EPIDEMIOLOGY OF LYMPHOCYTIC CHORIOMENINGITIS IN A MOUSE STOCK OBSERVED FOR FOUR YEARS

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Choriomeningitis in a mouse colony is a favorable material for the study of the problem of the effect on a parasite of long association with a given host. The host animals can be easily maintained in large numbers in a relatively small space, and two or three generations can be secured in a year, so that progress is rapid for this type of work. A colony of infected mice has now been observed for 4 years and it is the purpose of this report to describe the changes that have taken place in the disease and its causative agent since the epidemiology was first studied (1).

Methods and Materials

The infected stock, which has been kept in a large metal breeding cage, consisted on the average of 12 to 15 mature females, 2 to 3 mature males, 10 to 20 mice just weaned, and a varying number (usually 1 to 4) of litters of suckling mice. The breeding mice selected from as many different litters as possible were kept for about 7 months and then replaced by animals that had just become sexually mature. Young mice not needed as replacements were discarded at the age of about 4 weeks. The mice removed from the colony were tested for circulating virus from time to time in order to be certain that the disease was still present. Cannibalism occasionally caused losses among the suckling mice. Fighting between the males has been rare. When it occurred the most aggressive animal was removed and replaced by an immature one.

The fertility in the infected stock was considerably below that in the virus-free colony derived from the original infected stock late in 1934 (1). This fact, however, can hardly be attributed to the disease alone, since it has been found that mice breed less regularly when a large number are kept in one cage. In the virus-free colony 1 male and 4 females are kept together in a smaller cage, and under such conditions the mice breed very regularly.

The virus-free mouse stock just mentioned was built up on 6 uninfected mice

(3 males and 3 females) from the mouse colony in which choriomeningitis was discovered in 1934. The progeny of these 3 pairs of mice were carefully tested for the absence of the infection and then cross-bred. This new stock of mice has since remained free from the disease. The mice are uniformly susceptible to choriomeningitis and there is no evidence that their susceptibility has changed since 1935, when the first epidemiological experiments were performed. The health and general condition of the colony are very good.

The infected and the virus-free mouse stocks have been quarantined as strictly as possible. No new mice were added to the colonies, and the diet as well as the environmental conditions was not changed until May, 1938, when the infected stock was taken to Germany.

Further technical details will be given in the text.

A Change in the Mode of Transmission of the Disease

Two modes of transmission of the virus from mouse to mouse were observed in 1935, intrauterine and contact infection. Pregnant females that continued to carry virus in the blood after clinical recovery often transmitted the virus to their embryos. Other mice which did not become infected *in utero*, because their mothers had got rid of the infection, contracted the disease by contact soon after they were born.

Choriomeningitis was essentially a disease of young mice of which many became severely ill and some died during the first month of life. Other mice contracted a subclinical infection. In the period between October 7 and December 11, 1935, for instance, the rate of infection was practically 100 per cent, the morbidity amounted to about 20 per cent, and the mortality to 4.4 per cent in terms of the total mouse population, which then numbered some 150 animals. If the rates of morbidity and mortality had been expressed in terms of the number of immature mice present in the colony, they would have been considerably higher.

The mice infected *in utero* were the only ones to show definite symptoms, while the animals infected by contact shortly after birth merely showed a slightly decreased growth rate. They looked like normal mice to any one not familiar with the exceptionally rapid growth and the large size of the strain of mice used. These observations are based upon the results of a large number of careful tests.

When the epidemiological studies were resumed in 1937, intrauterine infection had become the only mode of transmission of the virus.

This was probably due to the fact that all of the stock mice, young and old, were carriers of virus. A life-long infection was demonstrated in a number of cases, while the other animals were discarded before the duration of their infection could be determined.

A Decrease in the Severity of the Disease and Its Possible Causes

The most striking change that occurred gradually between 1935 and 1937 was a marked decrease in the severity of the disease. Since it is now subclinical, infected young mice can no longer be distinguished with certainty from uninfected ones in spite of the fact that their tissues contain about the same amounts of virus as before the change occurred.

In view of the fact that certain other virus diseases sometimes change in a similar manner, an attempt was made to determine the cause for the present mildness of the disease. Such an attempt was possible because the mice from the virus-free colony, which had been used for intrauterine infection with stock virus in 1935, were available for comparative experiments on intrauterine infection with virus freshly isolated from the infected stock. On the basis of numerous other observations it could be assumed that the susceptibility of these mice had not appreciably changed since 1935, when intrauterine infection produced a severe disease in the majority of them.

