MICROGLIA-LIKE CELLS AND THEIR REACTION FOLLOWING INJURY TO THE LIVER, SPLEEN AND KIDNEY *

HENRY S. DUNNING, M.D., AND LEWIS STEVENSON, M.D.

(From the Department of Pathology, Cornell University Medical College, and the New York Hospital, New York City)

Certain cells in the nervous system which had hitherto been difficult to stain were included by Cajal[1] in 1913 under the term "third element" of the central nervous system, and were suspected by him to be of mesodermal origin. Somewhat later del Río-Hortega[2-5] was able to show that this "third element" consisted of two entirely different kinds of cells: one kind was the oligodendroglia of ectodermal origin; and the other kind he called microglia, the latter, in his opinion, being of mesodermal origin. The central nervous system was made up, therefore, of neurones (the first element), neuroglia consisting of astrocytes and oligodendroglia (the second element), and microglia (the third element). Del Río-Hortega's papers describing the microglia were published between 1919 and 1921. Today del Río-Hortega[6] believes, along with others, that the microglia represents the reticulo-endothelial system in the central nervous system.

In 1921 del Río-Hortega and Jiménez de Asúa[7] demonstrated phagocytic cells in tumors, tubercles, liver lesions, normal human kidney, and in lymph follicles stained by silver carbonate. In this paper the authors drew attention to their belief that phagocytosis in the nervous system is the function of the microglia and that the studies of del Río-Hortega on the microglia constitute a concrete example of the general problem of the histogenesis of the macrophages.

In 1927 Jiménez de Asúa,[8] using silver carbonate, demonstrated macrophages in a normal spleen and in tumors, and stated that in morphology, staining properties and in function they resemble the microglia of the nervous system and, finally, that microglia cells are members of the reticulo-endothelial system.

Cone,[9] in 1928, using del Río-Hortega's method for microglia, illustrated a phagocyte with a close resemblance to transitional microglia in a degenerating area of a hypernephroma.

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Dorothy Russell [10] in 1929 demonstrated intravital staining of microglia with trypan blue and concluded that such intravital staining identifies microglia with the rest of the reticulo-endothelial system and that microglia is a mesodermal element.

Wells and Carmichael [11] in 1930 made a study of microglia by means of tissue culture and vital staining and concluded that microglia is of mesodermal origin and is analogous to the resting histiocytes or fixed macrophages of the reticulo-endothelial system. They also found microglia-like cells in cultures from embryonic chick periosteum and limb-bud and found that these cells reacted to vital dyes *in vitro* in the same manner as wandering cells.

Visintini [12] in 1931 demonstrated microglia-like cells in the heart, voluntary muscle and urinary bladder by the method of Bolsi.

Belezky [13] in 1931, using silver carbonate, demonstrated cells in the spleen which he regarded as reticulo-endothelial cells. These cells, in our opinion, resemble microglia cells.

The microglia cells appear in the nervous system at about the time of birth. They migrate to all parts of the brain and spinal cord and remain there inactive until the advent of some disease. When this occurs the first pathological change that can be noticed in certain types of lesions is the sudden and tremendous activity of the microglia. They multiply, migrate in great numbers to the damaged area and become phagocytic, devouring the broken-down nervous tissue and other débris and apparently removing it to the nearest perivascular spaces, where they appear in the form of large, rounded, compound granular corpuscles loaded with fat (*Gitterzellen*). Thus they clear the way for the astrocytes to lay down a scar in place of the destroyed tissue.

A similar phenomenon might occur in other tissues of the body. With this possibility in mind experiments were performed on rabbits, using del Río-Hortega's original silver carbonate method of specific staining for microglia in the liver, spleen and kidney after producing a destructive lesion in these organs.

