Acta virol. **13** : 135—138, 1969

EXPERIMENTAL RESPIRATORY INFECTION INDUCED BY RABIES VIRUS VARIANTS ADAPTED TO TISSUE CULTURE

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Received June 12, 1968

Summary. — Street and fixed rabies viruses adapted to primary Syrian hamster kidney cell culture differed from neurotropic variants of street and fixed rabies virus and possessed a marked infectivity for mice when administered intranasally (*in*). In mice, the tissue culture variants reached titres from 2.6 to 3.5 log LD_{50}/0.03 ml on *in* and from 2.4 to 6.0 log LD_{50}/0.03 on intracerebral (*ic*) inoculation. Intranasal administration of brain suspension of neurotropic variants caused disease in 3 or 4 out of 10 mice. At the height of the disease in mice infected *in* with tissue culture variants, it was established that the virus accumulated in the lungs and brains; a low amount of virus was also found in the blood. The specificity of the infection was confirmed by immunofluorescence and neutralization test with commercial antirabies gamma-globulin.

It could be expected a priori that long-term serial passaging of rabies virus in extraneural tissue culture is able to cause a decrease in neurotropic and an increase in the viscerotropic activities of the virus. To study the viscerotropic characteristics of rabies virus variants adapted to tissue culture, the *in* route of infection was chosen. Data are available on *in* infection of mice with street rabies virus, when the incidence rate in response to a high concentration of the virus did not exceed 1/3 (Svet Moldavskaya, 1957). These results were explained by the penetration of the virus through the mucosa of the upper respiratory ways. As far as "canine" street rabies virus or neurotropic variants of fixed rabies virus are concerned, there are no reports on the accumulation of virus in lung tissue which could be interpreted as a respiratory infection. Atanasiu (1965) showed that tissue culture-adapted strains of rabies virus are pathogenic for Syrian hamsters on aerogenous or *in* inoculation and that the virus accumulates in the lungs of infected animals.

Fixed rabies virus (Sad strain) in the form of mouse brain suspension after 25 alternate passages in Syrian hamster kidney cell (SHKC) culture and in mice (Sad SHKCM-25) or in the form of culture fluid after 12—15 additional serial passages in SHKC culture (Sad SHKC 12—15); the brain variant of the original street rabies virus (Mochalin strain) in the form of rabbit brain suspension and its tissue culture variant in the form of virus-containing culture fluid after 9—38 passages in SHKC culture (Mochalin SHKC 9—38); mouse brain suspension of street rabies virus (Fomin strain) isolated from man; and fixed rabies virus (Moscow strain) in the form of rabbit brain suspension were used. For the origin of Mochalin, Sad and Moscow strains see Aksenova *et al.* (1967) and Mikhailovsky and Selimov (1967).

Mice weighing 5—6 g were infected *in* with 0.03 ml of 10% brain suspension or culture fluid (undiluted or diluted 10-fold) containing the respective rabies virus strain. The amount of the virus in lungs, brains and blood was estimated by *ic* inoculation of 5—6 g mice; the suspensions of these organs were centrifuged at 1000 rev/min for 5 minutes. The virus titres were calculated according to Reed and Muench.

To confirm the diagnosis of rabies, the mouse brain smears were investigated by the direct fluorescent antibody technique (Kluyeva *et al.*, 1967).

To study the pathogenicity for mice on *in* inoculation of culture and naturally occurring neurotropic variants of the street rabies virus and of neurotropic variants of the fixed virus, 8 experiments were carried out. Their results did not differ substantially.

Table 1. Intranasal infection of white mice with tissue culture and brain variants of rabies virus

Virus strain	Source of the virus inoculated	Titre log LD_{50}/ 0.03 ml	Incidence of rabies*	Average incubation period (in days)
Sad SHKCM-35 SHKC-15	Undiluted culture fluid	4.3	10	7.8
Sad SHKCM-35 Mochalin	10% mouse brain suspension	5.0	3	13.3
SHKC-37	Undiluted culture fluid	> 6.0	10	8.0
Mochalin, original	10% mouse brain suspension	3.3	3	10.5
Fomin	10% mouse brain suspension	3.5	4	18.5
Moscow	10% rabbit brain suspension	> 7.0	3	16.5

* Number of infected out of 10 inoculated mice.

