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STUDIES ON THE PATHOLOGY OF HEARTWATER [*COWDRIA (RICKETTSIA) RUMINANTIUM*, COWDRY, 1926]. I. NEUROPATHOLOGICAL CHANGES

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INTRODUCTION

From a summary of the literature on heartwater by Henning (1956) it appears that few publications on the pathology of this disease have appeared subsequent to Steck's (1928) original report. Nervous symptoms are a striking and typical feature of heartwater, particularly in the bovine species, and have been described in detail by Alexander (1931) and Henning (1956). Steck (1928) found no significant lesions other than leucostasis in the brain. Daubney (1930) confirmed this and in addition recorded some evidence of perivascular infiltration, subarachnoidal cellular exudation and ' satellitosis '. Clark (1962) studied the pathological physiology and concluded that the nervous symptoms could not be ascribed to any changes in the blood constituents examined by him nor to the circulatory collapse appearing subsequently.

In the present report the pathological changes in the central nervous system are described. These are probably related to the nervous symptoms of heartwater.

MATERIAL AND METHODS

The entire brains from 11 cattle, 12 sheep and one goat, submitted for diagnosis after natural death were studied. The carcasses selected for study showed mild or no post mortem changes. The majority of these animals had received intravenous or intramuscular injections of oxytetracycline or chlortetracycline before death. To supplement the above material three head of cattle were injected intravenously with infected blood and left untreated.

The brains were fixed *in toto* in a large volume of 10 per cent formalin or buffered 10 per cent formalin. Coronal sections, 4 mm in thickness, were cut from each brain and various blocks from the following areas were selected for microscopic study: choroid plexus, medulla oblongata, pons, cerebellum, midbrain, thalamus, cerebral peduncles, corpus striatum and cerebrum. The hippocampus, corpus callosum, optic tracts, spinal cord and fornix were also included occasionally.

Paraffin embedding was used and microscopic sections were cut at 3 to 6 μ thickness, stained with haematoxylin-eosin (H. & E.), haematoxylin-phloxin (H.P.), and the Hotchkiss periodic acid-Schiff (P.A.S.) technique combined with Mallory's phosphotungstic acid haematoxylin (M.P.A.H.) method.

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RESULTS

Macroscopic findings

The majority of the brains showed no striking macroscopic changes except for congestion of the meningeal vessels. In a few instances in both cattle and sheep, many petechial haemorrhages were noticeable on the outer surface of the cerebellum. On section, petechiae, ecchymoses and even larger haemorrhages were seen in the thalamus, hypothalamus, midbrain, cerebellum, medulla oblongata, hippocampus, cerebral peduncles, isthmus of the pyriform lobe and less frequently in the corpus striatum of some cattle (Plates 1, 2 and 3).

Brains showing leptomeningitis microscopically had dull and slightly oedematous meninges macroscopically. In several brains there appeared to be an accumulation of excess fluid in the subarachnoid space over the sulci. The choroid plexus was frequently thickened and dull greyish in appearance.

Microscopic findings

The incidence and distribution of the most significant histopathological lesions encountered are summarized in Tables 1 and 2.

Haemorrhages were found in the medulla oblongata, cerebellum, midbrain, thalamus, cerebral peduncles, hippocampus (Plates 1, 2 and 3) and less frequently in the corpus striatum, cerebrum and pons. These haemorrhages were usually confined to the Virchow-Robin (V.R.) spaces (Plate 5, C) and adjoining brain substance. More extensive haemorrhages were occasionally encountered, mainly in cattle. A necrotic bloodvessel (Plate 5, C) or a focus of necrotic brain tissue was sometimes visible in the centre. Smaller bloodvessels and capillaries were more commonly affected than larger vessels and showed pycnosis, karyorrhexis and fibrinoid changes.

Accumulations of a slightly eosinophilic substance were noticeable in some of the V.R. spaces in 57 per cent of the cattle and 38 per cent of the sheep of which 21 per cent and 31 per cent respectively represented very mild cases with only infrequent involvement of vessels. Some of these cases showed an extension of this fluid into the surrounding brain substance which was almost invariably affected by a loosening or rarefaction of the tissue. These changes are demonstrated in Plate 4, C and F.

The neuroglial cells showed striking changes. Enlargement and increased vesicularity of the nuclei, which frequently adopted a reniform or irregular shape, were observed in a few cases (Plate 8, A). The cytoplasm increased in volume and eosinophilia (Plate 7, A to C). Small intracytoplasmic granules and larger globules were noticeable around the nuclei (Plate 8, C to F) and within the various processes of these cells (Plate 8, H and C), even those attached to the bloodvessels, and within the sucker feet (Plates 8, G and 7, D). The granules and globules varied in size and staining reaction, the smaller ones being rather pale pink and the large globules more deeply eosinophilic and as an exception even finely vacuolated. Their increase in number and size invariably coincided with eccentrically displaced nuclei, pycnosis and ultimate karyorrhexis (Plate 8, D to F). These changes were most frequently observed in the cerebellar white matter and thalamus. The impression was gained that this glial activation, swelling, granularity, degeneration and necrosis were confined mainly to those cells with the larger and more leptochromatic, oval nuclei. These were regarded as astrocytes.

TABLE 1.—*The incidence of lesions in the brain*

Lesion	Cattle (14 cases)	Sheep & goats (13 cases)	Total (27 cases)
	%	%	%
Haemorrhages	64 (7)	61 (8)	63 (7)
Leucostasis	71 —	69 (23)	70 (11)
Vasculitis	100 (28)	54 (46)	74 (37)
Perivascular or meningeal cell infiltration	93 (64)	38 (38)	67 (52)
Changes in choroid plexus	86 (43)	92 (38)	89 (41)
Focal necrosis of bloodvessels	50 —	54 (8)	52 (4)
Oedema	57 (21)	38 (31)	48 (26)
Rarefaction	57 (14)	0 0	30 (7)
Swollen glia	71 (28)	61 (15)	67 (22)
Granular glia	57 (35)	69 (31)	63 (33)
Perivascular globules	93 —	77 (23)	85 (11)
Swollen axis-cylinders	86 (7)	69 (8)	74 (7)
Microcavitation	50 —	61 —	55 —
Necrosis of granular layer of cerebellum	28 —	38 —	33 —
Gliosis	7 —	15 (8)	11 (4)

The first figure in each column represents the percentage of brains which showed the lesions listed and the one in brackets represents very mild cases only.

TABLE 2.—*Distribution of the most significant lesions in the brain*

	Medulla oblongata	Cerebellum	Midbrain	Thalamus	Corpus striatum	Cerebrum
	%	%	%	%	%	%
Cattle (14)						
Haemorrhages	43	50	35	50	21	14
Swollen axis-cylinders	43	35	35	64	57	14
Microcavitation	21	14	21	14	7	7
Perivascular globules	43	64	64	86	57	43
Glial changes	43	78	35	64	43	50
Sheep & Goats (13)						
Haemorrhages	46	38	38	31	8	8
Swollen axis-cylinders	38	38	31	61	54	8
Microcavitation	23	46	46	46	31	8
Perivascular globules	46	69	54	46	46	15
Glial changes	38	69	31	46	46	38
Total (27)						
Haemorrhages	44	44	37	41	15	11
Swollen axis-cylinders	41	37	33	63	55	11
Microcavitation	22	30	33	30	18	7
Perivascular globules	44	67	59	67	52	30
Glial changes	41	74	33	55	44	44

The figures represent the percentage of brains which showed the lesions concerned. Those brains with very mild lesions are also included. The numbers in brackets on the left side represent the number of brains which were studied.

