IMMUNITY OF MICE FOLLOWING SUBCUTANEOUS VACCINATION WITH ST. LOUIS ENCEPHALITIS VIRUS

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Of the few known central nervous system virus infections of man with an immunology that can be studied experimentally in animals, St. Louis encephalitis in mice lends itself most readily to quantitative analysis. This virus infection, unlike poliomyelitis, can be studied in selectively bred mice which can be used in large numbers and in which the majority of disturbing variables can be eliminated or controlled. Already the pathogenesis of the infection *via* the nasal passages has been studied both in mice of high inborn susceptibility (1) and in closely related mice of high inborn resistance (2). The former succumb to an overwhelming infection of the brain; the latter remain well in spite of small, local foci in the brain. The differences in response of these innately susceptible and resistant mice appear to rest largely in a difference in the brain tissue itself, no other organ or tissue, including the blood, being concerned. Regarding this property of brain tissue as the significant factor in inherent resistance, we proceeded to analyze acquired resistance, especially that acquired resistance developed through exposure to the specific agent. The present studies describe the immunity of mice following subcutaneous or intraperitoneal vaccination with virulent St. Louis encephalitis virus.

Materials

St. Louis virus, strain 3, was passed as routine from brain to brain of susceptible mice. When required for tests, the virus-containing brain was removed from a prostrate susceptible mouse 4 to 5 days following an intracerebral injection. The brain, weighing approximately 0.4 gm., was triturated in a mortar, diluted with 3.6 cc. of hormone broth, pH 7.4, to make an approximately 10 per cent suspension, and centrifuged at 1,000 R.P.M. for 5 minutes. The supernatant of the 10 per cent

suspension was removed and further diluted in a tenfold series. Upon intracerebral injection, 0.03 cc. of the 10^{-7} dilution was fatal to 50 per cent or more of tested mice, hence 3×10^{-9} cc. were taken as the intracerebral titre of virus under these conditions. The brain is said to contain, therefore, approximately 10^9 intracerebral lethal doses of virus. Similarly, upon nasal instillation, 0.03 cc. of the 10^{-4} dilution was fatal to 50 per cent or more of tested mice, hence 3×10^{-6} cc. were taken as the intranasal titre.

Mice used in these experiments were the Swiss strain selectively bred for susceptibility to certain central nervous system viruses (1). When tested with St. Louis virus they were consistent and highly uniform in their reactions. When vaccinated with virulent virus given subcutaneously or intraperitoneally, all animals remained well. Consequently, their subsequent immunity was attributed entirely to the direct effect of the vaccine upon each individual with no disturbing possibility that part of the immunity of the batch might be the result merely of a weeding out of the susceptible individuals through the lethal effect of the vaccine.

EXPERIMENTS

Amount of Immunity Developed. Time of Appearance and Duration of Immunity.—Susceptible mice injected subcutaneously with virulent virus develop an immunity against both intracerebral and intranasal test inoculations. This immunity appears in about 4 days, is active against 10^5 intracerebral and 10^3 intranasal test doses within 1 week, endures for 8 weeks at this approximate level, and disappears in 12 to 24 weeks. The following protocol illustrates some of these findings (Table I).

Experiment 1.—Mice were given 0.5 cc. of virus diluted 1 to 1,000 subcutaneously in the groin. At 1, 7, 14, 20, and 24 weeks batches were tested for intracerebral and intranasal immunity, together with control unvaccinated mice of the same age. Table I shows that the intracerebral immunity was active in 1 week against 10^6 lethal doses, in 7 weeks against 10^5, in 14 weeks against 10^2, and that at 20 and 24 weeks the immunity had practically disappeared. Similarly the intranasal immunity was active in 1 week and at 7 weeks against at least 10^2 doses, in 14 weeks against 10 doses, and at 20 and 24 weeks was negligible.

Similar experiments have given comparable results. Some nasal immunity was detected as early as 4 days following vaccination.

