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Irwin M. Braverman
Yale University

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Study on the Pattern of Microglial Proliferation and Response in West Nile Encephalomyelitis

Irwin M. Braverman
B.A. Harvard University
1951

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the Degree of Doctor of Medicine

Department of Pathology
School of Medicine
Yale University
1955

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INTRODUCTION

In 1913, Cajal discovered a group of cells in the central nervous system different from the neurones and astrocytes which he called the "third element." These were "naked nuclei" without cell processes. Between 1919 and 1921, del Rio-Hortega undertook the study of these cells and showed them to be a mixture of oligodendroglia and microglia. The latter were the true "third element", and according to Hortega were probably of mesodermal origin, being the true macrophages of the central nervous system. Since that time many investigators have confirmed most of Rio-Hortega's original studies and conclusions concerning the microglia. (14)

There is general agreement on the following points:

Although the ectodermal or mesodermal origin of the microglia is still in dispute, it is generally agreed that the microglia precursors first appear in the brain at the height of its vascularization (in animals and humans). They are reticulo-endothelial-like (R.E.S.) cells which are the mesenchyma lining cells of the pia. They are seen to migrate a few days before to a few days after birth from certain "fountains" in the brain.

These fountains are in areas where the pia is in intimate contact with the brain substance, viz: choroid plexuses, the pia covering the cerebral peduncles and in the pial adventitia surrounding medium and large blood vessels. The microglial precursors in the brain tissue adjacent to these areas are identical histologically with those in the meninges. These cells develop pseudopodia and become amoeboid wandering cells. They can be traced passing along nerve tracts to all regions of the brain, where they lose their amoeboid character, and become the adult microglia cell. They elongate and send out processes that are beset with small lateral spines. These findings have been confirmed in recent years by Kershman (7).

The adult microglial cells are scattered randomly in the nervous tissue, but some are situated near nerve cells and vessels. Microglia accompanies vessels more frequently in the grey matter than in the white matter. They are applied to the adventitial walls or are arranged along the course of the capillaries.(14).

Not much is known about the normal function of microglia. However, in response to pathologic conditions in the brain, such as tumors and inflammation, Hortega (14), Penfield (12,13), Cone (1), and others have shown how the adult microglia cell retracts its processes, becomes hypertrophic; then globose and amoeboid; and finally migrates to the site of injury and phagocytizes the debris, thus forming compound granular corpuscles (gitter cells). It may stop short of the gitter cell and appear merely hypertrophic or rod cell shaped. Penfield (12) has shown that it probably migrates to the advential wall of the blood vessel and gives up its phagocytized material there and returns for further transport in a pre-gitter cell stage.

Because of these phagocytic properties, the microglia have been included in the reticulo-endothelial system. The additional criteria which they satisfy are (a) ingestion of blood pigment, as well as other debris, and (b) fixing and retaining colloidal particles. In addition to the tissue culture work of Costero cited by Rio-Hortega (14), in which he used explants of nervous tissue, and found microglia in all stages of development, including amoeboid movement, and

carmine red ingestion, experiments done in more recent times have gone far to link the microglia to the reticulo-endothelial system. Dougherty (2) has shown that the pial cells giving rise to the precursors of the microglial cell are identical by silver stain with the R.E.S. cells in the rest of the body. Other investigators (3,4,10) have shown that the phagocytes in the lungs, kidneys, and spleen of man and animals, stained with Hortega's silver stain, closely resemble and in many cases are identical with these gitter cells of microglial origin. In tissue explants of these same organs (3,16), it has been demonstrated that there are cells which will take up the silver stain and go through all the stages of "microglial" development, from the elongate cell with spiny processes to the gitter cell. In addition these cells will ingest trypan blue and carmine red. Injury to the liver, spleen, kidney (4) will bring about the proliferation of cells not unlike those of microglia.

The only two unusual factors that might possibly be thought to exclude the microglia from the reticulo-endothelial system are (a) the fact that these elements are not joined together in any syncytium like the R.E.S. cells seem

to be in other organs, and (b) that in normal intact animals, the parenteral injection of trypan blue is not taken up by the microglia, while the adventitial macrophages in the vessels in the brain, and R.E.S. cells elsewhere in the body, will. (8). The explanation for the latter phenomenon has been the postulation of the blood-brain barrier, which prevents the passage of colloidal substances into the brain. However, if sterile puncture wounds are made into the brain, and dye is given parenterally into the wound itself, the microglia will ingest it (8,15). However, several centimeters away from the wound, the microglia will not be found to have ingested the dye.

However, it seems that gitter cells have been derived not only from microglia but also from the adventitial cells of the blood vessels, and from cells of the dura mater (1,8); from the human blood monocyte in tissue culture, staining with the silver stain as additional proof (3); and finally from blood lymphocytes in the rabbit, in the earliest stages of puncture wounds of the brain (2). This latter point was demonstrated by Dougherty (2), who showed that the lymphocyte reacted in this way six to twelve hours after the initial

trauma, and that the microglia did not react until thirty hours later. All of these studies have been done under the experimental conditions of needle wounds of the brain. They have been done with the examination of the wound by the silver stain. There is general agreement that gitter cells formed from these various sources are indistinguishable from one another. Dougherty feels that those formed by lymphocytes look different (2). The investigators who have studied the multiple origin of the gitter cell are manifold; only a few have been mentioned.

Also, there is general agreement that astrocytes, and oligodendroglia do not form gitter cells, nor do the endothelial walls of the capillaries. Those that have claimed the origin of microglia from astrocytes and oligodendroglia were being confused by the regressive changes of the astrocytes and the acute swelling of the oligodendroglia reacting to injury (1).

However, when one begins to inquire into the formation of gitter cells and the role of the microglia in viral infections, one finds there is a great deal of controversial opinion.

The characteristic cellular changes found in the viral encephalitides caused by St. Louis encephalitis, western and eastern equine encephalitis, herpes simplex, Japanese B encephalitis and poliomyelitis viruses, etc. are perivascular cuffing of lymphocytes with focal and diffuse parenchymal infiltrations by these cells and occasionally by polymorphonuclear cells. Further, there has been described hypertrophy and proliferation of microglial cells within each focus of neurone cell degeneration, as well as a diffuse proliferation of microglia throughout the brain and spinal cord (17). There has also been demonstrated focal accumulations of predominantly elongated microglia-like cells, the so-called "glial" nodule. However, the source of this microglial proliferation is in dispute.

Hurst (6) in studying the lesions of experimental poliomyelitis found that the vast majority of the diffuse and focal exudates of the grey matter were microglial proliferation. They were in the "polyblastic" phase, i.e., amoeboid phase on their way to becoming gitter cells. He used the Hortega silver stain, and showed the complete transformation of microglia cells from rod shaped forms to the gitter cells.

However, Michels and Globus (11) in studying the small round cell infiltrations in human cases of polioencephalitis and acute epidemic encephalitis, but not using silver stains, felt that the perivascular lymphocytes which came from the blood stream infiltrated the parenchyma and gave rise to gitter-like cells, through transformation stages which they observed. They showed lymphocytes streaming through the vessel walls. They found no evidence that the endothelial or adventitial cells participated in the process of becoming phagocytes. They also felt that they saw large mononuclears (monocytes) undergoing the same transformation. The transitional forms of these cells that gave rise to these gitter cells had basophilic cytoplasm, so that these authors suggest that these are compound granular-like corpuscles. In addition, they describe "lymphoid foci" isolated from vessels, but also seen around capillaries, in which foci "gitter cells were found". They were inferred to have come from the "lymphoid foci". They did not dispute the fact that microglia in their sections underwent the complete transformation to gitter cells, but they imply that the transformation from lymphocytes make up the great proportion of gitter cells encountered.

Haymaker and Sabin (5) suggest in their paper on Japanese B encephalitis that the "polyblastic" forms in this disease are not Hortega cells as Hurst said they were in poliomyelitis, but were rather lymphocytes migrating from the blood stream. Further they mention that in their sections there were no reacting microglia cells in the cerebral cortex, but only foci of perivascular infiltrates and neuronophagic nodules both composed of mononuclear cells. "Hortega microglia cells were absent from the nodules, but cells advancing into the parenchyma and even those within perineuronal spaces had pleiomorphic nuclei, some of which bore a resemblance to the nuclei of Hortega cells. No gitter cells were observed. Therefore, the term "glial" nodule applied to nodular lesions is ill-advised". They feel that the brain tissue in Japanese B encephalitis reacts in the same way as poliomyelitis tissue does, and therefore they feel that they cannot agree with Hurst that the "polyblastic" forms are mobilized Hortega cells.

The purpose of this study is to determine whether resting microglia cells are able to proliferate and become hypertrophic, forming diffuse and nodular infiltrates, or

whether the elongated microglia-like hypertrophic cells and the so-called "glia" nodules seen in viral encephalitides are of hematogenous origin. Another possibility to be checked is whether these infiltrates may be derived from both sources.

The West Nile virus was chosen because Manuelidis (9) has shown that West Nile encephalitis shows occasionally, moderate perivascular cuffing and parenchymal infiltration and pronounced diffuse cortical proliferation of elongated microglia-like elements. In this disease, infiltrative phenomena with hematogenous elements would not obscure the potential proliferation of resting microglia, as is the case in poliomyelitis in the anterior horn of the spinal cord.

Hypophysectomized mice, were included in this experiment in the hope that they might reveal a different histological reaction to the West Nile virus, and perhaps disclose more information about the problems under consideration.

MATERIAL AND METHODS

Eighteen adult male albino mice were injected intracerebrally while unanesthetized, with 0.03 cc. of a 10% suspension of West Nile virus. The animals were obtained from the Endocrine Laboratories in Madison, Wisconsin. Nine of these mice were hypophysectomized and nine were normal. Two of the hypophysectomized mice died a few hours after the injections, presumably from the trauma of the injections. The animals were then sacrificed at the times shown in Table 1. Thus, one was able to have mice of both categories, from an asymptomatic stage to one of severe infection with the varying states in between. The animals were killed by excess ether inhalation. The brains and spinal cord were then removed, cut into smaller pieces and fixed in Brom-Formol. Twenty-four hours later, the brains and spinal cords were cut on a freezing microtome. Some of the sections from each block were stained by Penfield's second modification for oligodendroglia and microglia, utilizing a silver carbonate solution. The other sections from the same blocks were stained by routine hematoxylin and eosin (H & E). The corresponding sections were then easily matched up becoming the equivalents of serial sections.

The bases of the skulls of the hypophysectomized animals were decalcified and sections made for the examination of the sellae turcica. A pair of virus injected mice, one normal and one hypophysectomized were stained exclusively by H & E and a similar pair of non-infected mice were stained exclusively by the silver method to be used as controls. The silver stained and the corresponding H & E sections were then compared and studied for microglial and vascular reactions primarily.

RESULTS

The four animals, # 1,2,3, and 4, killed at 65 and 66 hours, showed similar findings: the H & E sections, with one exception to be described, in all these animals disclosed a moderate number of elongate, kidney-shaped or twisted cells that were distributed throughout the cerebrum without any obvious relationship to vessels. These cells were microglia-like by H & E stain (Fig. 1).