The brains, thoracic and abdominal organs of suckling mice from the infected stock were used as a source of virus. The females needed for the experiments were selected from 5 different litters of mice obtained from the virus-free colony and injected intranasally with stock virus at the age of 1 day. Mice infected in this manner carry virus in the blood for a very long time after clinical recovery (2). 9 females whose blood had been previously tested for virus were bred to 9 virus-free males. 2 young from each of the 9 litters obtained were sacrificed immediately after birth for a test for intrauterine infection. Their brains were removed aseptically and each suspended in 2 cc. saline. The brain suspensions were inoculated intracerebrally into virus-free mice in amounts of 0.05 and 0.05 $\times 10^{-3}$ cc. If these mice, particularly those receiving the higher dilution, developed the disease, it was assumed that the respective litters had become infected *in utero*. This assumption is justified by previous tests which showed that the virus is transmitted either to all of the embryos or not at all. The litters were watched daily for 2 months.

5 of the 9 litters became infected *in utero*, while the other 4 litters did not in spite of the fact that the mothers still carried the virus in the blood 2 weeks after

parturition. This fact suggests that the virus content of the blood may not have been alone responsible for the infection of the embryos of the other mice. The absence of detectable amounts of virus from the blood of another naturally infected mouse that transmitted the virus to its embryos (1, Table III) points in the same direction. It is possible therefore that the transmission of the virus takes place either in the ovaries, which contained relatively large amounts of virus in 2 carriers tested (2), or in the uterus, which likewise may be rich in virus (2), by "growth" through the placenta. The term "intrauterine infection" will be used for the sake of brevity, although it is realized that it may not always be accurate.

TABLE I

Intrauterine Infection in Mice from the Virus-Free Stock

Litter No.	Date of intranasal injection of mother at age of 1 day	Date of birth of litter	Number of young	Result
	1937	*1938*		
1	Oct. 20	Jan. 22	6	1 young became sick and recovered; the others showed decreased growth during the first 2 weeks of life
2	" 26	" 23	7	1 young became sick and recovered; another showed a markedly retarded growth for 4 weeks; the remainder developed normally
3	May 5	" 29	6	No symptoms, but slightly decreased rate of growth
4	Oct. 20	Feb. 1	3	1 young died on the 16th day; the others developed normally
5	" 20	" 22	9	2 young died on the 17th and 37th days; another became sick and recovered; the remainder showed no reaction

Table I shows that the majority of the mice from the 5 litters infected *in utero* failed to become sick. A few of the young however did show symptoms of the disease and 3 of them died. On the whole, the reaction of the animals was intermediate between the severe disease observed in 1935 and the extremely mild infection seen more recently.

The interpretation of this result is not easy. If one assumes that the susceptibility of the virus-free mice is still the same as in 1935, it seems that the pathogenicity of the virus for embryonic mouse tissue has markedly decreased since then. On the other hand, since the disease described in Table I was definitely more severe than that now prevailing in the infected stock, it may be that the mice from the latter

stock now show a higher degree of resistance to intrauterine infection than those from the virus-free colony. In 1935 the susceptibility to intrauterine infection of both kinds of mice was about the same.

The decrease in the severity of the disease resulting from intrauterine infection has been associated with an increase of the virulence of the virus for suckling mice infected by contact shortly after birth, as shown by experiments reported before (2) as well as later in this paper, whereas contact infection in mice older than 2 to 3 weeks is still subclinical as it was in 1935. It would have been of interest to study the effect of contact infection with the stock strain not only in young mice from the virus-free colony, but also in virus-free young from the infected stock. This has not been possible, however, since no mice of the latter type were found.

A Change in the Contagiousness of the Experimental Disease

Experiments on contact infection carried out in 1935 (1, and unpublished experiments) showed that the experimental disease was transmitted by mice of different ages, provided the period of exposure was sufficiently long. Since the disease induced by contact infection was subclinical, it had to be demonstrated by testing the blood for virus or by tests of immunity several weeks after exposure. When the experiments were resumed in 1938, different results were obtained in that the infection very rarely passed from mature mice infected experimentally to normal ones. Suckling mice infected by intranasal instillation of stock virus, however, still transmitted the virus to normal mice during the acute and chronic stages of the disease. Mice infected naturally, young ones as well as old carriers of virus, could likewise transmit the infection. To illustrate these observations some recent experiments will be given here in detail.

