Br. J. exp. Path. (1975) **56**, 72

THE OCCURRENCE OF VACUOLATED NEURONS IN THE BRAINS OF HAMSTERS AFFECTED WITH SUBACUTE SCLEROSING ENCEPHALITIS FOLLOWING MEASLES OR LANGAT VIRUS INFECTION

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Received for publication 19 September 1974

Summary.—Hamsters infected by the intranasal route with either hamster adapted Langat virus passaged at least 10 times in hamsters and the HNT strain of measles virus passaged 149 times in hamsters, developed subacute sclerotic lesions in the pyriform cortex, and beginning from 2 months after infection, were accompanied by neuronal vacuolation with ballooning of the cytoplasm, especially in parts of the brain in close proximity of the sclerotic lesions. The vacuolated neurons resembled those seen in scrapie, especially of sheep and goats, suggesting a similarity between the effects of slow and subacute viral infections.

In previous publications (Zlotnik, 1972; Zlotnik *et al.*, 1973) it was shown that Langat virus was able to initiate a subacute sclerosing encephalitis in hamsters that was leading to a protracted clinical condition with sclerosis and atrophy of parts of the brain. Later (unpublished), a similar condition was observed in hamsters infected with a neurotropic strain of measles virus (Burnstein, Jensen and Waksman, 1964). Subsequently, in a large scale study of subacute sclerosing encephalitis caused by either Langat or measles virus, full details of which will be published in the near future, it became obvious that after a number of i.c. passages in suckling hamsters, the above 2 viruses became more virulent for adult hamsters not only when inoculated i.c. but also after intranasal instillations. Histological examination of brains of hamsters that survived the acute phase of the disease and developed subacute sclerosing encephalitis revealed in hamsters infected with Langat virus of 10th and subsequent hamster passage material or measles virus of 149th passage in hamsters, vacuolated neurons with ballooning of cytoplasm in selected areas of the brain. In view of the fact that such vacuolation of neurons is highly characteristic, if not pathognomonic of scrapie disease of sheep and goats and that similar vacuolation is not usually observed in brains of animals infected with conventional viruses, it became obvious that an early publication of the above occurrence is warranted.

MATERIALS AND METHODS

Golden hamsters weighing 40–80 g and aged between 4 and 22 weeks were used for all the experiments.

The inocula consisted of Langat or measles viruses passaged in 4–5 day old suckling hamsters by i.c. inoculations, as follows: (a) virulent Langat virus (Smith, 1956) passaged 9 times in mice and then in suckling hamsters (Zlotnik *et al.*, 1973). The following passes were used in the present work: 2nd (TP_{21}/H_2), 6th (TP_{21}/H_6), 10th (TP_{21}/H_{10}), 14th (TP_{21}/H_{14}) and 19th (TP_{21}/H_{19}); (b) the HNT, neurotropic strain of measles virus (Burnstein *et al.*, 1964; Albrecht and Schumacher, 1971) passaged only in suckling hamsters. The following passes were used: HNT/117, HNT/122, HNT/127, HNT/136, HNT/142 and HNT/149.

Adult hamsters were inoculated only by the intranasal route. The dose for Langat virus intranasal instillations was 10^6 MLD_{50} (i.c.) in 0·1 ml of inoculum, while for the HNT strain of measles the dose was either 10^6 or 10^8 MLD_{50} (i.c.).

Infected hamsters were killed at various stages after infection from 1 to 26 weeks and as

a rule brains were removed immediately and fixed in 10% formol saline for paraffin embedding and conventional histology. Frozen sections for Cajal's staining of astrocytes were also prepared. Sufficient numbers of normal control hamsters of comparable age were also killed at various periods and their brains examined histologically.

