RESEARCHES ON VACCINE VIRUS *IN VITRO* WITH SPECIAL REFERENCES TO ITS AFFINITY FOR NERVE TISSUE *

NORIMASA HIRANO

(From the Nagasaki Medical College, Nagasaki, Japan, and the Department of Comparative Pathology, Harvard Medical School, Boston, Massachusetts)

It is well known that when a piece of fresh tissue is put at the bottom of a culture tube, chemical reduction of the culture medium takes place and at the same time several substances dialyze out of the tissue. However, the effect of tissue on micro-organisms under cultivation is still not understood.

The present study was undertaken with two objects in view, first to prove the effect of the tissue on vaccine virus in culture medium; and second to determine whether or not vaccine virus migrates along the tissue *in vitro*.

EXPERIMENTAL METHODS AND DATA

I. COMPARISON OF THE AFFINITY OF VACCINE VIRUS FOR THE KIDNEY AND THE SPINAL CORD

The cornea, skin and testicle of rabbits are suitable places for the growth of vaccine virus *in vivo*, of which the testicle seems to be the most favorable. This has been already proved by the experiments of Noguchi [1] and Henseval.[2] Recently Marie [3] reported that when the virus is inoculated into the brain of a rabbit, it causes the death of the animal after four or five days. Also Levaditi and Nicolau [4] showed that if vaccine virus which had been passed several times through rabbits by intratesticular inoculation is injected into the brain of a rabbit, it causes a fatal infection and they have succeeded in making passages from brain to brain in series. I have found that when a strain of vaccine virus which has been passed through rabbits by intratesticular inoculation without increasing its virulence, is injected into the brain of a rabbit, its virulence increases very quickly. In view of the above facts, there seems to be no doubt that the central nervous system is an especially suitable place for the propagation of vaccine virus. On the contrary, according to Ohta-

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wara [5] and Levaditi and his co-workers, the affinity of the virus for the kidney is said to be either completely lacking or else very slight *in vivo*.

Therefore, I have carried out the following experiments in order to obtain some knowledge as to the affinity of vaccine virus *in vitro* for these tissues, namely, kidney, spinal cord and testicle.

The culture medium employed throughout the experiments on the affinity of virus for tissue was 3 per cent glucose broth containing 1 per cent glycerin with a pH of 7.0 to 7.2.

In every instance the material employed for inoculation into culture medium was the testicular strains of vaccine virus which had been passed several times through rabbits. The stock emulsion of the virus was prepared by grinding up aseptically removed vaccinated testicle with 50 per cent glycerin solution containing 0.5 per cent carbolic acid in the proportion of 1.0 gm. of the tissue to 2.5 or 5.0 c.c. of the solution. After grinding, this emulsion was aspirated with a sterile pipette through sterile gauze and put into a sterile test tube. This test tube was allowed to stand in an icebox for a few days, thus permitting the coarse particles to settle. For the purpose of inoculation the supernatant glycerin was pipetted off and varying dilutions, 1: 2 or 1: 5 in physiologic salt solution, were prepared.

The method employed consisted in inoculating medium from two series of test tubes containing 10 c.c. of the culture medium mentioned above and a piece of fresh sterile tissue (either spinal cord or kidney) at the bottom. Into each tube 0.1 c.c. of the diluted vaccine virus was placed. After inoculation, the tubes were covered with a layer of sterile paraffin oil and placed in an incubator at 37 C. Every twenty-four hours during incubation the test tubes of this series were taken out of the incubator one at a time, and if the culture medium was free of contaminating bacteria, it was transferred into another sterile tube with a capillary pipette. The tissue at the bottom of each tube was washed several times with sterile salt solution and weighed to the milligram. An emulsion of the washed tissue was made by grinding with sterile salt solution in the proportion of 0.1 gm. of the tissue to 1.0 c.c. of salt solution, *i.e.*, 1: 10 dilution of the tissue. For the purpose of inoculation into a rabbit, dilutions of the culture medium and the emulsion of tissue were prepared with sterile salt solution as follows: culture medium, 1: 1, 1: 10 and 1:100; tissue, 1: 10 (original emulsion), 1: 100 and 1: 1000.

