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CLINICOPATHOLOGICAL STUDY ON EXPERIMENTAL *TRYPANOSOMA BRUCEI* INFECTIONS IN HORSES

PART 2. HISTOPATHOLOGICAL FINDINGS IN THE NERVOUS SYSTEM AND OTHER ORGANS OF TREATED AND UNTREATED HORSES REACTING TO NAGANA

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ABSTRACT

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A detailed description of the histopathology of the nervous system and a brief account of lesions in visceral and other organs of six horses experimentally infected with *Trypanosoma brucei* Plimmer & Bradford, 1899 is given.

Attempts to produce a chronic form of nagana in three horses by subcurative medications with Antrypol and Berenil were successful. The chronicity period was extended to 130 days in one and to approximately 9 months in the other two horses. The data on the histological findings on the three horses are listed in tabular form. The lesions in the central nervous system were characterized by a severe pleocytosis of the meninges, an extensive subpial gliosis corresponding in severity to the involvement of the overlying leptomeninges, segmental demyelination of optic tracts and some other areas of white matter as well as grey matter and extensive perivascular cuffing with lymphocytes, plasmocytes, large mononuclear and Mott cells in this order of descending frequency.

Comparison between lesions of the acute form of human sleeping sickness and those of the experimentally produced chronic form of equine nagana revealed that points of similarity are far greater than those of dissimilarity. The latter include a lymphophagocytosis in the meninges and brain of man, a higher incidence of Mott cells in the meninges of horses and the penetration of trypanosomes in the brain of man which was not seen at this site in horses.

With the exception of the pituitary of one horse, lesions of the nervous system of the remaining three horses were not striking. Histological changes in the visceral and other organs were neither pathognomonic nor of uniform occurrence.

INTRODUCTION

Part 2 of this article is chiefly concerned with the histopathology of the central nervous system and to a lesser extent with that of other tissues from six experimentally *Trypanosoma brucei* infected horses. Geldings 5484 and 5480 served as untreated controls, Gelding 2287 received subcurative treatments of Antrypol, while Mare 5588, Stallion 5259 and Gelding 5065 were subjected to repeated subcurative medications with Antrypol and Berenil. In Part 1 evidence was brought forward that the latter two horses not only showed a protracted nagana chronicity period, which persisted for approximately 9 months, but that the clinically recognizable nervous symptoms were accompanied by *T. brucei* in the cerebrospinal fluid and macroscopic lesions in the meninges and brain. It was therefore expected that systematic histological studies of the CNS would reveal lesions similar to those encountered by Manuelidis, Robertson, Amberson, Polak & Haymaker (1965) in the acute form of human sleeping sickness.

MATERIALS AND METHODS

Representative pieces of visceral and other organs as well as the whole brain were fixed in a large quantity of a 10% neutral buffered formalin solution. After adequate fixation, suitably-sized blocks from visceral and other organs were embedded in paraffin wax. From these blocks sections, 6 microns in thickness, were cut with a sliding microtome and stained with haematoxylin and eosin (HE).

From the brain of each horse a small number of pilot sections were cut first in order to determine to what extent additional ones would have to be prepared for the studies. Sections subsequently prepared from different brains varied in size. From Geldings 5484 and 2287 sections, not exceeding 30 mm in length, were prepared from representative areas of the brains. Coronal segments from selected levels of the brain were prepared from Geldings 5480 and 5065 and Mare 5588. The brain from Stallion 5259 was cut coronally into slices approximately 5 mm thick. These were transferred onto a piece of white cardboard in the order in which they were cut. They were photographed and thereafter small pieces were resected from the slices as indicated in Plate 1.

Selected brain sections were embedded in paraffin wax. The large coronal sections were cut at 8 to 10 microns and the smaller ones at 6 microns. They were routinely stained with HE, while replicate sections from Stallion 5259 and a limited number of those from other horses were stained with Luxol-fast-blue (LFB) and by the Holzer technique (Anon, 1960). Other special stains for various tissues included periodic-acid-Schiff (PAS) and ferrous iron (Anon, 1960).

RESULTS

A. *Histopathological findings in organs and tissues other than the nervous system*

Since emphasis has been placed on the histological studies of the nervous system only brief accounts on the pathology of other organs and tissues will be given.

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Gelding 5484

Four sections from different regions of the *heart* were all affected but the lesions varied in extent. The most extensive lesion, an interstitial myocarditis, overshadowed the mild epicarditis and endocarditis. These were widespread interstitial infiltrates consisting of both large and small mononuclear cells but the extent of the myocardial necrosis and degeneration was limited. In several areas, harbouring a mixture of histiocytes, lymphocytes and plasma cells, the cardiac muscle which had been destroyed was being replaced by fibrous tissue. The three cell types were diffusely, though not uniformly distributed between individual fibres of the myocardium as well as sites where there was no evidence of damage to the heart muscle [Plate 10 (70)]. Fibroblasts often appeared between layers of cardiac muscle. This was particularly noticeable where muscle layers crossed at right angles. Frequently a subendothelial infiltration of large and small mononuclear cells occurred along the course of blood vessels and blood spaces of the myocardium. Similar cell collections were present in the endocardium. Serous atrophy of the epicardial fat was accompanied by a moderate infiltration of large mononuclear cells and a few histiocytes.

The *lungs* revealed a number of small peripheral branches of the pulmonary artery with lesions consisting of focal areas of endarteritis and thrombi. There was also a marked pulmonary haemosiderosis with almost every capillary in the alveolar septa containing haemosiderocytes.

The *liver* showed an extensive centrolobular atrophy, degeneration and necrosis of the hepatocytes [Plate 10 (71)]. This was obviously preceded by blood stasis in the efferent veins and which had resulted in hypoxia. This lesion was very extensive and involved a third to a half of the hepatocytes of lobules. In addition there was a heavy infiltration of portal areas by large and small round cells. Vessels within the same area showed a vasculitis and perivasculitis with infiltrates of round cells. Some of the central and sublobular veins exhibited collections of small round cells beneath the endothelium. Bile stasis was present. A moderate haemosiderosis, with haemosiderocytes in the blood, was evident and hepatocytes and Kupffer cells contained haemosiderin.

The *kidneys* contained minute foci of small round cells in the interstitial tissues and around the blood vessels.

Many *lymph nodes* revealed a wide range of changes. Many of the lymph nodes were reactive but showed no perinodal involvement. There was frequently a marked lymphocytosis of the cortex in which lymphatic follicles could not readily be recognized. A few lymphocytes, many multinucleated giant cells and an extensive reticulo-endothelial cell hyperplasia were visible in the subcapsular and medullary sinuses [Plate 10 (72)]. Other nodes showed severe lymphoid hyperplasia of the medullary cords which became thickened and thereby impinging upon and constricting the medullary sinuses. Many plasma cells and an occasional Mott cell were present.

An extensive interstitial myositis of the *skeletal muscles* with infiltrates, similar to those described in the myocardium, was observed [Plate 10 (69)]. Mott cells were encountered in round cell aggregations consisting of lymphocytes and plasma cells. The cell types were especially common around blood vessels and in the muscular fascia.

Changes in the *tongue* included a glossitis, as evidenced by a mild leucocytic infiltration in the lamina propria, and an interstitial myositis. Leucocytes were represented by large and small mononuclear cells.

Gelding 5480

With the exception of lipofuscin pigment near nuclei of the cardiac muscle nothing unusual was seen in the *heart*. Haemosiderin was conspicuous within the haemosiderocytes of capillaries of the alveolar septa of the *lungs*. *Bone marrow* from the midshaft of the femur showed active haematopoiesis, especially erythropoiesis. The *liver* exhibited fatty metamorphosis in the centrolobular areas, biliary stasis and moderate haemosiderosis. The last condition was displayed by haemosiderin within hepatocytes, Kupffer cells and haemosiderocytes in the blood of afferent and efferent vessels and in that of sinusoids. PAS-positive material was associated with ferrous iron. Only mild focal round cell infiltrates were found interstitially and perivascularly in the *kidneys*. A fair number of small veins in the *large intestine* contained thrombi.

Gelding 2287

Mild subendocardial infiltrates of rather large histiocytes were the only visible changes in the *heart*. The *lungs* contained areas of patchy oedema. The capillaries of the alveolar septa showed a marked haemosiderosis, harbouring many haemosiderocytes. In the *liver* centrolobular congestion, accompanied by focal areas of atrophy and fatty metamorphosis of centrally-located cord cells, resembled changes seen with hypoxia due to a severe anaemia. Severe haemosiderosis was visible and the pigment mainly appeared in Kupffer cells and numerous free haemosiderocytes occurring in the blood of afferent and efferent veins and sinusoids. Small haemosiderin granules were visible within hepatocytes. Portal areas contained round cell infiltrates. The perivascular and interstitial tissues of the *kidneys* showed moderate, focal round cell infiltrates. Many *lymph nodes* were examined and all exhibited hyperplasia of lymphocytes, plasma and reticuloendothelial cells. Multinucleated giant cells were numerous in the sinuses. A perilymphadenitis, associated with infiltration of small round, plasma and several Mott cells in the capsular fat of nodes, was a common feature. Blood and fibrin appeared in some of the lymph vessels, while haemorrhagic areas occurred in the matrix. Lymphoid hyperplasia in Malpighian corpuscles of the *spleen* was conspicuous.

Gelding 5065

The extent of haemosiderosis of the *lungs* was as prominent as that described for the previous three horses. Thrombi occurred in several branches of the pulmonary artery and small round cells were present in the adventitia. The *liver* revealed a mild haemosiderosis. Small haemosiderin granules were harboured by hepatocytes while larger particles appeared within Kupffer cells and free haemosiderocytes of the blood. *Bone marrow* from the diaphysis of the femur exhibited active haematopoiesis, especially erythropoiesis. Several *lymph nodes* were hyperplastic and others showed a lymphadenitis with many eosinophiles, plasmocytes and Mott cells. A fairly large number of giant cells were present within the sinuses.

Stallion 5259

The *heart* showed a very mild, focal interstitial myocarditis with a predominance of plasma cells.

Changes in the *liver* were mild. Many Kupffer cells contained haemosiderin, and haemosiderocytes were present in the blood of sinusoids, afferent and efferent veins. Additional haemosiderocytes were found in the wall of blood vessels and in the connective tissue of portal areas; moderate infiltrates of round cells were also present at these sites. The hilus of the *kidneys* and the renal *lymph nodes* as well as the surrounding fat contained areas of old, focal haemorrhages and blood vessels with old thrombi. These areas were impregnated with a large amount of yellow haematoidin and golden brown haemosiderin. The fat harboured small round cell infiltrates while a number of small veins showed a mild panphlebitis in which round cells predominated. A few small blood vessels in the medulla of the *kidneys* had thrombi in their lumen and some tubules contained eosinophiles and proteinaceous casts with or without erythrocytes. Focal infiltrates of small and large round cells together with Mott cells were found in the interstitium of the cortex and medulla. Several localized round cell infiltrates were visible in the interstitium of the aspermatogenetic *testes* while regional veins, affected by focal endophlebitic lesions, contained many laminated thrombi. In the epididymis and pampiniform plexus the large veins, showing either phlebitic or periphlebitic lesions, harboured laminated thrombi. Small vessels of the epididymis showed vasculitis and perivasculitis. A mild hyperplasia of the white pulp of the *spleen* was accompanied by many plasmocytes and Mott cells in the red pulp.

Mare 5588

In the *lungs* organized thrombi occurred within the lumen of various branches of the pulmonary artery. The intima of many of these vessels was thickened by fibrous plaques containing haematoidin. Within the vessel wall and in the juxtavascular tissue were numerous haemosiderocytes. Cells of this type were also visible within the capillaries of alveolar septa. Diffuse but mild fatty metamorphosis, with localized necrotic groups of hepatocytes, characterized the *liver* changes. Haemosiderin was present in some hepatocytes and Kupffer cells but this manifestation was less prominent than that described for the previous five horses. A few haemosiderocytes were free in some of the larger types of veins. Haemosiderin was seen in the centre of some Malpighian corpuscles of the *spleen*.

B. *Histopathological findings in the nervous system*

Description of the histopathology of the CNS will be preceded by brief explanatory remarks related to the method employed for the presentation of the massive data from three of the six horses. Examination of the brain and meninges of Stallion 5259, Mare 5588 and Gelding 5065, which had received subcurative Antrypol and Berenil medications, showed that lesions were widely distributed and of such severity that a detailed account would be desirable. Quantitative as well as the more specific qualitative aspects of the extensive lesions were determined. For the sake of clarity and brevity, data on the former aspects have been presented in Tables 1, 2 and 3 for Stallion 5259, Mare 5588 and Gelding 5065 respectively. In contrast the response of the CNS of Geldings 5484, 5480 and 2287 to *T. brucei* was so mild that the observations could readily be included in the text.

Histopathological lesions observed in Stallion 5259 were far more extensive and severe than those which occurred in either the mare or gelding. A comparison between the degree of meningitis of the sulci and fissures with that of other meningeal areas in Stallion 5259 showed that these lesions were more pronounced at the former than at the latter sites. This was also evident in the mare and gelding but a detailed account of this manifestation was omitted in Tables 2 and 3 as identical observations had already been recorded in Table 1. Since the degree of vasculitis and peri-vascular cell infiltrates were two of the three factors on which the severity of the meningitis was assessed, they were combined in one column under the heading "Meningitis" and subheading "degree of", in Tables 2 and 3. It was considered advisable to retain a separate column for "Mott cells" under "Meningitis". Under the heading "Brain" in Tables 2 and 3, one column was devoted to "Subpial gliosis" but the one for "Gliosis" under "Other areas" in Table 1 was omitted. The last three columns were the same in all three tables. It was frequently necessary to compare lesions, seen in one brain section, with those present in another one from the same horse, and also to make comparisons between lesions occurring in different horses in order to rate the severity of changes as uniformly as possible.

Although the three tables present the quantitative nature of lesions on a comparative basis, it was considered necessary, for the sake of emphasis and clarity, to include additional information on both the quantitative and qualitative aspects of lesions for the individual horses in the text.

1. *Narrations on observations recorded in Tables 1, 2 and 3*

(a) *Narration* 1 (Stallion 5259)

Specific brain areas, selected for histological examination, were labelled as indicated on Plate 1. The letters in front of the numerals above, immediately around or on the coronally-cut brain segments correspond to those listed in columns 1 and 2 in Table 1.

(i) - Meninges

Quantitatively, the degree of involvement varied considerably from one part of the meninges to another. Qualitatively, the cellular infiltrates of affected areas of the leptomeninges were essentially the same over all surfaces of the brain. The exudate was of a non-suppurative nature and was essentially a pleocytosis, consisting of morula cells of Mott, histiocytes, plasma cells and lymphocytes [Plate 7 (48)] in that order according to ascending frequency of their occurrence.

With only few exceptions, the leptomeninges of the sulci [Plate 2 (6 and 8)] and fissures were more severely affected than those of gyri and smooth surfaces at the base of the diencephalon, mesencephalon and metencephalon. The leptomeningitis of the sulci and fissures was very severe over the dorsolateral and the latero-lateral ventral part of the occipital lobes of the cerebrum and even more so over the nodular lobe and paravermis of the cerebellum. With few exceptions the leptomeningitis in most sulci and fissures of other parts of the cerebral hemispheres was rated as "severe" and this was also the case for the rest of the cerebellum. The leptomeninges covering the anterior medullary velum were not severely affected [Plate 2 (10)]. At their mouths [Plate 4 (24)] and deepest portion, the leptomeninges of sulci were most prominently affected and thus the contrast between those at the mouths with those of the adjacent gyri was the greatest. The most outstanding contrast in the degree of involvement was seen between the sulci of the cerebellum, which rated "most severe", and other cerebellar areas, which rated "very mild". The leptomeninges of the ventral area

of the myelencephalon were either not involved or only mildly affected.