Amount of Virulent Virus Required for Immunization.—A subcutaneous injection of at least 500 intracerebral lethal doses of virus is required to immunize a mouse successfully (Table II).[1]

[1] Cox and Olitsky (*J. Exp. Med.*, 1936, **63**, 745) found that approximately this same amount of virulent equine encephalomyelitis virus is necessary to immunize a mouse.

TABLE I

Amount, Time of Appearance, and Duration of Immunity of Mice Following Vaccination with St. Louis Encephalitis Virus

Mice	Intracerebral test. Dilution of virus							Amount of immunity Intracerebral lethal doses	Intranasal test. Dilution of virus					Amount of immunity Intracerebral lethal doses
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	
Controls	—	—	—	6,* 6, 7	6, 7, 7	7, 7, 9	8, 8, 9	10^6	—	—	7, 9, 9, 11, 11	—	—	
Vaccinated (1 wk.)	S, S, S	S, S, S	S, S, S	S, S, S	—	—	—		S, S, S, S, S	S, S, S, S, S	S, S, S, S, S	—	—	10^2+
Controls	—	—	—	5, 5, 5, 6	5, 5, 6, 6	5, 6, 6, 7	6, 7, 8, 8		—	7, 7, 7, 8	7, 7, 7, 9	9, S, S, S	—	
Vaccinated (7 wks.)	8, 9, S	S, S, S	S, S, S	S, S, S	—	—	—	10^5	S, S, S, S, S	S, S, S, S, S	S, S, S, S, S	—	—	10^2+
Controls	—	—	—	4, 5, 5, 6	7, 7, 8, 9	8, 8, 10, S	S, S, S, S	—	—	—	5, 5, 6, 6, 6	—	—	
Vaccinated (14 wks.)	—	—	6, 7, 7	8, S, S	—	—	—	10^2	7, 8, 8, 10, S	—	—	—	—	10
Controls	—	—	—	—	—	5, 6, 6	6, 6, 7		—	—	9, 12, S	9, 12, S	S, S, S	
Vaccinated (20 wks.)	—	—	—	—	6, 6, 7	5, 9, S	5, S, S	1	—	10, 12, S	12, 12, S	9, S, S	—	1
Controls	—	—	—	—	—	7, 7	7, 7		—	—	7, 9	9, S	—	
Vaccinated (24 wks.)	—	—	—	—	—	7, S, S	7, S, S	1±	—	—	11, S, S	9, S, S	—	1±

* Day of death of mouse following injection.

S = mouse remained well 30 days.

— = dilution not tested.

Experiment 2.—A single subcutaneous injection of 1.5 cc. of mouse brain virus diluted from 10^{-3} to 10^{-7} was given to batches of Swiss mice. The 10^{-7} dilution contained approximately 50 intracerebral lethal doses, the 10^{-3} dilution, 500,000 doses. 3 weeks later part of the vaccinated mice, together with unvaccinated controls, were given test virus intracerebrally and part intranasally. The smallest intracerebral dose, 10^{-5}, used in the test contained 100 lethal doses, the smallest intranasal dose, 10^{-4}, contained not more than one nasal lethal dose as determined by many titrations. The test was therefore sufficiently sensitive to detect a small amount of immunity. Table II shows that vaccine containing 50 intracerebral lethal doses conferred no immunity, but that vaccine containing 500 or more units did induce the characteristic high immunity.

TABLE II

Amount of St. Louis Encephalitis Virus Required to Immunize Mice

Immunizing dose Number of intracerebral lethal doses	Intracerebral test Dilution of virus		Intranasal test Dilution of virus	
	10^{-4}	10^{-5}	10^{-3}	10^{-4}
None	4,* 5, 5	6, 7, 7	7, 8, 8	9, 9, 10
50	4, 5, 7	5, 5, 6	7, 8, S	9, S, S
500	S, S, S	S, S, S	9, S, S	6, S, S
5,000	6, S, S	S, S, S	S, S, S	S, S, S
50,000	S, S, S	S, S, S	S, S, S	S, S, S
500,000	S, S, S	S, S, S	S, S, S	S, S, S

* Day of death of mouse following injection.

