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LEUKOENCEPHALOMALACIA: A MYCOTOXICOSIS OF EQUIDAE CAUSED BY *FUSARIUM MONILIFORME* SHELDON

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ABSTRACT

MARASAS, W. F. O., KELLERMAN, T. S., PIENAAR, J. G. & NAUDÉ, T. W., 1976. Leukoencephalomalacia: A mycotoxicosis of equidae caused by *Fusarium moniliforme* Sheldon. *Onderstepoort Journal of Veterinary Research* 43 (3), 113–122 (1976)

When 2 horses were dosed with cultures of a *Fusarium moniliforme* isolate that had previously caused only hepatosis, 1 developed brain oedema and hepatosis, and the other only leukoencephalomalacia. A 3rd horse developed both leukoencephalomalacia and hepatosis after being dosed with another isolate obtained from maize which was associated with a natural outbreak of the nervous form of the disease. Since leukoencephalomalacia and hepatosis could be induced by the same culture material, it was concluded that both syndromes were manifestations of the same toxicosis. There was also some evidence that leukoencephalomalacia might be specifically induced by the administration of smaller doses of the culture material to horses over a longer period.

The clinical signs of nervous disorder included ataxia, paresis, apathy, hypersensitivity, frenzy, and other locomotory and psychic disturbances.

Autopsy showed that the brains were oedematous, and focal areas of liquefactive necrosis were present in the cerebral white matter. In 1 case the malacic areas were not confined to the subcortical white matter but were microscopically visible in the cerebral cortex as well. An histopathological examination of the areas bordering on the malacic areas revealed rarefication of the white matter, perivascular haemorrhages, oedema and cellular infiltration composed mainly of plasma cells and eosinophiles. Many of the macrophages in these areas contained lipofuscin-like granules, but these granules also occurred extracellularly in the neuropil. In the layers of the cortex nearest the malacic areas, satellitosis and neurophagia were commonly seen.

Résumé

LA LEUCO-ENCÉPHALOMALACIE: UNE MYCO-TOXICOSE DÛE AU FUSARIUM MONILIFORME *SHELDON ATTEIGNANTE LE CHEVAL*

A la suite de l'administration du champignon Fusarium moniliforme *isolé en culture à deux chevaux, un oedème cérébral et une hépatose se sont développées chez l'un et une leuco-encéphalomalacie seule chez l'autre. Auparavant ces cultures n'ont provoqué qu'une hépatose. Après l'administration d'un autre isolat de ce champignon provenant du maïs associé a un cas du type nerveux dans la nature, un 3 ème cheval a été atteint d'une leuco-encéphalomalacie et une hépatose. Comme la même culture a été à l'origine de la leuco-encéphalomalacie et de la hépatose, la conclusion a pu être tirée que les deux syndromes sont la manifestation de la même toxicose. Les auteurs se sont rendu compte de la preuve que la leuco-encéphalomalacie peut être provoquée chez le cheval d'une façon spécifique par l'administration de plusieures doses plus petites de la culture au cours d'une période plus longue.*

Les symptômes de type nerveux qui se manifestent sont l'ataxie, la parésie, l'apathie, l'hypersensibilité, la frénésie ainsi que d'autres perturbations locomotrices et psychiques.

A l'autopsie on constate un oedème cérébral et des foyers de leuco-encéphalomalacie. Dans un seul cas les foyers ramollis ont été présents non seulement dans la matière blanche sous-corticale mais aussi au niveau de l'écorce cérébrale. A l'examen de coups histologiques des régions voisinantes les foyers ramollis, une raréfaction de la matière blanche, des hémorragies perivasculaires, de l'oedème et une infiltration cellulaire aux plasmocytes et polynucléaires éosinophiles ont pu être mises en évidence. On a constaté la présence de granules de type lipofuchsine non seulement dans le cytoplasme de nombreuses macrophages, mais aussi en dépôt extra-cellulaire. Les couches corticales voisinantes les foyers ramollis ont fréquemment révélées de la satellitose pathologique et de la neuronophagie.

INTRODUCTION

The fungus *Fusarium moniliforme* Sheldon was recently shown to be the cause of equine leukoencephalomalacia (Wilson, 1971; Wilson & Maronpot, 1971; Wilson, Maronpot & Hildebrandt, 1973). These investigators reproduced the typical necrotic lesions in the white matter of the cerebrum in 1 donkey fed freeze-dried material of *F. moniliforme* cultured on autoclaved maize (*Zea mays* L.) kernels. The isolate of *F. moniliforme* used was originally obtained from mouldy maize from the Nile Delta where field outbreaks of leukoencephalomalacia had occurred.

An experimental mycotoxicosis in horses and donkeys caused by South African isolates of *F. moniliforme* from maize was described in a preliminary communication by Kellerman, Marasas, Pienaar & Naudé (1972). Cultures on autoclaved maize were dosed to 3 donkeys and 3 horses. In the 3 animals that died, the liver appeared to be the primary organ affected, no lesions being found in the nervous system.

This paper reports on the results of subsequen dosing experiments with *F. moniliforme* in 3 horses, and the pathology of the lesions produced in the liver and brain.

MATERIALS AND METHODS

Two isolates of *F. moniliforme* were used in the dosing experiments described here, viz. OP–32B isolated from a sample of maize meal from Klipfontein, Johannesburg (Kellerman *et al.*, 1972), and OP–124 isolated from ground maize associated with a field outbreak of leukoencephalomalacia in horses near Benoni during July, 1973. Cultures of both isolates were lyophilized as soon as possible after their primary isolation on a culture medium consisting of malt extract agar containing sodium novobiocin (Butler & Hine, 1958). Lyophilized conidia were resuspended in sterile distilled water and used to inoculate autoclaved yellow maize kernels in 2 litre Erlenmeyer flasks (400 g maize in 200 ml distilled water/flask). Cultures were incubated in the dark in a temperature-controlled room at 25 °C for 12 h/day, and 10 °C for 12 h/day, for 21 days. Thereafter the

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contents of the flasks were minced in a meat mincer, air-dried at room temperature, ground in a mill, and stored at 4 °C until used.

Watery suspensions of the culture material were dosed through a stomach tube to 3 horses. The horses were examined clinically every day, and routine chemical pathological determinations were done periodically on the blood.