On a specific section of brain the most severely affected areas of the meninges, as determined by the degree of vasculitis, perivascular infiltration and the incidence of Mott cells, were with few exceptions, usually in the meninges of the sulci and fissures. The findings have been recorded in Columns 5, 6 and 7 of Table 1. Some sulci were greatly distended by the hypercellularity of the inflamed meninges [Plate 2 (11)].

The adventitia of practically all meningeal vessels in areas of leptomeningeal involvement were heavily infiltrated with Mott cells, histiocytes, plasma cells and lymphocytes [Plate 2(7)]. These cell types were also frequent in the layers of the wall of veins. Numerous fibroblasts as well as fibrocytes and an excess of collagen, indicated the degree of chronicity of the leptomeningeal inflammation [Plate 2(9)]. The thickness of the leptomeninges appeared to depend upon both the degree and duration of involvement.

Continuous with the cells in the adventitia, there was a concentration of inflammatory cells in the meninges immediately surrounding the vessels. This was especially conspicuous where a vessel had been sectioned in a longitudinal plane [Plate 6(43)]. As a result of perivascular oedema in less chronically affected areas, the rather loose arrangement of cells in the meninges was occasionally associated with focal haemorrhages.

Mott cells were found anywhere in the leptomeninges from the junction with the dura mater and underlying neuroglial membrane, even the most superficial region of the arachnoid. They were very numerous in the highly cellular or most involved portion of the meninges. Their frequency and size varied at different sites in the meninges and brain. Generally speaking, most of them were in a size range from 10 to 20 microns. They harboured hyalin, cytoplasmic globules which varied greatly in size and frequency. These globules gave the cell a mulberry-like appearance from which the name, "morular cell", was derived (Mott, 1906, 1907). They were brilliantly stained with PAS and ranged from approximately 2 microns to a size which seemed to fill most of the cytoplasm of the largest Mott cells [Plate 7 (52)]. Large globules were apparently formed by coalescence of many small ones. Mott cells were seen within the lumen, the intima [Plate 7 (49)] and the adjacent portion of the vein wall [Plate 7 (50)]. Outside the vessels they appeared singly [Plate 7 (51)] or in groups [Plate 7 (46 and 47)]. In sections, their surfaces were either smooth or studded in appearance [Plate 7 (53 and 54)].

(ii) - Brain

A striking lesion involving the neuroglial, subpial limiting membrane of the brain, for practical purposes, was almost directly proportional to the degree of involvement of the overlying leptomeninges. This lesion, a severe gliosis, markedly altered the appearance and thickness of the subpial limiting membrane. Not only was there an increase in the number of glial cells and fibres but there were also many hypertrophic glia. They were abnormally large, bizarre-appearing cells which sometimes presented themselves in clusters causing the glial formations to simulate microminiature tumbleweeds [Plate 5 (31)] or bushes. At this stage of discussion, it will be necessary to draw the attention of readers to the histological feature of the subpial limiting membrane in mature, normal horses. Although this membrane, which is composed of neuroglia, is normally fairly prominent over the telencephalon [Plate 3 (12)] and over several other regions of the brain, it is apparently absent over the cerebellum. It could not be demonstrated in this locality in sections from the normal horse brain used for comparative studies [Part 1, Plate 2 (10 and 12)] nor was there any evidence of a subpial membrane of a horse in which there appeared a most severe involvement of the leptomeninges [Plate 4 (30)]. The exact distribution of the subpial neuroglial membrane in a normal horse is unknown to the writers nor could it be established whether or not anatomical studies on this tissue have been published.

Corresponding to the quantitative aspect of the overlying leptomeningitis, the subpial gliosis was most prominent over the cerebral hemispheres. To emphasize this feature a series of photomicrographs was prepared starting with the usual thickness of a normal subpial limiting membrane of a horse [Plate 3 (12)], for comparison, and running the gamut of the spectrum of the lesion [Plate 3 (13 to 23)]. As might be expected from the degree of the leptomeningitis, the subpial gliosis was very severe at the mouths of the sulci [Plate 4 (24)] and at their greatest depth. The occipital lobes of the cerebrum showed the most intense subpial gliosis [Plate 3 (23)]. Other lobes of the cerebrum were only slightly less involved and differed from one area to another as recorded in Table 1. Depending upon the degree of leptomeningeal involvement, the surfaces of the brain, devoid of either sulci or fissures, were also moderately affected by subpial gliosis.

The subpial gliosis had an isomorphous or an anisomorphous pattern but more often of the latter design. At some sites the gliosis resembled a hedge [Plate 3 (22)], at others it was of a bushy nature [Plate 4 (26 and 29)] and then again at some places it appeared to be composed of both patterns [Plate 3 (19)]. Both protoplasmic (plump) and fibrous (naked) astrocytes [Plate 5 (31 and 34)] occurred, the latter being far more numerous than the former. With Holzer stains the great increase in the number of fibres was clearly demonstrated so that the hypertrophic fibrous astrocytes had the appearance of miniature spiders [Plate 4 (25)]. Sucker attachments to vessels were frequently observed [Plate 5 (38)] and when highly magnified, the beaded or knobbly texture of the fibres became visible [Plate 5 (34)]. Isolated individuals or clusters of hypertrophic fibrous astrocytes were frequently situated between the subpial membrane and the pia mater [Plate 4, (25, 26 and 29)].

In the telencephalon, neuroglial fibres often extended from the subpial limiting membrane into the underlying zonal area [Plate 4 (27)] where they were accompanied by a marked numerical increase of discrete neuroglia [Plates 4 (27), and 5 (32)]. Significantly more neuroglia than are normally present were found in other deeper areas of the brain. The degree of their incidence has been recorded in Column 10 of Table 1. Fibrous astrocytes were responsible for nearly all the gliosis in these areas of which many were prominently affected. These included the lateral to the lateroventral part of the frontal lobe, especially the subcortical white matter [Plate 5 (35)], corona radiata, internal capsule, body of the fornix, pes hippocampus, hippocampal fimbria, hippocampal commissure, splenium of corpus callosum, pes pendicle, subthalamus, pyriform lobe, pulvinar, optic tract [Plate 5 (33)], lateral geniculate body, substantia nigra, central grey matter, brachia of the collicula aboralis, collicula oralis, occipital lobe, pons [Plate 5 (36)] and the anterior medullary velum [Plate 5 (37)]. Many fibrous astrocytes normally occur

within the subependyma but a greater number than normal was present at some sites of this region. In addition, a substantial numerical increase of these cells was encountered around vessels of the brain substance [Plate 4 (28)]. Ependymal cells seemed to be unaltered.

In association with the gliosis in many areas of the white matter, probably preceding it, there was a marked depletion of myelin. This manifestation was best illustrated in the optic tract [Plate 11 (73 and 75)] after applying the LFB stain. The affected tract had an achromatic appearance instead of a dark blue colour invariably acquired by normal tissue after staining. Although the degree of demyelination was pronounced in the right optic tract, it was less extensive in that of the opposite side. Diminution of myelin caused a discontinuity of the sheath as evidenced by the segmented demyelination of the optic tract. Holzer stains, applied to the same areas, revealed a striking degree of gliosis [Plate 11 (74)].

Damaged and depleted myelin sheaths were also encountered in the hippocampal fimbria, subcortical white matter, especially that of the gyrus cinguli, collicula oralis, body of the fornix, corona radiata and the septum pellucidum. It is possible that a more detailed survey on the extent of demyelination would have revealed that even more extensive areas were involved.

The remaining lesions of any significance in the brain involved the wall and the immediately surrounding vicinity of large and small blood vessels [Plate 6 (40)]. These included a vasculitis, perivascular cuffing and inflammation of the pia mater accompanying many of the vessels. In some of them it was difficult to determine what the sequence of events had been. The reactions were confluent and no distinct line of demarcation existed between the termination of the vasculitis [Plate 6 (39)] and the commencement of an extensive perivascular cuffing [Plate 6 (40)]. The inflammatory cells in the wall of vessels and perivascular tissues were identified as histiocytes, lymphocytes, Mott and plasma cells. The space-occupying cells in vein walls had often caused a marked stenosis of the lumen [Plate 6 (42)]. Besides the above-mentioned cell types, an occasional binucleated one appeared in the perivascular infiltrate and cellular proliferation. Although cells were sometimes distinctly and discretely suspended in a prominent Virchow-Robin space [Plate 6 (43)], it appeared that most vessels were surrounded by cells within a reticular network in which they were separated from each other by fibres. Many cells possessed projections with which they were attached to the network. Cells were found also beyond the Virchow-Robin space within the adjacent brain substance [Plate 6 (44 and 45)].

Cells were situated around the entire perimeter of large, medium and small veins but only in a linear pattern along one side of the venules. This arrangement caused affected vessels to be more prominent. An odd lymphocyte, plasma or Mott cell was situated next to a capillary or alternatively in the brain substance [Plate 7 (51)] in which capillaries were not visible.

Some vessels had a prominently infiltrated wall with numerous cells harbouring large nuclei, a conspicuous perivascular Virchow-Robin space containing an odd mononuclear cell and a highly cellular meningeal sheath [Plate 6 (41)]. Cells in the meninges were of the large mononuclear type.

For a uniform method of rating the extent to which specific brain regions, listed in Columns 11 and 12 of Table 1, had been affected, the degree of the severity of vasculitis and the thickness of perivascular cuffs, together with the number of affected vessels were selected as a suitable basis. It was fully realized that for comparative studies, it would be essential to apply the same standard when rating lesions exhibited by Stallion 5259, Mare 5588 and Gelding 5065. In Table 1 the sign "++" (moderate) reflected the highest rating given to any of the affected brain regions which included most of the frontal lobe, external capsule, corpus callosum, rostral commissure, gyri insulae, some areas of the corona radiata, body of the fornix, dorsomedial part of the thalamus, dorsolateral part of the parietal lobe, commissure of the subthalamus, pulvinar and lateral geniculate body. Both grey and white matter were represented in the cited areas. The impression was gained that the subcortical white matter was more affected than the cerebral cortex [Plate 9 (66)]. A comparison, based on the degree of vasculitis and perivascular cuffing, made it apparent that lesions in the mesencephalon, metencephalon and myelencephalon rated much lower than those in the telencephalon and diencephalon.

The distribution and concentration of Mott cells in the brain have been recorded in Column 13 of Table 1. Their presence within the lumen, vessel wall, Virchow-Robin space or adjacent to vessels too small to possess such a space, and as single cells in the brain substance, was a prominent feature in Stallion 5259. Mott cells were more numerous in the telencephalon and diencephalon than in other parts of the brain. They were frequently seen in the frontal lobe, external capsule, olfactory trigone, olfactory stria, internal capsule, gyri insulae, corpus callosum, dorsamedial part of the thalamus and the corona radiata. In addition they were often seen in the zonal layer of the cerebral cortices immediately beneath the subpial limiting membrane and the ependyma of the cerebral aqueduct [Plate 8 (56 and 57)].

(iii) Observations on other parts of the nervous system

Gasserian ganglion and *trigeminal nerve* - Mott cells and plasma cells were frequent in the gasserian ganglion [Plate 8 (61)] and in the endoneurium and perineurium of the trigeminal nerve [Plate 8 (60)].

Optic nerve - The meningeal sheath of the third cranial nerve was heavily infiltrated with lymphocytes and large mononuclear cells. Mott cells were present in the sheath [Plate 8 (59)] as well as between the nerve fibres [Plate 8 (58)].

Spinal cord - The spinal meninges of the cervical region were mildly infiltrated with lymphocytes and plasma cells [Plate 7 (55)]. Mott cells occurred also in the meninges and in the grey and white matter of the spinal cord.

Dura mater - The luminal surface of the wall of the dorsal longitudinal sinus was characterized by a pleocytosis; the lymphocytosis being accompanied by the presence of Mott cells [Plate 9 (64 and 65)].

Some of the arachnoid villi showed a villitis with numerous lymphocytes, plasmocytes and Mott cells [Plate 8 (62 and 63)]. At other sites the "visceral" surface of the dura was infiltrated with the same three cell types.

Tela choroides - This web-like structure of the fourth ventricle harboured lymphocytes, Mott and plasma cells. Very few of the last cell type were seen in the metaplexus.

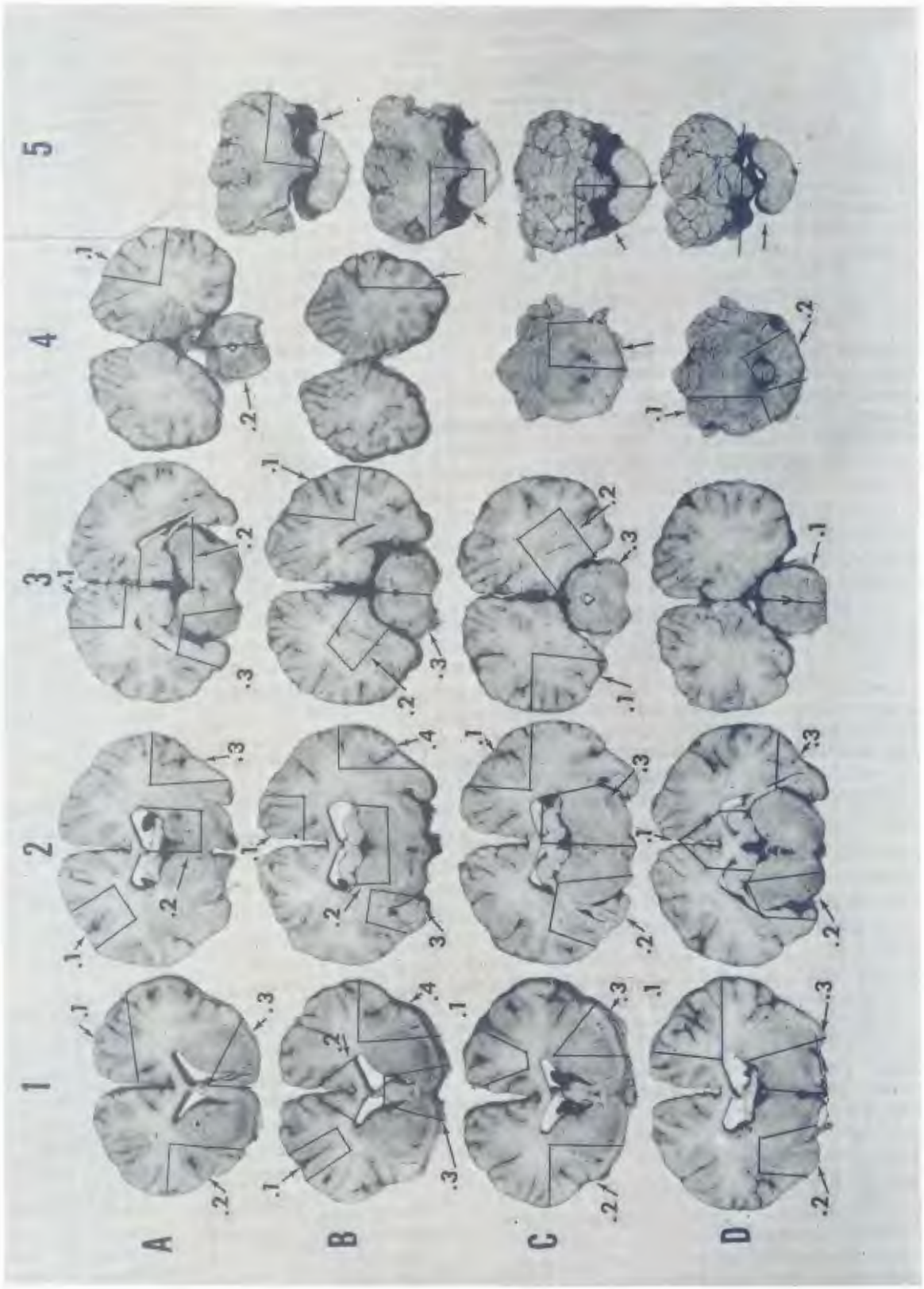

PLATE 1

1A to 5D. Coronal sections through the brain of Stallion 5259 showing the specific areas which were examined histologically (indicated by lines, figures and/or arrows). These numbers correspond to those in the first column of Table 1

