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Demonstration, Properties and Significance of Neutralizing Antibodies in Mature Mice Immune to Lymphocytic Choriomeningitis (LCM)

By

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The reaction to LCM virus is different in mice infected naturally in the embryonic stage and mature mice infected experimentally or by contact (12, 1). While the former are unable to produce demonstrable amounts of specific antibodies (18, 2, 17), the latter respond with the formation of complement-fixing (CF) antibodies (8, 9, 18) and, irregularly, with the production of neutralizing antibodies (5). The experiments described in this paper mainly concern the immunity of mice which contracted the infection as mature animals (5 weeks of age or older).

For a long time it was doubtful whether such mice would be capable of forming specific neutralizing antibodies. Different investigators failed to detect them in the serum using the intracerebral (i. c.) test in mice or the more sensitive subcutaneous (s. c.) test in guinea pigs (11, 18, 8, 2, 10). It was likewise not possible to demonstrate such antibodies in extracts of the retromediastinal lymph nodes of mice which had been infected by intranasal instillation of LCM virus (6, 7). Mice inoculated in the same manner with the viruses of influenza, vaccinia, Newcastle disease or tick-borne encephalitis gave good antibody responses. Simultaneous infection of mice with LCM and influenza viruses induced the formation of neutralizing antibodies for influenza but not for LCM virus (6, 10).

Rowe (5), in 1954, for the first time reported positive neutralization tests with the sera of mature mice infected experimentally with the virus under study. He was in the possession of a strain which regularly caused serous pleuritis and/or peritonitis in mice inoculated intraperitoneally (i. p.) and used the i. p. route in his neutralization and protection tests, measuring the amount of serous exudate present during the period of maximal symptoms as an indicator of the severity of the infection. *Rowe* concluded from his studies on the mechanism of the protective effect of the antibodies that the initiation of infection was not affected but the course of the disease was ameliorated. Growth of the virus appeared to progress at the same rate in mice treated with immune and normal serum, respectively, but the former were able to eliminate the virus earlier than the controls. Application of the

passive protection technique to tests on individual mice produced evidence of antibody in about half of the mice tested. No correlation was found between the presence of antibody and the carrier state or the status of cerebral immunity. The same technique applied to brain and organ suspensions of immune mice yielded consistently negative results. Antibodies were detected in the blood or serum of mice as early as 13 days and as late as 14 months after infection.

Own experiments (15, 17) have confirmed the formation of neutralizing antibodies by LCM-infected mature mice, but the weakness and irregularity of the reactions made the interpretation of their significance difficult. It was therefore decided to reinvestigate the problem and search for optimal conditions for the neutralization of LCM virus by homologous specific antibody *in vitro*.

In the present paper a method suitable for the demonstration of neutralizing antibodies in serum pools from LCM-immune mice will be described and an attempt be made to evaluate their role as a factor in cerebral immunity. Moreover, the question raised by *Rowe*'s observations as to whether these antibodies may differ in some respects from those resulting from infection with other viruses will be discussed.

Materials and Methods

Strains of virus: Strain W, isolated from a naturally infected wild mouse kindly furnished by Dr. *V. H. Haas*, National Institutes of Health, Bethesda, Md., U.S.A., was used in experiments recorded in Tables 2, 4, and 5 as well as for i. c. challenge inoculations because it regularly causes typical tremors and convulsions in 12 day-old and older mice infected by this route. It had been passed twice by i. c. inoculation in mice.

Strains WCP and WCC, obtained through the courtesy of Dr. *W. P. Rowe* of the same institute, had been transferred 365 times in mice by i. p. and i. c. inoculation, respectively.

Strain WCP regularly causes a severe disease characterized by serous pleuritis and peritonitis as well as by marked emaciation in mice inoculated i. p. In addition, some mice show tremors and convulsions but frequently recover from them in contrast to adult mice infected i. c. Mortality varies between 20 and 60 per cent and survivors do often not regain their original strength and vitality. This strain is suitable for i. p. neutralization tests (see Table 3).

Strain WCC is highly pathogenic when injected i. c. in 1 to 4 day-old mice as well as in adult animals. It was used in experiments listed in Tables 1, 4, and 5.

