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

I. Neutralizing Antibody in the Central Nervous System in Relation to Cerebral Resistance

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Neutralizing antibody in cerebrospinal fluid and brain extract of animals vaccinated with the virus of equine encephalomyelitis was studied in an attempt to clarify the apparent lack of correlation of antibody with resistance to intracerebral injection of active virus. Hurst (1) has reported that monkeys, after peripheral injection of active virus of Eastern equine encephalomyelitis (E.E.E.), developed neutralizing antibody. Certain of these animals succumbed, nevertheless, to subsequent intracerebral injection of active virus. The neutralization tests were carried out with undiluted serum. Such tests with undiluted serum have been found (2), however, incapable of differentiating, above a certain level, between the neutralizing capacity of different sera. When serum was titrated for antibody, using dilutions of serum and a constant amount of virus, it was found that resistance of vaccinated mice to intracerebral injection of active E.E.E. virus was associated only with a high level of antibody in the serum. The degree of resistance was correlated with antibody titer. Since the plasma does not have direct access to cells of the central nervous system, the high titer of antibody in the plasma suggested that it might be an indicator of antibody in the fluids of the central nervous system which would be more readily available to susceptible cells.

Antibody has been found by Freund (3) in the brain, spinal cord, and cerebrospinal fluid of rabbits vaccinated with typhoid vaccine. He has demonstrated, moreover, a constant relationship between the concentration of antibody in the central nervous system and that in the serum. Ramon and Descombey (4) have described a similar relationship of antitoxin in spinal fluid and serum. Mollaret and Stefanopoulo (5) have reported that monkeys surviving a subcutaneous injection of active yellow fever virus showed neutralizing antibody in their spinal fluid and resisted an intracerebral injection of active virus.

In rabbits vaccinated with equine encephalomyelitis virus we studied first, the relationship of antibody in spinal fluid to that in serum and second, its bearing on cerebral resistance to active virus.

Methods and Materials

Rabbits.—Rabbits of various breeds weighing from 2500 to 3500 gm. were obtained from dealers in the vicinity. They were usually kept in individual cages in the animal house for a week before use. For each group of test animals, controls were saved from the same lot. There were many animals used in addition to those which appear in the tables on which the record was not complete, but which, as far as tested, were consistent in their reaction with the others. One lot of animals had been bred and raised in our animal house; they will be mentioned again because of their unusual reaction.

Rabbits were vaccinated, usually subcutaneously, with active or formalin-inactivated Eastern (E.E.E.) or Western (W.E.E.) equine encephalomyelitis virus. The source of active virus (A.V.) was usually a saline suspension of infected 9-day-old chick embryo (sometimes infected mouse brain). Formalin-inactivated virus (F.V.) consisted of a 10 per cent suspension of infected chick embryo in 0.5 per cent formalin, which was non-infective by all tests available (6).

Cisternal Puncture.—Cerebrospinal fluid was obtained from the cisterna magna of rabbits anesthetized by intravenous injection of nembutal. An assistant firmly grasped the head of an anesthetized rabbit over the edge of a table with the top of the head vertical, the neck thus flexed at a right angle. A 22-gauge, 1¼-inch needle was then inserted in the midline, just caudal to the occipital protuberance. When in the proper position, with the needle at a depth of about ½ inch, the piercing of the dura could be easily felt and, when spinal fluid welled up into the hilt of the needle, it was drawn off with a capillary pipette. About 0.5 cc. of spinal fluid could be taken without risk. Only crystal clear spinal fluid was acceptable, in which no red blood cells were detectable after standing overnight in the refrigerator. Contamination with blood was more easily detected in this way than by cell count, for cells were visible in a slightly contaminated sample which showed less than one cell per microscopic field. With practice we were able to obtain clear spinal fluid from more than half the number of rabbits. Control rabbits were tapped at the same time as the test animals.

