EXPERIMENTAL ENCEPHALITIS (ST. LOUIS TYPE) IN MICE WITH HIGH INBORN RESISTANCE

A Chronic Subclinical Infection

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Plates 48 to 50

(Received for publication, February 26, 1936)

Previously we have described how the St. Louis encephalitis virus dropped into the nares of highly susceptible mice follows the route of the olfactory nerves to reach the brain within 24 hours. There it incites lesions within 3 days, multiplies rapidly, and sets up a fulminating encephalitis clinically apparent on the 6th day and fatal by the 10th day (1). These mice were generally uniform in their response to the virus.

Attention was next turned to highly resistant mice of the same stock, in fact, originating from the same parents as the susceptibles, and likewise generally uniform in their response to the virus. In these mice virus instilled nasally, although following the olfactory nerve route and reaching the brain promptly, gives rise there to an infection which is non-fatal, subclinical, and chronic. These findings and their possible implications are described in the present paper.

Materials

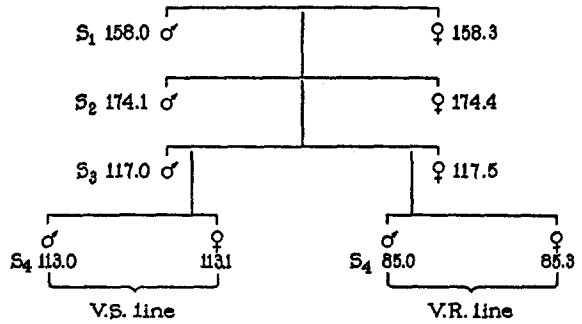
The resistant mice used in these studies were derived from the same hybrid stock as the susceptibles (2). In brief, two litters from the same parents were mated brother to sister. From the one litter-mating, the virus-susceptible line was developed; from the other, the virus-resistant line[1] (Text-fig. 1).

The susceptibles in general succumb to an intracerebral dose of 10^{-9} and to an intranasal dose of 10^{-6} gm. of mouse brain virus, while the resistants succumb to an intracerebral dose of 10^{-5} or greater and survive for the most part an intranasal dose of 10^{-3} gm. By either route, therefore, the resistants survive at

[1] These lines and their development will be described more fully in a forthcoming paper.

least 1,000 fatal doses for susceptibles. Mice of a given strain are not, however, entirely uniform in their response to a given exposure in spite of brother to sister inbreeding for twelve generations and standardization of environment. In comparing strains by means of a nasal instillation of 100 to 1,000 nasal lethal doses of virus for susceptibles, about 95 per cent of the susceptibles will die, as contrasted with 15 per cent of the resistants. Consequently a departure from expectancy of about 10 per cent was allowed for whenever indicated. We have for comparison, then, two lines of progeny from sibling litters, one line transmitting virus-susceptible, the other virus-resistant factors.

The test virus was strain 3 (1) passed as routine intracerebrally in susceptible mice.



TEXT-FIG. 1. *Mus musculus albinus*, Rockefeller Institute strain. Lineage of virus-susceptible and resistant lines from brother-sister matings of two sibling litters.

Limited Neurotropism of Virus in Resistant Mice

In resistant mice, virus shows the same sort of predilection for nervous tissue as in susceptible mice (1). Injected in maximum doses intracerebrally in resistants, its titre increases following an initial lag and lesions arise in the brain in the same manner as in susceptibles. Blood is contaminated immediately following injection and preceding death, and other organs are free of lesions (1). Small doses fatal to susceptibles but not to resistants bring about localized brain lesions in the latter and the virus persists but does not increase beyond a certain level. These findings are partly illustrated in the following experiment.

Experiment 1.—Jan. 14, 1936. Eight susceptible and eight resistant mice were each given 0.03 cc. of a 10^{-6} dilution of mouse brain virus intracerebrally.

This quantity is about ten lethal doses for susceptibles and is non-fatal for resistants. At the same time, eight additional resistant mice received 10,000 times this amount, intracerebrally,—0.03 cc. of a 10^{-2} dilution. At 6 hours and 3, 6, 9, 13, and 21 days after injection, one animal from each group was sacrificed and its blood, spleen, and various portions of its brain were tested for the presence of virus. The materials for testing were emulsified, prepared in serial, tenfold dilutions, and injected intracerebrally in 0.03 cc. quantities into two Swiss mice. The titre of virus in the test material was taken as the highest dilution fatal to at least one of the two injected mice expressed in numbers of intracerebral lethal doses on the basis that 0.03 cc. of 10^{-7} dilution is one fatal dose. The fully virulent brain, therefore, is said to contain 0.03×10^{7} fatal doses, or roughly 10^{9}. The titre is taken as the exponential value of the highest fatal dilution with sign changed increased by 2,—10^{-7} dilution, or 10^{9} titre.

The results are given in Table I. No virus was recovered from the susceptible mouse sacrificed at 6 hours. From the one examined at 3 days, virus was recovered in blood, spleen, olfactory areas, and in the remainder of the brain in 10^{-2} dilution, while from the one tested in convulsions on the 6th day, virus was found in blood and spleen and in whole brain diluted 10^{-7} times. The remaining five susceptibles died of encephalitis without being tested on the 5th and 6th days. All resistant mice receiving a like amount of virus remained well. At 6 hours virus was not recovered, at 3 days only from the piriform area, and at 9 days from olfactory areas and the remainder of the brain in 10^{-2} dilution. No virus was found at 13 and 21 days. Resistant mice receiving the large dose remained well, save for one dying on the 13th day, but showed virus in blood, spleen, olfactory areas, and the remainder of the brain in 10^{-2} dilution at 6 hours, spleen and olfactory areas at 3 days, olfactory areas on the 6th day, and in the remainder of the brain, besides, on the 9th day. On the 13th day, no virus was recovered.