Mice: All experiments were carried out with mice of Breed No. III of the Institute. This stock is of American origin. An intestinal infection with Theiler's virus is endemic in it, but this agent did not disturb the LCM experiments in any way. It has not contaminated LCM virus passed by i. c. inoculation.

Materials tested for neutralizing antibodies: Immune serum 1 was obtained from mice infected s. c. with strain W at the age of 6 weeks and hyperimmunized 9 times with infectious mouse brain suspension (17, Group 9).

The donors of the immune sera 2 and 5 were infected by contact with congenitally infected mice when 10 to 12 weeks old and hyperimmunized by one i. c. inoculation of infectious 10 per cent mouse brain suspension. The animals were exsanguinated 2 weeks later.

Immune serum 3 was furnished by congenitally infected mice hyperimmunized by 2 inoculations with 10 per cent mouse brain suspension (17, Group 2). It repeatedly gave negative CF tests.

Immune serum 4 was obtained from mice exposed to contact infection at the age of 10 to 12 weeks and bled 4 to 6 weeks after exposure. The animals were not hyperimmunized.

The immune blood and plasma tested in experiment 4 (Table 3) as well as the brains tested in experiments 5, 6, and 7 came from mice infected with strain W at the age of 5 to 6 weeks and hyperimmunized once by i. c. inoculation of 10 per cent mouse brain suspension. The materials were harvested 2 to 3 weeks after the last inoculation.

It should be mentioned in this connection that the strains of LCM virus used are uniform serologically.

Neutralization tests: Infectious 20 per cent mouse brain suspension from the 2nd i. c. passage of strain W served as source of virus in the i. c. tests (Table 2), while in the i. p. tests (Table 3) a 20 per cent suspension of spleen and lymph nodes from the 365th i. p. passage of strain WCP was used (experiments 1, 2, and 4). Later, liver tissue was added to spleen and lymph nodes after it had been found that this organ has the highest virus content in WCP-infected mice (experiments 3, 5, 6, and 7). The suspensions were prepared with Pyl's isotonic buffer pH 7.4 and kept deep-frozen in amounts of 0.2 ml.

In all experiments serial five-fold virus dilutions were made using as diluents the materials to be tested (serum, blood, brain extract or suspension from immune and normal mice). In some tests the materials were heated at 56° C for 30 minutes prior to use, in others heating was omitted (see tables). The brains tested in experiments 5 and 6, Table 3, were suspended in 4 volumes of heated normal mouse serum and those used in experiment 7 were extracted with 4 volumes of isotonic buffer solution. All materials contained 100 units penicillin and 1 mg. streptomycin per milliliter. The time and temperature of incubation of the virus dilutions before inoculation are indicated in Tables 2 and 3. In all tests six 4 to 5 week-old mice were used per dilution. The i. c. dose was 0.04 ml., the i. p. dose 0.15 ml. except in experiment 7, Table 3, in which 0.4 ml. were injected i. p. per mouse.

The test animals were observed for symptoms for 2 weeks and, in i. p. tests, the severity of the disease in individual mice was marked with plus signs in the protocols, + meaning mild, ++ moderate, +++ severe symptoms and ++++ a fatal infection. Survivors which had not shown definite symptoms were tested for immunity by i. c. injection of 2 per cent mouse brain suspension (strain W). Mice which had acquired cerebral immunity were counted as infected by the first inoculation and the severity of the disease was rated as zero in such cases.

The 50 per cent infectivity titer (ID_{50}) and the 50 per cent pathogenicity titer (SD_{50} according to *Rowe*) were computed using the Reed-Muench formula. In addition, the average severity of the disease in i. p. inoculated groups of mice was calculated by adding the number of plus signs recorded for individual animals and dividing the sum by the number of mice. Since some mice infected i. p. with strain WCP show little or no pleural exudate but severe meningeal symptoms, measurement of the amounts of serous fluid in the pleural and peritoneal cavities (5) would not have given a more accurate picture of the severity of the disease than the simpler method used in the present tests.

To obtain the number of ID_{50} or SD_{50} neutralized by an immune serum the logs of the infectivity or pathogenicity titers of the virus in the presence

of normal serum and the respective immune serum were subtracted and the corresponding antilogs recorded in the tables.

