COMPARATIVE STUDIES BETWEEN PATHOGENESIS OF STREET AND FIXED RABIES INFECTION*

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Electron microscopic observations have indicated that rabies virus multiplication is consistently related to the same type of characteristic matrix appearing in the cytoplasm of infected cells regardless of street or fixed virus infection (1–6).

On the other hand, the phenomenon that the capability of the street rabies virus for producing Negri bodies within neurons becomes lost after its "fixation," has been investigated with interest for almost a half century, with special emphasis on the biological role of the Negri body. Recently, we have demonstrated by the electron microscope that the acidophilic ground substance of the Negri body is identical with the matrix described above and, subsequently, that this inclusion body corresponds to the site of virus replication (7–9). These findings immediately raised the question of why fixed rabies virus cannot produce Negri bodies within the neurons of infected brains in spite of the fact that its replicating process is the same as that of the street rabies infection. From the evidence obtained by fluorescent antibody staining that the viral antigen has been seen as small foci identical in appearance to Negri bodies in nerve cells of Ammon's horn infected with fixed virus, it seems likely that there is no qualitative difference between the replication of street virus and that of fixed virus (10). Thus, our hypothesis in dealing with this question has been that the development of the matrix in brains infected with fixed virus might be usually confined to so small an extent in volume and number that matrices could not reach their full rate of growth, recognizable by the light microscope as cytoplasmic inclusion bodies.

This paper is an attempt to examine the hypothesis stated above, using light and electron microscopy. In connection with this problem, the comparative neuropathology caused by both street and fixed rabies virus was also studied in an attempt to understand the cytopathogenesis of this disease.

Materials and Methods

Two different rabies viruses were used, namely, the Komatsukawa strain of street virus and the HEP Flury strain of fixed virus. Origin and passage history of the former were de-

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scribed previously (9). The HEP Flury strain had had passages in chick embryo before it was obtained from Dr. Yoshino, Yokohama City University, and has since been passed in baby mouse brain in our laboratory.

Rabies virus infectivity tests were done on pooled brains. Infected brains were prepared as 10% suspensions in phosphate-buffered saline containing 3% calf serum and titrated in tenfold dilutions intracerebrally in suckling mice (2–3 days old) using six to eight mice per dilution. Infectivity end points for each virus were calculated by numbers of dead mice showing definitive signs of the disease, using the method of Reed and Muench (11).

Mice 5 or 6 days old, were inoculated intracerebrally with about $10^{7}LD_{50}$ of street (one passage in mouse brain) or $10^{8}LD_{50}$ of fixed (10 passages in mouse brain) virus respectively. The mice infected with street virus showed excitation 8–10 days after inoculation and then typical paralysis on the next day. On the other hand, those infected with fixed virus showed paralysis without excitation just 5 days after inoculation. At this time, specimens for both the light and electron microscope were prepared from these animals in the manner described previously (9). Briefly, preparation of specimens was done as follows: Tissues of the hippocampus were cut into 3-mm cubes, fixed in 2% osmium tetroxide buffered with phosphate containing 5.4 mg/ml of glucose (pH 7.4) at room temperature for 2 hr, dehydrated in graded alcohols, and embedded in Epon 812 (12). The other portion of the brain was fixed in Zenker's solution and embedded in paraffin. The sections embedded in paraffin were stained by Massignani's procedure (13).

The blocks of Epon-embedded tissues were trimmed to about 3-mm square so that the nerve cell band of the hippocampus might be easily identified and oriented in a suitable position. Thin (about 0.05 μ) and thick (about 1.0 μ) sections were cut with glass knives on a Porter-Blum microtome from the same blocks as close as possible. The former were stained with lead acetate (14) and observed in a Hitachi HU 11M electron microscope. The latter were stained with toluidine blue solution at pH 11.1 (15) and examined by the light microscope.

OBSERVATIONS

Comparison of Virus Titers between Street and Fixed Rabies Strains.—The infective titers of the Komatsukawa strain of street virus were nearly constant whenever adult or suckling mice were used and calculated to $10^{9.0}LD_{50}$/1 ml in this study. On the other hand, the values of titers of the HEP Flury strain of fixed virus varied widely when tested by adult mouse infective units. But the same sample of fixed virus, if titrated in suckling mice, gave almost constant values, being a titer of $10^{7.9}LD_{50}$/1 ml. From these results, it can be said that the viral concentration of the Komatsukawa strain yielded in suckling mice was higher than that of the HEP Flury strain.

Morphological Findings of the Street and Fixed Rabies Infection.—

In Fig. 1, toluidine blue–stained nerve cells of Ammon's horn infected with street virus are shown for comparison with the following Figs. 2–4, although similar evidence has already been illustrated in our previous papers (7–9). Of particular importance is the appearance of Negri bodies. They are recognized as cytoplasmic inclusion bodies which do not have the same affinity for the stain as does the nuclear sap, and contain one or more inner bodies, an important constituent of the Negri body, which stain as darkly as the nucleolus. Except for the presence of Negri bodies, no other pathological change can be found within the nerve cells. In contrast to the case of the street rabies infection, one of the most conspicuous find-

ings seen within the nerve cells of Ammon's horn infected with fixed virus, is varying grades of degenerative changes. As illustrated in Fig. 2, the characteristic regular arrangement of the cells within the nerve cell band is disordered, and most of the intercellular spaces are widened because of various extents of cellular shrinkage. The most striking alteration is that of the nucleus, showing rough internal structures stained intensely basophilic with hematoxylin. These basophilic structures increase in number and occasionally seem to have contracted into nuclear fragments of varying sizes. The nucleus in this stage of degeneration becomes shrunk and exhibits pyknosis, and its membrane is hardly recognizable. A number of nuclei lose their stainability to basic dyes, so that many necrotic nerve cells tend to stain diffusely with eosin. The cytoplasm of the degenerative nerve cells decreases markedly in volume as does that of the nucleus, and loses its characteristic granular structure. In the preparation embedded in Epon (Fig. 3), the cytoplasm also stains homogeneously by toluidine blue, and, owing to a marked decrease in the amount of Nissl's bodies, is similar in appearance to that of the nerve cell in paraffin-embedded tissue (Fig. 2). One pathological formation is that boardlike or spherical darkly stained structures are occasionally recognizable within the nucleus or at the periphery of the cytoplasm (Figs. 3 and 4). The extensive degenerative cells stain as a whole so darkly by toluidine blue that the nucleus becomes eventually indistinguishable from the cytoplasm. Only their characteristic shape and localization makes it possible to judge them as nerve cells. An increase in the number of glia cells is also found in this area. Several round granules in varying sizes are observed within the cytoplasm of these glia cells showing different stainability. These degenerative changes could already be observed 3 days after inoculation with fixed virus in some localized areas of the nerve cell band of Ammon's horn. Besides necrosis, a remarkable change seen within these nerve cells is the presence of inclusion bodies, the appearance of which is very similar to that of Negri bodies seen in Fig. 1. They are recognizable within the cytoplasm of degenerative as well as nondegenerative neurons embedded in Epon (Figs. 3 and 4), whereas the inclusion body can not be discernible in the paraffin-embedded preparation (Fig. 2). In general, these inclusion bodies are consistently smaller than Negri bodies and contain no internal structure. With this light microscopic evidence at hand, specimens obtained from the same source were examined by the electron microscope.

Fig. 5 is a part of Ammon's horn infected with street virus, taken from the same block as Fig. 1. In this figure are seen seven nerve cells, in most of which the nucleus cannot be seen. The fine structures of neurons in the nerve cell band of Ammon's horn have been described previously (9). Briefly, they are located close to each other without any intercellular gap. The cytoplasm contains rough surfaced endoplasmic reticulum of irregular disposition and numerous ribosomes in rosette or chainlike arrangement. Well defined Golgi complexes and many mitochondria are also scattered throughout the cytoplasm. No sign of degeneration is seen either in the nucleus or in the cytoplasm. There are moderately electron-opaque Negri bodies of varying sizes which replace the cytoplasmic components at the periphery of nerve cells, sometimes three in a single cellular profile. Within or contiguous to them are found clumps of street virus particles in association with the cytoplasmic constituents.