The mice infected experimentally as well as the uninfected animals exposed to infected ones were obtained from the virus-free breeding colony, while the naturally infected mice came from the infected stock. As in previous experiments, the mature mice used were all females in order to eliminate the possible sexual transmission of the disease, or the infection by biting in males.

The experiments were conducted as follows: Mice infected naturally or experimentally as indicated in the tables were placed in the same cage with some uninfected animals on the 3rd or 4th day after inoculation or removal from the infected stock. Care was taken that the mice which died from the disease were not de-

voured by their cage mates, since we have recently been able to infect 1 of 6 mice by feeding with virulent mouse brain given on bread. Unless otherwise stated, the animals were kept together for 4 to 5 weeks, after which time they were tested for immunity by intracerebral inoculation with highly virulent virus. The animals that failed to show any reaction after the test of immunity were assumed to have become infected by contact, while the mice that showed characteristic symptoms or died were counted as negative. If the injected mice, to which the normal animals were exposed had shown no signs of illness, for instance, after intranasal or subcutaneous injection with virus, they were also tested for immunity at the same time as the exposed mice to make sure that they had become infected. Control mice of about the same age as the tested animals were included in each immunity test. About 90 per cent of these died, while the remainder developed typical, non-fatal choriomeningitis with characteristic tremors and convulsions. There was not a single control mouse that failed to become sick.

Exposure of Normal Mice to Mature Mice Infected Experimentally.—The details of these experiments are recorded in Table II which shows that the 5-week-old mice usually failed to transmit the infection, no matter by which route they were inoculated.

Since mice injected intracerebrally with virus often do not survive for the period of time that would be necessary for the transmission of the disease (1), the 6 animals injected in this manner with small amounts of virus in Experiment 1 were each given 0.25 cc. hyperimmune guinea pig serum intravenously 3 hours before the virus inoculation. While such serum treatment usually does not prevent the disease, it often renders it non-fatal. Virus was demonstrated in the nasal washings but not in the urine of some serum-treated mice that were sick and ultimately recovered. The mice used in Experiment 4 received no immune serum. They were still sick but evidently recovering when placed in contact with a litter of normal mice on the 9th day after inoculation.

The Influence of the Age at the Time of Inoculation on the Ability of Mice to Transmit the Disease.—The fact that the mothers of litters from the virus-free colony which had been inoculated intranasally with virus at the age of 1 to 7 days always became immune suggested that young mice infected experimentally would transmit the disease more readily than mature mice. That this was the case is shown in Table III.

Mice injected intranasally with virus at the age of 1 day continue to transmit the disease as they grow up in spite of the fact that they show symptoms for only 3 to 4 weeks. This is shown by the experiment recorded in Table IV which was made with 2 mice left over from

the 4th experiment of Table III. They were sick for about 3 weeks, recovered, and appeared quite healthy at the age of 58 days when they

TABLE II

Exposure of Normal Mice to 5- to 6-Week-Old Mice Infected Experimentally with Stock Virus

Experiment No.	Injected mice			Exposed mice	
	Number of mice	Route of inoculation	Reaction	Age at time of exposure	Number of mice infected as evidenced by acquired immunity
1	10	ip*	4 died; 6 became very sick and recovered	5 wks.	1/8†
	4	sc	None; immunized	5 "	0/4
	6	Immune guinea pig serum iv, virus ic	3 died; 3 became sick and recovered	5 "	0/4
2	6	iv	4 died; 2 became very sick and recovered‡	5 "	0/7
3	3	iv	Exposed on 13th day after inoculation when 2 mice had recovered and the 3rd still appeared sick	5 "	0/7
4	2	ic	Just recovering from typical disease	1 day (4–5 mos., mother)	0/5 0/1
5	4	iv	Very slight symptoms followed by quick recovery	1 day (4–5 mos., mother)	0/10 0/1

* ip = intraperitoneally. sc = subcutaneously. iv = intravenously. ic = intracerebrally.

† 1 out of 8 mice became infected.