Virus titration in suckling mice descending from immune and normal mothers: The immune mice whose litters were tested for susceptibility to i. c. infection were immunized by s. c. inoculation with 0.2 ml. of a 2 per cent mouse brain suspension, which caused no visible reaction, and hyperimmunized 2 weeks later by i. c. injection of 0.04 ml. of a similar suspension. The animals were solidly immune, while the controls died in typical convulsions. Two weeks after challenge the immune females were bred to normal males. It was known from previous experiments (16) that intrauterine infection of the embryos would not occur under such conditions because mature mice infected i. p. and challenged i. c. eliminate the virus rapidly.

In preliminary susceptibility tests with normal litters i. c. infection with strain WCC was almost always fatal in 1 to 6 day-old mice but somewhat less severe in 8 to 14 day-old animals, while the reverse was true for strain W. Consequently, strain WCC was used to test the susceptibility of 4 day-old litters and strain W for tests with 12 and 14 day-old sucklings.

In the titrations decimal dilutions of 20 per cent mouse brain suspensions were inoculated i. c. in etherized baby mice of the respective age (see Tables 4 and 5) using one immune and one normal litter per dilution. As a rule, the litters comprised 7 to 10 animals each. Every litter was nursed by its mother and kept in a separate cage. The tests were usually confined to the dilution range of 10^{-2} to 10^{-7} in which the endpoints were expected. After an observation period of 4 weeks the surviving infantile mice, together with their mothers, were challenged i. c. to detect the animals which had contracted an inapparent infection.

The ID_{50}, SD_{50} and LD_{50} (50 per cent mortality titer) were calculated according to the method of *Reed* and *Muench*.

Rate of elimination of i. c. injected virus from the brains of cerebrally immune mice

Rowe (5) and *Haas* (2) failed to obtain evidence of multiplication of LCM virus after i. c. inoculation into mice which had previously resisted an i. c. challenge injection. Since materials from mice challenged i. c. were used predominantly in the neutralization tests described below, it was of interest to investigate the strength of their immunity as indicated by their reaction to challenge and the rate of elimination of reinoculated virus.

Twenty-four mice were injected s. c. each with 0.2 ml. of a 10 per cent mouse brain suspension (strain WCC). Two weeks later they were solidly immune to i. c. challenge with a 2 per cent mouse brain suspension, which was fatal in the controls. The second i. c. challenge inoculation with 2 per cent mouse brain extract (strain WCC) was made 3 weeks after the first i. c. injection including 24 normal controls of the same age and sex in the test. As can be seen in Table 1, the animals were sacrificed different periods of time after inoculation (two immune and two normal mice at each interval) and their brains tested individually for infectivity injecting 20 per cent suspensions i. c. each into 3 normal mice. The two remaining immune mice showed no symptoms and the normal mice were all dead on the 6th day so that no tests could be made on the 7th day.

As Table 1 shows, the decrease in brain infectivity during the first 12 hours was about the same in immune and normal mice. There was only a very slight neutralizing effect, if any, in the immune brains. Formation of new virus started in the brains of the normal mice between the 12th and the 24th hour, but no virus multiplication took place in the immune mice. The traces of virus detected in two immune brains 48 and 72 hours, respectively, after inoculation presumably were residues of the injected virus. The result of this test is similar to that of a corresponding

Table 1. Fate of intracerebrally injected LCM virus in cerebrally immune and normal mice

Hours after i. c. inoculation	i. c. infectivity tests with brains from											
	immune mice						normal mice					
	Series 1			Series 2			Series 1			Series 2		
½	+10*	i**	i	+9	i	−†	i	i	i	+7	+7	+8
3	+8	+8	+8	i	i	−	+10	+10	i	+7	+8	+8
6	+8	+9	−	i	−	−	+8	−	−	−	−	−
12	−	−	−	−	−	−	−	−	−	i	−	−
24	−	−	−	−	−	−	+6	+6	+6	+6	+6	+6
48	−	−	−	+7	+9	−	+5	+5	+6	+5	+5	+6
72	−	−	−	i	−	−	+5	+6	+6	+5	+5	+6
96	−	−	−	−	−	−	+5	+6	+6	+5	+5	+6
120	−	−	−	−	−	−	+5	+5	+5	+5	+5	+6
144	−	−	−	−	−	−	+5	+5	+5	+5	+5	+5
168	−	−	−	−	−	−						

* mouse died in convulsion on 10th day after inoculation
** mouse failed to show definite symptoms by acquired cerebral immunity
† no symptoms and no immunity

experiment reported by *Rowe* (5) except that the so-called eclipse phase came out more clearly in the present test due to the fact that less virus had been injected i. c.