Table 1 presents the results of one of these experiments. When the animals were infected *in* with tissue culture variants of street and fixed rabies virus (Mochalin and Sad strains), the disease had a short incubation period (7.8 to 8 days) in all the animals tested. However, as a result of *in* administration of the neurotropic street and fixed virus variants in the form of brain suspension, 3 or 4 out of 10 animals fell ill with rabies, the incubation period being longer, namely 16.7—18.5 days. The low *in* activity of brain variants of rabies virus did not depend on their original titre. Brain suspension of the Mochalin strain with a titre of 5.0 log *ic* mouse LD_{50}/0.03 ml, in experi-

Table 3. Virus levels in the organs and blood of white mice intranasally infected with rabies virus tissue culture variants

Virus strain	Virus titre (log LD_{50}/0.03 ml)		Virus in blood*
	Brains	Lungs	
Mochalin SHKC—37	6.0	> 3.0	4
Sad SHKC—15	> 6.0	3.0	5

* Number of infected out of 10 inoculated mice; the blood was diluted 1 : 10 with saline solution.

ments not shown in Table 1, also caused infection in 3—4 out of 10 mice inoculated *in*.

The high pathogenicity of culture variants of rabies virus for mice on *in* inoculation made it possible to titrate the virus in this way (Table 2). The titres determined *in* in mice reached vales up to 3.5 log LD_{50}/0.03 ml; they were somewhat lower than the titres found on *ic* inoculation.

This unusual phenomenon of the pathogenicity of culture variants of rabies virus for *in* infected mice arises a number of problems concerning the possibility of primary virus multiplication in lungs and other viscera and in respect to the route of virus penetration into the central nervous system.

Table 2. Intranasal and intracerebral titration of rabies virus tissue culture variants in mice

Virus strain	Titre (log LD_{50}/0.03 ml) determined	
	intranasally	intracerebrally
Mochalin SHKC-9	3.4	5.0
Mochalin SHKC-38	3.5	6.0
Sad SHKCM-35 SHKC-12	2.1	2.4
Sad SHKC-35	2.6	> 5.0

These problems are now being studied and will be the subject of our further reports. Here we present only data on virus accumulation in lung tissue of mice infected *in* (Table 3). These results offer evidence that adapted variants of rabies virus multiply in the lungs of *in* infected mice, although the amount of the virus in the brains was 1000 times as high as in the lungs. Some virus was found in the blood, suggesting viraemia.

In conclusion it is necessary to point out that clinical manifestation of the disease in mice and their death were associated with the development of rabies encephalitis. In all the experiments described above the specificity of rabies infection was confirmed by examining brain impression smears by the fluorescent antibody technique and by neutralization tests with anti-rabies gamma-globulin. The virus neutralized by anti-rabies gamma-globulin did not produce any disease in mice on *in* administration.

Our experiments on rabies virus accumulation in lungs and on the possibility of virus titration in mice infected *in* demonstrated marked changes in genetic properties of rabies virus strains adapted to cell culture of extraneural origin. These changes were characterized by a considerable increase of virus viscerotropic activity which was manifested, in particular, by the development of experimental respiratory infection in mice.

The data obtained are of especial interest with respect to the naturally changed American rabies virus of bats which, in contrast to European "canine" virus, possesses some viscerotropic activity and one extremely specific pathogenic feature: it accumulates in lungs, brown fat, kidneys and muscle tissue and is excreted for a long time through the salivary glands of clinically healthy bats. In recent years cases of respiratory transmission

of infection to man and some wild animals were recorded (Constantine, 1966).

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