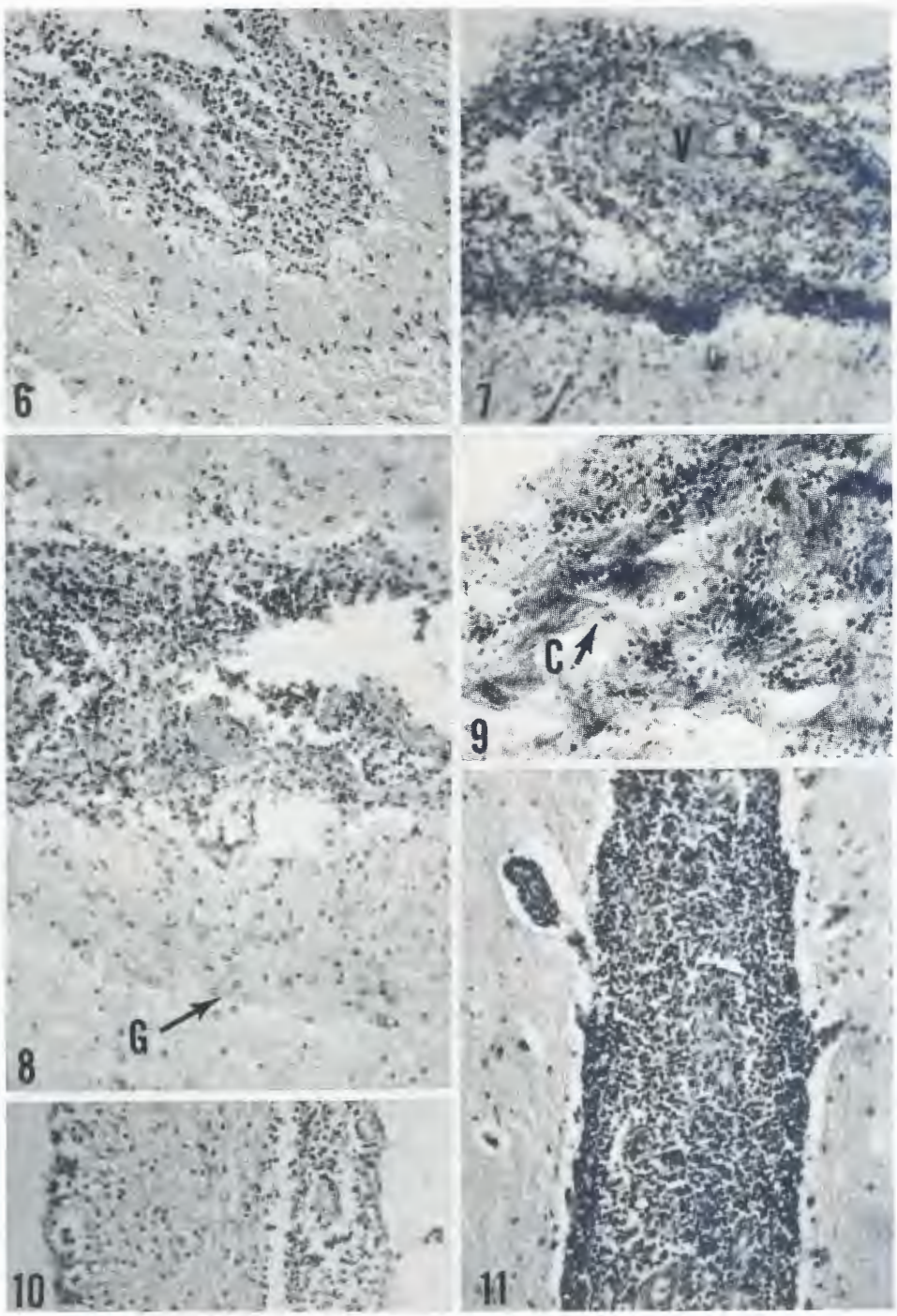

PLATE 2

6. Cerebral hemisphere. Leptomeningitis at depth of sulcus, × 75, HE
7. Cerebral hemisphere, level of olfactory trigone. Vein extensively involved in leptomeninges, × 150, LFB
8. Cerebral hemisphere. Severe involvement of leptomeninges of sulcus. Notice the thickening of the subpial limiting membrane (G arrow) due to gliosis, also apparent at top, × 150, HE
9. Leptomeninges, cerebrum. Collagenous fibres were more numerous in some areas of inflammation than in normal meninges, × 150, LFB
10. Anterior medullary velum. Round cell infiltration of the overlying leptomeninges, × 150, HE
11. Cerebellar hemisphere. Marked involvement of the leptomeninges, × 150, HE

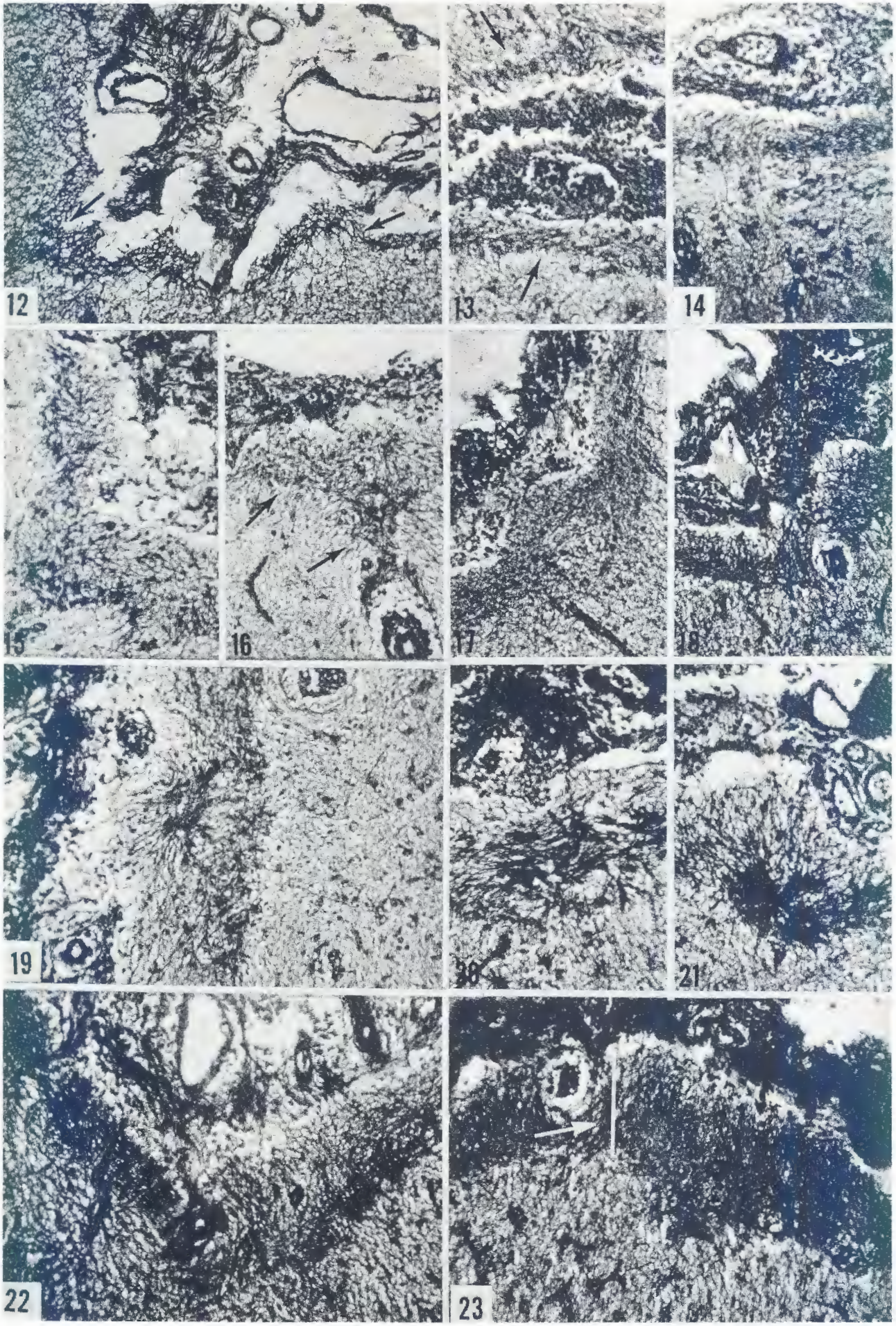
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PLATE 3

12. Cerebrum of a horse not affected for comparative purposes. Notice that the normal thickness of the subpial limiting membrane varies slightly depending on the sites (arrows) of sectioning, × 125, Holzer
13. Sulcus of dorsolateral part of frontal lobe. Leptomeningitis and thickening of the subpial limiting membrane (arrows) because of gliosis. Compare with the normal to the left (12). × 125, Holzer
14. Cerebrum. Slightly thicker subpial limiting membrane also with astrocytic processes extending into underlying zonal layer of cortex, × 125, Holzer
15. Dorsolateral part of parietal lobe. More thickened subpial limiting membrane due to hedge-like pattern of neurogliosis, × 125, Holzer
16. Mild leptomeningitis, cerebral gliosis extending from subpial limiting membrane along the course of vessel in the zonal layer of cortex, × 125, Holzer
17. Gliosis of subpial limiting membrane and leptomeningitis in a shallow sulcus of frontal lobe of cerebrum, × 125, Holzer
18. Sulcus of frontal lobe of cerebrum. In the deepest portion of the sulcus the subpial limiting membrane was the thickest due to extensive neurogliosis, × 125, Holzer
19. Cerebral hemisphere. An even thicker subpial limiting membrane because of both the bush and hedge-like patterns of neurogliosis, × 100, Holzer
20. Cerebral hemisphere. Thickened subpial limiting membrane resulting from hedge-like pattern of neurogliosis, × 100, Holzer
21. Frontal lobe of cerebrum. Thickened subpial limiting membrane resulting from bush pattern of neurogliosis, × 100, Holzer
22. Cerebrum. Very thick subpial limiting membrane along side of sulcus, × 100, Holzer
23. Occipital lobe, cerebrum. One of the thickest areas (arrow and line) of the subpial limiting membrane observed. Compare with the first illustration on this plate. All illustrations represent the same magnification, × 100, Holzer

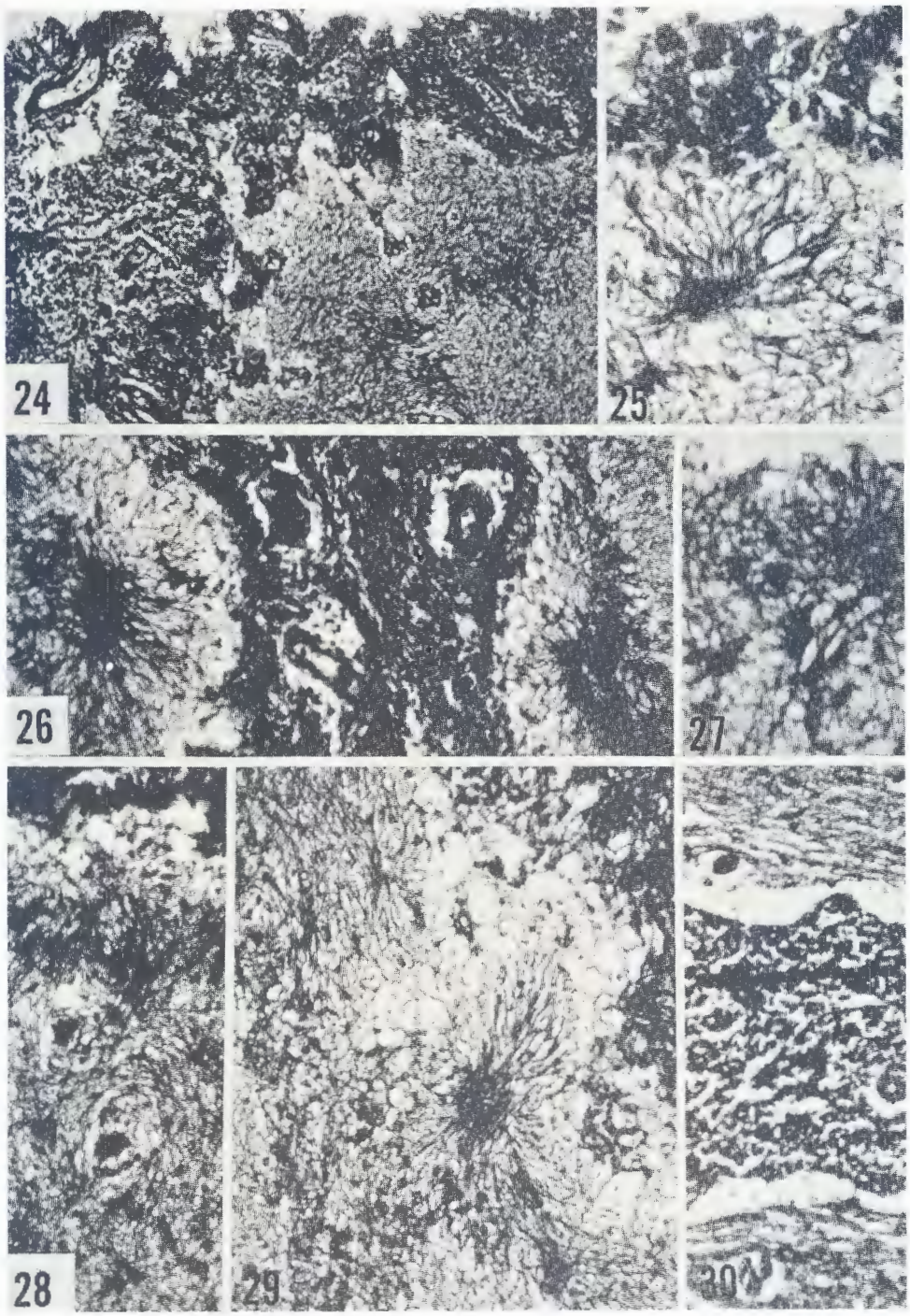

PLATE 4

24. Rostral part of parietal lobe. Involvement of leptomeninges of sulcus on the left compared to that of leptomeninges over gyrus at the top right. Notice also the thickened subpial limiting membrane situated right at the mouth of the sulcus, × 75, Holzer
25. Leptomeninges and subpial limiting membrane. Large fibrous astrocyte or spider cell immediately beneath the pia, × 320, Holzer
26. Sulcus of cerebral hemisphere. Bushy gliosis in subpial limiting membrane on both sides of the leptomeningitis seen in central portion of picture, × 320, Holzer
27. Occipital lobe of cerebral hemisphere. Astrocytes extending from thickened subpial membrane at top into the underlying zonal layer of the cortex, × 320, Holzer
28. Frontal lobe of cerebral cortex. Gliosis in zonal layer of cortex particularly severe perivascularly, × 150, Holzer
29. Dorsolateral portion of occipital pole of occipital lobe. Combination of the bush and hedge pattern of gliosis of the subpial membrane, × 150, Holzer
30. Leptomeningitis of cerebellum. Notice the complete absence of subpial gliosis, × 150, Holzer

