# ACUTE EPIZOÖTIC LEUCOENCEPHALITIS IN HORSES.

By W. G. MacCALLUM, M. D.,
*Associate in Pathology, Johns Hopkins University,*
AND
S. S. BUCKLEY, V. S.,
*Veterinarian, Maryland Agricultural College.*

(*From the Pathological Laboratory of the Johns Hopkins University and Hospital.*)

PLATES VII AND VIII.

A recent epizoötic among horses in Maryland, resulting in the death of a great many animals after a very brief illness, has led to the post-mortem examination of a number of such animals with results which seem worthy of note.

The disease, which is popularly known in this region and probably elsewhere as " cerebrospinal meningitis," presents fairly characteristic symptoms, which when the cases appear in epizoötic form lead readily enough to a diagnosis. Prodromal symptoms are not always present, although in many cases a general malaise may be noted before the acute onset. The acute symptoms are in general such as may be referred to a cerebral lesion. There may be drowsiness associated with an impairment of sight. Partial or complete paralysis of the pharynx is often observed; twitchings of the muscles of the shoulders and thighs, coldness of the extremities, and a general condition of unsteadiness and weakness with a tendency to walk to one side or a staggering, objectless gait, arise early in the disease. The pulse is usually normal; the temperature varies between 96° and 103° F., an elevated temperature usually indicating a secondary complication.

The horse may then become gradually comatose, responding slightly or not at all to stimuli and soon sinking to the stable floor not to rise again. In other cases there is a wild delirium, the animal rearing about and rushing blindly against obstacles, and this may be followed by exhaustion and the comatose condition.

The duration of the disease varies from a few hours to a week, the

average being perhaps 72 hours. Horses which recover are said to become "dummies"—animals with a permanent cerebral lesion and defective intelligence.

The following pathological report is based on the examination of four brains, brought to the laboratory by one of us (Buckley), from animals dying in the acute stages of the disease. There was also one brain from a horse which was said to have had the disease some time before and to have recovered, dying afterward from some other cause.

Of the four brains from acute cases, three were hardened in formalin and one was fresh. Of these, none showed any signs of the presence of an inflammation of the meninges; there was at most a trifling hyperæmia of the pia mater. The surface of the fresh brain showed no localized or circumscribed alterations in color, but the normal level of the convolutions was not everywhere preserved. In the frontal region on each side, anterior to the motor region of the cortex, there was a slightly depressed area which was softly fluctuant, but not marked out by any superficial hyperæmia or discoloration. On cutting through this brain a glairy fluid with small granular pulpy masses of whitish tissue flowed out from the softened area, and the rather thin roof composed of the meninges with the grey cortex collapsed over the cavity thus left. The lesion seemed almost entirely limited to the underlying white matter, which throughout an irregular area, perhaps 2 x 1 cm. in diameter in the left hemisphere, and a symmetrically placed focus 5 cm. in diameter in the right, was completely softened into a diffluent mass made up as described of shreds of softened, necrotic-looking, greyish white brain substance lying in a greyish, glairy or somewhat glutinous fluid. The portions of the brain substance forming the lining of the cavity could be fairly sharply outlined from the adjacent more normal white matter by its softness and raggedness, by its mottled greyish and yellowish opacity with translucent areas, and by the presence of numerous minute hæmorrhages sprinkled through it and adding to its mottled appearance. The remaining brain substance showed no apparent abnormality. The lining of the cerebral and olfactory ventricles was not congested nor inflamed. The blood-vessels were carefully traced and showed no thrombotic occlusion at any point.

Examined microscopically in the fresh state, the softened material showed necrotic cells and cell fragments of various forms; there were also beaded elongated fibrils thought to be axis cylinders with adhering myelin droplets. But few nuclei were found. No bacteria were found by the ordinary staining methods.

Cultures were made aërobically and anaërobically on various media—agar, glycerin agar, blood-serum agar, hydrocele-fluid agar, etc.—but all were negative. A rabbit inoculated with 1 cc. of an emulsion of the softened material into the ear vein remained well.