The presence of the virus in the various dilutions of culture medium and tissue was tested according to Groth's [6] method of intradermal inoculation, *i.e.*, 0.1 c.c. of each inoculum was injected intradermally into a normal rabbit previously shaved over the sides of the abdomen. Altogether twelve inoculations were made at one time at least an inch apart in a single animal. The rabbits were white healthy adults weighing usually from 1.5 to 2.0 kg. The emulsion of spinal cord and the spinal cord medium were injected on the left side and the emulsion of kidney and the kidney medium on the right side. Although an effort was always made to prevent any of the inoculum escaping into the subcutaneous tissues, it is likely that in some instances this happened to some extent. After inoculation, the skin was observed daily for signs of reaction until inflammatory processes had nearly disappeared.

The results of the experiments are shown in Table I. This table shows clearly that vaccine virus has an affinity for the spinal cord of rabbits but almost no affinity for the kidney. Twenty-four hours after inoculation, the virus was found to be present in both the

TABLE I

Affinity of Nervous Tissue for Vaccine Virus

Rabbit No.	Period of incubation	Inoculum	Dilution of inoculum	Reaction at various days after inoculation into skin					
				2	3	4	5	6	7
117	24 hours	Emulsion of spinal cord	1:10	+	+	++	++	++	R
			1:100	+	+	++	++	++	R
			1:1000	−	+	++	++	++	R
		Fluid medium	1:1	+	+	+++	+++	+++	R
			1:10	+	+	+++	+++	+++	R
			1:100	?	+	++	++	++	R
		Emulsion of kidney	1:10	?	+	++	++	++	R
			1:100	−	+	++	++	++	R
			1:1000	−	−	+	+	++	R
		Fluid medium	1:1	?	+	+++	+++	+++	R
			1:10	?	+	+++	+++	+++	R
			1:100	−	+	++	++	++	R

+ = visible lesion. The number of plus marks represents the size of the lesion, +++ being the maximum.

− = absence of a visible lesion.

R = inflammatory process retrogressing.

TABLE I (*continued*)

Rabbit No.	Period of incubation	Inoculum	Dilution of inoculum	Reaction at various days after inoculation into skin					
				2	3	4	5	6	7
118	2 days	Emulsion of spinal cord	1:10	+	++	++	+++	+++	R
			1:100	+	++	++	++	++	R
			1:1000	−	+	+	+	+	R
		Fluid medium	1:1	+	+++	+++	+++	+++	R
			1:10	+	+++	+++	+++	+++	R
			1:100	+	+	+	++	+++	R
		Emulsion of kidney	1:10	−	−	−	+	+	R
			1:100	−	−	−	+	+	R
			1:1000	−	−	−	−	−	R
		Fluid medium	1:1	+	+++	+++	+++	+++	R
			1:10	+	+++	+++	+++	+++	R
			1:100	+	+	+	+	+	R
119	3 days	Emulsion of spinal cord	1:10	+	++	++	++	R	
			1:100	+	+	+	+	R	
			1:1000	+	+	+	+	R	
		Fluid medium	1:1	+	+++	+++	+++	R	
			1:10	+	++	++	++	R	
			1:100	+	+	+	+	R	
		Emulsion of kidney	1:10	−	−	−	−	−	
			1:100	−	−	−	−	−	
			1:1000	−	−	−	−	−	
		Fluid medium	1:1	+	++	++	+++	R	
			1:10	+	+	+	+	R	
			1:100	−	+	+	+	R	
120	4 days	Emulsion of spinal cord	1:10	+	+	++	++	++	++
			1:100	+	+	+	++	++	++
			1:1000	−	?	+	+	+	+
		Fluid medium	1:1	+	+	++	+++	+++	+++
			1:10	−	?	+	++	++	++
			1:100	−	?	+	++	++	++
		Emulsion of kidney	1:10	−	−	−	?	?	+
			1:100	−	−	−	−	−	−
			1:1000	−	−	−	−	−	−
		Fluid medium	1:1	+	+	++	+++	+++	+++
			1:10	+	+	+	++	++	++
			1:100	−	?	+	++	++	++