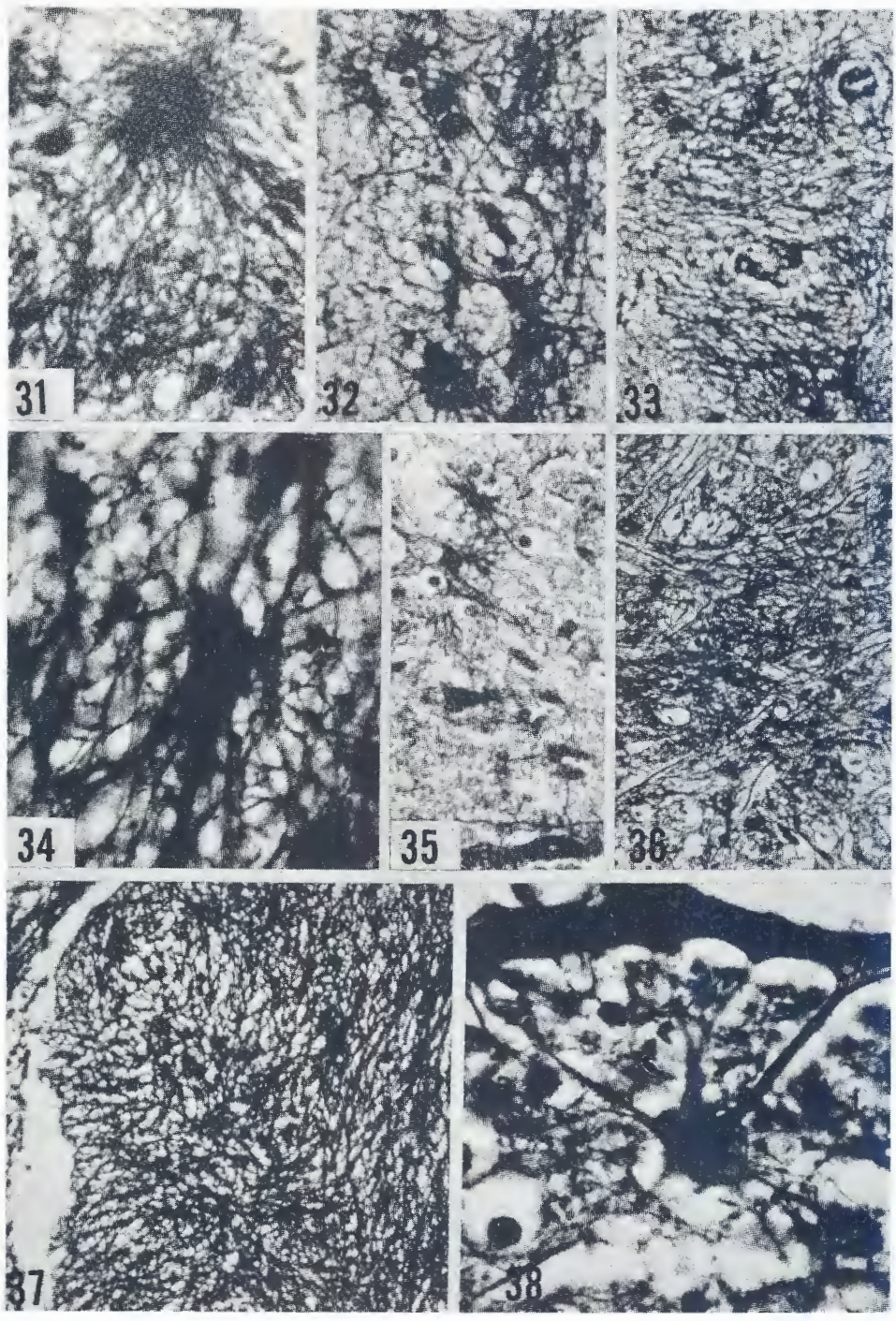

PLATE 5

31. Occipital lobe. Several fibrous astrocytes wound up in a mass and resembling a tumbleweed. These are located between the leptomeninges (out of the picture at the top) and the limiting membrane below, × 320, Holzer
32. High magnification of astrocytes in the zonal layer of the cerebral cortex, × 320, Holzer
33. Optic tract. Extensive gliosis. Note spiral pattern around blood vessels, × 150, Holzer
34. Oil immersion of the tangle of glial fibres in the zonal layer of the cortex just beneath the subpial limiting membrane, × 750, Holzer
35. Fibrous astrocytes in subcortical white matter, × 320, Holzer
36. Metencephalon. Gliosis in rostral portion of pons, × 150, Holzer
37. Anterior medullary velum. Excessive numbers of glial cells were present, × 150, Holzer
38. Blood vessel (top) with sucker plates of the astrocytic processes attached to wall (arrow), × 750, Holzer

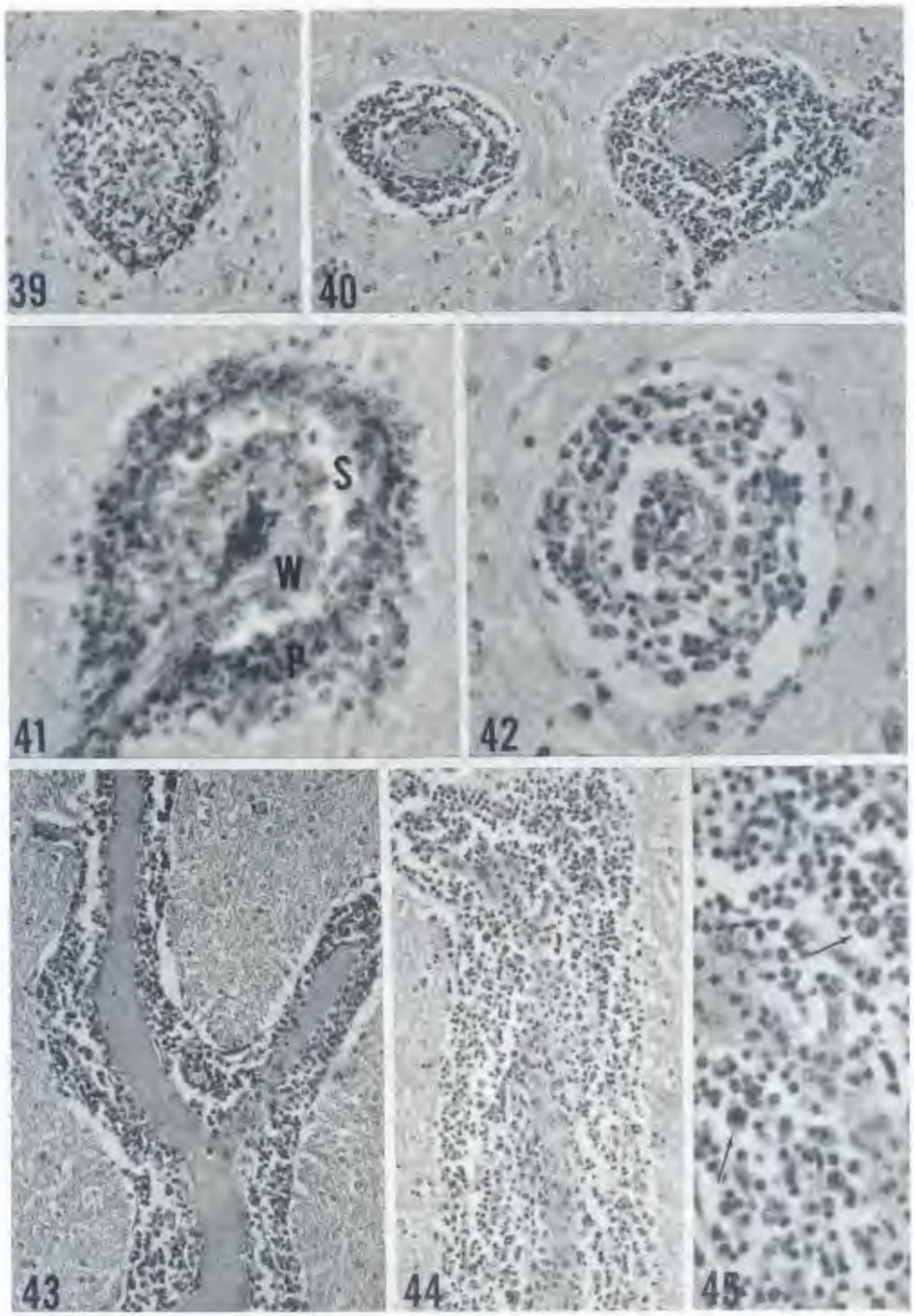

PLATE 6

39. Subcortical white matter, frontal lobe. Pronounced vasculitis but relatively mild perivascular cuffing, × 150, HE
40. Subcortical white matter, frontal lobe. Primarily thick perivascular cuffs surrounding two veins with little vasculitis, × 150, HE
41. Subcortical white matter, frontal lobe. Markedly thickened vein wall (W), few cells in the Virchow-Robin space (S) and greatly thickened pial membrane (P), × 320, LFB
42. Subcortical white matter, frontal lobe. Vein with greatly diminished lumen and thick wall due to vasculitis with thick cuff, × 320, HE
43. Subcortical white matter, frontal lobe. Longitudinally sectioned branching vein showing relative uniformity of the perivascular cuff, × 150, HE
44. Subcortical white matter of occipital lobe. Lymphocytes and large mononuclear cells extending beyond the Virchow-Robin space into adjacent white matter, × 150, HE
45. Higher magnification of preceding cells to show the large mononuclear cells (arrows) and lymphocytes, × 320, HE

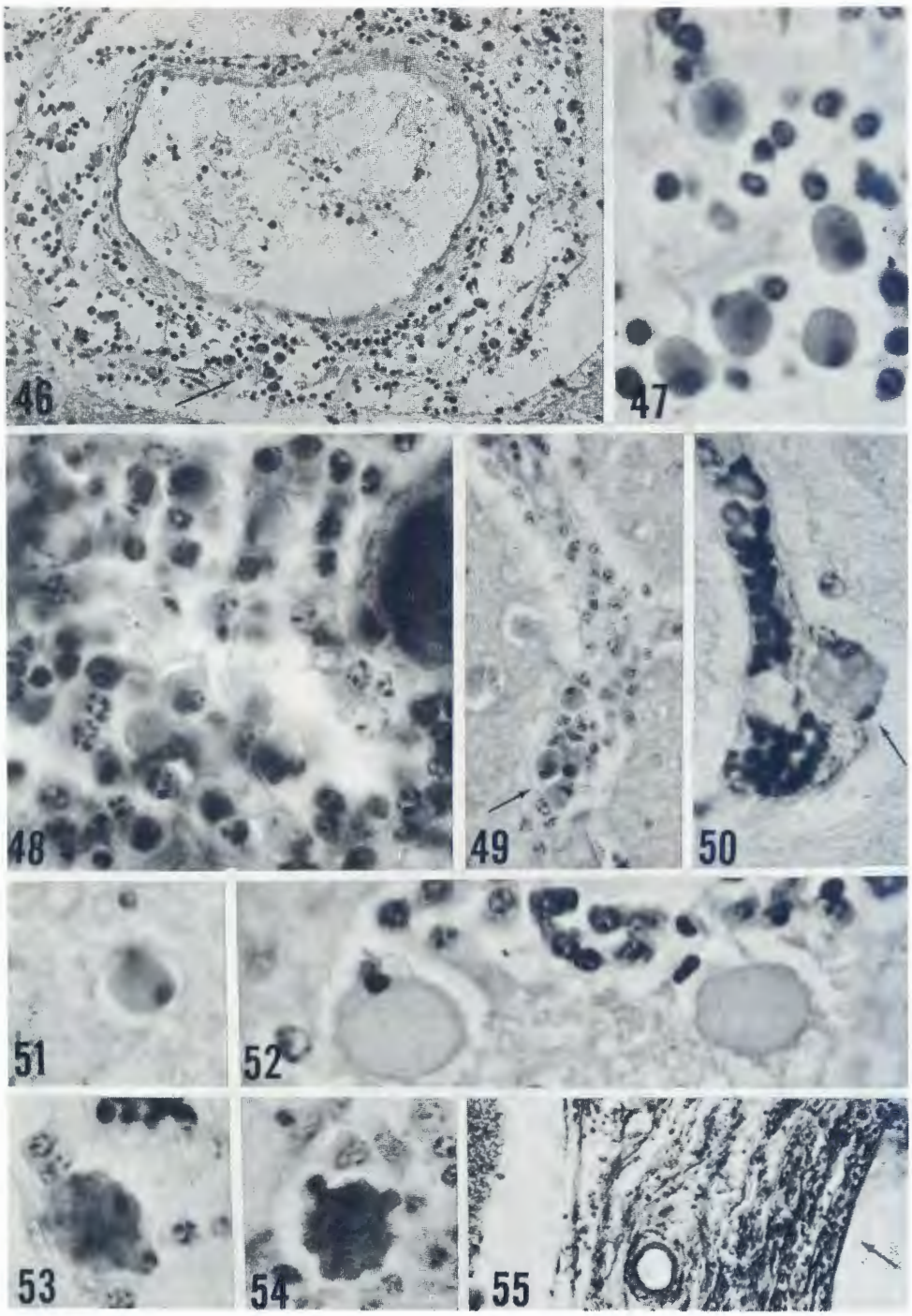

PLATE 7

46. Hippocampus. Perivascular oedema and cellular infiltrate composed of lymphocytes, plasma cells and morular cells of Mott (arrow), × 150, HE
47. Morular cells of Mott with higher magnification of group pointed to by arrow in preceding picture, × 750, HE
48. Lymphocytes, plasma cells and morular cells of Mott in leptomeninges, × 750, HE
49. Mott cells (arrow) in lumen of small vein, × 320, LFB
50. Mott cell just outside a small vein (arrow), × 750, LFB
51. Mott cell in subcortical white matter, × 750, HE
52. Two very large Mott cells outside small vein, × 750, HE
53 and 54. Very knobbly appearing surfaces of two Mott cells just outside small vessels, × 750, HE
55. Spinal cord meninges with meningitis (arrow), × 150, LFB