The appearance of the hardened brains corresponds very closely with that just described. Nowhere were any blood-vessels thrombosed or occluded in any way. Nowhere was there evidence of inflammation of the meninges. Section of the cerebral hemispheres showed irregular areas in the white matter of the occipital as well as the frontal lobes, and once in the temporal lobe, in which the brain substance had been softened and partly replaced by a translucent coagulated substance resembling agar. Shreds of greyish brain substance coursed through this clear gelatinous material. The adjacent greyish and opaque brain substance was studded with hæmorrhages through a thickness of about 3 mm. Where, as in some cases, the areas of softening were made up mainly of the greyish necrotic brain substance without much collection of fluid, the hæmorrhages were scattered throughout. In no instance did the cortical grey matter appear to be implicated, nor were the basal ganglia invaded.

Microscopically the lesions are practically identical in all the four cases except that while in all the process is quite acute, in one the destruction was less complete than in the others and the replacement of the necrotic material by coagulable fluid less extensive. A general view of a section carried through the cortex into the centre of such a focus shows the meninges practically normal, the elements of the grey cortex not notably altered, the nerve cells staining well, the blood-vessels patent and filled with blood. Passing inward the nervous elements begin rather abruptly to degenerate, disintegrate and disappear, and hæmorrhages begin to occur here and there; further toward the centre no more nerve cells are visible, axis cylinders are much degenerated, neuroglia cells stain badly, and the tissue has a much disintegrated appearance, being infiltrated with not very numerous polymorphonuclear leucocytes and fewer mononuclear round cells. Still further, and all evidences of tissue, except for small islands of necrotic substance, disappear in the highly refractive vacuolated hyaline material described (Plate VII, Fig. 1). We have then to consider in detail:

1. Changes in nervous elements.
2. Changes in neuroglia.
3. Changes in blood-vessels.
4. Changes in lymphatics.
5. Exuded fluid and cells.

The pyramidal ganglion cells which send down their axis cylinders through the degenerated area appear normal in the uninvolved portion of the cortex. The perigangliouic cells may perhaps be more than usually numerous. In the lower layers as one approaches the degenerated area the ganglion cells become swollen and granular, the nucleus stains less sharply, and the cell processes, so definite in the higher layers, have been lost or disappear after a very short course, forming mere projections from the outline of the cell. Many such cells take on a rounded outline and appear now as large, irregularly rounded, granular cells with rather diffusely staining nucleus. Indeed, as in Fig. 2 (Plate VII), such cells may be seen in the same field with their disintegrating processes which are slightly separated from the cell body; others still more degenerated have lost their nuclei. The much-degenerated cells lie in a tissue of axis cylinders and neuroglia which is thickly sprinkled with globules of various sizes of high refractive index and staining faintly bluish with hæmatoxylin. In specimens stained by Weigert's method these globules take the typical myelin stain.

The axis cylinders are somewhat swollen and thick and show evidences of disintegration (Plate VII, Fig. 3). They persist, however, fairly well into the completely necrotic substance, where they end abruptly. Throughout the degenerated area their myelin sheaths are broken up into the globules described above, many of which adhering to the axis cylinders give rise to the varicose appearances or bulbous swellings along the course of the fibril. In specimens prepared by Marchi's method such varicose beaded masses often stain black.

The neuroglia has also suffered severely. Traced by the aid of Mallory's special methods from the relatively normal cortex toward the centre of an area of softening, the dense matted feltwork of the outer region is seen to give place to a delicate network of finer deeply staining fibrils, which in their turn completely disappear further toward the centre, leaving the material there without any definite neuroglia stain and consisting of necrotic debris of cells and tissue without connecting supporting substance. Associated with this gradual disintegration of the neuroglia feltwork there are changes in the neuroglia cells. These lose the sharp contours of their nucleus, which comes to stain a diffuse greyish purple without any sharply stained chromatic particles; such nuclei become more and more indistinct and finally disintegrate.