Injected intraperitoneally, the virus behaves similarly in both sorts of mice. The following experiment illustrates the happenings.

Experiment 2.—Mar. 27, 1934. Resistant mice were given 0.5 cc. of a 1 to 200 dilution of virus intraperitoneally. At intervals from 10 minutes to 7 days after injection, individuals were sacrificed and their blood, brains, and spleens tested for the presence of virus, according to the technique previously described. An equal number of susceptible mice was given the intraperitoneal injection for comparison and three from each group were reserved as controls.

TABLE I

Distribution and Titre of St. Louis Virus in Susceptible and Resistant Mice Following Intracerebral Injection

Type of mouse	Dose of virus 0.03 cc. of dilution	Time after injection	Presence of virus. 0.03 cc. of dilution to two mice									
			Blood	Olfactory bulb	Piriform area	Remainder of brain						Spleen
			Undiluted	10^{-1}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
Susceptible	10^{-6}	6 hrs.						N.T.		N.T.	N.T.	
"	10^{-6}	3 days	7,* 8	7, 7	6, 8	8, 8	N.T.	"		"	"	6, 7
"	10^{-6}	†6 "	6, 7	N.T.	N.T.	N.T.	"	"	N.T.	6, 6	7, 7	7, 7
Resistant	10^{-6}	6 hrs.						N.T.		N.T.	N.T.	
"		3 days			7, 8		N.T.	"		"	"	
"		6 "					"		N.T.	"	"	
"		9 "		8, 10	7, 8	8	"		"	"	"	
"		13 "										
"		21 "										
Resistant	10^{-2}	6 hrs.	7, 8	8	8	8, 8	N.T.		N.T.	N.T.	N.T.	7
"		3 days		7, 7	6		"		"	"	"	7, 8
"		6 "		7	7	9	"		"	"	"	
"		9 "			9, 9	6, 8	"	8, 9				
		13 "										

* = duration of life of mouse in days.

N.T. = dilution not tested.

Blank spaces = mice remained well 21 days.

† Mouse in convulsions.

The controls remained well. Virus in the resistant mice was distributed in about the same manner as in the susceptible mice. 10 minutes after injection it was present in the spleen and at 20 and 60 minutes in the undiluted blood as well. It was absent from the blood at 3 hours and at all intervals tested thereafter. The spleens, however, contained virus at 3 and 9 hours and at 1, 2, and 3 days. Further tests showed that virus reached the blood as quickly in resistant as in susceptible mice but in somewhat less amounts and for shorter periods. Spleens and brains showed no lesions 2, 5, and 10 days following injection of virus.

Chronic Infections Following Nasal Instillation of Virus

The tests thus far described showed that resistants withstood many intracerebral fatal doses for susceptibles, that virus persisted at low titre for about 9 days and then disappeared, and that lesions following injection cleared up progressively. When it came to instilling the virus intranasally into resistant mice, the following distinguishing features were noted,—first, 1,000 intranasal doses fatal for susceptibles were not harmful to resistants; second, the titre of virus in the brain did not increase beyond a certain point; third, virus persisted in the brains as long as 4 weeks; fourth, lesions in the brain did not appear for 8 days but once present, were found for 3 months, while the animal remained quite well; fifth, these lesions closely resemble those found in human cases of encephalitis. These findings are described in the following experiments.

Intranasal Inoculation. Experiment 3.—Dec. 27, 1934. Batches of resistant and susceptible mice were given a nasal instillation of 0.03 cc. of a 1 to 100 dilution of virus. At intervals from 1 to 21 days after injection animals were sacrificed and their brains and spleens tested for content of virus as in Experiment 1. Twelve resistant and five susceptible mice were reserved as controls.

The results are shown in Table II. The susceptible controls died in 6 and 7 days; one resistant control died on the 9th day. No virus was found in brains or spleens of resistant or susceptible mice on the 1st day following injection. On the 2nd day, however, virus was present in brains of both resistants and susceptibles in 10^{-1} and 10^{-2} dilutions, respectively. On the 3rd day, virus was present in resistants in 10^{-1} and 10^{-2} dilutions, as contrasted with 10^{-4} and 10^{-6} in suscep-

TABLE II

Brain and Spleen Content of St. Louis Virus in Resistant and Susceptible Mice Following Nasal Instillation