The vessels in the sections stained with H & E, showed no proliferation of the cellular elements in their adventitial walls. There were neither perivascular round cell infiltrates in the Virchow-Robin spaces, nor were there nodular accumulations composed of lymphocytes or mononuclear cells in the brain tissue itself.

The silver stained sections disclosed many elongated, thickened microglial nuclei with many radiating processes beset with smaller laterally placed projections (Fig. 2-5). The microglia processes are predominantly located at the poles of the nuclei. Some elongated nuclei similar to those described above without any cytoplasmic processes were also observed (similar to those in Fig. 27).

Both type of cells were seen scattered throughout the brain and were independent of vessels. There were more of these cells in the grey matter than in the white matter.

In the H & E sections, microglia-like cells were seen in the molecular layer of the cerebellum and also in the medulla, independent of vessels. The silver stain confirmed these cells as being microglia, by demonstrating their processes. No vascular changes were seen. The spinal cord showed similar findings by H & E and silver stain.

The microglial cells seen in animals # 1, 2, and 4 were qualitatively and quantitatively similar, with animal #3 showing quantitatively less microglial cells.

Animal # 1 showed on a single H & E section, a loosely formed nodule composed of elongate, twisted and kidney-shaped cells in the temporal lobe near a vessel. The silver stain confirmed them to be microglia cells with cytoplasmic processes. The endothelial cells of this vessel were swollen, and the fixed tissue elements in its adventitial wall were slightly proliferated (Fig. 6).

Animal # 2 showed in one H & E section, a suspicious area which appeared to be the proliferation of fixed tissue

cells in an adventitial coat of a vessel, but without any perivascular infiltrate of mononuclear cells. The corresponding silver stain showed no microglia around this area.

Animals # 5 and 6, sacrificed at 87½ and 88½ hours, disclosed the following: there were more microglia-like cells by H & E and microglia by silver stain than in the previous animals; they were also independent of any vessel changes. The cerebellum, medulla, and spinal cord were the same as in animals # 1-4, except that there were more microglia present.

However, in addition, the H & E stain now disclosed occasional small perivascular cuffs in the Virchow-Robin spaces made up of small round cells and cells that appeared to be the same as the fixed tissue elements in the adventitia that were proliferating. There was no parenchymal invasion however with mononuclear cells.

The silver stain, in addition, also showed a vessel surrounded by microglial cells and their processes (Fig. 7). There was also seen another vessel surrounded by irregular elongate bodies that were present in the perivascular brain tissue. They were without processes, having the nuclear characteristics of microglia.

The molecular layer of the cerebellum in animal # 5 showed these elongated cells with and without cytoplasmic processes.

Animal # 6 disclosed fewer microglial cells than animal # 5.

In the animals killed at 98 hours, # 7 and 8, the cellular changes of the vessels and of the microglia were equal in intensity. There was a mild focal meningeal reaction (Fig. 8). There were a moderate number of vessels revealing changes in their walls. Some had proliferation of the elements in their adventitial walls only; some had, in addition, round cell infiltrates in the Virchow-Robin spaces (Fig.10-11); and some even had infiltrates in the surrounding brain tissue (Fig. 12-14). The parenchymal infiltrates were partially composed of elongate twisted microglia-like cells. These cells with silver stain did not reveal any processes. There were also small round cells in these infiltrates.

One H & E section in animal # 8 showed a parenchymal infiltration with elongate twisted elements very prominent at one pole of a vessel, where the vascular wall was particularly thickened and infiltrated (Fig. 15).

Some of these elongate cells in the silver stain have a single process at each pole and consequently appear sperm-like. They are present in the tissue infiltrates as well as occurring independently of vessels.

Animals # 9 and 10, killed at 118 hours, showed more microglial proliferation independent of vessels than previously, but also exhibited more vascular changes. There was more cuffing and more prominent parenchymal infiltrates of the elongate and also small round cells. In several instances, these elongate forms have processes by silver stain (Fig. 16). Animal # 9 had fewer cellular changes than animal # 10.

Animal # 11, killed at 123¼ hours, showed even more vascular changes with many infiltrates in the Virchow-Robin spaces and in the brain tissue. The brain tissue around the vessels disclosed a very pleomorphic group of cells. Some were elongate and straight; some elongate and twisted; some reniform; some multilobular; and some were small round cells (Fig. 17-19).

Animal # 12, killed at 122½ hours, showed minimal cellular changes similar to the 66 hour killed animal.

Animal # 13, killed at 137½ hours, shows the same picture as the previous animals, except that now one can see most clearly cells, apparently coming from the vessel walls, traversing the Virchow-Robin space, and invading the brain parenchyma (Fig. 20-21). These cells are elongated, reniform and have processes stained by the silver method (Fig. 22-23). Some of them are similar to the fixed tissue cells of the vessel adventitia.

One can also see microglia with their processes attached to a vessel wall (Fig. 24).

Many tissue infiltrates were seen in the medulla, made up of small round cells and cells resembling the adventitial cells. However, very few cytoplasmic processes were seen in the corresponding silver sections.

Many elongate cells were seen in the molecular layer surrounding vessels whose adventitial walls disclosed proliferating cellular elements. They were also seen around unchanged vessels. These cells were again microglia-like but showed no processes in the silver stain.

There were still many microglia observed which were independent of vessels (Fig. 25).

Animal # 14, killed at 137½ hours also, but stained only by H & E, showed many vessels with perivascular and parenchymal infiltrates. The latter were composed of round cells and microglia-like cells.

Animal # 15, killed at 185 hours, showed the same as the above last two animals, but none of the elongate cells in the tissue infiltrate could be shown to have processes by the silver stain as in animal # 13. Again, there were excellent examples of the microglial proliferation independent of vascular changes.

Animal # 16, killed at 208 hours, showed the same findings as # 14, except that the cellular reaction was far less in intensity.

Examination of the needle track with H & E stain revealed necrotic brain tissue surrounding the track with hemorrhage within the track as well as in the immediately surrounding brain tissue. Elongate twisted cell bodies were abundantly seen in this surrounding tissue.

In the silver stained sections of the same area, there was an amorphous black staining debris in the needle track and in the immediately surrounding tissue.

There were all stages of microglia from the hypertrophic forms with many processes and their lateral spines to the gitter cells, in the vicinity of the track. Many of the gitter cells were in very close proximity to the walls of moderate sized vessels and capillaries. However, there were also many elongate, twisted, and reniform cell bodies without processes intermingled with similar appearing cell bodies that did have processes (Fig. 26). In sections through the needle track of some of the animals there were no stainable processes associated with these elongate cells (Fig. 27). However, in these same areas there were many typical gitter cells with their spongy appearing cytoplasm.

There was a proliferation of capillaries around the needle track wound and there were a moderate number of microglia whose processes were in close proximity to the walls of these vessels.

The control animals, # 18 and 19, showed rare elongate and straight cell bodies with the silver stain in the brain and spinal cord. They appeared to be independent of vessels. These cell bodies were narrower and not hypertrophic as in the infected animals.

Only a very small percentage of these cell bodies had stainable processes associated with them. When these processes were seen, they were only faintly stained.

There were no changes seen in any vessel walls.

There were no histological differences between the control animals as far as could be determined by silver stain alone with respect to the microglia.

No pituitary remnants were found in any of the sections that were made through the sellae turcicae of the hypophysectomized animals.

DISCUSSION

The severity of the clinical picture in these experimental animals could not always be correlated with the severity of the histological changes. Some of the animals with mild clinical signs revealed severe histological lesions, and vice versa. The inflammatory reaction in general grew more severe the longer the animals remained alive after the intracerebral innoculation with the virus, regardless of how intense their symptoms may have been at the time of their deaths. There were no differences between the normal and hypophysectomized mice in their morphological reactions to the virus. However, the hypophysectomized mice succumbed very quickly once they began to show any signs of infection (4-6 hours), whereas the normal mouse survived for 12-24 hours before expiring.

The control animals which were stained with the silver method only, revealed narrow, non-hypertrophic microglial cell bodies, only a small percentage of which had stainable cytoplasmic processes. The vast majority of these processes were only faintly stained.

This may be explained on two possible bases: (a) the unpredictability and technical difficulties of the silver method which is well known to all neurohistologists; and (b) it is also generally recognized tha microglia as well as oligodendroglia stain best with the silver method only under pathological conditions, in which they swell up and become hypertrophic.

It should be stressed that all the following deductions and inferences are based upon observations made of microglial proliferation in areas of the brain tissue not visibly traumatized by the injection needle. The observations of microglia around the injection sites have served as collateral as well as confirmatory evidence for these conclusions.

The histological changes seen in the brains of the infected animals have led to the following theses:

There is a microglial proliferation to the virus without any significant changes in the vascular mesenchymal tissue before 87 hours post injection. These vascular changes include proliferation of fixed tissue cells in the adventitiae of the vessels, and infiltrates of these cells

plus small round cells in the Virchow-Robin spaces. Mitotic figures of microglia are also observed in this group of animals. Figure 25, shows two microglia in the process of division, and it is felt that prior to 87 hours post injection, the microglial response in West Nile encephalomyelitis is solely on the basis of this type of proliferation.

In the 66 hour animals, there were only two instances where any vascular changes as noted above were seen. These were found only after very careful search and consisted only of proliferation of adventitial cells. In one animal # 1, there was a loosely formed "glial" nodule in the temporal lobe in an H & E section (Fig. 6). On silver stain they were shown to be microglia with processes. Nearby there was a small vessel which had swollen endothelial cells and slight proliferation of the fixed tissue elements in its adventitia. However this nodule did not appear to arise from the vessel as convincingly as did other microglia that were observed in other animals to be discussed below. There was no evidence of any perivascular infiltrate around this vessel.

Animal # 2 showed in one H & E section, a suspicious area which appeared to be the proliferation of fixed tissue

cells in its adventitia, but without any perivascular infiltrate of mononuclear cells. The corresponding silver stained section showed no microglia around this area. It is felt that this questionable proliferation may be more apparent than real; i.e., it may actually represent an oblique cut through the vessel by the microtome.

At around 87 hours, and it became clearer after this time, there is an obvious participation by the vascular system. The changes enumerated above become readily apparent. The fixed tissue cells in the adventitia appear to infiltrate the Virchow-Robin space and thence, the brain tissue. There are also small round cells in both the Virchow-Robin space and brain tissue infiltrates. The elongate cells that appear in the brain tissue infiltrate resemble by H & E stain both microglial-like cells and the fixed tissue cells of the adventitia. Some of these elongate cells which are twisted and reniform and which are in the immediate vicinity of altered vessels have cytoplasmic processes stainable by the silver method, that makes them indistinguishable from the typical microglia cell.

The fact that many of the elongate twisted and reniform cell bodies do not have silver stainable processes should not speak against their being microglia or a transitional stage in the formation of the typical reactive microglia. For it has been demonstrated that (a) in the control animals very few processes were stained in the resting microglia for the reasons mentioned above; (b) in the sections in the infected animals where the needle track is included, one can observe these elongate cell bodies intermingled with similar appearing cell bodies that do have processes and look like typical microglia. In fact observations of these sections in the infected animals have disclosed an entire spectrum of stained cell bodies around the needle track. In some sections all the microglia have stainable processes; in others, only varying proportions of the cells have processes; and in still other sections, none of these elongate cell bodies have processes. Yet, in all these sections there are many typical gitter cells intermingled with these elongate twisted reniform cell bodies; (c) many investigators such as Cone (1), Hortega (14), Penfield (12,13) and Hurst (6) have shown that the microglia often retracts its

processes and becomes amoeboid as it travels and as it becomes transformed into the gitter cell; and finally (d) in the 66 hour animals where the microglial response was independent of vessel changes, there were many of these elongate cell bodies with and without processes present in the cerebral cortex. All these factors are felt to explain the absence of processes in these elongate cells, as well as supporting the thesis that these elongate twisted and reniform cell without processes do represent microglia.

