INDUCED RESISTANCE OF THE CENTRAL NERVOUS SYSTEM TO EXPERIMENTAL INFECTION WITH EQUINE ENCEPHALOMYELITIS VIRUS

III. Abortive Infection with Western Virus and Subsequent Interference with the Action of Heterologous Viruses

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The immunity resulting from vaccination of animals with formalin-inactivated Eastern (E.E.E.) or Western (W.E.E.) equine encephalomyelitis virus was found to be specifically directed against the strain used for vaccination (1, 2). The degree of immunity of vaccinated mice to active virus given by intraperitoneal (1) or by intracerebral (3) routes was shown to be correlated with the titer of neutralizing antibody in the serum. In addition, studies on vaccinated rabbits have revealed a constant ratio, due to physiological factors, between the titers of neutralizing antibody in serum, perfused brain tissue, and cerebrospinal fluid, the ratio being of the order of 300:3:1 (2). The relatively low concentration of antibody in the central nervous system may clear the apparent discrepancy reported by earlier observers, namely, that animals can have considerable amounts of antibody in their sera and yet have no resistance to intracerebral challenge doses (4).

In contrast to the specific immunity of vaccinated animals, mention has been made (2, 5) of a different type of resistance acquired by animals recovered from experimental encephalitis of either the Eastern or Western type. Such animals survived after intracerebral inoculation of either of the equine viruses, their resistance being independent of serological specificity or of the presence of neutralizing antibody.

Since laboratory animals rarely recover once they have developed manifest signs of encephalitis, a sufficient number was not available to study this phenomenon systematically. It was observed, however, that vaccinated rabbits responded to an intracerebral challenge dose of the homologous virus with fever, the height and duration of which were inversely proportional to the titer of neutralizing antibody and directly proportional to the amount of virus injected (2). Indeed, in borderline cases with relatively low antibody levels, the virus did occasionally produce neurological signs along with fever, and the immunity of such animals manifested itself only by their subsequent recovery. Thus it appeared that by adequate vaccination and by proper dosage of test

virus one could produce mild or abortive infections. It was found that animals prepared in this manner with Western virus could survive the intracerebral reinoculation, within a limited interval, of Eastern virus (5). Experiments will be presented to illustrate this type of resistance. The remainder of this paper will be devoted to a detailed study of the abortive type of infection of immune animals, which has revealed the operation of certain interesting mechanisms.

Materials and Methods

Most of the materials and methods used have already been described (1, 2, 6); certain other details of technique will be discussed in the text.

The *virus of vesicular stomatitis* (New Jersey strain) has been maintained in this laboratory for the past several years by occasional passage of lyophilized samples through mouse brains.[1] Its properties have already been investigated and reported (7).

Virus Titrations.—As a rule, intracerebral inocula consisted of infected brain suspensions derived from the homologous species; *e.g.*, guinea pig brain for inoculation into guinea pigs. 50 per cent death endpoints (LD_{50}) were calculated from titrations in mice. For titrations of virus in guinea pigs, at least two animals were employed per dilution, and one minimal lethal dose (M.L.D.) was defined as the highest serial dilution which killed one or more guinea pigs injected. Formalin-inactivated viruses were kept at 4°C. All other materials (virus or normal brain suspensions and sera) were stored in the dry-ice box at −70°C.

Neutralization tests were carried out by the intracerebral route in young adult Rockefeller Institute mice or Swiss mice bought from several dealers. Mixtures of serial virus dilutions and serial dilutions of materials to be tested for neutralizing antibody were incubated for 2 hours at 37°C. before inoculation.

Resistance of Guinea Pigs to Heterologous Virus Following Abortive Infection with W.E.E. Virus

Eleven 200 gm. albino guinea pigs received subcutaneously three 1 cc. doses of formalin-inactivated (0.5 per cent formalin) W.E.E. virus. A pool of their sera, obtained 1 week after the last dose of vaccine, in 1:300 dilution, neutralized 10 LD_{50} of W.E.E. virus. The same pooled serum fixed complement in the presence of Western antigen in 1:8 dilution, but failed to do so in the presence of Eastern or St. Louis encephalitis antigen.[2] Eight days after these sera were collected, five of the vaccinated guinea pigs received an intracerebral inoculation into the right hemisphere of 1,000 M.L.D. of Eastern virus, and the remaining six a similar dose of Western virus, in the form of infected guinea pig brain suspensions. Non-vaccinated animals of the same stock served as controls. Chart 1 illustrates the specificity of the immunity: No resistance was found to Eastern virus, while all vaccinated guinea pigs tested with Western virus survived. A fever spike on the first day following inoculation was observed in all but one of the latter.

Two weeks after the Western virus challenge inoculation the guinea pigs were bled from the heart and reinoculated intracerebrally on the left side with 10 or 1,000 M.L.D. of Eastern virus. Neutralization and complement-fixation tests with the second sera failed to reveal the presence of antibody reacting with Eastern equine virus. Nevertheless, as will be seen in Chart 2, the guinea pigs survived the inoculation of Eastern virus.

