CELLULAR INCLUSIONS IN CEREBRAL LESIONS OF LETHARGIC ENCEPHALITIS *

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In many of the articles dealing with encephalitis lethargica the previous work on the subject has been reviewed. Consequently no attempt will be made here to discuss the literature. Excellent summaries may be found in the papers of Zinsser,[1] von Economo,[2] and in the report of the Matheson Commission.[3]

Many investigators have implicated a filterable virus as the etiological factor in encephalitis lethargica, and some have claimed success in transmitting an encephalitis to experimental animals by the inoculation of brain or nasopharyngeal washings. In every instance, except one in which an encephalitis has been transmitted in series in experimental animals, the virus has been proved identical with that of herpes simplex. In the one exception the virus was that of rabies. Only nine strains of herpetic virus have been isolated from human encephalitis.[3] It has been the experience of most workers that repeated inoculations of material from encephalitic patients have uniformly led to negative results. Furthermore, Flexner and Amoss [4] have isolated a strain of herpes virus from the spinal fluid of a luetic patient in whom they found evidence neither of herpes nor of encephalitis. From the numerous negative results which have been published, and the large number which undoubtedly have not been published, it must be concluded that the isolation of a virus from cases of lethargic encephalitis infectious for laboratory animals is, at least, an unusual occurrence. The association of herpetic virus with human encephalitis seems, on the basis of available evidence, adventitious.

At the present time, diseases having filterable viruses as etiological agents are for the most part characterized by certain cytological changes in their specific lesions. These may be in the form of inclusion bodies within the nucleus or the cytoplasm, hyperplasia of cells

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or simply necrosis of individual cells. In spite of the fact that herpes simplex is characterized by definite intranuclear inclusion bodies, the workers who have implicated this virus in the etiology of human encephalitis have never demonstrated in human cases inclusions characteristic of herpetic infections. Herzog,[5] Da Fano,[6] and others have described vague intracellular bodies which are not at all typical of virus inclusions.

It is the purpose of this paper to present a case of human encephalitis, clinically encephalitis lethargica, in which intranuclear inclusion bodies were found. These inclusions are quite definite, and while they simulate in certain respects the inclusions associated with herpetic infections, there are certain morphological differences. Furthermore inoculations of brain tissue upon the cornea and intracerebrally in rabbits failed in every case to induce herpetic infection.

Report of Case

Clinical History: The patient, W. W., white, age 16 years, was brought to the Out-Patient Department July 18, 1931. His mother, who brought him to the clinic and gave the history, said that he had had a sunstroke. Additional points in the history were obtained by a social service worker.

Sixteen months ago the patient was found on the street in an unconscious condition and was taken home. No other details could be obtained. Twelve months ago, while attending a boys' camp, he is said to have had a sunstroke. He was taken to a hospital which recorded the presence of headache, sore throat, a diffuse rash over the body, temperature of 99° F, and clear spinal fluid under normal pressure with 14 cells. He recovered from this attack rapidly.

Eight months ago he had a light attack of influenza, but did not go to bed and had no doctor. Two weeks ago while working in the sun with his father he suddenly said, "I feel just like cussing you." A few nights later his sister came home and said that the patient was in a park, acting queerly and staring at trees. About this time he started walking about at night, refused to undress, was quite nervous and smoked a great deal. One week before admission he began having involuntary jerking movements of the arms and legs. Other information obtained without definite dates of onset was that he had gradually become very slow and deliberate in his movements and his memory had failed considerably. He was referred to as "the boy who slept standing up" by one of his recent employers. For some time he had had diplopia.

On admission to the Vanderbilt Hospital his temperature was 99° F, pulse 32, respirations 20. He was well developed and nourished. Voluntary movements and speech were slow and deliberate. The face had a typical mask appearance. During the examination he got out of bed several times and walked around the room. In walking the head was bent slightly forward. Occasionally there was an involuntary, convulsive jerking of the arms and legs. The head presented nothing of interest. The pupils were equal, regular and round. They reacted normally. The arms offered some rigidity to passive motion. The superficial

and deep reflexes were equal and hyperactive. There were no pathological reflexes. The neck was moderately stiff. There was a slight, fine tremor of the fingers. One observer noted the presence of "herpes" on July 29, eleven days after admission. How long these lesions had been present was not stated.