All the horses were autopsied immediately after euthanasia administered with an intravenous injection of pentobarbitone sodium. The entire brain and spinal cord, and specimens from other tissues were fixed in 10% buffered formalin. After fixation, serial coronal sections of the brain, approximately 3 mm in thickness, were made. Suitable blocks were selected from these and embedded in paraffin wax. Sections for microscopic examination were stained with haematoxylin and eosin. The following histochemical techniques were applied to brain and liver tissue: Hall's stain for bilirubin (Luna, 1968), Perl's stain for iron, the Hotchkiss periodic acid-Schiff technique, and Schmorl's stain for lipofuscins (Pearse, 1961).

Results

Toxicity trials

Horse 1, a 7-year-old gelding of 449 kg live mass, was gradually accustomed to maize by the addition of maize meal (1,5–2,5 kg/day) to the ration. The total consumption of maize meal in the days prior to the commencement of the experiment was 25,5 kg. No laminitis or other ill effects were observed.

The subject was then dosed daily (except at weekends and on public holidays) with culture material of isolate OP–32B at the rate of 5 g/kg live mass. Fifteen such doses, or a total of 33,67 kg of culture material, were given in 22 days.

The first clinical signs of intoxication became evident after approximately 10 days. These signs, which grew progressively more intense, included the following: icterus, petechial haemorrhages in the conjunctiva, intermittent anorexia and mild incoordination. Marked elevations in the serum glutamic oxaloacetic transaminase activity (382 King Units) and total bilirubin levels (7,4 mg/100 ml) were recorded in the blood. Early on the 22nd day of the experiment, the horse was found standing dejectedly in its stall with the head down, forelegs wide apart, covered in sweat, and trembling. It became aggressive when handled, pricking back the ears, lifting the head and baring the teeth. The horse was reluctant to move but managed to walk fairly normally to a crush pen for a routine clinical examination. In the crush pen it became violently agitated for no apparent reason. The eyes were wild, and marked blepharospasm and nystagmus were present; the facial muscles were contracted, the contraction flaring the nostrils and baring the teeth; and the animal thrashed about. Eventually it fell down in violent convulsions, passing dark urine and voiding faeces. As it lay on its side making ineffectual galloping motions, almost continuous clonic spasms racked the entire body.

Approximately 1½ h after the first signs were seen, the horse was destroyed by intravenous injection of pentobarbitone sodium.

Horse 2, a 20-year-old gelding of live mass 443,6 kg, developed severe laminitis after being dosed twice with a culture of isolate OP–32B at the rate of 5 g/kg live mass.

The laminitis was successfully treated by interruption of the dosing programme for 12 days, in the course of which an anti-histamine (500 mg promethazine hydrochloride) was administered intravenously on 2 consecutive days, and cold water was applied to the hoofs. Although a full recovery was made, similar attacks of laminitis frequently disrupted the experiment. Maize meal was sometimes fed during these interruptions both to accustom the horse to this diet and to establish whether a high maize intake was responsible for the laminitis.

Two days after dosing was resumed at 5 g/kg/day (given in conjunction with maize meal) laminitis again set in. The horse was then rested for 3 weeks. It is interesting to note that a mild relapse occurred after 9,5 kg maize meal had been fed in 1 week during this rest period.

Since the animal could not tolerate successive doses of 5 g/kg culture material, the regimen was altered to 2,5 g/kg given 3 times per week, viz. on Mondays, Wednesdays, and Fridays. After 16 doses in 42 days, the level of culture material was increased to 5 g/kg, but after only 1 such dose laminitis again set in.

The horse was now rested for 19 days before a new dosing regimen was introduced. This time 2,5 g/kg culture material was administered daily from Mondays to Fridays. In all, 31 such doses were given over a period of 45 days.

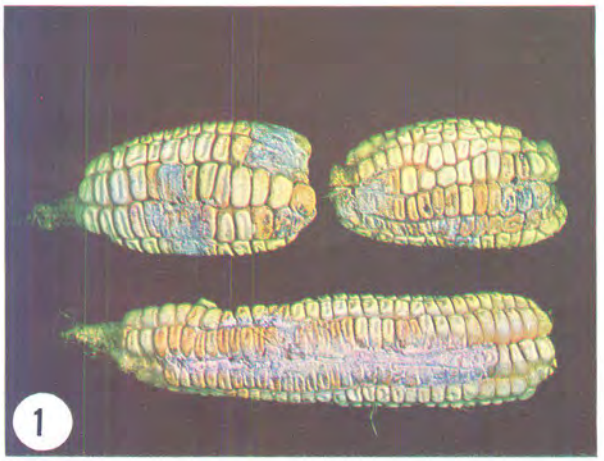
During the course of the entire experiment, which lasted 144 days, the horse received a total of 63,8 kg of culture material in 52 doses of 2,5–5,0 g/kg live mass.

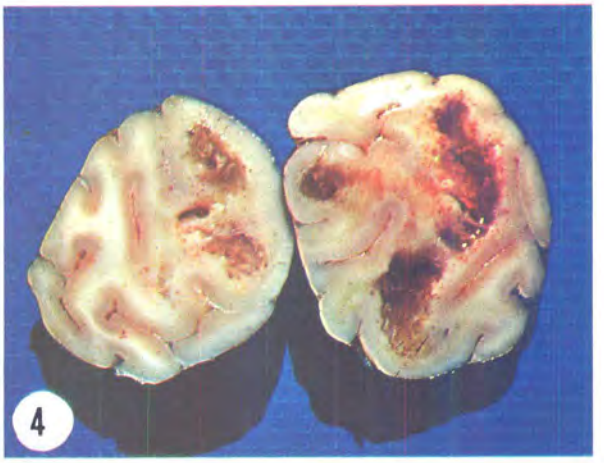
The clinical signs of intoxication abruptly appeared on the 145th day. At first the horse was merely apathetic and reluctant to move, but later it became wildly excited while being examined in a crush pen. No reason for the excitement could be found as the clinical examination was a routine procedure to which the animal was well accustomed. Contrary to its normal habit, the horse also refused to back out, but, instead, bore forward with such violence that it broke out of the closed front-end of the crush pen.

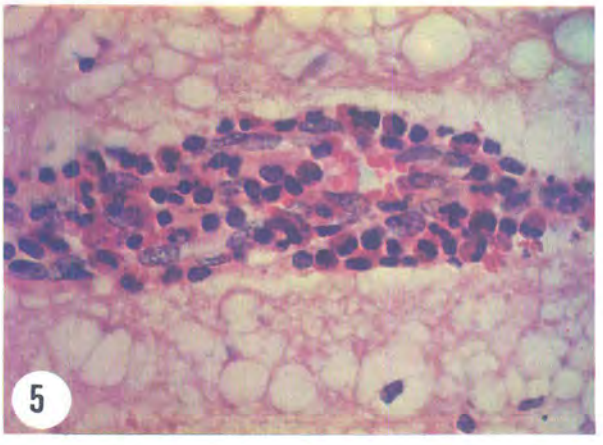
Back in the quiet surroundings of its own shelter, the horse calmed down somewhat, but it still persisted in pushing against the railings with its chest as if it was unwilling or unable to move backwards.