Tests for Resistance.—Tests for cerebral resistance of vaccinated rabbits were carried out by intracerebral or intracisternal injection of saline or broth dilutions of a suspension of mouse brain infected with W.E.E. or E.E.E.virus. Non-vaccinated rabbits from each lot served as controls of the infectivity of the virus.

Intracerebral Route.—A small hole was drilled with a trephine caudally to the frontoparietal suture to one side of the midline. 0.2 cc. of viral suspension was injected through a 27-gauge needle, ⅜ inch long, inserted vertically to the hilt. In this way, most of the virus injected went into the lateral ventricle, as confirmed by examination of brains immediately after injection of a dye (mixture of iron ammonium citrate and potassium ferrocyanide) with formalin.

Intracisternal Route.—A syringe containing suspension of virus was attached to the needle through which a sample of spinal fluid had been removed, thus injecting the virus directly into the cisterna magna whence it had free access to the entire subarachnoid space. In this way, when a clear sample of spinal fluid had been obtained, the virus did not come into contact with blood or with damaged cerebral tissue.

EXPERIMENTAL

Neutralizing Capacity of the Cerebrospinal Fluid

The spinal fluid of rabbits vaccinated with active or with formalin-inactivated virus was tested for neutralizing property.

Samples of blood and spinal fluid were taken from rabbits after subcutaneous vaccination with one or more doses of active or formolized, W.E.E. or E.E.E. virus. Samples were taken, as a rule, 2 weeks after the last dose of vaccine. Neutralization tests were carried out by the peritoneal test in young mice (7), using infected mouse brain. When testing for minimal amounts of antibody, the peritoneal test proved to be more sensitive than the cerebral neutralization test. Tests on spinal fluid and dilutions of serum of a given animal were made simultaneously and repeated whenever possible.

In the spinal fluid of rabbits sufficiently vaccinated, the capacity to neutralize the homologous virus could be readily demonstrated. Furthermore, when the neutralizing capacity of threefold dilutions from 1/10 to 1/1000 of serum of a given animal was compared with that of its spinal fluid, a dilution of serum of the order of 1/300 was found equivalent to spinal fluid. Table I shows the neutralizing capacity (as measured by difference in titer of virus in the presence of normal spinal fluid and that in test fluid) of spinal fluid and 1/300 dilution of serum of vaccinated rabbits.

In all cases, but one, shown in Table I, when 1/300 serum dilution neutralized virus, so also did the spinal fluid; when one failed to neutralize, so did the other. There was not more than a tenfold difference in neutralizing capacity when end-points were reached.

In the single exception, rabbit 35, the spinal fluid failed in a single test to neutralize virus, whereas the 1/300 serum dilution neutralized at least ten units of active virus on repeated test. This animal was from the group of cage-bred rabbits.

Just as has been reported for the ratio of antibody in spinal fluid to that in serum in response to a bacterial antigen (3) and to toxin (4), so also a ratio of neutralizing capacity of spinal fluid to serum of the order of 1/300 has been demonstrated in animals vaccinated with a viral antigen.

Neutralizing Capacity of Brain Tissue

The neutralizing capacity of a suspension of perfused brain of a rabbit vaccinated with formalin-inactivated W.E.E. mouse brain was compared with that of its spinal fluid and 1/300 serum dilution.

Rabbit 2-32 was vaccinated by means of thirteen subcutaneous injections of 2 cc. or more of 20 per cent mouse brain, infected with W.E.E. virus, in 0.5 per cent formalin over a period of 3 months. 5 days after the last dose, clear spinal fluid was obtained.