Under sterile conditions a cerebral hemisphere, the liver and the spleen of a rabbit were punctured by a hot trochar, ether anesthesia being used. At the end of 4 days the animal was killed and the brain, liver and spleen were fixed in Cajal's formol-bromide solution for about 24 hours. Frozen sections were cut from the traumatized portions of each of the three organs and stained at the same time by

del Río-Hortega's original silver carbonate method for microglia. In Figure 1 the area of brain destroyed by the hot trochar is surrounded by great numbers of microglia cells, forming a dark ring about the necrotic area. Figure 2 is a high power view of a portion of this ring. The microglia cells can be seen in different stages of metamorphosis from the almost normal cell with its spiked processes to the completely formed compound granular corpuscle loaded with fat. Figure 3 is a low power photomicrograph of a portion of the lesion in the liver. The margin of the necrotic area is marked by a dark ring of cells which in the high power view (Fig. 4) closely resemble the transitional microglia of the brain. These cells contain droplets of fat. Cells of the same character are present at the margin of the puncture wound of the spleen. The stages in the transformation of a cell with spiked processes into a swollen, rounded form are illustrated in Figures 5 to 10 by camera lucida drawings of six cells found at the margin of the necrotic area in the same spleen. All of the cells except the first contain droplets of fat, which appear black in the picture. We wish to draw attention to the close resemblance of the cells in this figure to the microglia cells illustrated in Plate 91 of Dorothy Russell's article, referred to above.

Using the same technique as in the first experiment a kidney of a rabbit was punctured by a hot trochar. The animal was killed at the end of 4 days and the injured organ was fixed in formol-bromide. Sections of the lesion were cut and stained in the same manner as were the brain, liver and spleen. Figure 14 is a high power view of fat-containing microglia-like cells in the process of swelling at the margin of the damaged area in the kidney. Some of these cells are elongated and have small spikes, very much like the microglia cells in a paretic brain (the "rod-cells" or *Stäbchenzellen* of general paralysis).

A few hours before a puncture wound of the spleen was made 10 cc. of a 1 per cent aqueous solution of trypan blue were injected into an ear vein of a rabbit. The animal was allowed to live 4 days, and during this period 35 cc. of the same solution of trypan blue were injected intraperitoneally. The spleen was fixed and sections of the lesion were cut and stained as before, with the exception that the silver impregnation was not toned in gold chloride because it tended to change the blue dye to purple. Figure 11 is a low power view of the damaged area outlined by a well marked dark ring of cells.

In the high power view of a portion of the ring (Fig. 12) many swollen cells, which closely resemble pathological microglia, can be seen, and all of the cells in this picture contain trypan blue in granular form. Figure 13 is a high power photomicrograph of a group of cells in a splenic nodule at some distance from the lesion. These cells closely resemble slightly swollen microglia, and small spike-like projections on their main processes can be seen, a feature characteristic of the microglia of the nervous system. Trypan blue could not be demonstrated in this group of cells. By staining two consecutive sections of the block, one with silver carbonate and the other with hematoxylin and eosin, the opposite halves of the same cells impregnated with silver were colored by the organic dyes. Stained by the more familiar method the cells pictured in Figure 12 have the following characteristics. Compared with the nucleus of the lymphocyte the nucleus of the argyrophilic cell is larger, less deeply stained by hematoxylin, and varies in shape, tending to be rounded, oval or kidney-shaped. It contains a nucleolus and a uniform distribution of fine granules of chromatin. The clear cytoplasm is pale pink, almost colorless, and contains large and small granules of trypan blue, masses of amber blood pigment and an occasional engulfed lymphocyte. The shape of the cell is as varied as the shape of the nucleus and its processes, which are so clearly impregnated with silver, cannot be distinguished in the section stained with hematoxylin and eosin.

The next step was to determine whether microglia-like cells are present in the normal liver, spleen and kidney as they are found in a resting state in the normal nervous system and whether they could be demonstrated by the same technique of staining. Accordingly, the liver, spleen and kidneys of normal rabbits were stained by del Río-Hortega's original silver carbonate method for microglia. Figure 15 is a high power photomicrograph of the normal liver of a rabbit. In the center of the field there is a triangular-shaped cell with three long processes extending between the liver cells. Typical of many others scattered throughout the liver and undoubtedly representing the nearly normal or very early transitional form of the cells demonstrated in Figures 3 and 4, it is morphologically similar to the microglia cells of the nervous system. In Figure 16, a high power photomicrograph of the normal spleen of a rabbit, one slightly swollen microglia-like cell is seen at the edge of a splenic nodule.