The presence of eosinophilic globules in the perivascular spaces was another fairly regular histopathological finding (Plates 8, I and 9, A to B). The variation in size and ability to be stained with eosin were similar to those of the globules within the cytoplasm of the glial cells, with the exception that those in the V.R. spaces were generally larger and more intensely eosinophilic. In some of the brains showing an abundance of globules, their distribution was not restricted to an area around the vessels but they were also present within the brain substance. In the sections stained P.A.S.-M.P.A.H., small terminal bipolar projections were noticed in some of the globules (Plate 8, J to K). These glial changes and eosinophilic perivascular globules were by no means constant or uniform in distribution, but were fairly well noticeable in 74 per cent of the brains examined and very mild in another 11 per cent. It was more prominent in cattle. The results of the histochemical examination of the globules are summarized in Table 3. Negative reactions were obtained for calcium, iron, lipids, phospholipids, lipofuscin, haemoglobin, acid-mucopolysaccharides and myelin. They proved to be P.A.S. positive and the staining intensity was in direct proportion to the size of the globules, the larger ones staining more intensely. This positive reaction was not influenced by diastase digestion. The tests applied for proteins were also positive. These findings indicate that the globules are of a mucoprotein or glycoprotein nature.

TABLE 3.—*Histochemical characteristics of the eosinophilic globules*

Test for Presence of	Reagents and Methods	Results
Minerals	Iron—Gomori's iron reaction (Gomori, 1953)	Negative
	Iron—Gomori's reaction for masked iron (Ibid)	do
	Calcium—Dahl's alizarin red S (Pearse, 1961)	do
	Calcium—Von Kossa's silver nitrate (Lillie, 1954)	do
Mucoproteins	P.A.S. (Gridley, 1960)	Positive
	P.A.S. after diastase digestion (Gridley, 1960)	Positive
	Alcian blue (Short method)—(Gridley, 1960)	Negative
Lipids	Sudan IV (Gridley, 1960)	Negative
	Oil red O (Lillie, 1954)	do
Phospholipids	Sudan black (Pearse, 1961)	do
Lipofuscins	Schmorl's method (Pearce, 1961)	do
	Ziehl-Neelsen—Long method, (Pearse, 1961)	do
Myelin	Luxol fast blue—M.P.A.H. (Margolis & Pickett, 1956)	do
Haemoglobin	Ralph's haemoglobin stain (Gridley, 1960)	Negative
Protein	Tetrazotized benzidine (Lillie, 1954)	Positive
	Dinitrofluorobenzene (DNFB)—(Thompson *et al.*, 1959)	Positive
	Tetrazotized benzidine after pretreatment with benzoyl chloride (Lillie, 1954)	Negative

Focal areas of swollen axis-cylinders and microcavitation were observed within the internal capsule, thalamus, midbrain, cerebellum, medulla oblongata, pons, cerebral white matter and cerebral peduncles (Plate 6). The optic tracts and brachium pontis were not regularly studied, but were found to be involved in some cases. Microcavitation was also noticed within the grey matter and rarely even in the cerebral cortex. Small areas of necrosis as evidenced by pycnosis, rarefaction, microcavitation and increased eosinophilia were present in the granular layer of the cerebellar cortex in 33 per cent of the cases studied (Plate 9, D and E).

Leucostasis was noticed in 70 per cent of the total number of brains examined and was more pronounced in cattle. In most of the specimens only isolated vessels were affected and occasionally, especially in cattle, this change occurred fairly uniformly throughout the brain. Since this reaction and the appearance of the cells involved have been described in detail by Steck (1928), Daubney (1930) and Jackson & Neitz (1932), no further attention will be given to it here.

A very mild focal vasculitis occurred in all the cattle in comparison with only 54 per cent of the sheep examined. Similar to the leucostasis it varied in incidence from isolated vessels to a more generalised distribution. Medium sized and small vessels were mainly involved. The walls were infiltrated with round cells, macrophages and a few polymorphonuclear cells. The nuclei of these infiltrated cells were frequently karyorrhectic. Various numbers of similar cells, including occasional 'gitter' cells, were also found in some of the V.R. spaces throughout the brain (Plate 5, E) as well as in the leptomeninges and more particularly over the cerebellum. Fairly widespread and prominent meningo-encephalitis, however, was seen in only a few cattle.

The choroid plexus within the various ventricles was regularly affected—only 11 per cent of the animals showed no changes whatsoever. A fibrinous exudate was usually noticeable in the delicate connective tissue core. This was extremely marked in a few cases (Plate 5, A). Fibrinoid thrombi were present in some of the small vessels. Congestion and haemorrhages were commonly seen and many of these cases showed a mild focal infiltration of polymorphonuclear leucocytes (Plate 4, E), small round cells and macrophages which appeared to be more confined to an area around the bloodvessels. This was occasionally very marked and diffuse (Plate 4, D). The macrophages contained acidophilic intracytoplasmic globules of various sizes (Plate 5, B). Focal necrosis of the epithelium was evident in certain instances (Plate 4, C). Colonies of *C. ruminantium* were observed in the endothelial cytoplasm of some bloodvessels.

In one of the cattle experimentally infected, small disseminated foci of gliosis were apparent throughout the brain (Plate 9, F). These foci were restricted mainly around small bloodvessels showing degenerative and necrotic changes. An area of microcavitation surrounded by gliosis was noticed in the midbrain (Plate 5, D). This case showed nervous symptoms and recovered without any treatment. A mild ataxia was still noticeable when the animal was electrocuted for examination, ten days after the initial rise in temperature. Focal gliosis was also observed in two of the thirteen natural cases in sheep.

A few swollen, eosinophilic Purkinje cells and other neurones with pycnotic and karyorrhectic nuclei were seen in the cerebellum, medulla oblongata and midbrain.

The spinal cord, which was not originally included in the survey, was later studied in a few sheep. In one, microcavitation, glial changes and a few swollen axis-cylinders were found.

No significant difference in the neuropathology was observed between treated and untreated cases.

Discussion

The previously described pathological changes in heartwater (Steck, 1928; Daubney, 1930), which include leucostasis, perivascular infiltration and subarachnoidal cellular exudation are ususally mild and inconstant and offers no explanation for the associated nervous symptoms.

It is well known that many cases of heartwater fail to recover in spite of specific chemotherapy. According to Clark (1962) some sheep in which the febrile reaction was controlled by chemotherapy afterwards pass into a state of collapse and may lie moribund for days with a normal to subnormal body temperature. Such cases usually occur when treatment has been delayed and nervous symptoms have been evident for some time. They invariably die. Clark studied three such cases in sheep and found that the plasma volume was not unduly reduced. No abnormalities were found in the blood constituents of experimental cases. The cerebrospinal fluid was found to be normal. He concluded that in these cases specific therapy had eliminated the infection in time to avert death from circulatory collapse, but that irreversible brain damage had occurred. This condition is described by Clark as a functional decerebration with the vegetative functions of the body proceeding more or less normally. Some of the lesions observed, such as microcavitation, swollen axis-cylinders and necrosis of the granular layer in the cerebellar cortex, confirm this theory and stress the importance of early chemotherapy. It is further evident that these lesions, including the glial and vascular changes, could be significant in the development of nervous symptoms in heartwater. No attempt will, however, be made at this stage to correlate the nervous symptoms with the distribution of lesions in the central nervous system, as this should be based on a topographical and symptomatological study.

