Br. J. exp. Path. (1969) **50**, 393.

THE PATHOLOGY OF EXPERIMENTAL VERVET MONKEY DISEASE IN HAMSTERS

I. ZLOTNIK AND D. I. H. SIMPSON

From the Microbiological Research Establishment, Porton, Salisbury, Wilts.

Received for publication March 7, 1969

SUMMARY.—Although lesions in the organs of hamsters resemble those of monkeys and guinea-pigs, the extension of the pathological process to the brain is a new feature, so far found only in hamsters.

The brain lesions in the hamster consist of capillary proliferations, haemorrhages, microglial changes and very severe astrogliosis. Necrosis of the needle track in i.c. inoculated hamsters is usually very pronounced.

Intracytoplasmic bodies believed to be the infective agent are found in hamsters not only in the liver but also in the kidneys and lung. These bodies are pleomorphic vary in size from 1–3·5 μ in diameter and they are either round or elliptical, and very commonly appear in the liver as ring-shaped structures.

THE transmission of vervet monkey disease (VMD) to hamsters (Simpson, 1969) supplied yet another susceptible laboratory animal and at the same time made possible a very close study of tissue changes caused by the slow adaptation of the agent to the new host.

Early attempts to transmit the disease from guinea-pigs did not meet with great success. Recently however VMD agent that has been passaged 9 times in guinea-pigs and 3 times in monkeys when inoculated into hamsters produced a clinical disease with mortality in suckling hamsters.

MATERIALS AND METHODS

The hamsters used in this work were of 3 age groups as follows: 4–9 days old sucklings; 3 weeks (newly weened), and 6–8 weeks old adults

The inocula consisted of 10 per cent suspensions of infected brain or liver in phosphate buffer (pH 7·2) with an addition of 0·75 per cent of bovine albumen. The route of inoculation was either i.p. or i.c. The dose for all animals irrespective of route was 0·02 ml.

The first attempts to transmit VMD to hamsters were made with infected guinea-pig livers of 4th or 5th guinea-pig passages. Two further hamster to hamster passages were carried out subsequently but these lines were abandoned in view of more uniform results obtained from passaging brain or liver from hamsters infected with monkey liver from animals infected after 9 consecutive guinea-pig passages followed by 3 passages in monkeys (Simpson, 1969).

Hamsters were killed with ether and organs were removed immediately after death. Brain, lung, heart, kidneys and livers were fixed in formol-saline solution, while spleens and lymph nodes were fixed in Susa. Paraffin sections were cut at 7 μ and stained with haematoxylin and eosin. Brain sections, cut on the freezing microtome, were stained with a modification of Cajal for the demonstration of astrocytes, with luxol fast blue for myelin and Sudan IV for fat. Selected sections of organs were also stained with Giemsa, Machiavello, iron-haematoxylin, phloxin-tartrazine, Feulgen, Von Kossa, periodic acid Schiff, and Sudan black.

RESULTS

Macroscopic changes

In all early hamster passages the incidence of overt clinical signs and mortality was limited to about 10 per cent of inoculated suckling hamsters, while in weaned and adult hamsters there were no signs of disease. From the 8th hamster passage the majority of suckling hamsters and a large proportion of adults developed signs within 5–9 days of inoculation.

Animals, both suckling and adult, that did not develop clinical signs sometimes had lesions mainly confined to the liver which was congested and enlarged. Brains of animals inoculated by the i.c. route showed a reddish or brownish discoloration along the needle track, and rarely thickening of the meninges in the vicinity.

All animals that developed signs had naked-eye lesions, but these were not very striking in most organs except in the livers of hamsters inoculated with 8th and 9th passage material, where very marked enlargement, discoloration and softening was invariably present. The spleen was seldom enlarged, and often appeared shrivelled, while the cut surface was hard and dark purple in colour. The kidneys of animals from early passages had no lesions whatsoever, but from 8th passage onwards they were congested: sometimes however they looked shrunk and the capsule was adherent. A large proportion of animals of late passages had definite lung lesions with foci of consolidation.

The brain of suckling hamsters was always affected, but those inoculated intracerebrally had much more severe lesions, with occasional necrotic patches, than those inoculated by the intraperitoneal route. The brain was invariably discoloured, reddish-brown, soft and all the meninges were highly vascular. The cut surface of the brain revealed either minute punctiform or large circumscribed haemorrhages in various parts. A feature of all suckling hamster brains was malacia around the tapetum. Brains of weaned or adult hamsters inoculated i.c. had lesions similar to suckling hamsters, except that the needle track was invariably necrotic and surrounded by a zone of haemorrhage. On the other hand brains from adult hamsters inoculated i.p. seldom had lesions and when present were in the form of circumscribed haemorrhages in the cerebral hemispheres.

