THE OCCURRENCE OF MITOTIC DIVISIONS IN GLOMERULI IN GLOMERULONEPHRITIS AND MALIGNANT SCLEROSIS*

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The purpose of this publication is to prove that, contrary to current opinion, mitotic divisions in glomeruli in glomerulonephritis and malignant sclerosis are not exceptional. Though microscopic examination of the kidneys could be performed in but a small percentage of our material, yet in 3 of 30 cases thus examined from a series of 140 autopsies, mitotic divisions in glomeruli could be readily demonstrated in epithelial and endothelial cells.

MATERIAL AND METHODS

In cases Nos. 1 and 3, autopsy was performed 45 minutes after death, and in case No. 2, 30 minutes after death. Slices of renal tissue, not more than 3 mm. thick, were cut for fixation. In case No. 1, Stieve's solution [1] (saturated solution of corrosive sublimate, 76 cc.; undiluted commercial formaldehyde solution, 20 cc.; glacial acetic acid, 4 cc.) was used as fixative; in cases Nos. 2 and 3, a mixture of equal parts of a saturated solution of corrosive sublimate and of Bouin's solution. This mixture, recommended by Petersen [2] for embryological material, gives excellent results with surgical and autopsy material and is, in our opinion, much superior to Bouin's fluid alone. The material from cases Nos. 2 and 3 was embedded in paraffin after treatment with Peterfi's methylbenzoate-celloidin mixture.[3] In case No. 1 it was deemed necessary to avoid as much as possible the shrinkage often apparent in paraffin sections. Therefore, the tissues were dehydrated in glycerin,[4] soaked for 3 days in a mixture of five parts of a 6 per cent celloidin solution and one part of oil of cedarwood; hardened in anhydrous chloroform and thereafter embedded in paraffin after passing through benzene and benzene-paraffin. Sections were cut 5 and 6 μ thick and stained with azocarmine-aniline blue and hematoxylin-azophloxin.

* Received for publication December 21, 1940.

Reports of Cases

Case 1

A colored girl, 16 years old, entered the hospital 3 weeks after giving birth to a full-term infant. The only anamnestic data available were that she had had convulsions. The patient died a few minutes after admission, so that clinical data are not available.

Gross Notes. Autopsy was performed 45 minutes after death. The most important findings were edema of the lungs, possible slight enlargement of the heart (270 gm.) and lack of significant changes in the internal genitalia. The kidneys weighed 340 gm. The capsules stripped very easily and the surface of the kidneys was pale, smooth and showed very few small hemorrhages. On section the cortex and medulla were well delimited, the medulla being a little darker than the cortex, and the glomeruli were enlarged, pale and plainly visible. The renal pelves showed nothing abnormal.

Microscopical Examination. The glomeruli were enlarged and very cellular. This cellularity was partly due to the presence of polymorphonuclear leukocytes, amongst which several eosinophilic leukocytes were found. The epithelium of the visceral layer of the capsule of Bowman was visible only on the outer surface of the glomerular loops and showed only a slight swelling and sometimes a few hyaline droplets. The outer basal membranes were clearly visible. The inner basal membranes were fused in many places; in others they were lying in close contact but were still visible as two separate membranes. In some places individual capillaries, sometimes containing a few erythrocytes, could still be distinguished. Many capillaries were fused and between the endothelial cells a surprisingly great number of very delicate, often branching, fibers were found. The fibers were much more delicate than those depicted by Bell [5] and could not be called hyaline. Real hyalinization was absent. It was interesting to note that in ordinary paraffin sections of the same material, stained in the same way, the number of the individual fibers seemed much smaller than in the material first embedded in celloidin, whereas in the paraffin sections the closely adjacent basal membranes were distinguished with great difficulty. In the much reduced capsular spaces no red blood cells or fibrin were found.