This cell is typical of many others found at the periphery of the nodules and these undoubtedly are the source of such cells as those demonstrated in Figures 5 to 10, 11 and 12. Figures 17 and 18 are high power photomicrographs of microglia-like cells in a normal kidney of a rabbit. Spike-like projections on their processes are well shown. Many similar cells were found scattered between the tubules throughout the kidney and they are undoubtedly the resting or early transitional forms of the cells demonstrated in Figure 14. In the normal organs that were studied all of the microglia-like cells were somewhat swollen, as if they were constantly being stimulated to activity.

SUMMARY

1. Cells have been demonstrated by del Río-Hortega's original silver carbonate method of specific staining for microglia in the liver, spleen and kidney of the rabbit that in morphology are identical with the nearly normal or very early transitional forms of microglia in the nervous system.

2. In their reaction to injury and to the intravital injection of trypan blue they have been shown to be identical with microglia.

3. These cells have been demonstrated in a transitional stage with spiked processes like microglia and containing droplets of fat or granules of trypan blue.

4. By the silver carbonate method of staining earlier transitional forms have been demonstrated that contain no visible amounts of fat or trypan blue.

5. A more advanced transitional form has been shown in preparations of the spleen of the rabbit to be a histiocyte or large mononuclear phagocyte without processes and containing droplets of fat, granules of trypan blue, blood pigment and engulfed lymphocytes.

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DESCRIPTION OF PLATES

Plate 91

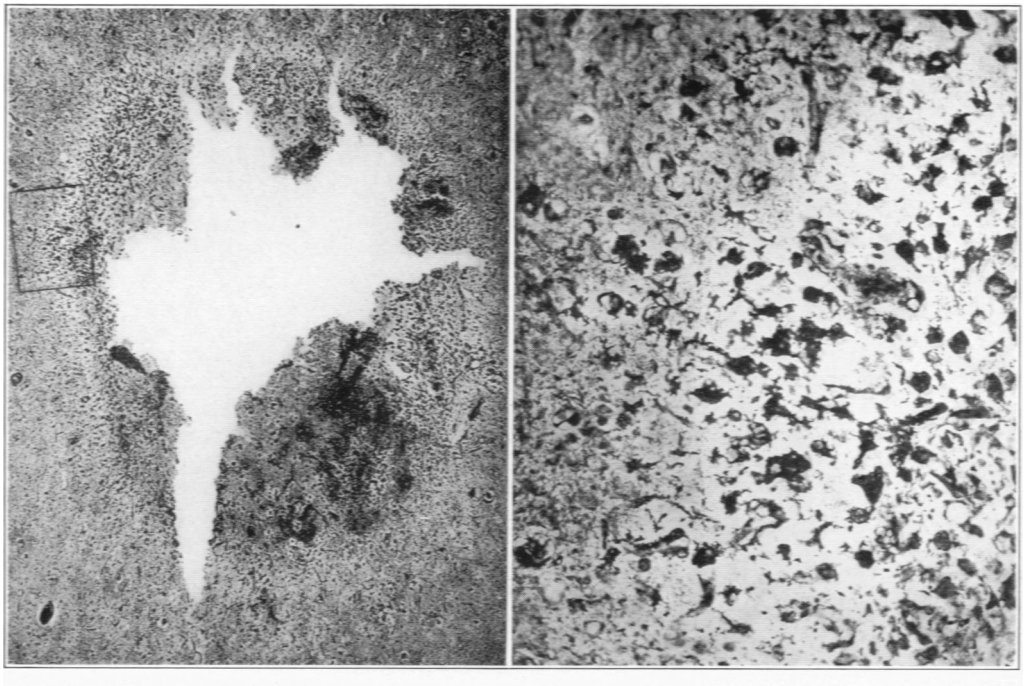
We are indebted to Mr. William S. Dunn for the photomicrographs illustrating this paper.

Fig. 1. Microglia cells in the cerebrum of a rabbit forming a dark ring about a necrotic lesion produced by puncture with a hot trochar. Silver carbonate stain for microglia. × 21.