Mice		Time interval injection to test	Content of virus. 0.03 cc. of each dilution to two mice							
			Brain							Spleen
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-1}
		days								
Resistant	1	1			N.T.	N.T.	N.T.	N.T.	N.T.	
	2	1			"	"	"	"	"	
	3	2	5,* 5			"	"	"	"	
	4	2	5, 5			"	"	"	"	
	5	3	5, 8			"	"	"	"	
	6	3	5, 6	6, 6		"	"	"	"	6
	7	4	5, 7	7, 7		"	"	"	"	6, 7
	8	4	5, 7	8		"	"	"	"	6, 7
	9	5	6, 6	6, 7	6, 6		"	"	"	6, 6
	10	5	6				"	"	"	
	11	6	6, 6	6, 7	6, 8			"	"	
	12	6	6, 6	6, 6	6, 8			"	"	
	13	7	6, 6	6, 7				"	"	
	14	7	5, 5	6, 6				"	"	6, 7
	15	12	6, 6	6, 7	6		"	"	"	
	16	12	6, 6	7, 8			"	"	"	
	17	18				"	"	"	"	
	18	18				"	"	"	"	
	19	21				"	"	"	"	
	20	21				"	"	"	"	
	21	21				"	"	"	"	
Susceptible	1	1			N.T.	N.T.	N.T.	N.T.	N.T.	
	2	1			"	"	"	"	"	
	3	2	5, 6	5		"	"	"	"	
	4	2	6, 9			"	"	"	"	
	5	3	5, 5	5, 5	5, 6	6, 8	6, 9	6, 9	"	7, 8
	6	3	5, 6	6, 6	6, 6	9, 10			"	
	7	4	N.T.	5, 5	8, 8				"	
	8	4	"	5, 5	5, 7	5, 7	8, 8	8	"	
	9	5	"	4, 6	6, 6	6, 8	7, 7	7, 7	"	5, 5
	10	5	"	4, 4	6, 6	6, 6	6, 7	6, 6	"	
	11	6	"	N.T.	5, 5	5, 5	5, 5	5, 6	6, 6	4, 4
	12	6	"	"	5, 6	7, 8	7, 8	8, 8		6, 8
	13	7	"	"	5, 6	6, 7	7, 7	7, 8	8, 8	8, 8
	14	7	"	"	5, 5	5, 5	5, 5	5, 5	7, 7	8, 8

* = duration of life of mouse in days.

N.T. = dilution not tested.

Blank spaces = mice remained well 21 days.

tibles. On the 4th, 5th, 6th, 7th, and 12th days, virus in the brains of resistant mice remained at the 10^{-1} to 10^{-3} dilution level, while in the susceptibles it reached a maximum tested of the 10^{-7} dilution on the 6th day. By the 7th day all susceptibles had died of encephalitis. The batch of resistants for testing remained well. At 18 and 21 days no virus was found in their brains. Spleens were irregularly positive in both groups from the 3rd to the 7th days inclusive.

Additional experiments on resistant mice gave results similar to the above together with one brain of ten positive at 4 weeks and with negative findings in the blood from 1 hour to 7 days after nasal instillation of virus. Moreover, spleens of twenty-one healthy, resistant mice were tested 4 to 5 weeks following nasal instillation of virus with four, or 19 per cent, positive and spleens of thirty-one mice after 6 weeks with four, or 13 per cent, positive.

Nasally infected, resistant mice were tested further with relation to the distribution of virus in various portions of the brain.

Experiment 4.—Jan. 27, 1936. A. Eight resistant mice received intranasally 0.03 cc. of a 1 to 100 dilution of virus of tested standard titre, 10^9. At intervals thereafter of 1 to 11 days animals were sacrificed, their brains removed, sectioned, and tested for content of virus. Olfactory bulbs, piriform area, cortex, cerebellum, pons, and medulla were each emulsified with alundum, diluted roughly one part to ten of hormone broth, and injected intracerebrally into two Swiss mice.

Jan. 31, 1936. B. Seven additional resistant mice were given virus in the same manner and sectioned for content of virus on the 7th, 10th, 14th, 16th, 18th, 21st, and 23rd days following instillation.

At the same time additional susceptibles and resistants were given virus intranasally, sacrificed at the stated intervals, their brains fixed in Zenker's acetic solution, sectioned, and stained for histological study. Reference to this material is made later.

The distribution of virus in these mice is shown in Table III. At 24 hours it was present in the olfactory bulbs. At 2 days and in one mouse at 3 days, no virus was recovered.

In the second mouse at 3 days and in the one mouse examined at 5 days, virus was recovered from olfactory bulbs, piriform area, and pons. At 7, 9, 10 (one mouse), and 11 days, it was present in all regions tested. At 13, 15, 18, and 21 days, no virus was recovered.

Evidently, therefore, virus traverses from nose to olfactory bulbs in resistant mice as promptly as in susceptible mice and spreads

throughout the brain. The striking difference lies in its failure to multiply readily in resistants and its ability to survive for periods as long as 3 to 4 weeks.

Other events in the intranasally instilled mice were the late appearance, long duration, and character of central nervous system lesions.

TABLE III

Distribution of St. Louis Virus in Brains of Resistant Mice Following Nasal Instillation

Mouse No.	Time interval injection to test	Content of virus. 0.03 cc. of 1 to 10 dilution injected intracerebrally into two mice					
		Olfactory bulbs	Piriform area	Cortex	Pons	Cerebellum	Medulla
	days						
Resistant 1A	1	7,* 7					
2A	2						
3A	3						
4A	3	6	9		9		
5A	5	5, 7			7		
6A	7	6, 6	6, 6	8, 8	6, 6	6, 8	6, 8
7A	9	5, 7	5, 6	5, 7	6, 7	6, 6	6, 7
8A	11	D, 6	6, 6	7, 7	6, 6	7, 7	7, 7
Resistant 1B	10	7, 7	8, 9	7, 7	7, 7	7, 8	8
2B	10						
3B	13						
4B	13						
5B	15						
6B	18						
7B	21						

* = duration of life of mouse in days.