Even more striking than these destructive degenerative changes in the nervous elements and the neuroglia cells and fibrils are the changes in the blood-vessels of the affected area.

It was stated above that examination of the vessels macroscopically and with scissors failed to reveal anywhere the presence of an occluding thrombus or embolus. Sections, too, made to pass through the blood-vessels in those brains already hardened when brought to the laboratory showed them to be filled only with blood. In the area of degeneration, however, wherever small vessels are left they may sometimes be found filled or partly filled with an elongated highly refractive hyaline mass, the free ends of which may be rounded off or pass over insensibly into the adjacent compressed and coalescing red blood-corpuscles. Such hyaline formations have been found mainly in the smallest vessels and in the degenerated area. Sometimes the lumen is only partly filled and the hyaline material may show gaps in which lie red corpuscles (Plate VIII, Fig. 5), or it may form a thick bluish-staining lining for the vessel in the lumen of which lie the red corpuscles.

The walls of the vessels in these areas show, however, extensive inflammatory changes. They are infiltrated (Plate VIII, Fig. 4) with cells of the type of the polymorphonuclear leucocyte for the most part, but occasionally mononuclear or so fragmented as to be difficult of diagnosis. This process affects arteries as well as the veins, and the infiltration extends throughout all the coats. The adventitial lymphatic sheath is in most cases distended and may contain masses of polynuclear and mononuclear cells with red corpuscles. Very often, however, this sheath contains only red corpuscles, but these in such numbers as to distend it to a diameter far greater than that of the blood-vessel. It seems most probable that this hæmorrhage has occurred by diapedesis, constituting one of the evidences of inflammation, but here and there there are apparently evidences of the direct rupture of the wall of a small vessel. The distended lymph sheath may also rupture; at any rate, in nearly every case there is a zone of hæmorrhage in the tissues round about it. Such extravasated red blood-corpuscles, like those within the sheath and the blood-vessel, are in a good state of preservation, indicating the extreme acuteness of the process. There is nowhere any definite accumulation of hæmatoidin or hæmosiderin to be found in the tissues or in the lymphatics—further evidence of the rapid course of the disease.

The small vessels lying in the centre of such hæmorrhages are very commonly such as are plugged with the rather blue-staining hyaline masses already described (Plate VIII, Fig. 5). Other vessels may contain a similar hyaline material and indeed hyaline is often found both within and surrounding the vessel. Especially is this true in the case of some of the larger vessels lying within those meningeal processes

which pass deep into the sulci. There the surrounding tissue is spread apart by the presence of this coagulated material.

The nature of the hyaline substance offers perhaps some difficulty of explanation. Leyden and Goldscheider[1] express themselves as follows:

> Sometimes in œdema, softening or acute inflammation of the cord one finds in sections structureless amorphous masses. These occur in the central canal, in the grey substance, less often in the white matter, often about the vessels. This phenomenon is explained in various ways: by some thought to be coagulated albuminous or fibrinous exudate, by others interpreted as a colloid, hyaline, mucoid or gelatinous degeneration of softened nerve substance or swollen and diseased neuroglia. It is this structureless mass which Lockhart Clarke described as "granular or fluid disintegration." According to that author it consists in a softening and destruction of the nerve tissue and its change into a granular mass which, with the exuded fluid, mixes to form a homogeneous substance. These masses take the carmine stain very weakly. Their nature is not yet settled; it is even questionable whether the material under discussion is everywhere the same. The perivascular masses are most probably exudate; whether this will hold for all similar forms is, however, uncertain. The attempts to determine the nature of the substances by various stains have so far not been successful.

