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EXPERIMENTAL INHALATION INFECTION OF MICE WITH VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS

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Summary. — Mice were infected by inhalation of 1600 guinea pig intracerebral LD_{50}, corresponding to approx. 16 mouse inhalation LD_{50}, of Venezuelan equine encephalomyelitis (VEE) virus. The virus in the organs was demonstrated by infectivity titrations in chick embryo cell cultures. The course of the infection was acute. The nasal mucosa could be considered as the site of primary virus multiplication and the source of viraemia and of the virus in the brain. The level of virus multiplication in the brain was higher than in other organs. By means of the direct fluorescent antibody (FA) method, the viral antigen was detected only in the brain. Histological changes developed predominantly in the central nervous system (CNS). The first signs of meningoencephalitis occurred 96 hours after infection.

Introduction

The pathological changes in mice after intranasal infection with VEE virus were described by Victor *et al.* (1956), those after intraperitoneal infection by Gleiser *et al.* (1962). The distribution of virus in the organs was followed quantitatively after intraperitoneal infection by Tasker *et al.* (1962), after subcutaneous and intracerebral infection by Kundin (1966) and Kundin *et al.* (1966). In the latter works attempts were made to detect the viral antigen by the FA method. Such studies, however, were not done after inhalation infection.

In this paper we would like to point out the difference in the course of infection in mice from that in guinea pigs (Hrušková *et al.*, 1969*b*) and to describe the picture of the disease as revealed by morphological and virological examinations.

Materials and Methods

The strain of VEE virus used in the form of mouse brain suspension and the technique of inhalation infection were the same as used in our previous work (Hrušková *et al.*, 1969*a*). For details of the other procedures see Hrušková *et al.* (1969*b*).

Mice weighing 16 g were infected by inhalation with a dose of 1600 guinea pig intracerebral LD_{50}, corresponding to approx. 16 mouse inhalation LD_{50}, of VEE virus. They were killed by chloroform. Materials for the determination of virus titres in the organs were taken 12, 24, 36, 48, 60, 72, 84, 96, 108, 120 and 144 hours after infection, those for histological and FA examinations at 24, 48, 96, 120 and 144 hours. The amount of infective virus was assayed by inoculating chick embryo cells with suspensions from the brain, spinal cord, nasal mucosa, trachea, lungs,

salivary gland, myocardium, liver, spleen, kidney, suprarenal gland, pancreas, large intestine, hilar and inguinal lymph nodes, blood serum and muscles. Pooled samples from 5 animals were used. In addition to the organs mentioned, we examined histologically also the sternal and femoral bone marrow and the thymus, always from 3 mice. By the FA method we investigated the brain, nasal mucosa, lungs, spleen, liver, pancreas, kidneys and salivary gland.

Results

Distribution of the virus

The results of virus titration are summarized in Table 1. At 12 hours the virus was present only in the nasal mucosa and in the large intestine. At further intervals, a rapid multiplication of the virus took place in the

Table 1. The titres of VEE virus in the organs from mice after inhalation infection

Material examined	Hours after infection											
	12	24	36	48	60	72	84	96	108	120	132	144
Brain	neg	2.7	2.0	4.0	7.0	7.5	8.3	7.7	7.7	8.3	8.5	7.0
Spinal cord	neg	neg	neg	3.0	4.3	5.0	6.0	6.5	7.0	4.7	6.7	5.5
Nasal mucose	1.7	5.3	2.5	5.3	7.3	7.3	6.0	6.5	7.0	6.0	6.0	5.3
Trachea	neg	1.7	2.5	3.5	4.7	5.5	3.7	4.3	4.7	2.5	2.3	4.0
Lungs	neg	2.0	4.3	4.0	4.3	4.0	3.0	1.7	3.3	2.0	2.7	4.3
Heart muscle	neg	1.7	3.3	4.7	3.5	5.0	4.5	2.3	2.7	4.0	3.0	3.0
Liver	neg	neg	3.0	5.3	4.3	4.3	2.5	neg	3.5	3.5	2.5	2.0
Spleen	neg	2.5	4.3	5.5	6.3	6.5	4.3	3.7	6.3	4.5	5.5	2.7
Kidney	neg	neg	2.3	4.7	4.5	5.0	3.5	1.7	5.0	5.7	4.7	6.0
Suprarenal gland	neg	neg	neg	4.3	3.5	4.7	2.7	2.3	3.3	2.3	3.5	4.7
Large intestine	1.7	2.5	2.5	3.5	2.5	2.5	4.3	2.5	2.7	2.5	3.0	2.5
Pancreas	neg	neg	2.5	4.7	5.5	4.5	4.0	3.5	5.5	6.3	5.3	7.5
Salivary gland	neg	3.5	3.5	6.0	5.7	6.5	4.0	4.0	6.3	2.7	3.5	2.5
Hilar lymph node	neg	neg	5.7	6.5	6.3	6.3	4.5	3.5	4.5	4.0	3.7	5.0
Inguinal lymph node	neg	neg	2.7	6.0	4.5	4.5	5.5	4.0	4.7	3.5	4.0	4.5
Blood serum	neg	1.7	4.5	4.5	6.0	7.0	—	3.7	4.5	3.7	2.3	1.3
Striated muscles	neg	neg	neg	3.5	4.0	2.7	3.0	2.0	4.7	4.3	4.0	3.7