S = mouse remained well. Discarded after 30 days.

Additional experiments gave comparable results. Moreover, not only fresh mouse brain virus but virus maintained under other conditions behaved in a similar manner. For example, virus grown in mouse embryo brain plus 10 per cent serum-Tyrode culture in our laboratory titrated 10^{-6}. This culture virus diluted to 10^{-3} to contain 1,000 intracerebral lethal doses immunized mice against the virulent mouse brain virus. Greater dilutions, however, containing less than 1,000 doses, did not protect. Again, brain virus kept in the frozen and dried state gradually decreased in titre until at 12 months, certain samples contained no virulent virus, others about 500 intracerebral lethal doses per 0.03 cc. tested, and still others, about 1,000 lethal doses. The immunizing potency of these preparations was tested in mice by administering a single subcutaneous dose of 0.5 cc. containing

in the first instance 1,000 intracerebral lethal doses, in the second instance 500 doses, and in the third instance non-virulent virus. The first preparations immunized the mice well, the second preparations gave both positive and negative results, while the third preparations, containing no virulent virus, failed to immunize.

Multiple doses of virulent virus gave no more prompt, no greater, nor more enduring immunity than a single dose containing about 10,000 intracerebral lethal doses.

Having found that a single subcutaneous or intraperitoneal injection of 1,000 intracerebral lethal doses of virus into susceptible mice immunized them regularly after 4 to 7 days against 10^6 intracerebral or 10^3 intranasal doses and that this immunity persisted 8 weeks and finally disappeared at about 20 weeks, we undertook further analyses of this immune state and of the immunizing process concerned. Our technique was guided by the view expressed recently by Goodpasture that in the voluminous literature on the subject of immunity there is a great deal about phagocytosis and antibodies and complement in general, but no scientific explanation of an acquired resistance to any infectious agent, due mainly, perhaps, to a lack of knowledge of the pathogenesis of the natural disease (3). Consequently we paid special attention to the question of pathogenesis of infection in the immune mouse as compared to pathogenesis of infection in the normal mouse.

Pathogenesis of Infection in Immune Mice.—The outstanding finding to date in these experiments has been that virus given nasally or directly into the brain of immunes does not gain a foothold in the brain tissue.

Experiment 3.—Forty susceptible mice were each given subcutaneously 0.5 cc. of fresh mouse brain virus diluted 1 to 1,000. 12 days later each received 0.03 cc. of fresh virus diluted 1 to 100 into the intact nasal orifices. Five unvaccinated mice were similarly inoculated. 1, 2, 3, 4, 6, 8, 10, 15, 25, 35, and 45 days after the test inoculation, three mice were sacrificed,—one for sections of the brain and two for testing for the presence of virus in the olfactory bulbs and in the brain. The remaining seven mice were observed for 30 days.

The unvaccinated mice receiving the nasal instillation of virus were dead by the 8th day; the seven vaccinated mice inoculated intranasally and set aside for observation remained well, indicating that the batch of forty test animals were immunized against a nasal

dose fatal to 100 per cent of non-vaccinated animals. None of the twenty-two animals sacrificed and tested for the presence of virus gave positive results and none of the eleven examined for lesions showed anything abnormal in the olfactory bulbs or brain. Apparently the virus instilled intranasally into immunized mice did not reach olfactory bulbs or brain in detectable amounts.

Finally, animals similarly immunized and given a test intracerebral injection of at least 1,000 lethal doses of virus disposed of the virus within 4 days and showed lesions related mainly to the puncture wound of the hypodermic needle used for the test injection.

