Br. J. exp. Path. (1972) **53**, 125.

ENCEPHALOPATHY IN MICE FOLLOWING INAPPARENT SEMLIKI FOREST VIRUS (S.F.V.) INFECTION

I. ZLOTNIK, D. P. GRANT AND DEE BATTER-HATTON

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

Received for publication September 16, 1971

Summary.—The avirulent " A8 " strain of S.F.V. gave rise to acute lesions of encephalitis in mice within 7 days of infection. After 6 weeks, however, all signs of disease disappeared from the brain, but when such animals were destroyed 2 years later they had advanced lesions of hydrocephalus, spongy degeneration and above all a severe astrocytic reaction in many centres of the brain. No such lesions were observed either in animals inoculated with formol-inactivated virus, or in uninoculated controls of the same age.

ALTHOUGH many strains of S.F.V. virus are known to give rise to fatal infections in laboratory animals (Smithburn and Haddow, 1944; Henderson, Peacock and Randles, 1967; Seamer and Zlotnik, 1970) the A8 strain replicates in the brains of mice without causing clinical signs or mortality (McIntosh, Brookworth and Kokernot, 1961; Bradish, Allner and Maber, 1971). Histological examination, however, of brains of mice infected either i.c. or i.p. with the avirulent strain showed that by the seventh day well established lesions of encephalitis were invariably present (Zlotnik, unpublished). The lesions consisted of inflammatory perivascular cuffings in various parts of the brain, degeneration of neurons in the olfactory lobe, pyriform cortex and hippocampus and a moderately severe astrocytic reaction in the thalamus, hippocampus and in the cerebral cortex. After 2 weeks, the lesions in the brain showed a tendency to regress and by the sixth week after infection almost all signs of disease disappeared, no virus was detected in the brain and no lesions were demonstrated in histological examinations.

In the present paper the results of histological examinations of brains of mice infected 26 months previously with the avirulent strain of S.F.V. are compared with those inoculated at the same time with inactivated virus and with brains of uninoculated controls of comparable age.

MATERIALS AND METHODS

Animals.—Weaned (3 weeks old) white mice of the Porton strain were used.

Virus.—The eighth mouse passage of the original AR 2066 strain of Semliki Forest Virus (McIntosh *et al.*, 1961) was prepared and stored as a 20 per cent suckling mouse brain suspension in borate buffer pH 9.

Inactivation of virus was carried out at room temperature by adding 0·2 per cent of formol to the 20 per cent brain suspension and stirring it with a magnetic stirrer for 3 days.

Inocula.—A dose 0·03 ml of a 10^{-1} dilution of live or inactivated virus was given i.c.

Experimental procedure.—Three groups of 20 mice each were used. One group received inoculation of live avirulent S.F.V., another was inoculated with the same virus, but inactivated with formol, while the third group was not inoculated.

Histological methods.—26 months after the inoculations all the mice, including the uninoculated controls, were anaesthetized and destroyed by decapitation. Brains were removed and divided sagittally, one half being fixed in 10 per cent formol saline for histological purposes, while the other half was used for virus assay and for subinoculations. From each brain a block of tissue was cut on a freezing microtome, while the remaining blocks were embedded in paraffin wax and cut on an ordinary microtome. Paraffin sections were stained either with H. and E., Nissl stain or Luxol fast blue, while frozen sections were stained with a modification of Cajal's gold chloride sublimate for the demonstration of astrocytes (Zlotnik, 1968).

RESULTS

During the 26 months under observation 29 mice died from unidentified causes. Of the 31 survivors there remained,

(a) Inoculated with live virus, 9
(b) Inoculated with inactivated virus, 13
(c) Uninoculated controls, 9.

