THE MECHANISM OF ACTIVE CEREBRAL IMMUNITY TO EQUINE ENCEPHALOMYELITIS VIRUS

I. Influence of the Rate of Viral Multiplication

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It has been found (1) that mice vaccinated with inactivated Western equine encephalomyelitis (W.E.E.) virus were less resistant to intracerebral challenge doses of the "R.I." strain than to comparable doses of other strains of W.E.E. virus. This observation could not be ascribed to antigenic or serologic differences between the R.I. and other strains, nor to differences in immunizing potency, since immunization with the R.I. strain protected mice more effectively against heterologous strains of W.E.E. virus than against the homologus strain. The latter had been subjected, over a period of many years, to many brain-to-brain passages in mice, and in the course of this treatment its lethal titer had increased and the survival time of inoculated mice was considerably shortened. It seemed most likely that the difficulty in protecting mice against the R.I. strain was connected with its ability to kill mice more rapidly. The hope seemed warranted that further comparative studies on the properties of this and one of the other strains would lead to a better understanding of factors involved in the mechanism of immunity to neurotropic viruses. The present paper deals in the main with studies on the comparative growth rates in the mouse brain of the R.I. and another strain of W.E.E. virus. In addition, further experiments will be described which illustrate the effect of strain differences on the reaction of immunized animals to intracerebral inoculation. The host factors which are responsible for variations in the response will be discussed in a subsequent paper (2).

Materials and Methods

Virus Strains.—The 2 strains of W.E.E. virus used were the R.I. strain and the Kelser strain previously described by Olitsky, Morgan, and Schlesinger (1). They differed in the manner already mentioned; *i.e.*, the R.I. strain had a higher lethal titer and, after intracerebral inoculation of comparable amounts, killed mice about twice as rapidly as the Kelser strain. The R.I. strain has been carried through many brain-to-brain passages in mice since 1933, while the Kelser strain has been subjected to only a few such transfers. The exact number of passages has not been recorded for either strain.

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Stock Virus Suspensions.—Brains were harvested from mice inoculated intracerebrally with about 100 LD_{50} of virus which were sacrificed when encephalitic signs were first seen. Crude 20 per cent homogenates of such brains were prepared in inactivated (56°C. for 30 minutes) normal rabbit serum diluted 1:2 in saline. The suspensions were kept frozen in sealed glass ampules in the dry ice chest. A fresh ampule was used for each experiment.

TABLE I

W.E.E. Virus, R.I. Strain: Frequency and Standard Deviations of Intracerebral Titers in Mice

Titer (X)*	Frequency (f) of titer X in sample				$f(X-\bar{X})^2$			
	A	B	C	Total	A	B	C	Total
8.5			1	1	0.00	0.00	0.25	0.49
8.6	1	1		2	0.36	0.36	0.00	0.72
8.7		1	1	2	0.00	0.25	0.09	0.50
8.8	2	1		3	0.32	0.16	0.00	0.48
8.9				0	0.00	0.00	0.00	0.00
9.0	6	1	2	9	0.24	0.04	0.00	0.36
9.1				0	0.00	0.00	0.00	0.00
9.2	1	1	1	3	0.00	0.00	0.04	0.00
9.3	4	2		6	0.04	0.02	0.00	0.06
9.4		1		1	0.00	0.04	0.00	0.04
9.5	3	3	1	7	0.27	0.27	0.25	0.63
9.6		1		1	0.00	0.16	0.00	0.16
9.7	1			1	0.25	0.00	0.00	0.25
9.8				0	0.00	0.00	0.00	0.00
9.9				0	0.00	0.00	0.00	0.00
10.0	1			1	0.04	0.00	0.00	0.04
No. of tests (N)	19	12	6	37	—	—	—	—
Mean titer ($\bar{X}$)	9.2	9.2	9.0	9.2	—	—	—	—
$\Sigma (X-\bar{X})^2$	—	—	—	—	2.12	1.30	0.63	4.33
S.D. (σ) $= \sqrt{\frac{\Sigma(X-\bar{X})^2}{N-1}}$	—	—	—	—	0.343	0.344	0.355	0.347

* Titers are expressed as log LD_{50}/0.03 gm. of brain tissue.

All dilutions of stock virus were made in saline containing 10 per cent inactivated rabbit serum. The intracerebral inoculum was 0.03 ml. per mouse.

Standardization of Stock Viruses.—Titers of the stock virus suspensions have been calculated according to the 50 per cent end-point method of Reed and Muench (3) and expressed in terms of LD_{50} per 0.03 gm. of brain tissue.