Fig. 2. A high power view of cells in the area of Fig. 1 outlined in black, showing the transformation of cells with spiked processes into rounded forms filled with droplets of fat. Silver carbonate stain for microglia. × 176.4.

Fig. 3. A portion of a necrotic lesion in the liver of a rabbit produced by puncture with a hot trochar. Microglia-like cells are gathered at its margin. Silver carbonate stain for microglia. × 46.20.

Fig. 4. A high power view of cells in the area of Fig. 3 outlined in black, showing cells with spiked processes and larger, irregular forms containing droplets of fat. These cells resemble the microglia at the margin of the lesion in the brain. Silver carbonate stain for microglia. × 149.24.



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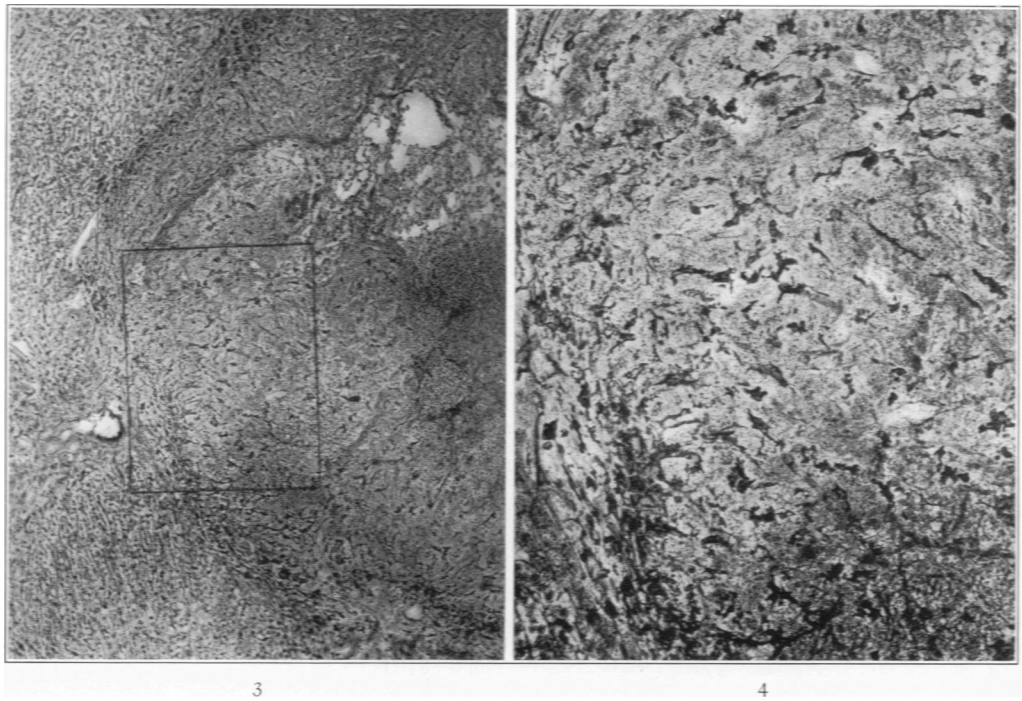

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PLATE 92

FIGS. 5–10. Camera lucida drawings of six cells found at the margin of a necrotic area produced by a hot trochar in the spleen of a rabbit, illustrating the stages in the transformation of a microglia-like cell with spiked processes into a large rounded form. Note the apparent transition of the processes into rounded projections resembling pseudopodia. All of the cells except the first contain droplets of fat, which appear black in the picture. Silver carbonate stain for microglia with Sudan III. × 950.