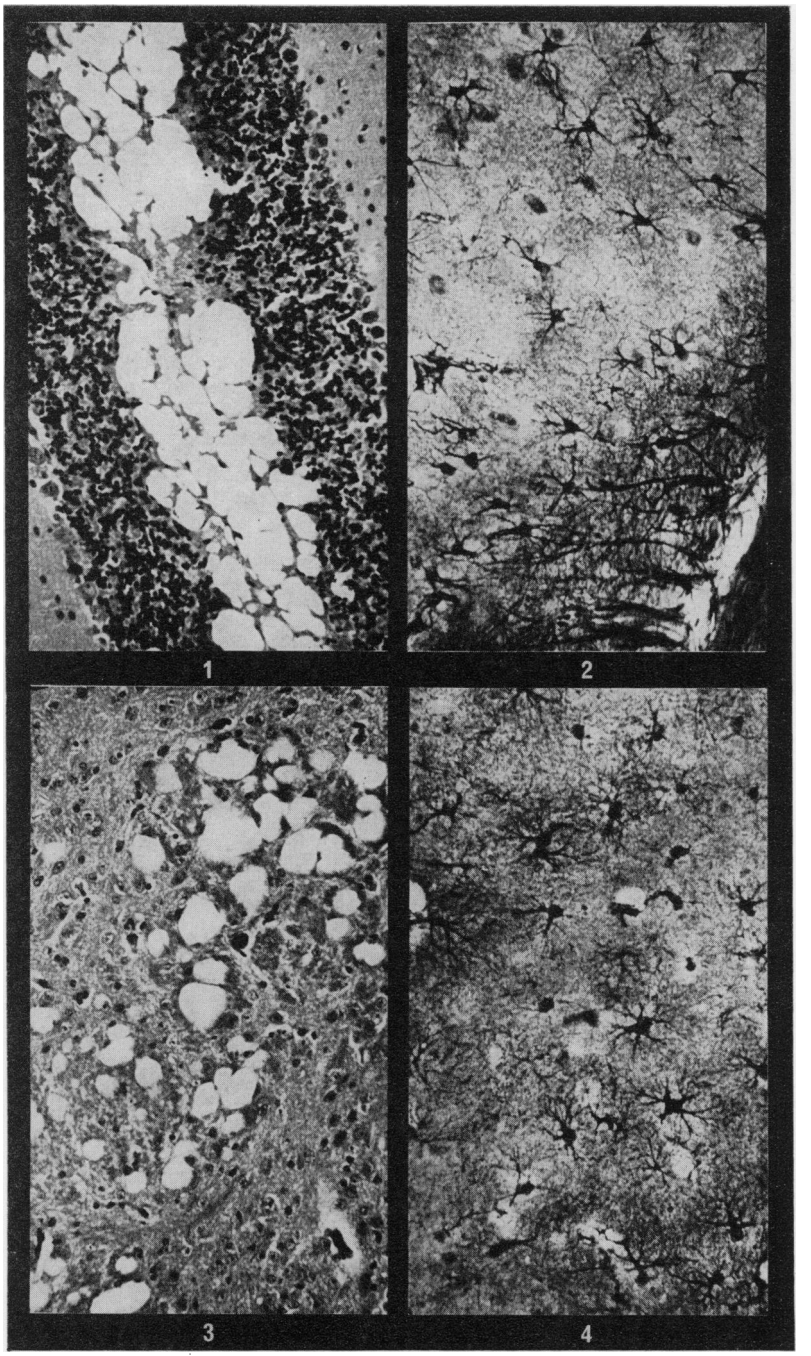
Although no definite signs of disease were observed in these mice, those inoculated with live virus showed very slight incoordination and ruffled coats. Histological examination of brains revealed a somewhat similar picture in both the uninoculated controls and those inoculated with inactivated virus, while those infected with live virus had definite subacute degenerative and astrocytic changes.