The horse was then taken to a large concrete-floored camp where it immediately started to circle aimlessly in an anti-clockwise direction. Its gait, apart from a slight tendency to stumble or to brush with the forefeet, was fairly normal, but in time the pace of the circling movements increased from a walk to a trot. Upon encountering foreign objects, such as droppings strewn across the path, the horse would stare uncomprehendingly at them for a long while before resuming the aimless circling movement (Fig. 3). Sometimes it collided with the fence or walked into objects, apparently through lack of comprehension rather than impairment of vision. After such minor collisions it would often recoil with violence out of proportion to the force of impact. Although the horse was now fairly apathetic, it became aggressive when handled and steadfastly refused to walk backwards.

In the early stages of the syndrome it attempted to eat hay, but the material hung unchewed from the mouth. Later the tongue became flaccid (Fig. 2) and the penis protruded from the sheath.







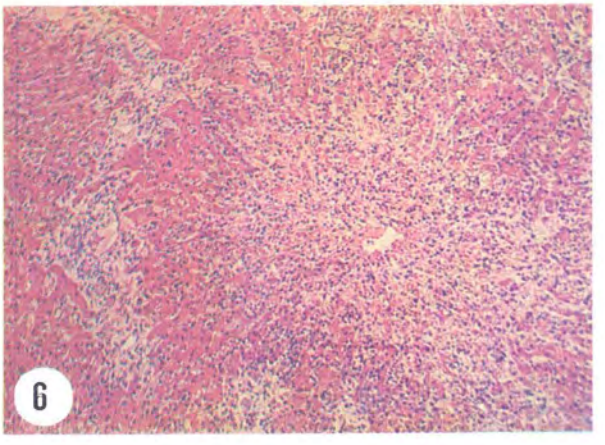
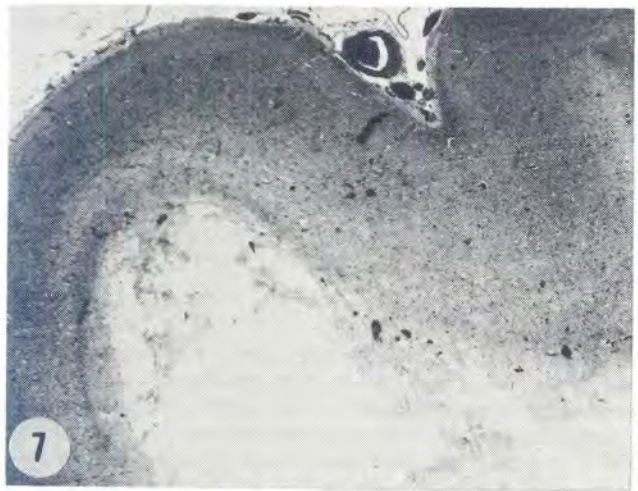
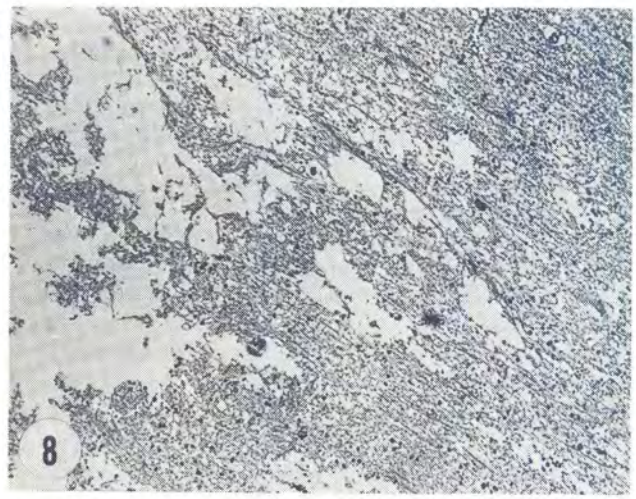
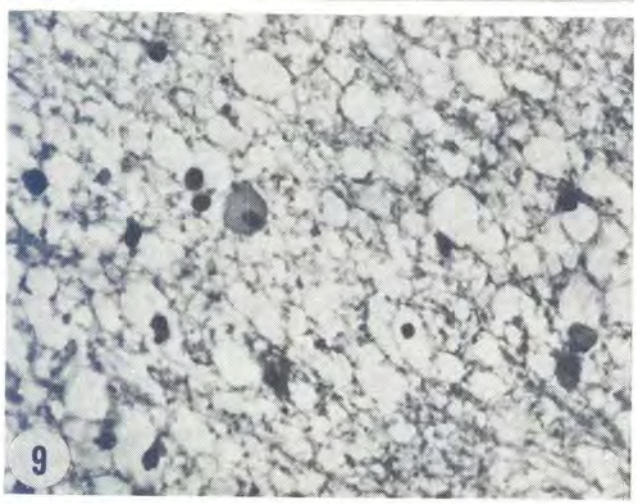
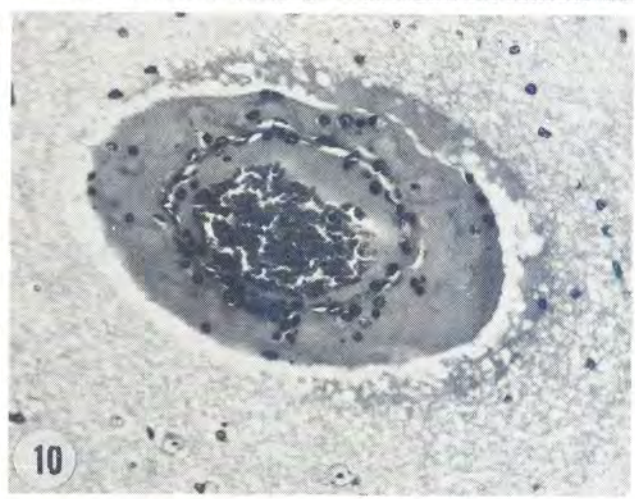