Fig. 6 *a* showing a part of Ammon's horn infected with fixed virus, was prepared from the same block as Figs. 3 and 4. In comparison with the morphological findings resulting from street rabies infection, the most obvious difference is the appearance of degeneration within nerve cells, as expected from the observation by the light microscope. At the center of this picture is found a nerve cell with nearly normal structures. Other cells generally show an increase in the electron density of the cytoplasm and some of them have the speckled appearance of varying opacity. In these cells, the rough surfaced endoplasmic reticulum and the Golgi complexes decrease in number. The neuron seen at the upper right corner partially loses its cell membrane so that its close contact with neighboring cells becomes incomplete, and irregular

intercellular gaps are formed around these cells. Lamellate myelin-like figures are found within the cytoplasm, especially near the cell membrane (Figs. 6 *a* and 13). Presumably, they correspond to the boardlike or spherical masses stained by toluidine blue in Fig. 4. The ultrastructural changes of the nucleus are more severe than those of the cytoplasm. It can be pointed out that there are several grades of nuclear abnormalities which are to a large extent parallel with those of the cytoplasm. A homogeneous increase in the electron density of the nucleoplasm is observed in a nerve cell (*A*), the cytoplasm of which shows only slight signs of degeneration. In a nerve cell (*B*) exhibiting marked degeneration of the cytoplasm, moderately advanced alteration is seen within the nucleus as follows. Two dense nucleoli showing somewhat abnormal structure and relatively electron-opaque masses of irregular shapes indicate that the characteristic homogeneity of the nuclear sap of the neuron has been lost. Its nuclear outline becomes rough. Ultimately, when the peripheral part of the cytoplasm has been shed from two neurons (*C*), distorted nuclear membranes are poorly preserved, and watery content of low density replaces the nuclear sap. A considerable number of masses of chromatin of varying shapes and some abnormal vesicles are scattered throughout these disintegrating nuclei. The nucleolus occasionally persists as a dense and coarse mass. As seen at the left part of Fig. 6 *a* (D), a necrotic neuron is also visible which is probably shrunk from its original size.

Another notable finding observed in Fig. 6 *a* is the appearance of moderately electron-opaque masses which replace the cytoplasmic ground substance in juxtanuclear or peripheral zones of nerve cells. The area enclosed by a rectangle in this micrograph is shown in Fig. 6 *b*. The homogeneous mass is composed of finely fibrillar, moderately electron-opaque materials. This morphological feature corresponds exactly to that of the ground substance of the Negri body (Figs. 9 *a* and 9 *b*). However, no virus particle is discernible within or around this mass. Hereafter the expression "matrix" is used for this mass in this study. The consideration that the inclusion body recognized in Epon-embedded specimens (Figs. 3 and 4) is consistent with the matrix should be supported by our proceeding data (8), although the observation of alternative thin and thick sections has not been attempted during this study.

As shown in Figs. 1 and 5, typical Negri bodies appear within nearly all the neurons in Ammon's horn. Generally speaking, Negri bodies tend to be small in size in all areas except the elected sites for their formation such as the hippocampus or cerebral cortex. On the contrary, matrices found in the nerve cell band are small in size, and they appear less frequently than those localized elsewhere (Figs. 3 and 6). As illustrated in Fig. 7, several matrices are sometimes located near by each other within the same cytoplasm. The most remarkable evidence is that few or no virus particles are found in association with the matrix (Fig. 6, and Figs. 10–13). Figs. 7 and 8, showing matrices within the neuron are prepared from a cortical tissue taken from the area surrounding the nerve cell band of Ammon's horn. A considerable number of virus particles are recognizable within the lumen of the swollen endoplasmic reticulum by which matrices are surrounded. At somewhat higher power in Fig. 8, double membranes are seen in these particles having a width of 80–90 mμ. Their outer limiting membranes are continuous at one end with the membrane of the endoplasmic reticulum, and both ends of particles are open to the matrix or the lumen. These morphological features resemble those of street viruses within or around the Negri body (Figs. 5 and 9 *a*). Fixed rabies virus of the Flury strain, therefore, may be considered to belong to the third type which has been described in detail previously (7, 9). Fig. 9 *a* shows again a typical inner body consisting of numerous virus particles, ribosomes, and few lysosomes. It is noticeable that all of these virus particles are classified as the third type. In Fig. 9 *b*, within the ground substance of the Negri body are seen three rods of the second type which are not associated with the endoplasmic reticulum and one particle of the third type within the swollen endoplasmic reticulum. The outer membrane of the particle (*A*) is continuous with the membrane of the endoplasmic reticulum and its intermediate membrane is also connected to the outer membrane of the particle (*B*) en-

closed in a lumen. This evidence may suggest the possibility of a developmental process from the second type to the third one as described before (7, 9).

Figs. 1 and 5 substantiate the fact that the nerve cells containing Negri bodies usually appear little damaged. In some electron micrographs of these neurons, however, Golgi complexes located near Negri bodies appear more prominent, due to both a dilation of their cisternae as well as to an increase in the number of vesicles, which are visible in rows or clusters (9). In the case of fixed virus infection as shown in Figs. 10–12, the same findings are also observed within neurons where no sign of severe degeneration is visible. Although such findings rarely appear within neurons of the nerve cell band of Ammon's horn, most of the cells showing a marked increase in the number of small vesicles are distributed elsewhere than the nerve cell band. Fig. 10 shows a slight increase in the number of vesicles of varying sizes, which may be originated from the Golgi complex. Typical micrographs of their diffuse distribution throughout the cytoplasm are shown in Figs. 11 and 12. In the former, a matrix and sheaves of neurofilaments are seen in the center, whereas the other portion shows not only many well defined Golgi complexes but also a lot of small vesicles and endoplasmic reticulum. At the higher magnification in Fig. 12, these small vesicles resemble the Golgi vesicles in respect to their shape, size, and electron opacity of their contents.

Figs. 13 and 14 illustrate further advanced degeneration of neurons which are located in the nerve cell band. The conspicuous findings were an increase in the electron opacity of the cytoplasmic ground substance and the appearance of numerous granules similar in entity to ribosomes. In contrast to the former figure, small vesicles, Golgi complexes, and the endoplasmic reticulum rather tend to decrease in number, and only electron-transparent vesicles are found. In addition, the lamellate, myelin-like structures are often found in the cytoplasm in the vicinity of the cell membrane (indicated in Fig. 6 *a*), occasionally in the nucleus (Fig. 14).

Figs. 15 *a* and *b* show low power micrographs obtained from the cerebral cortex surrounding the nerve cell band. In Fig. 15 *a* are seen four cells. From their morphological features, at least some of them can be classified as nerve cells. A considerable number of electron-opaque structureless bodies of varying sizes are included within the cytoplasm. They are also recognizable in thick sections stained darkly by toluidine blue (Fig. 15 *b*). They can be considered to be related to lysosomes which appear in the phagocytic or autodigestive processes. No prominent sign of other degeneration is visible in these cells.