With one exception, all the experiments to be described in this and the following paper (2) were done with a limited number of stock samples of the two strains. In Tables I and II are given the results of multiple titrations of these samples in mice and summaries of the cal-

culations of the standard deviations according to the formula previously used for other viruses by Lauffer and Miller (4) and by Horsfall and Curnen (5). It will be seen that the values found for individual samples were almost identical. Therefore, these values were combined, giving for the R.I. strain a mean titer of $10^{9.2}$ with a standard deviation of 0.347 log, and for the Kelser strain a mean titer of $10^{7.5}$ with a standard deviation of 0.345 log.

This standardization covers only the reproducibility of end-points on stock samples but not necessarily variables and sampling errors involved in determinations of the growth rate.

TABLE II

W.E.E. Virus, Kelser Strain: Frequency and Standard Deviations of Intracerebral Titers in Mice

Titer (X)*	Frequency (f) of titer (X) in sample			$f(X - \bar{X})^2$		
	A	B	Total	A	B	Total
7.0	1	1	2	0.25	0.16	0.50
7.1						
7.2	2	2	4	0.18	0.08	0.36
7.3	1		1	0.04		0.04
7.4						
7.5		3	3		0.03	0.00
7.6	2		2	0.02		0.02
7.7	1	1	2	0.04	0.09	0.08
7.8						
7.9		1	1		0.25	0.16
8.0	1	1	2	0.25	0.36	0.50
8.1	1		1	0.36		0.36
Total No. tests (N)	9	9	18	—	—	—
Mean titer ($\bar{X}$)	7.5	7.4	7.5	—	—	—
$\Sigma (X - \bar{X})^2$	—	—	—	1.14	0.97	2.02
S.D. (σ) $= \sqrt{\frac{\Sigma(X - \bar{X})^2}{N - 1}}$	—	—	—	0.377	0.348	0.345

* Titers are expressed as log LD_{50}/0.03 gm. of brain tissue.

Technique of the Experiments on Virus Multiplication.—Mice inoculated intracerebrally with various amounts of virus were sacrificed at appropriate intervals. Their brains were harvested and stored in the dry ice chest either whole or as homogenized suspensions in saline containing 10 per cent inactivated normal rabbit serum. In most experiments, pools of 2 or 3 brains were prepared for each interval. At the time of test, as a rule, 3.2-fold serial dilutions of the uncentrifuged brain homogenates were made over the suitable range, and at least 3 or 4 mice were inoculated intracerebrally with each dilution. For convenience, the 3.2-fold steps were considered as equal to 0.5 log-fold, and titers were calculated on that basis according to the method of Reed and Muench (3).

Vaccines.—Mice inoculated intracerebrally with about 100 LD_{50} of R.I. stock virus were

chloroformed after 40 hours when they began to show signs of encephalitis. Under deep anesthesia, the thoracic cavity was opened, the heart cut through, and the blood was drained onto absorbent cotton covered by gauze. The brains were removed, and excessive blood was washed off with saline. 4 ml. of cold saline was added per brain to make a 10 per cent suspension which was homogenized for 1 minute in a chilled Waring blendor. It was then filtered through several layers of gauze, and finally formalin was added to a final concentration of 0.3 per cent. The vaccine was shaken daily and kept in a refrigerator. It was used when proved non-infectious by intracerebral test in mice.

As a rule, immunization consisted of 6 intraperitoneal doses given in 2 courses of 3 daily injections 1 week apart. The stock vaccine was diluted in saline and the inoculum was 0.3 ml. The total amount of vaccine given is stated in the text in terms of equivalent amounts of undiluted vaccine. Challenge inoculations were given 2 weeks after the first dose of vaccine.

Mice.—Albino Swiss mice, weighing 7 to 10 gm., were obtained from 2 dealers. There was no difference between them in response to any of the procedures used.

EXPERIMENTAL

Comparative Rates of Multiplication of the R.I. and Kelser Strains of W.E.E. Virus

(*a*) *The Initial Yield of Virus in Relation to the Amount Inoculated.*—A finding common to all the present experiments was an initial decrease in the amount of virus recoverable from infected brains. Inoculation of a volume of 0.03 ml. into a brain weighing 0.4 gm. would result in 13.3-fold dilution. Hence, after inoculation of $10^n LD_{50}$, the expected yield prior to viral multiplication would be $10^{(n-1.12)}$ LD_{50} per 0.03 gm. of brain tissue. Actually, the mean yield at 1 hour after inoculation was only 3.5 per cent of the expected recovery in the case of the R.I. strain and 10.2 per cent in that of the Kelser strain. The disappearance from the brain of 90 to 96 per cent of the inoculum was independent of the amount of virus given, as shown in Tables III and IV. The significance of this loss is not clearly understood,[1] and for the purpose of studying the viral growth rate the extent of the loss is important mainly because it establishes the true starting point of multiplication.