RESULTS

Intranasal inoculations of hamsters with either hamster adapted Langat virus passaged less than 10 times in hamsters or the HNT strain of measles virus of less than 149 passages produced after the cessation of the acute phase of the disease, beginning from the 3rd week after infection, subacute lesions similar to those described previously (Zlotnik *et al.*, 1973) but situated mainly in the pyriform cortex and olfactory lobes. The subacute changes led almost invariably to sclerosis and atrophy of parts of the pyriform cortex and the disease process was usually extended over several weeks or months. The above changes were normally present in all hamsters irrespective of the period between infection and death, beginning from the first month after infection and up to one year. However, hamsters destroyed at least 2 months after infection with either the 10th, 14th or 19th hamster passage of Langat virus or the 149th passage of HNT strain of measles virus had, in addition to the above described subacute inflamma-

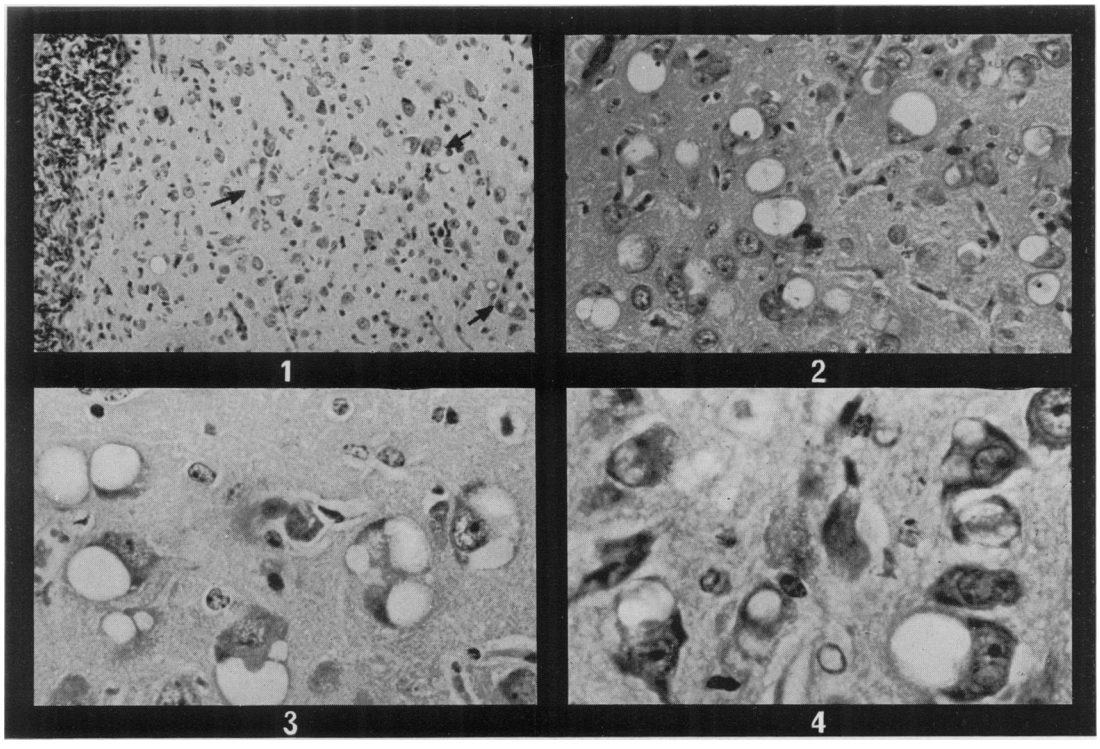

FIG. 1.—Vacuolated neurons scattered over a wide area in the vicinity of a large sclerotic lesion (measles virus infection). H. and E. × 102.

FIG. 2.—Large group of vacuolated neurons, some of which contain eosinophilic debris (Langat virus infections). H. and E. × 170.

FIG. 3.—Large vacuolated neurons: the cytoplasm is bulging into the vacuole of one neuron (measles virus infection). H. and E. × 525.