DISCUSSION

Our morphological investigations have demonstrated that the matrix is identical with the ground substance of the Negri body within neurons of the nerve cell band of Ammon's horn infected with street virus. However, two differences have been also shown between the matrix and the Negri body. First, in general, matrices remain smaller in volume and fewer in number than Negri bodies. A plausible explanation to account for this might be that the Flury strain of fixed virus injured the neurons of Ammon's horn so extensively that the full growth of the characteristic focus resulting from the virus-host cell interaction was limited to a relatively small extent. The second point is that only a very few particles of fixed virus are visible around the matrix, and that the inner body, being specific to the Negri body, has never been found within the matrix. Goodpasture (16) recognized Negri-like bodies containing no internal structure in the brains of rabbits infected with street virus and termed them "lyssa bodies" from the practical as well as theoretical importance of the dis-

tinction. He emphasized that lyssa bodies were mostly small in size. In our material infected with the Komatsukawa strain of street virus, a lot of small inclusion bodies containing no internal structure were also observed intermingled with typical Negri bodies having inner bodies. As a result of these findings, it seems likely that the presence of the inner bodies is not indispensable, but that they are finally brought about in the developmental process of Negri bodies which was discussed previously (9). Furthermore, many fluorescent bodies showing the same antigenicity as that of the Negri body have been demonstrated within neurons of Ammon's horn infected with fixed virus (10, 17). On the basis of these observations, it may be concluded that the matrix which appeared within neurons infected with fixed virus has essentially the same biological significance as that of the Negri body, regardless of the presence of inner bodies. In addition, small cytoplasmic inclusion bodies having the same affinity for toluidine blue solution as does the ground substance of the Negri body, were visible in the better preserved Epon-embedded tissue infected with fixed virus (Figs. 3 and 4), while no specific inclusion body was recognizable in paraffin-embedded tissue prepared from the same brain (Fig. 2).

Another striking finding is that the appearance of fixed virus within neurons of the nerve cell band of Ammon's horn was very rare, as shown in Fig. 6. Insofar as this study concentrated on an examination of the hippocampus area, most of the neurons containing matrices which were accompanied by fixed viruses, were located outside of the nerve cell band, and even in neurons around this area many matrices without virus particles were found. With such morphological differences between the replicating mode of street and fixed virus in mind, infectivity tests were done on the brains infected with rabies viruses of both strains. A higher yield of street virus (Komatsukawa strain) could be obtained than that of HEP Flury–fixed virus. However, there may yet remain some problems to be examined before the morphological evidence of both virus infections can be definitely correlated with their infective titers. For instance, there is the possibility of the selective vulnerability of viral replication of different groups of neurons in the brains. The electron micrographs of brains infected with fixed virus had been reported by Roots and Schultze (3) and Johnson and Mercer (6). Though they did not refer to the quantitative analysis of virus particles nor to the exact localization of brains examined, the former demonstrated a lot of elongated viruses within the lumen of the endoplasmic reticulum being adjacent to the matrix. Therefore, it can be only emphasized here that fixed viruses are produced in low concentration around the matrix within infected neurons of the nerve cell band of Ammon's horn which exhibit the critical cytopathology.

As has been frequently noted, nerve cells of the central nervous system infected with street rabies virus appear relatively well preserved, except for the occurrence of Negri bodies. Conversely, a variety of signs of degeneration was

observable in neurons undergoing the fixed virus infection. One of the signs of degeneration was the large number of small vesicles which appeared within the cytoplasm of infected nerve cells. Their morphological features were similar to those of the Golgi vesicles which increased often in number around the vicinity of Negri bodies (9) or matrices (Fig. 10). Recently, evidence has been accumulated from electron micrographs indicating that hydrolytic enzymes are initially synthesized in the endoplasmic reticulum and are then transferred to the Golgi apparatus (20), and that Golgi vesicles play a role as primary lysosomes in the phagocytic or autodigestive processes within the cells (18–20). In addition, acid phosphatase activity has been shown in the endoplasmic reticulum in the sciatic nerve following crush (21) or in Golgi saccules and vesicles of the normal nerve cells (18, 22). Considering these facts, the electron histochemical study in progress in our laboratory will try to find whether the small vesicles account for the increased formation of primary lysosomes so conspicuous in the fixed virus infection.

The occurrence of lysosomes showing varying features was the other alteration of cellular organelles. The lysosomes were scattered and intermingled with cytoplasmic organelles of neurons which were located in area surrounding the nerve cell band (Fig. 15). The significance of lysosomes in cytopathogenesis is not clearly understood. Because they have been found in metabolically active cells (23, 24) or reversely injured cells (18, 25), their appearance may not necessarily indicate severe damage leading to cell death. On the other hand, the most advanced degenerative changes involving both the nucleus and the cytoplasm were localized in neurons of the nerve cell band in the hippocampus. It has been well known that this group of neurons is one of the elected sites for the formation of Negri bodies. From these two points, it should be noted that the most remarkable pathological changes were produced in neurons of the nerve cell band of Ammon's horn regardless of whether they had been infected by fixed or by street virus.

As reported by Babes (26), degenerative alterations were already recognized by the light microscope in some areas of the nerve cell band 3 days after fixed virus infection. On the contrary, Johnson (27) demonstrated viral antigens within these neurons by fluorescent antibody staining, but no degenerative sign was seen around the area. This evidence, therefore, suggests that the degree of cytopathogenesis may also depend upon the strain of rabies virus used.

Lentz (28) first observed the structures which occur extracellularly, found among the nerve cells in the central nervous system of rabbits infected with fixed virus which now bear his name. Lentz bodies are similar to Negri bodies in that they are eosinophilic and contain blue inner structures. Although he considered this body to be the degenerative nucleus of neurons, because it has not been studied so intensively as the Negri body, its exact nature is at present still unknown. The light microscopic observation revealed that some degenera-

tive neurons reduced their volume and their cytoplasms stained intensely eosinophilic. These necrotic cells were found among normal or little damaged cells. It is difficult to compare these morphological features with those of Lentz bodies, since the description on the Lentz body was originally made after his sketch. In spite of this difficulty, it seems likely that the inner body-like structure corresponds to the necrotic nucleus, and therefore the Lentz body is identical with the severely degenerated nerve cell itself.

SUMMARY

Comparative neuropathology of Ammon's horn caused by both the street and fixed rabies infection was studied by combined light and electron microscopy. Neurons containing Negri bodies appeared comparatively little damaged. In striking contrast, in the case of fixed virus infection, neurons showed the following variety of degeneration. Lightly damaged neurons showed an increase in the number of small vesicles throughout the cytoplasm. A considerable number of lysosomes were also encountered within these nerve cells. Severe necrotic alteration involving the nucleus as well as the cytoplasm was found in the nerve cell band. The characteristic homogeneous foci (matrices) were discernible within these neurons. It appears that the matrix is morphologically identical with the ground substance of the Negri body, though its size is smaller than that of the Negri body. This evidence suggests a possibility that fixed virus injures neurons so extensively that they cannot bring about the full development of the characteristic matrix of the Negri body recognizable by the light microscope. Selective vulnerability was demonstrated among different groups of neurons in respect of cytopathogenesis of both street and fixed virus infection.

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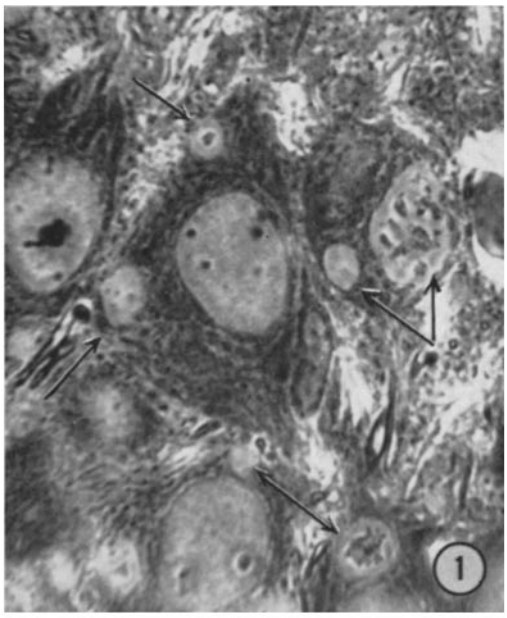
EXPLANATION OF PLATES