[1] All operations on animals were performed with the aid of full ether anesthesia.

[2] Complement-fixation tests were kindly carried out by Dr. J. Casals.

The outcome of this experiment was representative of a number of similar tests. Guinea pigs, which were prepared in the manner described with Western virus, were, with only few exceptions, refractory to Eastern virus within a

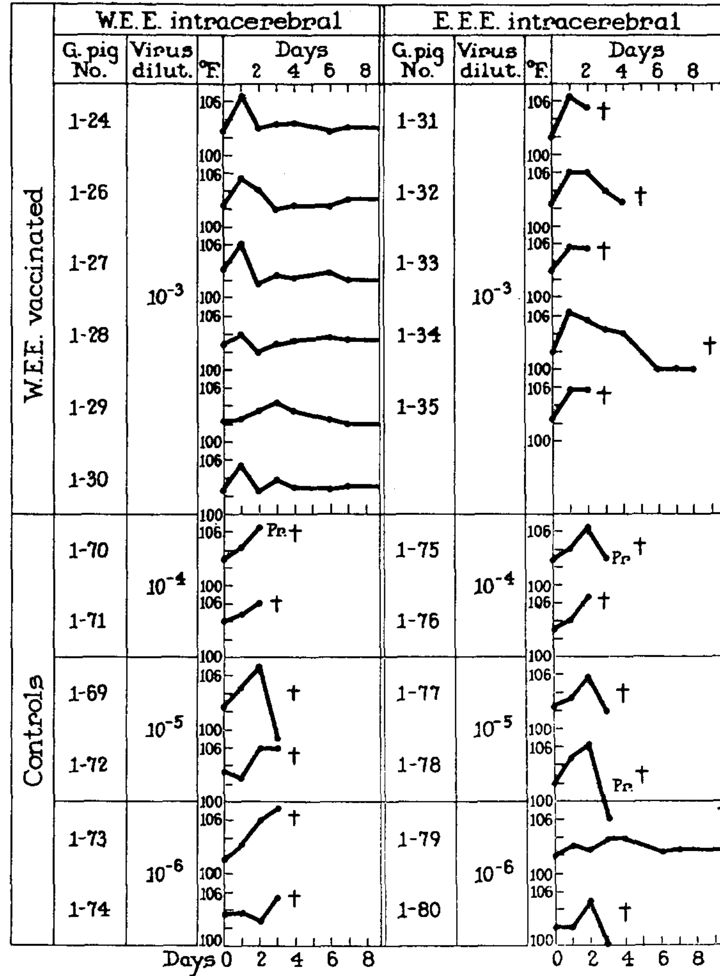

CHART 1. Specificity of immunity in guinea pigs vaccinated with formolized W.E.E. virus.

2 weeks' interval; after 3 weeks' interval they succumbed to Eastern virus, although after prolonged incubation periods. Moreover, vaccinated guinea pigs tested intracerebrally with homologous equine encephalomyelitis virus were reinoculated intracerebrally with 10 or 100 M.L.D. of the New Jersey strain of vesicular stomatitis virus. When the interval between the intra-