In many glomeruli, mitotic divisions were found, sometimes three in one glomerulus. The distribution of the mitoses over the different glomeruli was unequal. In some sections many glomeruli had to be examined before a mitosis was found; in other sections they were detected quickly and easily. The mitotic divisions were found in all stages, ranging from the spirem to the diaster. With the azocarmine stain the centrioles and achromatic spindles were easily demonstrated, so that it was impossible to mistake dark-staining, degenerating nuclei of leukocytes for mitotic figures. In some cases, as illustrated in Figures 1 and 2, the dividing cell could, by its location, be recognized as an endothelial cell, as it was lying just inside the outer basement membrane. In the epithelial cells mitoses could not be found. The tubules contained little granular material and the epithelial cells were swollen and sometimes showed hyaline droplets. Only in very few tubules were red blood cells found. The interstitial tissue was unchanged.

Epicrisis. This is a typical example of acute glomerulonephritis showing intracapillary fibers, polymorphonuclear leukocytes and proliferation of the endothelial cells. In the glomeruli typical mitoses could be demonstrated and in several instances the dividing cell could be recognized as endothelial.

Case 2

A colored man, 40 years old, entered the hospital and died 1 hour after admission. Clinical and anamnestic data were not available.

Gross Notes. Autopsy was performed 30 minutes after death. The most important findings were as follows: The kidneys were enlarged, the left kidney weighing 365 gm. and the right 315 gm. Their consistence was diminished, the capsule stripped easily and the surface was smooth and yellowish gray. On section, the cortex was widened, welled up above the cut surface, and its color was gray-yellow, with deeper yellow streaks. The liver was enlarged, weighing 2300 gm., and was yellowish light brown in color, with small yellow spots. The spleen was enlarged, weighing 285 gm., and showed on section many yellowish white spots on a red background. The heart was not enlarged and weighed 245 gm. The heart muscle was brown-red. Retroperitoneal lymph nodes were swollen, moist, and whitish. There was only slight edema.

Microscopical Examination. The glomeruli were not enlarged. The basal membranes in nearly all glomeruli showed thickening, which was in general not very pronounced but was more strongly developed in individual glomeruli or in some loops of a glomerulus. Sometimes it appeared that the basement membrane, especially in the peripheral parts of the loops, was split longitudinally and surrounded the endothelial cells. Also, that small short fibers split off from the basement membrane. The endothelial nuclei were distinctly increased. The epithelial cells of the visceral layer of the capsule of Bowman were very conspicuous. Their protoplasm and nuclei were swollen and they often contained vacuoles and hyaline droplets. In several glomeruli, mitotic divisions were found and it could easily be ascertained that the dividing cells were lying outside of the basement membrane and belonged to the visceral layer of the capsule. The tubules were often widened and contained granular and hyaline casts. Many epithelial cells were swollen and showed hyaline droplet degeneration; in other tubules the epithelial cells were flattened. In the epithelium of the tubules many mitotic divisions were found. The interstitium was edematous and contained very few lymphocytes and some histiocytes.

Epicrisis. On purely morphological grounds we believe this case to be an instance of glomerulonephritis closely related to lipoid nephrosis (Bell [6]). Mitotic divisions were found in the epithelial cells of the glomeruli.

Case 3

A colored man, 37 years old, entered the hospital complaining of dullness, headache which had been increasing for several days, and vomiting of blood. On admission the heart was enlarged and the pulse rate was 125 per minute. Examination of the ocular fundi was impossible. The blood pressure was 170 systolic and 120 diastolic. Examination of the urine showed the specific gravity to be 1006; albumin and glucose, negative; urobilin, positive; acetone, negative; red blood cells, none; casts, none. The blood showed the hemoglobin to be 65 per cent; red blood cells, 3,900,000; sedimentation rate, 54 to 86 mm. (Westergren); Wassermann's and Kahn's tests, negative; nonprotein nitrogen, 300 mg. per cent, and blood urea, 280 mg. per cent. The electrocardiogram showed a serious myocardial lesion, probably a bundle-branch block. Tests of kidney function could not be performed.