75 cc. of blood were drawn from the heart. The brain was perfused by injection of sterile saline through the carotid arteries until the fluid returning from the cut veins appeared blood-free. 500 cc. of saline were injected on one side, followed by 100 cc. on the other. A piece of brain was sectioned for histological study which revealed

TABLE I

Neutralizing Capacity of Spinal Fluid Compared with 1/300 Serum Dilution of Rabbits Vaccinated with E.E. Virus

Rabbit No.	Vaccination	Neutralizing capacity	
		1/300 serum	Spinal fluid
22-91	36 × A.V.-W.E.E.*	1,000	1,000
10-06	5 × A.V.-E.E.E.	100–1,000	>100‡
10-02	5 × A.V.-E.E.E.	1,000	>100
10-39	3 × A.V.-W.E.E.§	1,000	1,000
10-42	3 × A.V.-W.E.E.	1,000	1,000–10,000
10-03	3 × A.V.-E.E.E.	>10	10
10-14	3 × A.V.-E.E.E.	10	>10
2-32	13 × F.V.-W.E.E.‖	100	10–100
1-56	3 × F.V.-W.E.E.	10–100	>10
1-57	3 × F.V.-W.E.E.	10	>10
79 A	1 × A.V. 10^{-2}-W.E.E.	>100	>100
33	1 × A.V. 10^{-5}-W.E.E.	10–100	10–100
35	1 × A.V. 10^{-5}-W.E.E.	>10	0
83 A	1 × A.V. 10^{-5}-W.E.E.	0	0
1-30	3 × F.V.-W.E.E.	0	0
2-00	3 × F.V.-W.E.E.	0	0
2-03	3 × F.V.-W.E.E.	0	0
78 B	1 × F.V. 10^{-1}-W.E.E.	0	0
87 B	1 × F.V. 10^{-1}-W.E.E.	0	0
80 B	1 × F.V. 10^{-2}-W.E.E.	0	0
81 B	1 × F.V. 10^{-2}-W.E.E.	0	0

* 36 × A.V.-W.E.E. = 36 injections by intravenous or subcutaneous route of active W.E.E. virus, 10 to 20 per cent. The next two animals, 5 injections similarly of E.E.E. virus.

‡ >100 = difference in titer between control and test was at least 100-fold.

§ 3 × A.V.-W.E.E. = 3 subcutaneous injections; all other animals injected by same route; F.V. = formolized virus.

‖ Vaccinated with mouse-brain virus; all others with chick-embryo virus.

few red blood cells in the vessels. The remainder of the brain was ground in a mortar and made to a ⅓ suspension in saline. On centrifugation of this cream-colored suspension, no layer of red blood cells was visible. The supernate was drawn off and compared with spinal fluid and 1/300 serum dilution by repeated peritoneal neutralization tests (7) with mouse-brain W.E.E. virus.

From a vaccinated rabbit 1/300 serum dilution, 1/3 suspension of perfused brain, and undiluted spinal fluid each neutralized about ten units of W.E.E.

virus. This is similar to the finding of Freund that the ratio of titer of serum is to that of brain and to that of spinal fluid as 300:2.5:1.

Specificity of the Neutralizing Substance

Spinal fluid of an animal vaccinated with Western virus which could neutralize Western virus was not capable of neutralizing Eastern virus and *vice versa*. Since the neutralizing property of spinal fluid developed in response to vaccination parallels the serum antibody in the ratio described, and since the neutralizing substance reacted specifically with the homologous and not with the heterologous virus, it was considered antibody (6, 8, 9).

Correlation of Spinal Fluid Antibody with Resistance to Infection by the Cerebral Route

(*a*) *In Response to Active Virus.*—The spinal fluid (or 1/300 serum dilution) of thirty rabbits vaccinated subcutaneously with various doses of active chick-embryo virus was tested for neutralizing antibody to the homologous virus by the peritoneal neutralization test in young mice (7). The vaccinated rabbits, as well as normal controls, were injected intracerebrally with active, homologous, mouse-brain virus. The results are summarized in Table II.

Twenty-two rabbits showed neutralizing antibody in the spinal fluid (or 1/300 serum dilution), (Table II). Twenty-one of these survived an intracerebral injection of active virus in a dose 10 to 100 times the highest dilution lethal to normal controls. All eight rabbits having no demonstrable spinal fluid antibody succumbed after a typical course following intracerebral injection of active virus.

The single exception, an animal which died in spite of the presence of spinal fluid antibody, occurred in the cage-bred group. It may be recalled that it was in this group also that the single exception to the equivalence of spinal fluid antibody to 1/300 serum antibody was found.