spinal cord and the kidney tissue, each giving similar skin reactions; at the end of forty-eight hours' incubation in the test tube the virus in the spinal cord caused marked inflammatory processes in the skin, and four days later was found to be still active, whereas the incubated kidney showed a marked decrease in virus and an earlier disappearance.

The papules produced by the virus incubated in spinal cord were different from those caused by the virus in kidney or by the fluid portion of either medium. The papules during the active stages of the infection were redder and more prominent, the infiltration more intense and profound. Later they were sometimes covered with a crust which often remained for weeks.

The culture medium containing the spinal cord produced a more active reaction on the skin than that containing the kidney although the difference was by no means marked.

Repetitions of this experiment have in all cases given similar results.

The intradermal injection of an emulsion of a normal spinal cord caused no inflammatory reaction in the skin of rabbits.

Effect of Nervous Tissue on Vaccine Virus of Low Virulence

Vaccine virus of the lot previously used was employed in this experiment. It showed, however, a definite decrease of virulence in consequence of long preservation. Thus the inoculation of 0.1 c.c. of 1:100 dilution into the skin of a rabbit produced a definite though not intense inflammatory reaction.

The methods and culture medium employed to determine the effect of nervous tissue on virus of low virulence are similar to those previously used. The results of the experiment are summarized in Table II. It is quite evident that the skin reacted to the 1:10 tissue emulsion, while the medium failed to produce a visible lesion, although the medium of two days' incubation produced a papule. In this experiment, the vaccine virus incubated with the spinal cord for twenty-four hours produced a papule earlier than when incubated a longer time; its inflammatory processes, however, were not intense. On the contrary, the spinal cord which was incubated with the virus two or three days caused a papule later, but its infiltration was more intense and extensive.

TABLE II

Effect of Nervous Tissue on Vaccine Virus of Low Virulence

Rabbit No.	Period of incubation	Inoculum	Dilution of inoculum	Reaction at various days after inoculation into skin					
				5	6	7	8	9	10
104	24 hours	Emulsion of spinal cord	1:10	?	+	+	+	R	
			1:100	–	–	–	–	–	–
			1:1000	–	–	–	–	–	–
		Fluid medium	1:1	–	–	–	–	–	–
			1:10	–	–	–	–	–	–
			1:100	–	–	–	–	–	–
105	2 days	Emulsion of spinal cord	1:10	–	–	?	+	+++	+++
			1:100	–	–	–	–	–	–
			1:1000	–	–	–	–	–	–
		Fluid medium	1:1	–	–	–	–	++	+++
			1:10	–	–	–	–	–	–
			1:100	–	–	–	–	–	–
106	3 days	Emulsion of spinal cord	1:10	–	–	–	–	+	+++
			1:100	–	–	–	–	–	–
			1:1000	–	–	–	–	–	–
		Fluid medium	1:1	–	–	–	–	–	–
			1:10	–	–	–	–	–	–
			1:100	–	–	–	–	–	–

For legend see Table I.

The results indicate that the virus survives longer in the nervous tissue than in the medium, and it would appear that the virus diminishes in amount after incubation but increases in virulence.

II. COMPARISON OF THE AFFINITY OF VACCINE VIRUS FOR THE TESTICLE AND THE SPINAL CORD

The incubation was carried out at 37 C. and at room temperature (about 21 C.). In the case of the incubation at room temperature I gave my attention to obtaining evidence as to whether the tissue, especially the spinal cord at the bottom of the culture tube, had any effect on the virus suspended in the medium, simultaneously with my efforts to compare the affinity of the virus for the testicle and spinal cord.