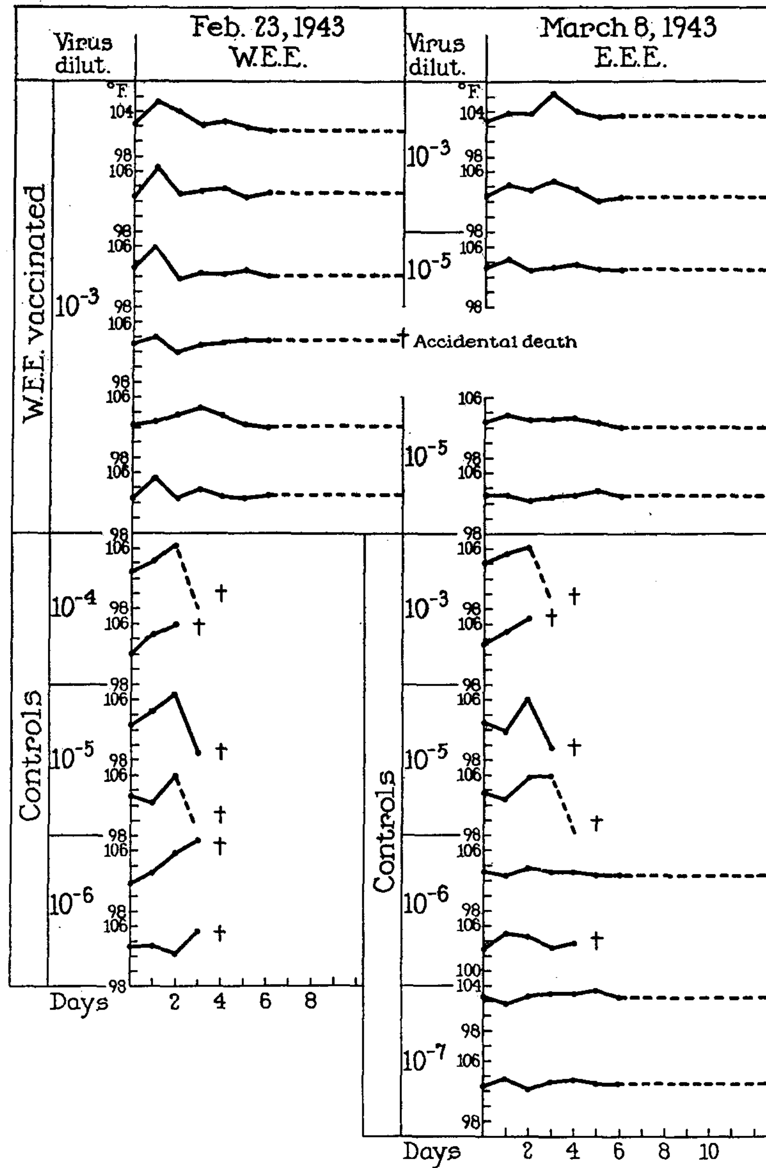

CHART 2. Temperature curves of guinea pigs vaccinated and tested with W.E.E. virus and subsequently reinoculated intracerebrally with E.E.E. virus.

cerebral inoculations was 4 days, guinea pigs survived; when 2 weeks, the average survival period associated with the vesicular stomatitis infection was prolonged from 6.5 days in the control animals to 13 days in the E.E.E.-conva-

lescent ones; when 3 weeks, full susceptibility to vesicular stomatitis virus was restored. On the other hand, guinea pigs injected intracerebrally with suspensions of normal guinea pig brain tissue or with formalin-inactivated W.E.E. virus failed to resist 11 to 15 days later even 10 M.L.D. of active W.E.E. virus given by the intracerebral route.

Several control tests were carried out in mice in which the intracerebral injection of (*a*) subinfective doses of active W.E.E. virus, or (*b*) W.E.E. virus inactivated by ultraviolet irradiation, or (*c*) W.E.E. virus inactivated by 0.2 or 0.5 per cent formalin, or (*d*) 10 per cent normal mouse brain in 0.2 per cent formalin, was followed at intervals ranging from 1 to 6 days by intracerebral inoculation of 10 to 32 LD_{50} of E.E.E. virus. No evidence of resistance was found. On the other hand, a certain number of mice vaccinated with inactivated virus and challenged by the intracerebral route with active W.E.E. virus were, after 4 or 10 days, resistant to as many as 100,000 LD_{50} of E.E.E. virus. Thus, observations in mice confirmed results obtained in tests on rabbits and guinea pigs.

As has been noted from Charts 1 and 2, vaccination against W.E.E. alone gave rise to strain-specific immunity, but it was the intracerebral challenge dose of W.E.E. virus which conditioned the animals so that their brains were not fully susceptible to the heterologous virus for a limited period of time. This suggested alterations in the susceptibility of the cells of the central nervous system brought about by contact with the first-inoculated virus. Three lines of investigation were followed in attempts to determine the extent of detectable changes associated with the abortive infection which was believed to result from this contact with the virus: (1) detailed study of the febrile reactions; (2) histopathology; (3) studies on the fate of virus and possible variations in the virus-neutralizing capacity of brain tissue.

Characteristics of Abortive Western Virus Infections in Immune Guinea Pigs

1. Febrile Reaction.—The two upper graphs in Chart 2 illustrate the average febrile course which followed intracerebral injection of 1,000 M.L.D. of W.E.E. virus into vaccinated and into non-vaccinated guinea pigs. For 20 to 30 hours following the inoculation, the behavior of vaccinated animals patterned itself after that of normal ones; thereafter the temperature returned to normal, while that of non-vaccinated guinea pigs remained elevated until encephalitic signs or death ensued about the 3rd or 4th day. No further manifestations have been observed in adequately immunized animals. If decreasing amounts of virus were injected, the maximum temperature during the first day was progressively lower in both vaccinated and non-vaccinated guinea pigs; following injection of 1 to 10 M.L.D., no significant rise of temperature was observed in vaccinated animals, while in non-vaccinated controls the temperature did not begin to rise until the 2nd or 3rd day.

Was the characteristic febrile response just described due to specific viral action on the tissue? The lower graph in Chart 3 gives the temperature curves

of a test in which the effects of intracerebral inoculations of infectious and of non-infectious materials were compared.