It may be seen from Table II that rabbits reacted to subcutaneous injection of active virus with either a high degree of immune response, with antibody in the spinal fluid and with resistance to an intracerebral injection of active virus, or else there was no antibody demonstrable, even in undiluted serum, and no cerebral resistance. However, even the immune animals were not entirely refractory; they reacted to intracerebral injection of active virus with febrile response lasting a few days. In general, animals with higher titer of spinal fluid antibody showed fever of shorter duration. This febrile response was interpreted as due to viral activity because the greater the test dose of virus, the greater the febrile spike on the first day following test injection, and because intracerebral injection of formalin-inactivated virus (at high concentration) induced no such reaction, even in normal animals. As might be ex-

pected, the full immune response occurred more frequently following multiple doses of virus or a large single dose. Thus it has been shown that cerebral resistance induced as a result of vaccination with active virus is associated with spinal fluid antibody.

The significance of this antibody which develops after vaccination with active virus is, however, not entirely conclusive, for: (*a*) After peripheral inoculation of active virus, the systemic phase of infection, accompanied by circulating virus, may be followed by a transitory invasion of the central nervous

TABLE II

Correlation of Spinal Fluid Antibody with Resistance to Intracerebral Injection of Active Virus in Rabbits Vaccinated Subcutaneously with Active Virus

Total No. of rabbits vaccinated	Vaccination subcutaneously	Antibody			Reaction to intracerebral injection	
		Serum		Spinal fluid		
		Undiluted	1/300		Fever.	Outcome
2	36 × W.E.E.		+	+	1-2 d.	Recovery*
2	5 × E.E.E.	+	+	+	1-5 d.	"
3	3 × E.E.		+	+	1-2 d.	"
2	1×10^{-1} W.E.E.		+	+	0-1 d.	"
2	1×10^{-2} W.E.E.	+	+	+	1-3 d.	"
4	1×10^{-3} W.E.E.		+	+	0-1 d.	"
3	1×10^{-4} W.E.E.		+	+	0-1 d.	"
3	1×10^{-5} W.E.E.		+	+	0-2 d.	"
1	1×10^{-5} W.E.E.	+	+	+	4 d.	Death
1	1×10^{-5} W.E.E.	+	+	0	4 d.	"
2	1×10^{-2} W.E.E.	0	0	0	2 d.	"
1	1×10^{-5} W.E.E.	0	0	0	4 d.	"
4	1×10^{-7} W.E.E.	0		0	2-3 d.	"

* 1-2 d. Recovery = 1 to 2 days of fever, followed by recovery; other symbols same as in Table I.

system, reflected by a marked rise in temperature, although the animal may show no clinical signs of involvement of the central nervous system and recover with full antibody response (10). (*b*) Following intracerebral injection of small doses of active virus, rabbits may also show only a transitory febrile reaction and may not develop demonstrable antibody. Such individuals subsequently resist intracerebral inoculation of the same virus, or even of heterologous virus. This type of non-specific resistance, independent of antibody, will be discussed in the next paper of this series (11). The possibility cannot be ruled out that a non-specific effect may play a part in the cerebral resistance after vaccination with *active* virus. Therefore we undertook vaccination with formalin-inactivated virus where by all tests available the possibility of the presence of active virus has been excluded.

(*b*) *In Response to Formalin-Inactivated Virus.*—In contrast to the "all or none" immune response induced by active virus, it was possible to obtain intermediate degrees of immune response only when we resorted to vaccination with formalin-inactivated virus.