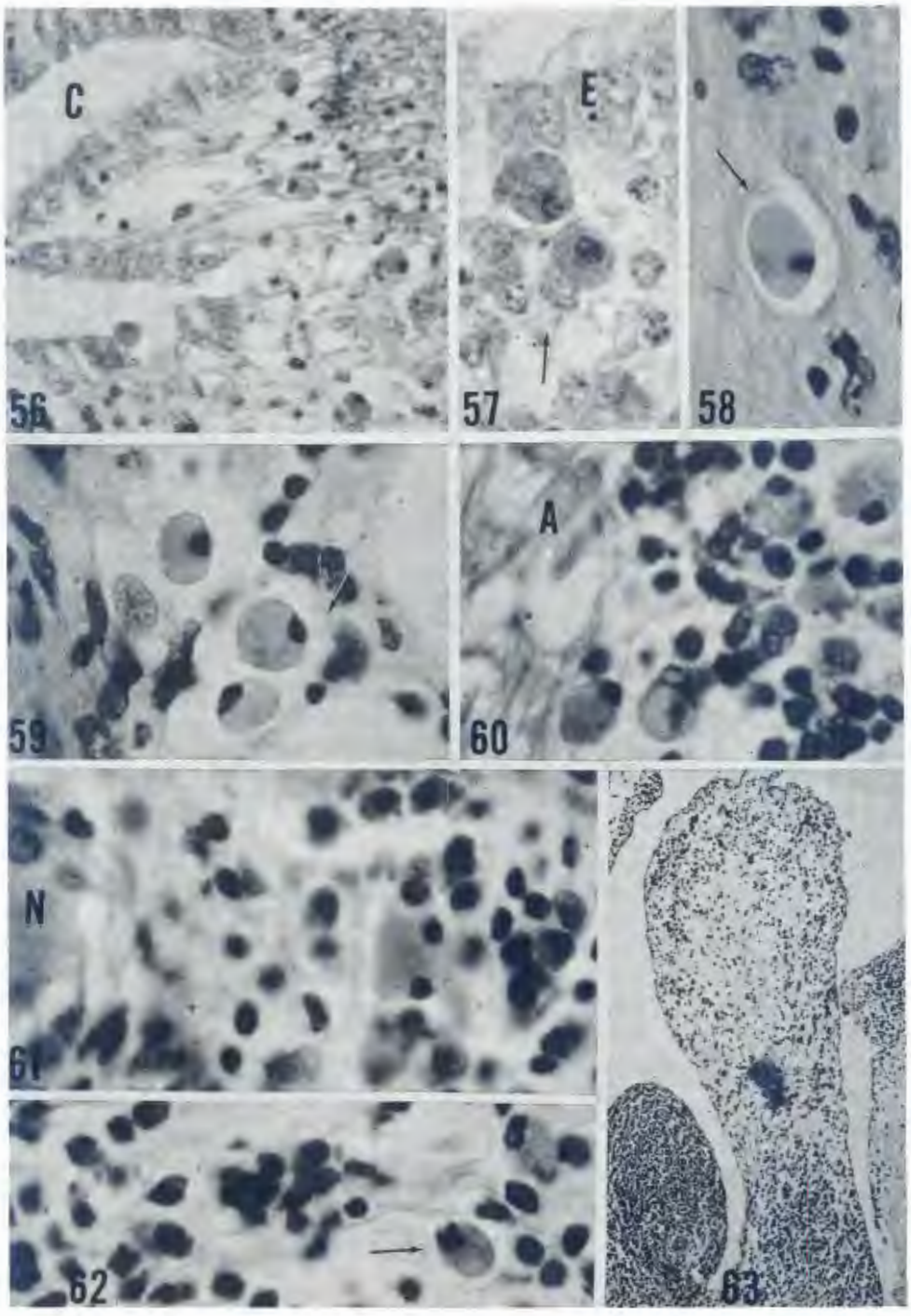

PLATE 8

56. Numerous Mott cells subependymally around the cerebral aqueduct (c), × 320, LFB
57. Higher magnification of Mott cells beneath the ependymal cells (E) of the cerebral aqueduct, × 750, LFB
58. Large Mott cell in optic nerve, × 750, HE
59. Mott cells in the subarachnoid space (arrow) of optic nerve, × 750, HE
60. Lymphocytes, plasma and Mott cells in the trigeminal nerve (A), × 750, HE
61. Ganglion cell (N), lymphocytes, plasma and Mott cells in the gasserian ganglion, × 750, HE
62. Lymphocytes, plasma and Mott cells within an arachnoid villus of the dorsal saggital sinus, × 750, HE
63. Arachnoid villitis in the dorsal saggital sinus, × 150, HE

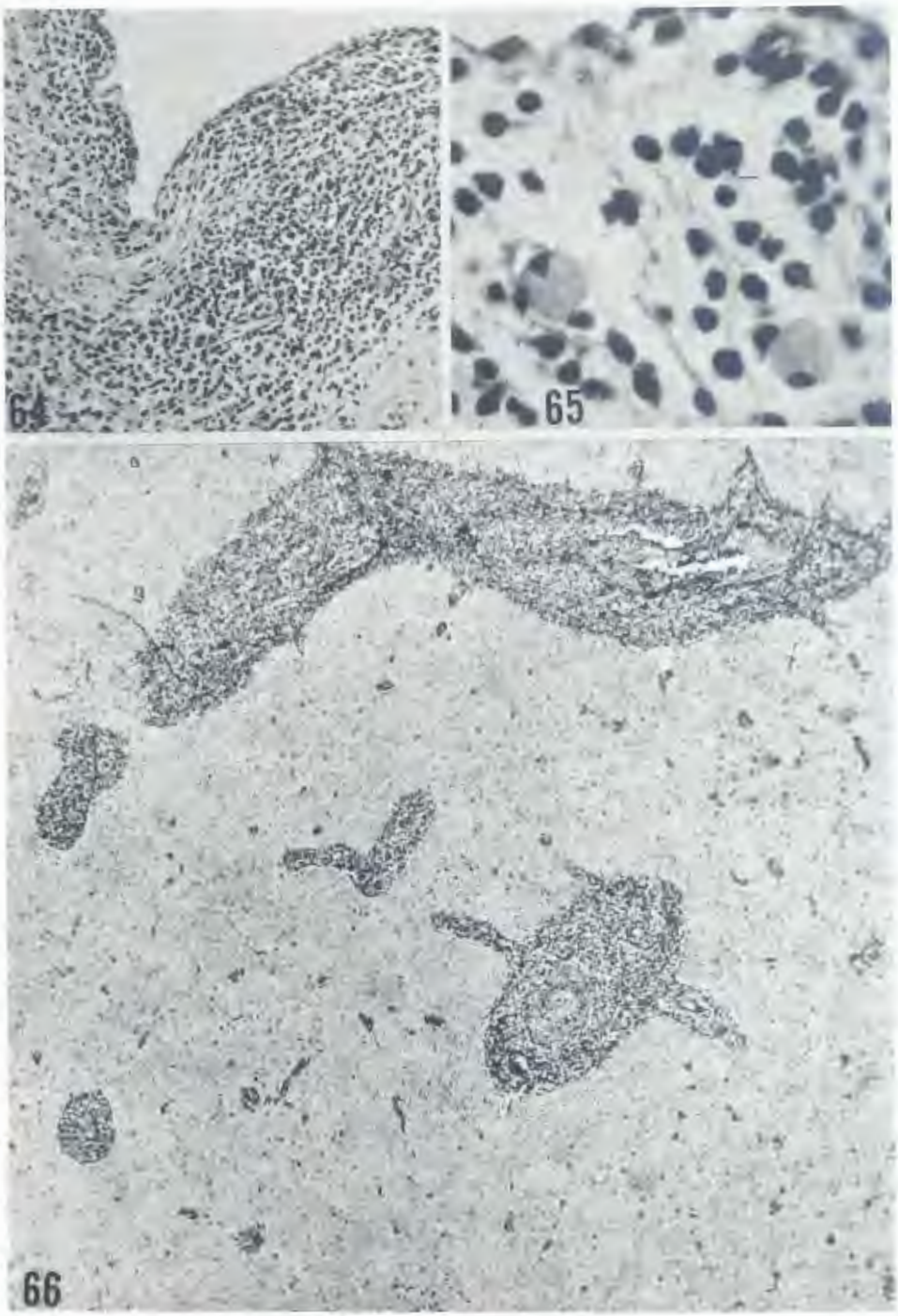

PLATE 9

64. Sinusitis of the dorsal saggital sinus, × 150, HE
65. Higher magnification of Mott cells and lymphocytes in the wall of the dorsal saggital sinus, × 750, HE
66. Low magnification to show the frequency of involved vessels in the subcortical white matter of cerebrum, × 75, HE

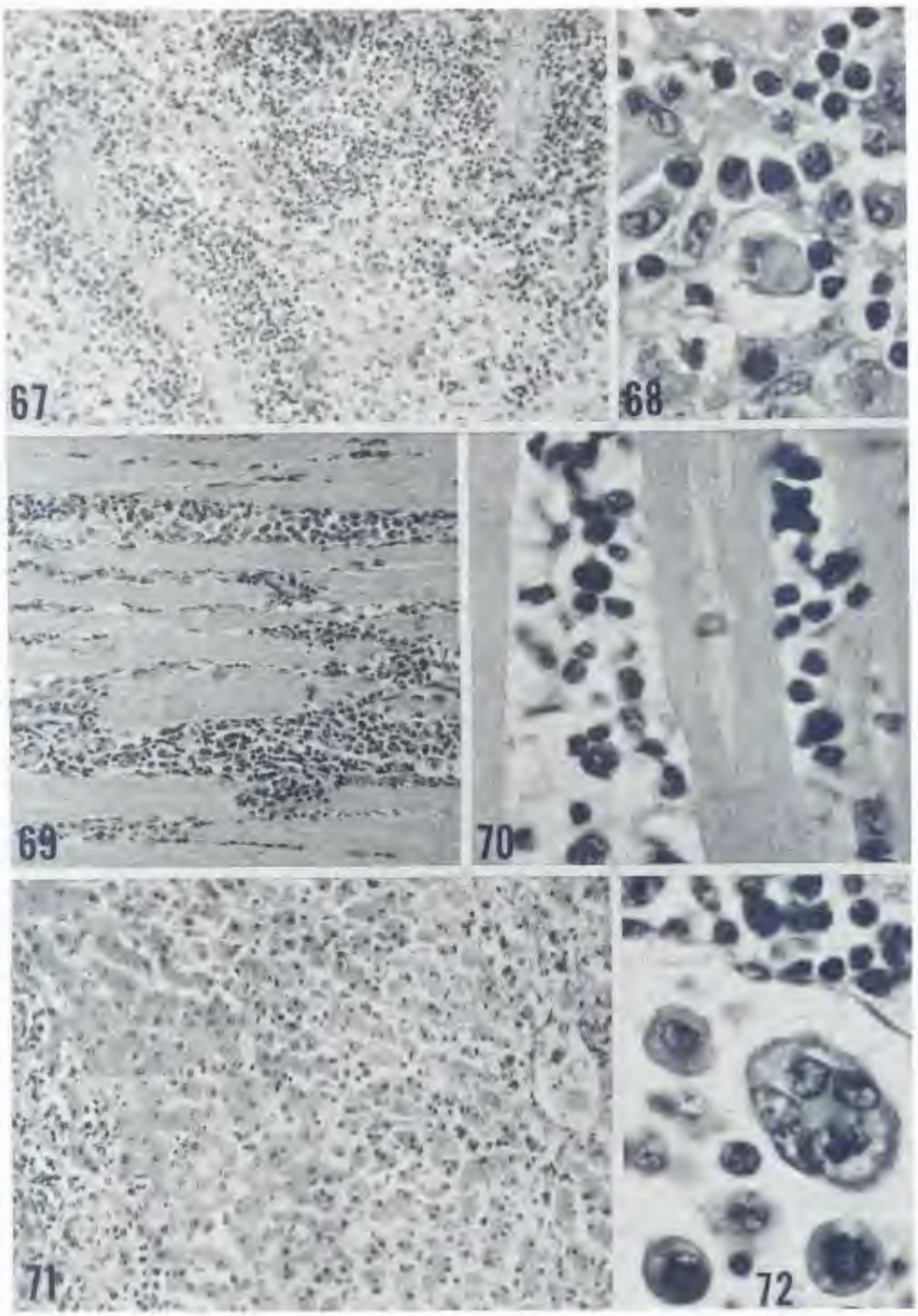

PLATE 10

67. Posterior pituitary. Heavy perivascular cuffing and round cell infiltration, × 150, HE
68. Posterior pituitary. High magnification of lymphocytes, and other mononuclear cells including a Mott cell, × 750, HE
69. Skeletal muscle. Interstitial myositis with numerous lymphocytes, plasma cells and large mononuclear cells, × 150, HE
70. Myocardium. Similar cells in diffuse interstitial myocarditis, × 750, HE
71. Liver lobule. Atrophy, degeneration and necrosis of liver cord cell centrolobularly as a result of chronic passive congestion. Notice also the round cell infiltrates in the periportal area, × 150, HE
72. Lymph node. Multinucleated giant cells in the medullary sinuses, × 750, HE

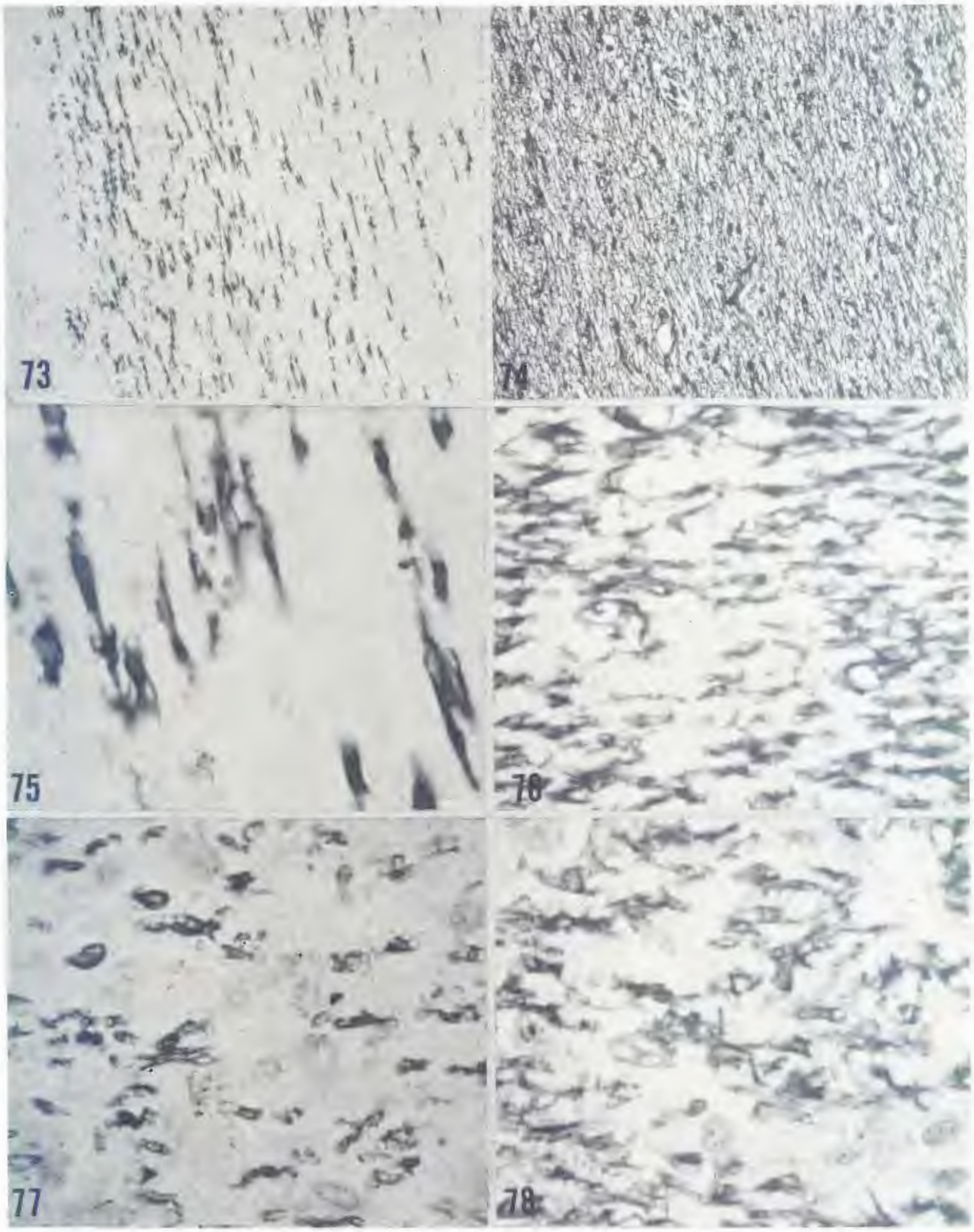

PLATE 11

73. Stallion 5259: Optic tract. Extensive segmental demyelination, × 150, LFB
74. Stallion 5259: Optic tract. Extensive gliosis with fibrous astrocytes predominating, × 150, Holzer
75. Stallion 5259: Optic tract. More highly magnified view of segmental demyelination, × 750, LFB
76. Mare 5588: Optic tract. Some demyelination of segmental nature, × 750, LFB
77. Gelding 5065: Left optic tract. Marked segmental demyelination, × 750, LFB
78. Gelding 5065: Right optic tract. Marked segmental demyelination, × 750, LFB