Laboratory Findings on Admission: Blood: White blood cells 6200, 73 per cent polymorphonuclear leukocytes, 24 per cent lymphocytes. Hemoglobin 14.5 gm. Wassermann negative.

Urine: Normal.

Spinal Fluid: Slightly increased pressure, clear, 14 cells, sugar 58 mg., globulin positive. Wassermann negative.

Course in Hospital: While in the hospital little was done for the patient with the exception of sedatives and forced feedings. During the first week the temperature chart showed variations from 98.6 to 100.8° F, usually reaching its peak at 4.00 or 8.00 P.M. Toward the end of the second week it reached 101.8° F. For two weeks it ranged from 99.6 to 102°; following this it showed fairly marked fluctuation with a gradual tendency to rise higher each day until he died on August 31. At this time his temperature was 106° F. His course was progressively down hill. He became more and more lethargic, and the convulsions and twitchings increased in severity and frequency. His expression became typically masked and he exhibited a definite lead-pipe rigidity of the extremities. Toward the end of his illness the convulsive twitchings became almost constant. Repeated examination of the spinal fluid showed little change except for a slight decrease of sugar and number of cells. Guinea pig inoculations and smears of spinal fluid were repeatedly negative.

Pathological Anatomy: Grossly, the brain showed no changes of a specific nature. The meninges were smooth, of normal thickness and glistening. There was no demonstrable increase in the amount of cerebrospinal fluid, nor was this fluid cloudy or blood-tinged. The vessels within the subarachnoidal space were definitely congested. Transverse sections through the brain showed a diffuse congestion and some edema. There were no definite areas of hemorrhage or necrosis.

Microscopic Examination

Microscopically, numerous changes are seen. These changes fall into two definite groups, the one affecting the blood vessels, the other individual cells. The sections studied were taken from all of the important cerebral structures, the spinal cord and the cerebellum.

The lesions associated with the blood vessels are congestion, hemorrhage and a lymphocytic infiltration of the adventitial coat of the smaller arteries and veins. These changes are seen in all the sections studied, with the exception of those taken from the cerebellum and spinal cord. In spite of their wide distribution these

changes are most evident in the sections from the cortex, the lenticular, caudate and red nuclei, and the thalamus. Examples of these changes are seen in the white as well as the gray matter. The congestion is the most constant finding, minute areas of hemorrhage and perivascular infiltration being somewhat less common. The areas of hemorrhage are always quite small and are seen around the capillaries chiefly. The cellular infiltration is composed almost entirely of lymphocytes. An occasional plasma cell is seen, but there are no polymorphonuclear leukocytes. While this exudate is rather scanty it is nevertheless quite definite. It is not a perivascular infiltration in the sense that the cells lie within the perivascular space. Close examination shows them to be enmeshed by the fibers of the vessel wall, particularly the adventitial sheaths. The smaller arteries are the vessels chiefly affected. No thrombi are seen within any of the blood vessels.

The changes affecting the cells are of four types. In eosin-methylene blue preparations they may appear in the form of eosinophilic intranuclear bodies entirely separate from the nucleolus of the cell, with or without irregular eosinophilic cytoplasmic bodies, or as simple degeneration and necrosis, or as phagocytosis of such cells. These changes are found in all parts of the cerebrum, but are most obvious in the left motor cortex, the red nucleus and the caudate nucleus. The intranuclear bodies may appear without any accompanying cytoplasmic bodies or any detectable degenerative changes. The intracytoplasmic masses are not seen in cells which do not show the intranuclear bodies and are almost invariably accompanied by degenerative changes of various types within the cell. Other cells may undergo degenerative changes without exhibiting bodies within the nucleus or the cytoplasm.

