HISTOLOGICAL STUDIES ON HOG CHOLERA

I. Lesions in the Central Nervous System*

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Plates 15 to 17

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Although symptoms that can be referred to the central nervous system have been observed clinically in hog cholera (1, 2, 3), papers dealing with the anatomical lesions are few and far from complete. Von Hutyra and Marek (2) state that the spasms, convulsions, staggering, and lethargy are due to hemorrhages in the meninges and brain substance. They further note that convalescent animals may suddenly die with symptoms of brain hemorrhage. Huguenin (4) found hemorrhages, edema, hyperemia, and inflammatory processes in various degrees in about 20 per cent of the cases studied. Later Brunschwiler (5) studied the central nervous system in various swine diseases. Among these were seven cases of hog cholera in which hyperemia, edema, hemorrhages in the meninges, and meningitis were found. In one case there were inflammatory lesions in the brain substance. He collected data on 61 cases of hog cholera, some of these cases being from the literature, and showed that brain lesions were present in 24 cases, or 39.3 per cent.

In the descriptions of lesions of the central nervous system that have been published there are no notes concerning the glia and nerve cells, and the distribution of the inflammatory reaction has not been studied. It is quite possible also that some of the cases were not virus hog cholera.

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TABLE I

Showing Source and Kind of Material Used

Swine No.	Route of infection	Virus strain	Nervous symptoms	Killed (k.) or died (d.) after infection	General pathological and anatomical diagnosis	Histological lesions			Remarks
						Encephalitis	Meningitis	Hemorrhages	
				days					
1	Intramuscular	1	+	6 (k.)	No typical hog cholera	+	+	+	
2	"	1	−	7 "	"	−	−	−	
3	"	1	+	8 "	Typical hog cholera	++	+	−	
4	"	1	?	8 "	"	++	+	+	
5	"	1	?	8 "	"	+	+	−	
6	"	2	−	8 "	"	++	++	−	
7	"	2	−	8 "	"	+	+	−	Very slight lesion
8	"	2	−	8 "	"	++	+	−	
9	"	1	+++	8 "	"	−	−	+	
10	"	1	+++	9 "	"	±	−	−	Congestion of blood vessels
11	"	1	+	9 "	"	−	−	−	
12	"	1	?	9 "	"	++	+	+	
13	"	3	+	9 "	"	+	+	−	
14	"	3	?	9 "	"	±	−	−	
15	"	1	+	9 "	"	++	+	−	
16	"	1	?	10 (d.)	"	++	++	+	
17	"	1	++	11 (k.)	"	+++	+	+	Marked infiltration
18	Contact	3	+	12 "	"	++	−	−	
19	"	4	+	12 "	"	+++	+++	+	
20	"	1	?	13 "	"	++	+	−	
21	"	2	+	13 "	"	+++	+	+	Glia proliferation
22	"	3	?	13 "	"	++	−	−	Many glia nodules
23	"	1	−	14 "	No typical hog cholera	±	−	−	

24	Intramuscular	1	?	15 "	Typical hog cholera	+++	++	+	Numerous glia nodules
25	"	1	+++	16 (d.)	"	+	+	++	
26	"	1	++	16 "	"	+	±	+	Very slight lesions
27	"	1	?	17 "	"	+	−	++++	Slight encephalitis, hemorrhages predominating
28	"	1	+++	17 (k.)	"	−	−	++	Marked hemorrhages in brain and spinal cord
29	"	1	+++	19 "	"	++	+	+	
30	"	1	++	20 "	"	+++	+	+	Pons and medulla oblongata especially involved
31	"	1	+++	20 (d.)	"	++++	+++	+	Marked lesions in the spinal cord. Some necrotic foci
32	"	1	++	20 (k.)	"	++	+	+	
33	"	1	+++	20 "	"	±	−	+	
34	"	1	++	24 "	"	+++	+++	+++	Clotted blood between dura and pia mater
35	"	1	+	29 "	"	+	+	−	Marked hyperemia
36	"	1	++	33 "	"	++	+	−	" "
37	"	2	+	34 (d.)	"	++	++	+	
38	"	3	?	35 "	"	++	+	−	Many glia nodules
39	"	1	+++	49 "	"	+++	+	+	
					Controls				
40	−	−	−	−	Normal	−	−	−	
41	−	−	−	−	"	−	−	−	
42	−	−	−	−	"	−	−	−	Previously infected with swine influenza, without developing the disease
43	−	−	−	−	"	−	−	−	
44	−	−	−	−	"	−	−	−	
45	−	−	−	−	Spontaneous enteritis	−	−	−	
46	−	−	−	−	Normal	−	−	−	