Neutralization tests with serum, blood, plasma, and brain suspensions from immune mice

In the following experiments and others not presented here the significance of certain factors which might influence the neutralization of LCM virus by specific antibody was studied. Such factors are: the route of inoculation, heating of the antibody-containing materials at 56° C for 30 minutes and the time and temperature of incubation of the mixtures of virus and antibody prior to injection. In addition, the relative neutralizing effect of whole blood and plasma from immune mice was investigated, since *Rowe* had obtained favorable results in neutralization and protection tests with whole blood.

It is evident from Table 2, in which two i. c. neutralization tests are recorded, that there was little, if any, neutralization when the mixtures were not incubated before inoculation but a definite neutralizing effect when they were kept at 37° C for 15 hours and, subsequently, in the refrigerator for 2 hours. Heated immune serum seemed to give slightly better results than unheated serum.

Table 2. Neutralization tests with unheated and heated mouse immune sera
(i. c. inoculation of mixtures)

Experiment No.	Incubation of mixtures	Serum	Unheated or heated at 56°C for 30 minutes	ID_{50} neutralized	SD_{50} neutralized
1	none	Immune serum 1	unheated	0	1
			heated	4	4
		Immune serum 2	unheated	0	0
			heated	1	1
2	15 hs. at 37° C 2 hs. at +2° C	Immune serum 1	unheated	10	4
			heated	>25	>20

Experiment 1 originally comprised mixtures incubated at 37° C for 24 hours and intended for comparison with the unincubated mixtures. However, the virus was inactivated by the incubator temperature both in the presence of immune and normal serum and the result therefore not conclusive. The time of incubation was reduced to 15 hours in experiment 2, but there still was so much thermal inactivation of virus in the control tubes that the amount of virus neutralized by the immune serum could not be accurately measured.

In the search for a substance which would protect this labile virus from the harmful effect of the incubator temperature chicken hemolysate, which exerts a marked protective effect upon Newcastle disease virus (13), and mouse hemolysate, which gave encouraging results with LCM virus (5), were tested without much success. Their efficacy was not better than that of undiluted normal mouse serum, heated or unheated.

The results of some i. p. neutralization tests are listed in Table 3. There again was little or no neutralization in mixtures not incubated

Table 3. Tests for neutralizing antibodies in serum, whole blood and brain from immune mice (i. p. inoculation of mixtures)

Experiment No.	Materials tested	Incubation of mixtures	ID_{50} neutralized	SD_{50} neutralized	Average severity of the disease in mice receiving	
					immune serum, blood or brain	normal serum, blood or brain
1	Immune serum 1, unheated	none	2	2	2.8+	3.3+
		24 hs. at 37° C	16	16	1.5+	2.8+
	dtto., heated	none	5	5	3.1+	3.6+
		24 hs. at 37° C	>12	>12	−*	2.9+
2	Immune serum 3, heated	24 hs. at 37° C	0	0	2.9+	3.1+
	Immune serum 4, heated	dtto.	>12	>12	−*	3.1+
3	Immune serum 5, heated	24 hs. at 37° C	250	320	2.9+	3.7+
		24 hs. at +2° C	79	79	3.7+	3.4+
4	Immune blood, unheated	22 hs. at 37° C	4	4	3.2+	3.3+
	Immune plasma, unheated	dtto.	>50	>50	−*	3.3+
5	Immune brain extract, heated	24 hs. at 37° C	0	1	3.1+	3.1+
6	Immune brain suspension, unheated	24 hs. at 37° C	1	1	3.4+	3.2+
		24 hs. at +2° C	1	2	3.0+	3.0+
7	Immune brain extract, heated	24 hs. at +2° C	0	0	3.3+	3.2+

* all mice not infected

before inoculation but a significant reduction of the infectivity titer in parallel mixtures incubated at 37° C for 24 hours (see experiment 1). Immune serum 3, originating from congenitally infected mice, completely failed to neutralize as was to be expected, whereas immune serum 4, obtained from mice infected by contact, contained neutralizing antibodies. Immune serum 5 had a marked neutralizing effect, which was greater in mixtures incubated at 37° C for 24 hours than in those kept in the refrigerator for the same period of time.