The intranuclear masses occur chiefly in the large ganglion cells, but are also seen rarely in neuroglial cells. There may be one or several within the nucleus, usually only one. They vary in size from those which are perhaps twice as large as the nucleolus to some which completely fill the nucleus. They may be round, oval or horseshoe-shaped. Associated with this variation in size there is a corresponding variation in appearance. The smaller bodies tend to be more eosinophilic and present a more granular appearance. The larger bodies are more homogeneous and in most instances tend to have a lavender color. In affected cells the chromatic material of the nu-

cleus is concentrated along the nuclear membrane. These intranuclear bodies are entirely separate and easily distinguishable from the nucleoli.

The cytoplasmic bodies occur only in ganglion cells. They are less definite in their appearance, are usually multiple, and vary in size from very small rounded masses to those which almost fill the cytoplasm. They are correspondingly irregular in shape. Their staining reaction is much more uniform. They appear as homogeneous, hyaline, pink-staining masses. As to distinctness of outline they again vary considerably. Many are rather sharply defined, but equally as many merge almost imperceptibly with the cytoplasm of the cells. In only a few instances are they surrounded by a halo.

The degenerative changes are frequently associated with the bodies just described. In many instances, however, one sees degenerating cells which are not so involved. Surrounding such cells one frequently sees accumulations of apparently healthy cells which vary in shape from round to spindle. These cells have rather scanty cytoplasm which stains lavender. Their nuclei are large as compared to the cytoplasm. Occasionally, one sees a necrotic ganglion cell which has been invaded by one of these cells. Such lesions are interpreted as pseudo- and true neuronophagia respectively. The nature of the phagocytic cells, whether neuroglial or microglial in origin, is unknown.

What appears as a later stage of the process of phagocytosis is the accumulation of cells, resembling the phagocytic cells, which are occasionally seen unassociated with degenerating or necrotic nerve cells. These phagocytic cells appear as rosettes. In some instances one is able to see small purplish fragments of material which resemble calcium in the center of such a rosette. These changes are seen most commonly in the pons, the lenticular and the caudate nuclei.

In addition to the changes found in the nervous system there was some bronchopneumonia, bilateral fibrinous pleuritis, and a few petechiae within the mucosa of the gastro-intestinal tract.

Discussion

The inclusion bodies found within the nerve cells and neuroglial cells are comparable morphologically to those seen in the lesions of herpes simplex, zona, varicella and virus III disease of rabbits. Of

these four types they resemble the inclusions seen in herpes simplex most closely. There are, however, several differences. The inclusions seen in this case never appear as extremely large, finely granular, lavender bodies which completely fill the nucleus. In herpetic lesions the intracytoplasmic masses are not seen. The inclusions in this case are more numerous in the cortex and are accompanied by a minimal amount of necrosis, while in herpetic encephalitis of rabbits the brain stem and mesencephalon are chiefly involved and there is widespread necrosis of nerve cells. One is able to conclude quite definitely that the cellular inclusions are not associated with the virus of herpes simplex because of the negative results of intracerebral inoculations of emulsified brain into rabbits.*

In view of the present state of knowledge as to the relation which exists between inclusion bodies and filterable viruses, it is suggested that in this particular case a filterable virus may have been associated with the lesions. That this is a case of encephalitis there can be no doubt. From the history, physical examination and laboratory findings, it fits quite well into the amyostaticakinetic form of encephalitis lethargica, the terminal attack being an acute exacerbation of a preëxistent infection of sixteen months duration.

A second case has recently come to us which presented a typical clinical picture of an acute lethargic encephalitis of only forty-eight hours duration. Postmortem examination of the brain revealed gross congestion, edema, microscopic hemorrhages and capillary thrombosis, unassociated with perivascular or epivascular lymphocytic infiltration. Careful examination of the nerve cells revealed extensive necrosis and some pseudoneuronophagia. No inclusions were observed.

