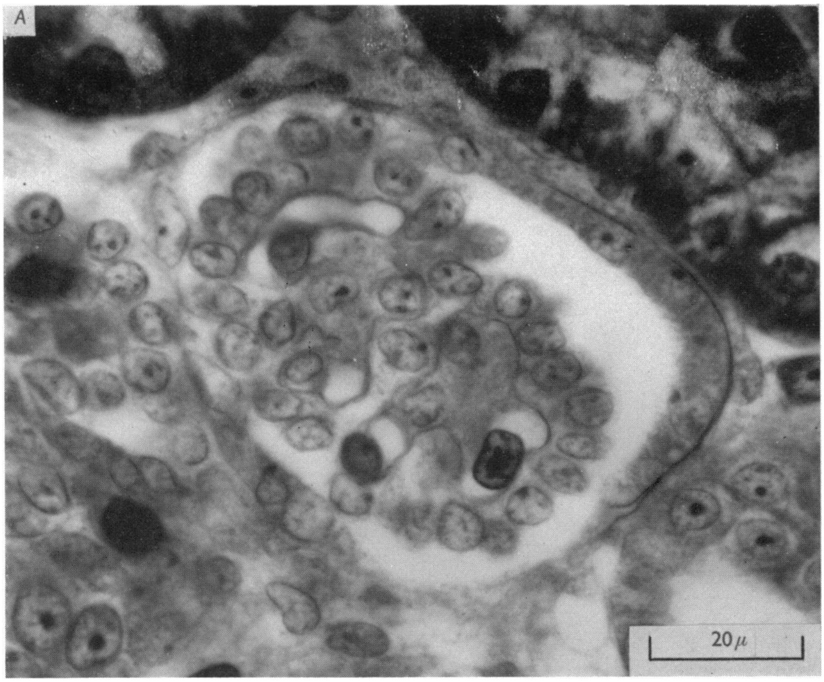
All photomicrographs are of sections 6 μ thick, stained with Azan, and photographed at a single magnification with a Beck 1·3 oil-immersion apochromat.

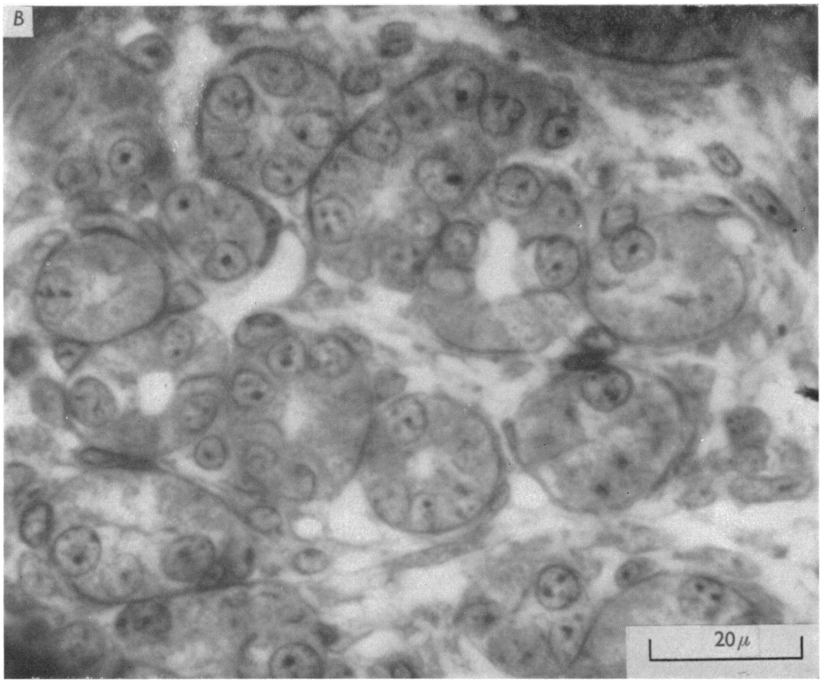
PLATE 1

A, Glomerulus in kidney of day-old rat. Note cuboidal epithelium lining Bowman's capsule. *B*, Part of the medulla from the same section as that shown in *A*. Tubules of cuboidal epithelium are separated by loose connective tissue.