Material

The material used for this investigation consisted of 39 cases of hog cholera that were used in other experiments on this disease. The animals were infected by intramuscular injection or by contact. Some were killed with chloroform in 6 to 49 days after infection while others died. Four different strains of virus were used; two were laboratory strains that had been passed artificially for a considerable period of time, and two were strains from fresh outbreaks in the Middle West. All infected animals had a characteristic temperature, and the majority showed what are regarded as typical lesions at autopsy, *i.e.*, hemorrhages in the kidney, lymph glands, oftentimes in the bladder and large intestine. Many of them developed central nervous system symptoms of varying degree. Seven normal pigs from the same source as the infected ones were used as controls. In Table I are summarized the more important data relating to these animals.

Methods

As noted above, chloroform was used for killing the animals and autopsy was done immediately after death. Most of the tissues from the central nervous system were fixed in 10 per cent formaldehyde. In some cases formaldehyde was injected through the carotid arteries in order to fix the entire brain. For special neuropathological methods, Zenker's and Mueller's fluids, alcohol, and bromformol were used for fixation. Hematoxylin and eosin and van Gieson and Mallory's methylene blue phloxin were used as routine stains. The following were used for special purposes: Nissl's stain, Spielmeyer's and Kulschitzky's stains for myelin-sheaths, Bielschowsky's stain for axis-cylinders, stains for glia fibers (Alzheimer-Mann, Heidelberger, Oppenheim, iron-hematoxylin), stains for neurofibers (Bielschowsky, Cajal); and furthermore the glia methods of Rio del Hortega and Cajal, the stains with methylgreen-pyronin, Weigert's fibrin and elastic tissue stains, iron and oxydase reactions, fat stain with Scharlach R and osmic acid, Klarfeld's tannic acid and silver method, Giemsa's stain, and Mann's, Lentz's, Stutzer's, and Hammerschmidt's stains for inclusion bodies.

Gross Pathology

Macroscopic changes of the central nervous system are not characteristic, except for a more or less marked congestion of the blood vessels. In a few cases pronounced hemorrhages and edema in the meninges, sometimes covering large areas of the brain and spinal cord, and in the brain substance are present. In such cases the liquor cerebri shows a slight yellowish red or red color.

Histology

The histological lesions may be divided into inflammatory and degenerative changes. They will be discussed under the headings of (*a*) mesodermal tissue, (*b*) glia, and (*c*) nerve cells.

(*a*) *Lesions of the Mesodermal Tissue.*—The most obvious and striking microscopical change is the mononuclear infiltration of the perivascular spaces of the parenchyma of the brain and spinal cord (Figs. 1 and 2). In the majority of cases the lesions in the brain substance are more marked than those in the meninges. We feel therefore that we are not dealing with a so-called "meningo-encephalitis" in which the infection spreads inward from the meninges, but that we have a true encephalomyelitis with a secondary involvement of the meninges (Figs. 3 and 4). The degree of perivascular infiltration which involves the smaller veins and arteries varies greatly. In early stages it may be slight or even absent, while in older cases regular perivascular "cuffings" with several layers of cells may be found. In some cases these cuffings are so large that they may be seen in stained sections with the unaided eye (Fig. 1). In an individual case the degree of infiltration varies and apparently is dependent upon the location of the area in the central nervous system. In general the lesions are more comparable with epidemic encephalitis in man than with Borna disease of horses in which the infiltration is more extensive.

The infiltrating cells consist largely of small lymphocytes and mononuclear elements, which are variously called polyblasts or macrophages, with a certain number of plasma cells and occasionally a few eosinophilic leucocytes. Polymorphonuclear cells are absent and the oxydase reaction is negative. Mitotic figures, fragmentation of nuclei, and other products of degeneration of the infiltrating cells are frequently found. The macrophages contain lipoids in varying amounts. Granulo-adipose cells with the form of a mulberry may be quite numerous. Iron pigment in the macrophages has been found only occasionally.