‡ The 2 survivors still discharged virus with the urine and nasal secretions at the end of the period of exposure.

were exposed to a newborn normal litter of mice to which they promptly transmitted the disease. At the age of 108 days the 2 mice were again exposed to normal mice of different ages, but for a shorter

TABLE III

The Influence of Age at the Time of Intranasal Infection on the Transmission of the Disease by Contact

Injected mice			Exposed mice	
Age at time of inoculation	Number of mice	Reaction	Age at time of exposure	Number of mice infected as evidenced by disease (in young mice) and acquired immunity
1 day	5	Slight illness; recovery	1 day	5/5
1 "	10	1 died; 5 became sick and recovered; 4 showed no definite symptoms	1 " Full grown mother	8/8 1/1
1 "	8	4 died; 4 showed retarded growth	1 day Full grown mothers 5 wks.	10/10 2/2 8/8
1 "	7*	2 died; 2 became sick and recovered; 3 showed only a retarded growth	5 "	7/7 (Mother of litter injected intranasally also became immune)
2–3 wks.	4	2 became sick and recovered; 2 showed no symptoms	2–3 "	0/6
2–3 "	3	1 became very sick and recovered; 2 showed no definite symptoms	5 "	1/5
5 "	5	None; immunized	1 day Full grown mother 5 wks.	0/7 0/1 0/8
5 "	6	1 became sick and recovered; 5 showed no symptoms; all became immune	1 day Full grown mothers	0/8 0/2
5 "	6	" "	5 wks.	0/8

* 2 females of this group were used in the experiment recorded in Table IV.

period of time. The disease passed to the majority of the young mice but not to the older ones. This result suggests that young mice contract the infection more readily than mature ones and confirms a previous observation (1).

Infection of Normal Mice by Exposure to Virus Carriers.—That full grown mice from the infected stock, which continue to carry virus in the blood and discharge it with the urine and nasal secretions, can

TABLE IV

Continued Transmission of the Infection after Recovery by Two Mice Injected Intranasally with Virus at the Age of 1 Day*

Age of 2 infected mice when placed in contact with normal mice	Exposed mice		
	Age at time of exposure	Period of exposure and method of testing for infection	Number of mice that became infected by contact
58 days	1 day	Tested for virus in blood on 19th day of exposure	5/5 (All young sick when tested)
	Full grown mother of this litter	Tested for immunity on 25th day of exposure	1/1 (Showed no symptoms)
108 "	1 day	Exposed for 13 days; tested for immunity 2 wks. later	6/8 (The infected mice had shown symptoms)
	5 wks.	" "	0/8

* See footnote to Table III.

transmit the disease to healthy mice has already been reported. This still is the rule, as Table V shows. The disease readily passes from carriers to normal mice of different ages. The majority of the carriers used in these experiments came from litters infected *in utero* that were used in previous experiments (2, Text-fig. 1). They looked quite healthy and could not be distinguished from normal animals.

Comparative Experiments on Contact Infection with Naturally Infected Carriers and Mature Mice Infected Experimentally.—In the following experiments an attempt was made to determine why the disease is often transmitted by healthy appearing carriers but rarely by mature mice infected experimentally. It was not unlikely that the virus content of the nasal secretions, which appears to be more important

for the transmission of the disease than the urine (1), had some connection with this discrepancy. To test this possibility the virus content of the nasal washings from mice infected either naturally or experimentally was determined before and after exposure to normal animals. Each infected female mouse was placed in the same cage with 5 virus-free 5-week-old females, which were tested for acquired immunity after an exposure for 32 days.

TABLE V

Infection of Mice by Exposure to Old Carriers

Old female carriers infected *in utero* to which normal mice were exposed	Age of carriers at time of exposure	Exposed mice			
		Number	Age when exposed	Number of mice that	
				Showed symptoms	Became immune
	mos.				
2 of Litter C*	10	4†	1 day	2	4
		Mother	4–5 mos.	0	?‡
2 " " D*	10	4†	1 day	2	4
		Mother	4–5 mos.	0	1
3 " " B*	10	1 ♀	4 "	0	1
2 " " "	13	4 ♀	5 wks.	0	4
2 full grown from infected stock	4–5	4 ♀	5 "	0	4

* See Text-fig. 1 in a previous paper (2).

† Virus demonstrated in pooled blood from each group of suckling mice on 19th day of exposure. Mothers not tested for circulating virus.

‡ Death from injury by intracerebral test inoculation.