N.T. = dilution not tested.

Blank spaces = mice remained well 21 days.

D = mouse died of trauma following injection.

Lesions Following Nasal Instillation of Virus

Experiment 5.—Mar. 26, 1935. Resistant mice were given an intranasal instillation of 0.03 cc. of a 1 to 100 dilution of virus. On the 3rd, 6th, 8th, 10th, 15th, and 20th days following injection, two mice were sacrificed and the virus content of their brains titred in the usual manner. Three additional mice were sacrificed at each time and their brains studied histologically for the presence of lesions. From the brain of each mouse about fifty sections were prepared of olfactory bulbs

and ten each of piriform area, anterior cerebrum, posterior cerebrum, mid-brain, cerebellum, and medulla, cervical, thoracic, and lumbar cord. The sections, 5 to 10μ in thickness, were mounted in strips of four to ten per slide, and stained for the most part with eosin-methylene blue. Virulence of the virus was checked by intracerebral and intranasal titrations in susceptible mice. All resistant mice remained well.

3 Days after Nasal Instillation.—Brains of two tested mice contained virus in the 10^{-2} dilution (Table IV). The sections of brains of three mice appeared normal.

TABLE IV

Brain Content of St. Louis Virus in Resistant Mice Following Intranasal Instillation

Mice	Time interval injection to test	Brain content of virus. 0.03 cc. of each dilution to two mice			
		10^{-1}	10^{-2}	10^{-3}	10^{-4}
	days				
Resistant 1	3	6,* 7	8, 10		
2	3	8, 8	9		
3	6	6, 6	6, 8	8, 9	10
4	6	6, 8	6, 9	10	
5	8	6, 7			N.T.
6	8	9			"
7	10	7, 7	8, 9		"
8	10	5, 5	7, 8		"
9	15	6			"
10	15	6			"
11	20			N.T.	"
12	20			"	"

* = duration of life of mouse in days.

N.T. = dilution not tested.

Blank spaces = mice remained well 21 days.

6 Days after Nasal Instillation.—Brains of the two tested mice showed virus in the 10^{-3} and 10^{-4} dilutions respectively, but the sections of brains of the three mice examined for lesions showed nothing abnormal.

8 Days after Nasal Instillation.—Brains of two mice tested at this time were positive only in the 10^{-1} dilution. Of three mice examined for lesions in the brain, one was negative and two showed inflammatory changes in the ventral, medial portions of the olfactory bulbs and piriform lobes similar in every respect to the primary lesion seen in susceptible mice on the 3rd day (1). Collected in the perivascular or subpial spaces, or scattered superficially nearby, were round cells and occasional polymorphonuclear leucocytes (Fig. 1). Small blood vessels in the vicinity were congested but all nerve cells appeared normal.

10 Days.—Virus was present in the brains of the two tested mice through the 10^{-2} dilution.

All mice appeared well and one of the three examined for brain lesions showed nothing abnormal. In the second mouse the inflammatory lesion was marked on the ventral aspect of olfactory bulb and piriform areas (Fig. 2). Leucocytes were collected in considerable numbers about the superficial blood vessels and were scattered throughout the neighboring tissue. They were also present around the deep blood vessels of the cortex. Hyperplasia of the pia, noted in susceptible mice on the 6th day, was conspicuous over the ventral, anterior piriform area. The third mouse showed these lesions in a more advanced state, plus necrosis of a few pyramidal nerve cells in the ventral piriform area. The necrosis was sharply localized to this group of cells but appeared similar in all respects to that seen in the susceptible mice on the 5th day. Some cells were normal save for swollen, eosin-staining cytoplasm. The cytoplasm in others was eosin-staining and granular, or shrunken and surrounding nuclei in various stages of pycnosis. A few cells were entirely destroyed.

15 Days.—Virus was recovered from the two tested brains in the 10^{-1} dilution only. The remaining mice appeared well and one of three studied for brain lesions showed nothing abnormal. The other two showed perivascular accumulations of round cells throughout the brain, plus necrosis of pyramidal cells of olfactory bulbs and piriform areas, more extensive than in the 10 day mouse (Fig. 4). The cell exudate in the neighborhood was marked and there was present a glial cell proliferation not seen in sections of susceptible mice (Fig. 3).

20 Days.—No virus was recovered from the whole brains of the two tested mice but the two examined for brain lesions showed inflammatory changes in both superficial and deep tissue. Vessels were quite generally collared with round cells and both beneath the pia and deep in the brain, foci of round cells were scattered about the blood vessels.

Experiment 6.—Oct. 3, 1935. Thirty-one resistant and 118 susceptible mice were given an intranasal instillation of 0.03 cc. of virus diluted 1 to 100. 116 (98 per cent) of the susceptibles died of encephalitis within 9 days; four (12.9 per cent) of the resistants died on the 6th, 10th, 13th, and 25th days respectively.

Oct. 26, 1935. Twenty-two resistant and twenty-five susceptibles were given a similar intranasal instillation of virus resulting in the death from encephalitis of one (4.5 per cent) of the resistants on the 16th day and twenty-four (96 per cent) of the susceptibles by the 10th day.

The forty-eight resistant mice which survived these two tests were examined at intervals of 27 to 117 days after injection for brain and spleen lesions and also for the presence of active virus in the brain.

