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SUBCUTANEOUS AND INHALATION INFECTION OF GUINEA PIGS WITH VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS

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Summary. — Guinea pigs were infected with Venezuelan equine encephalomyelitis (VEE) virus in the form of mouse brain suspension. In case of inhalation infection the dose was 3000 guinea pig intracerebral (ic) LD_{50} (= 857 guinea pig inhalation LD_{50}); in case of subcutaneous (sc) infection it was 50 guinea pig ic LD_{50}. The sc infection was characterized by a rapidly developing and high viraemia lasting up to the death of the animals. In inhalation infection, the virus was present in the early phase in the upper and lower respiratory tract, reaching high levels especially in the nose; as distinct from sc infection, the onset of viraemia was delayed and the increase in titres was slower. The upper respiratory tract mucosa and the lungs served as sources of virus for the viraemia. After either mode of infection, the virus multiplied or accumulated in several parenchymatous organs. There was no obvious neurotropism of virus. Viral antigen was demonstrated by the direct fluorescent antibody technique only in the pancreas. Histological examination revealed prevalent involvement of the lymphatic system, but none of the central nervous system (CNS).

Introduction

Infections of laboratory personnel presumably due to aspiration of VEE virus aerosol have been reported (Lennette and Koprowski, 1943; Casals *et al.*, 1943; Koprowski and Cox, 1947; Shubladze *et al.*, 1959). The course of aerosol infection of pigeons with VEE virus has been described by Miller (1966*a*, *b*). Victor *et al.* (1956) described morphological changes following administration of VEE virus by different routes.

There are no reports, however, on the course of morphological changes or on the virological findings in VEE virus-infected guinea pigs. The aim of the present study was to obtain information on the course of inhalation and sc infection of guinea pigs with VEE virus, using virological and morphological methods.

Materials and Methods

The VEE virus used in the form of mouse brain suspension was the same as previously described (Hrušková *et al.*, 1969).

Subcutaneous infection. Guinea pigs weighing on the average 450 g were inoculated on the back with 0.1 ml virus suspension containing 50 guinea pig ic LD_{50} of virus.

Inhalation infection. Guinea pigs weighing on the average 300 g were subjected to inhalation infection as described previously (Hrušková *et al.*, 1969). The dose was 3000 guinea pig ic LD_{50}, equivalent to 857 guinea pig inhalation LD_{50}.

Virus assay. In sc infected guinea pigs, urine, serum, brain, spinal cord, lungs, heart, liver, spleen, lymph nodes of the pulmonary hilus and inguinal nodes, kidneys, adrenals, great intestine, skeletal muscles, pancreas and salivary glands were examined for the presence of virus. In guinea pigs infected by inhalation, the examinations included also the trachea and nasal mucosa in addition to serum and the organs mentioned. Pools of organs from 5 guinea pigs killed by chloroform at intervals indicated in Tables 1 and 2 were examined.

Virus titrations were carried out in suspension cultures of chick fibroblasts grown in medium 199 supplemented with 3% calf serum (Institute of Sera and Vaccines, Prague). The cytopathic changes were read after 3—5 days at 37° C. The virus titres expressed in TCD_{50} values were calculated by the method of Reed and Muench. Urine was assayed for virus by sc inoculation of mice.

Immunofluorescence. Using the direct technique, the brains, spinal cords, lungs, spleens, livers, pancreas, salivary glands, lung hilus and inguinal lymph nodes, and in case of inhalation infection also the nasal mucosa, were examined at 24, 48, 72, 96 and 108 hours post infection (p.i.) (and also at 156 hours after inhalation infection). Sections from frozen organs were fixed for 10 minutes at room temperature with acetone. Fluorescein isothiocyanate-labelled gamma-globulin from hyperimmune rabbit serum was used. The preparations were contrast-stained with rhodamine sulfofluoride-albumin, mounted into TRIS-glycerol (Mrenová and Albrecht, 1966) and examined in an ML2 microscope.