The pathogenesis of infection in immune mice following nasal or intracerebral injection of virus differs sharply, then, from that in non-immune mice. In the immune mouse the test virus fails to survive in the brain; in the non-immune mouse it reaches the olfactory bulbs within a few hours following nasal instillation, spreads and increases rapidly, overwhelming the mouse (1). These differences appear to be due directly to conditions in the brain tissue or associated fluids.

Lack of Antiviral Properties in Blood Serum of Immune Mice.—The fluids of immune mice, of which blood serum is probably the most representative, may theoretically act directly against the virus which has reached its tissue of predilection, namely, the brain and cord, or in some sort of brain tissue combination, or again they may be entirely inert. The first possibility found no support in a large series of tests in which blood serum from immune mice vaccinated as above, when combined with virus and injected directly into the brain of susceptible mice, in no way altered the pathogenesis of the resulting infection. The serum from immune animals neither hastened destruction nor reduced titre of the virus. The following protocol illustrates the equally high titre of virus mixed with sera or with optimum broth diluent (Table III).

Experiment 4.—Susceptible mice were divided into two batches. One received an immunizing dose of fresh mouse brain virus, 0.5 cc. of 1 to 1,000 dilution intraperitoneally; the other remained untreated as controls. After 1 week, part of the vaccinated animals were tested for immunity and part were bled for serum for a neutralization test. Equal parts of serum were mixed with equal parts of mouse brain virus in various dilutions in broth. The mixtures were incubated for 2 hours

at 37°C., left standing for 2 hours at 23°C., and finally, each dilution was injected in 0.03 cc. amounts intracerebrally into four Swiss mice. Virus was also mixed with serum from unvaccinated mice and tested in a similar manner.

Table III shows that the virus was as active when mixed with serum from immune mice as when mixed with serum from unvaccinated mice or with broth alone.

Tests for antiviral substances in the sera of animals vaccinated as above have been made throughout the 10 to 20 week span of immunity (4). During the 4 to 8 week period, when the immunity of the mice

TABLE III

Absence of Neutralizing Antibodies in Sera of Immune Mice Following Vaccination with St. Louis Encephalitis Virus

Virus diluted with	Mice injected with 0.03 cc. mixtures of serum plus virus diluted		
	10^{-5}	10^{-6}	10^{-7}
Broth	5,* 5, 5, 5, 5	5, 5, 5, 7, 7, S	11, S, S, S, S
Serum, normal mouse	5, 5, 5, 6	5, 6, 6, S	7, S, S, S
Serum, vaccinated mouse (1 wk.)	5, 5, 5, 6	6, 6, 9, S	—

Immunity of Mice from Which Test Sera Were Obtained

Mice	Mice injected with 0.03 cc. virus in dilutions					
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Normal mice	—	—	—	5, 5, 5, 6	5, 6, 6, S	S, S, S, S
Vaccinated mice (1 wk.)	8, S, S	S, S, S, S	S, S, S, S	S, S, S, S	—	—

* Footnotes same as in Table I.

to a test intracerebral or intranasal injection of virus was high, antiviral activity of their sera was not detected; subsequently, however, as the immunity of the mice decreased and finally disappeared, antiviral activity of their sera appeared and increased progressively. In short, not only is antiviral activity of the serum undetectable during the period of maximum immunity of the animal, but it becomes detectable when the active immunity disappears.

Although sera from immune mice do not act against the virus under the above somewhat artificial conditions, it is possible that they may be effective under more natural conditions; this has been

difficult to test. If virus is injected intravenously into immune and non-immune animals, it disappears so promptly that no differences can be detected. Virus injected intraperitoneally, however, into immunes and non-immunes appears in the circulating blood in slightly higher titre and for a slightly longer interval in non-immunes than in immunes. Whether this small difference is attributable to blood or tissues of immune mice is not clear.