TABLE 1 *Microscopic findings in the central nervous system of Stallion 5259*

Coronal section No.	Microscopic preparations corresponding to those areas indicated on Plate 1	Meninges							Brain			
		Meningitis					Subpial area		Other areas			
				*Most severely affected areas			Gliosis					
		of sulci or fissures	of other areas	Vasculitis	Perivascular infiltrate	Mott Cells	of sulci or fissures	Non-sulcial and/or non-fissural parts	Gliosis	Vasculitis	Perivascular cuffing	Mott Cells
1A	Through cerebral hemispheres (telencephalon) at level of the olfactory trigonum 1 – Dorsal cerebral hemisphere, frontal lobe	++	+	+	++	++	++	±	±	++	++	++
	2 – Lateral to lateroventral portion of frontal lobe	++	±	+	++	+	++	±	±	++	++	+
	containing external capsule. .	•	•	•	•	•	•	•	+	++	++	++
	and putamen	•	•	•	•	•	•	•	±	+	+	±
	3 – Ventral to ventrolateral portion of frontal lobe	•	++	+	+	+	•	++	+	++	++	++
	including olfactory trigone, . .	•	•	•	•	•	•	•	+	++	++	++
	olfactory stria	•	•	•	•	•	•	•	—	++	++	++
	and internal capsule	•	•	•	•	•	•	•	±	++	++	++
1B	Through cerebral hemisphere at level of the rostral commissure 1 – Dorsolateral part of frontal lobe	++	+	+	+	+	±	±	±	++	++	++
	2 – Cingulate gyrus, ...	++	+	±	+	++	++	+	±	+	+	+
	corpus callosum, ...	•	•	•	•	•	•	•	+	++	++	±
	and septum pellucidum . . .	•	•	•	•	•	•	•	+	—	—	—
	3 – Genu of corpus callosum . .	•	•	•	•	•	•	•	+	—	—	—
	and rostral commisure . . .	•	•	•	•	•	•	•	+	+	++	+
	4 – Lateral to lateroventral part of the frontal lobe	++	±	±	+	+	++	±	++	±	±	±
	containing the rostral part of insulae gyrus	++	±	±	+	+	++	±	+	++	++	++

1C	Through cerebral hemisphere at the level of the rostral commisure and rostral end of the optic commisure 1 – Dorsal longitudinal superior gyrus of the frontal lobe . . .	++	±	±	+	++	++	±	+	+	+	+
	and containing the corona radiata	•	•	•	•	•	•	•	+	+	+	±
	2 – Lateral to lateroventral part of frontal lobe	+	±	+	+	+	+	±	+	+	+	+
	containing putamen, ...	•	•	•	•	•	•	•	—	±	±	±
	external capsule, ...	•	•	•	•	•	•	•	+	+	+	+
	claustrum, ...	•	•	•	•	•	•	•	±	±	±	±
	insular	•	•	•	•	•	•	•	±	±	±	±
	and lateral olfactory stria . .	•	•	•	•	•	•	•	±	—	—	—
	3 – Lateroventral part of frontal lobe	•	±	—	—	—	•	—	+	+	+	+
	containing globus pallidus, . .	•	•	•	•	•	•	•	±	+	+	+
	putamen, ...	•	•	•	•	•	•	•	±	±	±	±
	external capsule	•	•	•	•	•	•	•	++	—	—	—
	and lateral olfactory stria . . .	•	•	•	•	•	•	•	±	—	—	—
1D	Through the cerebral hemispheres (telencephalon) and the diencephalon at the level of the tuber cinerium and intermediate mass of thalamus 1 – Dorsolateral rostral part of parietal lobe with corona radiata . .	++	±	+	+	+	++	±	++	+	+	+
	2 – Rostral part of pyriform lobe	++	±	+	+	++	++	±	—	+	+	+
	and optic tract	•	•	•	•	•	•	•	++	—	—	—
	3 – Rostral part of thalamus, . .	—	—	—	—	—	—	—	±	+	+	+
	rostral part of intermediate mass	•	•	•	•	•	•	•	±	+	+	+
	and tuber cinerium	•	•	•	•	•	•	•	±	±	±	±

LEGEND FOR TABLES 1, 2 AND 3

— = negative
± = very mild
\+ = mild
++ = moderate
+++ = severe
++++ = very severe

* May be of sulci, fissures, or other areas

TABLE 1 *Microscopic findings in the central nervous system of Stallion 5259*

Coronal section No.	Microscopic preparations corresponding to those areas indicated on Plate 1	Meninges							Brain			
		Meningitis					Subpial area		Other areas			
		of sulci or fissures	of other areas	*Most severely affected areas			Gliosis		Gliosis	Vasculitis	Perivascular cuffing	Mott Cells
				Vascu-litis	Perivascular infiltrate	Mott Cells	of sulci or fissures	Non-sulcial and/or non-fissural parts				
2A	Through cerebral hemispheres (telencephalon) and through the diencephalon at the level of the caudal part of the intermediate mass, part of thalamus, hypothalamus and infundibulum 1 – Dorsolateral part of parietal lobe	++	±	+	+	±	++	±	±	+	+	+
	containing corona radiata . .	•	•	•	•	•	•	•	±	+	+	+
	2 – Body of fornix, ...	•	•	•	•	•	•	•	+	±	±	±
	body of caudate nucleus, . .	•	•	•	•	•	•	•	—	+	++	+
	rostral part of thalamus . . .	•	•	•	•	•	•	•	—	+	+	±
	and internal capsule . . .	•	•	•	•	•	•	•	++	+	+	±
	3 – Parietal lobe in the area of the lateral fissure, ...	++	±	+	+	+	+	—	±	+	+	+
	lateroventral part containing the insular gyrus	++	±	+	+	+	+	—	±	+	+	+
2B	Through cerebral hemispheres (telencephalon) and the diencephalon at the level of the caudal part of the intermediate mass part of the thalamus, hypothalamus, infundibulum and rostral part of the mammary body . . 1 – Dorsal part of the parietal lobe .	+	±	+	+	++	++	±	+	+	+	++
	2 – Body of the fornix	•	•	•	•	•	•	•	++	++	++	+
	and dorsomedial part of the thalamus	•	•	•	•	•	•	•	±	++	++	++
	3 – Pyriform lobe, ...	+	±	+	+	+	+	Sub-ependymal ++	++	+	+	±
	apex of lateral ventricle,, pes hippocampi	•	•	•	•	•	•	Sub-ependymal ++	++	++	+	+
	and optic tract	•	•	•	•	•	•	•	++	±	±	±
	4 – Lateroventral part of the temporal lobe	+	±	+	+	+	++	±	+	±	±	±

2C	Through cerebral hemispheres (telencephalon) and the caudal part of the diencephalon through the habenulae nuclei 1 – Dorsolateral part of the parietal lobe - the ectosylvius gyrus in the region of the ectosylvius sulcus .	++	±	+	++	+	++	±	+	+	++	+
	2 – Pyriform lobe containing pes hippocampi, ...	•	•	•	•	•	•	•	++	+	+	+
	fimbria	•	•	•	•	•	•	•	++	+	+	+
	and ventral horn of lateral ventricle, ...	•	•	•	•	•	•	•	•	•	•	•
	lateral part of the thalamus, . .	±	—	—	—	—	±	—	—	±	+	±
	hippocampal fissure and optic tract	±	±	—	—	—	±	±	+++	+	+	+
	3 – Caudal part of thalamus through mammary body, ...	•	—	—	—	—	—	—	+	±	+	±
	internal capsule, ...	•	•	•	•	•	•	•	+	±	±	±
	optic tract, ...	•	—	•	•	•	•	—	+++	+	+	+
	body of the fornix	•	•	•	•	•	•	•	++	±	±	+
	and hippocampus	•	•	•	•	•	•	•	++	+	+	+
2D	Through the occipital lobe of the cerebral hemispheres (telencephalon) and just rostral to the border between the diencephalon and the mesencephalon 1 – Splenium of the corpus callosum,	•	•	•	•	•	•	•	++	++	++	+
	commisure of the hippocampus .	•	•	•	•	•	•	•	++	++	++	+
	lateral and third ventricle, cingulate gyrus	++	±	+	++	++	++	±	±	++	++	+
	2 – Pes hippocampi, ...	•	•	•	•	•	•	•	++	+	+	+
	hippocampal fissure, fimbria, .	•	•	•	•	•	•	•	±	+	+	+
	laterocaudal part of the thalamus,	•	++	+	++	++	•	++	+	++	++	+
	pes pendicle, ...	•	—	—	—	—	•	—	++	—	±	±
	subthalamus	•	•	•	•	•	•	•	++	++	++	+
	and substantia nigra	•	±	±	±	+	•	++	+	+	+	±

TABLE 1 *Microscopic findings in the central nervous system of Stallion 5259*

Coronal section No.	Microscopic preparations corresponding to those areas indicated on Plate 1	Meninges							Brain			
		Meningitis					Subpial area		Other areas			
				*Most severely affected areas			Gliosis					
		of sulci or fissures	of other areas	Vasculitis	Perivascular infiltrate	Mott Cells	of sulci or fissures	Non-sulcial and/or non-fissural parts	Gliosis	Vasculitis	Perivascular cuffing	Mott Cells
	3 – Ventral horn of the fourth ventricle, pyriform lobe, ...	+	±	+	+	+	±	±	++	+	+	+
	pes hippocampus, ...	•	•	•	•	•	•	•	+	+	+	+
	fimbria, ...	•	•	•	•	•	•	•	+	±	±	+
	ventrotemporal lobe	±	±	±	±	±	+	±	+	±	±	±
3A	Through the occipital lobe of the cerebral hemispheres (telencephalon) and border of the diencephalon and mesencephalon 1 – Dorsomedial part of the occipital lobe - cingulate gyrus, .	+	±	±	+	+	++	±	+	+	++	±
	entolateral gyrus, lateral gyrus .	+	±	±	+	+	++	±	+	+	++	±
	and corona radiata	+	+	±	+	+	++	±	++	+	++	±
	2 – Lateral ventricle, ...	•	•	•	•	•	•	•	•	•	•	•
	fimbria, ...	•	•	•	•	•	•	•	±	+	+	+
	hippocampus, ...	•	•	•	•	•	•	•	++	+	++	+
	pulvinar of dorsal thalamus . .	±	±	±	±	±	+	±	++	+	++	+
	and lateral geniculate body . .	±	—	—	+	—	—	—	++	+	++	+
	3 – Pes pendicle, ...	±	—	—	—	—	•	+	±	±	±	±
	substantia nigra, ...	•	•	•	•	•	•	•	±	±	±	±
	medial geniculate body . . .	±	±	±	±	±	+	—	+	+	+	+
	and pes hippocampus . . .	•	•	•	•	•	•	•	++	+	+	+
3B	Through the occipital lobe of the cerebral hemispheres (telencephalon) and the mesencephalon through the rostral colliculus oralis, 1 – Dorsolateral part of the occipital lobe, ...	+++	+	+	++	++	+++	+	+	+	++	+
	ectosylvian gyrus, ...	+++	+	+	++	++	+++	+	+	+	++	+
	corona radiata	•	•	•	•	•	•	•	+	+	+	+

	2 – Occipital lobe, ...	±	•	±	±	±	±	•	+	±	+	+
	corona radiata, ...	•	•	•	•	•	•	•	++	±	+	++
	lateral ventricle, hippocampus .	•	•	•	•	•	•	•	++	±	±	±
	3 – Cerebral aqueduct, pes pendicle .	•	±	±	±	±	•	+	+	±	±	+
	substantia nigra, ...	•	•	•	•	•	•	•	±	—	—	±
	medial geniculate, ...	•	±	+	++	+	•	+	++	—	++	±
	red nucleus, ...	•	•	•	•	•	•	•	±	—	—	—
	central grey matter	•	•	•	•	•	•	•	++	+	+	±
3C	Through the occipital lobe of the cerebral hemispheres (telencephalon) and the mesencephalon through the caudal part of the colliculus oralis 1 – Laterolateral ventral part of occipital lobe, ...	+++	±	++	+++	++	++	+	+	+	+	+
	corona radiata	•	•	•	•	•	•	•	++	+	+	+
	2 – Occipital lobe, ...	±	±	±	±	±	+	+	+	+	++	+
	lateral ventricle, hippocampus .	•	•	•	•	•	•	•	++	±	±	±
	3 – Cerebral aqueduct, brachium of colliculus aboralis, ...	•	±	±	±	±	•	±	++	±	±	±
	colliculus oralis, ...	++	++	+	+	+	++	++	++	+	+	+
	red nucleus, ...	•	•	•	•	•	•	•	±	—	—	—
	substantia nigra, ...	•	•	•	•	•	•	•	±	+	+	+
	central grey matter	•	•	•	•	•	•	•	++	±	±	±
3D	Through occipital lobe of cerebral hemispheres (telencephalon) and the mesencephalon on the border of the colliculus oralis and aboralis 1 – Cerebral aqueduct, colliculus oralis, ...	+	+	±	±	+	±	±	++	+	+	+
	brachium of aboralis, . . .	•	±	±	±	+	•	±	++	±	±	±
	pes pendicle, ...	•	±	±	±	+	•	±	++	±	±	+
	red nucleus, ...	•	•	•	•	•	•	•	±	—	—	—
	substantia nigra	•	•	•	•	•	•	•	±	±	±	+
	and central grey matter . . .	•	•	•	•	•	•	•	++	±	±	±

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		of sulci or fissures	of other areas	Vascu-litis	Perivascular infiltrate	Mott Cells	of sulci or fissures	Non-sulcial and/or non-fissural parts	Gliosis	Vasculitis	Perivascular cuffing	Mott Cells
4A	Through the occipital lobe of the cerebral hemispheres (telencephalon) and the mesencephalon through the colliculus aboralis 1 – Dorsolateral portion of the occipital pole of the occipital lobe .	+	±	±	+	+	++	±	+	±	±	±
	2 – Colliculus aboralis	•	++	+	+	+	•	++	—	±	±	±
	pes pendicle, ...	•	±	±	±	±	•	±	++	±	±	±
	substantia nigra, ...	•	•	•	•	•	•	•	±	±	±	±
	cerebral aqueduct and central grey matter	•	•	•	•	•	•	•	++	±	±	±
4B	Near the caudal pole of the occipital lobe 1 – Lateroventral part of gyri of the occipital pole, occipital lobe . .	+	±	±	±	++	++	±	++	+	+	+
4C	Through metencephalon through the rostral part of the pons and the rostral part of the cerebellum 1 – Pons, ...	+	—	—	—	—	+	+	++	±	+	±
	dentate nucleus, ...	•	•	•	•	•	•	•	—	—	—	—
	interpositus nuclei, ...	•	•	•	•	•	•	•	—	—	—	—
	fourth ventricle brachium conjunctivum, ...	•	•	•	•	•	•	•	—	—	—	—
	lingula of the vermis	++	±	+	+	±	—	—	—	±	±	±
4D	Through metencephalon through the rostral part of the pons and cerebellum 1 – Vermis, ...	++	±	+	+	+	—	—	±	±	±	±
	paravermis, ...	++	±	+	+	+	—	—	±	±	±	±
	dentate nucleus	•	•	•	•	•	•	•	—	±	+	±

