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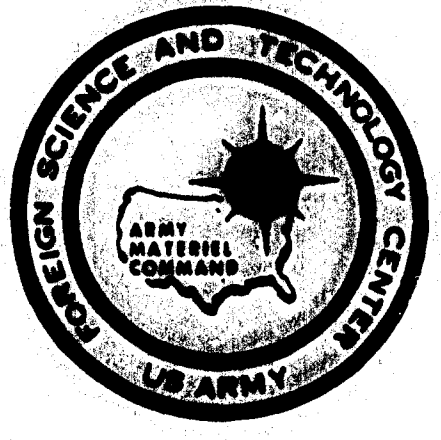

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KIDNEY TISSUE CULTURE

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GROWTH OF STREET RABIES VIRUS IN PRIMARY PUPPY KIDNEY TISSUE CULTURE

by
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GROWTH OF STREET RABIES VIRUS IN PRIMARY PUPPY KIDNEY TISSUE CULTURE

According to data in the literature successful serial passages of street rabies virus in cultures of extraneural tissues were accomplished in monolayer primary cultures of Syrian hamster kidney [3, 5] and an overlapping culture of hamster embryo fiberglass--VNK-clone 13 [6]. In the present report data are presented on the cultivation of street rabies virus in primary monolayer culture of puppy kidney--the natural host of rabies virus.

Materials and Methods

Monolayer culture of puppy kidney cells (PSKh) 1 to 5 days old were prepared according to the generally accepted method of trypsinization using lactalbumin hydrolysate in Hanks solution pH 7 with 10% normal bovine serum as the growth medium. Infection of the monolayer with virus, cultivation of virus and its titration were carried out according to the previously used method [3]. The standard deviation, LD_{50} and confidence of the differences in titers was determined by formulas [1, 2].

Results

Results are presented below of experiments in cultivation of strains of street virus (the original "cerebral" strains) in puppy kidney culture in which was studied the dynamics of accumulation of the Mochalin strain in serial passages, and also data of a comparative study of characteristics of propagation of two variants of the Mochalin strain--the original ("cerebral") and a preliminary adaptation to the PSKh cell.

A comparative study in tissue cultures of the original strain showed more active propagation of Mochalin strain in comparison with the Kar strain. As can be seen in Table 1 if the Mochalin strain was

regularly detected in three samples of culture fluid taken on the 20th, 24th and 29th day from the moment of infection then the Kar strain was detected less regularly--on the 15th and 19th day and was not detected on the 20th and 24th day after infection. Culture fluid containing the Mochalin strain towards the end of the experiment (29th day) caused a loss of all infected mice, both nonpropagating and propagation 10^{-1}, and culture fluid containing the Kar strain variant only in nonpropagating forms caused the loss of only one out of five infected mice.

Table 1
Propagation of Two Strains of Street Rabies Virus in Puppy Kidney Tissue Culture

Virus Strain	Day from Moment of Infection						
	5th	10th	15th	20th	24th	29th	
						Sample	Titer
Mochalin	0/10	0/10	1 +/10	2 +/10	3 +/5	5 +/5	1,0
Kar	0/10	0/10	2 +/10	0/5	0/5	1 + 4 -/5	0

Note: In the numerator the number of mice trapped; in the denominator the general number of infected mice.
Key: + confirmed diagnosis of rabies; - diagnosis of rabies eliminated.

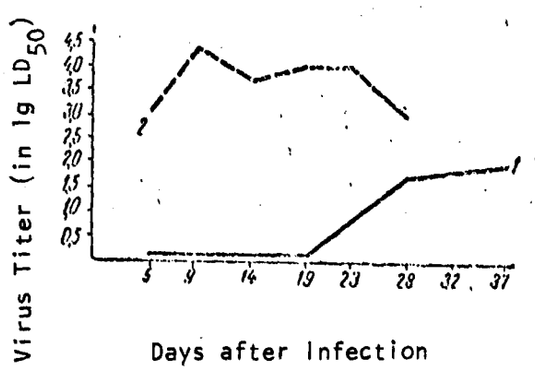
In the course of the entire experiment (29 days) degenerative changes in the cultures infected with Mochalin and Kar strains were not observed.

Further experiments (cultivation of virus and its serial passages) were carried out with the Mochaiin strain. These results are presented in Table 2. From this table it can be seen that in the first passage after infection of the culture by the original strain the virus is not detected in the culture fluid in the course of three weeks from the moment of infection and regularly appeared in it from the 23rd to 37th day inclusive while the mean titer of cultured virus was $10^{1,9}$. In the second passage viruses already detected after the fifth day from the moment of infection and on subsequent passages (3 to 9th) it was detected in the culture fluid on the fourth to fifth day of infection and regularly was detected in it in the period from 3 to 5 weeks of cultivation, while the level of cultured virus in the 3rd to 9th passages was higher than in the first to second.