Torack, Terry & Zimmerman (1960), in their studies on cerebral fluid accumulation, suggested that the clear glial cells of uncertain identiy could be involved in electrolyte transfer, nutrient supply and waste removal. They produced cerebral swelling by the application of solid carbon dioxide to the exposed brains of mice. Electron microscopic study of this material revealed enlargement of the entire cytoplasmic volume of the clear glial cells. With the light microscope a P.A.S. positive granular substance, proportional in amount to the severity of the oedema, was observed within the cell bodies, perivascular spaces and brain substance. They concluded that the clear glial cells have a vital role in fluid transport and that their plasma membrane is an important component of the blood-brain barrier.

Lumsden (1958) reported the occurrence of eosinophilic granules and larger inclusions in the perikaryon of adult astrocytes in tissue culture. The larger inclusions presumably developed from aggregations of the granules. The structures were strongly P.A.S. positive and were seen to migrate along the cell processes to appear in their terminal expansions and be discharged in a secretory-like manner.

Glial changes similar to those described by Torack *et al.* and Lumsden were found in most of the cases of heartwater that were studied. An initial response by the glia was evident from their enlargement and their nuclei becoming vesicular. This seemed to be followed by an increase of cytoplasm and the development of fine granules in the perikaryon, cell processes and sucker feet. The perivascular glial processes were more prominent than in normal animals. The granules subsequent-

ly either coalesced or increased in size to form larger globules which stained more intensely eosinophilic. The aggregation of proteinaceous globules within the glial elements was apparently followed by their eventual rather loose distribution in the V.R. spaces. Globules showing delicate terminal processes at opposite poles were noticed in P.A.S.-M.P.A.H. preparations. This was regarded as strong evidence that some of the apparently free globules were still located within fine glial processes. These observations and series of changes strongly indicated that the perivascular globules, so frequently encountered, originated from the neuroglia. In more advanced cases random distribution within the brain substance was apparent. Contrary to the findings of Lumsden, the granular and globular changes in the neuroglia in heartwater seemed to coincide with degenerative and necrotic changes in these cells. This suggests an erroneous metabolic transport mechanism either from the bloodvessel to the glia or *vice versa* rather than a secretory function. The observation by Torack *et al.* that the amount of P.A.S. positive granular substance was proportional to the severity of the oedema, indicates that it is related to some interference with the fluid transport function of the glial cells.

It seems reasonable to assume that the presence of *C. ruminantium* within the endothelial cells could affect the function of these cells. The possibility that a toxin produced by the organisms may be involved merits consideration. Jackson & Neitz (1932) investigated this possibility, but arrived at no definite conclusion. The necrotic changes observed in some of the bloodvessels in the cases presented, nevertheless, are very suggestive of damage by *C. ruminantium* and could explain the focal haemorrhage and oedema observed. Due to the close relationship between the endothelium and some glial cells by means of their sucker feet, it can be expected that a disturbance in endothelial function could also affect the glia. The series of glial changes observed in the present cases possibly indicate such an involvement.

It therefore seems justified to apply the classical term of 'brain swelling' to some cases of heartwater. This would indicate a less advanced stage where the changes are confined to the glial cells with a minimum of ruptured glial membranes. Brains with typical intercellular oedema, which may be accompanied by haemorrhages, represent the more severe and acute form. Swollen axis-cylinders and microcavitation were more frequently concomitant with perivascular globules and granular glial changes than with haemorrhages, intercellular oedema or swollen glia, and were interpreted as being present in more advanced cases. Gliosis appeared to be the aftermath of haemorrhages and necrosis.

SUMMARY

The most significant pathological changes in the central nervous system of 27 cases of heartwater are described. In addition to leucostasis and cell infiltration in the perivascular and subarachnoid spaces described previously, the following changes were noticed:—

Swollen axis-cylinders, microcavitation and focal necrosis of the cerebellar cortex; degenerative and necrotic changes in the neuroglia accompanied by the formation of P.A.S. positive intracytoplasmic granules and globules; the accumulation of P.A.S. positive globules in the V.R. spaces; choriomeningeal oedema and fibrinous choriomeningitis; haemorrhages, oedema and vascular changes.

Evidence is submitted in favour of the perivascular globules being proteinaceous and of glial origin.

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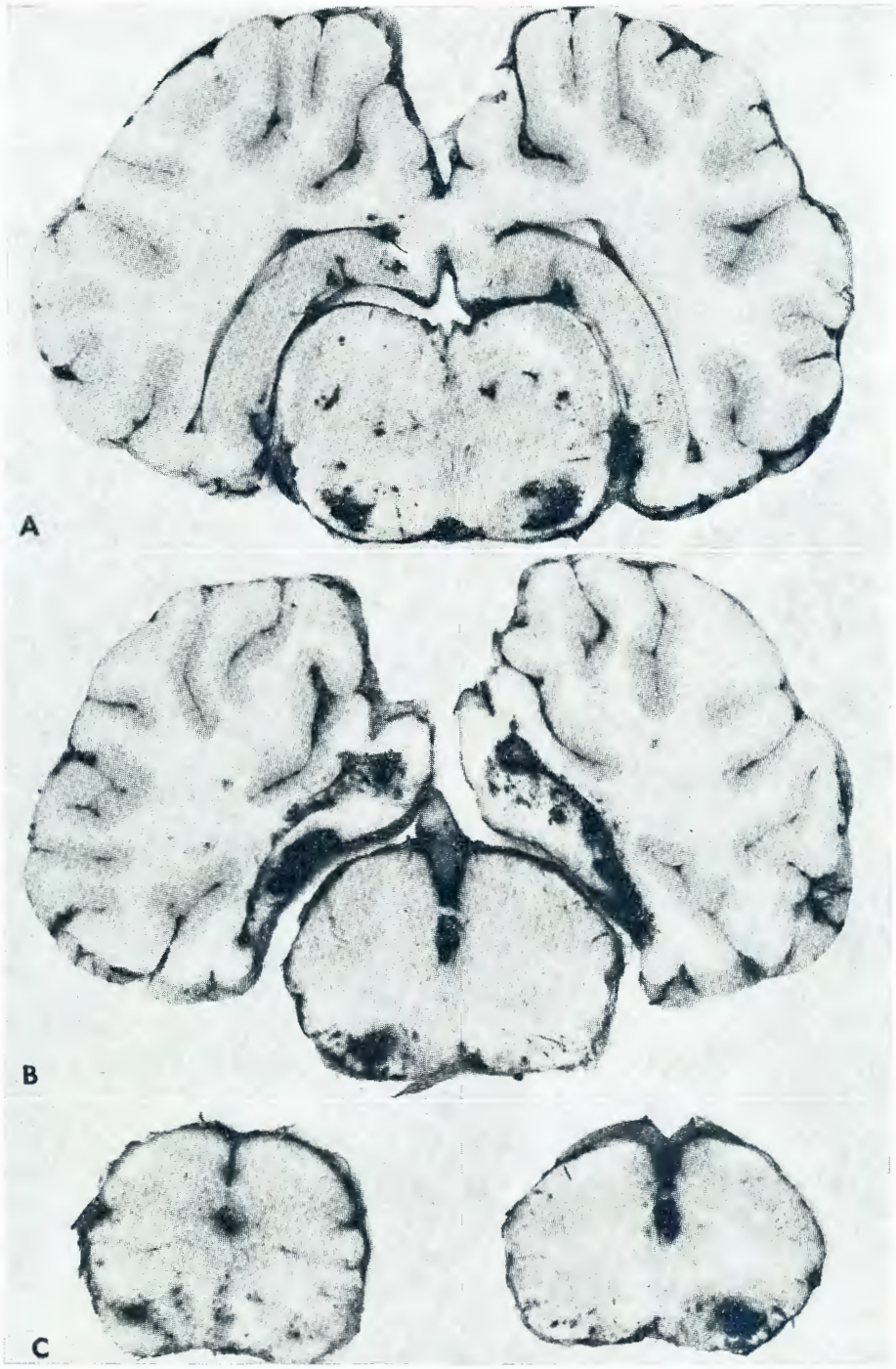