The result of experiment 4 is surprising inasmuch as heparinized whole blood effected hardly any neutralization, while heparinized plasma from the same animals neutralized more than 50 ID_{50} of the virus. It may be significant that, in this test, the incubator temperature inactivated 16 times more virus in the presence of normal plasma than in the presence of normal whole blood. The cells appear to have protected the virus to some extent from thermal inactivation as well as from specific neutralization by antibodies.

The immune mouse brain extracts tested in experiments 5 and 7 and the brain suspension used in experiment 6 gave practically negative results, since neutralization of less than 5 ID_{50} of the virus cannot be regarded as significant. Moreover, the severity of the disease was not affected by the materials tested.

It is noteworthy that the incorporation of liver tissue besides lymph nodes and spleen in the virus suspensions made accurate titer calculations possible even when relatively potent immune sera were used, as this was the case in experiment 3 and other tests not recorded in the table.

The results presented in Tables 2 and 3 show that neutralizing mouse immune sera not only decreased the severity of the disease (5) but also reduced the infectivity titer of the virus.

Relative susceptibility of suckling mice from immune and normal mothers for i. c. infection with LCM virus

The experiments reported in this section are part of a study of the significance of the neutralizing antibody as an immunity factor in LCM-immune mice.

Preliminary experiments with the viruses of influenza (FM 1) and eastern equine encephalomyelitis (EEE) had shown that actively immune mothers transmit a high degree of passive immunity to their descendents as this is the rule in many other virus diseases.

In the case of influenza, baby mice born and nursed by immune mothers had a high degree of pulmonary immunity when tested by intranasal instillation of virus at the age of 18 days. Quantitative tests were not made. The hemagglutination-inhibition titer of the mother was 1 : 80 and that of the suckling mice 1 : 160 on the 18th day after birth. In the baby mice the titer dropped

to 1 : 40 on the 32nd day, 1 : 10 on the 42nd day, and below 1 : 5 on the 81st day. In the mother it still was 1 : 80 at that time. It is clear therefore that the immunity of the infantile mice was a passive one.

EEE virus was titrated in suckling mice from cerebrally immune and normal mothers on the 12th day of life. The ID_{50} was $10^{-8.2}$ in normal litters and $10^{-2.9}$ in immune litters, the difference of 5.3 log indicating a high degree of passive immunity. The hemagglutination-inhibition titer of the serum was followed in one immune litter and its mother. Serum mixtures obtained from the young had titers of 1 : 200 on the 12th day, 1 : 24 on the 31st day, and zero on the 46th day. The respective values for the mother were 1 : 140, 1 : 140 and 1 : 240.

Table 4. Intracerebral titration of strains WCC und W in litters from immune and normal mothers

Experiment No.	Strain of virus	Age of litters (days)	Mothers of litters	ID_{50}	SD_{50}	LD_{50}
1	WCC	4	immune	$10^{-4.8}$	$10^{-4.8}$	$10^{-4.8}$
			normal	$10^{-5.4}$	$10^{-5.3}$	$10^{-5.3}$
2	WCC	4	immune	$10^{-5.0}$	$10^{-5.0}$	$10^{-5.0}$
			normal	$10^{-5.6}$	$10^{-5.4}$	$10^{-4.9}$
3	W	12	immune	$10^{-4.6}$	$10^{-4.5}$	$10^{-4.5}$
			normal	$10^{-4.7}$	$10^{-4.6}$	$10^{-3.2}$
4	W	14	immune	$10^{-4.5}$	$10^{-4.4}$	$10^{-4.4}$
			normal	$10^{-5.0}$	$10^{-4.8}$	$10^{-4.3}$