On the day of admission the patient vomited a few black coagula and some fresh blood. Thereafter the vomiting of blood stopped, but the condition of the patient deteriorated rapidly. On the fourth day Cheyne-Stokes' breathing appeared and the patient died 7 days after admission.

Gross Notes. Autopsy was performed 45 minutes after death. The principal findings were as follows: The heart was enlarged, weighing 545 gm. There was extensive necrosis in the wall of the left ventricle and in the papillary muscles, less extensive in the wall of the right ventricle, and coronary sclerosis, especially of the ramus descendens anterior sinister, of which the lumen was very much reduced. Typical syphilitic lesions were present in the aorta and in the innominate and subclavian arteries. The kidneys were enlarged, the right and left kidneys weighing respectively, 255 and 215 gm.; their consistence was diminished, the capsules stripped easily, the surface was nearly smooth and the color was grayish brown with the admixture of some yellow, showing, in addition, very numerous irregular red spots of the size of a pinhead, sometimes even a little larger. On section the cortex welled up, the demarcation between cortex and medulla was not distinct and many small irregular red spots were visible. The pelvis showed nothing of significance. The blood vessels on section did not project and their lumina were open. No gross lesion, which could have caused the vomiting of blood, was found.

Microscopical Examination. Most arterioles showed an extensive hyalinization and fatty degeneration. In many places arteriolonecrosis or endarteritis was observed. The glomeruli showed a variety of lesions; glomerulonecrosis, hemorrhages, necrosis of some capillary loops and in many instances alterative or proliferative glomerulitis. Others showed a progressive hyalinization, although still others appeared quite normal. In the glomeruli showing alterative or proliferative glomerulitis, the epithelial cells were swollen and sometimes giant cells with three or more small nuclei were found. Hyaline droplet degeneration was quite common. In the glomeruli with proliferative glomerulitis the endothelial nuclei were increased. Mitotic divisions were found in epithelial and endothelial cells. Many tubules were filled with erythrocytes or with hyaline or granular casts. Hyaline droplet degeneration was quite frequent. Small areas with atrophic tubules and infiltrates of lymphocytes were found.

Epicrisis. We believe this to be a case of malignant sclerosis, complicated by luetic aortitis and infarction of the heart. That the blood pressure was not so high as usual was probably due to the cardiac failure. In the glomeruli, mitoses were found in both endothelial and epithelial cells.

Discussion

As Bell [5] stated, it is generally agreed that the essential lesion in glomerulitis is an increase in the number and size of the endothelial cells. On the other hand, most authors agree on the absence of mitotic divisions in the endothelial cells; neither are mitoses in the epithelial cells mentioned in recent publications. Bell therefore concluded that if cell division actually occurs, it is largely of the amitotic type. As experienced cytologists either consider amitosis very rare in mammals (Levi [7]) or doubt the existence of real amitosis (Maximow and Bloom [8]), this is not a satisfactory solution of the problem. Other authors state simply that there is an increase in the number of the endothelial cells (Kimmelstiel and Wilson [9]) without telling how this increase is brought about, and this is true also of many textbooks (Aschoff,[10] Hueck,[11] Fishberg,[12] Hadfield and Garrod [13]). Van Waveren [14] assumed that the endothelial nuclei always outnumber epithelial, though Bell [5] and especially von Möllendorff,[15] the latter using the most excellent histological technic, came to directly opposite conclusions. As one of us worked in the same laboratory as van Waveren and performed part of the autopsies from which he obtained his material, we are in a position to confirm Bell's [5] opinion that the material and methods of van Waveren were quite unsuited for these investigations. Only Kaufmann [16] records the finding of mitoses in "adventitial cells" of the capillaries of the glomeruli.