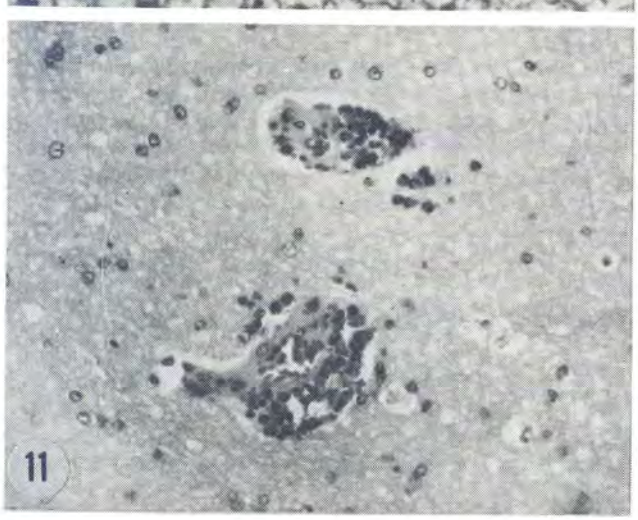
FIG. 1 Maize cobs infected with *Fusarium moniliforme*
FIG. 2 Horse 2. Somnolent appearance with tongue protruding
FIG. 3 Bewildered horse staring incomprehendingly at dung pads in front of it
FIG. 4 Large cavities in subcortical white matter of frontal area of cerebrum
FIG. 5 Large numbers of eosinophiles in and around a vessel. HE×400
FIG. 6 Liver showing fibrosis around a central vein. HE×50











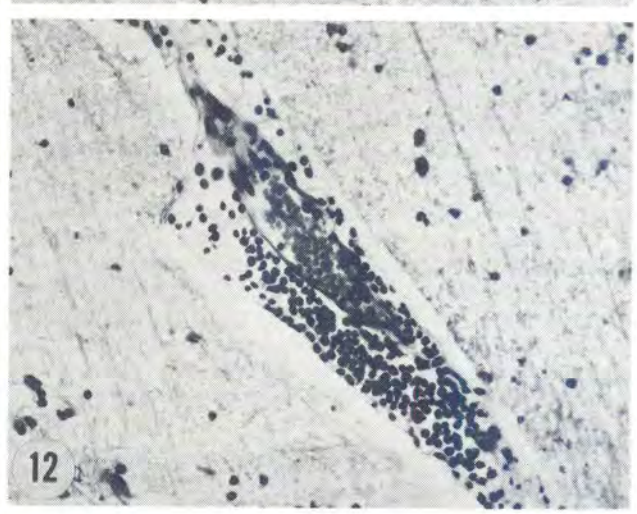


FIG. 7 Cerebrum. Total disintegration of subcortical white matter. The overlying grey matter is intact. HE×12
FIG. 8 Severely rarefied white matter on the edge of an encephalomalacic area. HE×75
FIG. 9 Higher magnification of rarefied area in Fig. 8, showing pyknosis of glial nuclei. Some glial cells show enlarged eosinophilic cytoplasms. HE×500
FIG. 10 Blood-vessels with marked perivascular oedema. HE×200
FIG. 11 Two blood-vessels with a mild perivascular infiltration of eosinophils. Note eosinophils in adjoining neuropil. HE×200
FIG. 12 Blood-vessel with a perivascular infiltration of plasma cells. HE×200

As time passed the clinical signs became more pronounced. The horse walked into objects with increasing frequency, until eventually it failed almost completely to recognise obstacles in its path. Sometimes it stood for long periods mindlessly pushing against the fencing, apparently unable to free itself by moving backwards. Finally, it repeatedly galloped full tilt into fences, grievously injuring itself in the process. At this stage the animal was frenzied, extremely hypersensitive, and completely unmanageable. Fortunately, it responded well to sedatives (50 mg acetylpromazine V*; 250 mg Rompun**) injected intramuscularly during pushing episodes, and could be destroyed by intravenous injection of pentobarbitone sodium.

The course of the disease, from the time that signs were first observed to the destruction of the animal, was 6½ h.

Horse 3 was an emaciated 13-year-old gelding that periodically suffered from the heaves. In the beginning it had a live mass of only 350 kg, but during the experiment it gained 45 kg and completely recovered from the respiratory disorder.

The experiment was in 2 parts: in the 1st, the horse was fed on mouldy maize kernels from a farm where leukoencephalomalacia had occurred. A total of 177,0 kg of these kernels was fed in 67 days at the rate of 0,5–3,5 kg/day. When the supply of kernels was exhausted, 7,0 kg of mouldy cobs from the same source was also fed to it in 3 days. No clinical pathological changes or other untoward effects were observed.

The horse was then kept under observation for 135 days before the 2nd part of the trial was started. This involved dosing cultures of the relevant *F. moniliforme* isolate (OP 124) at the rate of 5 g/kg live mass 3 times per week. Altogether 33 such doses, or a total of 29,6 kg of culture material, were given in 90 days.

After the first 5 doses, the horse developed mild icterus and, although the total bilirubin level in the blood was only 2,4 mg/100 ml, it was decided to discontinue dosing for 12 days for fear of precipitating a fatal hepatic crisis. The remaining 28 doses were given without incident.

The onset of leukoencephalomalacia was, in this case, heralded by mild icterus and petechial haemorrhages in the conjunctiva, which appeared on the 87th day of the experiment. On the 89th day, the horse had a marked bilirubinaemia (3,2 mg/100 ml), ate little, and the level of serum glutamic oxaloacetic transaminase activity in the blood was elevated (274 King Units). By the 90th day it was obviously sick.

Early in the morning of that day, the horse was found standing quietly with the back legs slightly apart, swaying in the hindquarters, and moving the tail to keep its balance. It was covered in sweat and trembled noticeably, especially in the flanks.

Despite the dejection, the horse would still turn its head to observe passing objects or prick up the ears in response to sound stimuli. At this stage the affection appeared to be primarily locomotory in nature.

The horse would stand immobile for at least 45 min. When it was forced to move, pronounced ataxia especially of the hindquarters was evident. During locomotion the rump sloped down steeply and the animal walked awkwardly, with the hindlegs tucked under the body. Short steps were taken and the hind feet were lifted high off the ground in a goose-step motion.

Approximately 3 h after the signs were first noticed, the horse was destroyed by intravenous injection of pentobarbitone sodium. The decision to destroy the horse before the clinical signs were fully expressed was taken for humane reasons.

Pathological lesions

Gross lesions

Brain. Oedema of the brain, seen as an accumulation of clear fluid underneath the meninges, and a few petechial haemorrhages in the cortex of the cerebellum were the only gross changes observed in the brain of Horse 1. However, there were obvious lesions of encephalomalacia in the brains of Horses 2 and 3.