Another case examined at autopsy was a typical clinical encephalitis lethargica coming on after extensive burns over the body, several weeks elapsing between the burns and the development of the symptoms referable to the central nervous system. Grossly, little could be demonstrated other than congestion of the basal ganglia. Microscopically, there were numerous lymphocytes within the arterial walls, extensive necrosis, a diffuse mononuclear exudate, and

* The material used for animal inoculation consisted of small fragments of basal ganglia which had been preserved in 50 per cent glycerine-saline solution and kept at freezing temperature for 1 month and 16 days. This material was washed, ground and emulsified in saline and inoculated intracerebrally into 2 monkeys (*M. rhesus*), 9 mice, 1 guinea pig, and 17 adult rabbits. One rabbit was inoculated in the testis.

little hemorrhage. The lesions were almost entirely confined to the basal ganglia. Here again no inclusions were demonstrated, and animal inoculations were negative.

These three cases may well be included in the group of encephalitis lethargica. Each is distinctly different from the other clinically, as well as pathologically. In fact, they present only a few points of similarity. In only one of them have inclusion bodies been demonstrated. In this particular case there is strong morphological evidence which points to a filterable virus as the etiological agent. Careful study of the other two cases revealed no inclusions, and it is difficult to believe that other investigators could have overlooked them in cases previously reported. It must be concluded that the case here described differs from others heretofore recorded in this essential feature.

If, then, in this small series there is evidence pointing to at least one cytological difference between cases which are called at present epidemic or lethargic encephalitis, it may be concluded that encephalitis lethargica is probably not a clinical or pathological entity, but that it represents an ill-defined group characterized by varied clinical and pathological findings and probably caused by various agents.

Experimentally, considerable work was done with material from all three of our cases, using fresh and glycerinated tissue from the basal ganglia, cortex and medulla as inocula. Material from the three cases was washed, ground, emulsified in saline and inoculated into a total of four baby rabbits, thirty-one adult rabbits, baby mice, adult mice, two puppies, three monkeys and several guinea pigs and cats. In most instances the emulsified brain was inoculated intracerebrally, although several attempts were made to infect the rabbit's cornea. In rabbits, monkeys and guinea pigs, 0.5 to 1 cc. of a thick emulsion was inoculated. Following Flexner's work on poliomyelitis attempts were made to infect animals by following the intracerebral inoculation with intraperitoneal injections of emulsified brain. The animals were watched for varying periods. Examination of their brains revealed not the slightest suggestion of any inflammatory process comparable to that seen in encephalitis.

Many of the animals survived without manifesting any clinical changes. These were observed over periods varying from three to six months. Careful temperature charts were kept on all animals.

In none of these animals was there any significant fever. Microscopic examination of the brains of animals which survived several weeks and were sacrificed showed no significant changes.

In another case, clinically diagnosed encephalitis lethargica (brother to Case 2), spinal fluid was inoculated intracerebrally into rabbits in 0.5 and 1 cc. quantities with negative results. This child survived the acute attack. At the present time, six months after onset, the child shows a reversal of the sleep cycle and some salivation, which is interpreted by the clinicians as evidence of encephalitic sequellae.

Summary and Conclusions

1. A case of lethargic encephalitis is reported in which intranuclear and intracytoplasmic "inclusions" occur.

2. It is suggested, on the basis of the presence of cellular inclusions, that this case of encephalitis may have been due to a cytotropic virus.

3. Two other cases of encephalitis are mentioned in which no inclusions were found.

4. It is further judged from an etiological standpoint that lethargic encephalitis may not be a distinct entity.

5. Animal inoculations with material from each case induced no demonstrable infection.

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DESCRIPTION OF PLATES

PLATE 3

FIG. 1. Longitudinal section through small venule in the lenticular nucleus. The lymphocytes are seen in the adventitial coat. Hematoxylin and eosin stain. × 100.