Experiment 5.—Fifteen mice were vaccinated with 0.5 cc. of a 10^{-3} dilution of virus given subcutaneously. Fifteen similar mice were left untreated as controls. 1 week later, when the vaccinated mice were known to be immune, they, together with the controls, were given 0.5 cc. of a 0.5×10^{-2} dilution of virus intraperitoneally. At frequent intervals thereafter, vaccinated and control mice were bled from the heart. Undiluted blood and blood diluted 10^{-1} and 10^{-2} were injected in 0.03 cc. amounts intracerebrally into two Swiss mice to titrate the amount of virus present.

The virus content of the blood of immune and non-immune mice is shown in Table IV. Following the intraperitoneal injection of about 2,000,000 intracerebral doses of virus, the blood of both immunes and non-immunes showed about 3,000 doses per cc. within 20 to 30 minutes. This quantity was recovered at intervals during a period of 3 hours and 40 minutes from non-immunes; from immunes the quantity dropped progressively to less than 30 doses per cc. (or 1 per 0.03 cc. which was the limit of sensitivity of the test) at 5 hours. None was recovered from immunes thereafter, although the non-immunes showed 300 doses at 5 hours and 30 at 6 hours.

A search for indirect evidence of activity of serum upon the virus in the animal was made by means of endothelial blockade and splenectomy respectively. Trypan blue was injected intravenously into susceptible mice in various doses ranging from a toxic to a barely coloring level. At different periods thereafter, from 4 to 24 hours, the mice were vaccinated and tested 3 weeks later for their immunity to an intracerebral injection. According to theory, the dye, by blocking the endothelial cells' phagocytic activity, interrupts the development of antibody formation. In these tests, however, despite the injection of dye, the animal developed the usual high grade of intracerebral immunity. Similarly, batches of animals were splenectomized 24 hours before vaccination and 1, 2, and 3 days following vaccination. 3 weeks later their immunity to an intracerebral injection was tested. Again the procedure of splenectomy, calculated to interrupt antibody

formation, did not disturb the development of the standard high level of intracerebral immunity.

At present evidence indicates that the essential difference between the immune and non-immune mouse rests in the changed condition of

TABLE IV

Titre of St. Louis Virus in Blood of Vaccinated and Unvaccinated Mice Following Intraperitoneal Injection

Time after intraperitoneal injection of virus	Source of blood tested	Fate of mice injected with 0.03 cc. blood in dilutions			Titre of virus in blood Lethal intracerebral doses per cc.
		Undiluted	10^{-1}	10^{-2}	
10 min.	Vaccinated	—	—	—	—
18 "	Unvaccinated	6,* 6	6, 6		330
24 "	Vaccinated	6, 6	6, 9	6, 13	3,300
28 "	Unvaccinated	6, 6	8, 9	8	3,300
50 "	Vaccinated	6, 6	8		330
50 "	Unvaccinated	4, 4	6, 6	9, 6	3,300+
1 hr. 45 min.	Vaccinated	8, 8			33
1 " 52 "	Unvaccinated	6, 6	6, 8	6, 6	3,300
3 hrs. 38 "	Vaccinated	8	13		33+
3 " 41 "	Unvaccinated	6, 6	6, 8	6	3,300
5 "	Vaccinated				Less than 33
5 "	Unvaccinated	6, 8	9		330
6 "	Vaccinated				Less than 33
6 "	Unvaccinated	6, 8			33
7 "	Vaccinated				Less than 33
7 "	Unvaccinated				" " "
25 "	Vaccinated				" " "
25 "	Unvaccinated				" " "
30 "	Vaccinated				" " "
30 "	Unvaccinated				" " "
48 "	Vaccinated				" " "
48 "	Unvaccinated				" " "
72 "	Vaccinated				" " "
72 "	Unvaccinated				" " "

the brain tissue itself, the tissue to which the virus has a special predilection.