	2 – Fourth ventricle, lingula of vermis	++	±	+	+	+	—	—	—	±	±	±
	sulci medians and limitans, vestibular nucleus	•	•	•	•	•	•	•	—	±	+	±
5A	Through the caudal part of the cerebellum in the metencephalon and through the cranial part of the medulla oblongata 1 – Nodular lobe of vermis of cerebellum, ...	++	±	+	+	±	—	—	±	±	±	±
	anterior medullary velum, . .	•	±	±	±	±	•	+	++	—	—	+
	solitary nucleus, ...	•	•	•	•	•	•	•	—	±	+	±
	vestibular nucleus	•	•	•	•	•	•	•	—	±	+	±
5B	Through the caudal part of the cerebellum in the metencephalon and further caudal in the medulla oblongata	•	—	—	—	—	•	±	±	±	±	±
	1 – Hypoglossal nucleus, ...	•	•	•	•	•	•	•	—	±	±	±
	solitary nuclei, ...	•	•	•	•	•	•	•	—	±	±	±
	nucleus of the spinal root of the fifth cranial nerve, ...	•	•	•	•	•	•	•	—	±	±	±
	nodular lobe of the paravermis, .	++++	±	+	+	+	—	—	±	+	+	+
	anterior medullary velum . .	•	±	±	±	+	•	+	++	—	—	+
	and part of the vermis . . .	++++	±	+	+	+	—	—	±	±	±	±
5C	More caudal in the cerebellum and medulla oblongata 1 – Nodular lobe and paravermis of the cerebellum, ...	++++	±	+	+	±	—	—	+	±	±	±
	anterior medullary velum, . .	•	±	±	±	±	•	+	++	—	—	±
	choroid plexus of the fourth ventricle, olive, ...	•	•	•	•	•	•	•	—	±	±	±
	pyramid, ...	•	±	±	±	±	•	±	—	—	—	—
	medial lemniscus	•	•	•	•	•	•	•	—	±	±	±
5D	1 – Medulla oblongata	•	±	±	±	±	•	—	±	+	+	±

TABLE 2 *Microscopic findings in the central nervous system of Mare 5588*

	Microscopic section		Meninges		Brain			
			Meningitis					
No.	General area of the brain -	- containing the following specific anatomical structures	degree of	Mott cells	Subpial gliosis	Vascu-litis	Perivas-cular cuffing	Mott cells
A	Through lateral, latero-ventral, ventral and medial telencephalon	Lateral to lateroventral frontal lobe,	± to +	—	— to ±	—	—	—
		rectal gyrus,	—	—	—	—	—	—
		subcallosal gyrus,	±	—	—	—	—	—
		genu of corpus callosum,	•	•	•	+	+	—
		lateral ventricle, body of caudate nucleus,	•	•	•	—	—	—
		extreme, external and internal capsules,	•	•	•	—	—	—
		claustrium,	•	•	•	—	—	—
		putamen	•	•	•	+	+	—
		and olfactory tract	—	—	—	—	—	—
B	Through telencephalon	Olfactory tracts,	±	—	—	+	+	—
		claustra,	•	•	•	—	—	—
		external and internal capsules,	•	•	•	+	+	—
		lateral ventricles, caudate nuclei,	•	•	•	—	—	—
		subcallosal areas,	—	—	—	—	—	—
		genu of corpus callosum,	•	•	•	+	+	—
		radiatio corporis callosi	•	•	•	+	+	—

C	Through cerebral hemispheres and the diencephalon at level of caudal part of intermediate mass of thalamus, hypothalamus and infundibulum	Corpus callosum,	•	•	•	+	+	—
		body of fornix,	•	•	•	++	++	—
		intermediate mass of thalamus	•	•	•	±	±	—
		internal capsule,	•	•	•	++	++	—
		hippocampus,	•	•	•	±	±	—
		pyriform lobe,	—	—	—	++++	++++	—
		amygdaloid body,	—	—	—	++++	++++	—
		hypothalamus,	•	•	•	—	—	—
		external capsule,	•	•	•	+++	+++	—
		optic tract,	—	—	—	—	—	—
		infundibulum	—	—	—	—	—	—
D	Through lateral ventricles, hippocampus (telencephalon) and thalamus (diencephalon)	Corpus callosum,	•	•	•	+	+	—
		septum pellucidum,	•	•	•	+	+	—
		commissura fornicus,	•	•	•	—	—	—
		hippocampal fimbria,	—	—	—	—	—	—
		pes hippocampi,	•	•	•	—	—	—
		pulvinar,	•	•	•	++	++	—
		posterior commissure,	•	•	•	±	±	—
		crus cerebri,	—	—	—	—	—	—
		optic tract,	—	—	—	—	—	—
		lateral geniculate body	•	•	•	++	++	—
E	Through occipital lobe of cerebral hemisphere (telencephalon)	Corona radiata,	•	•	•	+++	+++	—
		hippocampus,	•	•	•	—	—	—
		occipital lobe, grey matter,	± to +++	—	± to +++	±	±	—
		occipital lobe, white matter	•	•	•	+++	+++	—

TABLE 2 *Microscopic findings in the central nervous system of Mare 5588*

	Microscopic section		Meninges		Brain			
			Meningitis					
No.	General area of the brain -	- containing the following specific anatomical structures	degree of	Mott cells	Subpial gliosis	Vascu-litis	Perivas-cular cuffing	Mott cells
F G H	Through mesencephalon at different levels	Colliculi oralis,	—	—	—	+++	+++	—
		colliculi aboralis,	—	—	—	+++	+++	—
		brachia colliculus aboralis	—	—	—	+	+	—
		central grey matter,	•	•	•	++	++	—
		pes pendicles,	±	±	—	±	±	—
		red nuclei,	•	•	•	—	—	—
		substantia nigra,	•	•	•	±	±	—
		lateral and medial lemnisci,	•	•	•	—	—	—
		transverse pontis fibrae	±	—	—	—	—	—
I	Through rostral part of pons and cerebellum (metencephalon)	Pons,	—	—	—	—	—	—
		pyramids,	—	—	—	—	—	—
		lingula	+	—	—	—	—	—
		vermis, grey matter,	++++	—	—	±	±	—
		vermis, white matter,	•	•	•	++	++	—
		brachia conjunctivum,	•	•	•	—	—	—
		dentate nuclei,	•	•	•	—	—	—
		anterior medullary velum,	—	—	—	—	—	—
		paravermis, grey matter,	++++	—	—	±	±	—
		paravermis, white matter	—	—	—	+++	+++	—
J	Through caudal cerebellum (metencephalon) and cranial medulla oblongata (myelencephalon)	Nodular lobe, grey matter,	+++	—	—	±	±	—
		nodular lobe, white matter,	•	•	•	++	++	—
		paravermis, grey matter,	++++	—	—	±	±	—
		paravermis, white matter,	•	•	•	++	++	—
		pyramids,	—	—	—	—	—	—
		medulla oblongata	—	—	—	—	—	—

(*b*) *Narration 2* (*Mare 5588*)

The quantitative aspects of the findings in the meninges and brain have been presented graphically in Table 2.

(i) Meninges

Meningeal cellular infiltrates had an extremely erratic distribution in the mare. Over the greater portion of the brain they were either "absent" or "mild". In the frontal lobe the degree of infiltration varied from "very mild" to "mild", the latter type being of a more localized nature. Over the occipital lobes, the involvement by infiltrations of the leptomeninges ranged from "very mild" to "severe", the latter areas occurring primarily in the extreme depths of sulci. Such infiltrates were irregularly distributed. Some of the most severely affected areas, encountered during this entire study, were found in the meninges over the cerebellum. This was especially true over the vermis and paravermis but to a lesser extent over the nodular lobe. Over the remainder of the metencephalon, mesencephalon and myelencephalon, lymphocytic infiltrations were either rare or absent.

At all the sites, from the most severely to the very midly affected areas over the vermis and paravermis, lymphocytes predominated but large mononuclear cells were also present. Plasma cells were also visible but they did not constitute a prominent feature of the reaction. The incidence of Mott cells, in comparison with that observed in other horses, was not significant and thus this aspect was recorded as negative in Table 2.

(ii) Brain

Subpial gliosis occurred beneath the more affected areas of the meninges of the cerebral hemisphere. It was, however, absent below the extensively involved cerebellar meninges. The vasculitis and the perivascular cuffing was very erratic in the mare and varied from negative (—) to most severe (++++). The very severe and most severely affected areas were encountered in the following: pyriform lobe, including the amygdaloid body, external capsule, corona radiata, subcortical white matter of the occipital lobes, collicula oralis and aboralis and the white matter of the paravermal cerebellar folia. Only slightly less affected areas included the geniculate bodies, the central grey matter, body of the fornix, internal capsule and white matter of other parts of the cerebellar hemispheres.

Within the lesions lymphocytes were more numerous than large mononuclear cells. Although a few plasmocytes and Mott cells were present, the latter were recorded as negative in the last column of Table 2 because of their relative infrequency when compared with the very high incidence in Stallion 5259. The perivascular cuffs in this mare were some of the thickest seen in any of the brains of these horses. Frequently they extended beyond the boundary of the Virchow-Robin space and spread into the surrounding brain tissues. Ependymal cells appeared unaltered. Sections of the optic tract, stained with LFB, revealed a moderate degree of demyelination [Plate 11 (76)].

(c) *Narration 3* (*Gelding 5065*)

The microscopical findings in the meninges and brain are shown graphically in Table 3.

(i) Meninges

The meninges of the gelding were involved as severely as those of Mare 5588 but, with few exceptions, not to the same extent as in Stallion 5259. The exceptions included the meninges of the parietal and pyriform lobes which were the most severely affected areas in Gelding 5065 and even more so than those of the corresponding meninges of Stallion 5259. Mott cells were very rare in the meninges as compared with their high incidence in Stallion 5259. Plasma cells were absent. The degree of meningitis of the cerebellar hemispheres in this gelding rated next in severity.

(ii) Brain

The pattern of the most severe subpial gliosis along the sulci and fissures, as recorded in Columns 8 and 9 of Table 1 for Stallion 5259, could also be seen in Gelding 5065. The degree of gliosis in this gelding was less conspicuous but closely paralleled that of the overlying leptomeninges. The most severely affected areas were seen in the rostral portions of the parietal and pyriform lobes. As in the case of previous horses, subpial gliosis was absent over the cerebellar hemispheres.

Generally speaking, the extent of the vasculitis and perivascular cuffing was less severe than in Stallion 5259. Nevertheless some areas with an involvement identical to that of corresponding areas in Stallion 5259, included the corona radiata, intermediate mass of the thalamus collicula oralis, substantia nigra, central grey matter and the choroid plexus of the fourth ventricle. Mott cells were far less numerous than in Mare 5588. Some vessels, which appeared to have perivascu lar cuffs, on close examination showed that the meningeal sheaths were densely infiltrated with numerou lymphocytes and a few histiocytes. A single localize s malacic area, with an internal haemorrhage, was seed in the cerebrum. Ependymal cells appeared unalteredn LFB stained sections of both optic tracts revealed severe segmental demyelination which was more severe on the left than on the right side [Plate 11 (77 and 78)].

(iii) Observations on other parts of the nervous system

Gasserian ganglion and *trigeminal nerve* - An increase in the number of glia was visible in the gasserian ganglion. Lymphocytes were present in the endoneurium and perineurium of the trigeminal nerve.

Spinal cord - The spinal meninges were mildly infiltrated with lymphocytes.

Pituitary - The meninges contained focal infiltrates of lymphocytes and plasma cells. The only significant changes consisted of groups of lymphocytes and plasma cells and an odd Mott cell in the pars nervosa.

2. *Narrations on observations of the nervous system of the remaining three horses*

Compared to those of the three animals already described, the lesions of the meninges and brain were so slight in Geldings 2287, 5484 and 5480 that only a brief account of each one is necessary.

(a) *Narration 4* (*Gelding 2287*)

Sections of all major areas of the meninges and brain revealed only a mild involvement. The limited number of lymphocytes in the leptomeninges of the cerebral cortex and cerebellum was accompanied by a few perivascular cuffs in the brain substance, similar changes were present in the thalamus and hippocampus. Several of the infiltrated focal areas of the meninges were accompanied by a correspondingly greater prominence of the subpial membrane, except over the cerebellum. Lymphocytes and plasma cells were frequent in the dorsal midsagittal dura mater, the only area of the dura that was sectioned and examined.

Arachnoid villi contained a limited number of lymphocytes and plasma cells.

Lesions in the pars nervosa of the pituitary were striking, being more severe in this gelding than in any of the other horses. The infiltrates consisted of numerous lymphocytes, large mononuclear and plasma cells [Plate 9 (66)] but Mott cells were far less numerous [Plate 10 (67)]. Concentrations of infiltrated cells were greater around the venules and capillaries than in the surrounding tissues. A few vessels of the pars intermedia were also surrounded by lymphocytes. The meningeal covering of the pituitary contained a few small round cells.

(b) *Narration 5* (*Gelding 5484*)

Changes within the CNS were not striking. They included lymphocytic infiltrations of the meninges of the cerebrum, rated as "very mild", while those from the cerebellum rated from "mild" to "moderate". Other meningeal regions were not affected. A few lymphocytes occurred in the perivascular tissue of a limited number of blood vessels in the brain. Mott cells were absent in the CNS.

(c) *Narration 6* (*Gelding 5480*)

The cerebral meninges contained numerous large mononuclear cells but only a few lymphocytes and Mott cells. A correspondingly mild thickening of the neuroglial membrane was visible. Lymphocytes, large mononuclear and plasma cells occurred in the perivascular tissues of the olfactory tuberculum and mammary bodies. Other regions of the brain and meninges were free from lesions that could have been attributed to trypanosomes.

A small cholesteatoma in the choroid plexus of one of the lateral ventricles and ferrugation of the walls of some blood vessels antedated the experimental *T. brucei* infection.

Discussion

A. *Viscera and other assorted tissues*

Changes in the heart from four of the six horses varied in severity from mild to marked. Gelding 5484 had extensive lesions which included an epicarditis, an endocarditis and an interstitial myocarditis with mononuclear cell infiltrates and moderate necrosis and degeneration of the myocardium; Gelding 5480 showed an increased amount of lipofuscin near the nuclei of the cardiac muscle; Gelding 2287 revealed a mild subendocardial histiocytic infiltration; Stallion 5259 disclosed a mild interstitial myocarditis in which plasma cells predominated. Since these lesions were inconsistent they could not be considered to be of diagnostic value. It is nevertheless possible that investigations on a broader basis may reveal that cardiac involvement is not uncommon in horses naturally infected with *T. brucei*.

Haemosiderosis was found in the *lungs* of five horses. The amount of haemosiderin varied to some extent from one horse to another but haemosiderocytes were prominently displayed in all alveolar septa. A focal endarteritis and or thrombosis of pulmonary arterial branches were visible in three horses.

The marked degree of haemosiderosis of the *liver* and *lungs* was interpreted to be a reflection of the severe anaemia that appeared for varying periods in these horses (Part 1, Tables 1b to 6b). The anaemia was also responsible for the centrolobular changes resulting from hypoxia. The heart lesions in Gelding 5484 undoubtedly contributed towards the chronic passive congestion and thus the hypoxia of the liver. The round cell infiltrates in portal areas were interpreted as not only an indication of the host response to trypanosome toxins but also the katabolic products released by dead parasites in the hepatic blood circulation.

Grossly the longitudinal section of femurs revealed that the bone marrow was red from the proximal epiphysis to the midshaft of the diaphysis in Gelding 5480 while it was red throughout the length of the diaphysis in Geldings 2287 and 5065. Microscopic examination of the marrow from two horses confirmed the haematopoietic and especially the erythropoietic activity. These observations are contrary to those made by Jubb & Kennedy (1963) who stated that in bovine trypanosomiases hyperplasia of the bone marrow does not develop and that in long bones the marrow is always of the yellow type. They also expressed the view that this feature probably occurs also in the other animal species affected by nagana.