When the lesions are comparatively slight Klarfeld's tannic acid and silver method shows that the infiltrating cells remain limited to the membrana glia limitans (Fig. 5). In the more extensive lesions there are found in the immediate neighborhood of the involved vessels,

accumulations of cells the nature of which, whether glial or mesodermal, will be discussed later. Other changes in the vessels are hyperemia, swelling and degeneration of the endothelial cells, perivascular edema with suppuration of the connective and elastic tissue fibers, and more or less distension of the submarginal glia spaces. The formation of new capillaries has not been found in the inflammatory areas.

In addition to the above lesions there have been seen in a number of cases blood vessels in the brain and meninges that do not show perivascular infiltration but that are surrounded by a thin wall of red blood corpuscles. Similar microscopical hemorrhages have also been seen in the tissues unassociated with vessels (Fig. 1). It is worthy of note, however, that hemorrhages in the central nervous system are relatively infrequent while in other parts of the body they seem to be the predominating lesion. When found they are more pronounced in the cerebellum and spinal cord and they may be extensive enough to be seen with the naked eye.

Perivascular infiltrations may be found in the choroid plexus (Fig. 6) and the cerebrospinal fluid may contain mononuclear cells and red blood corpuscles, especially when there are hemorrhages in the meninges. Thrombi, emboli, and patches of softening have not been observed. In one case necrotic foci have been found, but these are apparently due to secondary invaders, for bacteria are present in great numbers in the capillaries and smaller vessels.

(*b*) *Lesions of the Glia.*—In hog cholera just as in Borna disease, dog distemper, and rabies, there is a more or less marked proliferation of the microglia and neuroglia which may be a prominent feature in the process. These glia proliferations vary according to the duration and probably also to the severity of the disease. The most common picture consists of small nodules often seen in the white matter, which are rather sharply outlined against the surrounding tissues (Fig. 7). In other cases there is a more diffuse proliferation of the glia cells (Fig. 8). In some cases these lesions are made up only of proliferating glia cells while in others mononuclear cells may also be found (Fig. 9). The latter type of lesions has been observed chiefly in the neighborhood of blood vessels, the walls of which are infiltrated with mononuclear cells. It has also been observed under the ependyma of the ventricles which

otherwise appear unchanged. Frequently a mobilization of neuroglia elements composed chiefly of cells of the Hortega type may be found around infiltrated vessels where they form areas having a peculiar circular arrangement. This is brought out in Fig. 10. In the areas of neuroglia proliferation mitotic figures are sometimes very numerous.

Satellitism is met with frequently in the gray matter of the brain, cerebellum, and spinal cord, and represents an increase of glia elements around ganglion cells. In some cases neuronophagia has been observed, as shown in Fig. 12. So-called "glial stars" or "glial roseknots" which are present in the gray matter may be a later stage of this process. Spielmeyer's "glial shrub-wood" had been found in some cases in the molecular zone of the cerebellum but not in a typical form.

Glia nodules were first described in rabies by Babes and are usually known as "nodules of Babes." For some time they were considered to be specific for this disease but it is now known that they appear in other types of encephalitis in man and animals.

The methods of Cajal and Rio del Hortega show that both the microglia and macroglia are involved in the proliferation process. The demonstration of microglia proliferation by Hortega's method was more difficult in swine than it is in rabbits. We have the impression that the microglia predominates in the acute and subacute stages of the disease while in the more chronic cases the macroglia cells are more noticeable. The latter are found exclusively in the proliferating areas surrounding the third and the fourth ventricle while accumulations near infiltrated vessels consist largely of microglia cells. Bacilli-like cells, or "Stäbchenzellen," which probably represent special types of microglia cells, have been found rather frequently.

There is very little increase of glia fibers, the nodules and other lesions being made up largely of the protoplasmic part of the glia cells. In some older cases a slight increase of glia fibers surrounding infiltrating vessels could be demonstrated (Fig. 11) but this is the exception rather than the rule. The increase of glia cells in hog cholera is not as prominent as in Borna disease or in epidemic encephalitis of man.