Histology. In addition to organs assayed for virus, also the bone marrow from the sternum and femur were examined. Three animals were investigated at each interval (the same as for immunofluorescence). The materials were fixed in 10% formol. Paraffin sections were stained with haematoxylin and eosin and by van Gieson's method with simultaneous demonstration of elastic fibres.

Results

Distribution of virus in infected guinea pigs as revealed by virus titration

The results of virus titrations are summarized in Tables 1 and 2.

Subcutaneous infection. At the first interval (6 hours p.i.) the virus was present in the blood, liver and pancreas. The findings of virus in the blood at 6 and 12 hours p.i. suggest resorptive viraemia, while the high levels of virus at 18 and 24 hours indicate that the virus either multiplied in the blood stream or was drained into the blood from other sites of multiplication.

The middle period of infection (from about 24 to 72 hours p.i.) was characterized by generally high levels of virus in the organs examined. The virus titres in CNS organs were not higher than those in parenchymatous organs or lymph nodes. In the final stages (96 and 108 hours p.i.) the virus titres in the brains and spinal cords were the lowest ones established. High levels of virus persisted mainly in the blood.

In the course of infection, especially in the middle period, the virus reached high levels in the kidneys, but we never demonstrated it in urine.

Inhalation infection. At the first interval (12 hours p.i.), the virus was detected in the portal of entry, i.e. in the nose and lungs. At 24 hours p.i., in addition to viraemia, the virus was demonstrated in the lymph nodes of the pulmonary hilus, pancreas and spleen. Increasing virus titres were observed starting from this interval in organs containing lymphatic tissue. The titres of virus in CNS organs were not higher than those in lymphatic tissues.

Starting from 48 hours p.i. till the end of the observation period, the

Table 1. VEE virus titres in guinea pigs subcutaneous infection

Material examined	Virus titres (log TCD_{50}) at hours p.i.												
	6	12	18	24	30	36	42	48	60	72	84	96	108
Brain	neg	neg	neg	2.3	3.3	3.0	5.5	5.0	5.3	6.0	5.3	3.0	2.0
Spinal cord	neg	neg	2.5	2.5	3.0	3.5	4.5	4.5	4.3	4.3	4.3	3.5	2.7
Lungs	neg	neg	2.5	3.5	—	3.5	5.5	5.7	6.3	5.0	4.0	5.5	3.7
Heart	neg	neg	3.5	2.5	4.0	2.3	5.0	4.7	5.7	6.0	4.0	4.0	2.5
Liver	2.5	2.3	4.0	3.0	4.0	3.0	5.5	5.5	6.0	5.5	3.5	3.0	4.7
Spleen	neg	neg	3.3	3.7	5.3	5.0	5.0	5.0	6.0	5.0	4.7	6.5	4.0
Kidney	neg	neg	neg	3.5	5.3	3.5	4.5	5.3	6.0	6.0	5.3	5.0	4.7
Adrenals	neg	neg	2.5	3.7	4.0	4.0	5.3	5.5	6.0	4.7	5.0	4.7	5.5
Great intestine	neg	neg	2.3	2.0	3.5	4.0	4.3	5.5	4.7	5.3	3.7	4.3	2.0
Pancreas	2.5	3.7	3.3	3.0	neg	neg	neg	2.5	neg	neg	2.7	3.0	2.7
Salivary gland	neg	neg	3.0	4.0	5.3	6.3	6.3	6.5	7.7	6.0	6.3	5.7	4.5
Hilar node	neg	neg	2.5	3.3	6.3	4.3	5.7	4.7	7.0	5.3	5.5	6.5	4.7
Inguinal node	neg	neg	2.0	4.7	4.3	5.0	5.3	6.5	5.7	5.3	4.7	5.7	5.0
Serum	1.0	1.0	2.7	5.5	5.7	6.0	7.5	7.5	8.7	7.0	8.5	6.0	6.6
Skeletal muscle	neg	neg	2.0	neg	4.0	2.7	neg	4.3	4.7	neg	3.3	3.0	2.3

The titres are given per gram of tissue or ml of serum. Neg = no virus detected in 0.1 ml of 10% organ suspension or undiluted serum.