A 10^{-2} suspension of brain tissue infected with W.E.E. virus, whether or not clarified by centrifugation at 10,000 R.P.M. for 25 minutes, produced the characteristic fever. Both formalin-inactivated W.E.E. virus and normal guinea pig brain tissue, clarified in the same

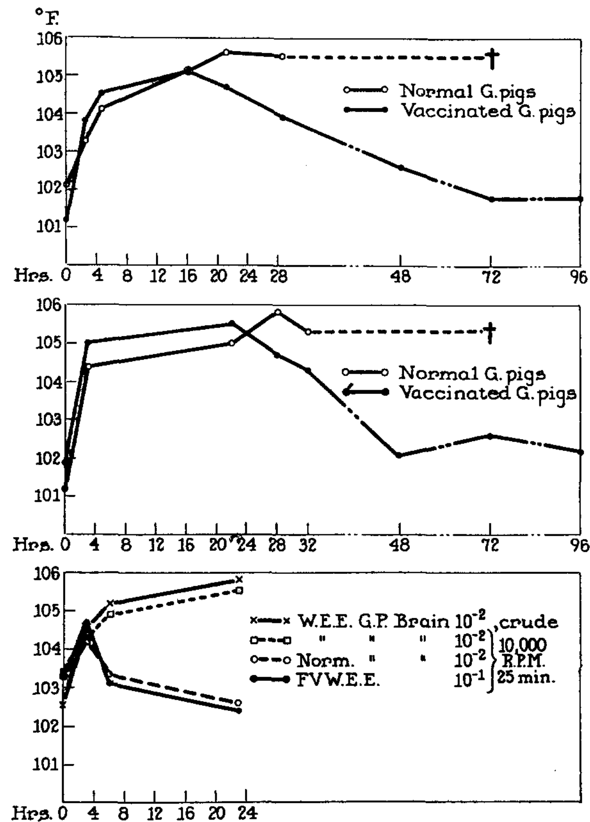

CHART 3. Average temperatures of normal and vaccinated guinea pigs after intracerebral inoculation of virus and control materials.

manner, gave rise within 3 hours to a febrile reaction which had subsided at 6 hours after inoculation. No further rise in temperature occurred. Untreated guinea pigs given ether anesthesia failed to develop fever.

Thus, it was seen that while the injection of non-infectious tissues evoked a brief febrile reaction, it was considerably shorter than that following injection of virus in immune animals.

2. Histopathology.—

The brains of thirty guinea pigs were studied histologically. Among them were those of non-vaccinated animals injected intracerebrally with active or formalin-inactivated W.E.E. virus or normal guinea pig brain suspension and sacrificed 24 to 28 hours later; others were from vaccinated guinea pigs receiving intracerebrally active virus and sacrificed 24 to 28 hours or 1 or 2 weeks later. Additional controls in this group comprised brains of normal stock animals and of a vaccinated one sacrificed when its mates were given an intracerebral challenge dose. Seventeen of these brains were removed after perfusion *in situ* with physiological saline solution and Zenker's fluid containing 5 per cent acetic acid. The latter was also used as subsequent fixative. Eighty to 150 semiserial sections of each brain were stained with eosin-methylene blue, phloxin-methylene blue, or gallocyanin-eosin.

The object was to find out whether lesions in the brains of immune guinea pigs at 24 to 28 hours after an intracerebral challenge inoculation of virus, *i.e.*, at the height of the febrile reaction, were comparable with those of non-vaccinated animals. This was found to be the case. The types of lesions were identical, although the intensity was on the whole somewhat greater in non-immune animals. The following lesions, as summarized, were observed:

(1) Hemorrhage along the needle tracks and petechial hemorrhages in the vicinity of, as well as at distances from the needle track. (2) Infiltration of the meninges; this had in some instances the appearance of purulent meningitis, in others the infiltration consisted of monocytes, lymphocytes, and predominantly polymorphonuclear cells; infiltration of the brain tissue with polymorphonuclear leucocytes which varied in degree. In some instances, scattered leucocytes were confined to the area neighboring the needle track and to periventricular areas including the matrix of the hippocampus. In others there was irregular infiltration of polymorphonuclear, rarely (two cases) mononuclear leucocytes throughout the brain. (3) Infiltration of perivascular spaces with polymorphonuclear leucocytes and a slight degree of swelling and proliferation of capillary and vascular endothelium; infiltration of the capillary network of the choroid plexus with polymorphonuclear leucocytes. (4) In the ependymal lining of the ventricles and of the choroid plexus, swelling and "blanching" of the nuclei with margination of the chromatin, the nuclei containing partially acidophilic (purple with polychrome stains) round, well defined bodies. (5) In a few instances limited, small areas of lysis of tissue involving neuroglia and cellular elements.

Small foci of necrosis of pyramidal cells of the hippocampus were seen in the brains of two vaccinated and two non-vaccinated animals. One of the former may not have been sufficiently immunized, since it already showed encephalitic signs at 24 hours after inoculation; in the other one, pyramidal cell necrosis was confined to the area of the needle track.

In the brains of immune guinea pigs, sacrificed 1 or 2 weeks following intracerebral injection of virus, no lesions were found except old hemorrhages with pigment deposits. No visible alterations from the normal were found in the brains of animals which had not been injected intracerebrally.