Lesions of glia cells resembling those in pseudosclerosis in man and

Schweinsberger disease in horses, characterized by marked hypertrophy of the nucleus and protoplasm, have been found. Sometimes three to six of these hypertrophied cells with their protoplasm connected have been demonstrated. Retrogressive changes are less common and are most frequently seen in cells which have previously shown the above lesions. They consist of degeneration of the nuclei and are characterized by partial or total hyperchromatosis, pycnosis, atrophy, etc. Karyorrhexis and fragmentation of the nuclei are seen only occasionally.

The inflammatory lesions, *i.e.*, the perivascular infiltration and the glia proliferation, have been seen in both the white and gray matter and there seems to be no special selection of the gray substance as in various types of encephalitis. Our preliminary studies show that the type of lesion as well as the distribution in hog cholera and dog distemper are quite similar (6).

(*c*) *Lesions of the Nerve Cells.*—The nerve cells show changes in the areas involved by inflammatory processes and also in parts of the central nervous system that are comparatively free from other lesions. The degree of change does not seem to be dependent upon the severity of the inflammatory process. Nerve cells in one area may show marked changes while adjacent cells may appear to be normal. In exceptional cases the large pyramidal cells seem to be involved more severely than the multipolar cells of the cerebral cortex and gray nuclei.

After examining a large number of preparations we feel that the changes found in the nerve cells are not diagnostic. They are chiefly of the degenerative type described by Nissl as "akute Zellerkrankung" and "schwere Zellveränderung," and are characterized as follows. The nucleus in an individual cell is swollen, is located peripherally, the chromatin is fragmented and the nucleolus absent. The protoplasm shows more or less marked tigrolysis; it contains vacuoles, the cellular membrane is denticulated, and Nissl's granules may be lost, so that only cell shadows are present. These lesions may be found in the cells of Purkinje but the so-called "homogenisierende Zellerkrankung" of Spielmeyer has been seen only rarely in these cells. The neuronophagia and glia nodules referred to in the previous section are often associated with this kind of cell degeneration.

The methods of Bielschowsky and Cajal have failed to reveal a

hypertrophy of the endocellular neurofibers such as is found in rabies and distemper. They show, on the other hand, an atrophy and fragmentation and a type of perinuclear accumulation. The extracellular fibers stain normally or may at times be slightly hypertrophied. Demyelinization in the parenchyma of the brain such as has been found in dog distemper by Perdrau and Pugh (7) has not been demonstrated in our sections. Sections of the spinal cord stained by Marchi's method show a degeneration of individual fibers throughout the white matter (Fig. 14).

Inclusion Bodies.—Uhlenhuth and his collaborators have found in the corneal epithelium in swine infected with hog cholera virus bodies simulating inclusions. According to a statement of Huguenin (4) inclusions could not be demonstrated in a variety of tissues including the central nervous system. Careful examination of our material by various methods has failed to reveal typical inclusion bodies in the central nervous system. In a number of cases intranuclear bodies resembling inclusions have been found in the nerve cells of various parts of the brain. In some instances they have been acidophilic, homogeneous, round, and sometimes surrounded by an unstained halo resembling closely Joest-Degen's inclusion bodies in Borna disease (Fig. 13). They have been found singly or in groups up to five in number. Their dimensions have varied from the limit of visibility to the size of a nucleolus. Most of them, however, take a basophilic stain, being somewhat lighter in color than the nucleus. Bielschowsky's method has revealed a sort of structure with argentophilic properties. These bodies have been found in only a small percentage of cases, and since they do not as a rule show the staining properties of inclusion bodies, we regard them as products of nuclear degeneration rather than true inclusions.

SUMMARY AND CONCLUSIONS

1. A more or less marked encephalomyelitis and meningitis was found in 33 out of 39 cases of virus hog cholera which had been infected either intramuscularly or by contact and killed between 6 and 49 days after infection.