It is a well known fact that after death the number of mitoses found in a given tissue decreases with time (Schmorl,[17] Mallory[18]) and it is therefore not surprising that in tissues, fixed many hours after death, the number of mitoses found may be small, even in rapidly growing tissues. Furthermore many mitoses become indistinct (Casey [19]) and it is difficult to distinguish them from the nuclei of degenerating cells. In connection with this it is interesting to note a quotation from Karsner, Saphir and Todd,[20] by MacMahon,[21] who was the first to describe the regeneration of heart-muscle fibers in infants. Failing to find mitotic figures in hearts of adults these authors remarked that this was perhaps due to the fact that the hearts were obtained *post mortem*.

In our cases the autopsies were performed a very short time after death, thin slices of tissue were fixed and rapidly penetrat-

ing fixatives were used. We believe that these factors enabled us to find the mitotic divisions. When we compare sections of other organs from our autopsy material, 50 per cent of which is fixed less than 1 hour after death, with those from other laboratories in which we have worked and where the interval between death and autopsy averaged more than 24 hours, the difference in the number of mitotic divisions is striking. The same is true of surgical material instantly cut into thin slices and fixed after removal from the body as compared with large specimens, often whole organs or large tumors, when left untouched for some time or placed in their entirety into a fixative. Such material is, of course, quite sufficient for diagnostic purposes but is not suitable for delicate histological work, a fact often forgotten by pathologists.

SUMMARY

A brief description of one case of acute glomerulonephritis, one case of subacute glomerulonephritis and one case of malignant sclerosis is given. In the first case mitotic divisions were found in the endothelial cells of the glomeruli, in the second case in the epithelial cells of the glomeruli, in the third case in both endothelial and epithelial cells.*

The importance of early and good fixation for the study of the glomerulus, and especially for the finding of mitotic divisions, is stressed.

* After submitting this paper we examined the kidneys of a man dying from septicemia with widespread metastatic abscesses. A few mitotic divisions were found in the endothelial cells of the glomeruli. The autopsy was performed 5 minutes after death.

REFERENCES

1. Stieve, H. Der Halsteil der menschlichen Gebärmutter, seine Veränderungen während der Schwangerschaft, der Geburt und des Wochenbettes und ihre Bedeutung. *Ztschr. f. mikr.-anat. Forsch.*, 1927, 11, 291–441.
2. Petersen, Hans. Histologie und mikroskopische Anatomie. J. F. Bergmann, München, 1935.
3. Romeis, Benno. Taschenbuch der mikroskopischen Technik. R. Oldenbourg, Berlin, 1932, p. 107.
4. Wolf, Jan. Die Glyzerinmethode der Zelloidineinbettung. *Ztschr. f. wissensch. Mikr.*, 1939, **56**, 57–62.
5. Bell, E. T. The early stages of glomerulonephritis. *Am. J. Path.*, 1936, **12**, 801–824. The pathology and pathogenesis of clinical acute nephritis. *Ibid.*, 1937, **13**, 497–552.