PLATE 1.—Bovine: Natural case. A. Marked bilateral haemorrhages especially in the cerebral peduncles. B. Bilateral haemorrhages in the hippocampus, one cerebral peduncle and the isthmus of the pyriform lobe. C. Haemorrhages in the midbrain

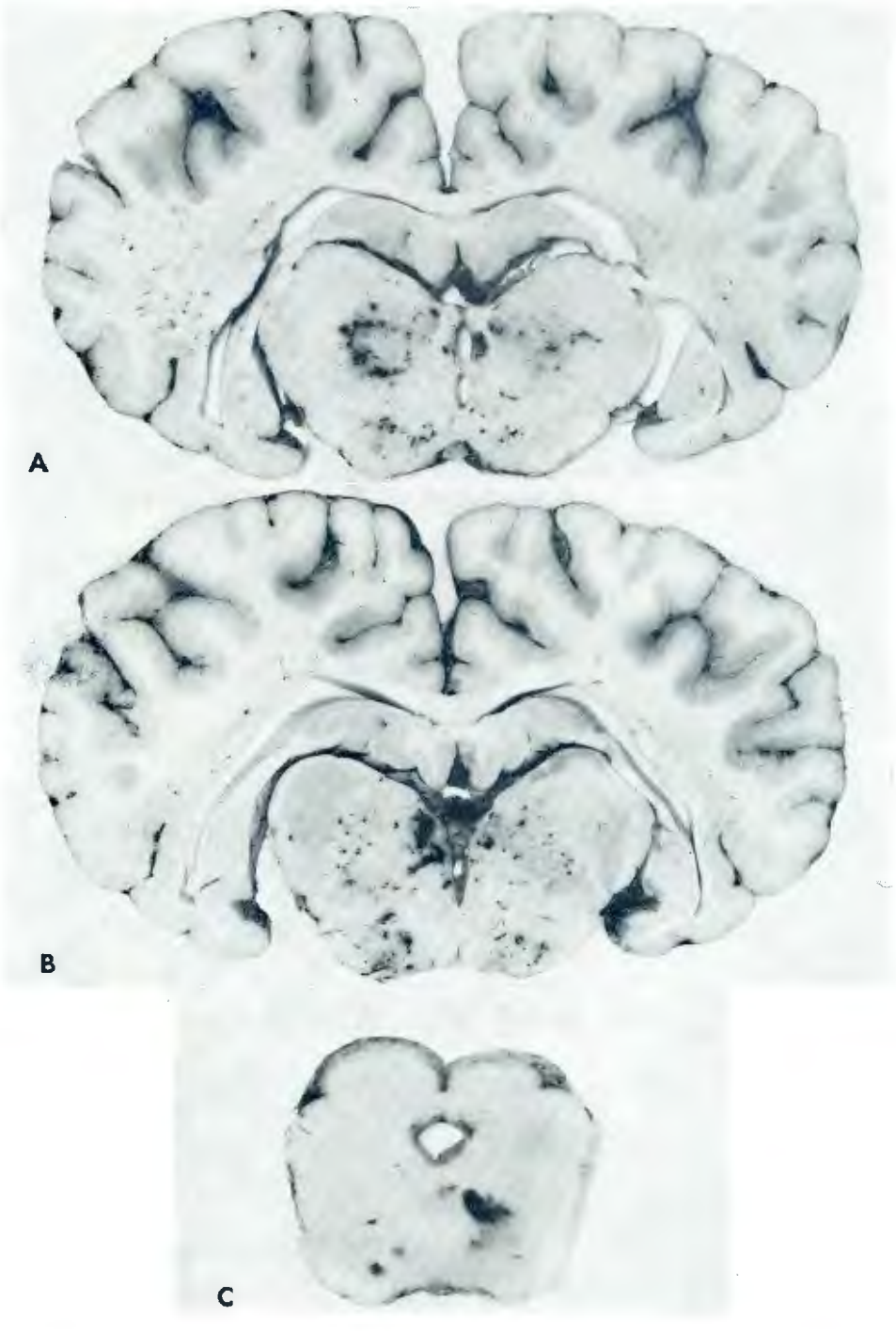

PLATE 2.—Bovine: Experimental case. A. and B. Haemorrhages in the thalamus. C. Haemorrhages in the midbrain