TABLE III

Comparison of the Affinity of Nervous and Testicular Tissues for Vaccine Virus
(Incubation at 37 C.)

Rabbit No.	Period of incubation	Inoculum	Dilution of inoculum	Reaction at various days after inoculation into skin					
				2	3	4	5	6	7
30	24 hours	Emulsion of spinal cord	1:10	+	+	++	++	R	
			1:100	−	+	+	++	R	
			1:500	−	−	−	+	R	
		Fluid medium	1:1	+	++	+++	+++	R	
			1:10	?	+	+++	+++	R	
			1:50	−	−	++	++	R	
		Emulsion of testicle	1:10	−	+	++	++	R	
			1:100	−	−	?	+	R	
			1:500	−	−	−	+	R	
		Fluid medium	1:1	+	++	+++	+++	R	
			1:10	+	+	+++	+++	R	
			1:50	−	−	++	++	R	
31	2 days	Emulsion of spinal cord	1:10	−	−	+	+	++	+++
			1:100	−	−	−	+	+	++
			1:500	−	−	−	−	−	−
		Fluid medium	1:1	−	−	−	+	++	+++
			1:10	−	−	−	+	++	+++
			1:50	−	−	−	−	+	++
		Emulsion of testicle	1:10	−	−	−	−	?	++
			1:100	−	−	−	−	−	−
			1:500	−	−	−	−	−	−
		Fluid medium	1:1	−	−	+	+	++	+++
			1:10	−	−	−	+	++	+++
			1:50	−	−	−	−	+	+
32	3 days	Emulsion of spinal cord	1:10	+	++	++	R		
			1:100	+	+	+	R		
			1:500	−	−	−	−	−	−
		Fluid medium	1:1	−	−	−	−	−	−
			1:10	−	−	−	−	−	−
			1:50	−	−	−	−	−	−
		Emulsion of testicle	1:10	−	−	−	−	?	−
			1:100	−	−	−	−	?	−
			1:500	−	−	−	−	−	−
		Fluid medium	1:1	−	−	−	−	−	−
			1:10	−	−	−	−	−	−
			1:50	−	−	−	−	−	−

For legend see Table I.

The strain of vaccine virus employed in the experiments was inoculated once into the brain of a rabbit and afterwards passed several times through rabbits by intratesticular inoculation. Otherwise it was prepared in the same way as in the previous experiments.

The culture medium and the methods are also similar to those of the previous experiments with the exception that testicular tissue was used in the place of the kidney. In some cases the tissue emulsions were inoculated also into the testicle of a living rabbit. The dilutions of each inoculum were prepared with sterile salt solution as follows: culture medium, 1: 1, 1: 10 and 1: 50; tissue, 1: 10, 1: 100 and 1: 500.

The results of the experiments are shown in Tables III to VI.

According to Table III, the spinal cord incubated with the virus twenty-four hours at 37 C. produced visible lesions on the skin earlier than the testicle produced them. In addition to this, the spinal cord diluted 1: 100 caused a papule on the skin even after three days' incubation, while the testicle similarly incubated produced no visible lesion even when diluted only 1: 10. The activity of both mediums whether containing a piece of spinal cord or testicle was the same. Three days after incubation the virus disappeared from the medium. In other words, as soon as the virus inoculated into the medium containing testicular tissue died out in the medium after incubation, it also disappeared in the tissue. On the contrary, when the virus which was inoculated into the medium containing the spinal cord died out in the medium, it still remained active in the spinal cord even to the following day.

Similar tests for the activity of virus by inoculation into the testicle *in vivo* showed results comparable with those obtained by intracutaneous inoculation as summarized in Table IV, namely, the testicle incubated three days with the virus caused only a very small induration in the testicle while the spinal cord emulsion still produced an intense inflammatory process.