Virus was not recovered in the brains of any of these survivors tested 1 to 3 months after nasal instillation. Lesions, however, were marked on the first examination, 27 days, and were still present although resolving at 117 days.

At 27, 36 to 38, and 51 to 54 days, the seventeen mice examined showed a diminishing number of round cells collected beneath the pia and surrounding the

neighboring blood vessels. Another type of lesion became conspicuous, however, namely, collections of round cells in the Virchow-Robin spaces of vessels deep in the caudate nucleus and in neighboring areas, associated with glial cells and one or two degenerating nerve cells (Figs. 5 and 6). This type of lesion, so characteristic of the human disease and of lethargic encephalitis as well, became most marked at about 5 weeks, shortly after active virus could no longer be recovered. At the same time other vessels scattered through the cortex, pons, and medulla showed perivascular cuffs of round cells. At 51 to 54 days, the same lesions were present but fewer in number.

At 72 to 77 days, four of ten mice examined showed the above changes but still less extensive. At 97 days, one of three brains showed a few round cells beneath the pia and near neighboring vessels. At 117 days three of eight mice showed a similar slight exudate of leucocytes.

Similar studies on additional batches of resistant mice (Experiment 4) confirmed the above results.

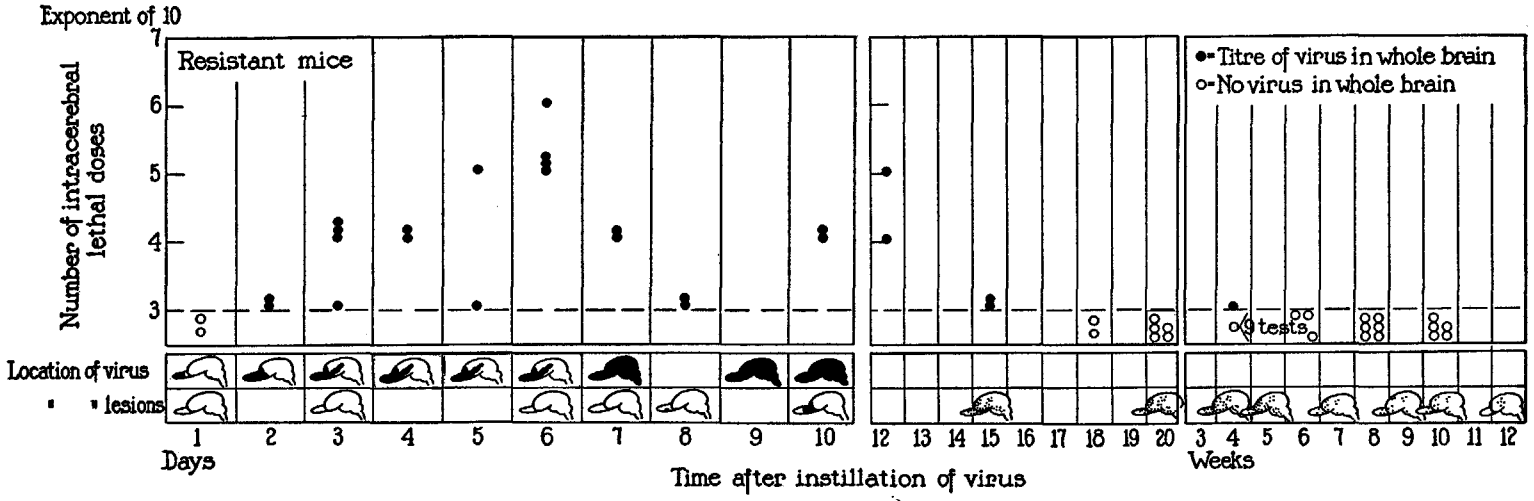
In brief, resistant mice given virus intranasally remained well but showed brain lesions from 8 to 117 days after infection, while virus was recovered from 1 to about 28 days (Text-fig. 2). Lesions at first consisted of an exudate of mononuclear plus a few polynuclear leucocytes beneath the ventral pia of the olfactory bulbs and piriform area and in the Virchow-Robin spaces surrounding neighboring blood vessels. In some instances the pia was hyperplastic. In two cases only, examined on the 10th and 15th days, was nerve cell necrosis conspicuous. After 3 weeks, leucocytes were found deeper in the brain substance, collected about blood vessels in the cortex and medulla and in foci especially in the caudate nucleus, accompanied by glial cells and occasionally by a necrotic nerve cell. After 8 to 12 weeks, lesions became less apparent, some brains appeared normal, while others showed only an occasional collared vessel deep in the caudate nucleus region or a few leucocytes beneath the pia.

These changes, unlike those in susceptible mice (1), resemble closely those in the human disease and in lethargic encephalitis as well, and certain cases of atypical encephalitis and bulbar poliomyelitis.

Alteration in Amount of Virus by Passage through Resistant Mice

The routine virus is altered by passage through resistant mice.