The severity and variety of histological changes resulting within 24 to 28 hours from the inoculation of virus might suggest that they were due to specific virus action. On the other hand, brains of animals sacrificed at the same interval after inoculation of formalin-inactivated virus or of normal brain suspensions showed essentially the same types of lesions as described, although of

milder degree. Thus again the changes resulting at this early stage from the inoculation of virus differed, as did the febrile reactions, only quantitatively from those resulting from injection of non-infectious materials.

Observations similar to those recorded here were made by Alexander and Campbell (8) who found quantitative differences only between the lesions in the brains of normal and of sensitized guinea pigs following intracerebral injection of horse serum. The types of lesions seen by them 24 to 30 hours after injection were essentially similar to those just described and, as in the present series, after 1 week the changes had subsided except for residual hemorrhage, pigment deposits, and mild glial reaction. They mentioned no ependymal changes. In the present series, the appearance of the nuclei of the ependymal lining (swelling, margination of chromatin, and acidophilic intranuclear bodies) was suggestive of virus effect; it was, however, similar in brains inoculated either with virus or with non-infectious control materials. This suggests that such changes may represent a reversible, and possibly functional, reaction of the ependymal cells to the stimulus of an intracerebral inoculum. It had been found earlier that the bulk of such an inoculum is deposited or seeps into the ventricular system (2).

Widespread neuronal destruction, characteristic of the well developed disease, was absent at the 24 to 28 hour interval from the brains of vaccinated as well as non-vaccinated animals. Apparently, then, the relative "virulence" of an inoculum could at that interval be measured only by the relative degree of the non-specific types of lesions described. In this respect, as already stated, no essential difference could be seen between the brains of vaccinated and those of non-vaccinated guinea pigs. The histological findings, therefore, supported the inference that the febrile reaction of immune animals signified an abortive infection.

3. Fate of W.E.E. Virus and Variations in Neutralizing Capacity of Brain Tissue after Intracerebral Challenge Inoculation in Immune Guinea Pigs.— The fate of W.E.E. virus inoculated into the brains of both vaccinated and non-vaccinated guinea pigs was studied.

Nineteen guinea pigs were vaccinated subcutaneously with four doses of 0.5 cc. each of formalin-inactivated W.E.E. virus (10 per cent mouse brain). Six days after the last dose, they received intracerebrally 0.1 cc. of 10^{-3} W.E.E. virus-infected guinea pig brain (1,000 M.L.D.). Twelve non-vaccinated control animals were similarly injected. One or two animals of each group were sacrificed at various intervals after inoculation, as indicated in Table I. Each animal sacrificed was exsanguinated, following which the head was perfused with sterile physiological saline solution until the returning fluid was clear. The brains were then removed and frozen until tested. Of the nine remaining animals in the vaccinated group, seven survived the observation period of 2 weeks, at the end of which one (22-23) was sacrificed. One died after 7 days (incubation period in control group, 3 days), presumably of W.E.E. virus infection; the last one died on the 13th day, but on passage of its brain, in the form of a 33 per cent suspension, into mice, no virus was isolated.

Thus, with these possible exceptions, the guinea pigs employed in this test proved resistant to the challenge dose, and the results of the tests for the presence of virus in the various brains indicate the pattern of a true immune reac-

tion. The second graph in Chart 3 shows the average temperatures of the guinea pigs used in this experiment.

Table I shows the outcome of the tests for the presence of virus in the brains of the sacrificed animals.

Brain suspensions in broth, in the tabulated concentrations, were injected intracerebrally into mice and guinea pigs. It will be seen that the demonstration of virus, even in the brains of non-vaccinated, fully susceptible guinea pigs, during the earlier phases of infection, proved difficult. No virus was unequivocally detected in the two animals 2½ or 3 hours after inoculation, and only traces were demonstrable at 6½ hours. At 17½ hours, multiplication had definitely taken place, and from then on until the time of death virus was present in all dilutions tested. In the vaccinated group, on the other hand, no similar multiplication of virus was observed. This finding may serve as additional evidence in favor of the assumption that the group as a whole was well immunized and would have survived the challenge dose. Traces of W.E.E. virus were, however, isolated from the brains of both animals at 3½ to 4 hours, and from those of one each at 7 and at 28 hours after inoculation.

TABLE I

Results of the Search for W.E.E. Virus and for Antibody in Brains of Guinea Pigs Sacrificed after Intracerebral Inoculation of 1,000 M.L.D.