Fate of Virus Employed for Vaccine.—The question of how the brain tissue becomes altered is being investigated. Does the virus in the vaccine circulate in the blood, penetrate the brain capillaries, and come into intimate contact with brain cells, or does it localize else-

where and produce an immunizing substance which then reaches brain tissue through the circulation? Previous tests (1) showed that virus injected as a vaccine subcutaneously or intraperitoneally was present within 10 minutes in the blood stream in large amounts but that rarely after 1 and never after 5 days was it found in blood or organs except the spleen. Moreover, the mice remained well and showed no brain lesions. Since mice invariably contract a fatal encephalitis if as little as 1/1000 cc. of virus-containing blood is injected directly into their brain, or if it is provided access to brain cells through trauma following a sterile intracerebral needle puncture (1), it is unlikely that the brain cells of vaccinated mice were exposed to the virus in the blood.

Persistence of Virus in the Spleen.—Following disappearance from blood and organs, virus remains in the spleen and increases somewhat in titre (1). Moreover, it can be recovered, although with increasing irregularity, up to but not later than 34 days following injection. Throughout this time the immunity of the mouse is at a maximum; shortly afterwards, however, it commences to decline. There would appear to be some relation, therefore, between duration of virus in the animal body and duration of immunity. Further experiments on this question are in progress.

DISCUSSION

The experiments described above are limited strictly to the immunity which follows vaccination with virus by some artificial route. Only to this extent, therefore, may the immunity produced by St. Louis encephalitis virus be compared with experimental immunity produced by other human viruses having special or exclusive predilection for the central nervous system. St. Louis virus, in the first place, is a relatively powerful immunizing agent, protecting the animal against a subsequent injection of a large amount of virus directly into the brain. Thus the St. Louis-immunized mouse will withstand 100,000 lethal doses given intracerebrally, the Japanese B and rabies-immunized mouse about 1,000 lethal doses, whereas the poliomyelitis-immunized monkey fails to withstand even one lethal dose with regularity. Secondly, the immunity of the St. Louis-vaccinated mouse, like that of the Japanese B and rabies-vaccinated mouse, is not permanent. Immunity to St. Louis virus is maximum for 4 to 6 weeks

and disappears by the 10th to 20th week; immunity to rabies is maximum for about 6 months and disappears after 12 months. Finally, the duration of immunity of the St. Louis-immunized mouse may have some connection with the persistence of virus in the tissue. This possible relationship is being studied further.

These studies also bear upon the question of whether immunity to this group of viruses is humoral or tissue in nature. Recent English reviewers of immunity to virus diseases (5–7) regard all acquired specific immunity as the result of a corresponding circulating antibody. This view seems to rest largely on the fact that in many infections, immunity, whether produced in nature or by artificial means, is associated with specific circulating antibody not otherwise present and that this antibody acts in one way or another against the specific agent.

Not always does this simple relation hold, however, and notable exceptions have recently been studied in the group of central nervous system virus infections. For example, the monkey vaccinated with poliomyelitis virus develops neutralizing antibodies but is not regularly immune (8–10); the same is true for the mouse vaccinated subcutaneously with rabies virus (11). Conversely, the mouse vaccinated with St. Louis virus becomes immune but shows no circulating neutralizing bodies. Therefore, these two sets of contradictions to the general theory above require consideration.

Bedson attempts to reconcile the data on antibody without immunity (6) by assuming that the poliomyelitis neutralizing bodies in the monkey without immunity are derived from resistant tissues and therefore play no part in resistance. In Sabin and Olitsky's opinion, however (10), there is no evidence that neutralizing antibodies from the poliomyelitis-vaccinated, non-immune monkey differ from those in the convalescent immune monkey. A further example not cited by Bedson is found in our report of mice vaccinated with rabies virus (11). If the mouse is vaccinated subcutaneously it develops high titre neutralizing antibodies but no immunity; if vaccinated intraperitoneally with the same suspension, it develops neutralizing antibodies of similar titre plus immunity. The circulating neutralizing antibodies in the non-immune have not been distinguished from those in the immune mouse. Hence, with no reason to believe that anti-

bodies in non-immunes differ from those in immunes, the fact remains that the presence of high titre circulating neutralizing antibodies in an animal does not necessarily render it immune.