Table 2. VEE virus titres in guinea pigs after inhalation infection

Material examined	Virus titres (log TCD_{50}) at hours p.i.									
	12	24	36	48	60	72	84	96	108	156
Brain	neg	2.5	neg	—	5.5	4.5	5.0	4.7	5.0	5.0
Spinal cord	neg	neg	neg	4.0	4.0	4.5	4.3	2.0	2.7	4.0
Nose	2.5	neg	1.7	5.7	6.5	7.0	7.0	7.0	5.0	3.0
Trachea	neg	2.3	neg	3.5	5.5	3.7	5.5	5.5	5.0	4.7
Lungs	1.7	3.0	3.5	5.5	6.0	5.5	4.7	5.5	5.0	5.0
Heart	neg	neg	neg	5.7	5.0	5.3	5.5	4.5	3.7	6.3
Liver	neg	neg	neg	5.0	7.5	4.0	5.7	5.8	2.5	5.3
Spleen	neg	2.5	2.7	4.7	4.7	4.5	5.3	6.5	6.7	6.5
Kidney	neg	2.3	neg	6.3	5.5	4.0	6.0	3.7	3.7	4.5
Adrenals	neg	1.7	neg	4.5	5.0	4.5	5.0	5.5	3.7	5.3
Great intestine	neg	neg	neg	2.7	4.5	3.0	4.7	4.0	3.7	neg
Pancreas	neg	2.5	3.3	2.0	2.5	2.5	neg	3.7	2.0	3.7
Salivary gland	neg	4.3	neg	7.5	6.5	5.3	6.7	6.5	neg	3.5
Hilar node	neg	2.3	3.5	5.5	6.5	5.3	7.3	5.3	7.3	5.5
Inguinal node	neg	neg	neg	7.0	5.5	6.5	5.5	4.5	6.5	4.5
Serum	neg	3.3	1.7	3.0	7.5	6.7	7.3	—	4.5	6.7
Skeletal muscle	neg	neg	neg	2.3	4.5	2.7	3.7	2.3	3.7	—

For explanation see Table 1.

results offered evidence of a massive invasion by the virus of all organs and in principle did not differ from the picture found with sc infection.

Demonstration of viral antigen by immunofluorescence

The results of immunofluorescence investigations on organ sections were surprisingly poor. At 24 and 48 hours p.i. none of the organs showed definite specific fluorescence. At 72 hours p.i. the latter was observed only in the pancreas, namely of the cytoplasm of single small cell groups in the acini. At 96 hours p.i. their frequency and size increased (Fig. 1). At later intervals the findings were similar.

A comparison of the virus levels in the organs with these findings showed that the immunofluorescence method was little sensitive for revealing antigen distribution in the course of the infection. At the same time it became obvious that positive fluorescence was independent of high virus titres in the organs.

Histological changes

At the first interval (24 hours p.i.), no microscopic changes were found in sc infected animals. At 48 hours p.i. we observed involvement of the lymphatic system, namely in lymph nodes of the pulmonary hilus and inguinal nodes and in the spleen, in which we found fresh necroses of the germinal centres of lymphatic follicles. These necroses, characterized by a rough granular disintegration of the chromatin of lymphoblasts, were not, however, always limited to the germinal centres but involved also the marginal zone of the follicle. It must be stressed that some lymphatic follicles both in the node and in the spleen remained intact.

At 72 hours p.i., the histological picture was similar. One of three animals examined displayed no changes in the spleen, while necrosis of various intensity occurred in the nodes of all 3 guinea pigs.

The character and the intensity of the changes remained basically the same also at 96 hours p.i. The necrosis of lymphatic follicles of the nodes and spleen occurred in all 3 animals examined; its intensity varied. In one case the necrosis in the inguinal node had spread outside of the follicle (Figs 2 and 3).