The experiments in which tissues were incubated at room temperature (21 C.), as will be seen in Tables V and VI, gave further evidence of the retention of the virus in the spinal cord. It was noted that the spinal cord incubated with the virus even for twenty-one days was very active and produced a more intense reaction on the skin than when incubated for sixteen days. In other words, the virus in the spinal cord remained very active notwithstanding the

TABLE IV

Comparison of the Affinity of Nervous and Testicular Tissues for Vaccine Virus (Incubation at 37 C.)

Rabbit No.	Period of incubation	Inoculum	Size of dose	Gross appearance of reaction at various days after inoculation into testicle					
				4	5	6	7	8	9
31	2 days	Emulsion of spinal cord	0.2 c.c. (1: 10)	–	Slight induration appeared	Induration increased	Induration and swelling	Increasing fullness and firmness	
		Emulsion of testicle	0.2 c.c. (1: 10)	–	Slight induration appeared	Induration increased	Induration and swelling	Induration and swelling slightly increased	
32	3 days	Emulsion of spinal cord	0.2 c.c. (1: 10)	Slight induration appeared	Induration increased	Marked induration	Fullness and firmness increased	Induration somewhat less than on the previous day	Similar to the previous day
		Emulsion of testicle	0.2 c.c. (1: 10)	–	–	–	Indurated spot appeared	Similar to the previous day	Induration disappearing

long incubation. The virus in the testicular tissue, however, became so weak after twenty-one days' incubation that it produced only a slight inflammatory process on the skin and in the testicle, even with an emulsion diluted 1: 10.

When the spinal cord which was incubated with the virus was inoculated into the testicle of rabbits, as will be seen in Table VI, there took place at the end of two or three days, swelling and induration of the organ, which increased in size and density for five or six days.

TABLE V

Comparison of the Affinity of Nervous and Testicular Tissues for Vaccine Virus (Incubation at Room Temperature)

Rabbit No.	Period of incubation	Inoculum	Dilution of inoculum	Reaction at various days after inoculation into skin				
				2	3	4	5	6
38	5 days	Emulsion of spinal cord	1: 10	+	++	++	++	R
			1: 100	+	++	++	++	R
			1: 500	−	+	+	+	R
		Fluid medium	1: 1	+	+	++	R	
			1: 10	+	+	++	R	
			1: 50	−	+	+	R	
		Emulsion of testicle	1: 10	+	+	++	++	R
			1: 100	−	+	++	++	R
			1: 500	−	−	+	+	R
		Fluid medium	1: 1	+	+	++	R	
			1: 10	−	+	++	R	
			1: 50	−	−	+	R	
39	7 days	Emulsion of spinal cord	1: 10	+	++	+++	+++	R
			1: 100	?	++	++	++	R
			1: 500	?	+	+	+	R
		Fluid medium	1: 1	+	+++	+++	R	
			1: 10	+	+	+	R	
			1: 50	?	+	+	R	
		Emulsion of testicle	1: 10	?	++	+++	+++	R
			1: 100	?	?	?	+	R
			1: 500	?	−	−	?	−
		Fluid medium	1: 1	?	++	+++	+++	R
			1: 10	?	+	+	+	R
			1: 50	?	+	+	+	R

For legend see Table I.

TABLE V (*continued*)