The problem before us is somewhat similar. The hyaline material within and about the meningeal vessels looks at times as if it had been produced by the coalescence of red corpuscles, but in general it is too abundant and homogeneous to be so explained. It is rather denser and more refractive than coagulated plasma would appear, and with water blue it stains brilliantly. In its general appearance and reaction it agrees fairly well with the larger hyaline masses in the areas of necrosis. Such hyaline material occurs also scattered about among the tissue elements, but nearly always about a vessel except in the most degenerated areas where the tissue becomes necrotic and entirely gives place to the structureless mass. There is even difficulty at times in outlining this necrotic substance from the hyaline material. Highly refractive as elsewhere it shows here, too, the tendency to contract and leave vacuoles, probably as the effect of the hardening reagent, so that the great central mass has, as a rule, an appearance almost like the cut surface of a Gruyère cheese (Plate VIII, Fig. 6). Often in such vacuoles a delicate coagulum can be made out, suggesting the presence there of a fluid of less density. The highly refractive substance is somewhat denser about the vacuoles. It is apparently very brittle in

[1] Die Erkrankungen des Rückenmarks und der Medulla oblongata, in Nothnagel's Spec. Path. u. Therap., Bd. X, Wien, 1897.

the sections and shows cracks and fissures here and there. It stains with eosin, taking a fairly bright pink color; Congo red tinges it brick red. Van Gieson's stain leaves it pinkish yellow—neither definitely red nor definitely yellow—with water blue and fuchsin it stands out sharply from the adjacent substance by its bright deep blue color; so also do the masses in and about the vessels. With Mallory's phosphotungstic acid hæmatoxylin it stains a rather pale purplish pink; with his modified stain for connective tissue as applied to the nervous system, it takes a dense deep purple color. With methylene blue, carbol fuchsin, Weigert's fibrin stain, etc., it is hardly tinged at all. Osmic acid does not stain it; in a Marchi preparation it is just visible as a smoky area.

The material stains therefore with acid dyes, in which respect (according to the hypothesis of P. Ernst) it corresponds to that form of hyaline derived from epithelial cells. Nervous elements being of epiblastic origin, might perhaps furnish the great mass of hyaline in the centre of the focus. There would be difficulty, however, in thus explaining the presence of a substance staining in exactly the same way in and about the arteries as well as the veins, and we must probably consider this one of the exceptions to the rule, as is the colloid of the thyroid which, although derived from epithelium, stains red with Van Gieson's stain.

In the smaller vessels in the neighborhood of the most intense degenerations the hyaline masses described above stain rather bluish with the hæmatoxylin and eosin stain, which seems to indicate that they are not quite identical in nature with the remaining hyaline substances described.

As stated above, the central hyaline mass in each focus is bounded by ragged edges of necrotic substance with here and there free islands of such tissue. Nowhere are there any evidences of the least pressure on this tissue, which becomes gradually rarefied toward the margin, where it quite disappears. This mass is, therefore, in all probability the result of the breaking down of the brain substance—perhaps added to also by exudation of fluid from the vessels.

The exudation of leucocytes is not very abundant in the sections. Beside the infiltration of the walls of the small vessels and the tissue surrounding them, leucocytes are found sprinkled in considerable num bers through the most degenerated tissue in the focus where it borders upon the hyaline material. These leucocytes are easily distinguished by their sharp staining from the greyish purple degenerated neuroglia nuclei which persist there.

Besides the leucocytes there are a few somewhat larger round cells with small single round nucleus and granular protoplasm. These appear to be analogous to the fat granule cells which are so common in inflammatory diseases of the nervous system of longer standing, they are however rather scarce, and although in a Marchi preparation they can be made out to contain blackened fat droplets, they are by no means a prominent feature in the section.

The process is therefore predominantly a destructive rather than an exudative one. To resume, we have an acute disease, rapidly fatal, producing large areas of complete destruction of the brain substance in which the anatomical elements are disintegrated and largely replaced by a colloid-like material. In the neighborhood the blood-vessels are acutely inflamed, there is exudation of leucocytes into the vessel walls, and throughout the adjacent tissue, with passage of the red corpuscles into the perivascular lymph sheath and into the adjacent tissues, these focal extravasations giving the inflammatory process its hæmorrhagic character.