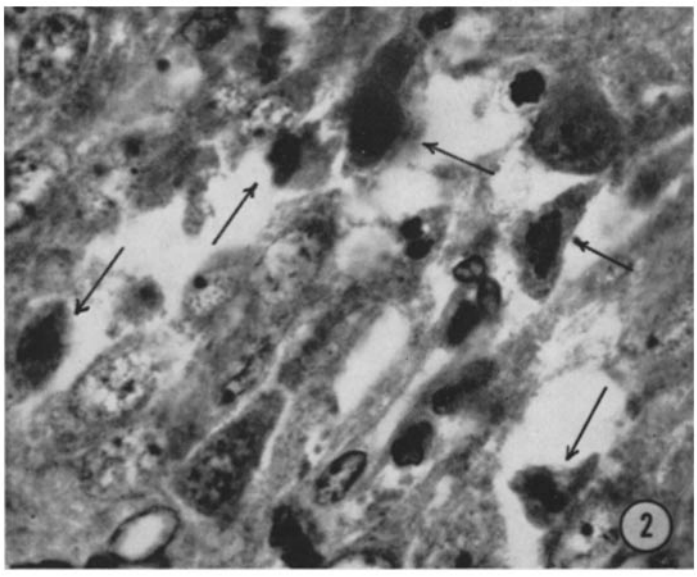
FIGS. 1, 5, and 9 were taken from Epon-embedded hippocampus infected with street virus, whereas all other figures were prepared from fixed virus-infected brains.

PLATE 53

FIG. 1. A phase-contrast micrograph of a part of Ammon's horn stained by toluidine blue. Several Negri bodies, some of which contain inner bodies, are indicated by arrows. × 1700.

FIG. 2. A part of Ammon's horn prepared from a paraffin-embedded block (Massignani's stain). Pyknotic or fragmental nuclei of necrotic neurons are indicated by arrows. Their cytoplasms are shrunken and lose their internal granularity. Vacuolization in intercellular spaces is prominent. × 1000.



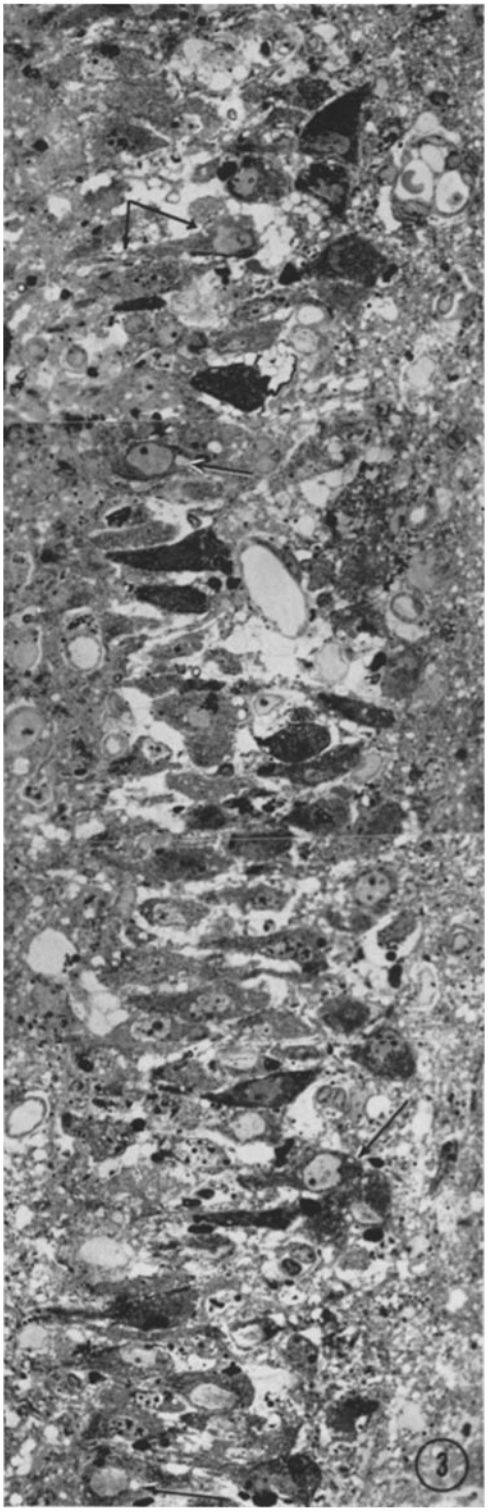


(Miyamoto and Matsumoto: Pathogenesis of street and fixed rabies infection)

PLATE 54

FIG. 3. A survey micrograph of the nerve cell band in Ammon's horn showing marked disarrangement of neurons and the formation of vacuoles in varying sizes. A considerable number of neurons are so dark that their nuclei become indistinguishable from their cytoplasms. Lightly stained inclusions in small size are found within relatively slightly damaged neurons. Representative inclusions are indicated by arrows. Toluidine blue stain. × 350.

FIG. 4. Darkly stained structureless masses are located within cytoplasms as well as a nucleus (at the center of figure). One inclusion is visible in the juxtanuclear position (arrow). × 1700.



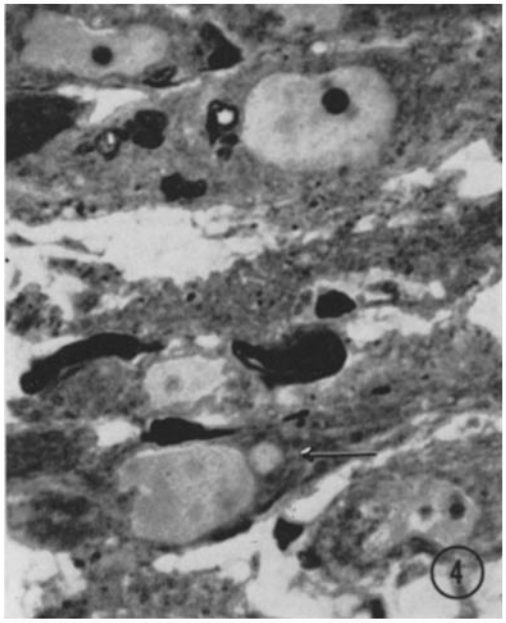


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PLATE 55

FIG. 5. Fig. 5 (and Fig. 6 *a*) are constructive maps of electron micrographs showing portions of Ammon's horn. In this figure many neurons having a normal appearance contain Negri bodies, in some of which clumps of virus particles are recognizable. × 4500.