Uninoculated controls

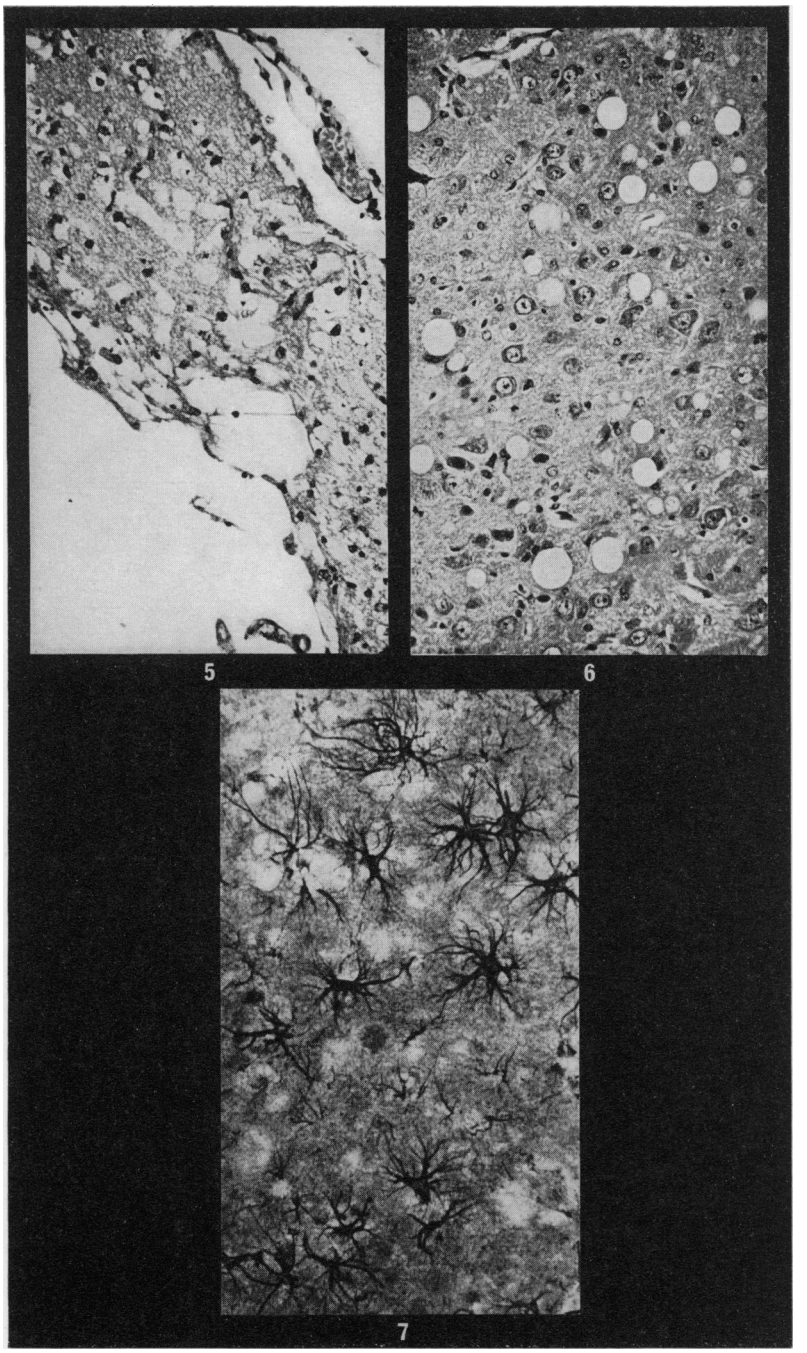
There were no macroscopic changes in any of the brains except that they were easily removed from the skulls because there was a definite space between the brain and the bony case. Histologically no abnormality could be found in the grey matter, but the white matter was affected in all 9 mice. The most affected part was the white matter of the cerebellum where very marked oedema was invariably present in the form of huge vacuoles (Fig. 1). Less obvious changes were seen in the inferior cerebellar peduncle, in the basis pedunculi of the mesencephalon, in the capsula interna and in the corpus callosum. The astrocytes in the grey matter showed a very slight hypertrophy and a slight increase in the number and size of the cell processes. In the white matter, however, the astro-