Guinea pig No.		Sacrificed. Hours after inoculation	Dilution of brain injected into:				LD_{50} W.E.E. neutralized by 1:3 brain
			Mice			Guinea pigs 1:3	
			1:3	1:30	1:300		
Vaccinated guinea pigs	21-49	4	0/3, 0/5*	0/3, 0/5	0/3	**1/3***	0
	21-98	3½	**3/5**	0/5		2/3?	0
	21-99	7	0/3, 0/5, 0/10	**1/3**, 0/5	0/3	0/3	0
	22-17	16½	0/3	0/3	0/3	0/3	0
	22-18	17	0/10				0
	22-13	28	0/3, **1/5**	0/3, 0/5	0/3	1/3?	0
	22-14	29	0/10				**10**
	22-15	51	0/3	0/3	0/3		0
	22-16	50	0/10				0
	22-22	73	0/3	0/3	0/3		0
	22-23	2 wks.	0/5				**32**
Non-vaccinated guinea pigs	22-31	3	0/3, 0/5	0/3, 0/5	0/3	2/3?	
	22-32	2½	0/5	0/5			
	22-33	6½	0/3, **3/5**	**1/3**, 0/5	0/3	**2/3**	
	22-39	17½	**3/3**	**3/3**	**3/3**		
	22-34	28½	**3/3**	**3/3**	**3/3**		
	22-36	51½	**3/3**	**3/3**	**3/3**		

* Fractions indicate number dead or sick of virus infection/number of mice or guinea pigs used. More than one fraction indicates the number of separate tests with the same material. ? is added in those instances where death or illness could not be definitely identified as being due to W.E.E. virus. Proved positive virus results are in bold face numbers.

The chances of detecting virus during the early phases following inoculation in the brains of immune or non-immune animals were, therefore, equally small. The following calculation explains the fortuitous character of the results of inoculation tests in the search for virus. The challenge dose consisted of 0.1 cc. of W.E.E. virus-infected guinea pig brain, diluted 10^{-3}. This amount equalled 800 LD_{50} for mice. Inoculation into a 4.0 gm. brain of a guinea pig, and subsequent passage of 0.03 cc. of a 33 per cent suspension thereof into mice reduced the number of LD_{50} to $\frac{800}{40 \times 3.3 \times 3.0} = 2$. On this basis it is fair to assume that the few instances of recovery of virus were indicative of a general pattern, while the inability to demonstrate virus in others did not establish its absence. It was concluded from these results that virus was not prevented from gaining a foothold in the brain of an immune animal.

An attempt was made to supplement these results by studying possible concomitant changes in the neutralizing capacity of brain tissue. For it was to be expected that in the presence of excess virus homologous antibody should not be demonstrable.

The neutralization tests were carried out as described, except that 33 per cent test and control brain suspensions spun in the Pickels angle centrifuge at 10,000 R.P.M. for 30 minutes, were substituted for serum; the mixtures were incubated at 37°C. for 45 minutes before intracerebral inoculation into mice.

The results are included in Table I. Definite neutralization of 32 LD_{50} was obtained with the brain of guinea pig 22–23, removed 2 weeks after intracerebral inoculation. All other specimens, with the exception of that derived from guinea pig 22–14 obtained at 29 hours, failed to neutralize virus.

A second experiment was carried out in which, after subcutaneous vaccination with seven doses of formolized and one dose of active W.E.E. virus, seventeen guinea pigs received an intracerebral challenge dose of 1,000 M.L.D., while two were sacrificed to determine whether antibody was present before the challenge dose was given. Of those inoculated, four were killed on the 1st, 8th, and 14th day, and two on the 3rd day after inoculation. The remaining three survived the observation period of 2 weeks. The serum of each animal sacrificed as well as the perfused brains of two animals of each group were frozen. The remaining brains, also perfused, were used for histological examination, the results of which have already been discussed. Each of the stored brains, with the exception of those obtained at 2 weeks after inoculation (Nos. 23–31 and 23–32), was passaged into ten mice in the form of 33 per cent suspensions. No virus was isolated from any of them.

Variations in the relative antibody concentration in the brains were determined by simultaneous titrations of the neutralizing capacity of brain suspension and serum of each animal. Serial 10- or 3.2-fold dilutions were tested against a constant amount of W.E.E. virus. The mixtures were incubated at 37°C. for 2 hours before injection into mice. The neutralizing titers of brain suspensions were not affected by clarification in the angle centrifuge at 10,000 R.P.M. for 30 minutes.

In Table II, the titers of brain tissue extracts and of sera are expressed in terms of the highest dilutions which neutralized 10 to 32 LD_{50} of virus. The lowest dilutions of brain tissue available were 33 per cent suspensions, some of which failed to neutralize.

It would appear from the results of the first experiment (Table I) that, with one exception at 29 hours, the brains of animals removed during the first

3 days following intracerebral inoculation failed to neutralize W.E.E. virus. It is noteworthy that this was true also for brain suspensions which yielded no detectable amounts of W.E.E. virus. In the second test, at the time intervals indicated in Table II, none of the brains contained detectable virus, and no alteration in the average virus-inactivating capacity of brain tissue from that of the preinoculation phase was noticeable at 1 and 3 days following injection of virus. A marked increase in this capacity was evident, however, at 1 week after inoculation, while no appreciable change occurred in the neutralizing

TABLE II

Neutralizing Titers of Brains and Sera of Vaccinated Guinea Pigs before and after Intracerebral Inoculation of 1,000 M.L.D. of W.E.E. Virus

Guinea pig No.	Sacrificed. Days after inoculation	Neutralizing titer*		Ratio: Brain/Serum
		Brain	Serum	
23-22	0	1:<3†	1:320	1/>100
23-23	(Not inoculated)	1:3	1:1000	1/300
23-24	1	1:3	1:320	1/100
23-26		1:<3	1:320	1/>100
23-27	3	1:3	1:320	1/100
23-28		1:<3	1:100	1/>30
23-29	8	1:1000	1:1000	1/1
23-17		1:100	1:1000	1/10
23-31	15	1:10	1:1000	1/100
23-32		1:32	1:3200	1/100

* Neutralizing titer = highest dilution of serum or brain suspension which neutralizes 10 LD_{50} of W.E.E. virus.