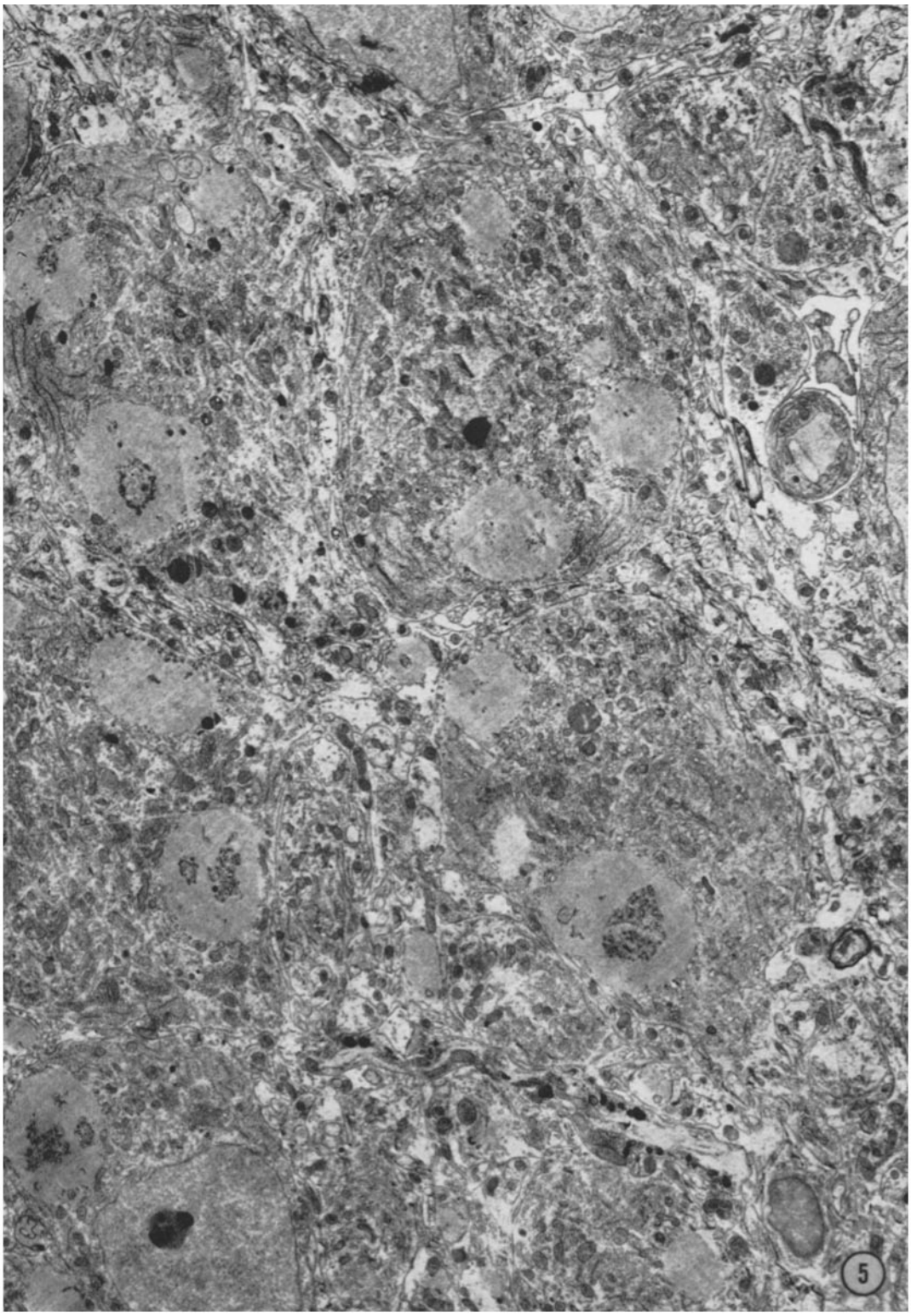

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PLATE 56

FIG. 6 *a*. In contrast with Fig. 5, degenerative changes of varying grades are evident in neurons seen in this figure. Densely osmiophilic neurons are apparent; intracellular detail is hardly distinguishable. Advanced necrotic neurons (*C*) have nuclei showing that coarse granules and watery content of low density replace the nuclear sap and nuclear membranes are disrupted in places. Cytoplasmic ground substance disappears in the vicinity of the cell membrane. A lamellate formation is visible near the cell membrane of a necrotic cell (*B*). The nuclear outline cannot be recognized in a necrotic cell (*D*) which is probably shrunken from its original size. Relatively small matrices are visible within these neurons. One of them enclosed by a rectangle in a neuron (*A*) is shown in Fig. 6 *b*. × 4500.

FIG. 6 *b*. The homogeneous matrix composed of fine fibrillous ground substance. This feature resembles that of Negri bodies shown in Fig. 5. Note the absence of virus particle associated with this matrix. × 18,200.