The encephalomalacic lesions were noticed on the external surface of the left cerebral hemisphere of Horse 2 as ill-defined but distinctly swollen areas which fluctuated on palpation. The swollen areas were located sagittally in the dorsal middle and medially in the anterior frontal part of the hemisphere. The gyri overlying the swollen parts had a flattened appearance and were tinted faintly yellow. On coronal sectioning of the brain, large cavities, representing complete liquefaction of the subcortical white matter (Fig. 4), were found in both cerebral hemispheres. The borders of these cavities had a frayed and irregular appearance, with numerous small haemorrhages in the white matter surrounding them. The white matter immediately surrounding these cavities had a bright yellow colour which changed to a light peagreen after fixation in formalin.

Cross sections, after fixation, of the brain of Horse 3 revealed a number of light-green foci, 1–2 cm in diameter, in the subcortical white matter of the frontal poles of both cerebral hemispheres. They were randomly distributed and were located directly underneath the cortex. No obvious softening could be seen in these green foci, though very fine petechiae were associated with some of them. Similar greenish-discoloured areas were also discovered unilaterally in the ventral portion of the hippocampus and in the medial geniculate. Larger foci, showing definite encephalomalacia but not yet complete liquefaction of brain tissue, were present bilaterally in the dorsolateral aspects of the middle portion of both cerebral hemispheres. Numerous petechial haemorrhages were scattered in and around these malacic foci and the adjoining white matter had a green colour. In one of the inferior colliculi a number of small haemorrhages could be seen.

The external surface of the cerebral hemispheres in Horse 3 appeared unaltered, except that a palpable softness was detected in the cortex overlying the larger areas of encephalomalacia. No definite swelling or discoloration was evident over these areas as was the case in Horse 2.

Other organs. In addition to the brain oedema, the autopsy on Horse 1 revealed a mild icterus, hepatosis, and subendocardial, subepicardial, and subpleural haemorrhages, as well as haemorrhages in the adrenal cortex and petechial haemorrhages in the mucosa of the last part of the jejunum and ileum. This part of the

* Boots (A. S. Ruffel)
** Bayer Agro-Chem

intestinal tract also showed a mild oedema of the mucous membrane. The liver was slightly swollen and the lobulation was accentuated by a light yellow-brown colour around the central veins. The periphery of the lobules, however, had a normal red-brown colour. Underneath the capsule, irregular olive-green discoloured patches, up to a few centimetres in diameter, were seen throughout the organ, and were thought to be focal areas of bile stasis. On section, similar olive-green patches were found in the depth of the liver, and the consistency of the organ also appeared to be slightly increased.

Except for the encephalomalacia and lesions of self-inflicted trauma, no gross pathological changes were present in the carcass of Horse 2. Horse 3 had a very mild icterus at autopsy and, although the liver was not enlarged, an increase in consistency could be detected.

Microscopic lesions

Brain. Large, irregularly-outlined empty spaces (Fig. 7) where the tissue had totally disintegrated marked the centre of the encephalomalacic areas in the brain of Horse 2. Immediately around these empty spaces, the white matter appeared severely rarefied (Fig. 8) owing to the breaking up of myelin sheaths, and no normal myelin could be recognized. Some of the axis cylinders were preserved, while most of the glial cells that were still intact showed pyknotic nuclei and enlarged eosinophilic cytoplasms (Fig. 9). Towards the periphery of the encephalomalacic area, the rarefied white matter gradually changed over to normal white matter without a sharp line of demarcation. However, many of the blood vessels situated in this transitional zone had perivascular haemorrhages, perivascular oedema (Fig. 10), or a cuffing by infiltrating cells. Usually 1 of these phenomena was present around a single vessel. The perivascular cell infiltration consisted of eosinophils and round cells, mainly plasma cells. Some vessels contained only eosinophils (Fig. 5 & 11) in their walls and in the Virchow-Robin (V.-R.) space, while others had predominantly plasma cells (Fig. 12) with isolated eosinophils sometimes present. Although only a small number of vessels in the vicinity of the encephalomalacic areas showed these perivascular cell infiltrations, this feature was constantly present near the necrotic foci in the brains of both Horse 2 and Horse 3. The intensity of this cell reaction varied from a few cells to a layer 3–4 cells deep. The eosinophils were not limited to the vessel walls and V.-R. spaces only, but could also be found in small numbers in the adjoining neuropil and in capillaries close to the lesions.

The encephalomalacic lesions visible in Horse 3 were represented microscopically as focal areas of microcavitation (Fig. 13). There was no total disintegration of brain tissue as was seen in Horse 2. Blood-vessels around the foci of microcavitation showed perivascular haemorrhages, oedema and cell infiltration similar to that seen in the brain of Horse 2. The focal green areas, discernible grossly after fixation in the brain of Horse 3, proved to be groups of vessels, in fairly close proximity to each other and with marked perivascular oedema (Fig. 14). No obvious microcavitation was present in these discoloured foci.

Apart from the oedema, another striking microscopic feature in these green foci was the presence of a light-yellow pigment. This was largely contained within macrophages present in the V.-R. spaces (Fig. 15) and in the vessel walls, but sometimes it also occurred extracellularly in these localities. An identical pigment was also associated with blood-vessels showing perivascular oedema in the brain tissue around the necrotic lesions in Horse 2, but it was less abundant. Negative results were obtained on staining the pigment with Hall's bilirubin stain, Perl's stain for iron, and the Hotchkıss periodic acid-Schiff technique, but Schmorl's staining method for lipofuscins gave a positive reaction.

In the deeper layers of the cerebral cortex overlying the malacic lesions in the white matter, satellitosis and neurophagia were frequently seen in Horses 2 and 3, a feature mainly of the neurones situated closest to the necrotic areas. Many of these neurones exhibited enlarged vesicular nuclei and tigrolysis, with fading of the staining intensity of the cell, which was interpreted as representing plasmolysis and karyolysis.

Necrotic lesions in Horse 3 were not confined exclusively to the white matter of the brain; a few small areas were encountered in which the entire depth of the cortex showed marked cavitation of the neuropil with pronounced widening of the perivascular spaces (Fig. 16). Numerous eosinophilic globules, varying in size and in staining intensity, occurred in the perivascular spaces, in the neuropil close to the vessels (Fig. 17), and around some glial nuclei. Similar eosinophilic globules were also observed to a lesser extent in the vicinity of the necrotic lesions in the white matter in both Horses 2 and 3. These globules were regarded as a pathological change resulting from increased vascular permeability, and were identical in appearance and staining characteristics with those previously reported in ruminants with brain oedema (Pienaar, Basson & Van der Merwe, 1966). Small haemorrhages occurred throughout these areas in the cortex, and a few neutrophils were also found in perivascular spaces and in the brain substance close to the vessels.