† 1:<3 = 33 per cent suspension of brain failed to neutralize 10 LD_{50} of virus.

titer of the serum. As shown in Table II and illustrated in Chart 4, the ratio $\frac{\text{Brain titer}}{\text{Serum titer}}$ shifted from about 1:300 to 1:1 or 1:10 1 week after inoculation, and back to 1:100 2 weeks after inoculation.

Specificity of Neutralizing Substance in Brain Tissue.—The specificity of neutralizing antibody to either Eastern or Western equine encephalomyelitis virus has been affirmed by others (9, 10) as well as ourselves in numerous tests over a period of 10 years and again in the present series of experiments. Yet, because of the coincidence of resistance to E.E.E. virus following recovery from an abortive W.E.E. infection with enhanced capacity of brain tissue to inactivate W.E.E. virus, the specificity of the neutralizing substance was investigated. 33 per cent suspensions of the brains of guinea pigs 23-17 and 23-29

which had the highest neutralizing titer against W.E.E. virus, as shown in Table II, were mixed with minimal infective amounts of E.E.E. virus. No neutralization was observed, thus the specificity of the neutralizing substance in brain tissue was demonstrated.

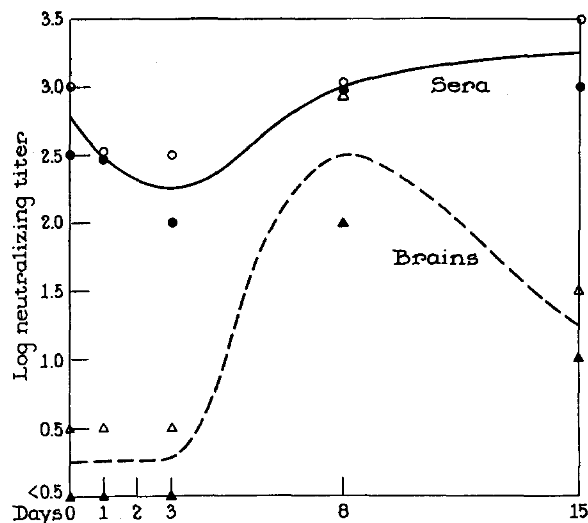

CHART 4. Neutralizing titers of brains and sera of vaccinated guinea pigs before and after intracerebral inoculation of 1000 M.L.D. of W.E.E. virus. Each pair of open or closed symbols indicates the values found for one animal at each interval, brain titers represented by triangles, serum titers by circles.

DISCUSSION

It has been shown that immunity, acquired by guinea pigs as a result of subcutaneous vaccination with inactivated W.E.E. virus, did not protect the central nervous system completely from the effects of homologous virus: intracerebral inoculation into such animals of sufficiently large doses of active W.E.E. virus was followed during the first 20 to 30 hours by an abortive infection characterized by fever and by morphological changes in the brain tissue simulating the reaction of non-vaccinated animals at that early stage. During the abortive infection, virus could be demonstrated in perfused brain tissue. Several days after the intracerebral challenge dose, a striking transitory increase in the specific virus-inactivating capacity of brain tissue was observed, the ratio between the neutralizing titers of brain tissue and serum being shifted from about 1:300 to 1:1 or 1:10. The chain of events so changed the nervous tissue of animals that it was refractory for about 2 weeks to the action of the serologically distinct Eastern equine virus and, to a lesser extent, to that of vesicular stomatitis virus.

The observation that immune animals are not completely protected against

the action of fully virulent homologous virus on susceptible tissue is not new. Thus, Bedson (11) found that intraperitoneal injection of active psittacosis virus into mice immunized with formolized virus did not lead to the frank disease, but virus established itself in the spleen where it persisted for several months and produced specific lesions which differed only quantitatively from those seen in non-immunized mice. This modified infection took place, although neutralizing antibody in an undefined amount was present in the serum of immune mice (11). In the study now reported, inoculation of 1,000 M.L.D. of W.E.E. virus into the brains of immune guinea pigs placed an excess of virus in highly susceptible tissue which had immediately available only a small fraction of the serum antibody (2). One must assume that, for some time, virus remained in excess over antibody and could infect cells. As subsequently more antibody became available, the progression of virus from affected cells was blocked, and the infection was arrested. Whether or not virus persisted in the central nervous system beyond that time cannot now be decided, since the methods used were not even sufficiently sensitive for the detection of virus in the brains of non-vaccinated animals, which were known to contain it. On the other hand, it had been noted earlier that administration of hyperimmune serum to guinea pigs infected by subcutaneous injection of W.E.E. virus led in a certain number of cases to a marked delay in the onset of the manifest disease (6). Such animals developed signs after a long period of apparent well-being and at a time when neutralizing antibody in their serum had fallen to a low titer. It was believed that virus had reached the central nervous system before serum was injected and had then been held in check until antibody within the central nervous system fell to an ineffective concentration. If this were the correct interpretation, then one may well suspect that in the nervous tissue of an immune animal the equine virus can persist for long periods of time.