With the participation of the vascular system, the microglia in the brain increased in number both around the vessels as well as seemingly independently of vessels. The latter group of microglia may be explained either on the basis of migration from the vessels or else as continued proliferation of resting microglia. The former possibility is very probable, since the entire increase in the number of microglia cannot be explained simply on the basis of proliferation of the resting microglia.

While the elongate twisted cells which are felt to be microglia seem to resemble the adventitial cells and are

felt to be possibly derived from them, the question of the lymphocytic origin of these cells must be answered. There were small round cells in the infiltrates that were not unlike lymphocytes. The elongate twisted cells in the infiltrate were more ovoid when seen in the Virchow-Robin space, and it is possible that lymphocytes in extruding themselves through the vascular wall as they have been shown to be capable of doing by Michels and Globus (11), may undergo a morphological transformation to this oval form as they become microglial cells. Among the elongate cells in the parenchymal infiltrate, there were oval cells which could not be distinguished from those in the Virchow-Robin space. This is a theory for which there is little proof other than the observations just cited.

There were also vessels where elongated twisted cells were partially in the Virchow-Robin space and partially in the vessel wall. There were also vessels which were not uniformly surrounded by infiltrates and where the presence of elongated microglia-like cells in the brain tissue was proportionally related to the severity of the perivascular infiltration (Fig. 15).

It is felt that the adventitial cells are a more likely source of the microglia, since in addition to other observations noted above, they resemble in their proliferating state many of the elongate cells seen in the brain tissue infiltrates.

In the section on results, it was mentioned that animal # 12 reacted histologically like a 66 hour animal even though it had survived 122½ hours. This observation coupled with the evidence of possible vascular changes in two of the 66 hour animals is explained most probably by the biological variation of response in living organisms, and in no way destroys the clinico-pathological correlations cited above, namely, the clinical signs bear no relationship to the survival time, but the severity of the histological response does. The failure of animal # 12, to disclose a more severe histological response may also be explained by the injection of too little virus, since the injection of 0.03 cc. is subject to some variation from animal to animal.

To the histological characteristics of the viral encephalitides belongs the "glial" nodule. The term "glial" is questioned by Haymaker and Sabin (5) who feel that these

formations are of hematogenous origin. In the present study, only one such nodule, which was loosely formed, was observed. This nodule was made up of microglia cells (Fig. 6). Nearby there was a vessel which had slight changes in its endothelial and adventitial cells as described previously. From this single observation, one cannot take a definite stand concerning the origin of the cells participating in this formation. It seems, however, that the term "cell" nodule introduced by Haymaker and Sabin is more appropriate because some of the cells do not bear any resemblance to microglial cells but rather are small round cells.

There is enough circumstantial and collateral evidence elsewhere in these sections to establish with a high degree of probability that these elongate reniform cells are truly microglia and that they do come from the vascular system, and that the adventitial cells are the likely origin of them. However, in West Nile encephalomyelitis at least, the early phase of the microglial reaction is independent of any vascular changes, arising from resting microglial cells, but that beginning about 87 hours post injection, the vascular system,

possibly the adventitial cells, begin to take part in the formation of microglia.

Accordingly it is felt that Hurst (6) is correct in his contention that in polioencephalomyelitis, the diffuse and focal aggregates are mobilized Hortega cells. However, he is not altogether correct when he implies that they are all proliferation of resting microglia, and denies the contribution from the vascular tree.

Likewise, Haymaker and Sabin (5), Michels and Globus (11) are partially correct in their contention that the vascular system is the origin of these microglial cells. However, it is not the source of all of them. Although from the experimental material in this study, one has the impression that these microglial cells arise from the adventitial cells, one could not completely deny the lymphocytic origin of the microglia.

The controversy in the literature is based upon the fact that not all investigators used silver stains to evaluate their sections, and perhaps also because they were looking at the cellular reactions at different times in their evolution.

This could very well have happened in West Nile encephalomyelitis if one individual studied the disease process at 66 hours, and another at 180 hours. In addition, the immense perivascular and parenchymal infiltrates seen in other viral encephalitides such as poliomyelitis, Japanese B encephalitis, etc., may have obscured the proliferation of the resting microglia.

It is generally known that the inflammatory response in viral encephalitides does not undergo marked variations in the histological qualities of the participating cellular elements. Therefore, the results gained by examining animals infected with West Nile virus reveals a pattern of cellular response that may be common to all the viral encephalitides in respect to the reaction of the microglia cells and their source of origin.

SUMMARY

1. It has been demonstrated that in West Nile encephalomyelitis, the reacting microglia may be with or without processes, independent of their relationship to infiltrated or resting vessels.
2. In West Nile infection, the resting microglia first proliferate independently of any vessel activity.
3. At about 87 hours, the vascular tree, by the proliferation of fixed tissue cells in its adventitiae,becomes active in the formation of cells which invade the brain tissue and become microglia. The reacting microglia are the result of the proliferation of resting microglia and of the vascular elements.
4. Not enough evidence was obtained that lymphocytes may be the source of microglia.
5. Only one "glial" nodule loosely formed was found which was composed almost entirely of microglial cells. The origin of "glial" nodules was discussed.

6. Hypophysectomized and normal mice react in the same manner to the virus. The severity of the cellular response is proportional to the time of survival after the injection, but not to the clinical symptoms.
7. Hypophysectomized mice succumb faster to the virus infection than do normal mice.
8. The pattern of microglial reaction seen in West Nile encephalomyelitis was compared with other viral encephalitides and its significance discussed.

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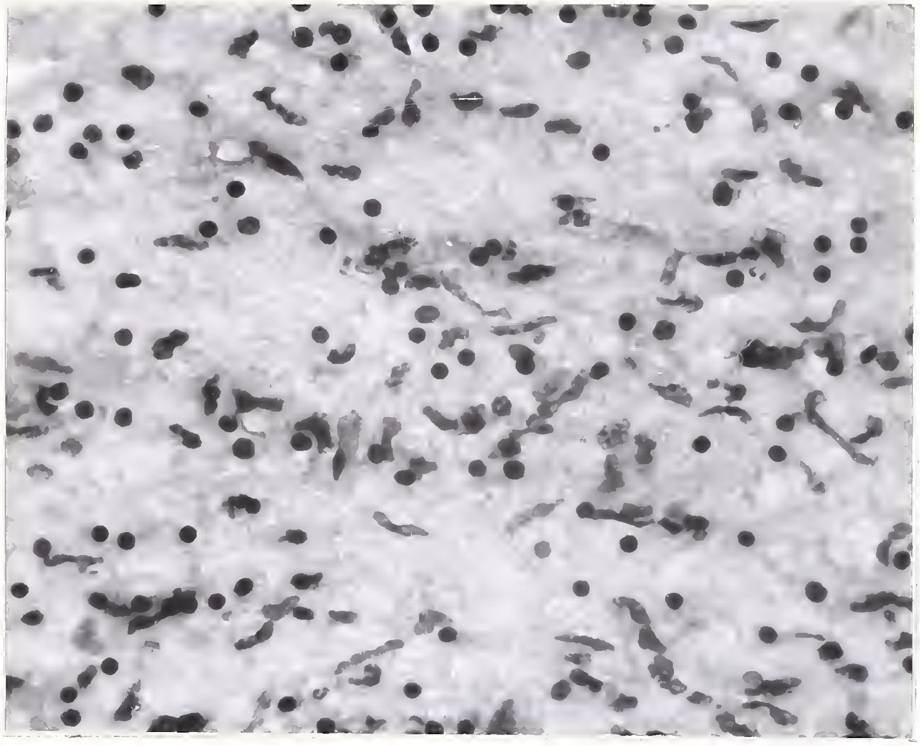
Fig. 1. Cerebral cortex. Elongated kidney-shaped and twisted hypertrophic microglia cells. Hematoxylin and eosin. X450.

Fig. 2. Cerebral cortex. Elongated microglia cells with many processes and laterally placed spines. These cells are independent of vessels and perivascular infiltrates. Hortega silver method. X450.



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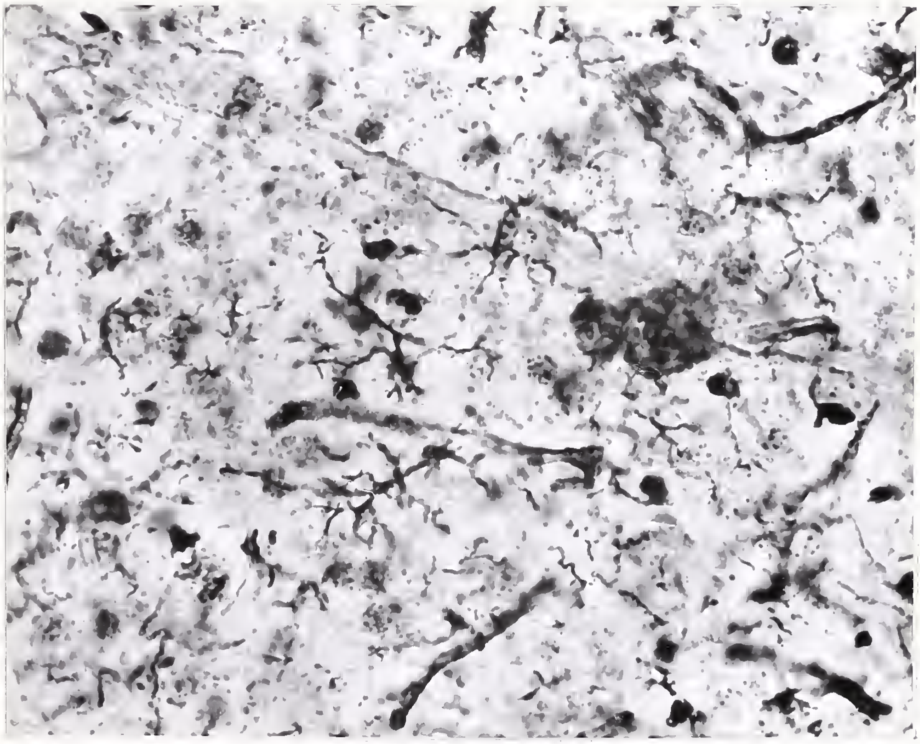