6. Bell, E. T. A clinical and pathological study of subacute and chronic glomerulonephritis, including lipoid nephrosis. *Am. J. Path.*, 1938, 14, 691–736.
7. Levi, Giuseppe. Trattato di Istologia. Torino, 1935, p. 177.
8. Maximow, A. A., and Bloom, William. A Textbook of Histology. W. B. Saunders Co., Philadelphia, 1930, ed. 1, p. 18.
9. Kimmelstiel, Paul, and Wilson, Clifford. Benign and malignant hypertension and nephrosclerosis. A clinical and pathological study. *Am. J. Path.*, 1936, 12, 45–81.
10. Aschoff, Ludwig. Pathologische Anatomie. G. Fischer, Jena, 1936, Bd. 2.
11. Hueck, Werner. Morphologische Pathologie. G. Thieme, Leipzig, 1937.
12. Fishberg, A. M. Hypertension and Nephritis. Baillière, Tindall and Cox, London, 1939.
13. Hadfield, Geoffrey, and Garrod, L. P. Recent Advances in Pathology. P. Blakiston's Son & Co., Philadelphia, 1938, ed. 3.
14. van Waveren, E. K. Over de Anatomie en histologische Diagnostiek van Glomerulonephritis. Van Gorcum & Comp., Assen, 1935, 1–213. (Cited by Bell.)
15. von Möllendorff, Wilhelm. Stöhr's Lehrbuch der Histologie. G. Fischer, Jena, 1930, p. 304.
16. Kaufmann, Eduard. Spezielle Pathologische Anatomie. Walter de Gruyter & Co., Berlin, 1922, p. 1044.
17. Schmorl, C. G. Die pathologisch-histologischen Untersuchungsmethoden. F. C. W. Vogel, Leipzig, 1922, p. 128.
18. Mallory, F. B. Pathological Technique. W. B. Saunders Co., Philadelphia, 1938, p. 108.
19. Casey, A. E. A study of some lymphatic diseases by means of a new yardstick—the mitosis count. (Abstract.) *Am. J. Cancer*, 1937, 30, 586–588.
20. Karsner, H. T.; Saphir, Otto, and Todd, T. W. The state of the cardiac muscle in hypertrophy and atrophy. *Am. J. Path.*, 1925, 1, 351–371.
21. MacMahon, H. E. Hyperplasia and regeneration of the myocardium in infants and in children. *Am. J. Path.*, 1937, 13, 845–852.

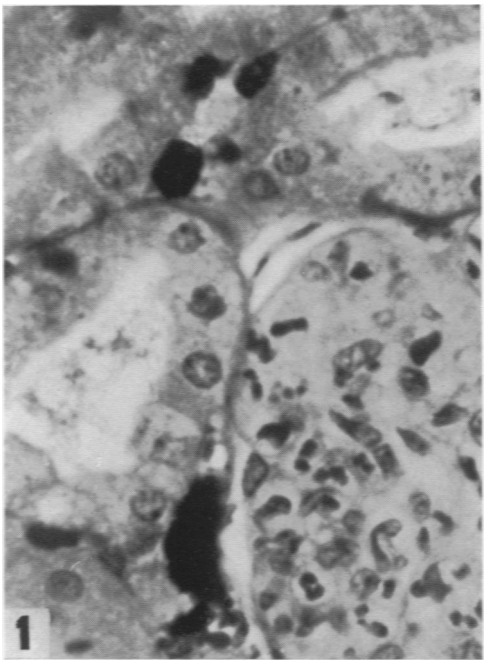
DESCRIPTION OF PLATES

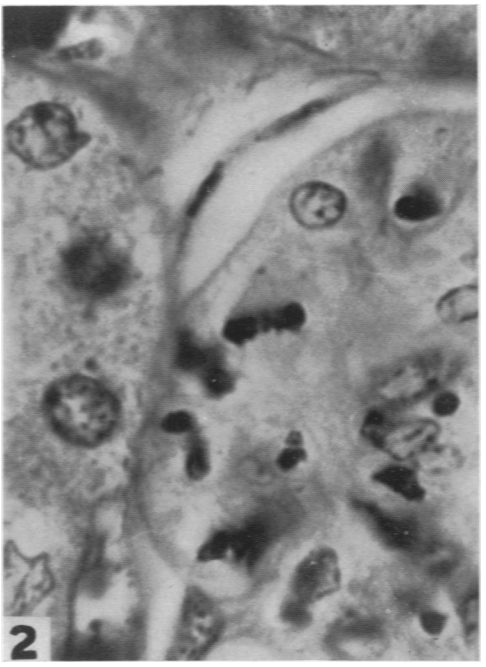
PLATE 105

FIG. 1. Case 1. A glomerulus with a mitosis in its upper left corner and in the center of the field. × 716.

FIG. 2. Case 1. The same mitosis at a higher magnification. The dividing cell is lying inside the basement membrane and must be considered as endothelial. One centrosome is visible. × 1500.