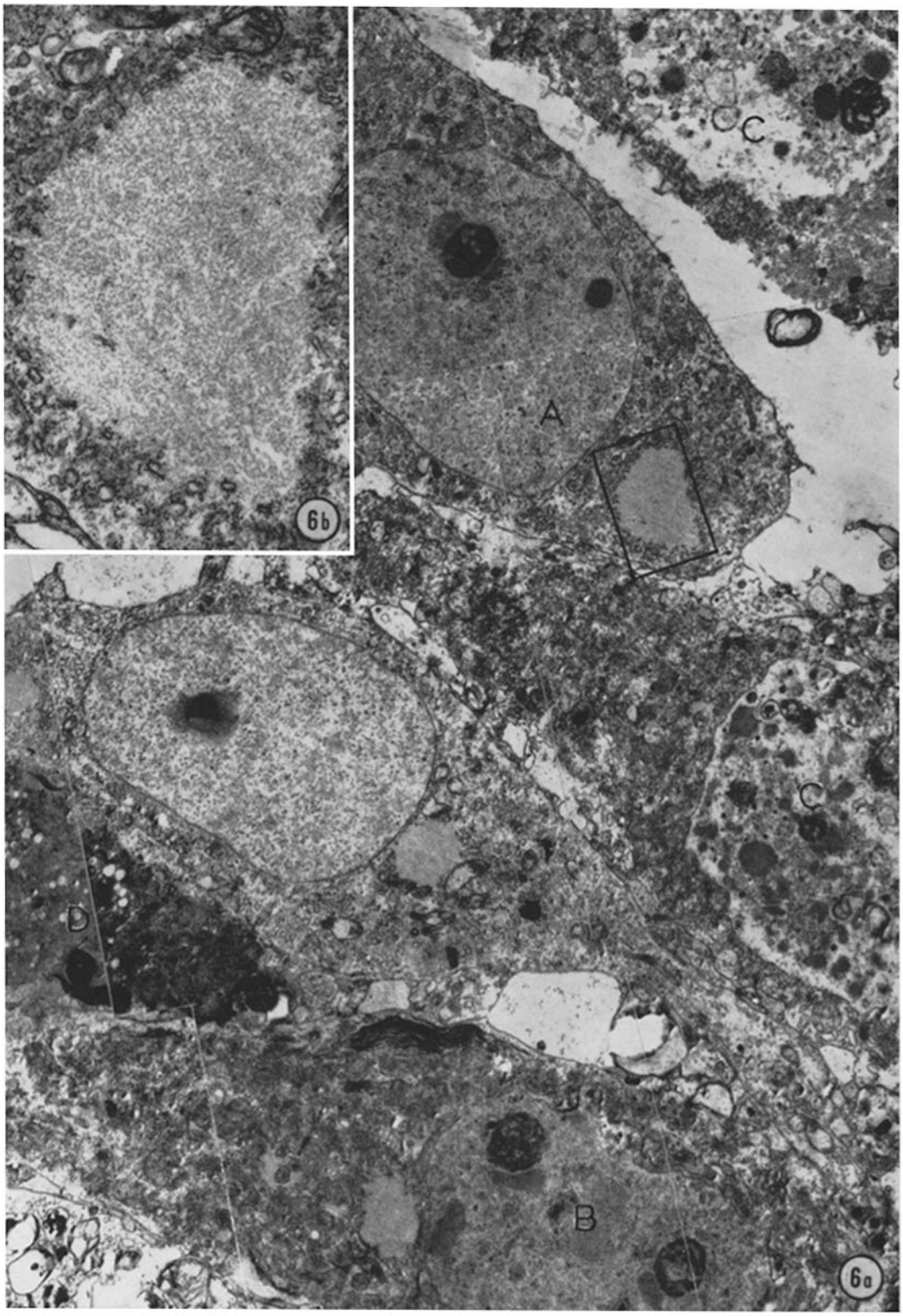

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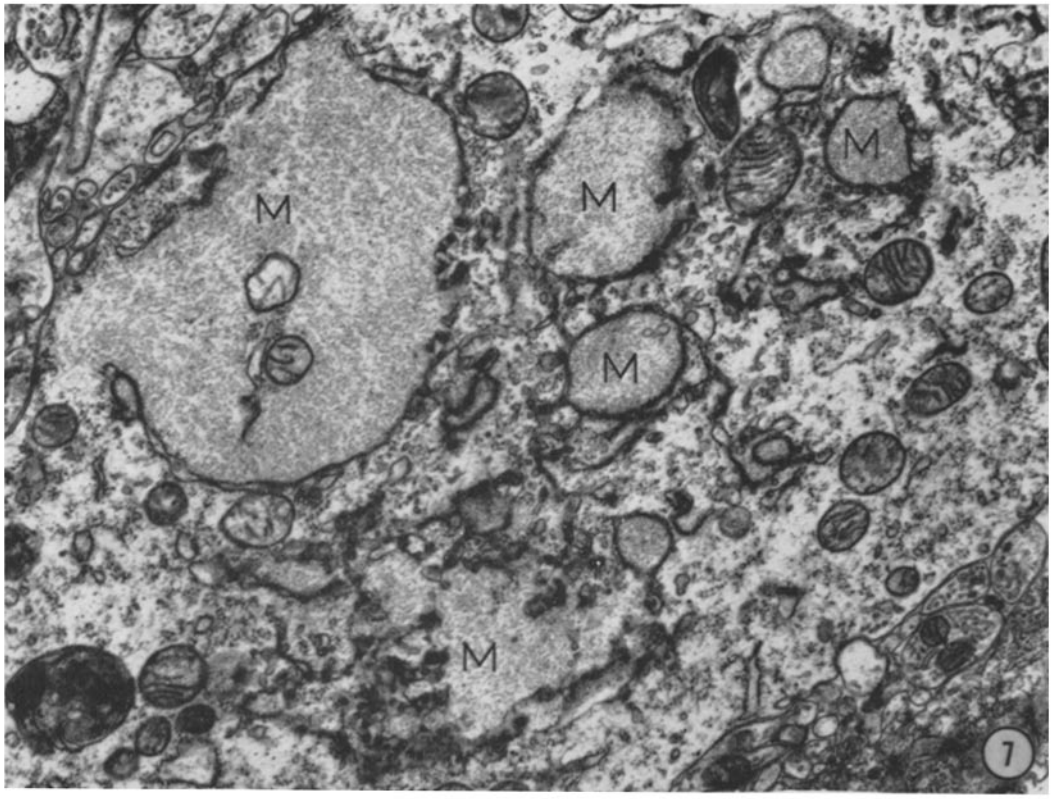
PLATE 57

FIG. 7. A portion of a neuron obtained from a cortical area near the nerve cell band. Several matrices (*M*) are partially or entirely surrounded by the endoplasmic reticulum. Virus particles are located within the lumen of the endoplasmic reticulum. × 13,000.

FIG. 8. A part of a matrix (*M*). Protrusion of virus particles from membranes of the endoplasmic reticulum. × 46,000.



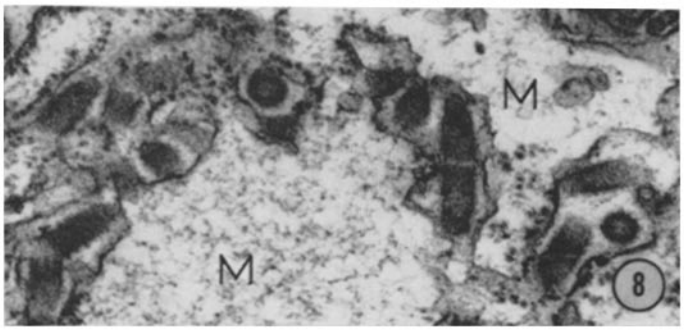


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PLATE 58

FIG. 9 *a*. A typical inner body composed of a large number of virus particles and some amount of cytoplasmic organella. × 46,000.

FIG. 9 *b*. A part of a Negri body. Three virus particles (type 2) are directly buried within the ground substance of this inclusion. On the other hand, one particle (type 3) is covered by the endoplasmic reticulum. Arrows show the continuity of structure between the outer membrane of a virus (*A*) and the endoplasmic reticulum, and between its inner limiting membrane and the outer membrane of a virus (*B*). × 46,000.

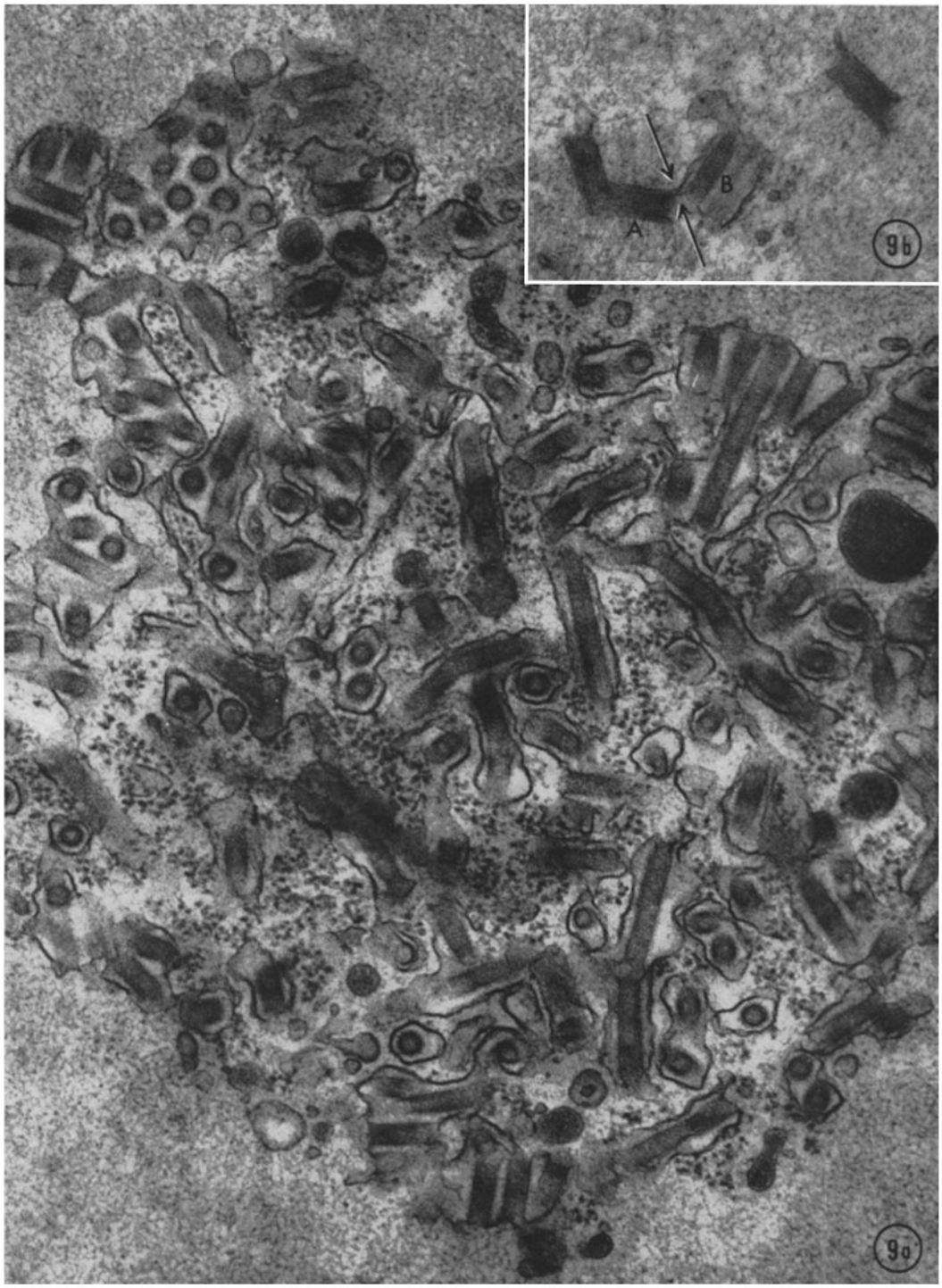

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PLATE 59

FIG. 10. A part of a neuron. A matrix (*M*) is accompanied with no virus particle. A considerable number of small vesicles are scattered between the matrix and the Golgi complex (*G*). × 35,700.