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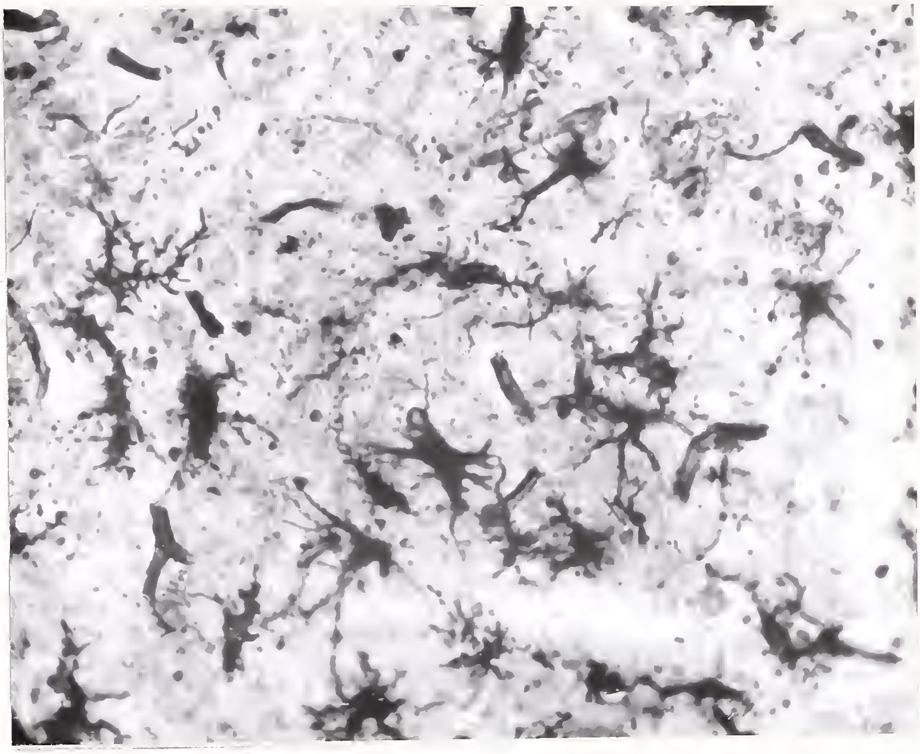
Fig. 3. Cerebral cortex. Microglia cells are more numerous more hypertrophic, and possess thicker processes as compared with Fig. 2. Microglia are independent of vessels. Hortega silver method. X450.

Fig. 4. Same as Fig. 3.



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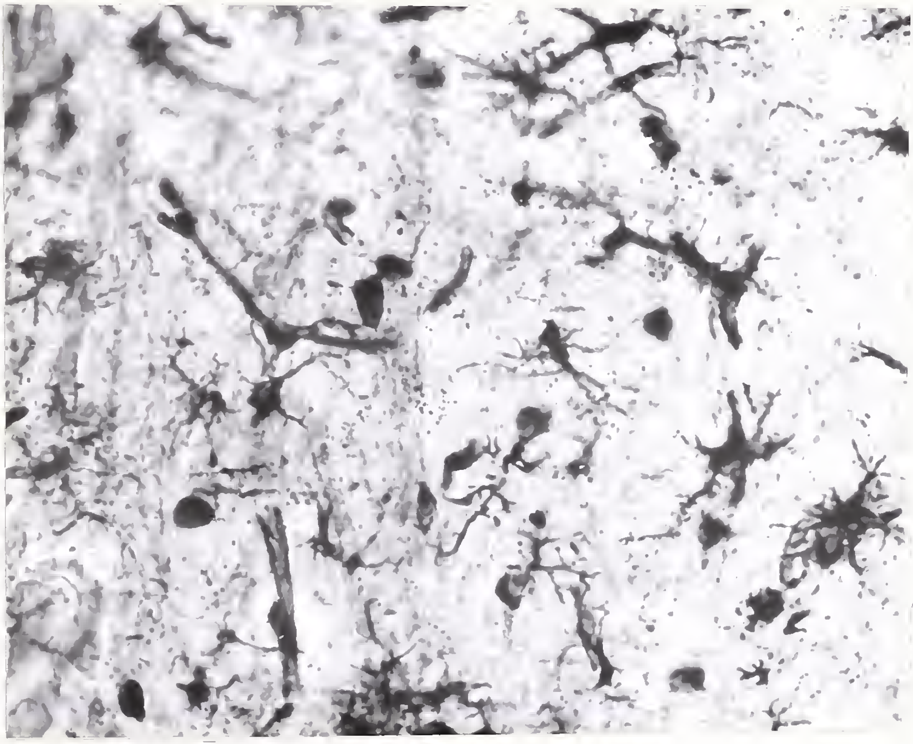

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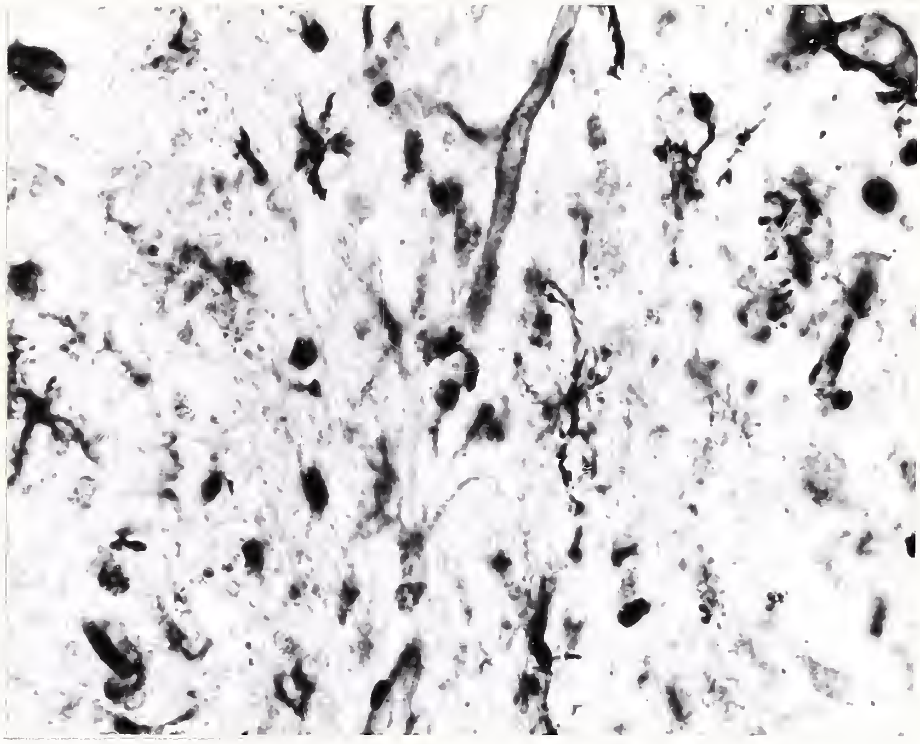
Fig. 5. Cerebral cortex. Isolated hypertrophic microglia cells. Hortega silver method. X450.

Fig. 6. Cerebral cortex. Loosely formed nodule composed of elongate to oval microglia-like cells. Thickened vessel with swollen endothelial cells is in the vicinity. Hematoxylin and eosin. X450.



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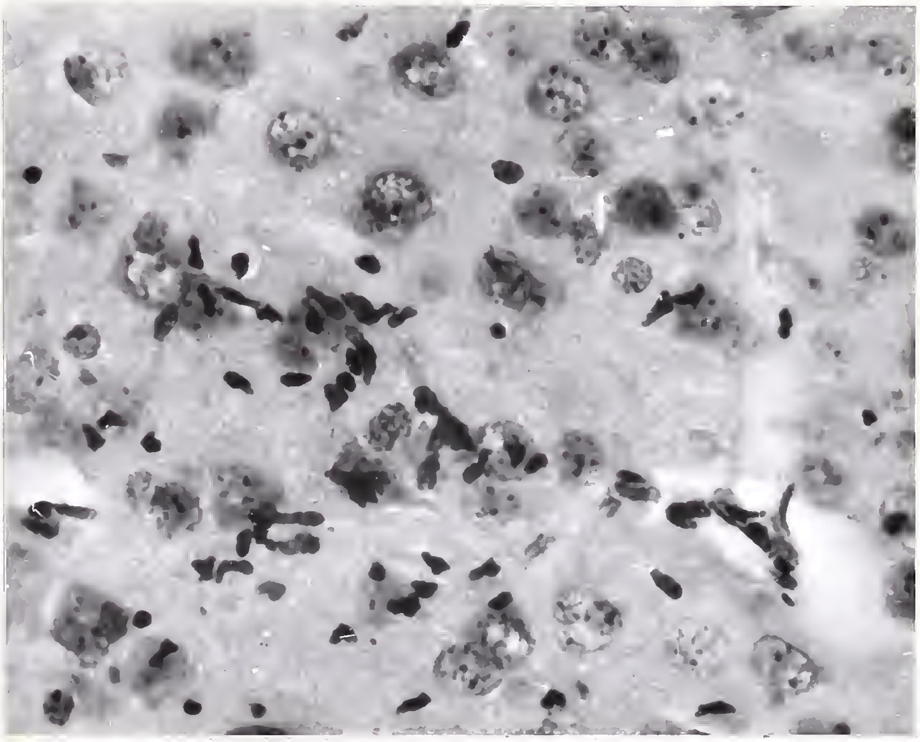

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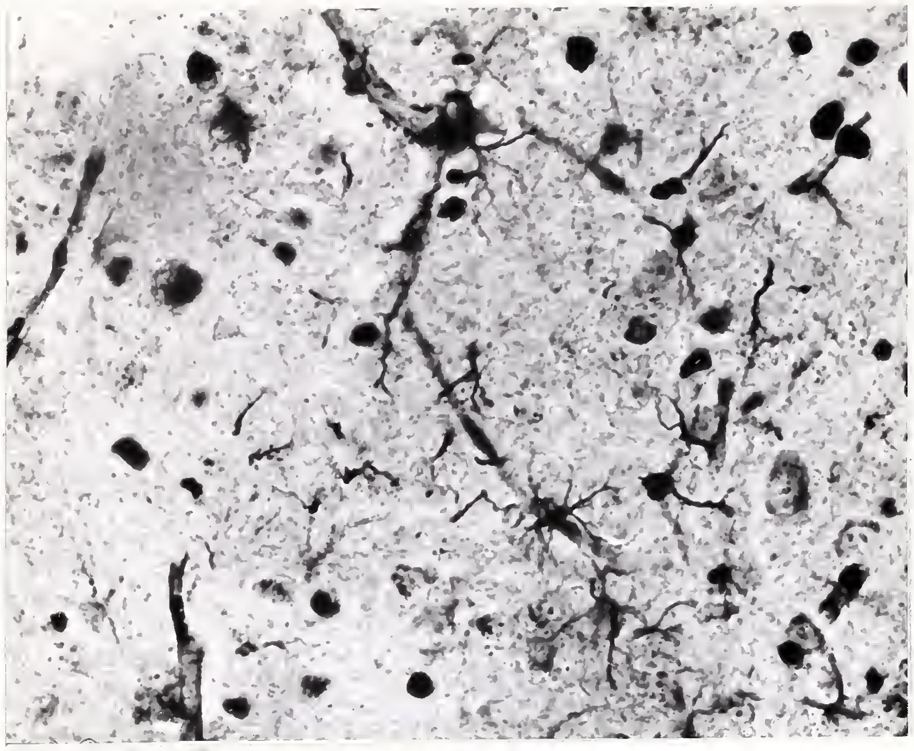
Fig. 7. Cerebral cortex. Microglia with processes, some independent and some in close relation with the vascular wall. Hortega silver method. X450.