TABLE III

Antibody Response and Resistance Induced by Formalin-Inactivated W.E.E. Virus

Rabbit No.	Vaccination subcutaneously	Antibody				Test for resistance		
		Serum			Spinal fluid	Route	Fever.	Outcome
		Undiluted	1/200	1/300				
2-89	7 × 20 per cent*			+	+	Intracerebral	3 d.	Recovery
1-56	3 × 10 " "			+	+	Intracisternal	0	"
1-57	3 × 10 " "			+	+		2 d.	"
1-53	3 × 10 " "			+			3 d.	"
1-30	3 × 10 " "		+	0			2 d.	"
1-24	1 × 10 " "	+	0	0			4 d.P.P.‡	"
1-27	1 × 10 " "	+	0				5 d.	"
94	1 × 10 " "	+	+	0		Intracerebral	3 d.	"
97	1 × 1 " "	+	0	0			3 d.	Death
87 B	1 × 10 " "	+	0	0	0		3 d.	"
2-00	3 × 10 " "	+	0		0		3 d.	"
2-03	3 × 10 " "	+	0	0			3 d.	"
1-54	3 × 10 " "	+	0	0		Intracisternal	6 d.	"
1-55	3 × 10 " "	+	0	0			2 d.	"
1-18	1 × 1 " "	+	0		0		3 d.	"
1-19	1 × 1 " "	+	0		0		3 d.	"
1-25	1 × 1 " "	+	0		0		2 d.	"
1-28	1 × 1 " "	+	0				5 d.	"
1-29	1 × 1 " "	+	0		0		5 d.	"

* 7 × 20 per cent = vaccinated by 7 subcutaneous injections of formolized, 20 per cent chick-embryo vaccine of W.E.E. virus.

‡ 4 d.P.P. = 4 days of fever, with posterior limb paralysis.

Rabbits were vaccinated by one or more subcutaneous injections of chick-embryo W.E.E. virus inactivated by formalin. Samples of spinal fluid and blood were taken usually 2 weeks after the first dose. Cerebral resistance was tested either by injection of active mouse-brain virus into the cisterna magna through the needle used for withdrawal of fluid, or by intracerebral injection on the following day. Antibody in the spinal fluid and in serum dilutions was measured by the peritoneal neutralization test (7). Fluids in a given series were tested simultaneously. The results are shown in Table III.

It may be noted from Table III that it is possible to induce resistance to intracerebral injection of active virus in rabbits by vaccination with formalin-inactivated, chick-embryo W.E.E. virus. Similar results were obtained using F.V.-mouse-brain vaccine. Individuals which had developed antibody demonstrable in spinal fluid and in 1/300 serum dilution survived an intracerebral or an intracisternal injection of active virus. Temperatures read daily following test injection were normal or indicated fever lasting up to 3 days. At the other extreme, when antibody could not be demonstrated in the spinal fluid, such injection of active virus led to death from typical encephalitis, in spite of demonstrable antibody in the undiluted serum.

Intermediate between these two groups were individuals, like rabbit 1-30, which showed antibody at 1/200 but not at 1/300 dilution of serum and recovered after 2 days of fever. In this case, no sample of spinal fluid was available, but since 1/300 serum dilution did not reveal antibody, our experience indicates that an equal volume of spinal fluid would also be negative; however, since the serum at only a slightly higher concentration did show antibody, so also a larger sample of spinal fluid might be expected to be positive. The demonstration of antibody at 1/200 serum dilution in this individual may be contrasted to its absence at the same dilution of serum of rabbits 1-24 and 1-27 of the same series, which, although they survived, showed a prolonged course of fever, and even posterior limb paralysis in one. Also in the same series were rabbits 1-18 through 1-29, with no antibody at 1/200 serum dilution and no cerebral resistance. Two additional rabbits of interest are 94 and 97, vaccinated simultaneously with different doses; the former showed antibody at 1/200 serum dilution and was resistant; the latter did not, and succumbed.

The immune response and result of subsequent intracisternal injection of active virus in the series of rabbits 1-53–1-57 are shown in Fig. 1.

The three rabbits which showed antibody in the 1/300 serum dilution, and in spinal fluid when tested, resisted the test dose, whereas the two in which no antibody was demonstrable in the 1/300 serum dilution succumbed to it.

In summary, rabbits which had antibody demonstrable in the spinal fluid were found to resist active virus introduced into the central nervous system; lack of spinal fluid antibody was associated with lack of such resistance.