With regard to the second contradiction, immunity in the absence of circulating antibodies, Bedson (6) and Burnet (7) postulate a mechanism of local formation and functioning of undetectable antibodies, again without basis on fact. Burnet suggests further that this postulate might be tested in a nasally induced neurotropic virus infection in a host in which infection rate is high but morbidity low. Studies on pathogenesis of such an infection have already been reported (2) with no evidence of local formation or functioning of antibodies conditioning infection or morbidity. Rather the initial degree of innate resistance of the brain tissue appears to condition pathogenesis throughout. Hence with no evidence of local formation or functioning of circulating antibodies, the fact remains that animals may show a high degree of immunity to certain central nervous system virus infections in the absence of detectable circulating antibodies.

The present experiments with St. Louis encephalitis virus in mice have special weight in that they express quantitative relations between a very high degree of immunity and a negligible amount of antibody activity, and over periods of time when both immunity and antibodies are changing. The blood and extracted brain tissue of immune animals fail to protect the brain of susceptibles against one lethal dose of virus, while the brain in the living immune mouse resists a direct injection of 100,000 lethal doses of virus. Moreover, immunity and neutralizing antibody titre vary in opposite directions in that antibody is minimum when immunity is maximum and increases as immunity decreases. Finally, studies on pathogenesis of infection in susceptible mice following nasal or intracerebral inoculation of virus show a prompt spread of virus and lesions in close association with nervous tissue. In immune mice, however, 1,000 to 100,000 lethal doses of virus are blocked and destroyed at once, indicating a highly potent antagonism not found in serum. This blocking of a tissue type of pathogenesis in the immune mouse, together with the failure to detect antibody activity in the sera of immunes, and the quantitative variation of immunity and antibody titre in opposite directions indicate that the immunity of the mouse to St. Louis

virus following vaccination rests largely on the acquired refractory state of the brain tissue itself.[2]

At the moment, therefore, immunity in certain of the central nervous system virus infections is not explainable in terms of a circulating antibody acting directly against the virus.

CONCLUSIONS

1. Susceptible mice injected subcutaneously or intraperitoneally with 15,000 intracerebral lethal doses of St. Louis encephalitis virus develop an immunity in 4 to 7 days to 1,000 to 1,000,000 lethal doses given either intracerebrally or intranasally.

2. This immunity persists 4 to 6 weeks, then decreases gradually and disappears after 8 to 12 weeks.

3. More than 1,000 intracerebral doses of virus given as a vaccine do not materially increase the amount or duration of the immunity; less than 1,000 doses give little or no immunity.

4. Test virus injected intracerebrally into immunized mice induces few lesions and is rapidly destroyed; instilled intranasally, it rarely reaches the olfactory lobes or brain.

5. While immunity is maximum, circulating neutralizing antibodies are not detectable. Moreover, the immunity is not affected by endothelial cell blockade or by splenectomy.

6. A few moments after the immunizing virus is given, it can be recovered from the blood in relatively high concentration. After 24 hours, the blood no longer contains demonstrable virus nor do any organs thus far tested except the spleen. The brain and cord remain entirely normal. The spleen, however, becomes enlarged and harbors virus for as long as 30 days.

[2] The immunity of mice to St. Louis encephalitis infection following vaccination may or may not be similar to that accompanying convalescence from the disease. The same is true of poliomyelitis immunity in the *Macacus* monkey. Hence analogous findings in the St. Louis-vaccinated immune mouse and the poliomyelitis-convalescent immune monkey are, at the moment, of questionable significance. Attention may merely be directed to the fact that in both the vaccinated mouse and convalescent monkey (10, 12), test virus instilled nasally or injected intracerebrally is promptly destroyed in the absence of detectable antibodies.

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