The various forms of acute hæmorrhagic encephalitis in man as described by Wernicke, Strümpell, Friedmann and others seem, as a rule, to progress less rapidly and to be much less violently destructive than this form. Anatomically, however, the conditions are analogous.

In horses the disease is apparently fairly well recognized. Friedberger and Fröhner,[2] giving the bibliography, summarize the results of investigation into the pathology of acute encephalitis about as follows:

> Local non-purulent encephalitis occurs in irregular, round foci, mostly of the size of a pea to that of a hen's egg, sometimes even involving a whole lobe of the brain, but not sharply limited. At first the place is slightly diffusely reddened, this being soon followed by a swelling and softening from serous exudation, when, according to Schütz, the cells of the neuroglia and the ganglion cells are swollen and granular, and finally undergo fatty degeneration; the axis cylinders are varicose and the glia tissue infiltrated with small cells. The focus undergoes maceration, swelling and liquefaction, resulting finally in a softened mass consisting of disintegrated and fatty glia and ganglion cells, leucocytes and free fat-globular cells, and is spoken of as simple inflammation of the brain or inflammatory softening of the brain, distinguishable from ischæmic

[2] Lehrb. d. spec. Path. u. Therap. d. Hausthiere, Bd. ii, p. 79, 2te Aufl., Stuttgart, 1889.

encephalomalacia by the exudation of leucocytes. This may be all, but often there are complicating hæmorrhages giving rise to hæmorrhagic inflammation of the brain. With the decomposition of the hæmoglobin in such a focus the color disappears gradually and becomes yellowish. Then, as the mass of disintegrated tissue and exudate becomes more fluid, there is formed either a grey gelatinous mass or cyst, or finally a scar arises.

This description would apply to the cases described above fairly well except that the gelatinous fluid mass appears only at the end where the process is on the way to healing, whereas in our cases the brain substance throughout a large focus is quickly reduced to a gelatinous, structureless mass of necrotic and hyaline material.

The single case of our series in which recovery from the disease had occurred showed in the frontal lobe of one hemisphere a depression which on section of the brain corresponded with an elongated, grey, translucent scar which ran deep into the substance of the brain. This microscopically showed only a loose granulation tissue with numerous cells resembling the fat granule cells. Of course, whether or not it was really the end product of such a condition as described above depends on the accuracy of the diagnosis, but as the symptoms are fairly characteristic and the scarred condition of the brain about what might be expected as the final result of the anatomical process, it seems probable that this was an instance of recovery from the affection here described.

*Addendum.*—Since the above was sent to press there has occurred another outbreak of the disease in southern Maryland in the course of which great numbers of horses have died. We were able to make three autopsies on animals in which the symptoms during life were such as are described above. The two horses when seen were comatose while the third animal—a mule—had died after a short but violent delirium. As the horses were obviously dying they were killed, but the autopsies revealed no recognizable macroscopic lesion. Microscopically, however, the vessels in the substance of the brain show in many places an acute inflammatory affection of and around their walls, and here and there in their neighborhood there is infiltration of the tissue with mononuclear, polymorphonuclear and eosinophilic leucocytes. No widespread destruction such as that described for the previous cases was found in these cases, and it is clear that they represent an earlier stage of the affection than that described above.

Bacteriological examination in these cases led also to no satisfactory results. Cultures from the organs of the horses were sterile except for occasional obvious contaminations. A rabbit inoculated with an emulsion of the brain substance of the mule, which had been dead 48 hours, died with a general infection with a bacillus probably of the hog-cholera group and very virulent to rabbits. Further study of this organism will be made but it is not likely that it has any relation to the disease in question.

## DESCRIPTION OF PLATES VII AND VIII.

### PLATE VII.

Fig. 1. Photograph of a section through part of a focus of encephalitis showing the disintegration of the white matter, and the central hyaline substance.