Experiment 7.—Nov. 18, 1935. Ten virus-resistant mice were given a nasal instillation of 0.03 cc. of a 1 to 100 dilution of freshly prepared mouse brain virus as described in Experiment 3. At intervals thereafter, from 1 to 7 days, mice



TEXT-FIG. 2. Distribution of virus and lesions in resistant mice following nasal instillation of St. Louis virus.

were sacrificed and tested for content of virus in various portions of the brain. At the same time, the nasal titre of the virus for susceptibles was checked. On the 7th day, three mice were sacrificed, their brains pooled, titred in susceptible mice both intracerebrally and intranasally, and passed again intranasally into eight more virus-resistant mice. Each day, one of these second passage, virus-resistant mice was sacrificed for tests on distribution of virus in brain and on the 7th day, three were sacrificed, brains pooled, titred intracerebrally and intra-

TABLE V

Alteration in Amount of St. Louis Virus after Nasal Passage through Resistant and Susceptible Mice

Source of virus Type of mouse	Virus titre tested in susceptible mice										
	Intracerebral titre 0.03 cc. to two mice							Nasal titre 0.03 cc. to two mice			
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Susceptible	N.T.	N.T.	N.T.	N.T.	N.T.	4,* 5	5, 6	N.T.	N.T.	6, 6	6
Resistant	"	"	7, 7	12 (A)		N.T.	N.T.			N.T.	N.T.
1 passage											
Resistant	"	"				"	"		N.T.	"	"
2nd passage											
Resistant		"	N.T.	N.T.	N.T.	"	"		"	"	"
3rd passage											
Resistant 1 passage plus Susceptible 1 passage (A)	N.T.	"	"	"	"	6, 6	7, 8	7, 8	8, 8	8	"

* = duration of life of mouse in days.

N.T. = dilution not tested.

Blank spaces = mice remained well 21 days.

A = brain of susceptible mouse used for titration.

nasally as in the first passage, and finally passed for the third time intranasally into virus-resistant mice.

The virus carried as routine in brains of susceptible mice dropped 1,000-fold in amount after one intranasal passage in resistant mice. At the outset (Table V) it was fatal in 10^{-4} dilution intranasally and 10^{-7} dilution intracerebrally. This virus instilled into the noses of resistant mice (first passage) and recovered from their brains was not fatal for susceptible mice when given intranasally and was

fatal to them by intracerebral route not beyond the 10^{-4} dilution. Again, this first passage virus in brains of resistant mice, when again instilled into the noses of resistant mice, second passage, failed to reach their brains and when these brains were pooled and tested, failed to kill susceptible mice either intranasally or intracerebrally. At the same time, when first passage virus in brains of resistants was placed in brains of susceptibles, and the brains of these susceptibles A, were titred after the animals had succumbed to encephalitis, the titre reverted at once to normal.

This experiment was repeated on three occasions with the uniform result that after one intranasal passage in resistants, the virus did not kill susceptibles when given intranasally in highest concentration and fell in titre 1,000-fold when injected intracerebrally in susceptibles. After a second intranasal passage in resistants, another 1,000 reduction in titre took place, rendering the second passage material generally non-infective for susceptibles.

These intranasal passage experiments were paralleled by intracerebral passages of virus in resistant mice.

Experiment 8.—Jan. 13, 1936. Virus was injected intracerebrally in 10^{-2} dilution into one susceptible and two resistant mice and at the same time was titrated intracerebrally and intranasally in susceptible mice. 6 days later, at a time when brain content of virus was presumably at a maximum, the animals were sacrificed, brains removed, pooled, and titrated intracerebrally and intranasally in susceptible mice. Finally, the brain of a susceptible mouse A, succumbing to the intracerebral injection of virus from the brain of a resistant mouse, was likewise titrated in susceptibles to determine whether one passage in susceptibles would restore the titre to maximum.

The results are given in Table VI. At the outset, the virus was fatal to susceptibles intracerebrally in 10^{-7} and intranasally in 10^{-4} dilutions. The brain of the test susceptible mouse receiving the virus showed a similar titre but that of the resistant mouse was fatal intracerebrally not beyond the 10^{-5} dilution and intranasally not at all. Finally, the brain of a susceptible A, which had succumbed to the 10^{-5} dilution of resistant brain virus given intracerebrally, was titrated in susceptibles and found to contain the usual amount of virus.

Serial intracerebral injections and passages in resistant mice were carried out twenty-two times and virus was tested for as above at

the eleventh, fourteenth, and seventeenth passages. On each occasion, resistant brain virus injected into susceptibles intracerebrally was fatal not beyond the 10^{-4} or 10^{-5} dilution and intranasally was harmless.

In summary, virus in susceptible mice, when transferred to resistants, loses in quantity approximately 1,000-fold. This loss is such that after one passage in resistants the virus becomes non-infective

TABLE VI

Alteration in Amount of St. Louis Virus after Intracerebral Passage through Resistant and Susceptible Mice

Type of mouse	Brain titre of virus after intracerebral injection										
	Intracerebral titre 0.03 cc. to two or three mice							Nasal titre 0.03 cc. to three mice			
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Susceptible	N.T.	N.T.	N.T.	N.T.	N.T.	4,* 5	5, 6	N.T.	N.T.	6, 6, 6	6, 8
Susceptible	"	"	"	"	"	7, 7	7, 7	"	"	8, 8, 8	
1 passage Resistant	"	"	"	8, 8, 8	8, 9, 9						
1 passage Resistant					(A)						
1 passage plus (A) Susceptible 1 passage	"	"	"	5, 6	6, 6	6, 6	6	N.T.	8, 8, 9	8, 9, 9	9

* = duration of life of mouse in days.

N.T. = dilution not tested.

Blank spaces = mice remained well 21 days.