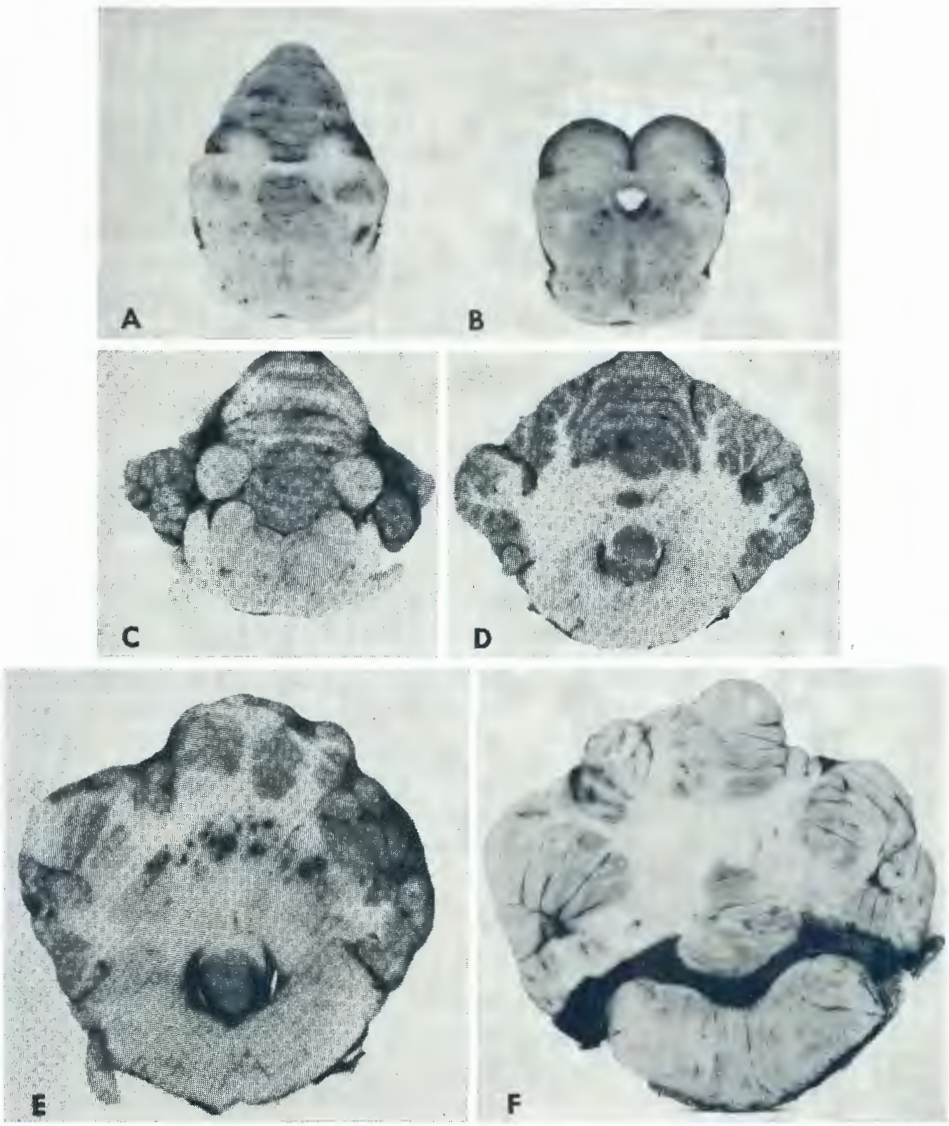

PLATE 3.—A, B, C, D: Bovine: Natural case showing numerous petechial haemorrhages in the midbrain and cerebellum. E. Cerebellum of the experimental case illustrated in Plate 2 with haemorrhages in the vicinity of the nuclei situated in the dorsal white matter. F. Bovine: Another experimental case with a very prominent fibrinous choriomeningitis

PLATE 4.—A, C, D, E: Natural cases. B, F: Experimental case. A. Bovine: Sub-pial haemorrhage into the molecular layer of the cerebellum. H. & E. × 30. B. Bovine: Two enlarged necrotic neurones. H. & E. × 192. C. Bovine: Bloodvessel in the white matter of the cerebellum with an accumulation of fluid in the V.R. space. Rarefaction of the white matter can also be seen. H. & E. × 192. D. Ovine: Chorio-meningitis with a marked round cell infiltration. H. & E. × 75·6. E. Bovine: Marked fibrinous exudation in the choroid plexus with a mild polymorphonuclear cell infiltration. H. & E. × 192. F. Bovine: Hippocampus with a vessel on the left showing accumulation of fluid in the V.R. space, rarefaction and a haemorrhage on the right. H. & E. × 75·6

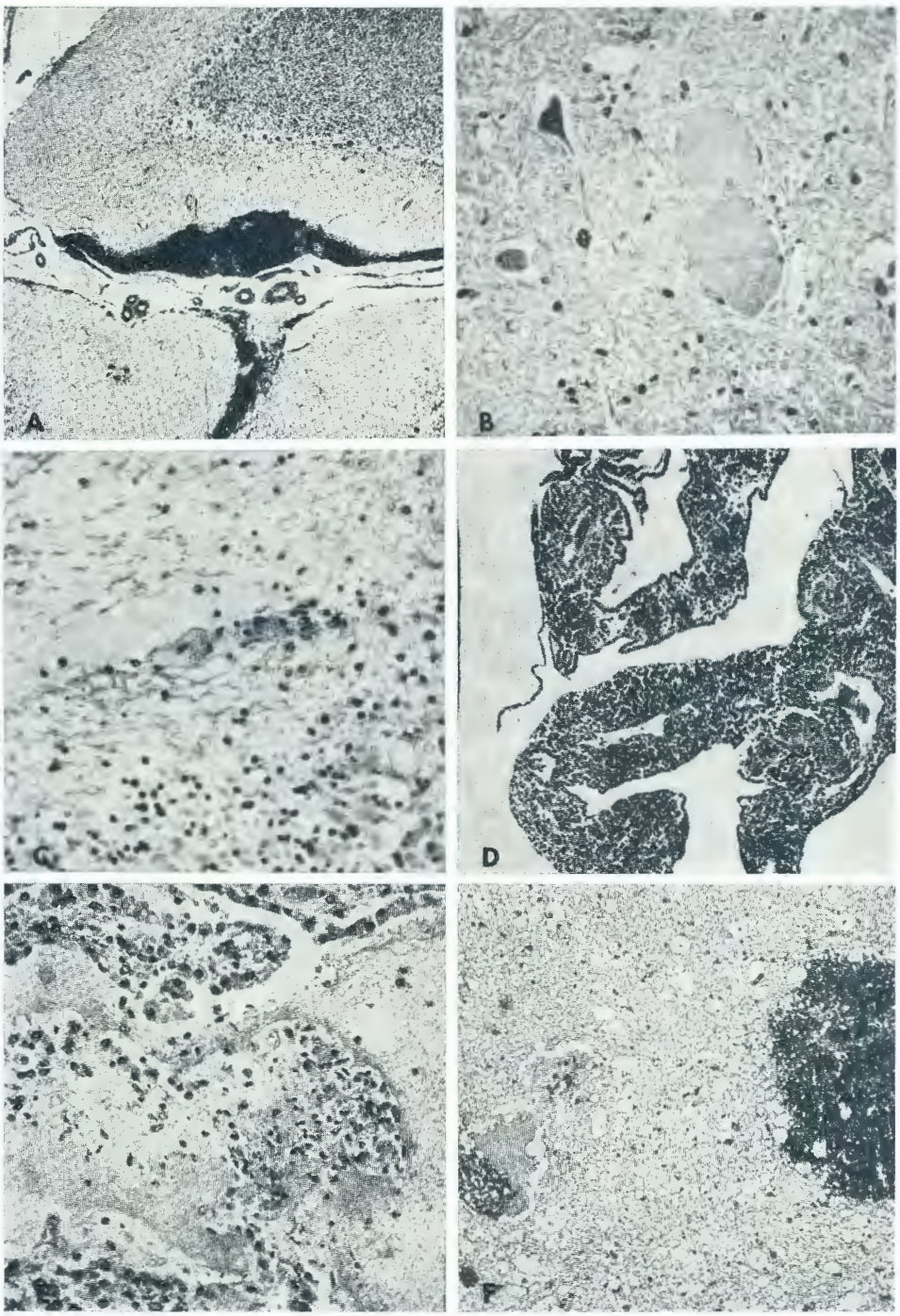
A
B
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PLATE 5.—A Bovine: An experimental case with a very marked fibrinous exudation in the choroid plexus. Scattered macrophages can be seen and a focal round cell infiltration is present in the left upper corner. H. & E. × 95·3. B. Bovine: As A, showing a fibrinous exudate with macrophages containing intracytoplasmic eosinophilic globules. H. & E. × 480. C. Bovine: Experimental case. Necrotic bloodvessel in the hippocampus with karyorrhectic material, scanty cell infiltration and haemorrhage into the V.R. space. H. & E. × 288. D. Bovine: Recovered experimental case. Focal microcavitation in the midbrain surrounded by gliosis. H. & E. × 192. E. Bovine: Natural case. Perivascular round cell infiltration in the cerebellum. H. & E. × 192