(*b*) *Correlation between Rate of Virus Increase and Course of Disease in Mice.*[2]—Of 45 mice inoculated intracerebrally with $10^{2.6}$ LD_{50} of the R.I. strain, 30 were sacrificed in pairs at 2 to 4 hour intervals. Definite signs of disease, *i.e.* spontaneous convulsive seizures or continuous circling, were first seen 38 hours after inoculation. All remaining mice died after 42 to 48 hours. 64 mice received intracerebrally $10^{3.4}$ LD_{50} of the Kelser strain, and of these 48 were sacrificed in pairs at 4 hour intervals. Here, definite encephalitic signs began 74 hours after inoculation and the survival time of the remaining mice varied from 3 to 7 days with an average of 3.7 days.

[1] Losses of similar magnitude were encountered when *E. coli* phages T1 or T7 were inoculated intracerebrally in mice.

[2] The samples of the 2 strains of virus used in this test have not been included in Tables I and II.

The rates of multiplication of the 2 strains are presented in Fig. 1. The titer of the R. I. strain rose logarithmically up to 32 hours after inoculation. By that time, its increase was 1,000 times greater than that of the Kelser strain. The R.I. strain reached its maximum titer about 6 hours before onset of definite encephalitic signs. The growth rate of the Kelser strain appeared to slow

TABLE III

W.E.E. Virus, R.I. Strain: Difference between Expected and Actual Yield of Virus from Mouse Brains 1 Hour after Intracerebral Inoculation

Test No.	Inoculum (a)*	Yield at 1 hr.		Difference $(b) - (c)$*	Deviation from mean difference (d)	$(d)^2$
		Expected $= (a) - 1.12$ (b)*	Actual (c)*			
1	3.2	2.08	1.12	0.96	−0.49	0.240
2	3.7	2.58	0.70	1.88	+0.43	0.185
3	3.7	2.58	0.95	1.63	+0.18	0.032
4	4.2	3.08	1.5	1.58	+0.13	0.017
5	4.2	3.08	1.0	2.08	+0.63	0.397
6	4.2	3.08	1.5	1.58	+0.13	0.017
7	4.2	3.08	1.7	1.38	−0.07	0.049
8	5.2	4.08	3.0	1.08	−0.37	0.137
9	5.2	4.08	2.73	1.35	−0.10	0.010
10	7.2	6.08	4.75	1.33	−0.12	0.014
11	7.2	6.08	4.88	1.20	−0.25	0.063
12	8.2	7.08	5.95	1.13	−0.32	0.102
13	8.2	7.08	5.37	1.71	+0.26	0.068
Total (Σ)	68.6	54.04	35.15	18.89	3.48	1.331
Mean $\frac{\Sigma}{N}$	5.277	4.157	2.704	1.453	0.268	—

Standard deviation $(\sigma) = \sqrt{\frac{\Sigma(d^2)}{N-1}} = \sqrt{\frac{1.331}{12}} = 0.333$

* All figures given as log LD_{50}.

down beginning about 36 hours after inoculation and came to a standstill after about 62 hours or 12 hours before onset of definite encephalitic signs.

Thus, the difference between the 2 strains with regard to incubation and survival periods reflected a difference of similar magnitude between their rates of multiplication.

(*c*) *Latent Phase Preceding Multiplication of the R.I. and the Kelser Strains.*— As is shown in Fig. 2, no viral growth was demonstrated for about 3 hours in the brains of mice inoculated with $10^{3.7}$ LD_{50} of the R.I. strain. In the case of the Kelser strain, after inoculation of $10^{4.5}$ LD_{50}, there was no significant multiplication for 5 hours (Fig. 3). After these latent periods, there was

growth at a fairly constant rate of the R.I. strain, while that of the Kelser strain appeared to come to a standstill between 7 and 10 hours after inoclation. Between 3 and 10 hours after inoculation, there was a 1,000-fold increase in titer of the R.I. strain, and only a 10-fold increase in that of the Kelser strain.

(*d*) *Influence of Variations in the Size of the Inoculum on the Rate of Viral Multiplication.*—After inoculation of various amounts of W.E.E. virus in excess of 100 LD_{50} there was relatively little variation in the survival time

TABLE IV

W.E.E. Virus, Kelser Strain: Difference between Expected and Actual Yield of Virus from Mouse Brains 1 Hour after Intracerebral Inoculation

Test No.	Inoculum (*a*)*	Yield at 1 hr.		Difference (*b*) − (*c*)*	Deviation from mean difference (*d*)	$(d)^2$
		Expected = (*a*) − 1.12 (*b*)*	Actual (*c*)*			
1	2.5	1.38	<0.70	>0.68	>0.31	0.096
2	2.5	1.38	<0.50	>0.88	>0.11	0.012
3	4.0	2.88	2.50	0.38	−0.61	0.372
4	4.0	2.88	2.50	0.38	−0.61	0.372
5	4.0	2.88	1.25	1.63	+0.64	0.409
6	4.5	3.38	2.62	0.76	−0.23	0.053
7	4.5	3.38	2.38	1.00	+0.01	0.0001
8	4.5	3.38	2.50	0.88	−0.11	0.012
9	4.5	3.38	1.66	1.72	+0.73	0.533
10	4.5	3.38	1.91	1.47	+0.48	0.230
11	6.5	5.38	4.12	1.26	+0.27	0.073
12	6.5	5.38	4.50	0.88	−0.11	0.012
Total (Σ).....	52.5	39.06	27.14	11.92	4.22	2.173
Mean $\frac{\Sigma}{N}$.....	4.375	3.255	2.261	0.993	0.352	—