Focal oedema of the leptomeninges (Fig. 18) was encountered in Horses 2 and 3. Blood-vessels associated with the oedematous changes in the meninges sometimes contained perivascular cell infiltrates similar to those seen in the cerebral white matter. The molecular layer of the cerebellum underneath the meninges was also frequently infiltrated by oedematous fluid, markedly so in some instances.

No lesions of encephalomalacia were present in the brain of Horse 1 on microscopic examination. The only significant finding was oedema of the leptomeninges and of the underlying brain tissue. This oedema, occurring in a focal manner, was not a generalized change. Similarly, single vessels scattered in the white matter of the brain had oedematous fluid in their perivascular spaces and adjoining neuropil. A very slight infiltration of neutrophils and of a few round cells was in some instances associated with the vessel walls in these focal areas of oedema. No eosinophils were encountered. Blood-vessels throughout the brain were congested.

Liver. An histopathological examination of the livers of Horses 1 and 3, which had a bilirubinaemia clinically and gross liver lesions at the post mortem examination, revealed a fibrosis which occurred intralobularly in a zonal pattern involving the centrilobular area uniformly throughout the organ.

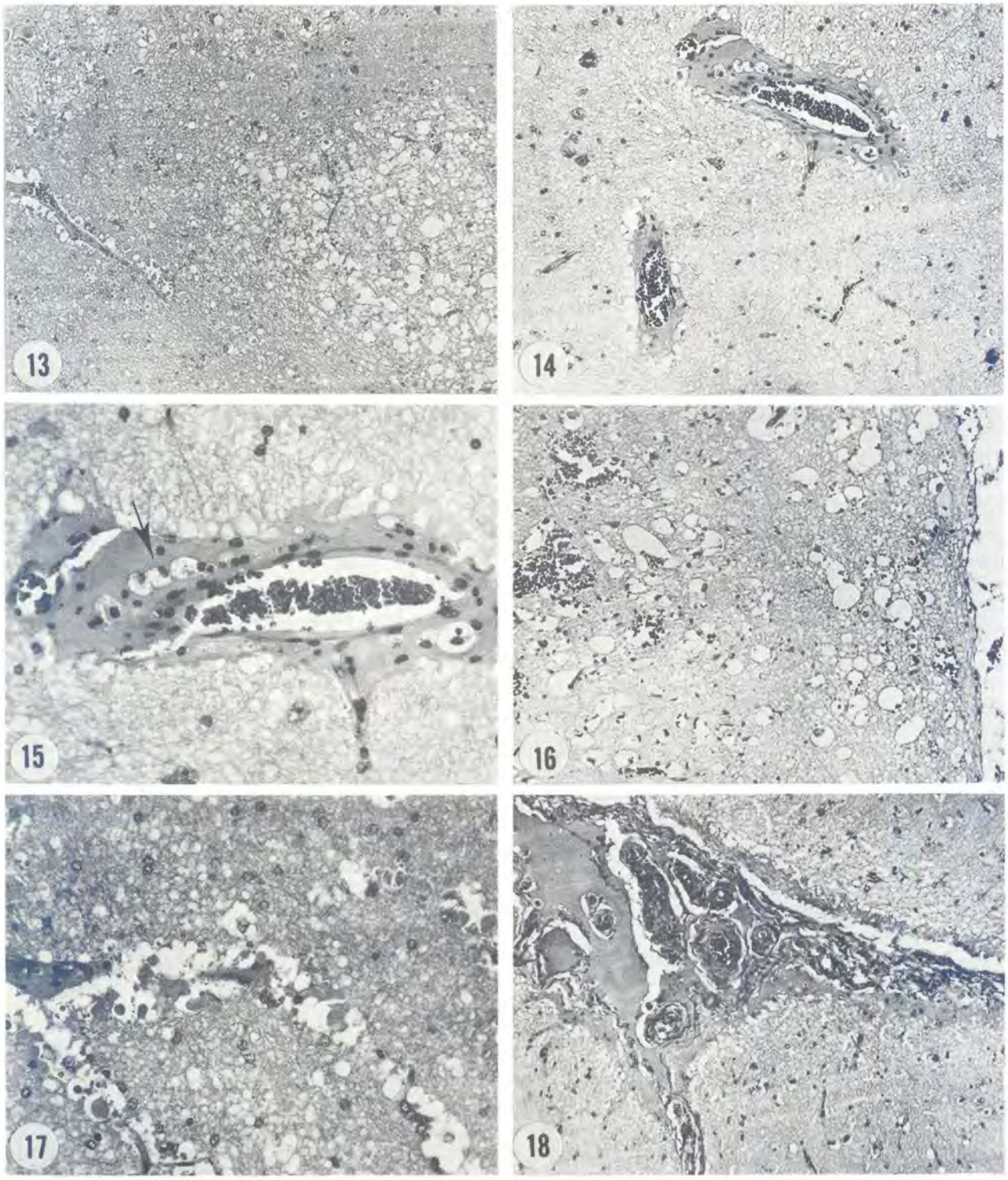
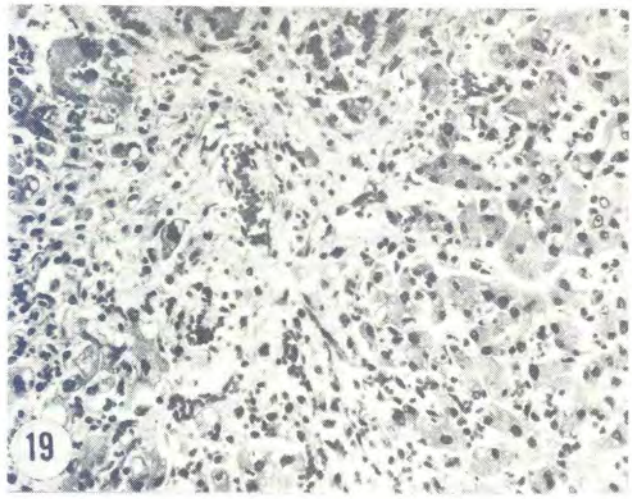
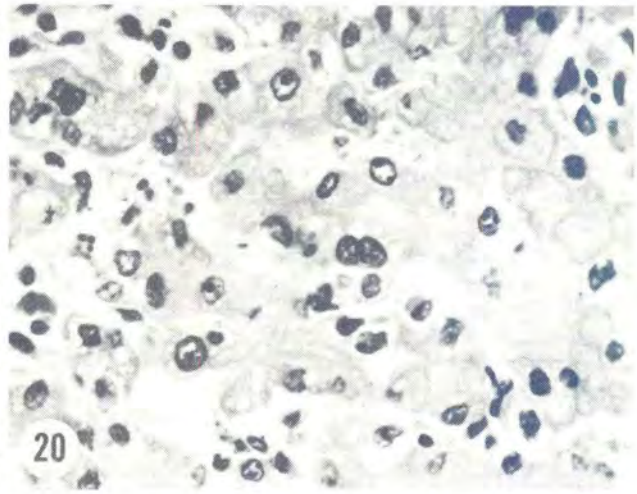
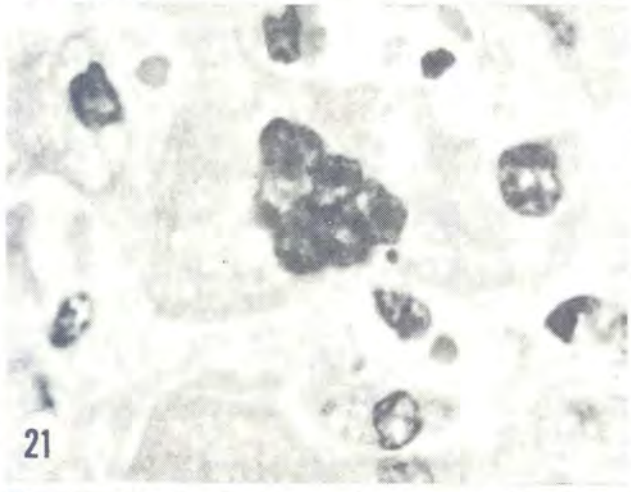
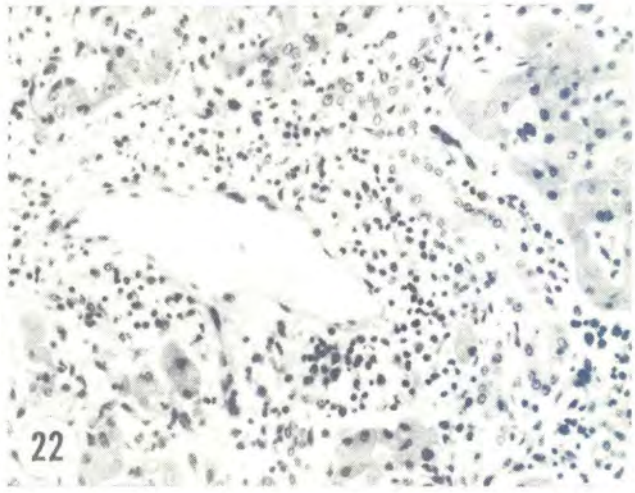