PLATE 2

A, Glomerulus at 11 days. The epithelium is now squamous. *B*, Part of the outermost medulla from the same section as that shown in *A*. The field corresponds in position in the kidney to that included in 1*B*. Tubules are smaller than in 1*B*. Intervening loose connective tissue is still present.





J. M. N. BOSS, HELENA DLOUHÁ, M. KRAUS AND J. KŘEČEK

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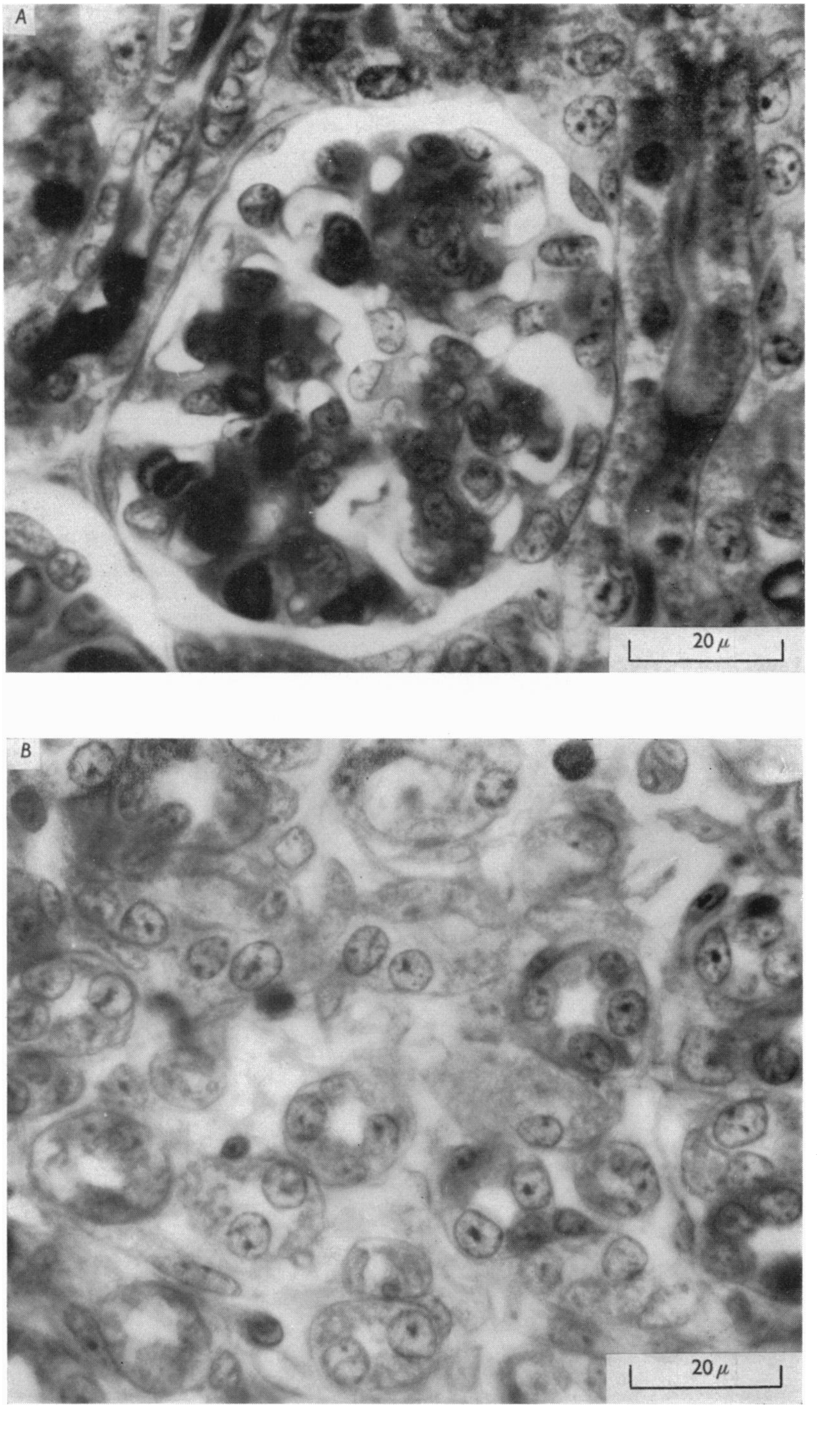

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