Histological changes

Irrespective of passage material used and the route of inoculation, lesions were invariably present in many organs including brains of suckling hamsters. In adult hamsters however, brain lesions were noted in all i.c. inoculated but only rarely in those infected by the i.p. route. Although lesions were present in organs of hamsters from all passages including early guinea-pig passages the intensity of lesions in many organs varied. However, from the 9th passage, all organs of inoculated suckling hamsters were equally affected.

Liver.—Hamsters inoculated with early hamster or guinea-pig passage material had lesions evenly distributed, affecting all parts of the liver. The most striking changes in these hamsters were hypertrophy and swelling of Kupffer cells and single cell necrosis of liver cells. Only very occasionally small foci of 2–3 necrotic cells were seen. Some of the necrotic cells were rounded, hyalinized and stained red with eosin resembling Councilman bodies. The liver sinusoids were greatly

enlarged and in many cases showed proliferation of their lining cells. In many portal spaces accumulations of proliferating mononuclear cells were present. These cells were large, reticular in character and resembled sarcomatous cells. Some of them were found undergoing necrosis with the formation of small foci of spindle shaped cells. Occasionally livers were found undergoing fatty degeneration (Fig. 1).

In hamsters inoculated with 8th or 9th passage material, liver lesions were often very severe and widespread. The necrotic cells formed large confluent foci while masses of macrophages appeared within the sinusoids laden with nuclear debris. While many liver cells and infiltrating mononuclear cells were undergoing necrosis, many other liver cells were actively dividing (Fig. 2). Many livers contained clumps of intracytoplasmic granules within the liver cells and Kupffer cells. The granules were of various sizes and were either small round structures or elongate ring-shaped bodies. Their sizes ranged from $1 \times 1{\cdot}2\ \mu$–$2{\cdot}5 \times 3{\cdot}5\ \mu$ in diameter. They were either scattered within the cells or formed large aggregates as if cemented together by a matrix. These clumps or bodies usually disappeared from necrotic cells, leaving behind a few scattered granules (Fig. 3 and 4).

The histochemical properties of the intracytoplasmic bodies called here for convenience VMD bodies were as follows: they were basophilic, stained reddish-purple with Giemsa, bright red with Machiavello, brown with von Kossa and were fluorescent in U.V. light. They were PAS positive, Feulgen weakly positive and did not stain with Gomori's aldehyde fuchsin. Incubation at 60° in normal HCl for 10 min. abolished von Kossa's reaction, but did not affect the PAS reaction. It appears therefore that the bodies were coated with a gluco- or muco-protein which may have become impregnated with calcium (von Kossa).

Spleen.—Irrespective of passage material used, all spleens showed severe proliferation of the reticulo-endothelial system with large numbers of mitotic figures always present. In addition there was a progressive depletion of white pulp elements but until the 8th hamster passage no obvious necrotic changes could be seen. Usually all the sinuses were filled with large numbers of free macrophages, many of them containing cellular debris (Fig. 5). In hamsters inoculated with 8th or 9th passage material, the spleens showed progressive degeneration and necrosis of lymphocytes usually starting at the periphery of the Malphigian bodies (Fig. 6).

Lymph nodes.—Starting from the 8th hamster passage, many lymph nodes, especially those of the neck and mediastinum, showed large areas of necrosis, affecting not only lymphocytes but also reticulo-endothelial cells.

Kidneys.—Hamsters inoculated with early passage material invariably had parenchymatous changes in the convoluted tubules, dilatation of capillaries, and very occasionally mononuclear interstitial infiltrations. In hamsters inoculated with high passage material, degenerative changes in the kidney were much more pronounced. Many glomeruli appeared contracted, and cystic dilatation of tubules was of common occurrence. In some cases inoculated with 9th passage material, in addition to degenerative changes, interstitial mononuclear infiltrations were found in the medulla and clusters of intracytoplasmic VMD bodies were found, both in the tubular epithelium and within the endothelial cells of the capillaries. Although clusters of VMD granules in epithelial cells did not provoke any cellular reaction, when present in the capillaries they were usually surrounded

by a zone of mononuclear cells. The granules within these clusters were usually very small, darkly stained, about 0·5 μ diameter. Only very rarely were the large ring-shaped forms seen in the kidneys (Fig. 7).