2. This hog cholera encephalitis is characterized by a varying amount of vascular and perivascular infiltration with small lympho-

cytes, mononuclear elements, a few plasma cells, and occasionally a few eosinophilic leucocytes. The glia shows a proliferation surrounding infiltrated vessels or forming small nodules or more diffuse foci. Satellitism and in a few instances true neuronophagia have been observed. Both microglia and macroglia participate in this process. There is no essential increase of glia fibers. In nearly all parts of the central nervous system degenerating lesions of the nerve cells such as tigrolysis and degeneration of the nucleus, including a slight atrophy of endocellular neurofibers, are encountered. No demyelinization has been observed. Specific inclusion bodies in the nerve cells are absent. In addition, in a certain number of cases microscopic and macroscopic hemorrhages are present in the brain, spinal cord, and meninges.

3. These lesions in varying degrees have been found in swine infected with four different strains of hog cholera virus. Two were laboratory strains and two were obtained from fresh field outbreaks.

4. Histological changes in the central nervous system were found as early as 6 days after infection before the animal showed central nervous system symptoms. In two cases which were paralyzed no lesions in the central nervous system could be demonstrated.

5. The lesions in the central nervous system are considered to be the anatomical substratum for the various nervous symptoms commonly found in hog cholera.

REFERENCES

1. Fröhner, E., and Zwick, W., Lehrbuch d. speziellen Pathologie u. Therapie d. Haustiere, Ferd. Enke, Stuttgart, 1925, **2**, 255.
2. von Hutyra, F., and Marek, J., Spezielle Pathologie u. Therapie d. Haustiere, G. Fischer, Jena, 1922, **1**, 305.
3. Uhlenhuth, P., Miessner, H., and Geiger, W., in Kolle, W., Kraus, R., and Uhlenhuth, P., Handbuch d. pathogen Mikroorganismen, G. Fischer, Jena, and Urban and Schwarzenberg, Berlin and Wien, 1929, **9**, 281.
4. Huguenin, F., *Schweiz. Arch. Tierheilk.*, 1923, **65**, 41; *Centr. Bakt., 1. Abt., Orig.*, 1929, **110**, suppl., 189.
5. Brunschwiler, K., *Z. Infectionskrankh. Haustiere*, 1925, **28**, 277.
6. Seifried, O., Pathologie neurotroper Viruskrankheiten der Haustiere, *Ergebn. allg. Path. u. path. Anat.*, 1930, **24**, in press.
7. Perdrau, J. R., and Pugh, L. P., *J. Path. and Bact.*, 1930, **33**, 79.

EXPLANATION OF PLATES

PLATE 15

FIG. 1. Medulla oblongata. Perivascular "cuffings," infiltration of the tissue, glia proliferation, hemorrhages. Hematoxylin-eosin. Swine 32. ×35.

FIG. 2. Midbrain. Perivascular infiltration and gila mobilization surrounding the aqueduct. Nissl stain. Swine 12. ×85.

FIG. 3. Cortex of cerebellum. Marked meningitis. Sulcus. Hematoxylin-eosin. Swine 32. × 35.

FIG. 4. Midbrain, cross section through corpora quadrigemina. Marked meningitis and encephalitis. Hematoxylin-eosin. Swine 32. × 35.

FIG. 5. Wall of an infiltrated vessel showing infiltrating mononuclear cells between the separated and extended connective tissue fibers. Klarfeld's tannic acid silver method. Swine 25. × 570.

PLATE 16

FIG. 6. Choroid plexus. Congestion and perivascular infiltration of the blood vessels. Hematoxylin-eosin. Swine 21. × 60.

FIG. 7. Neighborhood of third ventricle. Glia proliferation forming a so-called glia nodule. Nissl stain. Swine 21. × 145.

FIG. 8. Midbrain, substantia nigra. Diffuse glia proliferation, with many cells of the Hortega type, in the surroundings of ganglion cells. Nissl stain. Swine 22. × 100.

FIG. 9. Immediate neighborhood of third ventricle. Diffuse proliferation, mononuclear infiltration, and hemorrhages. Hematoxylin-eosin. Swine 39. × 100.

FIG. 10. Midbrain, surroundings of aqueduct. Infiltrated vessel with perivascular glia proliferation. Nissl stain. Swine 25. × 85.