Standard deviation (σ) = $\sqrt{\frac{\Sigma(d^2)}{N-1}} = \sqrt{\frac{2.173}{11}} = 0.444$

* All figures given as log LD_{50}.

of mice (1). This similarity in the course of the disease is matched by the observation that the growth curves after inoculation of different amounts tended to converge so that the maximum titer was reached at about the same time regardless of the size of the inoculum. This is illustrated for the 2 strains in Figs. 4 and 5. It will also be seen that after inoculation of relatively small amounts of the R.I. strain (see Fig. 4), there was a tendency for the growth rates to parallel each other.

On the basis of these findings regarding the growth rates of the 2 strains,

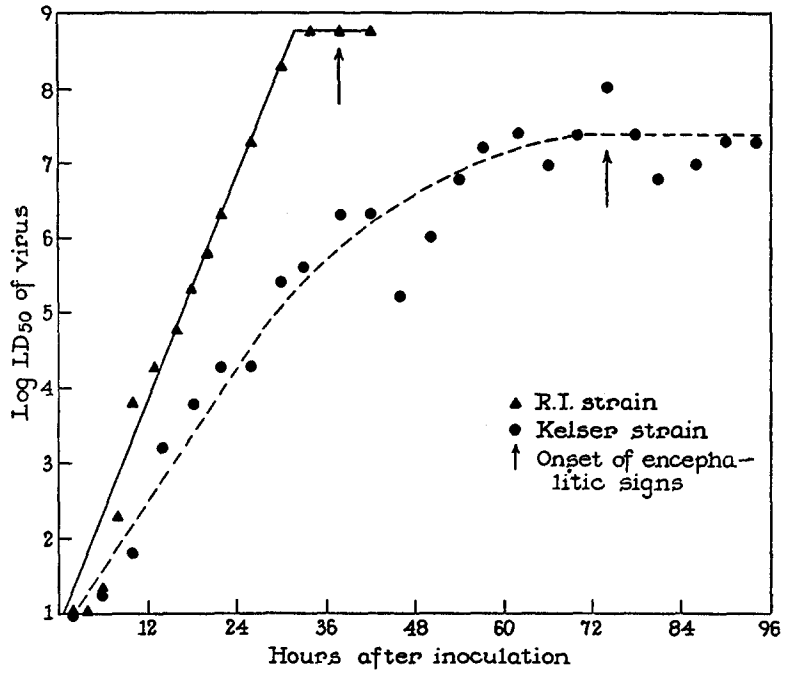

FIG. 1. Comparative rates of multiplication of R.I. and Kelser strains of W.E.E. virus in brains of normal mice.

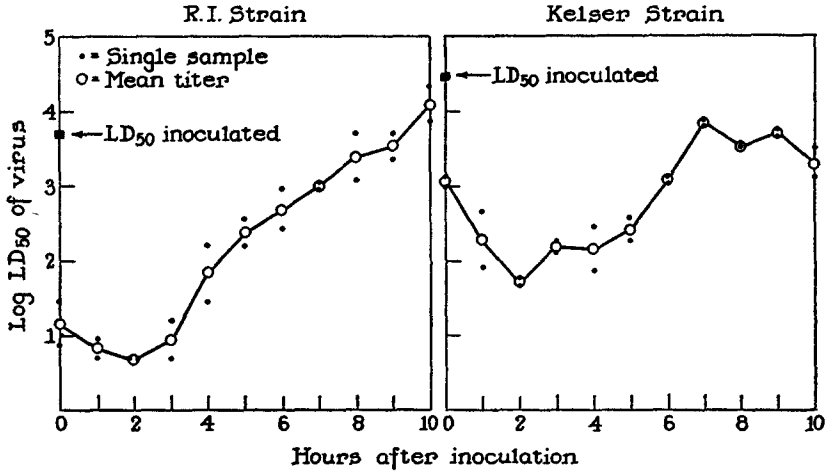

FIG. 2 FIG. 3

FIGS. 2 and 3. Rate of multiplication of W.E.E. virus in brains of mice after intracerebral inoculation.