Lung.—Although small foci of interstitial pneumonitis were found in almost all lungs examined, at least half of the hamsters under experiment (140/260) had various degrees of true interstitial pneumonitis. In many animals large foci of granulomatous mononuclear cells were found scattered throughout the lung. The bronchi were very often filled with a granular eosinophilic material and contained large amounts of desquamated epithelial cells. The small arterioles were as a rule affected by hypertrophy of the muscular wall and had evidence of endarteritis. Small venules usually contained coagulated blood rich in leucocytes, but some of them had fibrinous clots. The air sacs were very often empty but in the vicinity of foci of cuboidal metaplasia they were usually packed with macrophages containing cellular debris and varying numbers of polymorphs.

In many lungs of the 9th hamster passage, endothelial cells of the alveolar walls were greatly distended and contained either dispersed or clumped together small basophilic granules similar to the VMD bodies of the liver and kidneys. In some lungs desquamated endothelial cells undergoing degeneration were found within some of the bronchioles (Fig. 6).

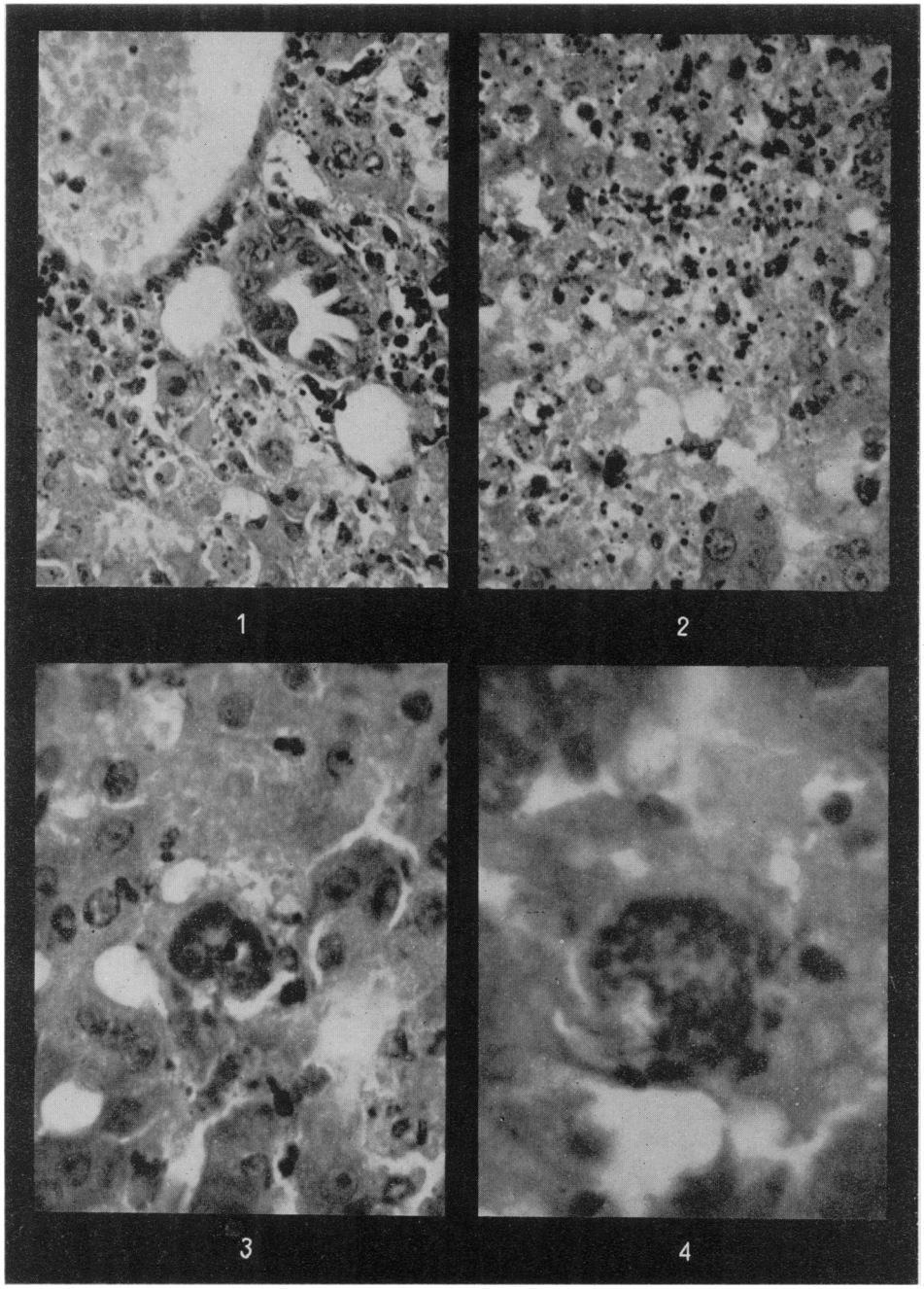
Brain.—Lesions were present in all suckling hamsters inoculated either i.p. or i.c.; however in weaned or adult hamsters, brain lesions were confined to those inoculated i.c. Adult hamsters inoculated i.p. seldom had brain lesions and when present were either in the form of small haemorrhages into the cortex, or as microglial proliferations around the haemorrhagic areas.