The nasal washings were taken as already described (2) and tenfold dilutions of them were made in saline. These as well as the undiluted materials were inoculated subcutaneously in amounts of 0.5 cc. into 5-week-old mice from the virus-free stock, one mouse being used for each dilution. It was not practical to titrate the nasal washings by intracerebral inoculation because of the bacteria ordinarily present in them. These were without effect when injected subcutaneously. Since mice inoculated subcutaneously with choriomeningitis virus never show symptoms, the number of infected mice was determined by intracerebral immunity tests made 2 weeks after inoculation.

TABLE VI

The Transmissibility of the Disease by Contact in Relation to Virus Content of Nasal Washings

Experiment No.	Infected mice (♀)				Number of exposed mice (5-week-old ♀) infected as evidenced by acquired immunity
	No.	Mode of infection	Titration of nasal washings		
			Before exposure	After exposure	
1	1	Healthy appearing mouse infected *in utero* and carrying virus in blood for over 1 year	10^{-1} i* 10^{-2} i 10^{-3} i 10^{-4} ni* 10^{-5} ni	10^{0} i 10^{-1} i 10^{-2} i 10^{-3} i 10^{-4} i	5/5
	2	" "	10^{-1} i 10^{-2} i 10^{-3} i 10^{-4} ni 10^{-5} ni	10^{0} i 10^{-1} ?† 10^{-2} i 10^{-3} i 10^{-4} i	3/4†
	3	Mouse injected iv with stock strain 9 days previously. Still sick but recovering when exposed on 9th day	10^{-1} i 10^{-2} i 10^{-3} i 10^{-4} ni 10^{-5} ni	10^{0} i 10^{-1} ni 10^{-2} ni 10^{-3} ni 10^{-4} ni	1/5
	4	" "	10^{-1} i 10^{-2} ni 10^{-3} i 10^{-4} ni 10^{-5} ni	10^{0} ni 10^{-1} ni 10^{-2} ni 10^{-3} ni 10^{-4} ni	0/5
2	5	Healthy appearing carrier from infected stock, 4–5 mos. of age	10^{-1} i 10^{-2} i 10^{-3} i 10^{-4} ni 10^{-5} ni	10^{-1} i 10^{-2} i 10^{-3} i 10^{-4} ni 10^{-5} i	4/4†
	6	" "	10^{-1} i 10^{-2} i 10^{-3} i 10^{-4} i 10^{-5} ni	10^{-1} i 10^{-2} i 10^{-3} i 10^{-4} ni 10^{-5} ni	1/5
	7	Same as Nos. 3 and 4	10^{-1} i 10^{-2} ni 10^{-3} ni 10^{-4} ni 10^{-5} ni	10^{-1} i 10^{-2} ni 10^{-3} ni 10^{-4} ni 10^{-5} ni	0/5
	8	" " " " " "	10^{-1} i 10^{-2} i 10^{-3} ni 10^{-4} ni 10^{-5} ni	10^{-1} ni 10^{-2} ni 10^{-3} ni 10^{-4} ?† 10^{-5} ni	0/5

* i = immunized; ni = not immunized. † Mouse died from injury after immunity test.

The details of the experiment are given in Table VI which shows that naturally infected mice (Nos. 1, 2, 5, and 6) in general discharged large amounts of virus over a longer period of time than the other animals infected experimentally (Nos. 3, 4, 7, and 8). This may be the reason why the former mice transmitted the disease more readily than the latter animals.

The urine of the infected mice, which may also play a minor rôle in the transmission of the disease, was not titrated because it was often impossible to obtain more than a few drops of it, and these would not have been sufficient for exact titrations. In other experiments, however, the virus content of the urine often ran parallel with that of the nasal washings, and the same may have been the case in the present tests.

Influence of the Strain of Virus on the Communicability of the Disease.—Since the change in the communicability of the experimental disease may have been due to a change of the virus, it was decided to test this possibility in the following series of experiments. Unfortunately it was not possible to compare the stock virus of 1935 with that of 1938 under the same experimental conditions, because we have not succeeded as yet in preserving choriomeningitis virus in mouse tissue for several years without resorting to animal passage. The latter may markedly alter some characteristics of the virus. In fact, no more than 8 intracerebral passages in mice were necessary to change the pathogenicity for guinea pigs of passage strain B (3). Its virulence for mice likewise differs from that of the stock virus (2). In the following experiments the communicability of the infection induced in mice by the passage strain will be compared with that of virus freshly isolated from the infected stock.