Infection of mice in the brain by culture fluid of the first through ninth passages produced in them typical experimental rabies with the characteristic clinical symptoms (agitation, convulsions, paralysis) with incubation of 5 to 10 days, while an increase of titer and the cultured virus produced a shortened incubation period in the infected mice. In the brains of mice stricken from infection by cultured virus of the first through ninth passages, typical Babes-Negri were regularly observed. Investigation of the brains of these mice by the immunofluorescent method also yielded positive results.

During cultivation of the cerebral virus no destructive changes the monolayer were observed either in the first passage or in subsequent (2-9th) passages.

In the figure data are presented of a comparative study of propagation of two variants of the Mochalin strain--the original ("cerebra and the cultured (adapted to PSKh culture "variant 7PSKh").



Puppy Kidney Tissue Cultures. Multiplication of Two Types of Mochalin Strain--Initial (1) and Adapted to PSKh Culture (2). First Passage.

As can be seen in the figure, the original variant strain was detected in the culture fluid only after the third week from the moment of infection, then as preliminary adaptation of the virus to PSKh it was regularly observed in the medium from the fifth day of infection. The average titer of this virus (3.5 lg) exceeded the average titer of the original variant in the culture fluid (1.9).

Table 2
Serial Passages of Mochalin Strains in Puppy Kidney Culture and Virus Titer in Culture Fluid

Passage	Day from Moment of Infection of Culture																Geometric Mean of Virus Titer in Culture Fluid (in lg LD_{50})
	4th	5th	7th	9th	10th	14th	15th	16th	19th	21th	23th	25th	28th	30th	32th	35th	37th	
1st		—		—		—			—		$\frac{1}{20}$		1,7		$\frac{6}{6}$		2,0	1,9
2nd		$\frac{2}{5}$		1,9														
3rd	$\frac{1}{10}$		$\frac{4}{10}$		$\frac{10}{10}$	$\frac{10}{10}$			$\frac{10}{10}$		3,7		3,0		3,0		1,6	3,0
4th		2,5		3,0		3,8			3,7		3,0							3,2
5th		2,5			2,3		3,1		4,3									3,0
6th				1,0		2,8												
7th		$\frac{2}{10}$		$\frac{10}{10}$				3,0		2,8		3,0						2,9
8th		$\frac{9}{10}$		2,5		3,0			3,3		3,7			3,6				3,2
9th		1,9			3,5		4,4			3,0		2,6						3,0

Typical experimental rabies with an incubation of 5 to 6 days was produced in mice by intracerebral injection of culture fluid containing cultured variant of the virus. The diagnosis of rabies was confirmed by detection in the mouse brains of Babes -Negri bodies and by immunofluorscences.

In the course of the entire period of cultivation of the cultured variant of the Mochalin strain in puppy kidney culture (four weeks) destructive changes of the infected tissue was not noted.

Discussion

Thus the results of the experiments carried out indicated that the puppy kidney tissue culture--extraneural tissue of the natural host of the rabies virus--is susceptible to rabies virus. In a comparative study in culture of two variant strains of the street rabies virus it was observed that they possessed different degrees of affinity to the tissue culture. The more active propagation of the Mochalin strain noted above was apparently determined by individual characteristics of the strain, because the conditions of infection and cultivation of the Mochalin and Kar strains were identical. This differential capacity of the street virus strains to propagate and culture was also noted by Kissling [5], who succeeded in adapting only one out of three strains of street rabies virus to PSKh culture.

As a result of serial passages of the Mochalin strain in primary puppy kidney culture a shortening of the latent period of infection was noted and an increase of its titer in the culture which attested to the adaptation of the strain to the culture indicated.

The propagation of the cultured variant of the virus in puppy kidney culture is distinguished from propagation of the original variant by a shorter latent period and higher virus content in the culture fluid. Such characteristics as propagation of the cultured variant in puppy kidney cells may be caused by a change in genetic composition of the strain as a result of its preliminary adaptation to PSKh culture. Similar changes of culture properties were noted with fluorine KhE 11 sixth virus, which after adaptation to the diploid Wl-38 culture acquired this ability to propagate rapidly in green marmaset kidney culture [7], and also in Aueski virus whose cultivation in L cell cultures gave it the ability to actively propagate and KEM-LA culture initially resistant to it [4].

Results

1. Successful serial passages of street rabies virus in primary puppy kidney cell culture was achieved.

2. The adaptation of street virus to culture was characterized by shortened latent period of infection and increase in the titer of the cultured virus.

3. Preliminary adaptation of street virus to PSKh culture gave us the capacity to actively propagate in puppy kidney cultr

4. The propagation of street virus in puppy kidney culture was not accompanied by cytopathic effect.

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13. ABSTRACT

Street rabies virus was successfully grown in a primary culture of puppy kidney cells. Data are presented comparing growth of the "Mochalin" and "Kar" strains over a 29-day period. The "Mochalin" strain which had been adapted to the culture by nine serial passages multiplied much more actively. Adaptation of the virus to the culture was characterized by a shortened latent period of infection and by an increase in the virus titer, ($10^{-1.9}$ at the first passage, $10^{-3.2}$ at later passages). No destruction of the cell monolayer was observed during virus propagation.

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