Fig. 8. Cerebral cortex. Focal meningitis composed of round cells. Hematoxylin and eosin. X450.



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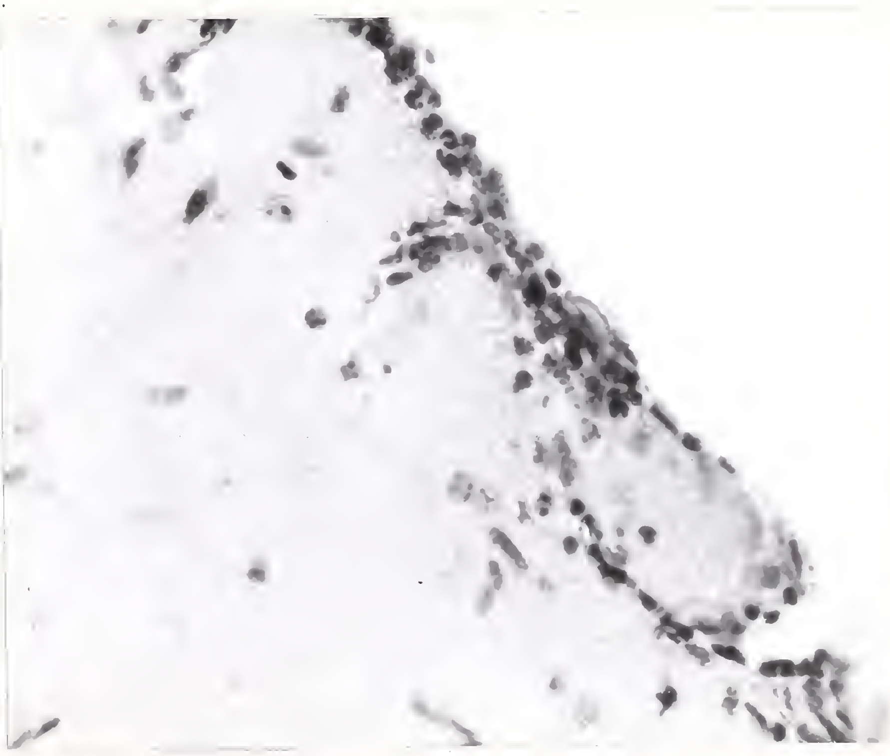

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Fig. 9. Cerebral cortex. Thickening of the vascular wall and early infiltration of the Virchow-Robin space. Hematoxylin and eosin. X450.

Fig. 10. Cerebral cortex. Same as Fig. 9. In addition, in the upper right half of the picture, oval to elongated microglial-like nuclei are present. These are more obvious in Fig. 11, where some are in the immediate vicinity of the Virchow-Robin space. Hematoxylin and eosin. X450.

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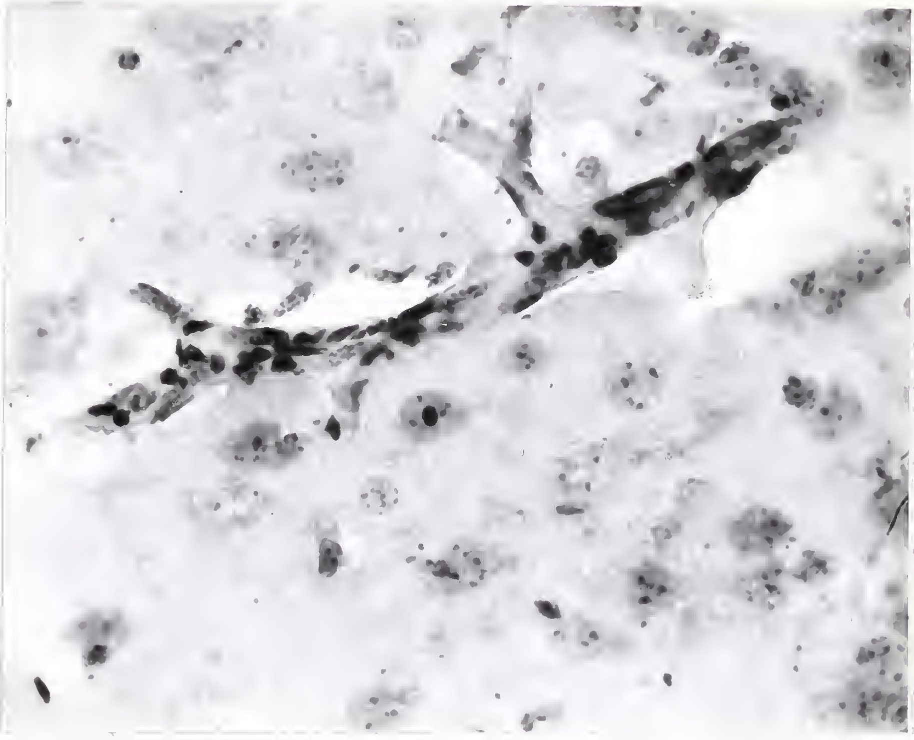

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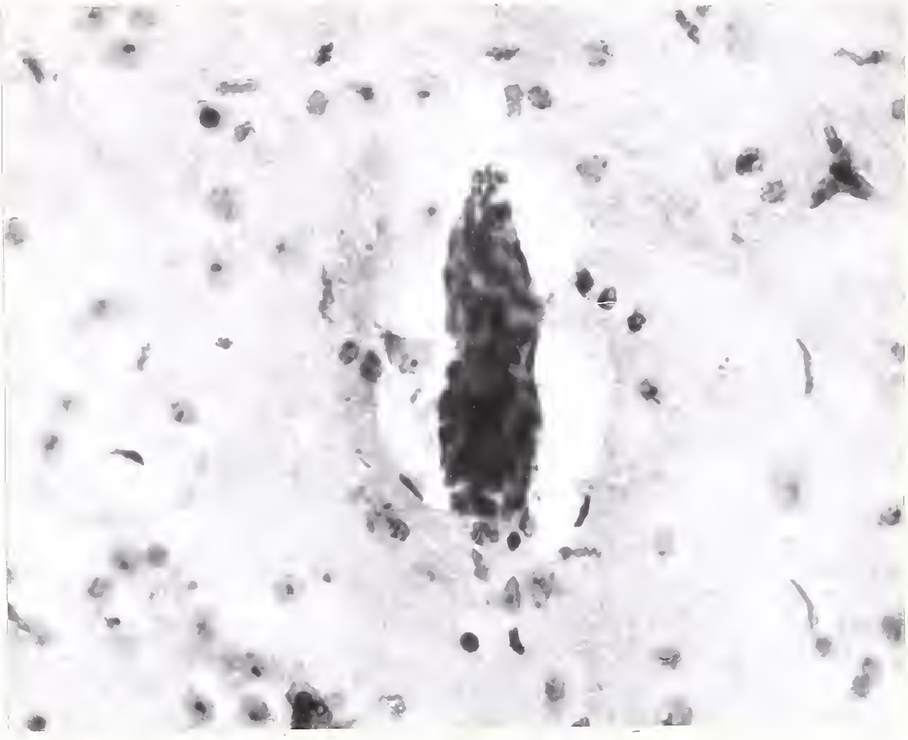
Fig. 11. Same as Fig. 10.

Fig. 12. Cerebral cortex. Small vessel with perivascular and parenchymal infiltrate. Among the latter, there are round and microglia-like cells. Hematoxylin and eosin. X450.



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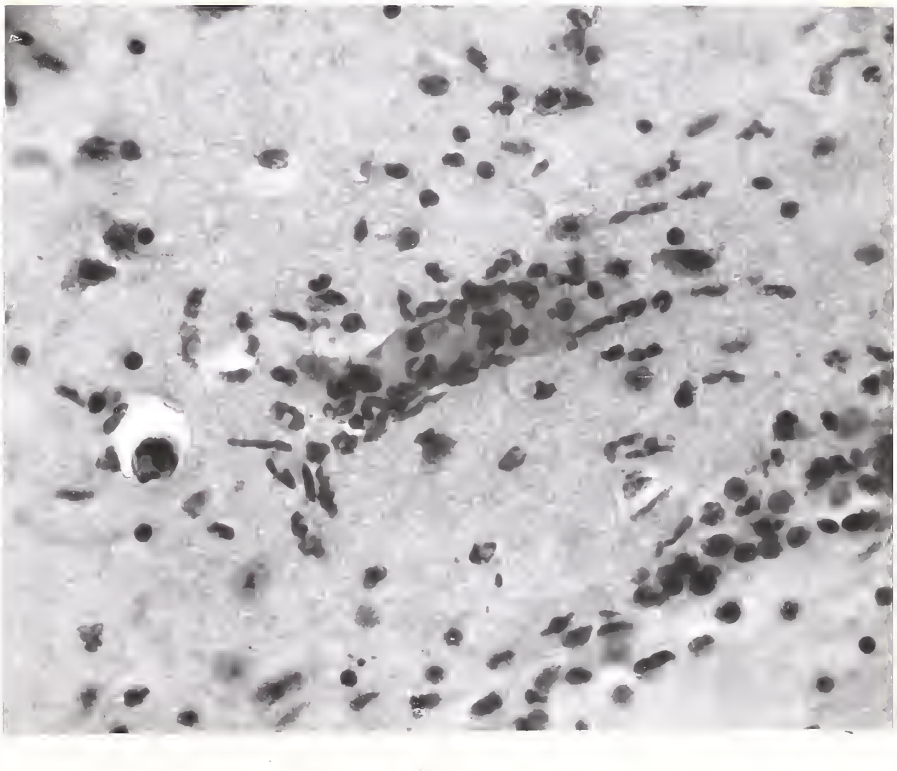

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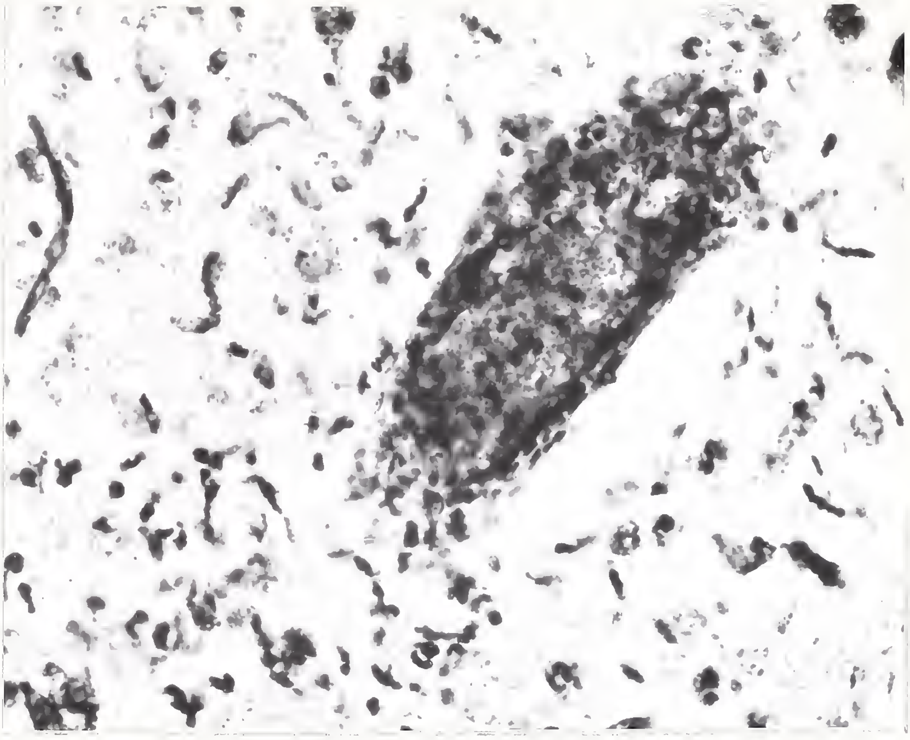
Fig. 13. Same as Fig. 12. In addition, one can see among the parenchymal infiltrates microglia cells which do not reveal any processes. Hortega silver method. X450.