The lesions in brains of hamsters could be classed as meningo-encephalitis, however, in no case was the exudate in the meninges or in the brain substance composed of lymphocytes. The infiltrating cells were as a rule either mesodermal mononuclear cells or astrocytes. In suckling hamsters inoculated i.c. diffuse meningo-encephalitis usually started around the needle track, which appeared in

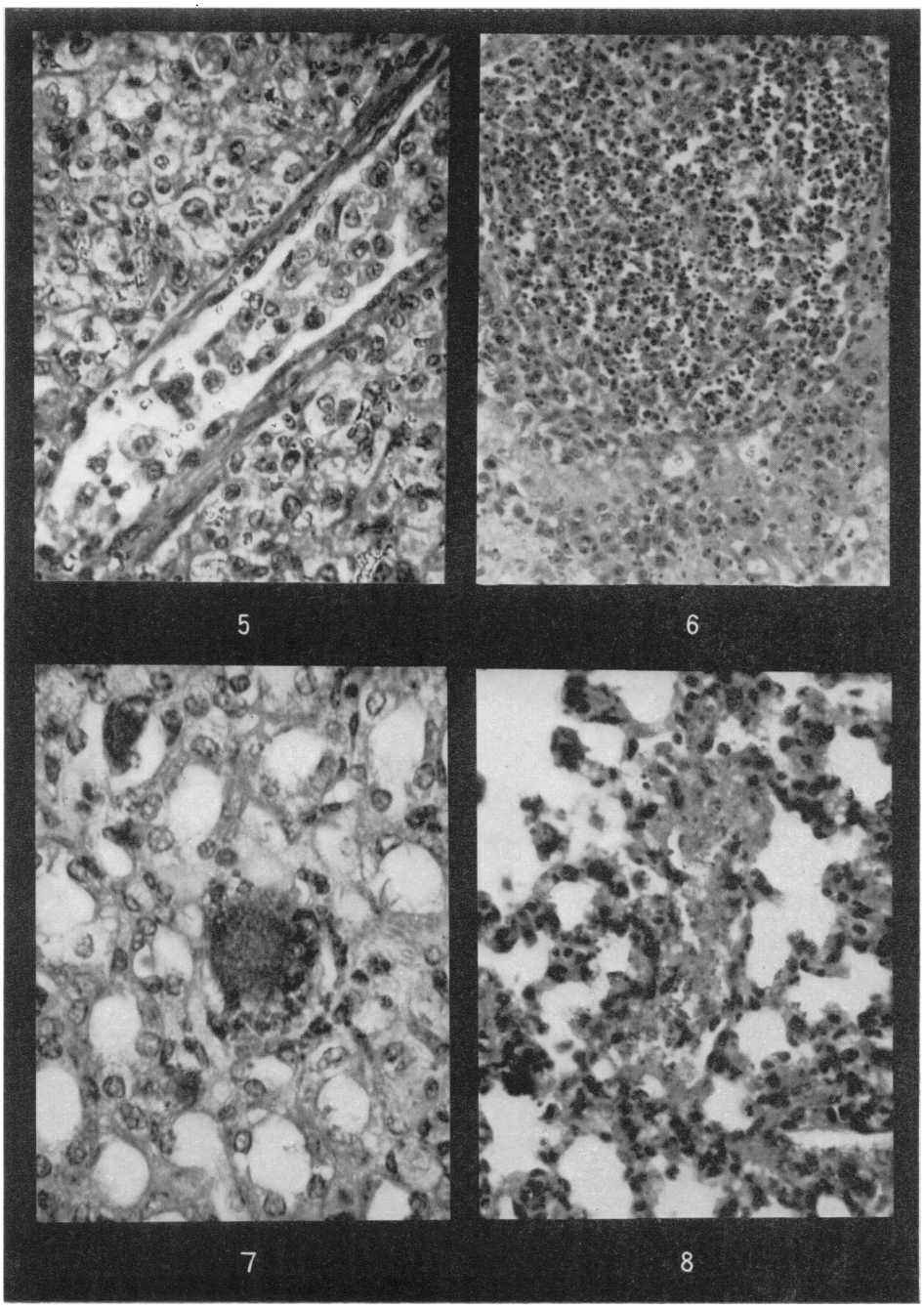
---

EXPLANATION OF PLATES

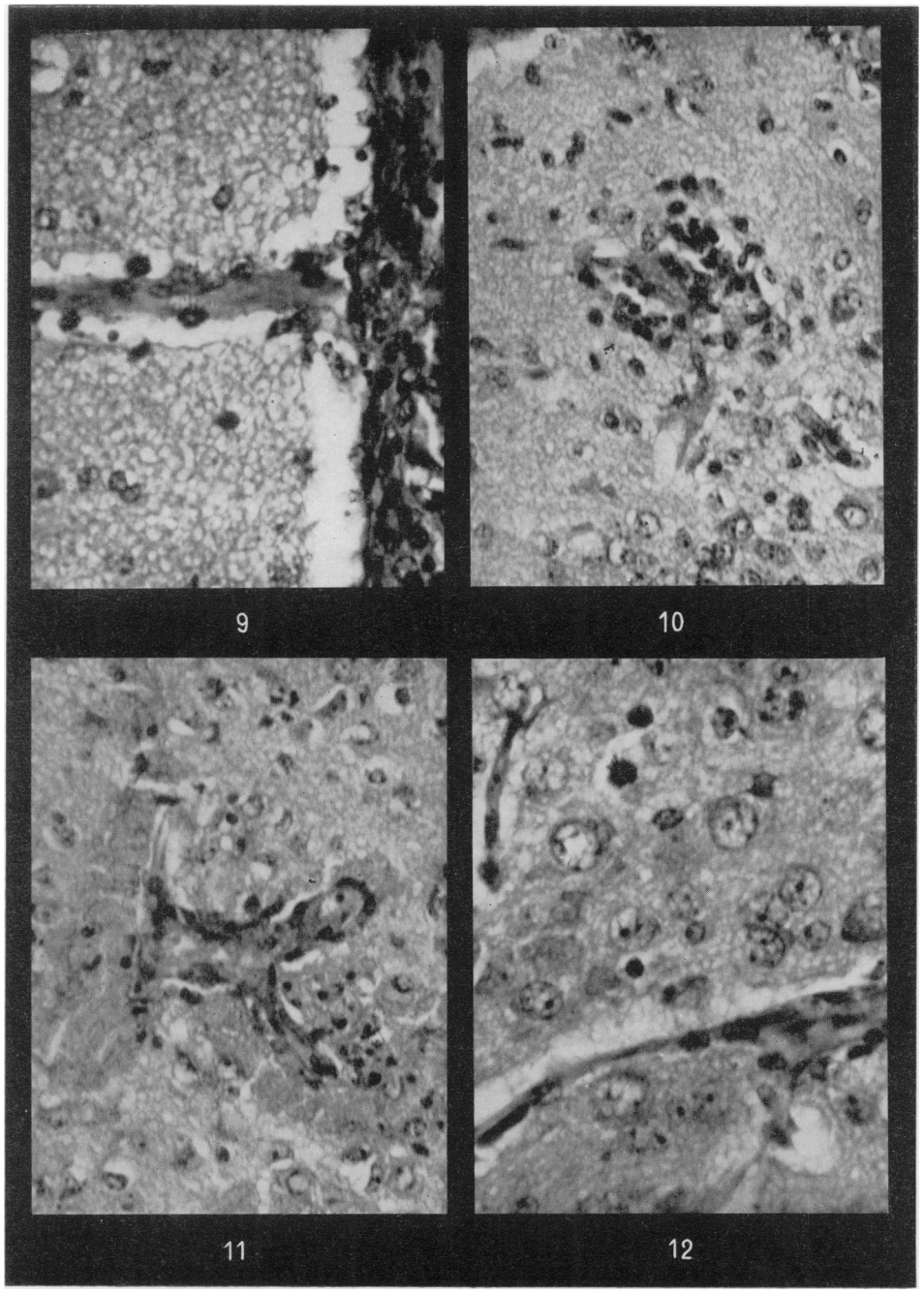
FIG. 1.—Liver—Periportal necrosis. H. and E. ×340.
FIG. 2.—Liver—Severe necrosis of liver cells. H. and E. ×340.
FIG. 3.—Liver—Intracytoplasmic bodies clumped together within a liver cell. H. and E. ×570.
FIG. 4.—Liver—High power magnification of intracytoplasmic body (VMD). H. and E. ×1050.
FIG. 5.—Spleen—Proliferation of reticulo-endothelial cells. H. and E. ×340.
FIG. 6.—Spleen—Necrosis within a Malpighian body. H. and E. ×190.
FIG. 7.—Kidney—Large clumps of VMD bodies within the medulla. H. and E. ×380.
FIG. 8.—Lung—Interstitial pneumonitis (note VMD granules within endothelial cells). H. and E. ×190.
FIG. 9.—Brain—Cerebral cortex (meningeal infiltration and capillary endothelial proliferation). H. and E. ×340.
FIG. 10.—Brain—Cerebral cortex (pericapillary microglial infiltration). H. and E. ×230.
FIG. 11.—Brain—Hippocampus (small haemorrhage in the vicinity of capillaries). H. and E. ×230.
FIG. 12.—Brain—Capillary proliferation within the cortex (note mitotic division of a cell). H. and E. ×380.
FIG. 13.—Brain—Astrocytosis—cerebral cortex of i.c. inoculated hamster. Cajal. ×230.
FIG. 14.—Brain—Astrocytosis—cerebral cortex of i.p. inoculated hamster. Cajal. ×230.
FIG. 15.—Brain—Astrocytosis—hippocampus of i.c. inoculated hamster. Cajal. ×230.
FIG. 16.—Brain—Astrocytosis—hippocampus of i.p. inoculated hamster. Cajal. ×230.