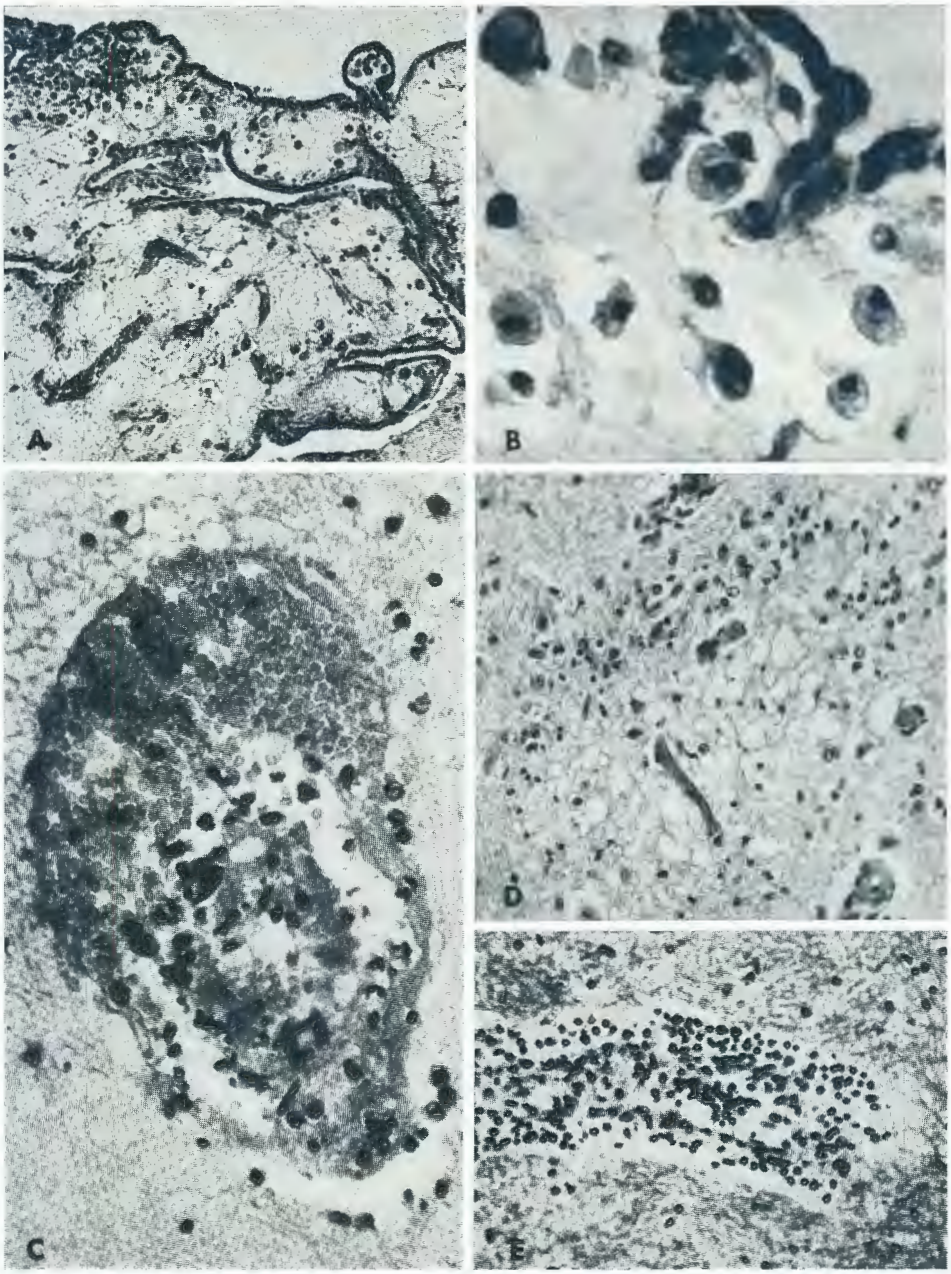

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PLATE 6.—Natural cases. A. Bovine: A tract within the internal capsule with microcavitation and rather inconspicuously swollen axis-cylinders. H. & E. × 75·6. B. Bovine: An area of microcavitation in the white matter of the cerebellum. A small haemorrhage and the granular layer of the cortex are visible on the left. H. & E. × 76·6. C. Ovine: The midbrain showing a focus of microcavitation. H. & E. × 75·6. D. Bovine: Thalamus with a small area of swollen axis-cylinders. H. & E. × 192. E. Bovine: A large area of swollen axis-cylinders in the medulla oblongata. H. & E. × 480. F. Bovine: Another area of swollen axis-cylinders in the midbrain of the same case illustrated in A and E. H. & E. × 480

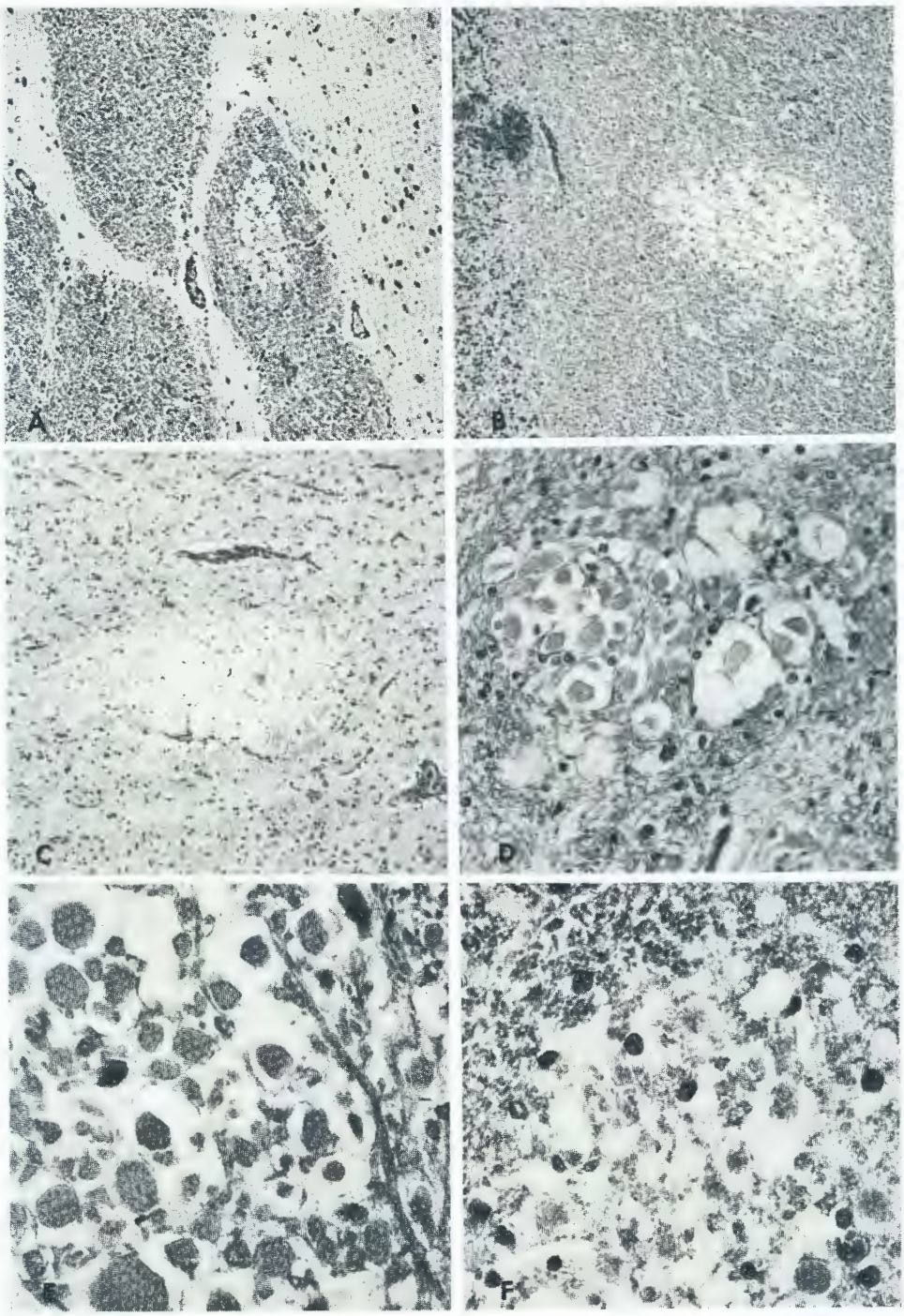
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PLATE 7.—A, B, D: Natural cases. A. Bovine: The white matter of the cerebellum showing enlarged glial cells. H. & E. × 192. B. Bovine: Two enlarged glial cells, presumably astrocytes, in the white matter of the cerebellum. H. & E. × 480. C. Bovine: Experimental case. Hippocampus with four swollen glial cells. H. & E. × 480. D. Bovine: A small bloodvessel in the thalamus showing several globules within the glial processes. P.A.S. × 1200