Fig. 14. Same as Fig. 13.



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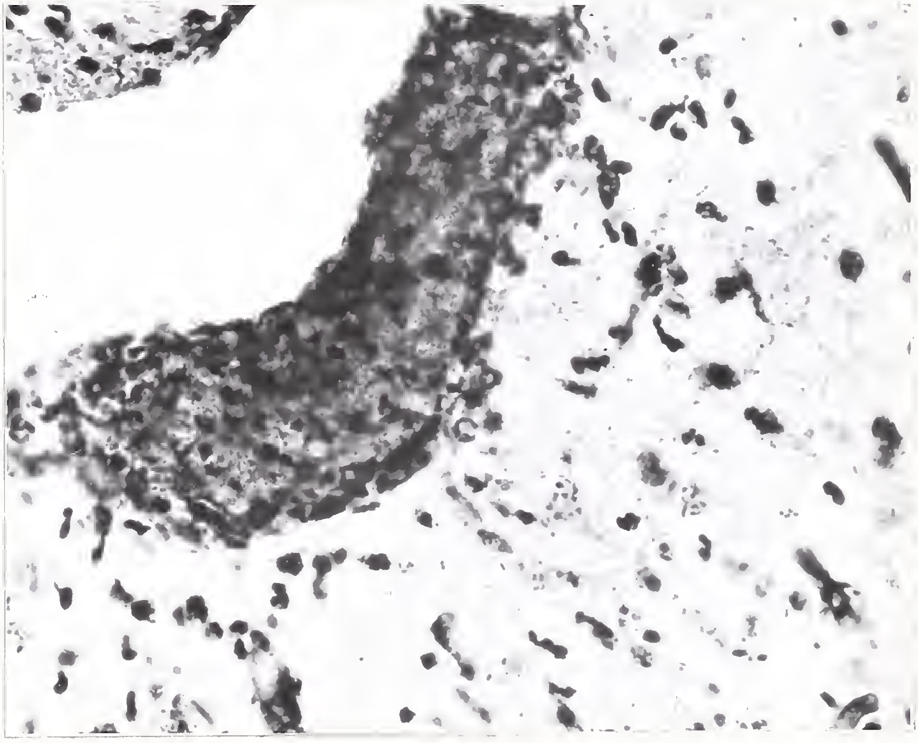

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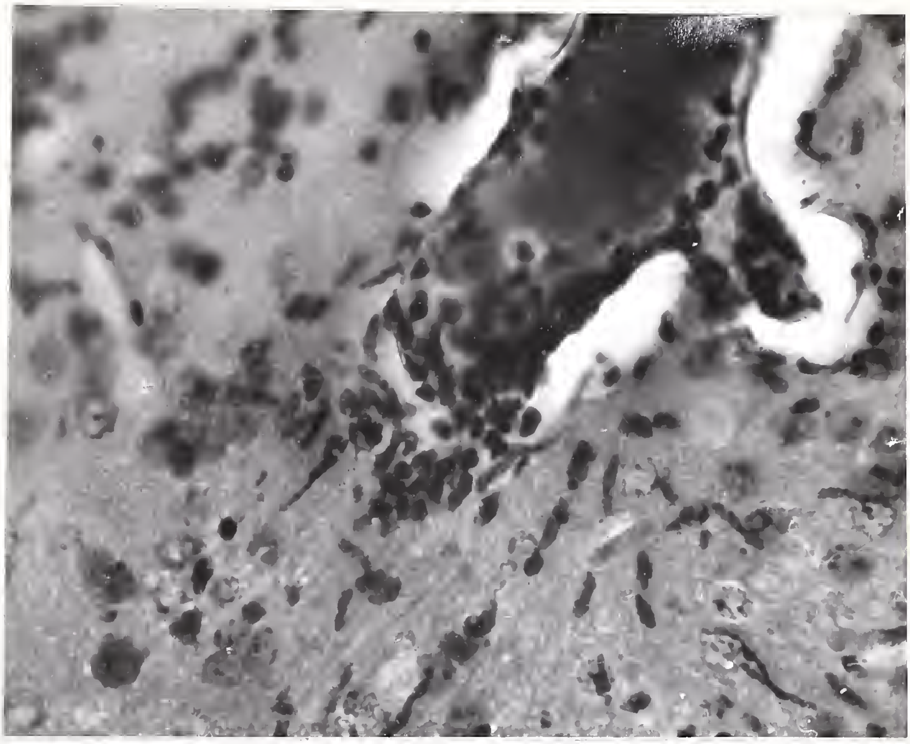
Fig. 15. Cerebral cortex. Vascular wall showing thickening and infiltration. The perivascular infiltrate is in continuity with the parenchymal infiltrate which is partially composed of elongate microglial-like cells. Hematoxylin and eosin. X450.

Fig. 16. Cerebral cortex. Infiltrated vessel with many elongated microglial cells in its immediate vicinity, some of which possess processes. Hortega silver method. X450.



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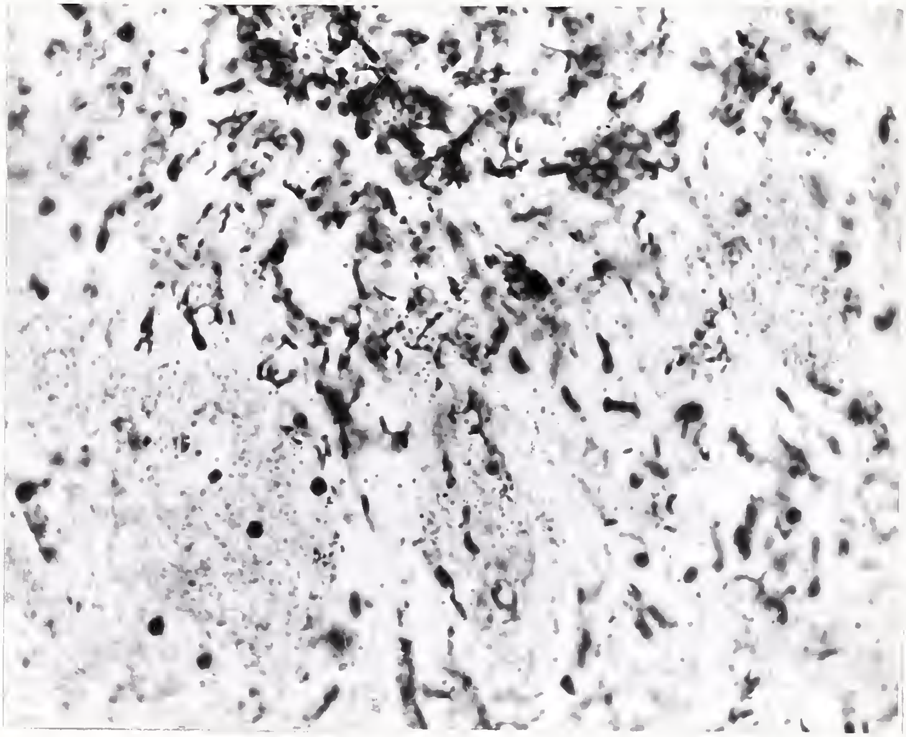

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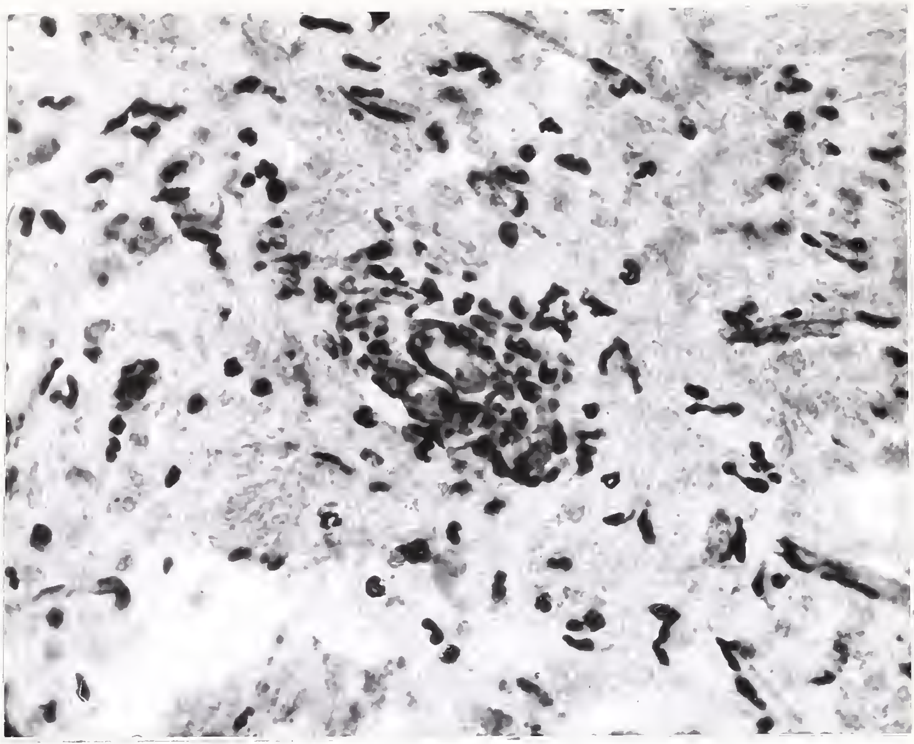
Fig. 17. Cerebral cortex. Vessel with pleomorphic parenchymal infiltrate consisting of elongated, twisted, reniform, and multilobular elements. Small round cells are also present. Hortega silver method. X450.

Fig. 18. Same as Fig. 17.