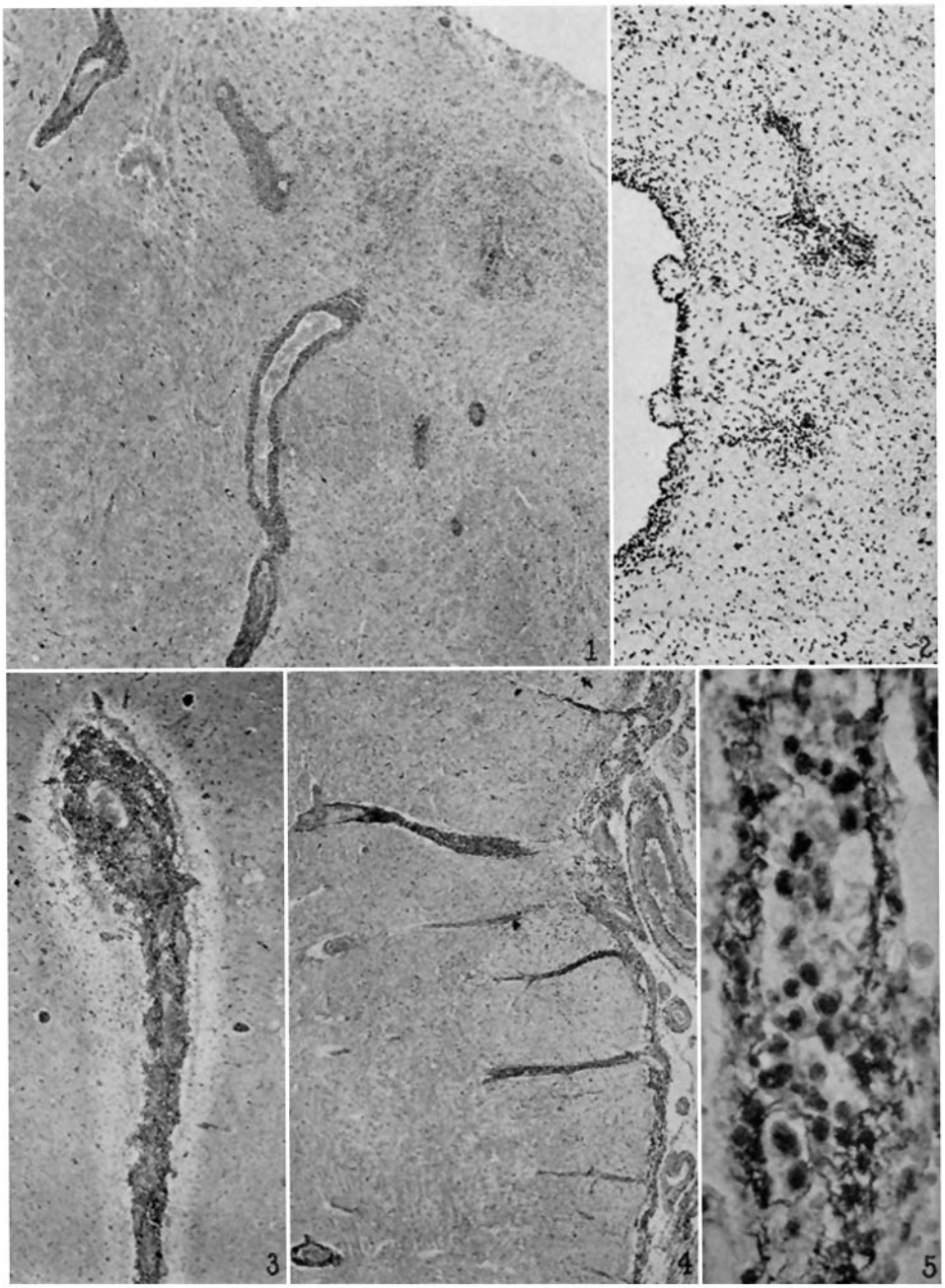
PLATE 17

FIG. 11. Infiltrated vessel with extension of submarginal glia spaces and slight increase of glia fibers. Iron-hematoxylin. Swine 17. × 660.