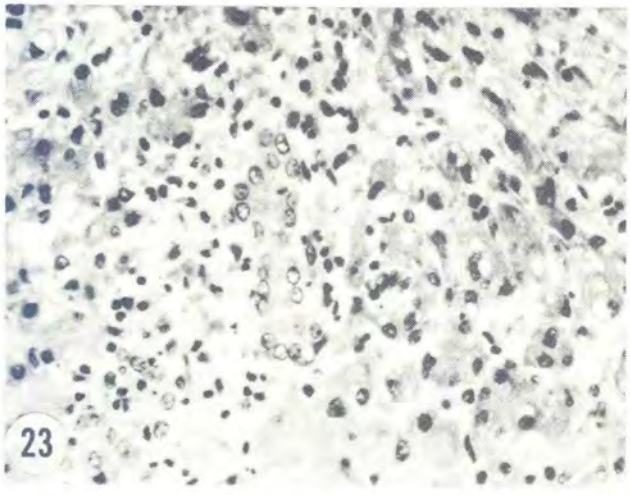
FIG. 13 Focal area of microcavitation in the subcortical white matter of the brain. HE×75
FIG. 14 Blood-vessels in close proximity to each other, with obvious perivascular oedema. HE×120
FIG. 15 Higher magnification of top blood-vessel in Fig. 14. Note macrophages filled with pigment (arrow). HE×200
FIG. 16 Microcavitation in cerebral cortex. Small haemorrhages are also present. HE×75
FIG. 17 Numerous eosinophilic globules in perivascular spaces of blood-vessels on the edge of encephalomalacic area. HE×200
FIG. 18 Oedema of leptomeninges. Note also oedema of outer surface of molecular layer. HE×75











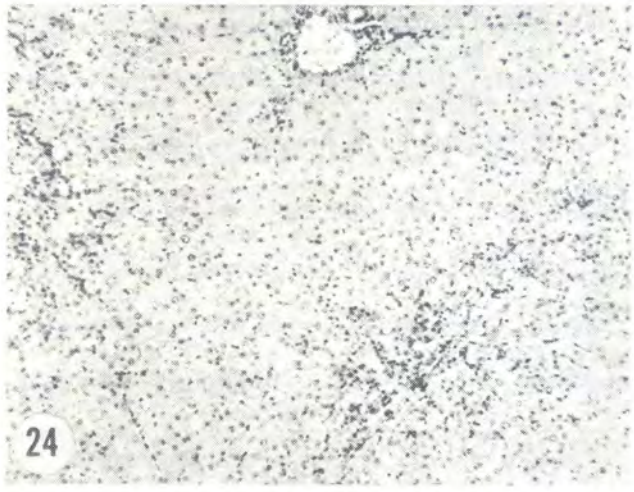


FIG. 19 Centrilobular fibrosis in liver. HE×75
FIG. 20 Binucleated cell. Liver. HE×500
FIG. 21 Multinucleated hepatocyte. HE×1 200
FIG. 22 Portal triad, showing slight fibroplasia and round cell infiltration. HE×200
FIG. 23 Bile duct epithelium forming ductular structures on edge of centrilobular fibrotic areas. HE×200
FIG. 24 Increased cellularity around central veins in liver of Horse 3. HE×75

In Horse 1 the centrilobular parenchyma was largely destroyed and replaced by fibrous tissues (Fig. 6 & 19) which radiated in the form of thin bands connecting the centrilobular fibrous tissue of adjoining lobules. This fibrous tissue was infiltrated by moderate numbers of leukocytes, consisting mainly of neutrophils, some eosinophils, and a few round cells. Many macrophages and Kupffer cells located in it contained a yellow-brown pigment which gave a positive reaction with the Schmorls' staining method for lipofuscins.