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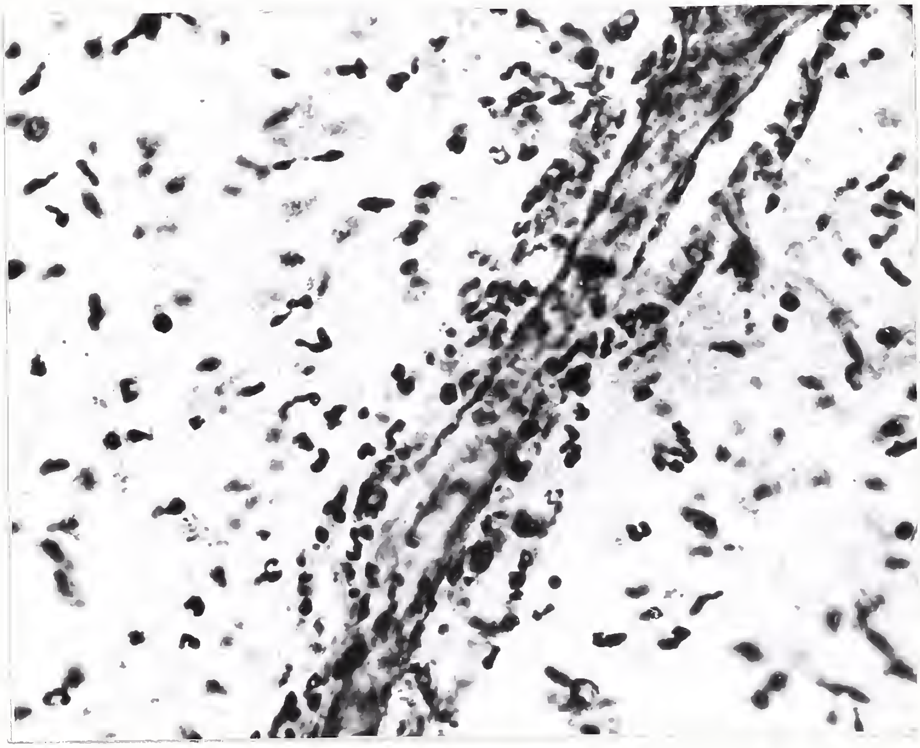

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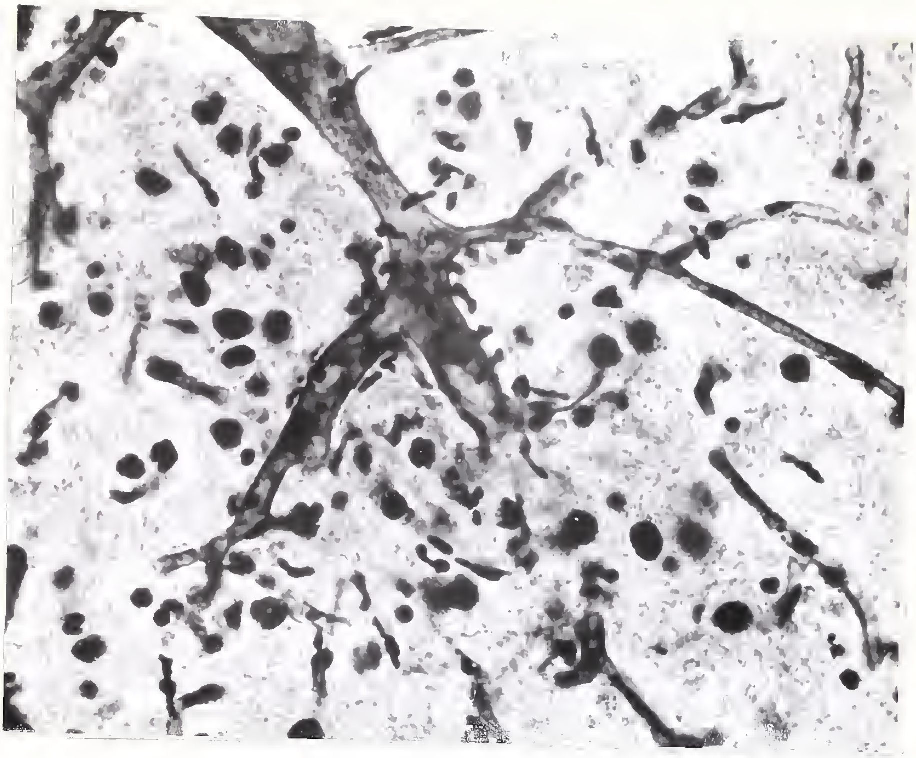
Fig. 19. Same as Fig. 17.

Fig. 20. Cerebral cortex. Accumulation of cells in the Virchow-Robin space invading the brain tissue. Some of the elongate cells which are present in the infiltrate look similar to the adventitial elements. Hortega silver method. X450.



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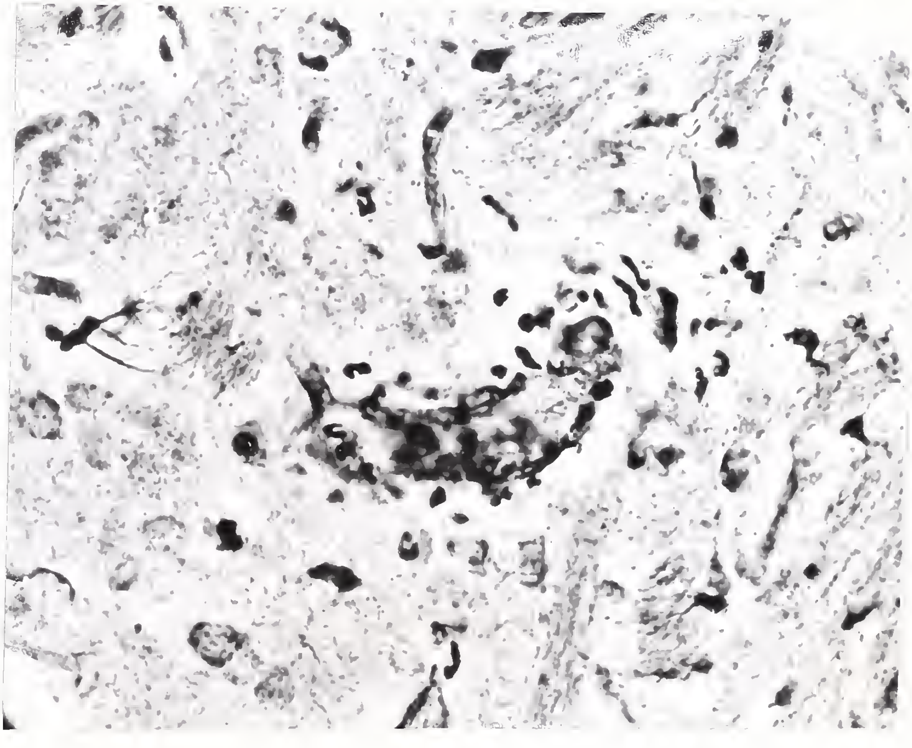

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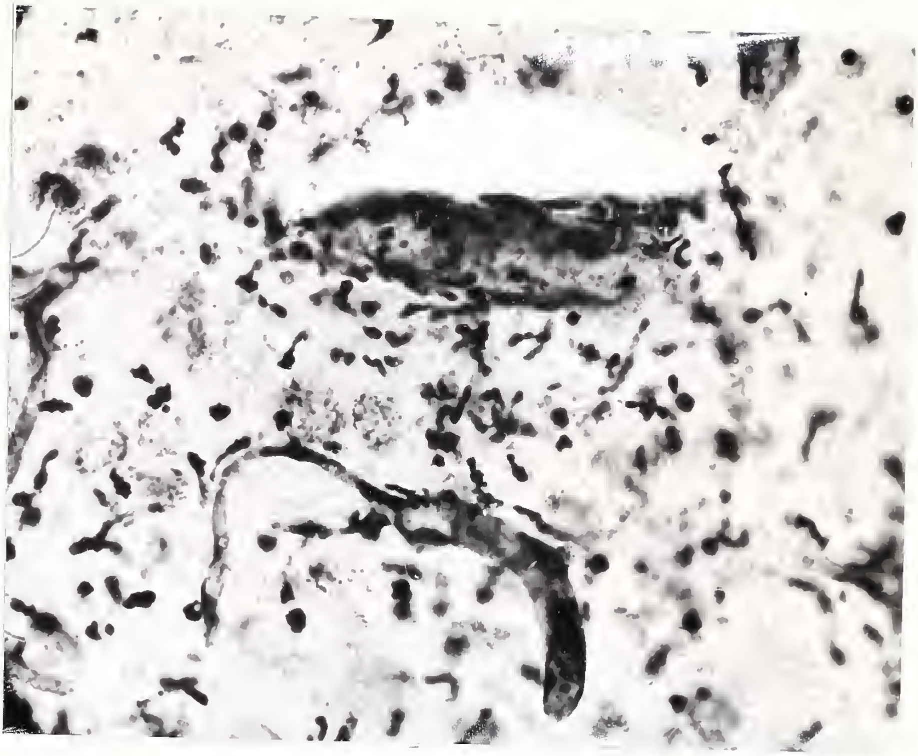
Fig. 21. Same as Fig. 20.

Fig. 22. Cerebral cortex. Marked parenchymal infiltrate with many elongated microglia cells some of which show cytoplasmic processes. Hortega silver method. X450.



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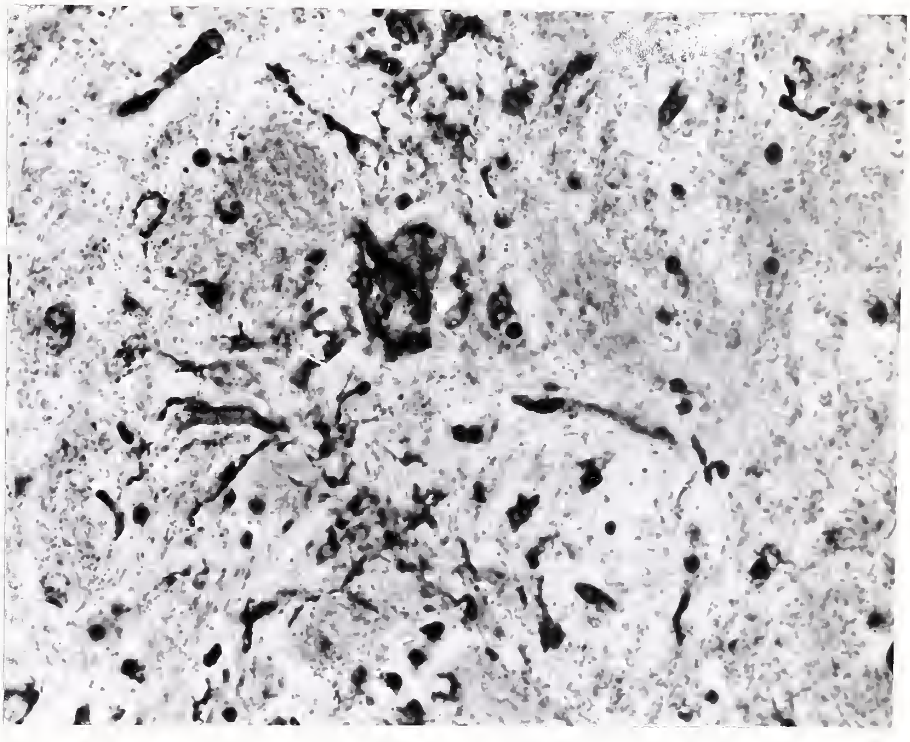

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Fig. 23. Same as Fig. 22.

Fig. 24. Cerebral cortex. Microglia whose processes are in very close relation with the vessel wall. Hortega silver method. X450.