The results of the titrations of LCM virus in litters from immune and normal mothers are summarized in Table 4 which shows that, in contrast to EEE, the passive immunity in descendents of immune mothers, which had a high degree of cerebral immunity themselves, was of a very low order, the largest amount of virus neutralized in their brains being equivalent to $10^{0.6}$ ID_{50}. There was no marked difference between the ID_{50} and the SD_{50}, and the average rate of mortality was even higher in litters from immune mothers than in normal ones. This difference is brought out more clearly in Table 5 in which comparative figures are given. Special attention was paid to convulsive symptoms, which have been correlated with an antigen-antibody reaction (3).

It is evident from Table 5 that the number of baby mice which contracted an inapparent infection as indicated by acquired cerebral immunity was quite small. As usual, such infections occurred only in the endpoint

dilution range. The possibility cannot be completely excluded that some of the sucklings which became immune without showing symptoms were infected by contact with infected litter mates, since some of the mothers of the normal litters also acquired immunity (1 of 2 mothers tested in experiment 1, Table 4, five out of 6 in experiment 2, three of 5 in experiment 3 and none of 4 in experiment 4). In Table 5 the mortality from LCM was therefore expressed in per cent of the baby mice showing symptoms because these were no doubt due to the experimental infection as shown by the length of the incubation period.

Table 5. Morbidity and mortality in progeny of immune and normal mothers

Age of litters (days)	Mothers of litters	No. of suckling mice which			
		became infected	showed definite signs of disease	showed typical convulsions	died
4	immune	50	50	28 (56 per cent*)	50 (100 per cent*)
	normal	64	61	12 (20 p. c.)	56 (92 p. c.)
12—14	immune	50	48	48 (100 p. c.)	48 (100 p. c.)
	normal	75	72	72 (100 p. c.)	51 (71 p. c.)

* of the number of mice showing symptoms

The rate of mortality was always higher in litters from immune mothers than in those from normal ones. The difference was greatest in mice infected with strain W at the age of 12 or 14 days. This result is surprising in view of *Rowe*'s observation, confirmed by data recorded in Table 3, that immune serum ameliorates the course of the disease in adult mice.

With regard to the frequency of convulsions there was no difference between immune and normal litters infected with strain W when 12 or 14 days old, but in 4 day-old mice infected with strain WCC convulsions were definitely more frequent in the animals descending from immune mothers. Both in experiments 1 and 2 (Table 4) the incidence of characteristic tremors and convulsions was highest and the average survival period shortest in sucklings receiving a 10^{-4} dilution of virus. This result does not seem to be accidental.

That the immunity of baby mice born and nursed by immune mothers is a passive one was shown by a study of the CF titers in mother and

young. CF antibodies, if present in the mother's serum, were regularly transmitted to the sucklings. The titer of their serum was slightly higher than that of the mothers on the 15th day of life and dropped to zero between the 21st and the 51st day, whereas the CF titers of the mothers remained high. Sometimes they showed a slight rise after the litters had been weaned.

From the results obtained with litters from females immune to influenza, EEE or LCM it appears that, at the age of about 2 weeks, infantile mice may have some mechanism enabling them temporarily to accumulate antibodies obtained from the mother.

The way in which antibodies are transmitted from mother to young was not investigated. In view of the observation just mentioned and other known facts it is likely that transmission occurs through the milk rather than transplacentally.

Discussion

Based on the results listed in Tables 1 and 2, our standard method for the demonstration of neutralizing antibodies in the sera of LCM-immune mice now comprises the following features: use of an organ suspension containing liver, spleen and lymph node tissue from WCP-infected mice as source of virus; serial five-fold dilution of the virus in heated immune and normal serum; incubation at 37° C for 24 hours; i. p. inoculation (5) into 4 to 5 week-old mice using 6 animals per dilution; i. c. challenge of the survivors which failed to show definite symptoms 2 weeks after inoculation; calculation of the ID_{50} of the virus in the presence of immune and normal serum according to the *Reed-Muench* formula.