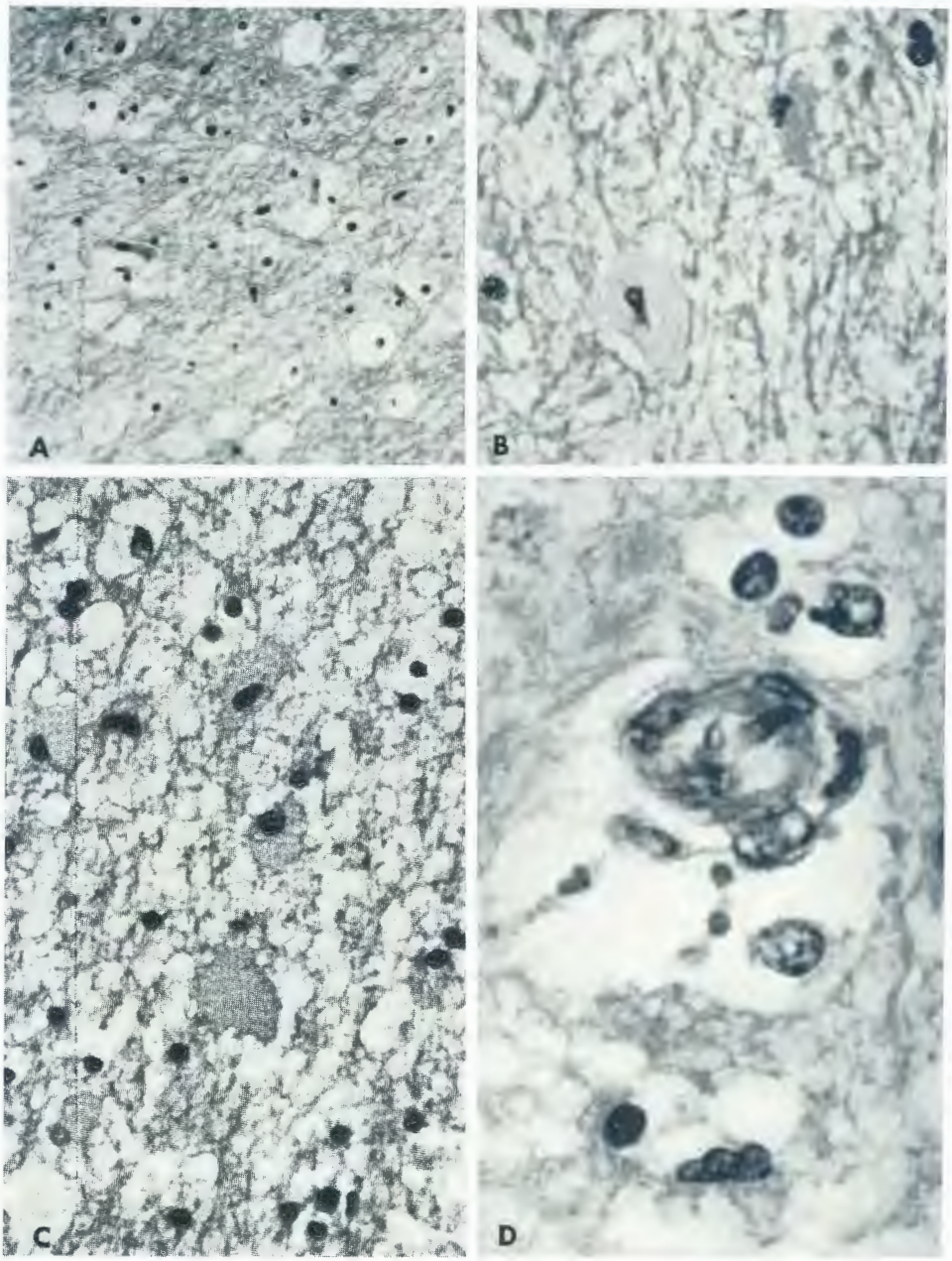
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PLATE 8.—Natural cases. A. Ovine: An activated glial cell, probably an astrocyte, within the cerebral white matter. H. & E. × 1200. B. Ovine: A glial cell in the cerebellum showing hyperchromasia and a mild increase of cytoplasm. H. & E. × 1200. C. Ovine: Cerebral peduncle with glial cell containing a small number of intracytoplasmic granules. H. & E. × 1200. D. Bovine: Glial cells in the medulla oblongata. The nuclei are irregularly shaped, very pycnotic and peripherally displaced. A further increase of cytoplasm and granules can be seen. H. & E. × 1200. E. Ovine: A markedly pycnotic glial nucleus surrounded by large globules (Cerebellum). H. & E. × 1200. F. Ovine: The cerebellum of the same case illustrated in B and E, showing a karyorrhectic glial nucleus and granular cytoplasm. H. & E. × 1200. G. Bovine: Perivascular process and sucker feet of a glial cell containing eosinophilic globules (indicated by arrow) in the thalamus. H.P. × 1200. H. Bovine: Glial cell in the midbrain containing globules in the perikaryon and processes. P.S.A.-M.P.A.H. × 1200. I. Bovine: Numerous globules in the V.R. space of a blood vessel. (Midbrain.) P.A.S.-M.P.A.H. × 480. J. & K. Bovine: Globules showing delicate terminal projections (Midbrain). P.A.S.-M.P.A.H. × 1200