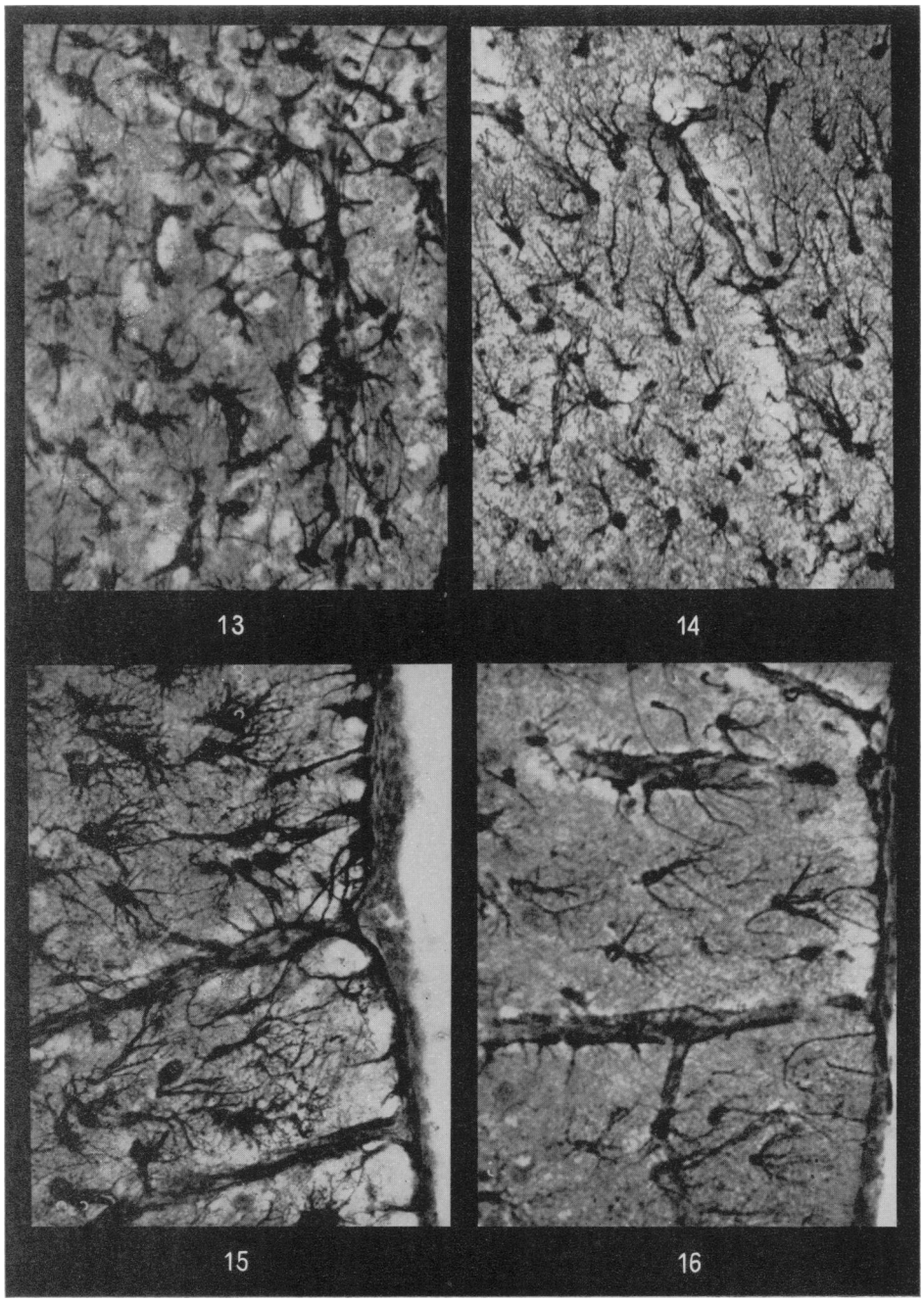
Zlotnik and Simpson.