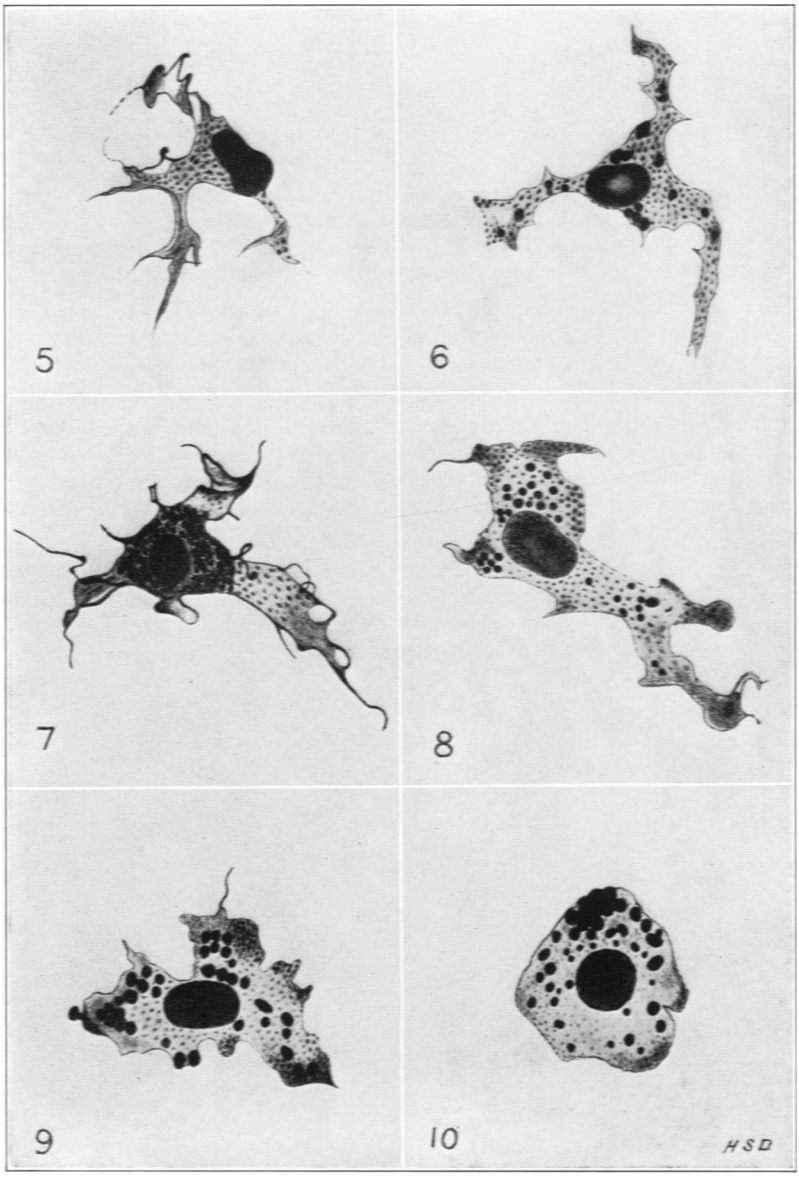
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PLATE 93

FIG. 11. A necrotic lesion outlined by a dark ring of cells produced by a hot trochar in the spleen of a rabbit injected intravitally with trypan blue. Intravital trypan blue and silver carbonate stain for microglia. × 12.6.

FIG. 12. A high power view of cells at the margin of the lesion in Fig. 11 in the area outlined in black. Granules of trypan blue are present in microglia-like cells with spiked processes and in the larger rounded forms. Intravital trypan blue and silver carbonate stain for microglia. × 149.24.

FIG. 13. Cells resembling slightly swollen microglia in a splenic nodule at a distance from the lesion in the spleen pictured in Fig. 11. Note the numerous spikes on their main processes. Trypan blue could not be demonstrated in this group of cells. Intravital trypan blue and silver carbonate stain for microglia. × 149.24.

FIG. 14. A high power view of fat-containing microglia-like cells in the kidney of a rabbit gathered at the margin of a necrotic lesion produced by a hot trochar. Note the elongated forms with spikes resembling the rod-cells in a paretic brain. Silver carbonate stain for microglia. × 126.