FIG. 2. Large ganglion cell from left motor cortex showing several dense cytoplasmic inclusions surrounded by halos, and a single, poorly defined intranuclear inclusion. Phagocytic cells are beginning to accumulate around it. Eosin-methylene blue stain. × 1100.

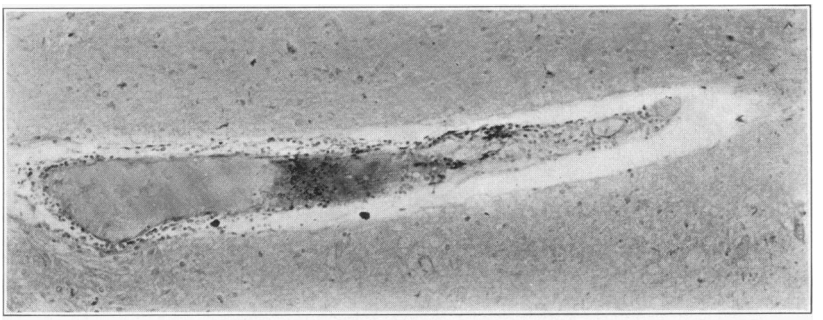
FIG. 3. Pseudoneuronophagia of a ganglion cell from the caudate nucleus. The cell contains a definite intranuclear inclusion and a single pale, rounded cytoplasmic inclusion near the upper pole of the nucleus. Eosin-methylene blue. × 1100.

FIG. 4. Ganglion cell from left motor cortex showing the characteristic large granular inclusions and three poorly defined, homogeneous, cytoplasmic inclusions. This cell shows the characteristic condensation of the chromatic material along the nuclear membrane. Eosin-methylene blue. × 2500.

FIG. 5. Necrotic ganglion cell from pons surrounded by phagocytic cells. Eosin-methylene blue. × 1100.

FIG. 6. Ganglion cell from left motor cortex showing a granular intranuclear inclusion and a large homogeneous cytoplasmic inclusion. Eosin-methylene blue. × 2500.

FIG. 7. Two swollen neuroglial cells from caudate nucleus showing small intranuclear inclusion. Eosin-methylene blue. × 1100.



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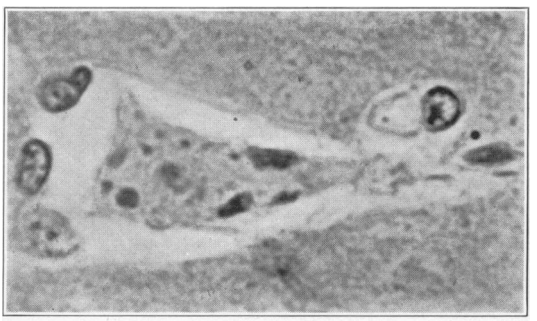

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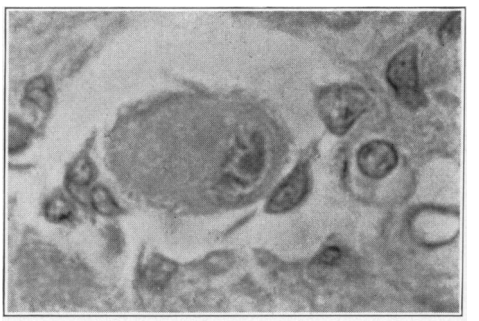

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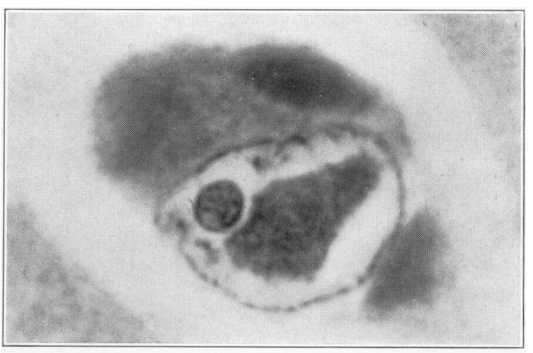