EXPLANATION OF PLATES

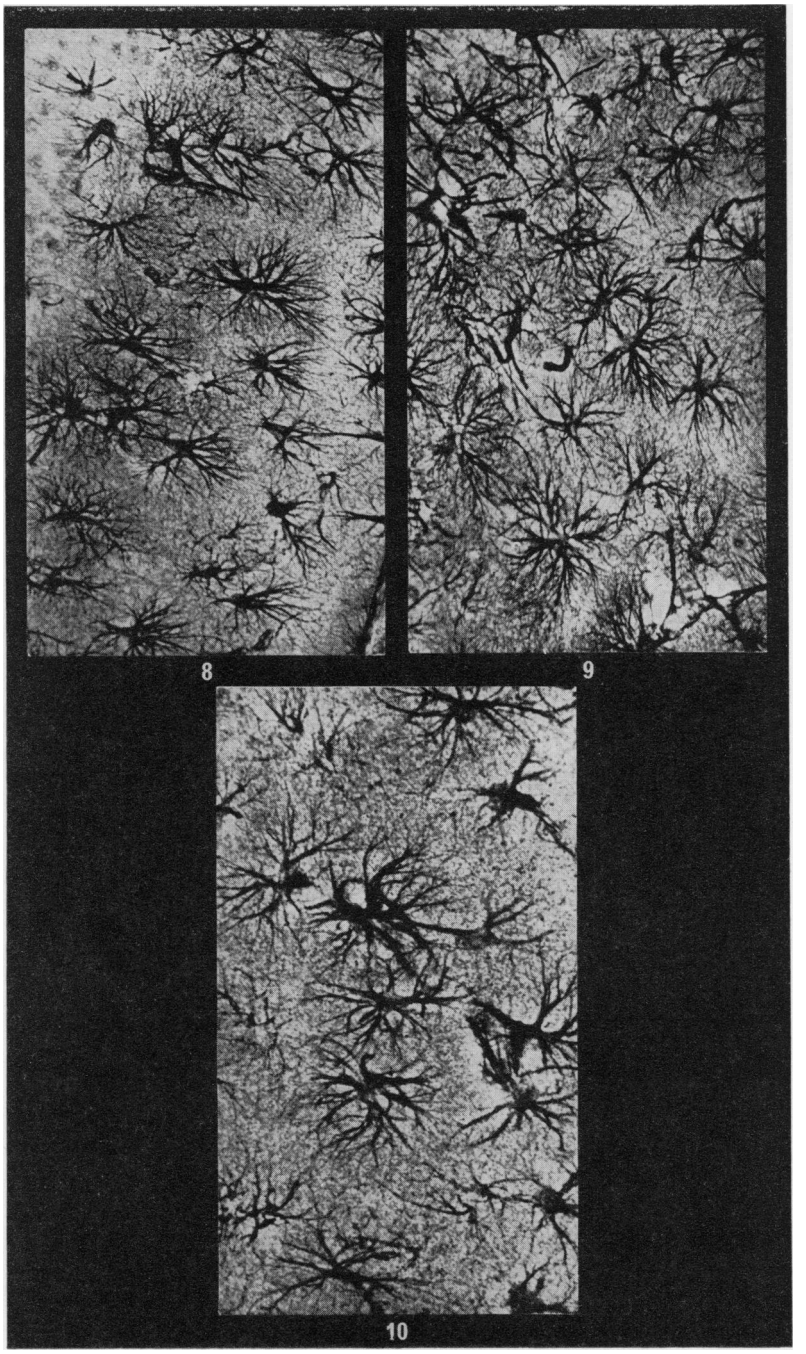
FIG. 1.—Severe oedema in the cerebellum of a 27 month old uninoculated mouse. H. and E. × 250.
FIG. 2.—Astrocytes in the grey and white matter of the hippocampus; (normal 27 months old mouse). Cajal. × 400.
FIG. 3.—Severe oedema in the inferior cerebellar peduncle of a mouse inoculated with inactivated S.F.V. virus. H. and E. × 250.
FIG. 4.—Astrocytes in the hippocampus of a mouse inoculated with inactivated S.F.V. virus. Cajal. × 400.
Figures 5–10 are from brains of mice inoculated with live " A8 " strain of S.F.V. virus.
FIG. 5.—Hydrocephalus—note severe atrophy and thinning of the brain cortex. H. and E. × 250.
FIG. 6.—Spongy degeneration in the mesencephalon. H. and E. × 250.
FIG. 7.—Marked astrocytic reaction in the cerebral cortex. Cajal. × 400.
FIG. 8.—Astrocytic proliferation and hypertrophy in the hippocampus. Cajal. × 400.
FIG. 9.—Astrocytosis in the hippocampus. Cajal. × 400.
FIG. 10.—Giant cells in the hippocampus. Cajal. × 400.



Zlotnik, Grant and Batter-Hatton.



Zlotnik, Grant and Batter-Hatton.



Zlotnik, Grant and Batter-Hatton.

cytes showed marked proliferation and thickening of cell processes giving rise to a very dense network of fibres (Fig. 2).