Other findings included not very advanced hepatic steatosis along with small focal necroses of hepatocytes. These changes were also frequent in control animals and we consider them, therefore, as not directly related to experimental infection. No pathological changes were detected in the other organs examined, including the CNS and pancreas.

The changes after inhalation infection were in principle similar. At 48 hours p.i. we found necroses of germinal centres; in one animal they occurred both in the nodes and spleen, in the second only in the spleen and in the third they were absent. At 96 hours p.i. the necroses were found in the nodes and spleen; in one animal, however, the spleen was unaffected.

In addition to limited steatosis of the liver and focal necrosis of hepatocytes, which should be interpreted as above, we observed involvement of the respiratory tract. In the trachea there occurred focal desquamation of the epithelium; limited mixed inflammatory cellulisation, hyperaemia and

leukostasis in the veins was seen in the lamina propria of the mucosa. In the lungs, both the parenchyma and bronchi were involved; peribronchial changes were also observed. In the bronchi, like in the trachea, the epithelium desquamated and the lumen contained sparse exudate with an admixture of neutrophils. Round cell infiltrates occurred in the walls of bronchi and occasionally also peribronchially. In the parenchyma itself there were foci of somewhat thickened septa, containing mononuclear elements; the alveolar capillaries were dilated and contained numerous neutrophils. In some alveoli there occurred groups of macrophages, partially of a foamy character. The other organs examined displayed no pathological changes.

Discussion

In our study we attempted to elucidate the course of sc and inhalation infection of guinea pigs with VEE virus by simultaneous virological, immunofluorescence and histological examinations and thus supplement the knowledge about pathological changes as described by Victor *et al.* (1956).

We found that our mouse brain-adapted virus strain displayed a minimal neurotropism in guinea pigs after both sc and inhalation infection. In accordance with Victor *et al.* (1956) we observed a marked involvement of the lymphatic tissue, in which the virus also multiplied to high levels.

The course of infection following sc inoculation or inhalation of virus differed only in the early phase. In inhalation infection, the primary sites of virus multiplication were the nasal mucosa and lung parenchyma. Although on inhalation infection the virus multiplied rapidly in the nasal mucosa, thus offfering the possibility of virus spread via the fila olphactoria, we obtained no evidence that the effect of virus on the CNS would increase in guinea pigs infected by inhalation. We assume, therefore, that in either way of infection the multiplication, transport and pathogenic action of virus in lymphatic tissue and in the lymph and blood vessels was of decisive importance for pathogenesis.

Of interest was the positive immunofluorescence in pancreas cells, which was not accompanied by any visible morphological involvement or a high titre of virus. A relation of VEE virus to the pancreas was detected by immunofluorescence in mice after both sc and intracerebral infection (Kundin *et al.*, 1966; Kundin, 1966). We are of the opinion that the positive immunofluorescence can be explained in particular by the fact that excretory cells of the pancreas were not destroyed by the action of virus and that the viral antigen could form detectable structures, as distinct from cells of lymphatic tissues, in which the virus caused destruction and in which immunofluorescence was negative in spite of a high infectious titre. This assumption is supported by our experience with immunofluorescence of VEE virus in HeLa cells and chick fibroblasts. HeLa cells, substantially less susceptible and sensitive to VEE virus infection, yield much stronger immunofluorescence than chick fibroblasts.

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Explanation of Photomicrographs:

Fig. 1. Section from the pancreas of a guinea pig, 96 hours after infection with VEE virus. Fluorescent group of acinus cells.

Fig. 2. Lymph node from a guinea pig, 72 hours after infection with VEE virus. Nuclear debris in the central part of lymphatic follicle. Van Gieson, ×400.

Fig. 3. Spleen from a guinea pig, 96 hours after infection with VEE virus. Necrosis of Malpighian follicle, involving also the marginal zone in the greater part of the circumference. Haematoxylin and eosin, ×160.