The amount of virus expressed in $\log_{10}$ $TCID_{50}$ per gram of tissue or 1 ml serum.
neg = no virus detected in 10% organ suspension or in undiluted serum (0.1 ml of inoculum per tube).

nasal mucosa. The virus level in the trachea and lungs increased starting from 24 hours after infection, but it did not reach values as high as in nasal tissues. The rapid increase of the virus in the brain should be considered important. The virus level in the brain was considerable at 24 hours after infection, although the viraemia at that time was relatively low. Virus accumulation in the lymphatic tissue was very intensive.

Virus detection by the FA method

Positive results were obtained only in the brain. Single neurons showed positive fluorescence in their cytoplasm after 120 hours (Figs 1 and 2). At 144 hours the fluorescing neurons formed larger groups, which in longitudinal sections were situated along the small arterioles forming a cylindrical bordering from viral antigen-containing cells around the arterioles.

Histological examination

No pathological changes were found at 24 hours after infection. After 48 hours, dilatation of capillaries in alveolar walls of the lungs of all three animals investigated was seen. The mild epithelial desquamation in nasal and bronchial mucosa was not accompanied by inflammatory reaction. Single necrotic cells were detected in the germinal centers of splenic follicles. At later intervals, no marked changes were found in the respiratory tract mucosa, whereas conspicuous lesions developed in the lymphatic system. The monocellular necroses (Fig. 3) in the splenic follicles became more widespread and destruction of small groups of reticular cells of the germinal centers occurred.

The first rather sporadic inflammatory changes in the CNS occurred in one animal at 96 hours after infection. The small perivascular cuffings situated in the cerebrum and cerebellum consisted predominantly of polynuclear leucocytes. After 120 hours the changes in the CNS became more widespread (Figs 4 and 5). The meninges were diffusely infiltrated with polynuclear and mononuclear leucocytes. In numerous cuffings the cells partially infiltrated also the vessel walls. At 144 hours, meningoencephalitis developed in all the mice examined.

Discussion

Our investigation on experimental inhalation infection with VEE virus in mice showed, as distinct from guinea pigs, a development of meningoencephalitis. The virus spread towards the brain rapidly and its occurrence there preceded the increase of viraemia. This supports the possibility of virus travel along the olphactory route. The travel of VEE virus along the olphactory route has not yet been directly proved. Our experiments also failed in this respect, probably due to the lower sensitivity of the FA method. The morphological findings, on the other hand, stressed the importance of blood vessels for the distribution of virus in the CNS. We judge so according to the perivascular cuffings, which coincided with the localisation of the fluorescent antigen.

In agreement with Kundin's and our previous results in guinea pigs, the FA method is relatively little sensitive for immunofluorescent detection of the VEE virus antigen in visceral organs.

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Explanation of Photomicrographs:

Fig. 1. Group of neurons in a mouse brain section, 120 hours after infection with VEE virus. Viral antigen in the cytoplasm of neurons and their processes. ×140.

Fig. 2. As in Fig. 1, but ×400. Bright specific fluorescence in the cytoplasm of a ganglion cell.

Figs 3—5. Histological changes in organs from mice infected by inhalation with VEE virus. Haematoxylin and eosin, ×400.

3 — Degenerating cells in a splenic lymphatic follicle.

4 — Inflammatory infiltrate in the meninges, consisting mainly of polynuclear leucocytes and of mononuclear cells, and accumulating around the pial vessels.

5 — Perivascular cuffing and scarce infiltration of polynuclear and mononuclear cells in the brain white matter in the vicinity of a vessel.