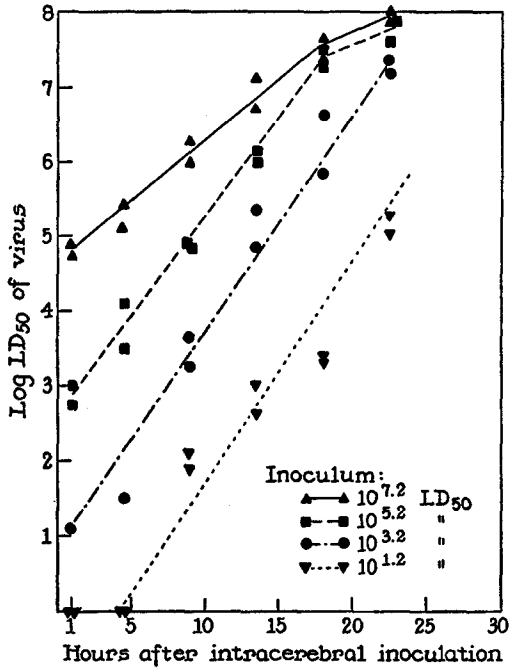

FIG. 4. Rates of multiplication of W.E.E. virus, R.I. strain, after intracerebral inoculation of different amounts.

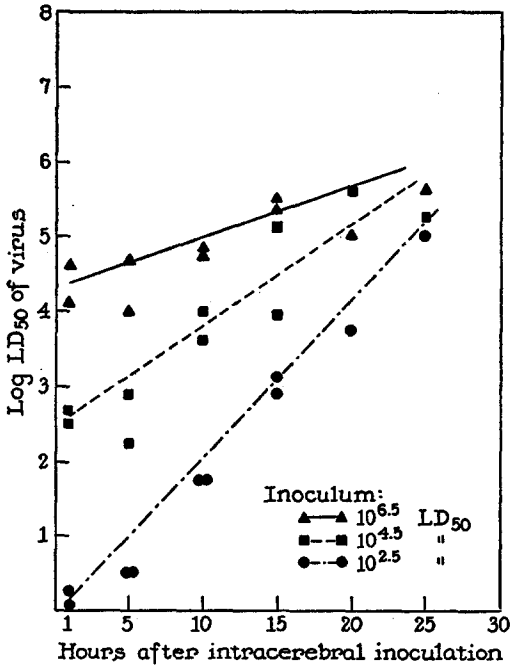

FIG. 5. Rates of multiplication of W.E.E. virus, Kelser strain, passage 3, after intracerebral inoculation of different amounts.

the difference in response to them of immunized mice was reexamined. The primary purpose of the experiments to be described in the following sections was to define this difference quantitatively and to establish a baseline for an investigation into its immediate cause.

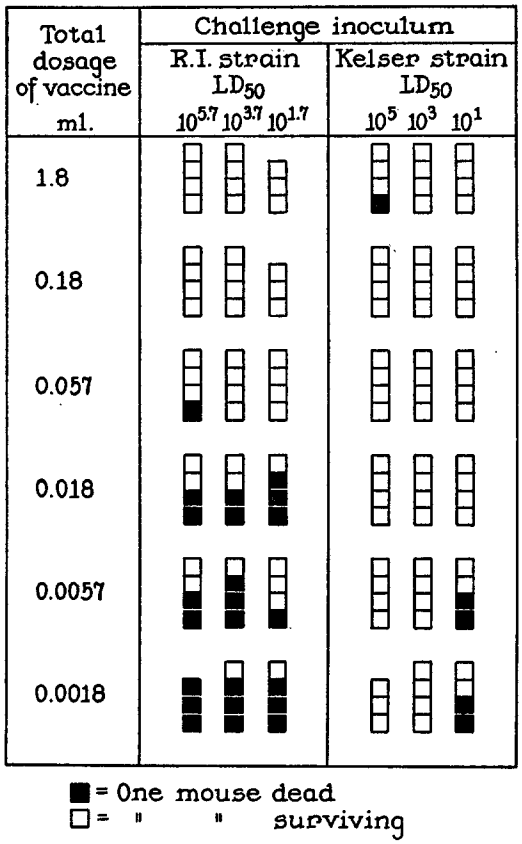

FIG. 6. Comparative degrees of resistance of immunized mice to intracerebral challenge doses of two strains of W.E.E. virus.

The Response of Immunized Mice to Intracerebral Challenge Doses of the R.I. and Kelser Strains

Fig. 6 presents the results of an experiment in which mice immunized with different amounts of vaccine were challenged by the intracerebral route with comparable graded amounts of the R.I. or the Kelser strain of W.E.E. virus. It is clear that after immunization with relatively small doses of vaccine (less than 0.057 ml.) there was a much greater degree of resistance to the Kelser

than to the R.I. strain. This difference in response was masked when a larger dosage of vaccine was used.[3]

It is noteworthy that four of eight mice immunized with the smallest amounts of vaccine (0.0057 and 0.0018 ml.) succumbed to a challenge dose of 10 LD_{50} of the Kelser strain while all the mice challenged with 10^3 or 10^5 LD_{50} survived. This "paradoxical" type of response was found to be characteristic of mice with low degree of immunity and will be discussed in a subsequent paper (2).

