STUDY OF ENCEPHALITOGENIC ACTIVITY OF *Salmonella typhi* IN GUINEA PIGS

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Experiments on guinea pigs showed that *Salmonella typhi* possesses encephalitogenic activity similar to that of *Mycobacterium tuberculosis*. The difference is that the allergic encephalomyelitis caused by *S. typhi* has a more protracted and remittent course, rarely terminating in death; in addition, the effect can be reproduced by injection of *S. typhi* cells without brain tissue.

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Numerous investigations [1-5, 7] have shown that when animals are sensitized with nerve tissue mixed with Freund's adjuvant experimental allergic encephalomyelitis (EAE) regularly develops. In this connection some interesting reports have been published indicating that certain Gram-negative bacteria also apparently possess an action of this character [14].

The object of the present investigation was to study under experimental conditions the possibility that heat-killed *Salmonella typhi* cells possess an encephalitogenic action and to compare this activity with that of *Mycobacterium tuberculosis*.

EXPERIMENTAL METHOD

The encephalitogenic properties of *S. typhi* strain O_{901} were investigated. A culture of the microorganisms was heated to 56° for 1.5 h and suspended in sterile mineral oil. Suspensions containing 12.5, 2.5, and 0.5 mg/ml of bacterial mass (expressed as dry weight) were prepared.

The encephalitogenic activity of *S. typhi* was investigated in experiments on noninbred male guinea pigs weighing 300-350 g. The encephalitogenic mixture was prepared by Waksman's method [6]. Fresh, homologous nerve tissue was emulsified in the ratio 3:2 in mineral oil containing different numbers of *S. typhi* cells. The mixture was injected intradermally in a dose of 0.2 ml per guinea pig. Some guinea pigs were injected with *S. typhi* cells in mineral oil without brain tissue. The encephalitogenic action of *S. typhi* was also compared with the analogous action of BCG vaccine.

Suspensions of *M. tuberculosis* cells were prepared in mineral oil containing 5, 2.5, 0.5, and 0.05 mg/ml of bacterial mass. Guinea pigs were sensitized with encephalitogenic mixture both with and without homologous nerve tissue.

The severity of the EAE was assessed by a six-point scale as suggested by Waksman [6]. The guinea pigs were sacrified at the height of development of EAE or on the 30th day after sensitization.

Sections of different parts of the central nervous system (cerebrum, medullar, lumbar, and thoracic portions of the spinal cord) were cut for histological investigation and stained with hematoxylin-eosin.

EXPERIMENTAL RESULTS

The first clinical manifestations of the disease (paresis of the hind limbs, disturbance of movement coordination) appeared in the guinea pigs sensitized with encephalitogenic mixture containing 12.5 mg *S. typhi* cells/ml (series I) on the 9th-13th day after sensitization. The experimental animals developed pareses of the muscles of the hind limbs and their disturbances of movement coordination became more severe. The signs of the disease became less marked during the next 7-10 days.

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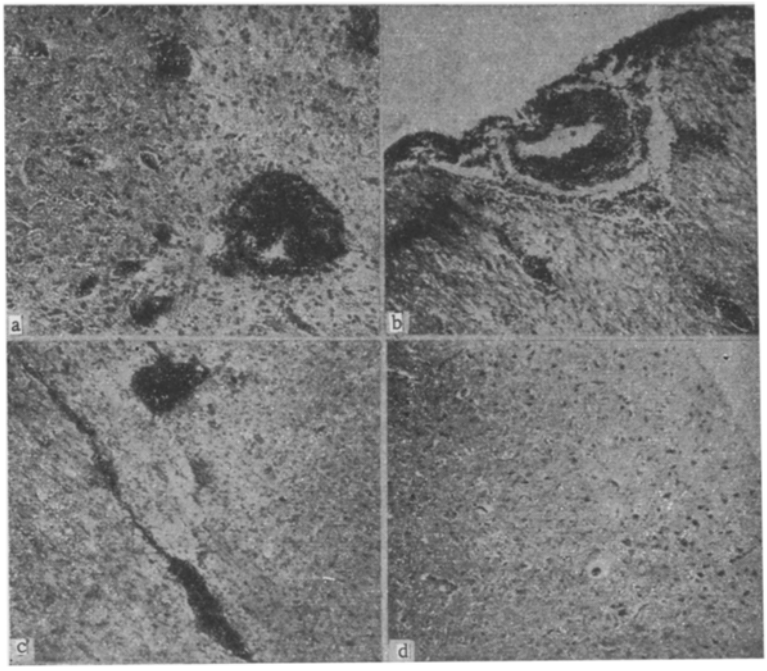

Fig. 1. Vascular and inflammatory changes in the brain of guinea pigs sensitized with an encephalitogenic mixture including S. typhi cells with (a) or without (b) homologous nerve tissue or M. tuberculosis cells with (c) or without (d) homologous nerve tissue. Hematoxylin-eaosin. 90×.

On the 25th-30th day after sensitization the disease again became more severe. The clinical manifestations were most marked during this period. Characteristic lesions were observed (see Fig. 1) in the white matter of the brain and spinal cord (dilatation and congestion of the blood vessels, thickening of their walls, formation of perivascular foci of round-cell infiltration in the form of "cuffs" and so on).

When the guinea pigs were sensitized with a mixture containing S. typhi cells in a concentration of 2.5 mg/ml (series II), the first signs of the disease appeared on the 13th-18th day. The course of the disease and the pathological changes in the central nervous system were indistinguishable from the picture described in the preceding series of experiments.

Reducing the dose of the bacterial component to 0.5 mg/ml (series III) led to a considerable decrease in the encephalitogenic activity of the mixture. The first signs of the disease appeared later in the guinea pigs of this group, on the 20th-28th day after sensitization. The histological changes in the central nervous system also were less marked.

Unexpected results were obtained with guinea pigs sensitized by a suspension of S. typhi cells in mineral oil without brain tissue (series IV and V).

Characteristic clinical signs of the EAE (pareses of the muscles of the hind limbs, disturbances of movement coordination) appeared in the experimental animals on the 19th-25th day. The disease could not be confirmed histologically in all cases. The severity of the clinical picture and the intensity of the histological changes were dependent on the dose of S. typhi cells.

Injection of a suspension of M. tuberculosis cells in mineral oil without brain tissue was not followed by development of clinical or pathological signs of EAE in the guinea pigs.

Hence, the action of S. typhi is to a certain extent unique. In the overwhelming majority of animals the disease was protracted and remittent in character. Despite the well-marked clinical and pathological manifestations of EAE in the guinea pigs, death of the animals was very rare. We consider that the encephalitogenic activity of the S. typhi cells when injected in mineral oil without brain tissue is an important factor. In this respect the action of S. typhi differs in principle from that of M. tuberculosis.

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