FIG. 4.—Vacuolated neurons: 2 small vacuoles at the periphery of the cytoplasm in one cell (measles virus infection). H. and E. × 450.

tory lesions, degenerative changes mainly in the form of discrete vacuolation of neurons in areas of the brain that were not affected by inflammation but situated in the close vicinity of the sclerotic changes. Although no vacuolation was seen in brains of hamsters destroyed less than 2 months after infection, vacuolated neurons were present in brains of hamsters until 6 months after infection, *i.e.* the longest period that such brains were available for examination.

As already pointed out, the most severe subacute sclerotic lesions in the intranasally infected hamsters were present apart from the olfactory lobes in the pyriform cortex, beginning usually at the periphery and extending from the meningeal border to the amygdaloid nuclei and the corpus striatum. Vacuolated neurons, although occasionally found within the sclerotic lesions, were however regularly situated in otherwise non-affected areas but in close proximity of the subacute changes (Fig. 1). They were most commonly present in the neurons of the pyriform cortex, area amygdaloidea anterior, nucleus amygdaloideus centralis and occasionally also in the corpus striatum. Such vacuolated neurons were found singly, situated amongst normal neurons, or a few scattered over a large area (Fig. 1) or, finally, in close proximity of other vacuolated neurons in groups of

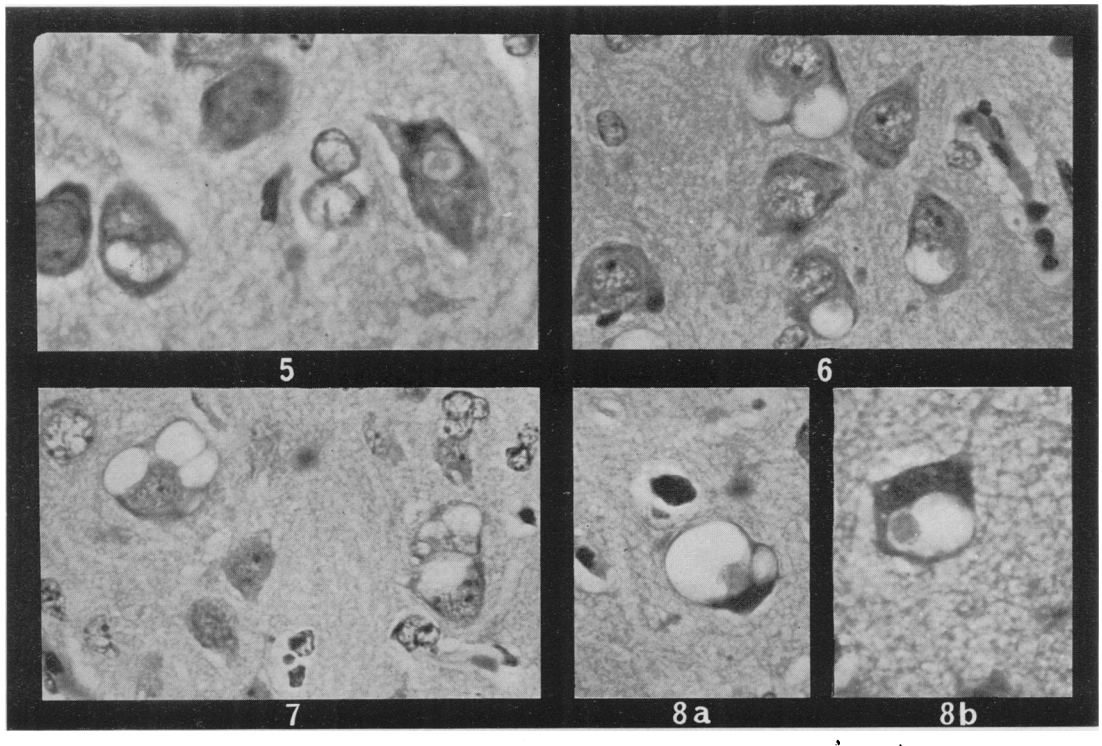

FIG. 5.—Stellate eosinophilic body in the vacuole of one neuron and 3 small vacuoles at the periphery of another neuron (measles virus infection). H. and E. × 900.