FIG. 11. A part of a nerve fiber. A matrix (*M*) is visible at the center. An increase in the number of small vesicles is prominent throughout the cytoplasm. Neurofilaments (*F*). × 9600.

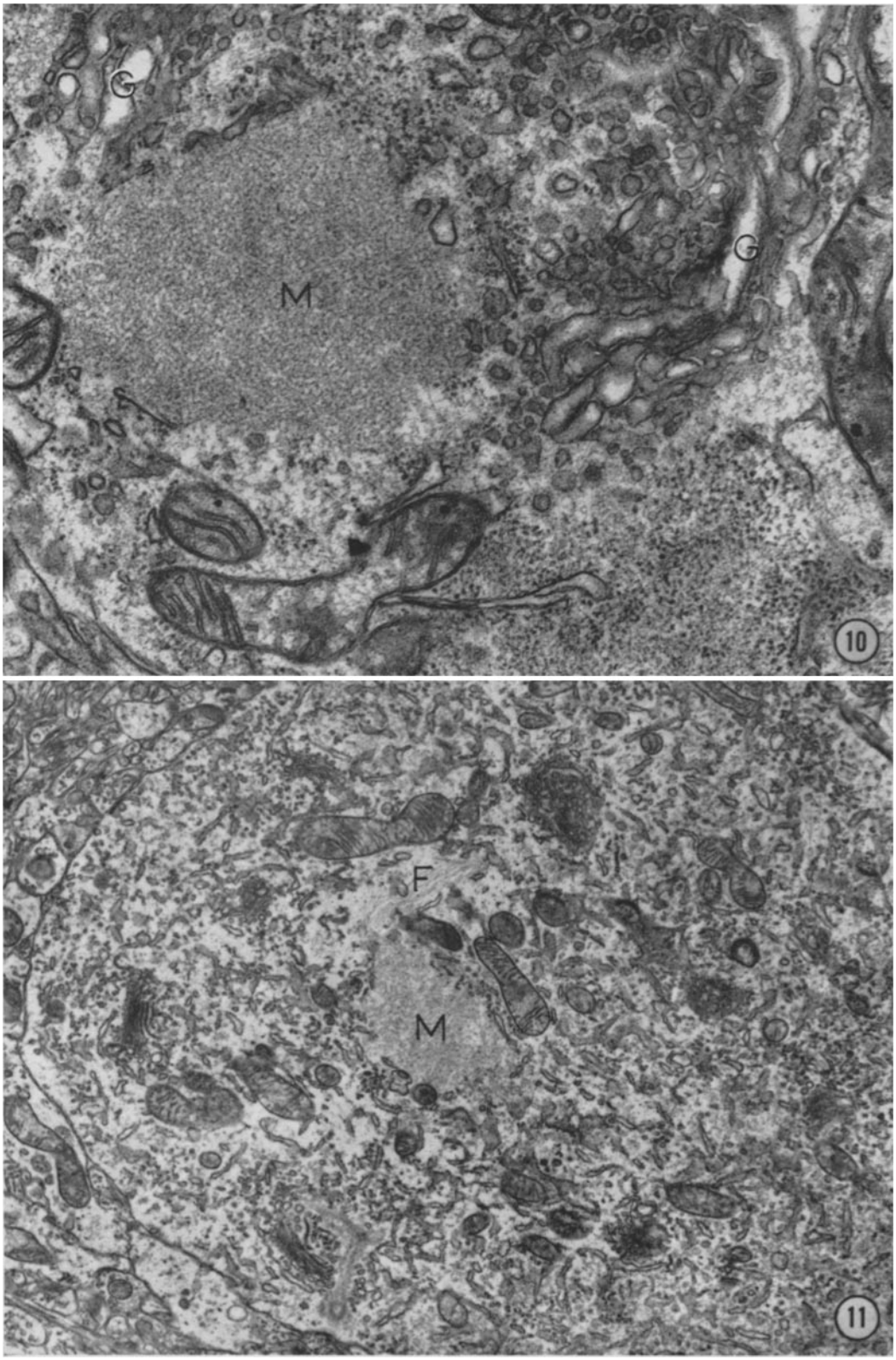

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PLATE 60

FIG. 12. A part of a neuron containing a matrix (*M*) accompanied with no virus. A large number of small vesicles are accumulated within the cytoplasm. These vesicles are very similar with Golgi (*G*) vesicles seen in this picture. × 32,200.

FIG. 13. A part of a degenerative neuron having a matrix (*M*) at the juxtanuclear position. Electron opacity increases throughout the cytoplasm and the nucleus. Ribosomes appear to be crowded together. Lamellated membranes are visible at the right lower corner (arrow). × 18,000.

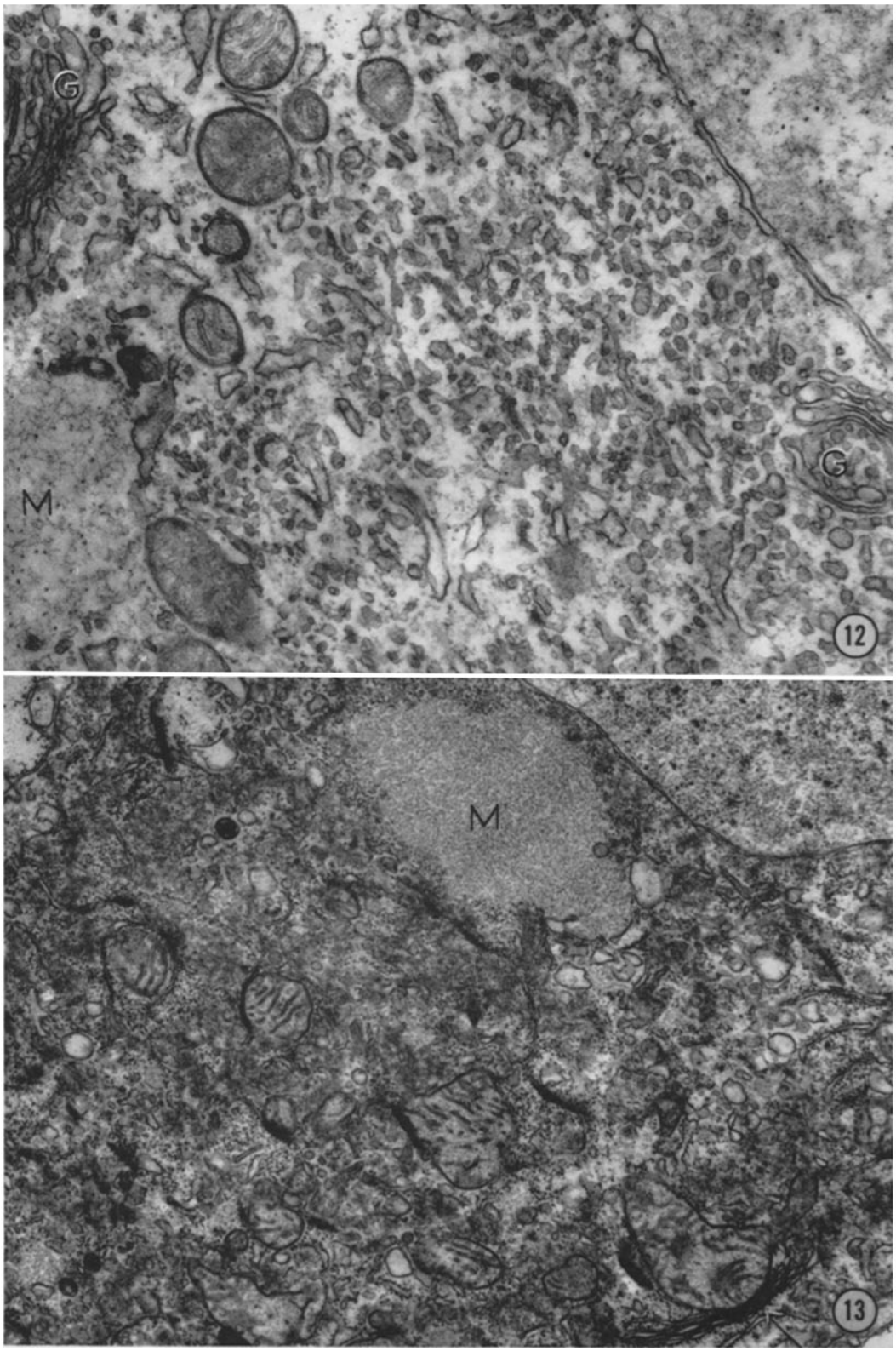

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PLATE 61

FIG. 14. A markedly degenerative neuron. A myelin-like formation is seen within the nucleus showing a rarefied zone beneath the nuclear membrane and complicated nuclear indentation. × 11,400.