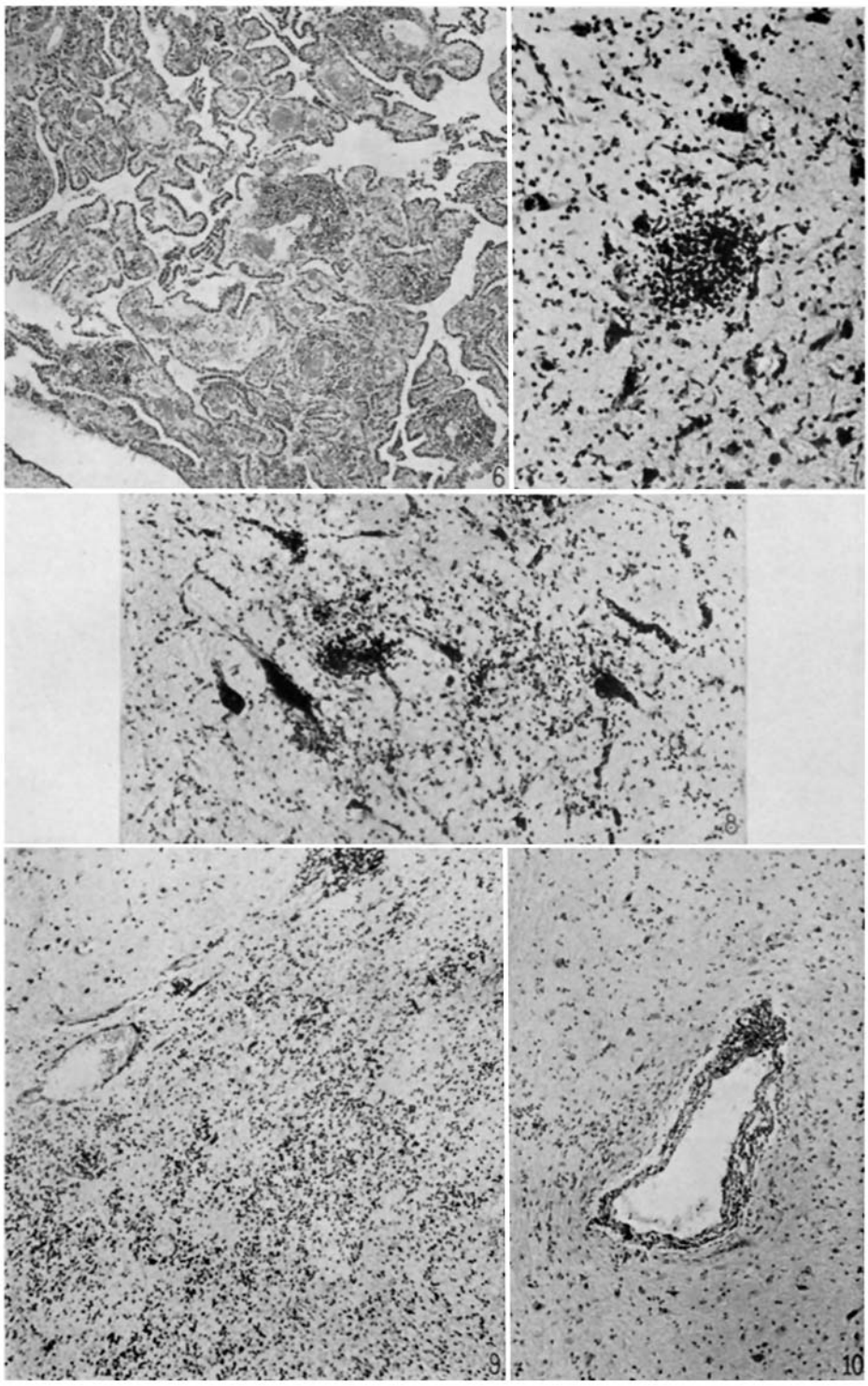
FIG. 12. Interbrain, neighborhood of third ventricle. Neuronophagia. In the center of the cell accumulation a degenerating nerve cell. Nissl stain. Swine 21. × 660.

FIG. 13. Medulla oblongata. Degenerating nerve cell with an intranuclear body simulating an inclusion body. Giemsa stain. Swine 21. × 1000.

