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Observations on Immunological Tolerance and "Immunity" in Mice Infected Congenitally with the Virus of Lymphocytic Choriomeningitis (LCM)

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Mice infected *in utero* with LCM virus carry large amounts of active virus in their blood and organs for many months and discharge considerable quantities of the agent with their secretions and excretions (11). This observation indicates continuous multiplication of the virus in the organs of such animals, which look like normal individuals in spite of their chronic infection. Intrauterine infection leads to a stable equilibrium between virus and body. It was also observed that mice infected *in utero* would develop practically no specific antibodies, while the sera of mature mice infected experimentally or by contact at least gave positive complement-fixation (CF) tests (9, 14).

Attempts to demonstrate neutralizing antibodies in the sera of such animals using customary techniques gave essentially negative results (for references see 13). More sensitive methods, however, have made the demonstration of such antibodies possible (7). Recent experiments have shown that their titer is relatively low and their antiviral action exceptionally slow (13). The failure to recognize this fact no doubt accounts for the negative results obtained earlier by different investigators. For several reasons it is still doubtful whether these antibodies represent the immunity factor of prime importance in mice recovered from experimental infection (10, 7, 13). The solid immunity of mice infected *in utero* is obviously not due to antibodies, since none could be demonstrated with sensitive methods even after repeated inoculations of virus (12, 13) and since life-long persistence of large amounts of active virus in the body would hardly be compatible with the antibody theory.

Burnet and *Fenner* (1) have regarded the condition prevailing in mice infected *in utero* with LCM virus as immunological tolerance and all of the available evidence suggests that this interpretation is correct (12). The case probably is no longer unique among infections caused by animal viruses, since it is not unlikely that similar relationships exist between certain tumor

viruses and their respective hosts (12). Moreover, the opinion has been expressed that human serum hepatitis and equine infectious anemia may fall in this category as well (5).

Immunological tolerance regularly arises in mice infected in the embryonic stage and many mice infected neonatally also become tolerant and long-time carriers of LCM virus (11, 5, 12), whereas this is not the case in mature mice infected experimentally or by contact.

It was pointed out recently that two distinct mechanisms are operative in embryonic infection with LCM virus (12). In tolerant mothers infection of the ova occurs either in the ovaries, the oviduct or the uterus, transovarian transmission being most likely in view of the high virus content of the ovaries. In this case, all embryos become infected with great regularity. On the other hand, mice infected experimentally during the first week of pregnancy transmit the virus transplacentally to their embryos in an irregular fashion. Some embryos may escape infection in this case. We have had litters in which only 1 out of 9 or 12 embryos contained virus at birth, while in other cases all embryos were positive. Embryos infected in either way were tolerant towards LCM virus later in life.

As long as transovarien transmission of the virus has not been proved, we shall refer to the former mode of transmission as congenital infection and to the latter mechanism as transplacental infection. The tolerant mice used in this study all became infected congenitally.

The present paper concerns the mechanisms of immunological tolerance and of the strong "immunity" of tolerant mice in the absence of antibodies. An attempt will be made to correlate tolerance with the antigen content of the lymphatic system and the "immunity" of tolerant animals with the interference phenomenon.

Materials and Methods

Strains of virus: The tolerant mice were carriers of strain W, which was isolated from a naturally infected wild mouse kindly sent to us by Dr. *V. H. Haas*, National Institutes of Health, Bethesda, Maryland, U.S.A. Congenital and transplacental infections caused by this strain are usually inapparent (12). Females infected in this manner will regularly transmit the virus to their progeny in successive litters. Experimental neonatal infections also take a mild course in the majority of the animals. Mature mice inoculated intracerebrally (i. c.) show characteristic fatal convulsions with fair regularity. Intraperitoneal (i. p.) infection is frequently followed by serous pleuritis and peritonitis, while subcutaneous (s. c.) infection fails to cause definite symptoms.

Strains WCC and WCP were obtained through the courtesy of Dr. *W. P. Rowe*, National Institutes of Health, Bethesda, Md. In contrast to strain W, strain WCC is highly pathogenic for 1 to 4 day-old suckling mice inoculated i. c. (see Tables 5 and 6). It had gone through 365 i. c. passages in mice. Strain WCP, passed 365 times by i. p. inoculation, regularly causes disease (pleuritis and peritonitis, associated with marked emaciation and, sometimes, with typical convulsions) in adult mice infected by this route. It is particularly suitable for i. p. neutralization tests.