A = brain of susceptible mouse used for titration.

for susceptibles by the nasal route. If passed in series in resistants intracerebrally, the resistant brain virus remains active to the 10^{-4} dilution. Its nasal infectivity, however, is lost at once due probably to the fact that the required infecting dose, which is about 10,000 times the intracerebral infecting dose, is not contained in the resistant brain. Virus passed in resistants appeared to undergo no qualitative change and was restored to titre by one passage in susceptibles.

DISCUSSION

In discussing the implications of these findings, it must first be made clear that we are using two inbred strains of mice of identical parentage which differ widely and quantitatively in inborn resistance to the virus. At the same time, the individuals within each strain are mostly uniform in their response, indicating that variables not only genetic but also environmental have been controlled. A second point is that we deal here with resistance not alone to artificial infections following a parenteral injection of virus but to the more natural infections resulting from placing the virus on the external nares. And finally, we have sought to throw light on the resistance mechanism by analyzing extremely resistant and extremely susceptible individuals whose responses are generally predictable and applying the knowledge to the problem in average mice.

The experiments show that, *ceteris paribus*, inborn resistance factors determine the amount of mortality, type of clinical disease, persistence of virus, and type of pathology. Mice with inborn low resistance die promptly with fulminating encephalitis and show extensive destruction of the brain; mice with high resistance generally remain well and show microscopic focal lesions in the brain closely resembling those in the human disease. Finally, virus persists at least 3 weeks in the brains and spleens of resistant mice.[2]

The pathological findings suggest that there is, besides an etiological specificity of lesions, a host specificity. For example, the lesions in resistant mice following nasal instillation of St. Louis virus are extraordinarily like those in man. But they resemble also those in human cases of lethargic encephalitis, unnamed encephalitides, and certain types of bulbar and encephalo-poliomyelitis. Again, lesions in susceptible mice given St. Louis virus are entirely different from those in man but closely resemble those in mice following nasal instillation of louping ill and equine encephalomyelitis viruses, and are similar in general to those of yellow fever and vesicular stomatitis in mice. The character of a given lesion, therefore, is affected as much by host factors as by those of the specific agent.[3]

[2] A similar controlling effect of inborn resistance on the character of *B. enteritidis* mouse typhoid has been reported (3).

[3] Rake has separated quantitative differences in lesions in mice following nasal instillation of pneumococci dependent on inborn resistance factors (4).

A further point previously stressed (1) in this connection is that the development and extension of lesions do not bear a simple relationship to the presence and quantity of virus. Virus given intranasally to resistant mice is demonstrable in the brain 7 days before lesions are recognized and disappears 2 months before lesions clear up. In fact, the foci and neighboring perivascular accumulations of leucocytes which are the outstanding and characteristic lesions of the disease in monkeys and man do not usually appear in the caudate nuclei until virus is no longer present. This dissociation of virus and lesions may well account for the failure to recover a specific agent from human cases in which lesions of this sort are conspicuous.

We have no definite conception of the essential difference between these resistant and susceptible mice save that the central nervous systems of the latter appear highly susceptible to a number of the encephalitis-producing viruses.

With regard to the St. Louis virus itself, the present experiments amplify those reported with susceptible mice (1), indicating that the virus is chiefly neurotropic, traveling from nose to brain by the olfactory route. Whether passage takes place by axons or perineural spaces is still uncertain, however, since lesions in resistant mice are an unreliable index of the presence of virus. The predominating picture is one of subpial and perivascular exudate of leucocytes and little or no conspicuous nerve cell involvement.

The 1,000-fold loss of titre of virus when passed from a susceptible to a resistant host and its prompt restoration when returned to a susceptible probably has a counterpart in less well controlled reactions between other viruses and hosts, such as the poliomyelitis virus and the monkey. Finally, in nature this salutary rôle of the resistant host in limiting multiplication of virus must be largely offset by the more dangerous one of acting as a reservoir of virus. Transfer of virus from a resistant to a susceptible with consequent increase in titre, together with reverse transfer from susceptible to resistant host with similar decrease may represent phenomena of special epidemiological significance.

CONCLUSIONS

1. St. Louis encephalitis virus injected intracerebrally or intraperitoneally in maximum doses in resistant mice is distributed and is

effective in a manner generally similar to that in susceptible mice. The minimum infecting dose is at least 1,000 times greater in resistant than susceptible mice and virus injected in the brain tends to remain at a relatively low titre, persist for a few days, and then disappear.

2. Virus dropped in the nares is demonstrable and progresses in the brains of resistant mice as in susceptible mice, but does not increase in titre beyond the 5th day, does not bring about fatal encephalitis, and persists for at least 4 weeks.

3. Lesions in the brains of resistant mice following nasal instillation of virus do not appear until the 8th day, reach a maximum at 40 days, and are still present, though resolving, at 3 months. The changes resemble those seen in the human disease and in other unnamed forms of encephalitis.

4. The quantity of virus drops 1,000-fold when recovered from resistant mice and becomes non-infective by the nasal route. Passage in susceptible mice promptly restores its full titre.

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EXPLANATION OF PLATES

Sections of brains of resistant mice at various intervals after nasal instillation of St. Louis encephalitis virus. Eosin-methylene blue stain.