FIG. 3. Case 2. A mitosis in an epithelial cell. Centrosomes and spindles are plainly visible. × 1500.





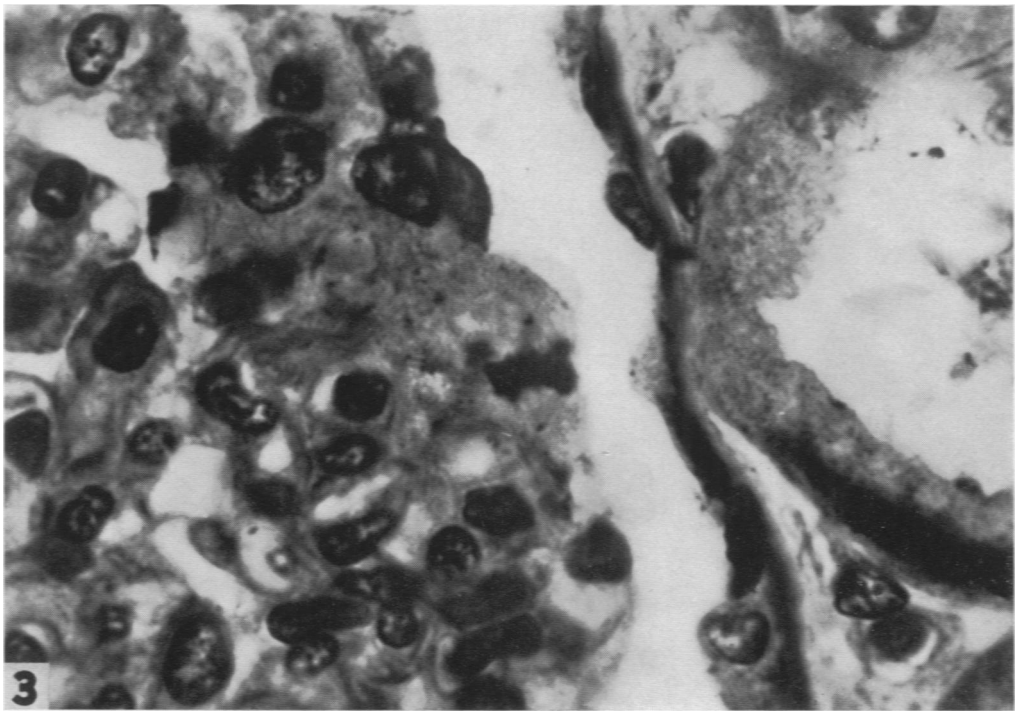


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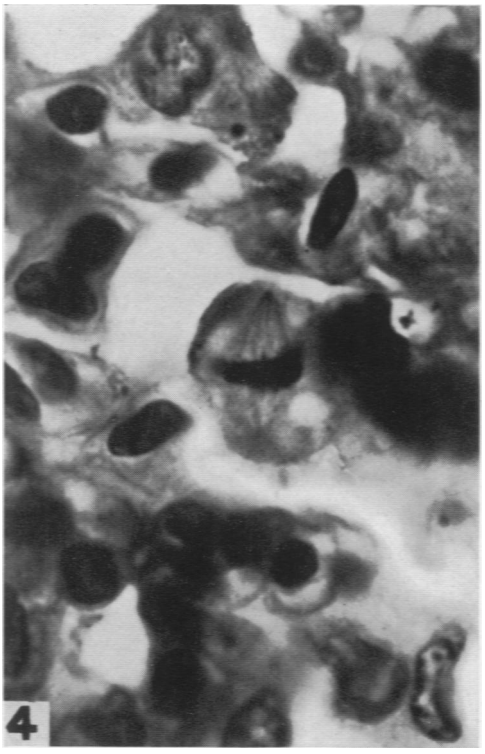
PLATE 106