Strain WE 3, a guinea pig passage strain highly pathogenic for this species, was kindly provided by Professor *W. Scheid*, Universitäts-Nervenklinik,

Cologne. It was used for the hyperimmunization of guinea pigs following primary infection with the mild strain W.

The EEE (eastern equine encephalomyelitis) virus used was strain S 18888, which is highly pathogenic upon i. p. inoculation in young mice just weaned.

Mice: All experiments on LCM were carried out with animals of Breed No. III of the Institute, which is not pure genetically but uniformly susceptible to the agent under study in spite of the fact that some animals have an intestinal infection with Theiler's virus.

The tolerant mice descended from two females which became infected shortly after birth by contact with a wild carrier mouse and later transmitted the virus congenitally to their offsprings. Such transmission occurred regularly in successive generations. We are maintaining a small colony of tolerant white mice, from which the animals needed for the present experiments were derived. All animals of this stock are carriers of LCM virus.

Infectivity titration: 10 or 20 per cent extracts (considered as 1 : 10 and 1 : 5 dilutions, respectively) of the organs to be tested were diluted serially in ice-cold phosphate-buffered saline pH 7.4 and the dilutions injected i. c. or i. p. into mice as indicated in the text, using 3 or 5 animals per dilution. Surviving mice were tested for immunity by i. c. inoculation of virus (strain W) two weeks later and immunized mice counted as infected in the ID_{50} calculations according to the well-known method of *Reed* and *Muench*.

Tests for CF antigen: 20 per cent organ extracts were spun in a refrigerated angle centrifuge at 15,000 r. p. m. for 15 minutes and the supernatant fluids tested undiluted and in twofold serial dilutions against 1 : 10 dilutions of hyperimmune and normal guinea pig serum, using the same sera in all tests. The immune serum was obtained from animals immunized with strains W (primary infection) and WE 3 (hyperimmunization). The sera were heated at 56° C for 30 minutes prior to use. Complement was used in amounts just sufficient to cause complete lysis in control tubes with the respective normal organ extracts. For fixation and lysis the tubes were incubated in a serological waterbath at 37° C for periods of 30 minutes. The highest dilution of an extract giving complete (++++) fixation was recorded as its titer.

Hemagglutination-inhibition tests: Such tests were carried out according to the method of *Casals* and *Brown* (2) as modified by *Mussgay* (6).

Antigen content of the organs in tolerant and non-tolerant LCM-infected mice

In previous experiments (11) the tissues of congenitally infected carrier mice were found to contain surprisingly large amounts of virus even in animals of advanced age. The virus content of their blood was about as high as that of experimentally infected mature mice during the acute stage of the disease. The titration method practised at that time was too inaccurate, however, to allow a precise comparison of the virus content of different organs.

The expense involved in accurate infectivity titrations, which require large numbers of mice, has prompted us to use the CF test for the demonstration of specific viral antigen in murine organs. Since there is a non-infectious soluble antigen associated with this virus (8), the CF test does

not accurately measure the infectivity of a tissue extract. Experience has shown, however, that a rough parallelism exists between the infectivity and CF titers of various organs in tolerant mice.

The result of an experiment with mice infected i. p. with strain WCP at the age of 5 weeks is recorded in Table 1. The materials obtained on the 6th day after inoculation were tested simultaneously for infectivity and CF antigen. In the infectivity titration serial five-fold dilutions of 20 per cent organ extract were inoculated i. p. each into 5 mice.

In this experiment the CF test detected the most and the least infectious extracts (liver and brain, respectively), but not the difference in virus content between the lymph nodes and the spleen. It is not clear in this case which test gave the more accurate result. On the basis of

Table 1. Comparison of infectivity and CF titers of different organs from mice infected i. p. with strain WCP at the age of 5 weeks

Titer	Brain	Spleen	Lymph nodes	Liver
CF	—*	1:4	1:4	1:32
ID_{50}	$10^{-5.8}$	$10^{-6.4}$	$10^{-6.9}$	$10^{-8.2}$

* negative reaction with undiluted 20 per cent extract

these and other tests not presented in detail we think that the CF test can give a rough idea of the relative virus content of different organs.

We then proceeded to a systematic study of the distribution of CF antigen in tolerant mice of different age and of non-tolerant animals infected experimentally at the age of 5 weeks. In the latter group, those infected with strain W received serum from tolerant mice either i. p. or s. c. (see Table 2), while those infected with strain WCP (see Table 3) were inoculated i. p. with organ extract from the 365th serial passage.