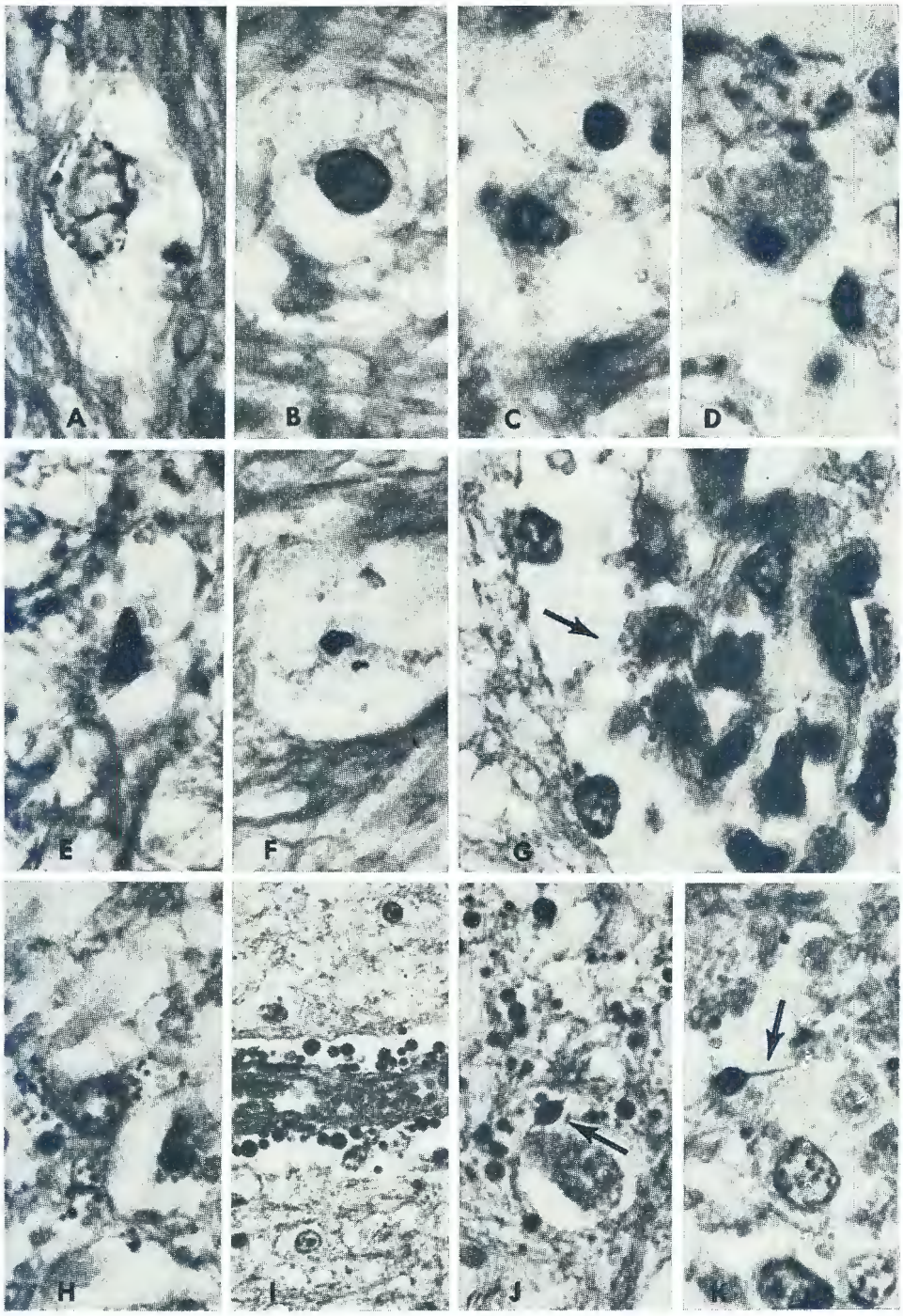
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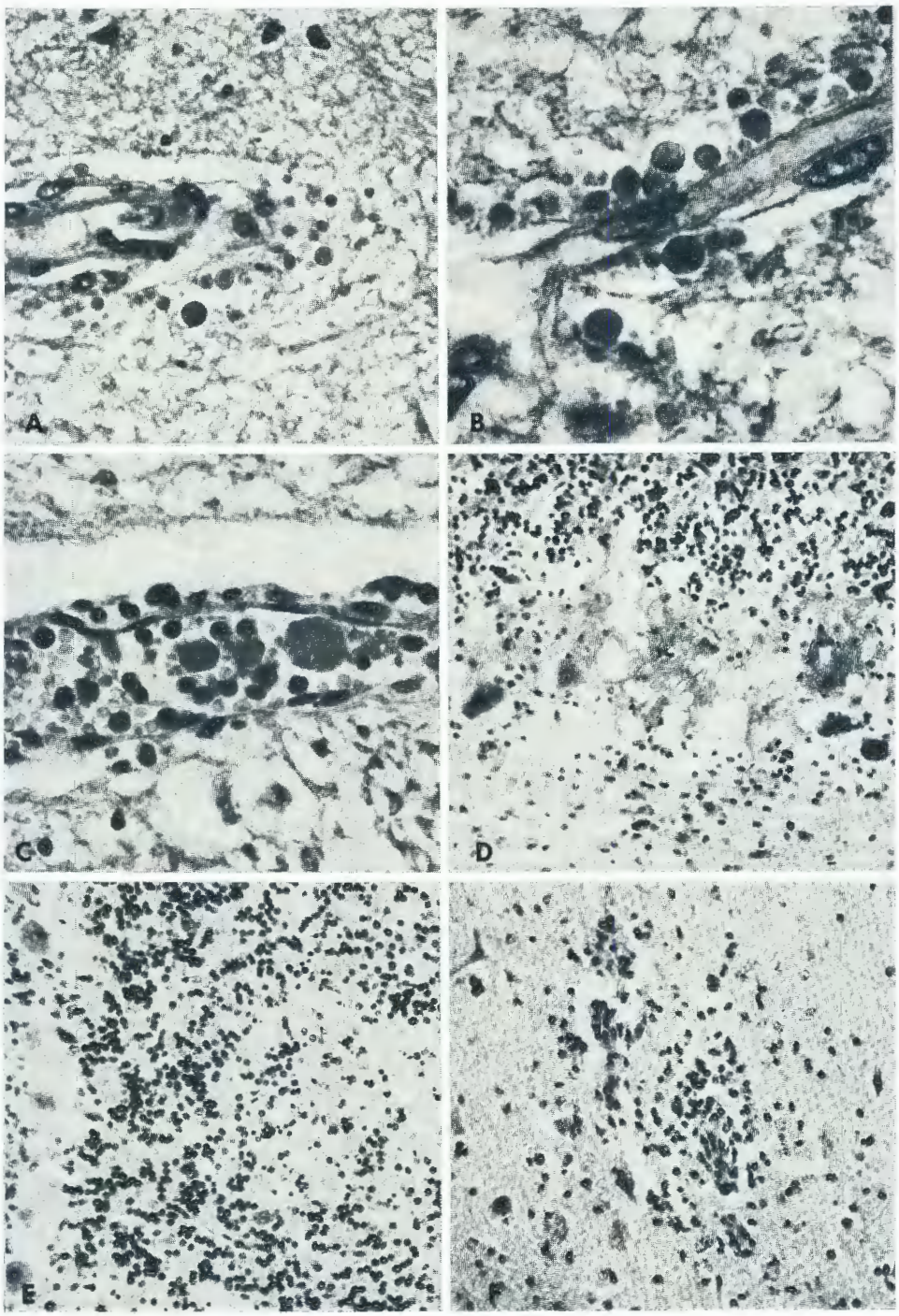
PLATE 9.—A, B, D, E: Natural cases. A. Bovine: Bloodvessel in midbrain. Globules are present in the V.R. space and adjoining brain substance. H.P. × 480. B. Bovine: Large globules surrounding a capillary in the midbrain. H.P. × 1200. C. Bovine: Experimental case. Bloodvessel in the cerebellum containing large globules in its lumen. H. &. E. × 480. D. Bovine: Necrotic focus and a small haemorrhage in the cerebellum. H. & E. × 192. E. Ovine: Focal necrosis in the granular layer of the cerebellum. H. & E. × 192. F. Bovine: An area of gliosis in the thalamus of an experimental case, 10 days after initial rise in temperature. H. & E. × 192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