Zlotnik and Simpson.



Zlotnik and Simpson.



Zlotnik and Simpson.

the form of a very narrow necrotic zone surrounded by small punctiform haemorrhages. Blood vessels and capillaries in the vicinity of the needle track had swollen endothelial cells and were surrounded by a few mononuclear cells. Sprouting of capillaries was widespread and in many capillaries there was definite evidence of endothelial proliferation and marked hypertrophy of astrocytic footplates with dense astroglial fibrosis. Astrocytosis was widespread throughout the brain, but the individual astrocyte cell bodies were much bigger in the cortex than in other regions and had long and tortuous filamentous processes (Fig. 10, 13 and 15).

Apart from the inflammatory changes, degeneration of neurones was very common in many brains, and haemorrhages in many regions were a constant feature. In the vicinity of these haemorrhages many neurones were found undergoing necrosis. In some instances a number of the mesodermal cells were actively dividing and mitotic figures could be seen (Fig. 12).

Adult hamsters inoculated i.c. had brain lesions similar to those of suckling hamsters, but were as a rule much more severe. Extensive haemorrhages were often present around the needle track and very large areas of the brain substance surrounding the needle track were undergoing necrosis and in most cases necrosis spread out to distant parts of the brain.

Vascular changes were very marked especially in the pyriform cortex extending from the meninges into the brain substance. Microglial perivascular infiltrations were found not only in the anterior parts of the brain but also in the brain stem and cerebellum. Diffuse astrocytosis with hypertrophy affected all parts of the brain irrespective of the actual distance from the needle track.

Intraperitoneal inoculations invariably produced brain lesions in suckling hamsters. The process usually began with active proliferation of capillaries within the meninges and at the periphery of the cortex, accompanied by a rather sparse cellular mononuclear exudate (Fig. 9). Very soon haemorrhages of various sizes appeared throughout the brain. These haemorrhages were most common around the ventricles and in the vicinity of the tapetum. The haemorrhages ranged from small extravasations of blood in the vicinity of a capillary, to widespread infiltration of erythrocytes into the brain substance (Fig. 11). Throughout the brain the capillaries showed active proliferation and branching and were very seldom found surrounded by microglial cells. In many cases, in the vicinity of large haemorrhages, peri-capillary malacia could be seen with the appearance of foamy brain macrophages. The area around the tapetum was especially prone to malacia and necrotic changes. The astrocytes as a rule showed widespread proliferation and hypertrophy, but the degree of hypertrophy was never as severe as in i.c. inoculated hamsters, although the peri-capillary astrocytic fibres were as abundant as in those inoculated i.c. (Fig. 14 and 16).

Although brain lesions were present in hamsters inoculated with early or late passage material, the degree of the cerebral damage in hamsters inoculated with early passage material was usually inverse to that of liver damage: thus hamsters that had widespread brain changes usually showed only slight liver lesions and *vice-versa*. In hamsters inoculated with late passage material there was no such correlation, and liver and kidney, lung, spleen and brain were very often equally affected. It is worth noting that in many brains small clumps of basophile granules were found within the capillaries, but it was impossible to decide whether these consisted of the same VMD bodies as seen in the liver and other organs, or were just nuclear debris.