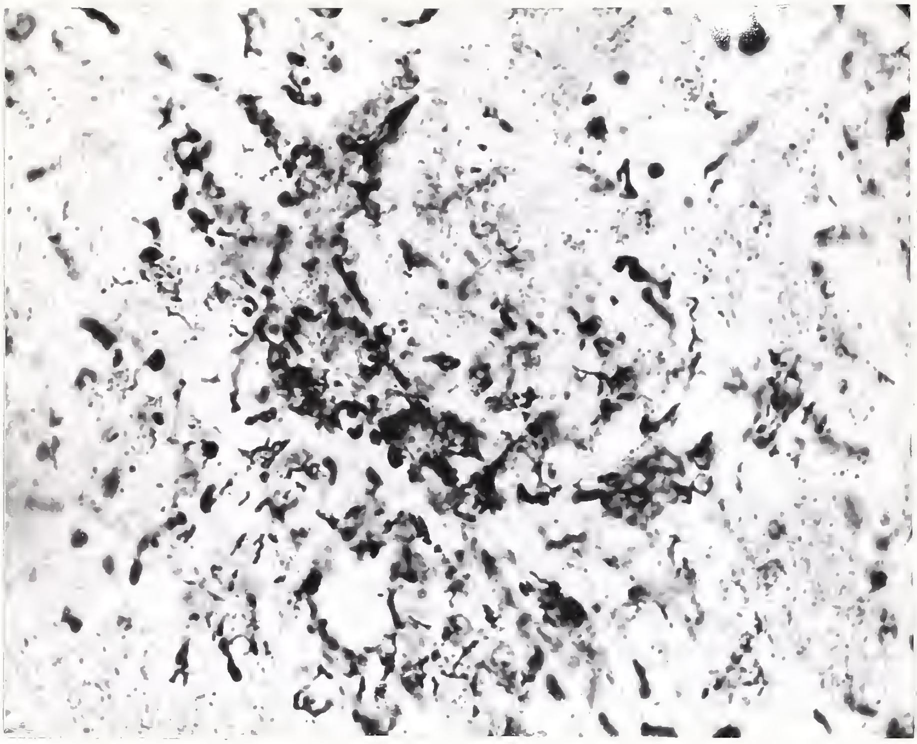

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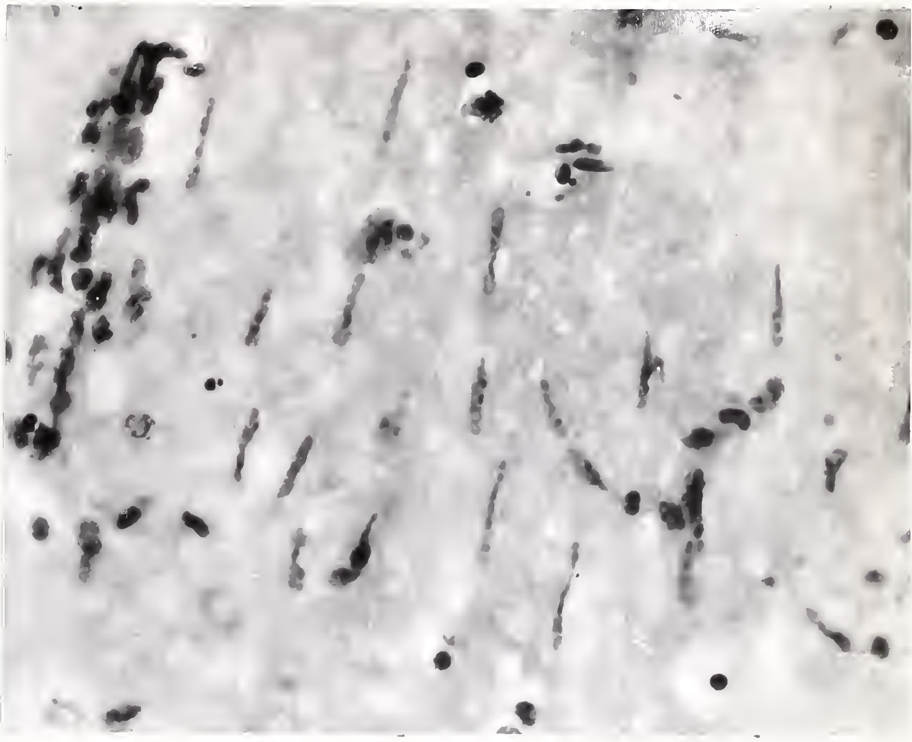
Fig. 25. Medulla. Microglia-like cells independent of vessels. Two pair of microglia cells in apparent division. Hematoxylin and eosin. X450.

Fig. 26. Cerebral cortex. Needle track showing elongate microglia with and without processes, round cell infiltrates and typical gitter cells. Hortega silver method. X450.



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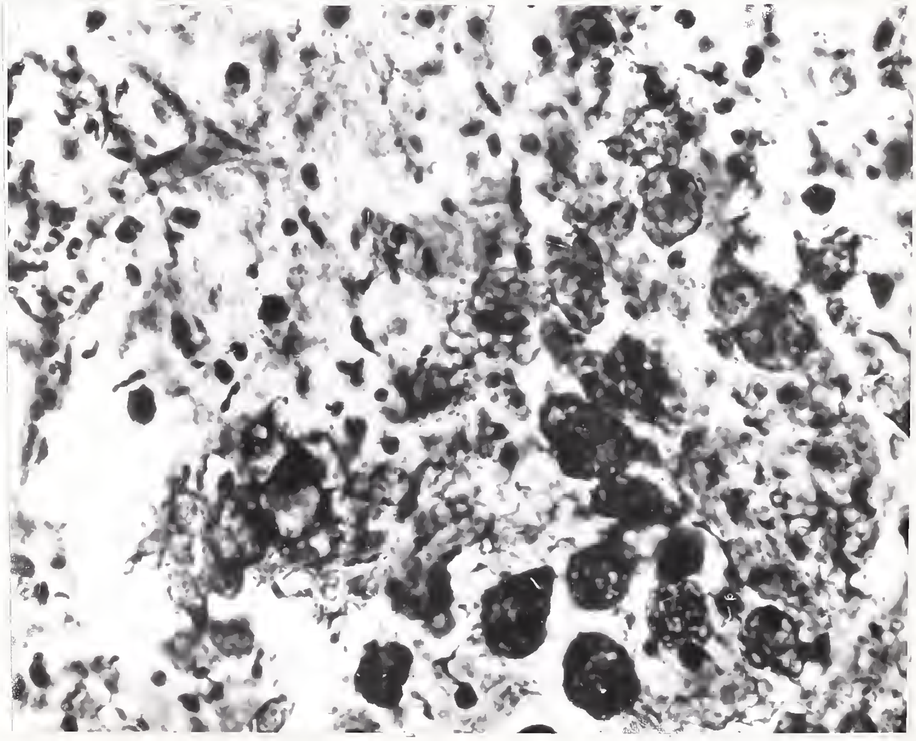

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Fig. 27. Same as Fig. 26, except that there are no stainable processes with these elongate cells. Hortega silver method. X450.

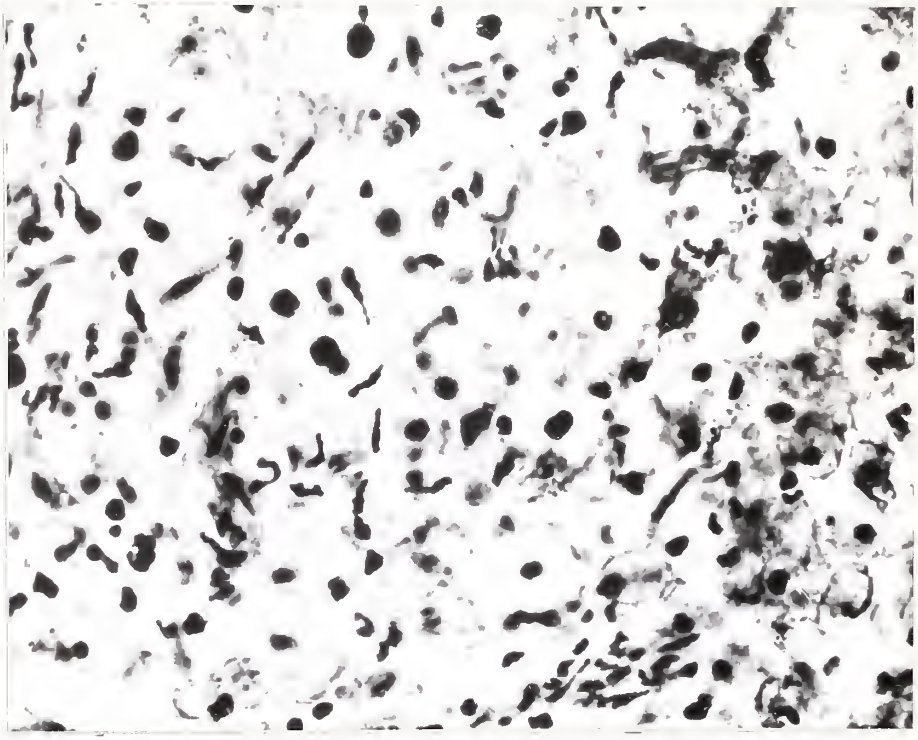

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TABLE I

No.	Animal	Hours sacrificed Post injection	Signs	Severity of Infection	Stains
1	H	65	Asymptomatic	0	AG, H & E
2	H	65	Paralyzed, respiratory distress opisthotonic	3+	AG, H & E
3	N	66	Asymptomatic	0	AG, H & E
4	N	66	Asymptomatic	0	AG, H & E
5	N	$87\frac{1}{2}$	Fur slightly ruffled. No neurological signs	1+	AG, H & E
6	H	$88\frac{1}{2}$	Fur slightly ruffled. Paresis left hind limb	2+	AG, H & E
7	H	98	Fur moderately ruffled. Paresis all limbs	2+	AG, H & E
8	N	98	Fur slightly ruffled. No neurological signs.	1+	AG, H & E
9	N	118	Fur moderately ruffled. Paresis all limbs.	2+	AG, H & E
10	N	118	Same as # 9	2+	AG, H & E
11	H	$123\frac{1}{4}$	Fur moderately ruffled. Paresis slight in all limbs. Gait slow.	2+	AG, H & E
12	N	$122\frac{1}{2}$	Paresis minimal. Slow gait. Fur slightly ruffled.	1+	AG, H & E
13	H	137 3/4	Convulsions. Respiratory distress Marked paresis	3+	AG, H & E
14	H	137 3/4	Same as # 13	3+	H & E
15	N	185	Fur markedly ruffled. Respiratory distress. Limbs paralyzed	3+	AG, H & E
16	N	208	Same as # 15	3+	H & E
18	N	Control	-	-	AG
19	H	Control	-	-	AG

EXPLANATION OF TABLE I

N = Normal Mouse

H = Hypophysectomized Mouse

AG = Hortega Silver Stain Method
(Penfield's Second Modification)

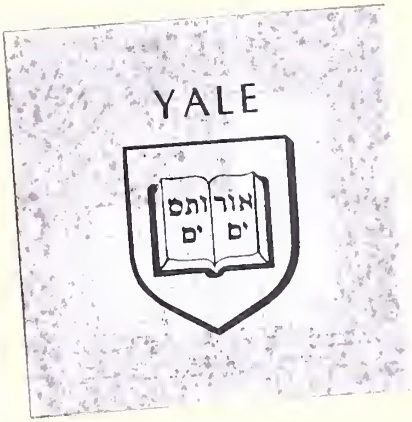
H & E = Hematoxylin and Eosin Stain

0 = No signs of infection

1+ = Slight or early infection

2+ = Moderate infection

3+ = Severe infection; animal in extremis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