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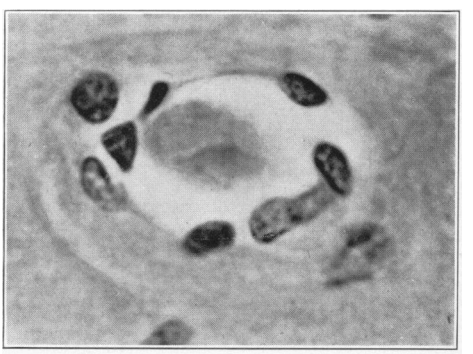

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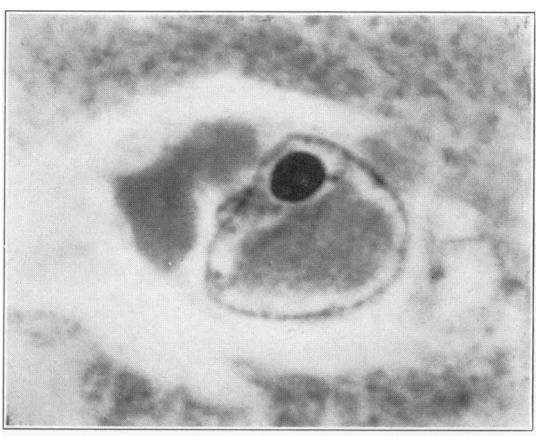

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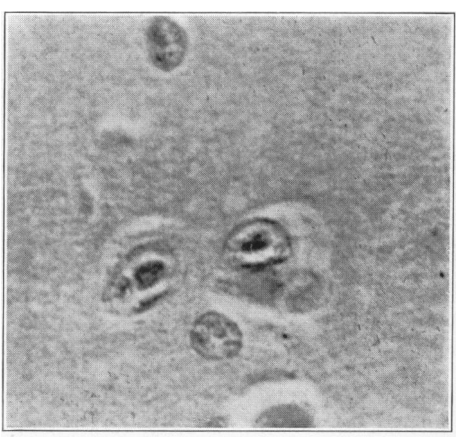

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Dawson

Cellular Inclusions in Lethargic Encephalitis

PLATE 4

FIG. 8. Ganglion cell from caudate nucleus showing an eosinophilic, granular, intranuclear inclusion and a poorly defined, round, homogeneous cytoplasmic inclusion. Surrounding the cell are many phagocytes. Eosin-methylene blue. × 2500. (See Fig. 3 for photomicrograph of this cell.)

FIG. 9. Ganglion cell from left motor cortex showing large, granular, eosinophilic intranuclear inclusion and three indefinite homogeneous cytoplasmic inclusions. The chromatic material is collected along the nuclear membrane. Eosin-methylene blue. × 2500. (See Fig. 4 for photomicrograph of this cell.)