That the temporary enhancement of the virus-inactivating capacity of brain tissue which was found 1 week after the abortive infection with W.E.E. virus had taken place, was due to specific antibody was manifested by the failure of the brain suspensions to neutralize Eastern virus. Thus this enhancement was apparently due to a local accumulation of strain-specific neutralizing antibody far out of proportion to the physiological ratio between brain tissue and serum antibody. Similar accumulation of neutralizing antibody in the brains of mice surviving intracerebral inoculation of 17 D yellow fever virus has been observed by Fox (12). While the ratio $\frac{\text{Brain antibody}}{\text{Serum antibody}}$ following extraneural (intraperitoneal) immunization was roughly of the same order of magnitude as determined by Freund (13) and ourselves (2), it averaged about 1:2 following intracerebral immunization.[3] This concentration of antibody in the brain was

[3] These comparable values have been obtained by multiplying the titers given by Fox (12) for brain tissue by 10, since he based his values on titrations in which 10 per cent brain suspensions served as starting materials.

associated with a much higher degree of resistance to intracerebral challenge doses than that attained after extraneural immunization, although the average serum antibody titer after the latter was higher. These findings by Fox have again furnished evidence for the important rôle of immediate availability of antibody to the exposed susceptible tissue in the mechanism of immunity.

Whether the accumulation of antibody in the brain is the result of increased permeability of the blood-brain barrier due to inflammatory processes or of local production of antibody is unknown, nor is it known whether it reflects a specific response to the presence of the homologous antigen in the brain, or a non-specific disturbance in the protein balance between plasma and interstitial fluid. Tests are under way in which guinea pigs immunized against both Eastern and Western viruses are to be challenged by intracerebral inoculation of only one of these viruses. It will then be possible to find out whether only homologous or, in addition, heterologous antibody as well is accumulated in the brain tissue. The work reported by Walsh and Cannon (14) would suggest that such concentration of antibody in tissues directly exposed to an antigen can be due to a specific local immune mechanism. In this connection, it is of interest that in the present series the highest antibody concentration in the brain was found at a time (1 week after inoculation) when the earlier infiltration of the brain tissue by cellular elements and the other tissue changes had subsided. Whatever the mechanism underlying the local accumulation of antibody may be, its occurrence, considered together with the other manifestations of the abortive infection, was evidence of profound transitory changes taking place in the brains of immune animals in response to the intracerebral challenge inoculation.

Further evidence in the same direction was the temporary resistance of the central nervous system of guinea pigs to heterologous viruses acquired as a result of the abortive infection with W.E.E. virus. The absence of antibody against these heterologous viruses from the serum as well as from the brains has led to the belief that the cells of the central nervous system are rendered temporarily insusceptible as a result of their contact with W.E.E. virus. The relationship between this type of "acquired cellular resistance" and the interference phenomena described in recent years for a variety of viruses (15) has already been pointed out (5). While the mechanism underlying these phenomena requires further study, the present investigations have thrown into sharp relief the fundamental difference between serologically specific humoral immunity and non-specific cellular resistance of the interference type.

SUMMARY

Although vaccination of guinea pigs with formalin-inactivated Western equine encephalomyelitis virus rendered them specifically immune to an intracerebral challenge dose of 1,000 M.L.D. of Western virus, it failed to protect

their central nervous system against the initial effects of the virus: the intracerebral challenge dose was followed by an abortive infection of 20 to 30 hours' duration characterized by fever and histopathological changes which simulated the response at that early stage of non-vaccinated control animals.

During the abortive infection of immune animals, virus could occasionally be demonstrated in their brains; indeed, it was detected with about the same frequency it was isolated from brains of similarly inoculated, non-immune guinea pigs during corresponding early phases of the infection. About one week after the abortive infection there was found a marked transitory accumulation of specific neutralizing antibody in the brain tissue. The ratio $\frac{\text{Brain antibody titer}}{\text{Serum antibody titer}}$ equalled at this time 1:1 to 1:10 instead of the value of about 1:300 found under physiological conditions.

Guinea pigs which had recovered from an abortive infection with Western virus were resistant for a limited period of time to the effects of intracerebral inoculations of the immunologically distinct viruses of Eastern equine encephalomyelitis or vesicular stomatitis.

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