DISCUSSION

Transmission of VMD to hamsters supplied not only a new susceptible laboratory animal but introduced several new features hitherto absent or so far unrecorded in other laboratory animals.

The disease in hamsters has many features in common with those described for guinea-pigs and monkeys and in some respects with human patients, (Smith, Simpson, Bowen and Zlotnik, 1967; Simpson, Zlotnik and Rutter, 1968; Hass, Maas and Ochlert, 1968; and Gedigk, Bechtelsheimer and Korb, 1968). Thus the liver, spleen and lungs were found to be equally damaged in the hamsters. The intracytoplasmic bodies described for guinea-pig livers, were found also in livers of hamsters and details of their structure could be studied and compared with those of other animals. It is quite possible that the ring-shaped forms visible in the livers of hamsters correspond to the electron microscopic appearance of the bulbous heads of the agent (Zlotnik, Simpson and Howard, 1968; Kissling, Robinson, Murphy and Whitfield, 1968; and Peters and Muller, 1968). The histochemical properties of the intracytoplasmic bodies seen in hamster livers correspond to those of guinea-pig livers. However, whereas in the guinea-pig the intracytoplasmic bodies were found only in the liver, in hamsters these bodies, either in small groups or large clusters, were present not only in the liver but also in the kidney and the lung. The kidneys appeared to be almost constantly affected and the possibility of contact transmission through the urine must be considered.

The hamsters provide a new aspect for the study of the pathogenesis of VMD, in that the agent almost invariably produces encephalitis. In suckling hamsters the encephalitic process does not depend upon the route of inoculation and it is probably the first organ to be affected in the process of the disease. The brain lesions, although closely similar to encephalitis have some interesting features which require special attention. To begin with, the tendency towards haemorrhage is very conspicuous and might explain some of the properties of the agent. These haemorrhages are very often seen in the brain in the vicinity of capillaries and it is quite possible that blood may escape into the brain substance without an actual breach in the continuity of the capillary wall.

The widespread degeneration seen around the haemorrhagic areas is not the only type of degeneration, as scattered neurones can be seen undergoing regressive changes without the presence of haemorrhage.

The inflammatory reaction is confined to a microglial proliferation and astrocytosis together with capillary sprouting and endothelial hypertrophy. The few infiltrating cells, present either in perivascular spaces or in the meninges, were always of a mononuclear type similar to macrophages and at no time were true lymphocytes seen. This shows that the depletion of lymphocytes seen in the spleen and in some lymph nodes must have some bearing on the reaction of the brain to the infection.

The generalized astrocytic reaction seen throughout the brain provides yet another instance of change in astrocytes under the influence of acute infective agents similar to that described for several arboviruses and rabies (Zlotnik, 1968).

In adult hamsters, brain lesions were a constant feature in i.c. inoculated animals, while in those infected by the i.p. route only limited haemorrhages surrounded by a small zone of reaction could be found. Of special interest,

however, is the great severity of lesions after i.c. inoculation, particularly the great tendency for extensive necrosis, starting around the needle track but spreading to further parts of the brain.

We wish to thank all the members of the Epidemiology and Experimental Pathology sections of this Establishment for valuable technical assistance.

REFERENCES

GEDIGK, P., BECHTELSHEIMER, H. AND KORB, G.—(1968) *Dt. med. Wschr.*, **93**, 590.
HAAS, R., MAASS, G. AND OEHLERT, W.—(1968) *Med. Klin.*, **35**, 1359.
KISSLING, R. E., ROBINSON, R. Q., MURPHY, F. A. AND WHITFIELD, SYLVIA G.—(1968) *Science*, **160**, 888.
PETERS, D. AND MULLER, G.—(1968) *Arzneimittelforsch.*, **65**, 1831.
SIMPSON, D. I. H.—(1969) *Br. J. exp. Path.*, **50**, 389.
SIMPSON, D. I. H., ZLOTNIK, I. AND RUTTER, D. A.—(1968) *Br. J. exp. Path.*, **49**, 458.
SMITH, C. E. G., SIMPSON, D. I. H., BOWEN, E. T. W. AND ZLOTNIK, I.—(1967) *Lancet*, ii, 1119.
ZLOTNIK, I.—(1968) *Br. J. exp. Path.*, **49**, 555.
ZLOTNIK, I., SIMPSON, D. I. H. AND HOWARD, D. M. R.—(1968) *Lancet*, ii, 26.