Mice inoculated with inactivated virus

The changes in the 13 brains from mice inoculated with inactivated virus resembled, in general, those of uninoculated controls, except that the oedema in the inferior cerebellar peduncle was much more marked, sometimes as severe as that in the cerebellar white matter (Fig. 3). The astrocytes resembled also those of uninoculated controls except that in the grey matter of the hippocampus occasional division of astrocytic cells was observed (Fig. 4).

Mice inoculated with live virus

In all 9 cases macroscopic changes were observed in the extracted brains. There was obvious hydrocephalus with differing degrees of thinning of the temporal cortex and as a rule the hemispheres were shrivelled and had corrugated surfaces. No live S.F.V. virus was demonstrated in these brains but histological examination revealed severe cerebral atrophy around the greatly distended ventricles and in 3 brains there was hardly any grey matter left (Fig. 5). In one brain there was a necrotic focus in the cortex and a cystic enlargement of the meninges and in all the other brains there was obvious degeneration of pyramidal cells in the atrophic cortex.

In addition to the hydrocephalus, there was oedema of the white matter, especially in the cerebellum, and a coarse type of spongiform degeneration affected many subcortical centres, such as the medulla, pons, midbrain, thalamus and corpus striatum (Fig. 6).

The astrocytes were affected in all 9 brains but whereas in 5 there was a diffuse marked hypertrophy and only foci of severe astrocytic swelling, especially in the cerebral cortex (Fig. 7), in the remaining 4 there was very severe hypertrophy and proliferation of astrocytes throughout the brain with changes both in the astrocyte bodies and in the processes (Figs. 8 and 9). In the hippocampus large foci of giant cells were found in 2 brains (Fig. 10).

DISCUSSION

Slow virus infections, and especially the part that virus may play in producing subacute progressive encephalopathy are still to a certain extent an enigma (Daniel, 1971; Field, 1969; Zlotnik, 1970). Initially it was difficult to accept spongy degeneration of the brain and subacute astrocytosis as the main lesions caused by scrapie " virus ". However, as other degenerative conditions such as Kuru, mink encephalopathy, etc. proved to be transmissible and were shown to produce similar lesions to those of scrapie, the concept of a slow virus infection became more acceptable (Eckroade *et al.*, 1970; Gajdusek, Gibbs and Alpers, 1967; Gibbs *et al.*, 1968; Zlotnik and Barlow, 1967). Recently, the problem of the scrapie type of lesion was raised again following a report that changes somewhat similar to those of scrapie could be produced in mice by treatment with Cuprizone, and obviously some doubts about the specificity of such lesions now arise (Pattison and Jebbett, 1971).

A study of the results of some viral infections of the C.N.S. and of the sequelae to such diseases showed that the astrocytic reaction is far from being only an

expression of chronicity of the process, but, on the contrary, is often the first result of invasion of the C.N.S. by a virus (Zlotnik, 1968). It was shown also that, following infections with Langat or W.E.E. virus, progressive changes both degenerative and astrocytic may take place long after the acute process is over and long after any live virus could be isolated from the brain (Zlotnik, 1971; Zlotnik *et al.*, 1972). In addition it is now obvious that degenerative and necrotizing lesions in the C.N.S. can be the result of viral infections in the complete absence of inflammatory changes in immunosuppressed animals (Zlotnik *et al.*, 1970, 1971). Thus it seems that viruses that cause acute infections may give rise also to changes both astrocytic and degenerative similar to those seen in scrapie and other slow virus infections. The question that remains is whether the lesions described in this paper, hydrocephalus, changes in the astrocytes and spongy degeneration which were found 26 months after S.F.V. infection, were a result of either the direct action of the virus of S.F.V. or of a virus-initiated process leading to such changes, or of an acute viral infection of C.N.S. which triggered off another latent infection. There is, however, a lesson that can be learnt from the present work. Lesions identical with those seen in slow virus infections can be demonstrated in the C.N.S. of mice 2 years after recovery from an inapparent S.F.V. infection.

The implication of the virus itself in the production of the lesions or of the acute process initiated by the virus is without any doubt, because no such subacute lesions were demonstrated in mice inoculated with inactivated virus. The only changes observed in the latter mice were those resulting from senility since they were very similar to abnormalities of the brain in uninoculated controls of the same age.