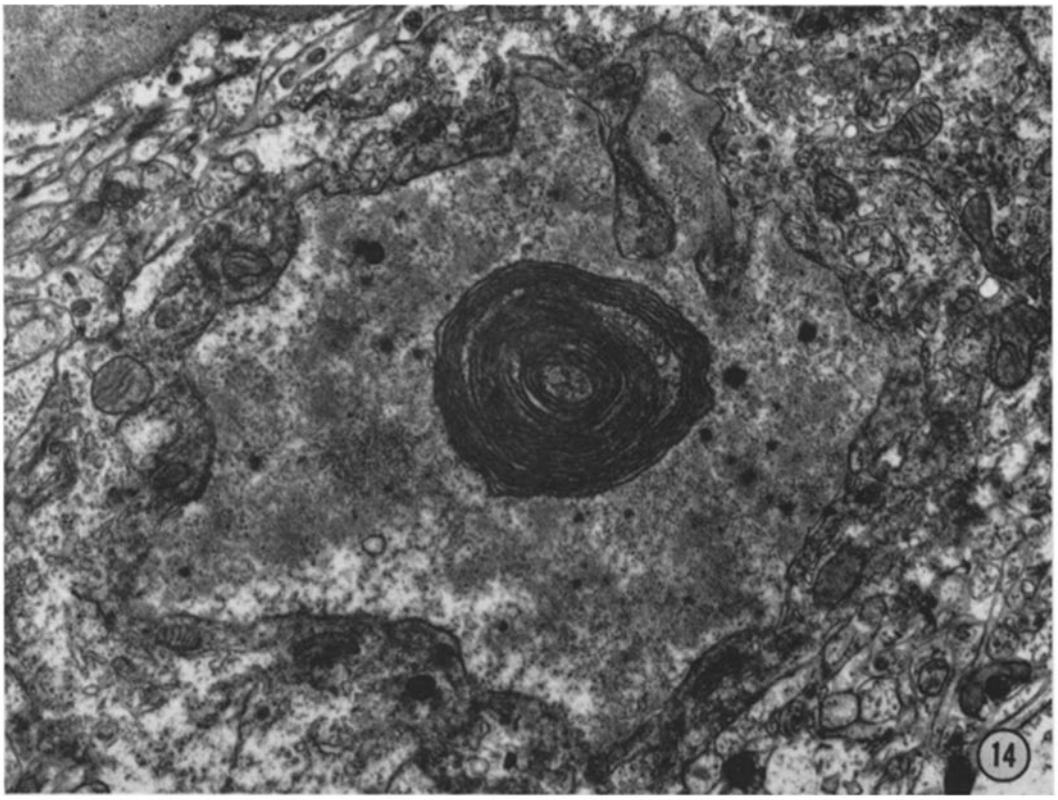

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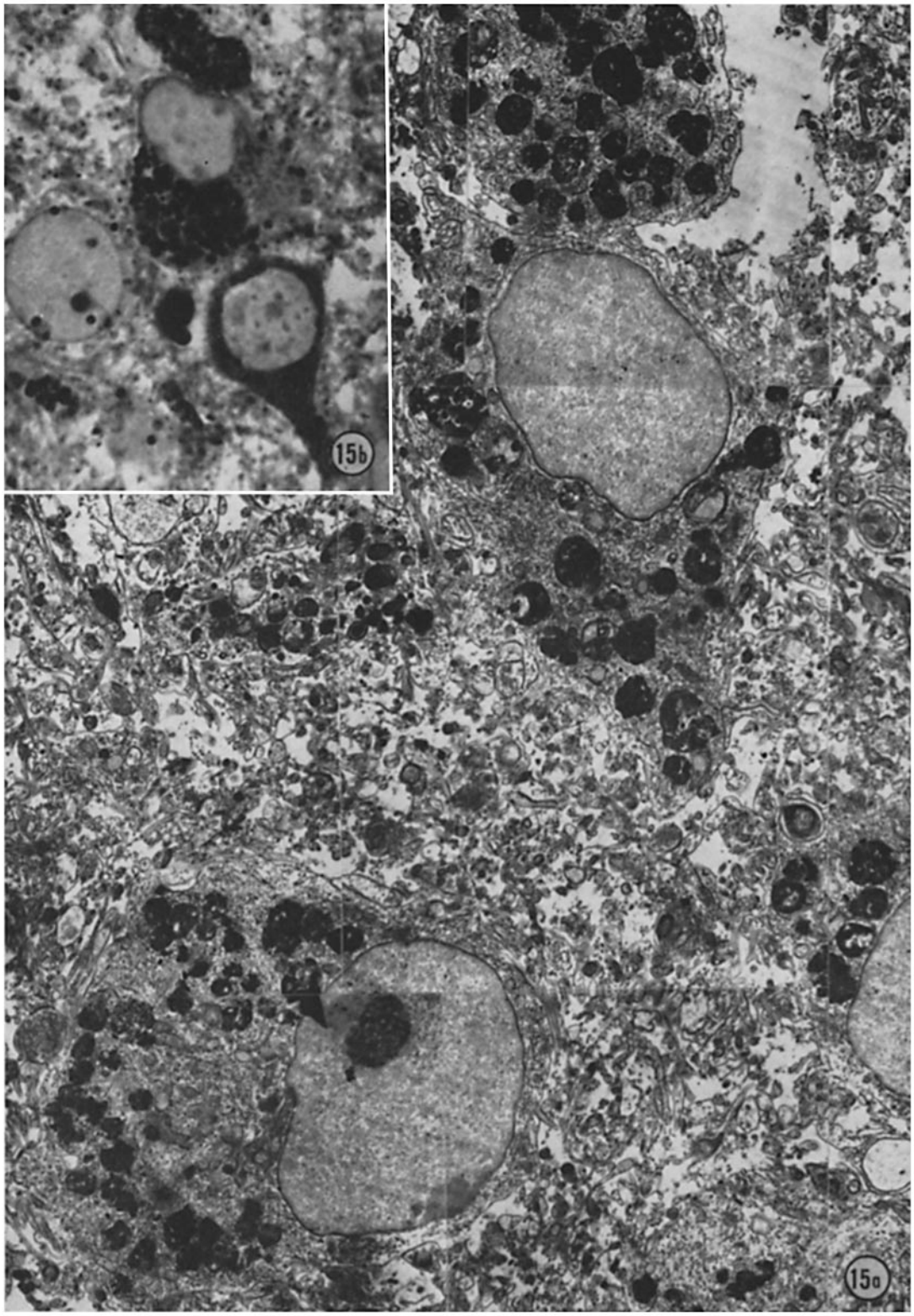
PLATE 62

FIGS. 15 *a* and 15 *b*. Electron and light micrographs of a group of neurons exhibiting numerous osmiophilic masses of varying sizes within the cytoplasm. Fig. 15 *a*, × 4500; and Fig. 15 *b* (toluidine blue stain), × 1700.



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