TABLE V

Comparison of Typical Titrations in Mice of Three Representative Samples of W.E.E. Virus

Sample of virus	Dilution of virus	Day of death		Total mortality	Log LD_{50}
		Individual mice	Average		
R.I. strain	10^{-7}	2, 2, 2, 2	2.0	4/4	9.2
	10^{-8}	3, 3, 4, 5	3.75	4/4	
	10^{-9}	3, 3, 0, 0	3.0	2/4	
	10^{-10}	4, 0, 0, 0	(4)	1/4	
Kelser strain passage 3	10^{-1}	6, 7, 7, 7, 8	7.0	5/5	>7.4
	10^{-2}	6, 7, 7, 7, 9	7.2	5/5	
	10^{-3}	6, 6, 6, 7, 9	6.8	5/5	
	10^{-4}	5, 7, 8, 8, 9	7.4	5/5	
	10^{-5}	6, 6, 7, 8, 9	7.2	5/5	
	10^{-6}	5, 6, 6, 7, 8	6.4	5/5	
	10^{-7}	5, 6, 8, 8, 0	6.75	4/5	
Kelser strain passage 40	10^{-6}	3, 3, 3, 3, 7	3.8	5/5	7.6
	10^{-7}	3, 3, 3, 4, 6	3.8	5/5	
	10^{-8}	4, 0, 0, 0, 0	(4)	1/5	
	10^{-9}	0, 0, 0, 0, 0	—	0/5	

Effect of Continued Brain-to-Brain Passages on the Behavior of the Kelser Strain

In an effort to show that the distinct properties of the R.I. strain had resulted from its continued propagation in the mouse brain, the slower Kelser strain was subjected to a rapid succession of brain-to-brain passages in mice.

After the virus had undergone a total of 40 passages in this laboratory,

[3] In earlier experiments (1), the difference between strains was demonstrable even in mice immunized with relatively large doses of vaccine. At that time, vaccines had been prepared from infected chick embryo and had been centrifuged. They were less potent than the ones used more recently. Studies on St. Louis and Japanese B encephalitis vaccines have shown that centrifugation may result in considerable loss of antigenic potency (6). The present studies, carried out with crude vaccines prepared from infected mouse brain, confirm the report of Ruchman (7) to the effect that there is no difficulty in effectively immunizing mice against the R.I. strain.

TABLE VI

Comparative Neutralization Test with Mouse Immune Serum against the R.I. Strain and Two Variants of the Kelser Strain of W.E.E. Virus

Log final dilution of serum	R.I. strain				Kelser passage 3				Kelser passage 40			
	LD_{50} in mixture			Neutralization index*	LD_{50} in mixture			Neutralization index	LD_{50} in mixture			Neutralization index
	$10^{3.3}$	$10^{2.3}$	$10^{1.3}$		10^{3}	10^{2}	10^{1}		$10^{3.3}$	$10^{2.3}$	$10^{1.3}$	
0.8	1/4‡	0/4	0/4	>3.53	1/4	0/4	0/4	>3.33	2/4	0/4	0/4	3.2
1.8	4/4	4/4	0/4	1.7	3/4	1/4	1/4	2.33	4/4	0/4	1/4	2.58
2.3	4/4	3/4	0/4	1.87	4/4	4/4	1/4	1.33	4/4	3/4	0/4	1.87
2.8	4/4	3/4	2/4	1.4	3/3	3/3	1/4	1.33	4/4	4/4	2/4	1.2
3.3	4/4	3/4	2/4	1.4	4/4	3/4	2/4	1.2	4/4	4/4	0/4	1.7
3.8	4/4	4/4	2/4	1.2	4/4	4/4	3/4	<0.7	4/4	4/4	4/4	<0.7
Neutralizing titer§	1.13	1.74	3.3	—	1.3	2.04	3.1	—	0.8	1.83	3.36	—

* Neutralization index, log LD_{50} of virus neutralized by indicated dilution of serum.

‡ Numerator, number of mice dead. Denominator, number inoculated.

§ Neutralizing titer, log of estimated highest dilution of serum which would protect 50 per cent of the mice in mixture with indicated amount of virus.