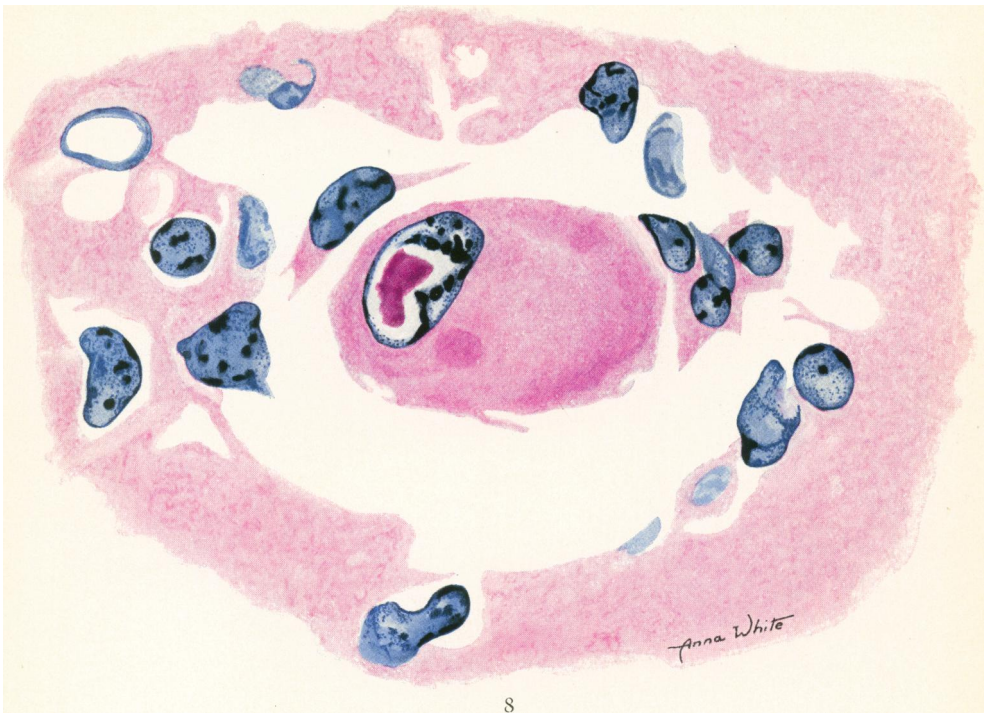
FIG. 10. Ganglion cell from left motor cortex showing characteristic intranuclear inclusion. Eosin-methylene blue. × 2500.

FIG. 11. Ganglion cell from left motor cortex showing several reddish orange, homogeneous, cytoplasmic inclusion bodies which are surrounded by halos. There is also a large granular intranuclear inclusion. Eosin-methylene blue. × 2500. (See Fig. 2 for photomicrograph of a similar cell.)

FIG. 12. Ganglion cell from left motor cortex showing a single intranuclear inclusion. This cell also illustrates the condensation of the chromatic material along the nuclear membrane. Eosin-methylene blue. × 2500.

FIG. 13. Ganglion cell from caudate nucleus showing a typical intranuclear inclusion. Eosin-methylene blue. × 2500.

FIG. 14. Ganglion cell from caudate nucleus showing an intranuclear inclusion with some condensation of the chromatic material along the nuclear membrane. Eosin-methylene blue. × 2500.



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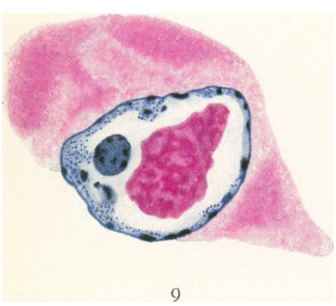

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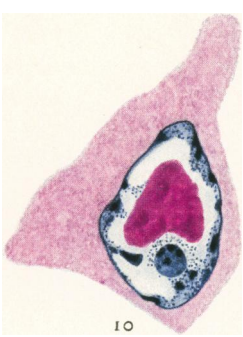

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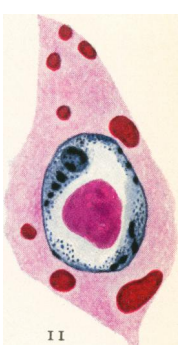

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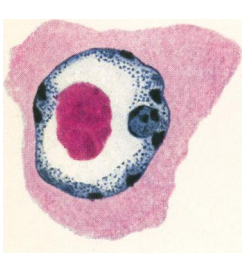

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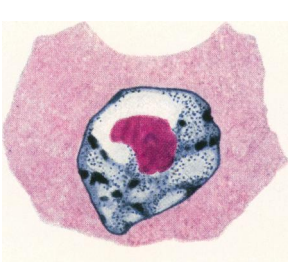

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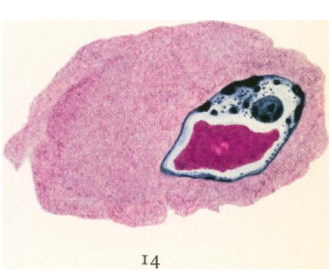

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