Specificity of Immunity Induced by Vaccination with Formalin-Inactivated Virus

Rabbits vaccinated with formalin-inactivated Western E.E. or Eastern E.E. virus were tested for cerebral resistance to Western E.E. virus.

Three rabbits were vaccinated by seven subcutaneous injections of 2 to 5 cc. of 20 per cent suspension of chick-embryo E.E.E. virus inactivated by formalin. Three rabbits were vaccinated with formolized W.E.E. virus. Six doses were given on alternate days and after a rest period of 4 weeks, a final dose was given. 5 days later, samples of blood and spinal fluid were taken and on the following day, the animals were injected intracerebrally with dilutions of mouse-brain W.E.E. virus. From six normal control rabbits, spinal fluid was obtained at the same time and they were injected with amounts of virus indicated in Fig. 2.

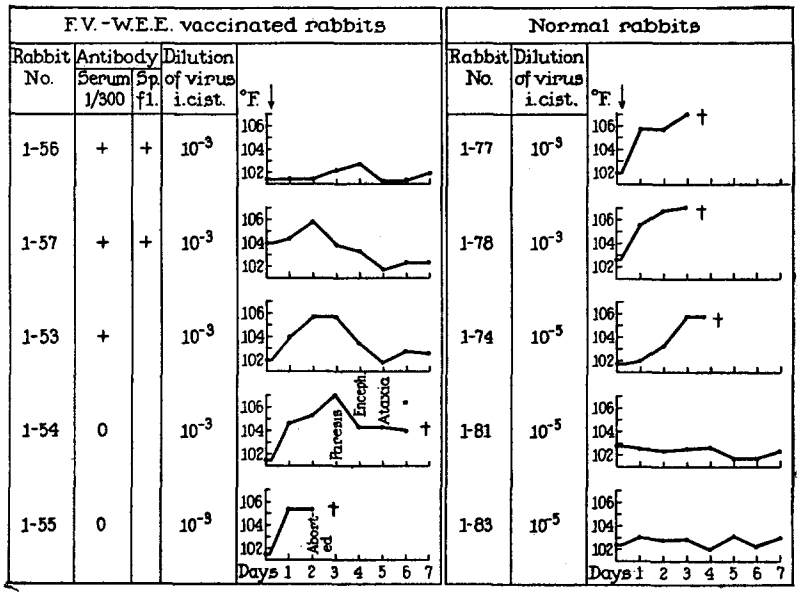

FIG. 1. Correlation of antibody in spinal fluid (or in 1/300 serum dilution) of F.V.-vaccinated rabbits with resistance to intracisternal injection of active virus.

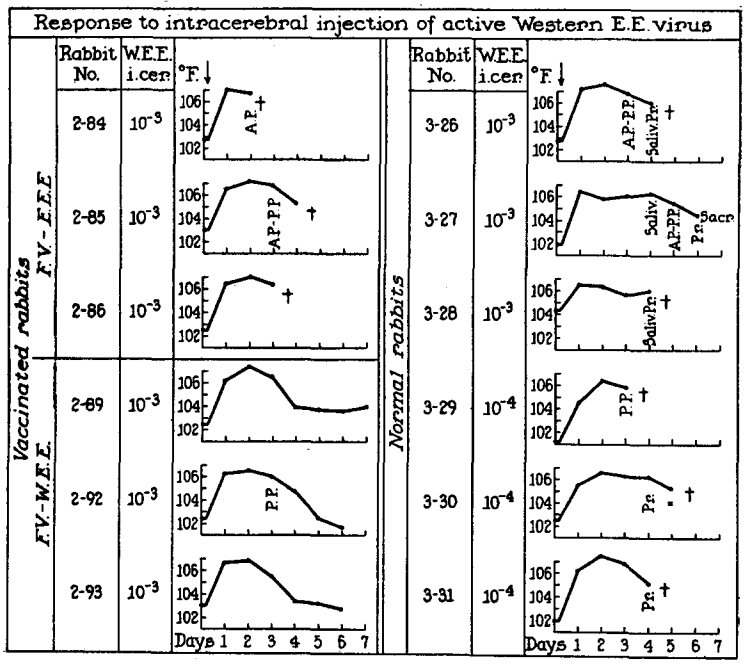

FIG. 2. Specificity of cerebral resistance induced in rabbits by vaccination with formolized Eastern or Western E.E. virus. A.P. = anterior limb paralysis; P.P. = posterior limb paralysis; Pr. = prostrate.