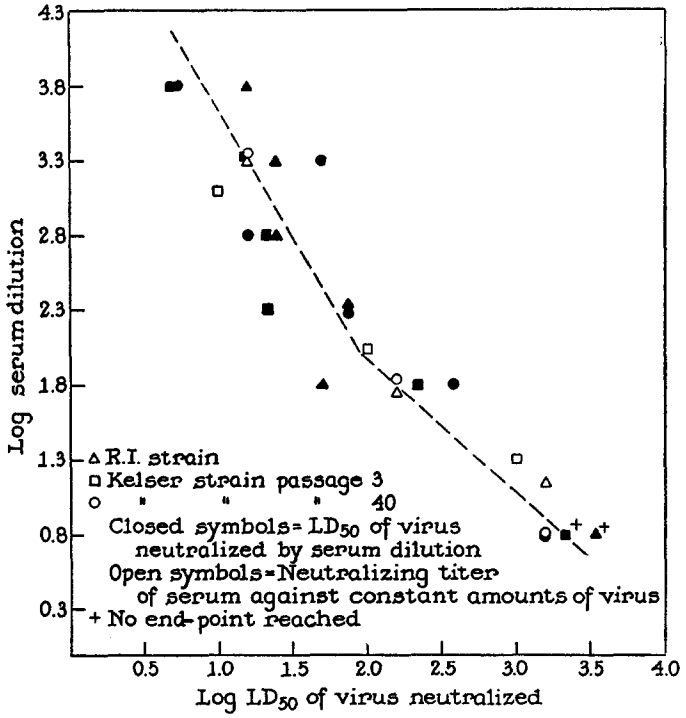

FIG. 7. Neutralization of W.E.E. virus by hyperimmune mouse serum.

convulsive seizures were regularly seen in all mice 2 days after inoculation of 10^{-2} diluted brain tissue. In Table V, typical titrations of the R.I. strain and of 3rd and 40th passage Kelser virus are presented. Although the titer of the 40th passage virus was still relatively low, the average survival period of mice infected with it had so decreased as to approach that of mice infected with the R.I. strain.

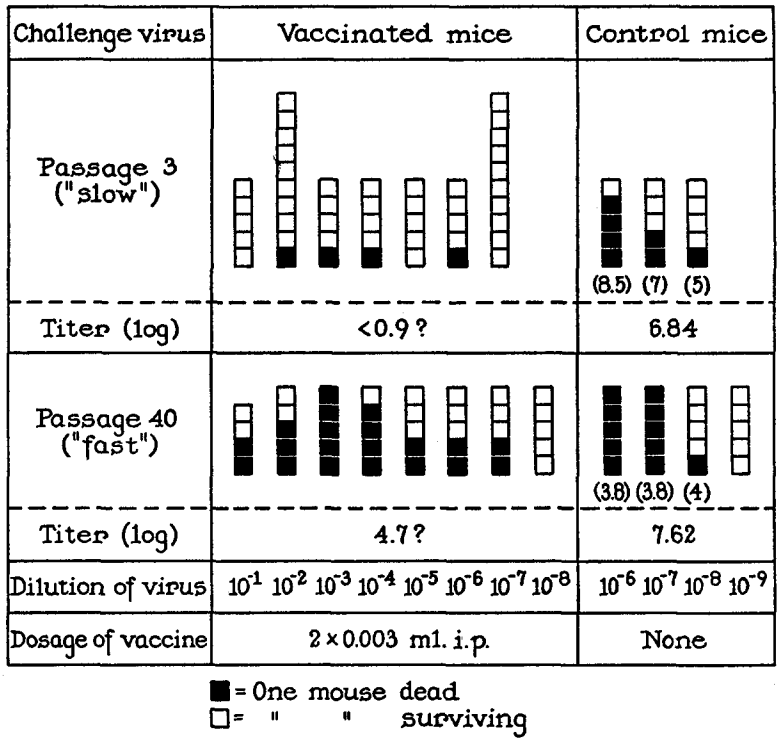

FIG. 8. Comparative degrees of resistance of immunized mice to intracerebral challenge doses of "slow" and "fast" variants of the Kelser strain.
Numbers in parentheses indicate the average survival periods in days.

The same three virus preparations were employed in a neutralization test for the purpose of establishing their serological identity.

Serial 0.5 log-fold dilutions of a W.E.E. R.I. immune mouse serum were mixed with approximately 10, 100, and 1,000 LD_{50} of each of the 3 virus samples. After 2 hours' incubation in the 37°C. water bath, the mixtures were injected intracerebrally into mice. The results of this test are given in Table VI. The data are summarized in Fig. 7 which shows that the 3 samples were neutralized equally well by the serum. Thus, no measurable serological changes

resulted from continued propagation of either strain of W.E.E. virus in the mouse brain.

It remained to be seen whether the "fast" derivative of the Kelser strain differed from its parent strain when used as challenge inoculum in immunized mice. After immunization with 0.006 ml. of vaccine, mice were challenged intracerebrally with graded doses of passage 3 or passage 40 samples of the Kelser strain. The outcome of this test is presented in Fig. 8. After 37 additional passages, the Kelser strain behaved like the R.I. strain in that mice vaccinated to an extent such that they resisted almost without exception even maximal amounts of the "slow" strain were only slightly protected against its "fast" derivative. In the latter group, deaths were scattered over the entire range of virus dilutions. This illustrates the inability to express intermediate degrees of immunity in mathematical terms. With the small number of animals employed, it appeared that the chance of survival was hardly greater after challenge with 4 than with 4 million LD_{50}.