On account of the high virus content of the liver a 24-hour incubation of such suspensions will not reduce the infectivity titer to such a low level as to render accurate calculation of the ID_{50} in the presence of immune serum impossible. This is often the case when suspensions of brain or spleen and lymph nodes are employed. It is not unlikely that the mode of incubation of the mixtures of virus and antibody before i. p. inoculation played a part in the experiments of *Rowe* (5) as well, who stated that, among other factors, the time and temperature of incubation required further study. Incubation for 2 hours has been found suitable in neutralization tests with human sera (4). In contrast to the results obtained in these experiments heating of murine immune sera did not decrease their neutralizing power. On the contrary, heated sera gave slightly better results than unheated ones.

The use of the improved technique has given conclusive results ever since but not invalidated the statement made elsewhere (15) that the

neutralizing power of the most potent sera from LCM-immune mice (e. g., immune serum 5, Table 3) is far inferior to that of murine antisera against other viruses, for instance, that of EEE.

It is clear from the results presented that neutralizing antibodies from LCM-immune mice not only reduce the severity of the disease but also the infectivity of the virus and that they do not differ from antibodies to other viruses in this respect. Their antiviral effect is exceptionally slow, but this property may be attributed to their low concentration. This conclusion is supported by the observation (14) that neutralizing antibodies can be demonstrated in extracts of washed blood cells from chickens immune to Newcastle disease virus only when mixtures of extract and virus are incubated for several days prior to inoculation into chick embryos. In contrast to such extracts, which merely contain traces of antibodies, the neutralization of this virus by undiluted chicken immune serum does not require incubation. However, prolonged incubation intensifies neutralization in this case also and brings about a firm union between virus and antibody.

The very low grade of passive immunity present in baby mice born and nursed by cerebrally immune mothers and the failure to detect neutralizing antibodies in the brains of mice solidly immune to i. c. inoculation of virus indicate that these antibodies are not the only immunity factor in such animals. It has long been suspected that a cellular factor of some sort plays a part in this immunity (11) and the theory, supported by much experimental evidence, has been advanced more recently that a change in cellular reactivity may be important (5).

The strange case that the antibodies which immune mothers transmit to their descendents slightly reduce the susceptibility of the baby mice to i. c. inoculation of virus but, at the same time, increase the lethal effect of the agent may be correlated with the hypothesis that the striking tremors and convulsions shown by mice infected i. c. with LCM virus are primarily caused by an antigen-antibody reaction (3). If this is correct, it is conceivable that convulsive symptoms will be more frequent in baby mice which have obtained antibodies from their mothers than in those descending from normal females. It has been shown that a certain percentage of the progeny of normal females becomes immunologically tolerant when infected with LCM virus during the first days of life (17). According to the hypothesis just mentioned such animals would not show convulsions since they cannot produce antibodies themselves, and this was actually the case in some of the mice infected i. c. with strain WCC at the age of 4 days (see Tables 4 and 5). The relatively high frequency of convulsions in litters from immune mothers infected with a 10^{-4} virus dilution suggests that a certain ratio of virus and antibody may favor the appearance of such symptoms.

It is obvious from the results reported that neutralization tests in a simple medium like tissue cultures cannot disclose the indirect effects which the antibodies may have on the course of the disease.

Summary and Conclusions

Neutralization of LCM virus by murine immune sera was intensified by prolonged incubation (24 hours at 37° C) of the mixtures of serum and virus prior to intraperitoneal inoculation into mice. The neutralizing capacity of the sera tested was very low compared with that of mice immune to other viruses.

With the improved technique neutralizing antibodies were regularly demonstrated in serum pools from mature mice infected experimentally or by contact but not in the brains of such animals or in mice infected *in utero*.

In contrast to the progeny of mice immune to influenza or EEE viruses, litters from LCM-immune females showed very little passive immunity when challenged intracerebrally at the age of 4 to 14 days and, in general, reacted more severely to the infection than normal controls. This phenomenon has been correlated with the hypothesis (3) that the symptoms of mice infected with LCM virus by the cerebral route are primarily caused by an antigen-antibody reaction.

The results obtained favor the theory that the immunity resulting from experimental infection of adult mice with the virus under study is not exclusively due to the presence of neutralizing antibodies (11, 5).

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