FIG. 6.—Vacuolated neurons containing amorphous debris: one vacuole consists of 2 compartments (measles virus infection). H. and E. × 560.

FIG. 7.—Multilocular vacuoles in the area amygdaloidea anterior (measles virus infection). H. and E. × 560.

FIG. 8a.—Large multilocular vacuole causing ballooning of the cytoplasm and containing eosinophilic debris (Langat virus infection). H. and E. × 600.

b.—Large vacuole containing a round eosinophilic body (measles virus infection). H. and E. × 900.

3–15 cells (Fig. 2–4). The vacuoles consisted of cavities within the cytoplasm, either in the form of small empty spaces, often at the periphery of the cell, or large vacuoles that caused ballooning of the cytoplasm and compression of the peripherally displaced nuclei (Fig. 2–4, 8a, b). Although the great majority of vacuoles formed single cavitations within the cytoplasm, many neurones contained also multilocular, complex vacuoles, ranging from 2 to 6 compartments (Fig. 3–7).

The vacuoles remained empty or contained varying amounts of eosinophilic material. The latter consisted most commonly of amorphous cellular debris (Fig. 6, 8a), or, less often either of actual cytoplasm bulging into the cavity (Fig. 3), or regularly shaped round bodies (Fig. 8b) or stellate bodies (Fig. 5). Although the vacuolar content was eosinophilic, when stained with phloxine-tartrazine it did not retain the red colour. The cytoplasm and nuclei of vacuolated neurons showed superimposed degenerative changes such as chromatolysis and pyknosis. However, in the neurons showing extensive ballooning the cytoplasm was often reduced to a dark narrow ring.

DISCUSSION

Vacuolation of neurons may be seen occasionally in various pathological conditions (Courville, 1950) at the periphery of degenerating neurons or, more specifically, in the form of ballooning of anterior horn cells with the accumulation of hyaline like material in ALS (Hirano, 1965). However, typical vacuolation with cytoplasmic cavitation and ballooning of the cytoplasm is of regular and specific occurrence only in the spongiform encephalopathies due to slow viral infections. In this group of diseases it is of diagnostic and pathognomonic significance only in scrapie, especially of sheep and goats (Zlotnik, 1958, 1961; Zlotnik and Stamp, 1961) and in mice infected with the ME strain of Suffolk sheep scrapie (Zlotnik and Rennie, 1962, 1963, 1965). In the other spongiform encephalopathies, such as kuru, transmissible mink encephalopathy and Creutzfeldt-Jakob disease, vacuolation of neurons forms only an additional phenomenon to the widespread spongy degeneration (Gajdusek and Zigas, 1959; Klatzo, 1965; Beck and Daniel, 1965; Zlotnik and Barlow, 1967; Marsh *et al.*, 1969; Barlow, 1972; Daniel, 1971, 1972). In view of the fact that vacuolation of neurons plays such an important part in slow viral infections and was hitherto unknown in conditions of the CNS caused by conventional viruses that give rise to encephalitis, the occurrence of vacuolated ballooned neurons in the vicinity of sclerotic lesions in hamsters infected with Langat or measles virus assumes special proportions and may point to similarities in the effects of subacute and slow vital infections.

The vacuoles present in the subacute forms of Langat and measles virus infections of hamsters appeared as true cavities and were most probably filled with fluid which could not, however, be identified in histological sections. On the other hand, a significant proportion of vacuoles contained material that could be classified either as amorphous cytoplasmic debris or as a bulge of cytoplasm into the vacuole, or as more regular bodies, round or stellate in shape. Thus, these vacuoles conform to Courville's (1950) definition of neuronal vacuoles as real cavities within the cytoplasm, filled either with liquid or with products of degeneration.

The distribution of vacuolated neurons, along the pyriform cortex and amygdaloid nuclei, follows closely the path of infection after intranasal instillations from the olfactory bulb to the olfactory cortex and the subsequent subacute pathological lesions.

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