All normal rabbits succumbed to intracerebral injection of active W.E.E. virus in dilutions indicated in Fig. 2, after showing signs of typical encephalitis. Of the vaccinated rabbits, the three vaccinated with Eastern virus succumbed typically, while the three Western virus-vaccinated animals showed a normal temperature after 3 to 4 days of fever, and recovered. In a reciprocal test, rabbits vaccinated with F.V.-W.E.E. virus succumbed to active Eastern, but resisted Western virus.

Thus cerebral resistance to virus was induced in rabbits by vaccination with formalin-inactivated vaccine of the homologous virus but not by the heterologous virus. This is in agreement with consistent evidence that Eastern and Western E.E. viruses are immunologically distinct, showing no cross-reactions, either by serum-neutralization test (8, 9) or by resistance tests in immune animals (6, 11).

DISCUSSION

It has been found that a definite ratio exists between the concentration of neutralizing antibody in the serum, brain tissue, and cerebrospinal fluid of rabbits vaccinated with equine encephalomyelitis virus. This confirms the earlier observation of Freund on agglutinin to typhoid bacilli. The results here reported were based chiefly on tests with spinal fluid rather than perfused brain extract, because (1) we could be more certain that there was no contamination with plasma; (2) the animal could be spared for resistance test; and (3) the concentration of antibody in the perfused brain extract was fully as high as that in the spinal fluid.

Whether spinal fluid obtained from the cisterna magna represents a fluid similar to intercellular fluid of the brain is still under discussion. Besides the well known source of spinal fluid in the choroid plexuses of the ventricles (12, 13, *cf.* 14), there is a possible extraventricular source. According to the experiments of Weed (15), there is a direct continuity of the intercellular spaces of the brain cells with pericapillary spaces and these in turn with the Virchow-Robin spaces surrounding the pial vessels and finally the subarachnoid space. Furthermore, he demonstrated a flow in the direction named, *i.e.*, from the capillaries to the subarachnoid fluid. Kafka (13) presents diagrams of this circulation. Since in mammals there is no evidence of a lymphatic system within the central nervous system, the circulation described may be considered analogous to that of the interstitial fluid of other tissues. Slight differences in chemical composition between ventricular fluid and subarachnoid fluid lend supporting evidence for an extraventricular formation of spinal fluid and for such a physiological function. Friedemann (19), on the other hand, has reviewed the evidence for his belief that the spinal fluid is not necessarily an intermediary between blood and brain cells.

Regardless of the relationship between the interstitial and the cisternal

fluids, antibody in the spinal fluid serves as an indicator of antibody in the brain tissue, since there is a constant ratio between them.

The finding of antibody in the spinal fluid in a constant, but low ratio to that in the serum is in accord with the recent report of Kabat, Landow, and Moore (16), that the proteins of concentrated human spinal fluid show an electrophoretic pattern similar to that of the plasma proteins, and that alterations in the composition of serum proteins produce similar changes in the spinal fluid patterns. The ratio reported of antibody in the spinal fluid to that in the serum is, moreover, similar to the total protein ratio. (Flexner (17) gives an average total protein ratio of 1:250, although the normal range in both fluids is wide.) A similar ratio of agglutinin in spinal fluid to that in serum was established in animals which received antiserum intravenously, *i.e.*, by passive immunization (3). For these reasons, we believe that there is no necessity for assuming local production of antibody (18), but that antibody in the spinal fluid merely reflects the antibody in the plasma in the ratio described.