Table 2 shows the striking difference in antigen content between tolerant mice infected congenitally with strain W and non-tolerant mature animals infected experimentally with the same strain. It should be recalled at this point that the latter animals can form antibodies, while the tolerant ones cannot. There was no decrease in the amount of antigen in the organs of tolerant mice in the course of time. It was greatest in the lymphatic system (spleen and lymph nodes), but several other organs had considerable titers as well. The results again show that LCM virus affects a variety of organs of different origin, structure and function. The salivary glands, which had a high virus content in previous titrations (11), did not give conclusive results in CF tests because of the very strong anticomplementary effect of their extracts. Of great significance

Table 2. Distribution of complement-fixing antigen in organs of tolerant and non-tolerant mice infected with strain W

Mice	CF titers (1 : ...)															
	B	LN	Th	H	Lu	Li	S	K	A	P	O	U	MG	Te	I	BF
12 tolerant ♀, 5 weeks old	2	32	16	1	4	4	32	1	2	8	4	4				1
2 tolerant ♀, 98 days old	—*	32	4	2	8	32	32	16	—	8	4	8				±
2 tolerant ♂, 98 days old	—	16	—	—	8	32	32	2	—	8				2		1
10 tolerant ♀, 4—5 months old	1	32	16	2	8	16	32	8	1	16	4	16			4	1
6 tolerant ♀, 6—7 months old, not lactating	2	32	16	±	16	8	32	16	—	8	8	8	2			1
3 tolerant ♀, 7—8 months old, lactating		32					32					16	8			
6 non-tolerant ♀, strain W i. p. at age of 5 weeks, sacrificed on 3rd day	—	±†	—	—	—	—	1	—	—	—	—	1				—
dtto., sacrificed on 5th day	—	4	—	—	—	—	4	—	—	1	—	1				—
dtto., sacrificed on 7th day	—	1	—	—	—	—	1	—	—	—	—	4				—
6 non-tolerant ♀, strain W s. c. at age of 5 weeks, sacrificed on 3rd day	—	—	—	—	—		—	—	—	—	—	—				—
dtto., sacrificed on 5th day	—	1	—	—	—		—	—	—	—	—	—				—
dtto., sacrificed on 7th day	—	2	—	—	—		2	—	—	—	—	—				—

Abbreviations used in Tables 2 and 3: B = brain; LN = lymph nodes; Th = thymus; S = spleen; H = heart; Lu = lungs; Li = liver; K = kidneys; A = adrenals; P = pancreas; O = ovaries; U = uterus; MG = mammary gland; Te = testes; I = intestines; BF = brown fat

* negative reaction with undiluted 20 per cent extract

† incomplete reaction with undiluted 20 per cent extract

epidemiologically is the marked affinity of the virus for female genital organs.

Compared with the large quantities of CF antigen detected in tolerant mice the antigen content was very meager in 5 week-old mice infected experimentally with the same strain. Specific antigen was demonstrated in the lymph nodes and in the spleen, but the titers were much lower than in tolerant mice. The uterus contained antigen in mice infected i. p., but not in those inoculated s. c. On the 7th day after inoculation all mice infected i. p. showed severe pleuritis and peritonitis, while s. c. infection failed to cause definite symptoms. The only change noted in the latter animals was moderate enlargement of the lymph nodes on the 7th day. It should be mentioned in this connection that swelling of

Table 3. Distribution of complement-fixing antigen in organs of non-tolerant mice infected i. p. with strain WCP at age of 5 weeks

Mice	CF titers (1 : ...)													
	B	LN	Th	H	Lu	Li	S	K	A	P	O	U	MG	BF
6 ♀, sacrificed on 3rd day	—	8	—	—	—	±	16	—	—	—	—	4	—	—
6 ♀, sacrificed on 5th day	—	4	8	—	2	16	8	—	—	—	8	16	—	—
6 ♀, sacrificed on 6th day	—	4	4	±	2	32	4	—	2	4	16	16	—	—
4 ♀, sacrificed on 8th day	—	2	8	2	2	32	8	±	16	8	8	16		1
6 ♀, sacrificed on 10th day	—	1	2	±	1	8	2	±	2	2	16	8		—
6 ♀, sacrificed on 14th day	±	±	1	±	±	2	±	—	8	—	2	4		1
6 ♀, sacrificed on 21st day	—	—	—	—	—	—	—	—	±	±	±	—		—

the lymph nodes is frequently seen in LCM-infected mice. The pathological picture is often similar to that in the beginning stage of lymphomatosis.