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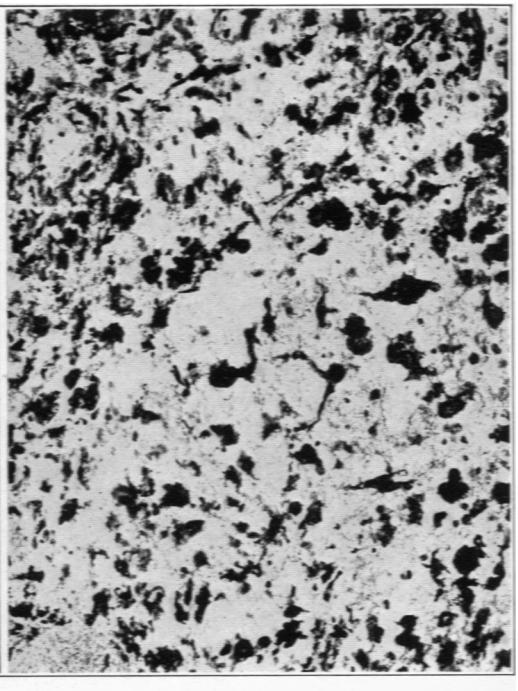

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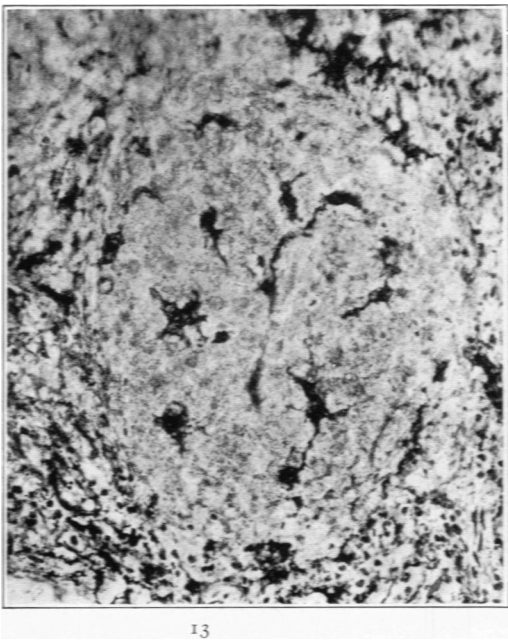

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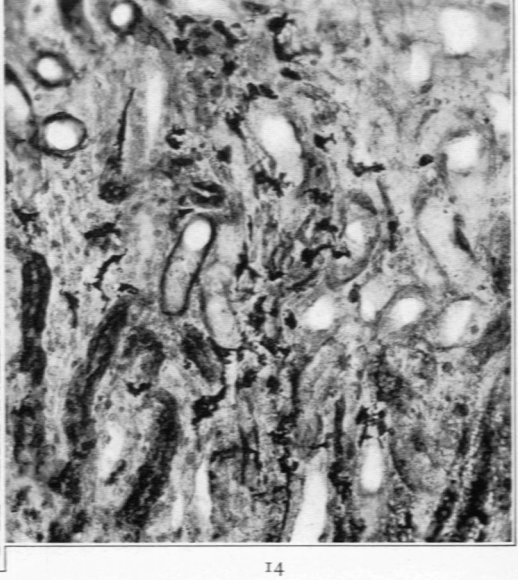

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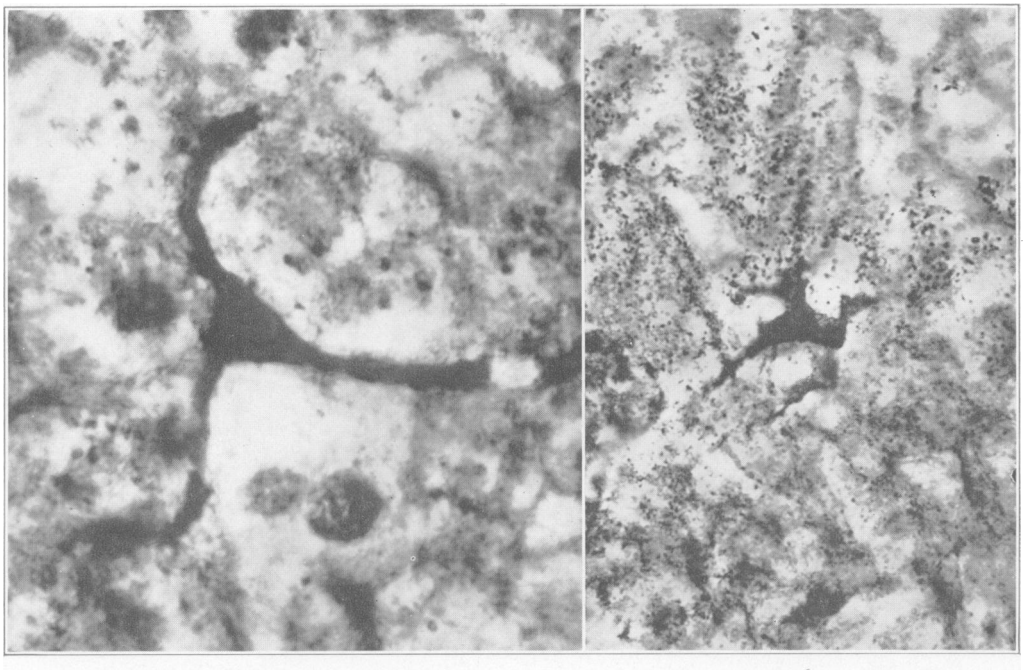
PLATE 94