Fig. 2. Ganglion cells which are losing their processes and becoming rounded—steps toward their complete disintegration.

Fig. 3. Nerve fibres undergoing degeneration. The myelin sheath forms droplets or varicosities along the axis cylinder. Other highly refractive droplets are scattered about in the tissue.

### PLATE VIII.

Fig. 4. Small vessel with cellular infiltration of the wall, the perivascular lymph sheath being distended with blood.

Fig. 5. Similar vessel with extravasation of blood into its lymph sheath. The vessel is partly filled with a hyaline material.

Fig. 6. Central portion of a large focus showing the margin of the necrotic material and the central hyaline substance with vacuoles.

FIG. 1.

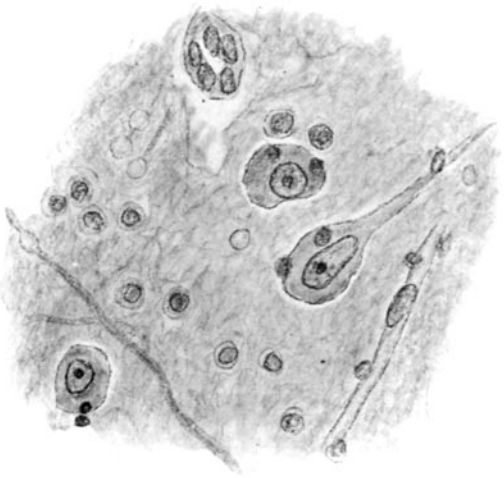

FIG. 2.

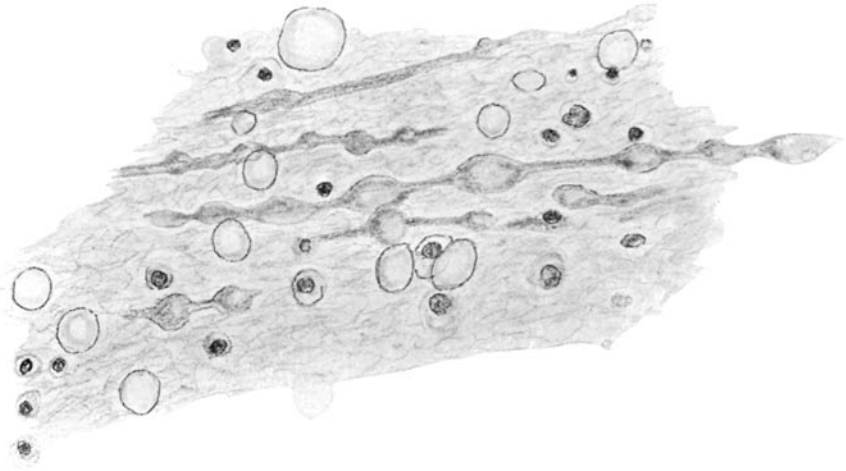

FIG. 3.