It is evident from Table 3 that mice injected i. p. with strain WCP carried much CF antigen in the spleen and lymph nodes on the 3rd day, but the titers fell off thereafter, while those of the liver, ovaries, and uterus increased. The highest titer (1 : 32) was reached by the liver. Antigen was also demonstrated in the thymus, lungs, adrenals, and pancreas. The antigen content of the organs of WCP-infected mice decreased steadily after the 8th day and was almost zero 3 weeks after inoculation. Very small amounts of antigen had remained in the adrenals, pancreas and ovaries only.

It was of interest to determine whether mice infected i. p. with strain WCP would be capable of antibody formation in spite of the temporary high virus content of their organs and their poor condition. Such animals as a rule show severe symptoms and those which recover do so very slowly. Viremia persisted for more than 5 weeks in 63 per cent of the mice tested and for at least 9 weeks in 18 per cent. Three months after inoculation the organs of 16 out of 18 mice were non-infectious, while

those of 2 animals still contained traces of active virus. All animals had formed CF antibodies. There was no evidence suggesting that their capacity to produce such antibodies had been impaired.

In another experiment concerning the time of appearance of CF antibodies in WCP-infected mice no antibodies were demonstrable in the serum on the 5th day after i. p. inoculation, but the majority of the mice had formed small amounts of such antibodies by the 8th day in spite of their very severe illness. On the 14th day all sera tested gave strongly positive reactions.

Behavior of tolerant mice towards EEE virus

Haas (4) has shown that mice infected *in utero* with LCM virus can produce neutralizing antibodies against the viruses of St. Louis encephalitis and influenza. In the present study the ability of tolerant and normal mice to form hemagglutination-inhibiting (HAI) antibodies against EEE virus was tested quantitatively.

Table 4. Interference of LCM with EEE infection in tolerant mice

Recipient mice (3 weeks old)	Result of i. p. inoculation of decimal dilutions of EEE virus							ID_{50}
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	
tolerant	10/11*	10/10	9/10	12/21	5/20	0/16	0/10	$10^{-6.2}$
normal	11/11	10/10	9/10	18/21	12/20	7/16	0/10	$10^{-7.3}$

* 10 of 11 injected mice developed encephalitis

Before starting the experiment an attempt was made to determine if and to which extent LCM virus would interfere with i. p. infection by EEE virus in tolerant mice. If interference between the two viruses did occur in cells of the antibody-forming system, one would expect lower HAI titers in tolerant than in normal mice.

In the interference test EEE virus (infectious 10 per cent mouse brain suspension, in the endpoint calculation considered as 10^{-1} dilution) was titrated in 3 week-old litters of tolerant and normal mice as indicated in Table 4. In the dilution range in which the endpoints were expected two litters of each group were used per dilution, otherwise one large litter only.

Table 4 shows that the ID_{50} values differed by 1.1 log in the two groups. This difference is significant in view of the considerable numbers of test animals used and indicates moderate interference. It is not known in which cell system such interference occurred.

For the HAI test 10 tolerant and 10 normal mice, all 5 to 6 weeks old at the start of the experiment, were immunized against EEE as follows:

1st inoculation: 0.5 ml. formolized 20 per cent suspension of infected mouse brains i. p. (the suspension contained 0.3 per cent formalin and was non-infectious after incubation at 37° C for 3 days);

2nd inoculation (2 weeks later): 0.5 ml. of the same material s. c.;

3rd inoculation (7 days later): 0.5 ml. 10^{-1} dilution of infectious 20 per cent mouse brain suspension i. p.

The animals were exsanguinated 8 days after the last injection and their sera tested individually for HAI antibodies.

The individual titers varied between 1 : 160 and 1 : 904 in the tolerant group and between 1 : 226 and 1 : 640 in the normal mice. The computed mean values (1 : 394 for the tolerant and 1 : 367 for the normal animals) show that tolerant mice can produce antibodies against EEE virus just as well as normal mice.