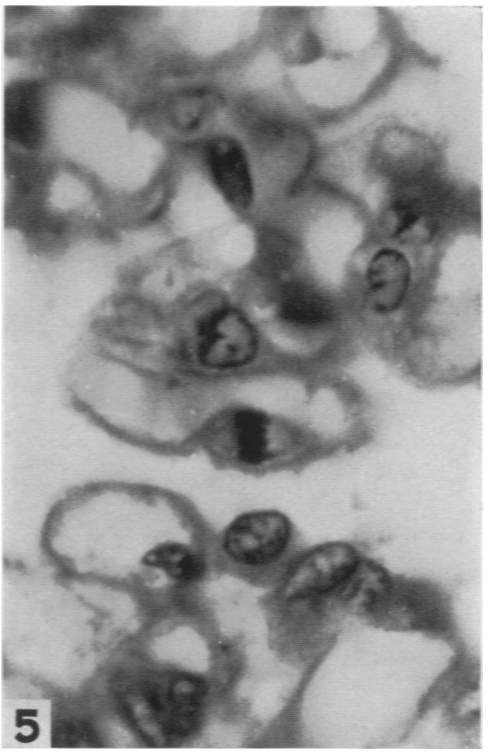
FIG. 4. Case 2. A mitosis in an epithelial cell. × 1500.

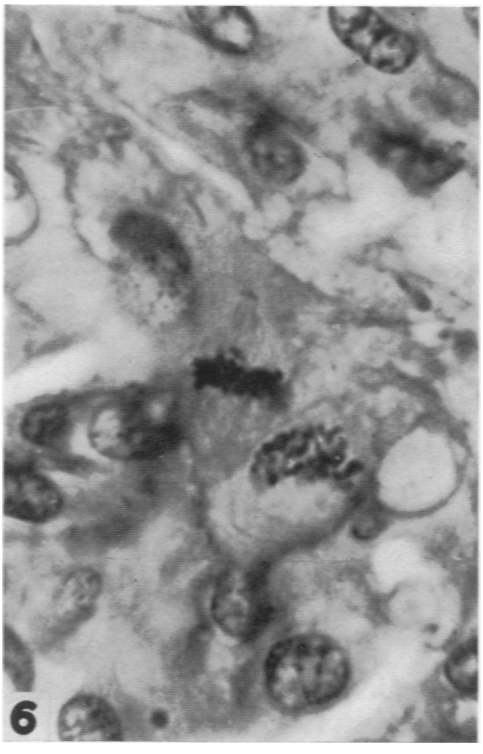
FIG. 5. Case 3. A mitosis in an endothelial cell. × 1500.

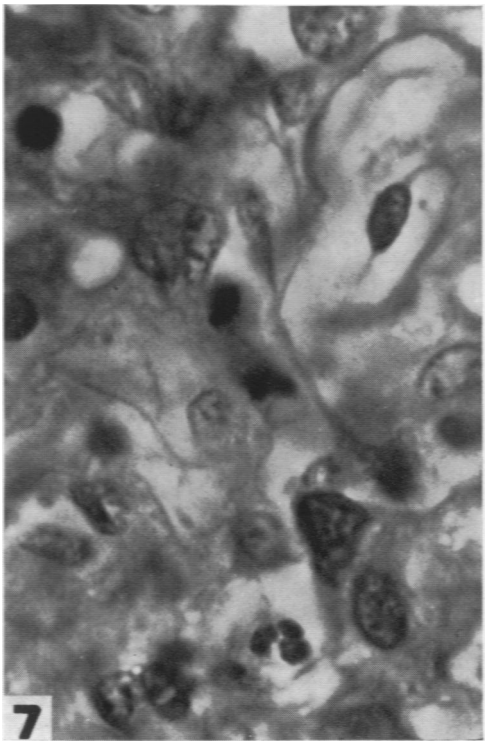
FIG. 6. Case 3. Two mitoses in epithelial cells at the vascular pole of a glomerulus. × 1500.

FIG. 7. Case 3. A diaster in a glomerulus. × 1500.









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