When strain B was isolated from a naturally infected stock mouse in 1935 it produced a contagious disease in 5- to 6-week-old mice. This statement is made with some reserve, however, because the number of mice tested was rather small. In one experiment made with virus from the 1st intracerebral mouse passage, 4 mice from the virus-free colony were inoculated intraperitoneally with virus and exposed to 8 uninfected mice for 32 days. The injected animals were ill from the 6th to the 10th days after inoculation and then recovered. Of the 8 exposed mice 6 became resistant to intracerebral inoculation with highly virulent virus.

The virus used in the present experiments had undergone from 30 to 42 passages in 5-week-old mice. The experiments recorded in Table VII were made with 5-week-old females. They are comparable to those given in Table II and therefore need no special description.

TABLE VII

Experiments on Contact Infection with the Mouse Passage Strain in 5-Week-Old Females

Experiment No.	Injected mice			Exposed mice
	Route of inoculation	Number of mice	Reaction	Number of mice infected as evidenced by acquired immunity
1	ic*	1	Dead on 6th day	0/5
	ip	3	None; immunized	
	ic	1	Dead on 6th day	0/5
	sc	3	None; immunized	
	ic	1	Dead on 7th day	0/5
	in	3	None; immunized	
2	Immune guinea pig serum iv, virus ic	5	2 dead on 7th or 8th day; 3 became sick and recovered	0/4
	ip	4	None; immunized	0/4
3	Immune guinea pig serum iv, virus ic	6	3 dead on 7th day; 3 became sick and recovered	0/4
	ip	5	None; immunized	0/4

* ic = intracerebrally. ip = intraperitoneally. sc = subcutaneously. in = intranasally. iv = intravenously.

Their results were uniformly negative and show that the infection with the mouse passage strain in 5-week-old mice is even less contagious than that with the stock strain. A comparison of the results obtained with the mouse passage strain in 1935 and 1937–1938 gives the impression that its communicability has changed in the course of the serial passages in mice.

In the experiment presented in Table VIII newborn mice were used. 2 litters of mice were injected intranasally with the stock strain and 2

others with the mouse passage virus. On the 4th day after inoculation each group of young together with their uninjected mothers was exposed for 4 weeks to 2 litters of virus-free mice which were tested for immunity at the end of this period by intracerebral inoculation with virus. It can be seen from Table VIII that the stock strain proved more contagious under such conditions than the mouse passage strain and likewise was more virulent for the mice infected by contact.

TABLE VIII

Comparison of Communicability of Stock and Mouse Passage Virus in Newborn Mice

Strain of virus	Mice injected intranasally with virus			Exposed mice	
	Age at time of inoculation	Number of mice	Reaction	Age at time of exposure	Number of mice infected as evidenced by signs of the disease or acquired immunity
Stock	1 day	18	5 died; 5 became sick and recovered; 4 showed only a retarded growth; the remainder presented no definite signs of illness	1 day	18/18 (9 mice showed symptoms, the others a decreased growth rate)
Mouse passage	1 "	18	11 died; 6 became very sick and recovered; 1 showed no signs of illness	1 "	4/13 (The 4 infected mice showed no signs of disease but were immunized)

Effect of a Change in the Environmental Conditions on the Course of the Epidemic

In May, 1938, a collection of mice from the infected stock together with some mice from the virus-free colony were taken to Germany. The animals were shipped in metal cages with screen covers, and these cages had to be kept close together on the trip. The fact that the mice failed to become infected is additional proof that the disease is not highly contagious.

The environmental conditions of the mice in Germany differ from those in America, especially as regards climate and diet. The diet in

America consisted of water, corn, powdered milk, white bread and biscuits, occasionally with lettuce or green alfalfa; whereas the present daily ration comprises water, corn, a special kind of dog biscuit, and rye bread. Green alfalfa is added during warmer weather. Milk is omitted. Both diets appear to be adequate. The method of keeping and handling the animals has not been changed.

The change in the environmental conditions seems to have had no influence whatever on the course and character of the epidemic. The disease is still subclinical. Intrauterine infection appears to be its only mode of transmission, and all of the mice from the infected stock tested have been carriers of virus.