FIG. 15. A cell in the liver of a normal rabbit resembling a nearly normal or very early transitional microglia cell. Note the manner in which its three processes insert themselves between the liver cells. Silver carbonate stain for microglia. × 950.

FIG. 16. A cell resembling a slightly swollen microglia cell at the edge of a splenic nodule in the spleen of a normal rabbit. Silver carbonate stain for microglia. × 600.

FIG. 17. A cell resembling an early transitional microglia cell in the kidney of a normal rabbit. Silver carbonate stain for microglia. × 1200.

FIG. 18. Another cell in the kidney pictured in Fig. 17 resembling a nearly normal or very early transitional microglia cell. Note the spikes on its main process and its position between the kidney tubules. Silver carbonate stain for microglia. × 950.



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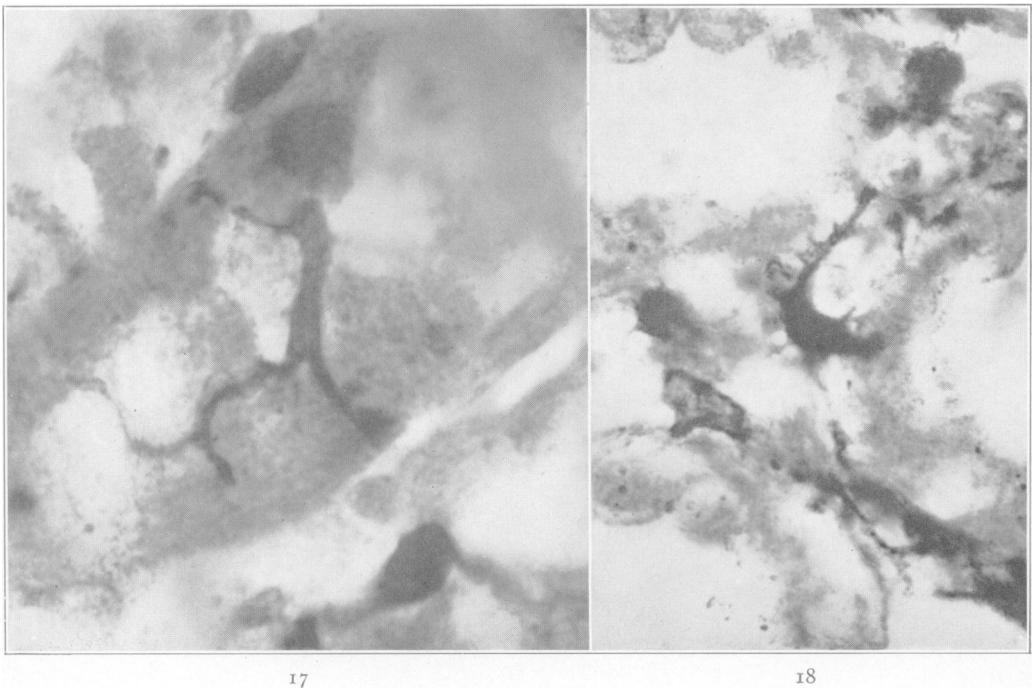

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