Surviving hepatocytes, within and on the edge of the fibrous tissue, had large fatty vacuoles in their cytoplasms and were often multinucleated. Binucleated cells (Fig. 20) were most commonly seen, while fewer cells had 3, sometimes 4, and occasionally 8 nuclei (Fig. 21). Hyperchromasia, obvious enlargement and bizarre shapes of nuclei were features frequently present in these cells. Around the portal tracts the hepatocytes showed marked fatty vacuolization, their cytoplasms filled entirely with small fat droplets, while slight proliferation of bile ducts could be seen in the portal triads. Some fibroplasia was present here but not to the same degree as in the centrilobular zones. A similar leukocyte infiltration, as was present in the centrilobularly located fibrous tissue, occurred in the portal triads (Fig. 22), and lipofuscin pigment was also found in macrophages.

An interesting histological change was the focal proliferation of bile duct epithelium to one side of most of the centrilobular fibrotic zones. These cells formed ductular structures, but no clear continuation of these with the proliferating bile duct in the portal tracts could be established (Fig. 23). The presence of a mild cholestasis was indicated by the occasional bile canaliculus plugged with bile, and by some of the larger bile ducts in the portal triads containing inspissated bile.

There was no large-scale replacement of hepatocytes by fibrous tissue in the centrilobular zones in the liver of Horse 3. These, however, stood out clearly owing to an increased cellularity around the central veins (Fig. 24). Fibroblasts, proliferating Kupffer cells, fairly numerous neutrophils, few eosinophils and a small number of round cells were responsible for this. The Van Gieson's stain showed a slight increase of collagen fibres between the hepatocytes in this area.

As in Horse 1, many of the Kupffer cells had phagocytized lipofuscin pigment. About $\frac{1}{3}$ of the hepatocytes appeared necrotic, as evidenced by their pyknotic nuclei and deeply eosinophilic cytoplasms. Some of the hepatocytes on the periphery of the lesions had large, round, eosinophilic hyalin-like bodies lying within a vacuole in their cytoplasms. A mild fatty vacuolization occurred in the hepatocytes throughout the rest of the lobules. Multinucleated hepatocytes were less commonly observed in this instance, binucleated cells with enlarged nuclei being much more common. Large bizarre hyperchromatic nuclei were also seen in hepatocytes, but much less frequently than in Horse 1. The distribution of the lesions was the same as in Horse 1, i.e. centrilobular with strands connecting neighbouring central veins. The only change in the portal triads in this case was a mild degree of bile duct proliferation. There was no evidence of cholestasis, cell infiltrate or fibrosis, as in Horse 1.

The liver of Horse 2 appeared unremarkable, microscopically, except that the Kupffer cells were rather prominent, a fair number of binucleated hepatocytes were encountered, there was variation in nuclear size and the hepatocytes appeared swollen, obscuring the normal sinusoidal pattern.

Discussion

In a previous study, Kellerman *et al.* (1972) described a hepatotoxicosis of equidae caused by *F. moniliforme*, and now leukoencephalomalacia, with or without liver involvement, has been produced by dosing cultures of the fungus to horses. Therefore, since either lesion could be produced by the same culture material, it is concluded that both syndromes are manifestations of the same toxicosis.

Although large quantities of culture material had to be dosed in order to produce either leukoencephalomalacia or hepatosis, the 2 syndromes appeared to be dependent on dosage. A dose equivalent to 0,67–1,94 kg culture material/day for 11–21 days was required to produce a fatal hepatosis (Kellerman *et al.*, 1972), while leukoencephalomalacia was precipitated by the equivalent of 0,33–0,44 kg/day for 90–144 days. From this it would appear that brain lesions can be specifically induced by dosing relatively small quantities of culture material over a long period. The data must be cautiously interpreted, however, as the number of experimental animals was small and factors, such as age, etc. (Badiali, Abou-Youssef, Radwan, Hamdy & Hildebrandt, 1968, and Wilson *et al.*, 1971) were not accounted for.

In both conditions, the onset of symptoms can be sudden; death can occur within days, or even hours. The appearance of an hepatic crisis was usually heralded by icterus and other appropriate chemical pathological changes in the blood, but the nervous signs of leukoencephalomalacia can occur without warning.

The name leukoencephalomalacia is derived from the siting of the malacic areas in the white matter of the brain, but in Horse 3 these lesions were also found in the cerebral cortex. This finding concurs with that of Iwanoff, Yuan & Fang (1957) who described foci of necrosis in the grey matter of the cerebral cortex in some cases. In view of this, they suggested that the disease be re-named "toxic or mycotoxic encephalomalacia".

Iwanoff *et al.* (1957) further reported satellitosis, neuronophagia and gliosis in cases living 4–5 days after the appearance of symptoms. We observed neuronophagia and satellitosis in Horses 2 and 3. The absence of gliosis in our cases can be explained by the fact that the horses were killed soon after the onset of the nervous symptoms. None of the previous workers described perivascular cell infiltrations in the vicinity of the encephalomalacic areas except Iwanoff *et al.* (1957), who mentioned cells, mostly granulocytes, visible in the oedematous fluid around vessels. However, they did not identify these cells any further. Perivascular infiltrations of eosinophiles and plasma cells, similar in distribution and intensity to those of the experimental cases reported here, were seen in the brains of natural cases of *F. moniliforme* poisoning in horses in South Africa (unpublished data).

No definite necrosis was present in the brain of Horse 1. Schwarte, Biester & Murray (1937) too, reported only congestion, oedema and haemorrhages in the brains of some of their experimental cases.

Yellow oedematous foci in the white substance of the brain, without obvious encephalomalacia, were described by Iwanoff *et al.* (1957). They speculated

that the discoloured areas may represent initial stages of the lesion. These discoloured foci were particularly prominent in the brain of Horse 3 which was destroyed shortly after the onset of clinical signs. In contrast to those of Horse 2, the encephalomalacic areas in the brain of this animal were also smaller and less numerous. Microscopic examinations showed that the discoloured foci consisted of groups of vessels with marked perivascular oedema that may indeed develop into areas of encephalomalacia. In this respect, Wilson *et al.* (1973) pointed out that although the exact mechanisms of degenerative changes in the brain are not clear in this disease, present evidence would suggest that damage to blood-vessels may be an important factor.

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