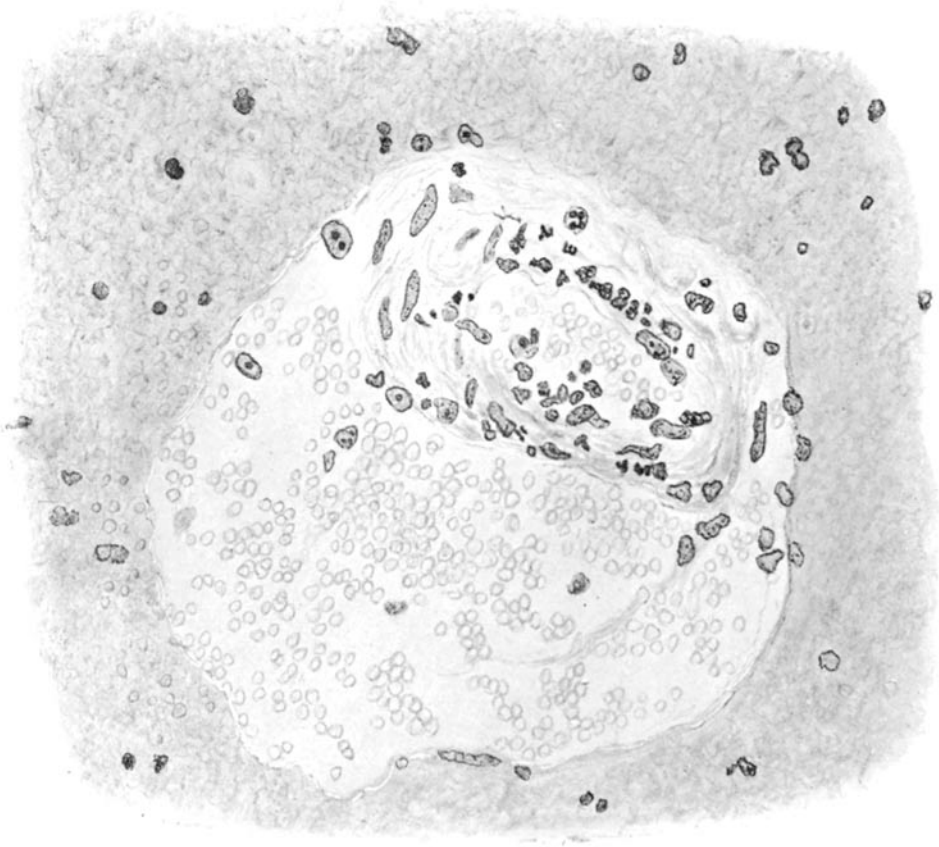

FIG. 4.

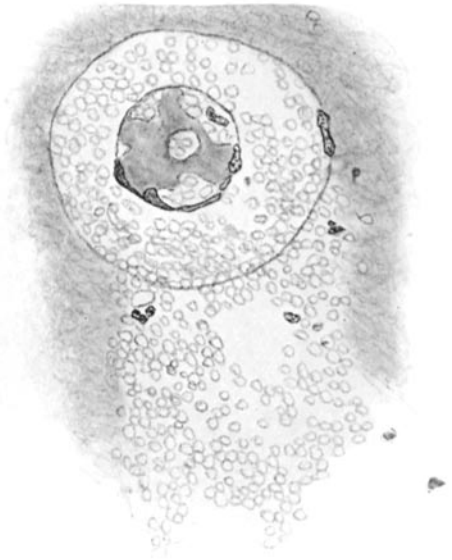

FIG. 5.

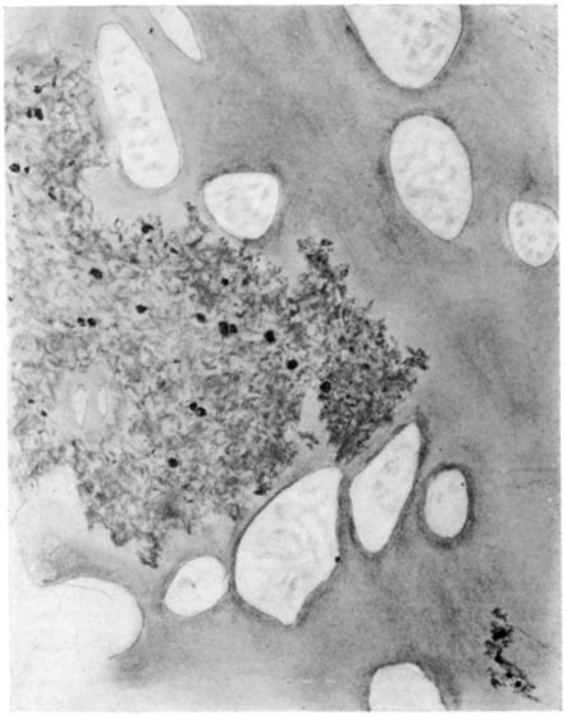

FIG. 6.