REFERENCES

BRADISH, C. J., ALLNER K. & MABER, H. B. (1971) The Virulence of Original and Derived Strains of Semliki Forest Virus for Mice, Guinea-pigs and Rabbits. *J. gen. Virol.*, **12**, 141.

DANIEL, P. M. (1971) Transmissible Degenerative Diseases of the Nervous System. *Proc. R. Soc. Med.*, **64**, 787.

ECKROADE, R. J., ZU RHEIN, GABIELE, M., MARSH, R. F. & HANSON, R. P. (1970) Transmissible Mink Encephalopathy: Experimental Transmission to the Squirrel Monkey. *Science, N.Y.*, **169**, 1088.

FIELD, E. J. (1969) Slow Virus Infections of the Nervous System. In *International Review of Experimental Pathology*, Vol. 8 Ed. G. W. Richter and M. A. Epstein. New York: Academic Press. p. 130.

GAJDUSEK, D. C., GIBBS, C. J. JR. & ALPERS, M. (1967) Transmission and Passage of Experimental " Kuru " to Chimpanzees. *Science, N.Y.*, **155**, 212.

GIBBS, C. J., JR., GAJDUSEK, D. C., ASHER, D. M., ALPERS, M. P., BECK, ELIZABETH, DANIEL, P. M. & MATTHEWS, W. B. (1968) Creutzfeldt-Jakob Disease (Subacute Spongiform Encephalopathy): Transmission to the Chimpanzee. *Science, N.Y.*, **161**, 388.

HENDERSON, D. W., PEACOCK, S. & RANDLES, W. J. (1967) On the Pathogenesis of Semliki Forest Virus (S.F.V.) Infection in the Hamster. *Br. J. exp. Path.*, **48**, 228.

MCINTOSH, B. M., BROOKWORTH, C. & KOKERNOT, R. H. (1961) Isolation of Semliki Forest Virus from *Aëdes* (*Aëdimorphus*) *argenteopunctatus* (Theobald) Collected in Portugese East Africa. *Trans. R. Soc. trop. Med. Hyg.*, **55**, 192.

PATTISON, I. H. & JEBBETT, JEAN, N. (1971) Clinical and Histological Observations on Cuprizone Toxicity and Scrapie in Mice. *Res. vet. Sci.*, **12**, 378.

SEAMER, J. & ZLOTNIK, I. (1970) Louping-ill and Semliki Forest Virus Infections in the Short-tailed Vole *Microtus Agrestis* (L). *Br. J. exp. Path.*, **51**, 385.

SMITHBURN, K. C. & HADDOW, A. J. (1944) Semliki Forest Virus. I. Isolation and Pathogenic Properties. *J. Immun.*, **49**, 141.

ZLOTNIK, I. (1968) The Reaction of Astrocytes to Acute Virus Infections of the Central Nervous System. *Br. J. exp. Path.*, **49**, 555.

ZLOTNIK, I. (1970) The Pathogenesis of Scrapie. In *6th Int. Congr. Neuropath., Paris*, Paris: Masson. p. 901.

ZLOTNIK, I. (1972) Virus Infection and Brain Development. In *The Brain in Unclassified Mental Retardation*, Study Group No. 3. Institute for Research into Mental Retardation (in press).

ZLOTNIK, I. & BARLOW, R. M. (1967) The Transmission of a Specific Encephalopathy of Mink to the Goat. *Vet. Rec.*, **81**, 55.

ZLOTNIK, I., SMITH, C. E. G., GRANT, D. P. & PEACOCK, S. (1970) The Effect of Immunosuppression on Viral Encephalitis with Special Reference to Cyclophosphamide. *Br. J. exp. Path.*, **51**, 434.

ZLOTNIK, I., CARTER, G. B. & GRANT, D. P. (1971) The Persistence of Louping-ill Virus in Immunosuppressed Guinea-pigs. *Br. J. exp. Path.*, **52**, 395.

ZLOTNIK, I., PEACOCK, S., GRANT, D. P. & BATTER-HATTON, DEE (1972) The Pathogenesis of Western Equine Encephalitis Virus (W.E.E.) in Adult Hamsters with Special Reference to the Long and Short Term Effects on the C.N.S. of the Attenuated Clone 15 Variant. *Br. J. exp. Path.*, **53**, 000.