Rabbit No.	Period of incubation	Inoculum	Dilution of inoculum	Reaction at various days after inoculation into skin				
				2	3	4	5	6
40	10 days	Emulsion of spinal cord	1: 10	++	++	++	++	R
			1: 100	−	++	++	++	R
			1: 500	−	−	+	+	R
		Fluid medium	1: 1	++	+++	+++	+++	R
			1: 10	++	++	+++	R	
			1: 50	+	+	++	R	
		Emulsion of testicle	1: 10	+	++	++	R	
			1: 100	−	?	+	R	
			1: 500	−	−	+	R	
		Fluid medium	1: 1	++	+++	+++	R	
			1: 10	+	++	++	R	
			1: 50	−	+	+	R	
41	16 days	Emulsion of spinal cord	1: 10	−	+	+	+	R
			1: 100	−	−	+	+	R
			1: 500	−	−	+	+	R
		Fluid medium	1: 1	−	−	−	+	R
			1: 10	−	−	−	−	−
			1: 50	−	−	−	−	−
		Emulsion of testicle	1: 10	−	−	+	+	R
			1: 100	−	−	?	+	R
			1: 500	−	−	?	?	R
		Fluid medium	1: 1	−	−	−	+	R
			1: 10	−	−	−	−	−
			1: 50	−	−	−	−	−
43	21 days	Emulsion of spinal cord	1: 10	+	+	++	++	R
			1: 100	−	+	+	++	R
			1: 500	−	?	+	+	R
		Fluid medium	1: 1	+	+	+++	+++	R
			1: 10	−	+	++	++	R
			1: 50	−	+	+	+	R
		Emulsion of testicle	1: 10	?	−	+	+	R
			1: 100	−	−	?	+	R
			1: 500	−	−	−	−	−
		Fluid medium	1: 1	−	+	+	++	R
			1: 10	−	?	+	++	R
			1: 50	−	-	+	+	R

At this time the color of the testicles was purplish red, spotted here and there with irregular yellowish areas of variable dimensions. In sections many polymorphonuclear leucocytes are seen to invade the interstitial tissues together with a serous exudate; the nuclei of cells within the infiltrated areas become karyorrhectic and some tubules are destroyed. The testicular cells are hydropic and fill up the tubular lumen. The virus in the testicle *in vitro*, however, gradually became attenuated after incubation. In consequence of this attenuation in every instance, the virus gave no intense reaction in the testicle with the exception of that of three days' incubation. The virus after incubating three days in medium containing testicular tissue or spinal cord gave almost the same reactions in the testicle up to six days after the inoculation. At the end of seven days, however, definite differences were noted, namely, the testicle inoculated with the testicular emulsion decreased in size, while that inoculated with spinal cord emulsion still showed an intense reaction. At the end of twelve days the former testicle was normal in size, pale and soft and few small grayish foci were recognized. The sections showed only a small infiltrated area. The latter testicle, however, was covered with a grayish fibrous mass adhering to the tunica vaginalis which was still edematous. The sections showed areas of necrotic tissue and extensive infiltration.

The results obtained in these experiments show that the nervous tissue seems to be endowed with more marked affinity for vaccine virus than the testicle. No marked difference was noted in the effect of the tissue of the spinal cord and that of the testicle on the virus suspended in the medium.

III. Effect of the Spinal Cord on Activity of Vaccine Virus

In view of the results obtained in the foregoing experiments it seems proper to raise the question whether the nervous tissue contains any substances which increase the activity of vaccine virus. To determine this question the following experiments were carried out.

A piece of fresh spinal cord and a piece of the spinal cord previously incubated in the medium (glycerin dextrose broth) for two or three days at 37 C. were separately ground with sterile physiologic salt solution in the proportion of 0.1 gm. of tissue to 0.5 c.c. of salt solution. These tissue emulsions were mixed with the same amounts

TABLE VI

Comparison of the Affinity of Nervous and Testicular Tissues for Vaccine Virus (Incubation at Room Temperature)