Round cell infiltrations and other lesions in the *kidneys* were very mild in four of the experimental horses. One of them showed casts and other tubular changes as well as thrombi in some vessels. The renal *lymph nodes* of this horse revealed limited necrosis and haemorrhage, very active lymphocytopoiesis in the cortex and medullary cords and hyperplasia of the reticulo-endothelial cells. Giant cells were frequently encountered within the peripheral and medullary sinuses. This manifestation was similar to that described by Mönckeberg & Simons (1918) who observed giant cells of the syncitial type in lymph nodes of dogs suffering from artificially induced *T. brucei* nagana.

An extensive interstitial myositis of the skeletal muscles with infiltrates, similar to those described in the myocardium, was observed in Gelding 5484. Additional lesions in assorted tissues appeared in large and small veins of the *testes*, *epididymis* and *pampiniform plexus*. The vessels were frequently found to be thrombosed and this undoubtedly contributed towards the development of marked praeputial oedema of Stallion 5259.

B. *Nervous system*

Innes & Saunders (1962) have given lengthy comments on the paucity of reports on the histopathology of the CNS in nagana-affected domestic animals. Since it is beyond the scope of this paper to consider all forms of nagana, attention will be paid only to relevant observations made on *T. brucei* infections.

Mönckeberg & Simons (1918) published observations on the histopathology of the nervous system from experimentally *T. brucei* infected dogs. They stated that some nerve cells throughout the CNS showed a varying degree of degeneration and that they were frequently situated close to apparently normal ones, thereby excluding the possibility that autolysis had been responsible for this change. A similar cellular degeneration, however, was not observed in any of the six experimental horses. Spielmeyer, according to Innes & Saunders (1962), noted selective degeneration of the posterior roots of the spinal nerve, trigeminal roots and optic nerves in dogs suffering from *T. brucei* infection. In the present investigations dorsal roots and their ganglia were not examined but studies on a number of gasserian ganglia failed to disclose neuronal necrosis.

Many articles have been written on sleeping sickness but with the exception of some of the earlier reports, notably those of Mott (1906a, 1907, 1910–11), there were, until recently, only a few dealing with the histo-

pathology of the nervous system. Detailed accounts of complete brain studies on *T. rhodesiense* encephalitis by Manuelidis *et al.* (1965) as well as systematic observations on the peripheral nervous system in sleeping sickness by Janssen, Von Bogaert & Haymaker (1956) have provided information on human cases for comparison with those of animals. The present studies on horses have advanced far enough to compare the histopathology of the CNS of equine *T. brucei* nagana with that of *T. rhodesiense* sleeping sickness and that of some lesions in the peripheral nervous system of these horses encountered at corresponding sites in fatal cases of African human trypanosomiasis.

In horses the involvement of the dura mater was slight in comparison with that of the leptomeninges. In the vicinity of the dorsal sagittal sinuses of one animal, the dura, at some sites, was infiltrated with lymphocytes, plasmocytes and a few Mott cells. The leptomeninges of the same animal were severely affected and this accounted for the meningeal opacity observed macroscopically and depicted in photographs of the brain of Stallion 5259 [Part 1, Plate 2 (9 and 11)]. According to Manuelidis *et al.* (1965), five human cases had an involvement of the leptomeninges but there was a considerable variation in the extent of changes. This was also true for the leptomeninges of horses; changes were slight in Gelding 5484, 5480 and 2287 but severe in Gelding 5065, Mare 5588 and Stallion 5259. The degree of leptomeningeal involvement, in the first three horses, was similar to that recorded by Manuelidis *et al.* (1965) for mild human cases.

The most severely affected human cases and the even more severely affected horses, particularly Stallion 5259, revealed many similar and several dissimilar features. Apparently, relatively few Mott cells were seen in man in comparison with their frequency in horses. They were very numerous in Stallion 5259, very frequent in Mare 5588 and frequent in Gelding 5065. Lymphophagocytosis, which was prominent in the meninges of one human case, was absent in all horses. Otherwise the meninges of human and equine cases were similarly affected. The cell types were essentially the same with many lymphocytes, plasma cells and histiocytes prominent in both species. They were distributed throughout the thickness of the leptomeninges with an extensive involvement of blood vessel walls and juxtavascular areas. The leptomeninges of the mouths of sulci were also sites of severe involvement. In horses the meningeal sulci, especially at their greatest depth, were all severely affected; the degree of meningitis of fissures was similar to that of sulci; the meninges covering adjacent gyri were less involved. The topographical distribution of the meningeal changes over cerebral and cerebellar regions showed that this was more extensive in horses than in man.

In both species the cellular infiltration extended from beneath the endothelium of meningeal blood vessels right through to the adventitia. In some instances this was associated with fibrosis of the vessel walls and an increased number of fibroblasts and collagen fibres at various sites in the leptomeninges.

By using special techniques for microglial studies, Manuelidis *et al.* (1965) were able to record the frequency of their incidence and their distribution in man. Such studies were not undertaken on horses and thus nothing can be stated about the occurrence of microglia in this species. Besides minor differences, the results of the investigations by Manuelidis *et al.* (1965) were similar to those established in horses. Lymphophagocytosis was seen in the human brain but never in that of horses. Mott cells ranging from none to quite a few around blood vessels and within the brain substance of man were evidently never as numerous as in some of the horses, especially Stallion 5259.

Subpial gliosis was found in association with foci of meningitis in a few human cases. It was particularly striking in Stallion 5259 and present to a lesser extent in all horses. The degree of gliosis was in direct relationship to that of the severity and extent of the infiltrates in the overlying leptomeninges. Other areas also showed gliosis, especially those around vessels which contained severely involved meningeal sheaths. There was a numerical increase of glia in the brain far removed from sheathed vessels of both grey and white matter, the latter being more severely affected. Stallion 5259 showed a marked gliosis of the optic tracts. The gliosis of the central nervous system of these horses, particularly Stallion 5259, appears to conform in all respects to that observed in chronic cases of human sleeping sickness, a chronic case of dourine in a horse and experimental cases of *Trypanosoma gambiense* in two monkeys observed by Mott (1906b, 1907). He stated ". . . prolonged trypanosome infection causes in all three conditions a marked proliferation and overgrowth of the subpial, septal, and perivascular neuroglia tissue." This was not the only common feature that these three diseases and chronic nagana share, but certainly one of the most significant.

Perivascular cuffing and infiltration of the meningeal sheaths of the cerebral cortices were prominent features but subcortical and other areas of white matter were even more severely affected in both species. This was particularly so in the white matter around the basal ganglia. The nerve cell masses were also more affected than the cortex and, in some cases of both species, as severely as the enveloping white matter. Cell types were essentially the same in both species but Mott cells were far more frequent in horses. In one or other of the human and equine cases these cell types were found to extend beyond the border of the meningeal sheaths.

Demyelination of the optic tracts of Stallion 5259 was more severe on the left than on the opposite side. Since this horse was either partially or completely blind in the right eye as a result of an injury, it was assumed that demyelination was due to the discontinued use of the tract. However, when it was determined that demyelination was bilateral, attention was paid to the condition of optic tracts in other horses. Examination revealed that there was some loss of myelin in the optic tracts of Mare 5588 and severe segmental demyelination in Gelding 5065. Consideration of the occurrence of demyelination in the vicinity of blood vessels in the brain of sleeping sickness victims (Manuelidis *et al.*, 1965) made it clear that the changes observed in these horses were undoubtedly a sequel to nagana. The investigators also pointed out that although some nerve cells were lost they were not the target of the primary damage in the disease.

Extensive infiltration of the pars nervosa of the pituitary was prominent in one horse but with less, yet similar involvement, in another one. Except for the loss of neurons, the cellular infiltrates and proliferations and other changes in the gasserian ganglia, optic nerves and roots of the trigeminal nerve were consistent with those described in human cases of African trypanosomiasis (Janssen *et al.*, 1956).

TABLE 3 *Microscopic findings in the central nervous system of Gelding 5065*

Microscopic section			Meninges		Brain			
No.	General area of the brain -	- containing the following specific anatomical structures	Meningitis		Subpial gliosis	Vascu-litis	Perivas-cular cuffing	Mott cells
			degree of	Mott cells				
A	Through cerebral hemispheres (telencephalon) at level of olfactory trigonum	Dorsal cerebral part of frontal lobe,	+	—	±	±	+	±
		lateral and lateral ventral frontal lobe,	+	—	±	±	+	±
		containing external capsule,	•	•	•	±	+	±
		corpus callosum	•	•	•	±	+	+
		and putamen	•	•	•	—	—	—
		Ventral to ventrolateral frontal lobe	+	—	±	±	+	+
		including olfactory trigone,	+	—	±	±	±	—
		olfactory stria.	+	—	±	—	—	—
		and internal capsule	•	•	•	—	—	—
B	Through cerebral hemisphere (telencephalon) and the diencephalon at the level of the optic chiasma	Ventrolateral rostral part of parietal lobe	+++	±	+++	+	+	—
		with corona radiata,	•	•	•	++	++	±
		corpus callosum.	•	•	•	+	+	±
		Rostral part of pyriform lobe,	+++	±	+++	+	+	±
		internal capsule,	•	•	•	+	+	—
		caudal part of caudate nucleus,	•	•	•	+	+	—
		intermediate mass of thalamus,	•	•	•	++	++	—
		optic chiasma	+	—	—	+	+	—
C	Through cerebral hemisphere (telencephalon) and the diencephalon at the level of the caudal part of the intermediate mass of the thalamus, hypothalamus and optic tract	Corpus collasum,	•	•	•	+	+	±
		lateroventral part of temporal lobe	++	+	+	+	+	—
		containing corona radiata,	•	•	•	+	+	—
		Pyriform lobe,	+	±	±	±	±	—
		external capsule,	•	•	•	+	+	—
		thalamus,	•	•	•	++	++	—
		optic tract	+	—	—	±	±	—

D	Through occipital lobe (telencephalon) and the mesencephalon through the rostral colliculus oralis	Corona radiata,	•	•	•	+	+	±
		ventral part of occipital lobe,	+	—	±	+	+	±
		lateral ventricles, hippocampus,	•	•	•	±	±	—
		splenium of corpus callosum,	•	•	•	+	+	—
		cerebral aqueduct, colliculus oralis,	+	—	—	++	++	—
		brachium of colliculus aboralis,	±	—	—	+	+	—
		red nucleus,	•	•	•	±	±	—
		substantia nigra,	•	•	•	++	++	—
		central grey matter,	•	•	•	++	++	—
		crus cerebri	±	—	—	—	—	—
E	Through rostral pons (metencephalon)	Fourth ventricle, anterior medullary velum,	±	—	—	—	—	—
		brachium conjunctivum,	±	—	—	±	±	—
		brachium pontis,	+	—	—	±	±	—
		trigeminal nerve roots,	±	—	—	—	—	—
		lateral and medial lemnisci,	•	•	•	—	—	—
		nuclei pontis,	•	•	•	—	—	—
		transverse fibres of pons	±	—	—	—	—	—
F	Through pons and cerebellum (metencephalon)	Vermis,	++	—	—	+	+	—
		paravermis,	++	—	—	+	+	—
		dentate nucleus	•	•	•	±	±	—
		fourth ventricle, lingula of vermis,	++	—	—	+	+	—
		brachium conjunctivum,	•	•	•	±	±	—
		pons,	±	—	—	+	+	—
		pyramids	±	—	—	+	+	—
G H I	Various levels of myelencephalon (medulla oblongata) and through vermis of cerebellum, fourth ventricle, choroid plexus, pyramids	Vermis,	++	—	—	+	+	—
		medulla oblongata,	+	—	—	+	+	—
		choroid plexus,	•	•	•	++	++	—
		pyramids	+	—	—	—	—	—

So far as the cellular proliferation or infiltrates in the brain and meninges of horses are concerned, it is obvious that they fall in the realm of immunopathological lesions. They are possibly associated with toxins liberated from trypanosomes. Lymphocytes, but perhaps even more so plasmocytes and Mott cells, support this view.

The gliosis cannot be explained so readily. It is undoubtedly a response to an irritant but its true nature is obscure. With stains used for staining brain sections, trypanosomes could not be demonstrated within the brain substance. The presence of trypanosomes within the CSF of Stallion 5259 and Gelding 5065 was evidence that they had reached the ventricular system. Lysis of dead trypanosomes would undoubtedly have released substances which could have possibly accounted for the gliosis in subpial and other regions. Hoare (1949) states that some nervous symptoms with human trypanosomiasis are probably due to the action of toxins released by trypanosomes present in the CSF after their destruction by lymphocytes. Other symptoms may be due to an invasion by trypanosomes of the blood vessels of the brain, whence the parasites penetrate the brain tissue and produce inflammatory lesions. In the present studies only circumstantial evidence was found that *T. brucei* had occurred anywhere in the brain tissue. Had this parasite been observed, it would possibly have helped to explain the pathogenesis of some of the lesions encountered in the brain.

Summary and conclusions

1. A detailed description of the histopathology of the nervous system and a brief account of lesions in the visceral and other organs of six experimentally *T. brucei* infected horses are presented.

2. They consisted of a group of two untreated controls and two groups of treated horses. Of the latter, one group contained a single horse which had received two subcurative doses of Antrypol and the other contained three horses which had acquired a chronic form of nagana following subcurative Antrypol and Berenil medication.

3. In both groups of treated horses, lesions in the visceral and other organs, were neither pathognomonic for nagana nor were they of uniform occurrence. The outstanding lesions included:

(a) various types of inflammatory reactions in the heart;
(b) haemosiderosis of the liver and lungs;
(c) moderate cellular infiltrations and tubular changes in the kidneys;
(d) active lymphocytopoiesis and hyperplasia of the reticulo-endothelial system in some but not in all lymph nodes;
(e) haematopoietic and especially erythropoietic activity of the bone marrow;
(f) thrombosis of blood vessels of the kidneys, testes, epididymis, pampiniform plexus, large intestine and branches of the pulmonary artery;
(g) myositis of the skeletal muscles.

4. With the exception of the rather impressive lesions in the pituitary of one horse, the changes in the nervous system of the two controls and the horse which was only treated with Antrypol were neither striking nor of uniform occurrence. They included:—

(a) a varying degree of infiltration by one or more cell types in the leptomeninges of the cerebellum and cerebrum, dura mater, arachnoid villi and perivascular tissue of the olfactory tuberculum and mammary bodies;
(b) perivascular cuffs in the brain substance of the cerebral hemispheres, thalamus and hippocampus;
(c) a varying degree of cellular infiltration in the pars nervosa, very mild perivascular infiltration in the pars intermedia and a few round cells in the meningeal covering of the pituitary.

5. The data on the extensive lesions in the nervous system of the three horses of the second treated group are listed in tabular form. The histopathology of the nervous system was characterized by a severe pleocytosis of the meninges, an extensive subpial gliosis corresponding in severity to the involvement of the overlying leptomeninges, segmental demyelination of optic tracts and other white matter as well as grey matter and extensive perivascular cuffing with lymphocytes, plasmocytes, large mononuclear cells and Mott cells in that order of descending frequency.

6. A comparison has been made between the lesions in the nervous system of human *T. rhodesiense* sleeping sickness and those of experimentally produced chronic *T. brucei* nagana.

7. Consideration of the lesions, enumerated under the heading "Discussion" in the text, makes it apparent that points of similarity are far greater than those of dissimilarity. The latter included a lymphophagocytosis in the meninges and brain of man, a higher incidence of Mott cells in the meninges of horses and the penetration of trypanosomes in the brain of man which was not seen at this site in horses.

8. It is concluded that by prolonging the survival period of a *T. brucei* infected horse by subcurative medication of trypanosomacidal drugs, an animal model suitable for the study of the nervous form of trypanosomiasis is produced. The hope is expressed that this chemotherapeutic approach for the production of such models, using solipeds or perhaps other animal species, will facilitate research on various aspects of trypanosomiasis.

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