DISCUSSION

The data presented in this paper have confirmed the fact that continued propagation of W.E.E. virus in the mouse brain may yield a viral variant with increased rapidity of action. The greater speed with which such adapted virus killed mice was paralleled by a corresponding increase in its rate of multiplication in the brain. This was associated with a shortened initial latent period during which there was no measurable increase in viral titer.

In mouse brains infected with W.E.E. virus, the titer of recoverable virus was 90 to 96.5 per cent lower than the theoretical yield, and it was at this reduced level that the virus maintained itself for about 3 hours in the case of the fast R.I. strain and for about 5 hours in that of the slow Kelser strain. The initial drop in detectable virus may have been due simply to leakage into other tissues, or it may have been caused by adsorption of a proportion of the inoculum onto host cells with resulting loss of infectivity. The observation that losses of similar magnitude followed the intracerebral inoculation of bacteriophage does not necessarily rule out the latter possibility. With the nature of this initial loss unexplained, it remains doubtful whether the latent period represented a temporary equilibrium between rate of disappearance from the brain and rate of virus increase, or whether it was due to a latent phase in the intracellular growth cycle comparable to the "constant periods" described for bacteriophage (8) and influenza viruses (9). A more conclusive interpretation of the growth experiments is difficult because of the inability to separate unadsorbed or newly liberated virus from infected host cells.

It is interesting, however, that after inoculation of various large amounts of either strain of W.E.E. virus, the rates of multiplication tended to converge, while after inoculation of the R.I. strain in amounts closer to the mini-

mal lethal dose they tended to parallel each other. This latter finding suggests that of the small amount of virus all was utilized in infecting host cells. Convergence in the higher range may indicate that an increasingly high proportion of the seed virus was in excess of the amount immediately utilized to initiate infection. It will be shown in the following paper (2) that this postulated excess may have an important function as free antigen in the mechanism of immunity in vaccinated animals.

The growth experiments here reported, while of some obvious interest in relation to the broader problems of virus-host relationship and viral adaptation, have their chief significance in connection with the difference in response of immunized animals to intracerebral challenge doses of the "fast" and the "slow" variants of W.E.E. virus. This difference was so striking that one may suspect serological heterogeneity. That minor serological changes may occur upon continued propagation of viruses in certain hosts has been suggested by studies on influenza virus (10). Careful investigation, however, has failed previously (1) and again in the present work to reveal detectable serological changes resulting from adaptation of W.E.E. virus to the mouse brain. The mechanism which enables vaccinated animals to survive infection with a "slow" strain but not with a "fast" derivative from it will be described in the following paper (2).

The different response of vaccinated animals to 2 serologically indistinguishable variants of the same virus may have some practical significance. It is conceivable that active immunity tests with variants of other viruses having similar differences in growth rates may have created the impression that they represented strains of different immunological types. This possibility should be considered especially in the case of poliomyelitis virus where immunological differentiation of strains has often been based on active immunity rather than on neutralization tests.

Similarly, certain standard potency tests to which vaccines prepared for medical and veterinary use are subjected involve intracerebral challenge inoculations in vaccinated mice. Habel and Wright (11) have recently recognized that various strains of rabies virus when used for challenge in such tests may cause wide variations in the results obtained, and on this basis they have recommended the use of aliquots of a single sample of standard challenge virus in all laboratories.

The results and conclusions presented in this and in the following paper (2) for W.E.E. virus may apply equally to similar variations encountered with other viruses.

SUMMARY

Continued serial brain-to-brain passage of strains of W.E.E. virus in mice has yielded variants which kill mice with increased rapidity. Their rate of

multiplication in the mouse brain has been found to be correspondingly increased.

At 1 hour after intracerebral inoculation of various amounts of W.E.E. virus, only 3.5 to 10 per cent of the expected amount of virus was recovered from the infected brains.

In infected mouse brains, the period of active viral multiplication was preceded by a latent phase which lasted a considerably shorter time in the case of a "fast" than in that of a "slow" variant.

In brains inoculated with various amounts in excess of minimal lethal doses the rates of multiplication tended to converge with the result that the maximum titer was reached after about the same period of time. After inoculation of smaller amounts, the rates of viral multiplication tended to parallel each other.

Vaccinated mice may be fully resistant to maximal intracerebral doses of a slowly multiplying strain while they are not at all or only partly protected against a rapidly multiplying one derived from it. This difference is demonstrable even though fast and slow variants are, as far as can be tested, serologically identical. The difference in response may be masked if animals are immunized with relatively large doses of vaccine.

The bearing of these findings on certain practical problems has been pointed out.

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