In an animal sufficiently vaccinated, antibody is present in the central nervous system, as determined by tests on spinal fluid. Such an animal survives an intracerebral injection of active virus which is lethal for non-vaccinated controls. That some cells may become infected, nevertheless, as an immediate consequence of intracerebral injection of active virus despite the presence of antibody in the central nervous system, is indicated by the febrile response following the injection. The mode of action of the antibody in the central nervous system may then be to neutralize virus deriving from infected cells and thus to prevent the spread of infection. Also, a certain amount of virus injected into a ventricle may be neutralized by the antibody present in the spinal fluid. By the experimental procedure of an intracerebral injection, virus is placed in direct contact with susceptible cells, in contrast to inoculation of a vaccinated animal by a peripheral route where antibody may have full access to the virus before it reaches the central nervous system (6).[1]

The significance of antibody in the course of infection following peripheral inoculation of virus (probably simulating more closely the natural mode of infection) has been discussed (10). After subcutaneous injection of active W.E.E. virus in adult rabbits, systemic infection, accompanied by virus in the blood stream, may be followed by virus invasion of the central nervous system, characterized by high fever but without apparent signs of involvement of the nervous system. Such infection takes place when antibody has already appeared in the blood stream. Defervescence and recovery

[1] H. B. Shumacker, Jr., and associates (*J. Immunol.*, 1939, **37**, 425; *Bull. Johns Hopkins Hosp.*, 1940, **67**, 92) have reported that in active and passive immunization in different species of animals, a higher level of antitoxin is needed for protection against tetanus toxin introduced into the spinal cord or medulla than when the toxin is given peripherally.

set in at a time when serum antibody titer reaches 1/300 (or, in other words, when antibody is present in the spinal fluid). On the other hand, young rabbits, after a similar injection of virus, die of encephalitis before this antibody level has been reached.

In human beings convalescent from infection with W.E.E. virus, Howitt (20) found neutralizing antibody in the spinal fluid of seventeen of twenty cases which showed serum antibody. In four monkeys which survived infection induced by intracutaneous injection of W.E.E. virus, antibody appeared in the spinal fluid.

It has been said that the central nervous system is impervious to antibody, because a vaccinated animal may show antibody in the serum and yet not resist an intracerebral injection of a neurotropic virus. The same apparent lack of correlation has led some to believe that antibody has nothing to do with immunity of the central nervous system. Having defined the conditions for availability of antibody, we believe that the rôle of antibody in immunity of the central nervous system is not unique but differs quantitatively from that of other tissues; *i.e.*, that it is necessary to have a high titer of antibody in the plasma in order to have a minimal amount present in the central nervous system. The concept of availability of antibody may be applied to immunity to other infections. Francis (21) has reported that serum antibody in influenza patients rose between the acute and convalescent phases, accompanied by a rise, at a considerably lower level, of inactivating capacity of the nasal secretion. He suggested the possibility that only those individuals essentially devoid of such inactivating substance in their nasal secretions need be considered susceptible to infection with the virus.

This definition of the relation of antibody to resistance allows immunity of the central nervous system to fall in line with the generally accepted principles of immunology.

SUMMARY

1. Neutralizing antibody to equine encephalomyelitis virus was found in the spinal fluid of rabbits sufficiently vaccinated with active or formalin-inactivated virus. Antibody was specific for the Western or for the Eastern virus.

2. Neutralizing capacity of spinal fluid was equivalent to that of a 1/300 dilution of serum of the same animal, and was of the same order of magnitude as that of perfused brain of a vaccinated animal.

3. Vaccinated rabbits which showed antibody in the spinal fluid resisted intracerebral or intracisternal injection of active virus. This immunity was specific, *i.e.*, there was no cross-reaction between the Eastern and Western virus after vaccination with formalin-inactivated virus. On the other hand, lack of antibody in the spinal fluid, even when antibody was demonstrable in the undiluted serum, was associated with lack of cerebral resistance.

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