DISCUSSION

The present mildness of the disease in the infected stock appears to have been brought about by a combination of two factors, namely, a change in the mode of transmission of the infection, and a shift in the severity of the disease with regard to the age of the mice at the time of infection. This shift, in turn, seems to have been caused by a decrease in the pathogenicity of the virus for embryonic mouse tissue, and a concurrent increase in the resistance of the stock mice to intrauterine infection. Shifts in the severity of the infection in relation to the age of the host also occur with other virus diseases. Some epidemics of poliomyelitis, for instance, are associated with unusually severe reactions in adults, while the disease in children is milder in contrast to its usual behavior.

If no other shift occurs in the future, one may expect the natural disease to remain mild as long as intrauterine infection represents its only way of transmission. The picture may change, however, when some litters are born virus-free and become infected by contact. In this case one might again find sick mice, unless the resistance to the virus of suckling stock mice has increased also.

The disease has reached a remarkable equilibrium. It no longer causes visible illness, nor is the virus markedly inhibited by the defensive forces of the body. If the virus were a living organism, one might call its present relationship to the host a "perfect parasitism." Theoretically, the mouse is an ideal reservoir host for the virus.

The cause for the change in the communicability of the experimen-

tal disease has not been determined. Since the contagiousness of the disease seems to depend entirely or in part on the virus content of the nasal secretions, it is not unlikely that in 1935 the virus had a greater affinity for the upper respiratory tract of mature mice from the virus-free colony, which decreased with the progressive adaptation of the virus to embryonic mouse tissue.

Hereditary factors, which may have played a part in the changes of the disease and are considered by some investigators to be of great importance in the epidemiology of infectious diseases, have not been studied, because it was not desirable for other reasons to interfere with the natural course of the epidemic by selective breeding, for instance, by establishing genetically pure mouse stocks. The possibility therefore exists that the genetic character of the mice has changed since 1935. In the course of extensive experimental work with mice from the virus-free colony the impression was gained that this stock has not changed genetically. It is not unlikely, however, that the above mentioned change in the resistance to intrauterine infection of the mice from the infected stock was of a genetic nature.

The immunological factors influencing the disease are fundamentally the same as in 1935. It has been noted, however, that the number of immune mice whose blood and viscera contained no demonstrable virus progressively decreased in the infected stock. Today, the very solid immunity demonstrable in all of the stock mice is invariably associated with infection. Their tissues and blood contain surprisingly large amounts of virus (2). This "infection immunity" is of the utmost importance for the epidemiology of the disease, because it permits the virus to be transmitted *in utero* with great regularity and no doubt is chiefly responsible for the long persistence of the disease in the infected stock. It is due to the extremely poor antibody response to the infection in mice as well as to certain other factors which have recently been studied (2).

SUMMARY AND CONCLUSIONS

A small mouse stock in which lymphocytic choriomeningitis is endemic has been observed over a period of 4 years. The disease has persisted during that time, but it has become so mild that it can no longer be recognized by clinical observation. In spite of this fact, all of the stock mice tested, both young and old, carried considerable

amounts of virus in their organs and blood. The females readily transmit the infection to their offspring. Intrauterine infection has become the only mode of transmission of the disease in contrast to the situation in 1935 when a certain number of mice were born virus-free and became infected by contact shortly after birth.

The present mildness of the disease appears to be due to two factors, namely, the change in its mode of transmission just mentioned, and a shift in the severity of the disease with regard to the age of the host at the time of infection. This shift has occurred gradually since 1935 when the mice infected *in utero* were the only ones to become sick. Since 1937, however, the virus is quite harmless for such animals and produces symptoms only in suckling mice from the virus-free stock exposed to contact infection. Evidence is presented which suggests that the shift in the severity of the disease was caused by a decrease of the pathogenicity of the virus for embryonic mouse tissue and a concurrent increase of the resistance to intrauterine infection of the mice from the infected stock.

Another change noted concerned the communicability of the experimental disease. In contrast to observations made in 1935 the experimental infection of mature mice from the virus-free colony is now very rarely transmitted by contact to healthy mice, young or old. Suckling mice from the same stock infected by intranasal instillation of virus, however, readily transmit the disease and continue to do so as they grow up. The same is true for mice infected naturally. The reason for this discrepancy has not been ascertained, but it has been shown that naturally infected mice capable of transmitting the disease in general discharge large amounts of virus through the nose for a longer period of time than mature mice infected experimentally which fail to transmit their infection. It may likewise be of significance in this connection that the virus can lose its communicability by animal passage.

A marked change (chiefly climatic and dietary) in the environmental conditions of the infected stock failed to influence the course and character of the epidemic.

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