Experiments concerning the mechanism of immunity in tolerant mice

Tolerant mice show neither symptoms nor lesions following i. c. inoculation with large amounts of virus (11). The mechanism of this strong "immunity" in the absence of antibodies was correlated hypo-

Table 5. Titration of brains of tolerant and normal mice various periods of time after i. c. inoculation with strain WCC

Time after i. c. inoculation of strain WCC	Mortality and average time of survival in suckling mice used for i. c. titration of brains from				ID_{50} of brains of normal controls
	tolerant mice*		normal controls		
	Mortality	survival time (days)	Mortality	survival time (days)	
5 minutes	2/21**	17.0	22/22	12.8	$10^{-2.4}$
6 hours	6/20	18.5	18/18	13.2	$10^{-2.0}$
12 hs.	1/21	20.0	20/21	13.5	$10^{-3.1}$
24 hs.	11/63	18.5	70/74	14.0	$10^{-4.7}$
48 hs.	17/41	18.0	40/40	14.1	$10^{-5.7}$
72 hs.	4/41	17.2	43/44	12.8	$10^{-6.1}$
96 hs.	4/33	17.7	31/31	13.8	$10^{-5.6}$
120 hs.	2/41	14.0	35/36	12.8	$10^{-5.6}$
144 hs.	13/45	19.2	32/34	13.0	$10^{-5.4}$
no WCC virus ...	6/45	16.5			

* ID_{50} ranging from $10^{-4.7}$ to $10^{-6.0}$ ** 2 of 21 infected mice died

thetically with the interference phenomenon (11). The demonstration of considerable quantities of viral antigen in a variety of organs of tolerant mice is strongly in favor of this interpretation.

Since it is possible to differentiate strain W with some degree of certainty from strain WCC on account of their different pathogenicity for 1 to 4 day-old suckling mice, an attempt was made to reinfect tolerant mice i. c. with strain WCC.

In the first experiment recorded in Table 5 ten tolerant and 10 normal mice were injected i. c. with 20 per cent mouse brain suspension from the 365th serial passage of strain WCC. The inoculated animals were sacrificed at intervals as indicated in the table (one tolerant and one normal mouse each time) and 20 per cent suspensions of their brains titrated i. c. in one day-old normal mice using one litter of 9 to 11 animals per decimal dilution. In the 24-hour test the brains of two pairs of mice were titrated. Two tolerant mice not inoculated with WCC were included in the tests to check the pathogenicity of strain W for new-born mice. The inoculated litters were observed twice daily and the symptoms and time of death recorded for every animal. Strain WCC will kill nearly all new-born mice infected i. c. in contrast to strain W, for which a mortality rate of only 13 per cent was recorded in previous experiments (12). Moreover, the average time of survival after inoculation is by a few days shorter in suckling mice infected with WCC. Survivors were tested for immunity by i. c. inoculation of virus (strain W) at the age of 4 weeks. It was possible in this manner to determine the number of infantile mice infected with strain W.

Table 6. Attempt to detect strain WCC in the brain of a tolerant mouse 48 hours after i. c. inoculation

Titrated brain originating from	ID_{50} in 2 day-old suckling mice	Mortality
(1) tolerant mouse injected i. c. with WCC	$10^{-5.4}$	2/49 (4 per cent)
(2) normal mouse injected i. c. with WCC	$10^{-5.9}$	49/53 (92 p. c.)
(3) tolerant mouse (litter mate of No. 1) not inoculated with WCC	$10^{-5.0}$	3/38 (8 p. c.)

Judging from the rate of mortality and the time of survival after inoculation, the results presented comprehensively in Table 5 suggest that strain W interfered with strain WCC in every case. There was no evidence for multiplication of the latter strain in the brains of tolerant mice with the possible exception of the 48-hour test in which some baby mice succumbed after short incubation periods and the rate of mortality was 41 per cent. This test was therefore repeated and the result given in Table 6 obtained. It does not indicate multiplication of strain WCC in the brain of the respective tolerant mouse.

It is evident from the last column in Table 5 that virus multiplication started in the brains of normal mice after a lag period of 6 to 12 hours and reached its maximum at 72 hours, slowly declining thereafter.

Discussion

The mechanism underlying immunological tolerance still is unknown. For theories and experimental attempts to explain it the reader is referred to a recent review by *Chase* (3).

The experiments presented above have shown that the organs of mice tolerant towards LCM virus contain more viral antigen than those of mature mice infected experimentally with the same strain and maintain their high antigen titers indefinitely. The antigen content is highest in the lymphatic system of tolerant animals. It is conceivable that their antibody-forming apparatus is clogged by specific antigen and thus prevented from antibody synthesis, a situation reminiscent of the "immunological paralysis" caused by inoculation of mature mice with large amounts of pneumococcal polysaccharide (for references see 3). This phenomenon is now regarded as an example of immunological tolerance (3).