Rabbit No.	Period of incubation	Inoculum	Size of dose	Gross appearance of reaction at various days after inoculation into testicle				
				3	4	5	6	7
37 *1	3 days	Emulsion of spinal cord	0.2 c.c. (1: 10)	Swelling and induration appeared	Similar to the last	Swelling and induration increased	Marked swelling and induration accompanied by edema of the scrotum	Similar to the last
		Emulsion of testicle	0.2 c.c. (1: 10)	Swelling and induration appeared	Similar to the last	Swelling and induration increased	Marked swelling and induration accompanied by edema of the scrotum	Marked reduction in size
38	5 days	Emulsion of spinal cord	0.2 c.c. (1: 10)	Slight induration appeared	Similar to the last	Induration increased accompanied by edema of the scrotum	Similar to the last	Marked swelling and edema of the scrotum
		Emulsion of testicle	0.2 c.c. (1: 10)	Slight induration appeared	Similar to the last	Similar to the last	Similar to the last	Induration not increased
40 *2	10 days	Emulsion of spinal cord	0.2 c.c. (1: 10)	Swelling and induration appeared	Swelling and induration increased	Marked swelling and induration accompanied by edema of the scrotum		
		Emulsion of testicle	0.2 c.c. (1: 10)	Swelling and induration appeared	No increase	No increase		
43 *3	21 days	Emulsion of spinal cord	0.2 c.c. (1: 10)	Slight induration	Marked induration	Marked swelling and edema of the scrotum		
		Emulsion of testicle	0.2 c.c. (1: 10)	No sign	Slight induration	Similar to the last		

*1 Twelve days after inoculation both testicles were removed. *2 and *3 Five days after inoculation both testicles were removed.
All these testicles were fixed in Zenker's fluid and sections cut in paraffin.

of a highly virulent virus (original mixture diluted 1: 100); these mixtures were further diluted 1: 10 with sterile salt solution. Then 0.1 c.c. of each mixture was inoculated into the skin of a normal rabbit. As control, the virus and the emulsion of spinal cord without vaccine virus similarly diluted with sterile physiologic salt solution, were inoculated.

The results of these experiments are shown in Table VII.

TABLE VII

Effect of the Spinal Cord on Vaccine Virus

Rabbit No.	Spinal cord and control	Inoculum	Dilution of inoculum	Size of dose	Reaction at various days after inoculation into skin				
					3	4	5	6	7
62	Fresh	Vaccine virus with spinal cord	1: 1 (original)	0.1 c.c.	+	+	+	+	R
			1: 10	0.1 c.c.	−	+	+	+	R
	Control	Vaccine virus without spinal cord	1: 1 (original)	0.1 c.c.	−	−	+	+	R
			1: 10	0.1 c.c.	−	−	+	+	R
		Emulsion of spinal cord	1: 1	0.1 c.c.	−	−	−	−	−
63	2 days incubated	Vaccine virus with spinal cord	1: 1 (original)	0.1 c.c.	+	+	+	R	
			1: 10	0.1 c.c.	+	+	+	R	
	Control	Vaccine virus without spinal cord	1: 1 (original)	0.1 c.c.	−	+	+	R	
			1: 10	0.1 c.c.	−	+	+	R	
		Emulsion of spinal cord	1: 1	0.1 c.c.	−	−	−	−	−
64	3 days incubated	Vaccine virus with spinal cord	1: 1 (original)	0.1 c.c.	−	−	+	+	++
			1: 10	0.1 c.c.	−	−	−	−	+
	Control	Vaccine virus without spinal cord	1: 1 (original)	0.1 c.c.	−	−	+	+	+
			1: 10	0.1 c.c.	−	−	−	−	?
		Emulsion of spinal cord	1: 1	0.1 c.c.	−	−	−	−	−

For legend see Table I.

The results show that the virus inoculated with the emulsion of spinal cord caused stronger inflammatory processes in the skin of a normal rabbit than the virus without spinal cord. In view of this fact, there seems no doubt that the spinal cord of a normal rabbit has a suitable substance for promoting the activity of vaccine virus.