FIG. 14. Spinal cord. Degeneration of myelin-sheaths of nerve fibers in cord. Marchi's method. Swine 34. × 310.



(Seifried: Histological studies on hog cholera. I)



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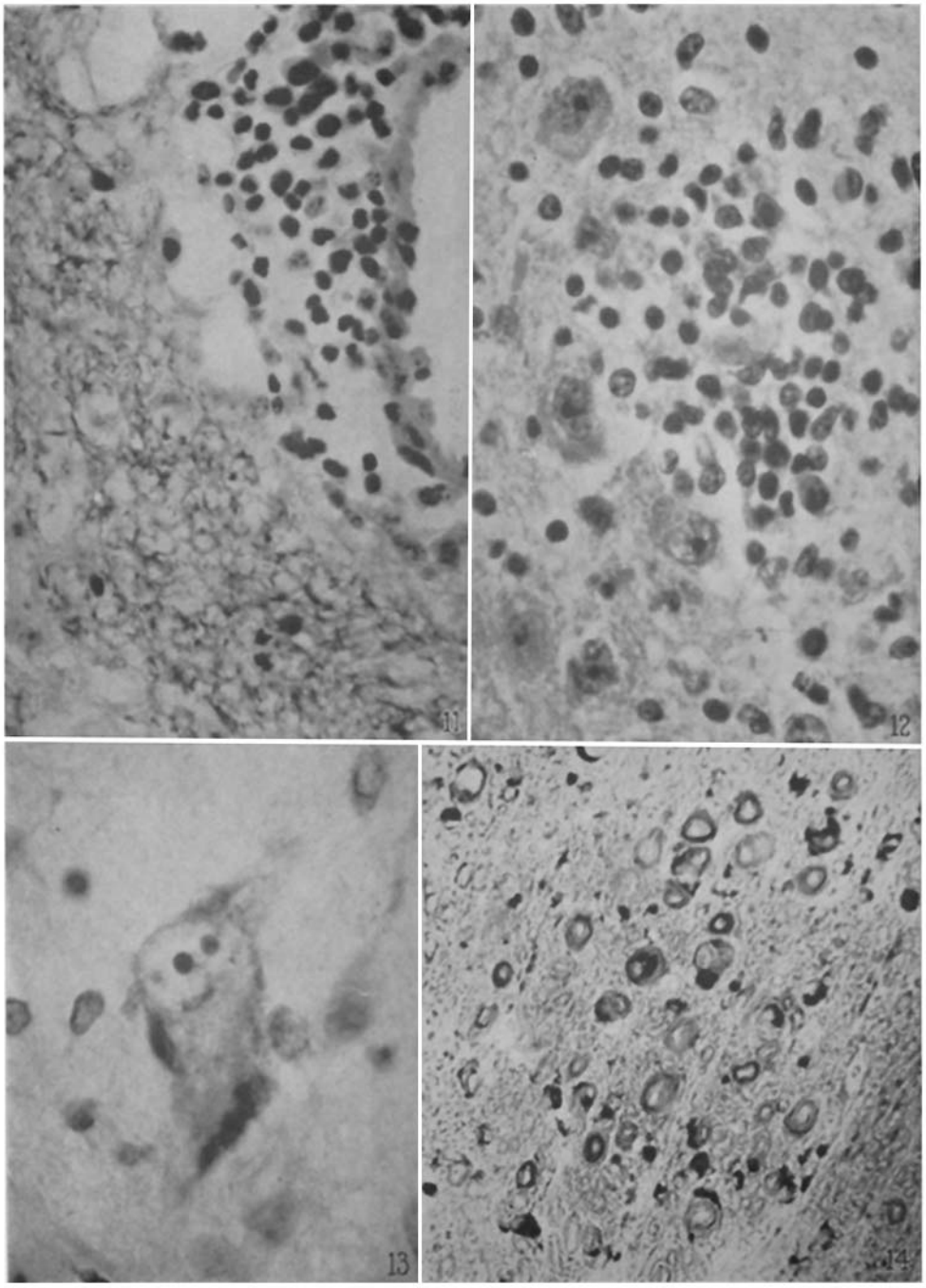

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