The quantities of specific antigen present in the organs of non-tolerant mice infected experimentally with strain W were surprisingly small, while i. p. inoculation of adult mice with strain WCP effected a transient high antigen content of certain organs, notably the liver. However, the amounts of antigen detected in the spleen and lymph nodes were inferior to those in tolerant mice and decreased slowly after the first week following inoculation. This may be the reason why WCP-infected animals can produce antibodies and get rid of the virus in the course of time.

It appears from the results obtained with tolerant and non-tolerant mice infected with strain W that embryonic mouse tissues are more susceptible to infection with LCM virus and better suited for its multiplication than those of adult mice. They maintain this quality as they mature, and this may be a decisive factor in the mechanism of immunological tolerance in this case. The cells obviously transmit the infection with this non-cytocidal agent (5) to their descendents in successive generations. It is doubtful whether there are any uninfected susceptible cells available at any time. Susceptibility to infection should be sharply differentiated, however, from susceptibility to the pathogenic action of the virus which, in the case of strain W, is minimal in embryos and low upon neonatal infection, but high in mature mice infected experimentally. This is not a general rule, however, since neonatal infection with strain WCC is highly fatal (12).

While tolerance of mice towards LCM virus can be satisfactorily explained in the same way as the "immunological paralysis" caused by pneumococcal polysaccharide, it is more difficult to account for the long duration of tolerance in animals injected in the embryonic stage or neonatally with antigens incapable of spontaneous replication and more

susceptible to enzymatic decomposition than polysaccharides. Although it seems probable that exceedingly small amounts of antigen in sensitive areas can block the immunological apparatus (3), one would expect only such antigens to cause long-lasting tolerance whose antigenically active groups are highly resistant against enzymatic degradation.

The ability of tolerant mice to form antibodies against EEE virus as efficiently as normal mice may imply either that EEE antibodies are produced by other cells than LCM antibodies or that cells may be blocked by antigen of one virus and still be fully capable of antibody formation against another agent. The first alternative appears unlikely. It would be difficult to imagine that a special set of antibody-producing cells might be available for every one of the many antigens with which the body has to deal.

Since embryonic and mature mouse tissues react differently towards certain strains of LCM virus and adult tolerant mice appear to behave like embryos in this respect, it was at first thought possible that cellular reactivity (7) might play an important role in the "immunity" of tolerant mice. However, their resistance towards strain WCC, which is equally pathogenic for newborn and adult mice, suggested that this is not the case. In view of the wide distribution of virus in the organs of tolerant animals it is more likely that their "immunity" is due to the interference phenomenon. *Rowe* (7) has shown that small quantities of a modified strain of LCM virus can interfere in non-tolerant mice with an unmodified strain. In the present experiments it has not been possible to recover strain WCC from the brains of tolerant animals at any time after i. c. inoculation. The failure to detect it even during the lag period (see 5-minute and 12-hour tests in Table 5) indicates that strain W interfered with strain WCC not only in the brains of the tolerant mice, but also in those of the infantile animals used for the brain titrations. If there had been no such interference, it should have been possible in the lag period to demonstrate the superimposed virus which, as the results obtained with the normal controls show, had not yet been inactivated at that time by the body temperature or other non-specific factors.

In a study of tolerance and interference in mice infected congenitally with LCM virus one is tempted to consider the possibility that both phenomena may have similar fundamental mechanisms. Special enzyme systems, necessary for antibody formation on the one hand and for viral synthesis on the other, may be blocked.

Summary and Conclusions

In a comparative study of the distribution of viral antigen in the organs of tolerant mice infected congenitally with LCM virus and of non-tolerant mature animals infected experimentally the complement-

fixation titer was used as an indicator of the intensity of the infection. Preliminary tests had shown that there is a rough parallelism between the complement-fixation and infectivity titers of murine organs.

The organs of congenitally infected mice, young or old, generally contained more antigen than those of mature mice infected artificially. The very high antigen content of the lymphatic system in tolerant animals has been correlated with the tolerance phenomenon and the possibility considered that their immunological apparatus may be blocked by specific antigen as in mice showing "immunological paralysis" following inoculation with large doses of pneumococcal polysaccharide.

Mice tolerant towards LCM virus were able to produce hemagglutination-inhibiting antibodies against EEE virus to the same extent as normal mice in spite of the fact that LCM infection interferes slightly with EEE infection in tolerant animals.

The results of interference tests with distinguishable strains of LCM virus favor the hypothesis that the strong "immunity" of tolerant mice in the absence of antibodies is due to the interference phenomenon.

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