IV. Is Vaccine Virus Capable of Migrating along the Spinal Cord *in Vitro?*

To determine this question the following experiments were carried out. A piece of the spinal cord about 6 cm. long was placed straight in a Petri dish. A mixture of 15 per cent agar (one part) and glycerin dextrose broth (one part) was poured into the dish until the piece of

Table VIII

The Question of Migration of Vaccine Virus along Spinal Cord in Vitro

Rabbit No.	Period of incubation	Inoculum	Method of inoculation	Result
54	3 days	1	Intradermal	+++
		2	"	–
		3	"	?
58*	3 days	2	Intratesticular	?
		3	Intradermal	?
59	4 days	1	Intradermal	+++
		2	"	–
		3	"	–

1 = emulsion of tissue of inoculated end.
2 = emulsion of tissue of uninoculated end.
3 = agar surrounding uninoculated end.
* Two weeks after first inoculation revaccination was made into the skin but no response was obtained.

spinal cord was totally covered. After the poured mixture hardened, one end of the piece of spinal cord was exposed by removing a little agar, and at this end a small drop of vaccine virus was inoculated. The dish was placed at room temperature (21 C.). After incubation for different periods of time 0.1 cm. was cut off each end of the spinal cord. These end-parts of tissue were separately emulsified by grinding with sterile salt solution in the proportion of 0.1 gm. of tissue to 0.5 c.c. of salt solution. A normal animal was inoculated with 0.2 to 0.3 c.c. of the emulsions and also with the agar surrounding the uninoculated end of the cord.

The results of these experiments, as summarized in Table VIII, show that the tissue emulsions of the uninoculated end failed to produce a visible lesion, while in two cases agar surrounding the tissue caused a doubtful reaction on the skin. In view of these facts it is evident that vaccine virus is incapable of migrating along the spinal cord *in vitro*, although there is a possibility that it may be transmitted through a space between the spinal cord and agar (capillary attraction?).

Discussion

According to the embryonic origin of tissues the spinal cord is an invaginated segment of the ectoderm, so that it can be inferred that the central nervous system of the rabbit affords as favorable a site for the multiplication of the virus as the skin. But when the virus is injected into the blood stream, the brain is either deprived or fairly free of vaccine virus. In this connection Levaditi [4] has announced that it is probable that the vascular endothelium (or the entire choroid plexus) prevents the passing of vaccine virus from the blood stream into the nervous system. On the other hand, he produced evidence that the virus grows abundantly in the brain when normal saline or broth is injected into the brain of an intravenously infected animal, because the irritation due to the intracerebral injection of saline or broth causes an aseptic meningitis and breaks down this resistance, thus offering to the virus an excellent culture medium, the brain itself.

My experiments *in vitro* show that the central nervous system of the rabbit is endowed with more marked affinity for the vaccine virus than the testicle. Furthermore it seems as if the virulence of the virus increases in the nervous tissue to some extent. Therefore it is probable that of all tissues the central nervous system contains the most favorable substances and soil for propagation of the virus although the virus does not multiply in the nervous tissue removed from the body.

Steinhardt and his co-workers [7, 8] applied the method of cultivating corneal tissues *in vitro* to the study of vaccine virus and stated that there was slight multiplication of the virus. Recently Parker [9] announced that he had succeeded in carrying vaccine virus through many generations in artificially cultivated testicular tissues. If the virus is really able to grow in tissue cultures, it would be expected

that the central nervous system, being endowed with a specific affinity for the virus, should be the favorable tissue for such a purpose. Experiments to decide this point are now under way.

SUMMARY

1. Vaccine virus has an affinity for the central nervous system but almost no affinity for the kidney *in vitro*, the latter indeed having a deleterious effect on the virus.

2. Vaccine virus has also an affinity for testicular tissue but this is less marked than its affinity for nervous tissue.

3. Vaccine virus survives in nervous tissue immersed in fluid medium after dying out in the latter.

4. Vaccine virus in culture medium containing spinal cord produces a more marked reaction on the skin of rabbits than the virus in medium containing kidney, although the difference is by no means sharp; however, there appears to be almost no difference in the effect of spinal cord and testicle on the virus suspended in the medium.

5. The vaccine virus emulsified with spinal cord causes more marked inflammatory reaction than control glycerinated virus. Therefore, it would appear that the spinal cord of a normal rabbit is favorable to the growth of the virus and tends to increase its virulence.

6. Vaccine virus appears to be incapable of migrating along the spinal cord *in vitro*.

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