PLATE 48

FIG. 1. 8 days. Earliest lesion. Olfactory bulbs. Ventral, medial, posterior surface. One of several areas showing an exudate of round cells beneath the pia and extending between the olfactory nerve bundles toward the pyramidal cell layers. Nerve cells appear unaffected. This lesion is similar in every respect to that appearing in susceptible mice on the 3rd day (Reference 1, Fig. 1). × 500.

FIG. 2. 10 days. Early lesion. Piriform area. Ventral, medial surface. Round cells are collected in considerable numbers beneath the pia and in the Virchow-Robin spaces of neighboring blood vessels. Capillaries are prominent. Nerve cells appear normal. This lesion was present in most mice examined at this time and resembled precisely that noted in susceptible mice on the 4th day (Reference 1, Figs. 2, 3). × 300.

PLATE 49

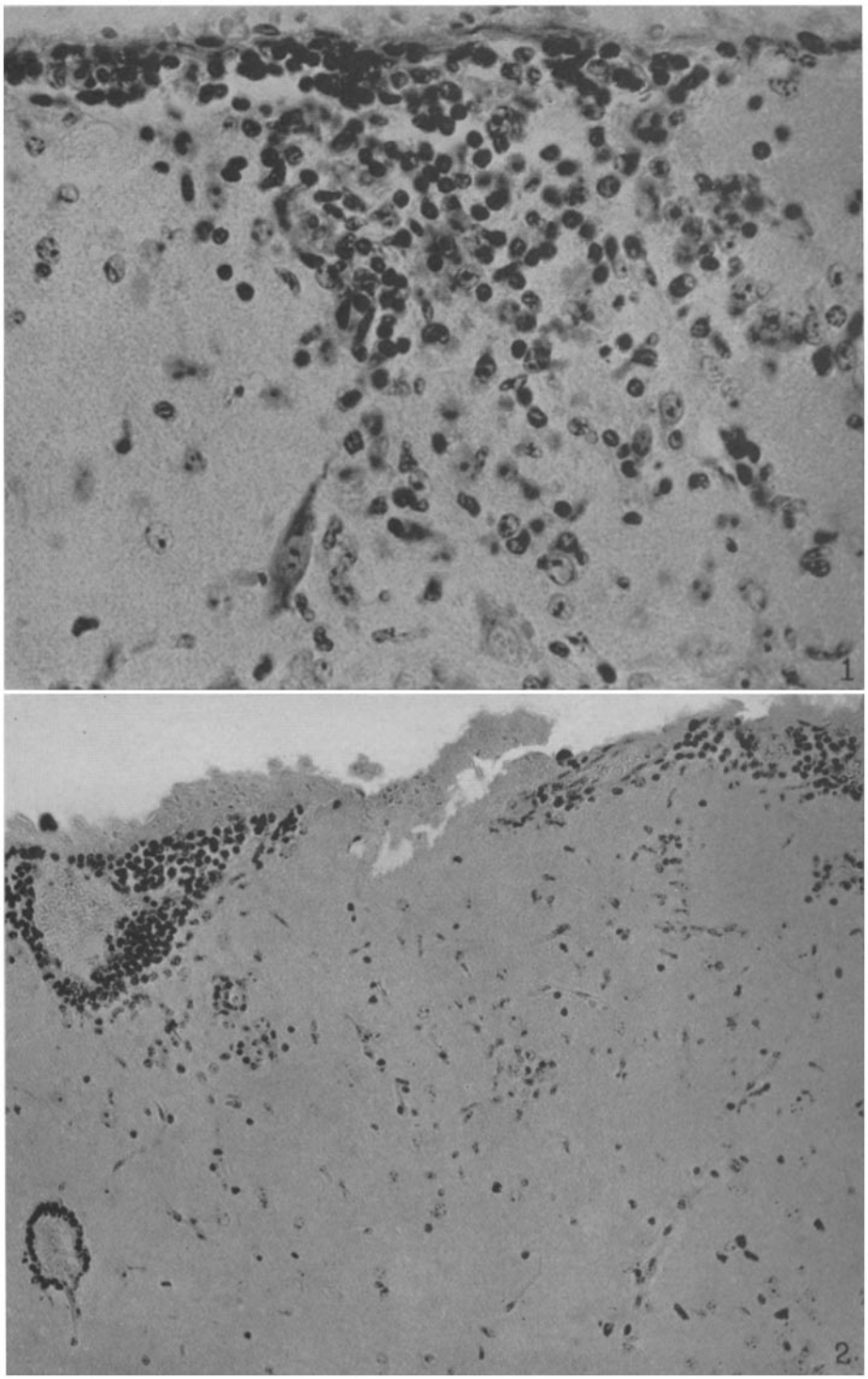
FIG. 3. 15 days. Piriform area similar to Fig. 2. Besides the subpial exudate, this unusual reaction was noted in two animals, consisting of round cells scattered more diffusely through the superficial layers, capillaries unusually conspicuous, a proliferation of glial cells, and scattered nerve cells undergoing necrosis. × 300.

FIG. 4. 15 days. Olfactory bulbs. Area similar to Fig. 1. The exterior band of pyramidal nerve cells is interrupted by an area, upper left in the figure, of necrosis. Nerve cells are absent altogether, or are shrunken, with deeply staining cytoplasm and pycnotic nuclei. Many nerve cells in the deeper molecular layers, lower left of figure, are also necrotic. This lesion was encountered in two mice. × 300.

PLATE 50

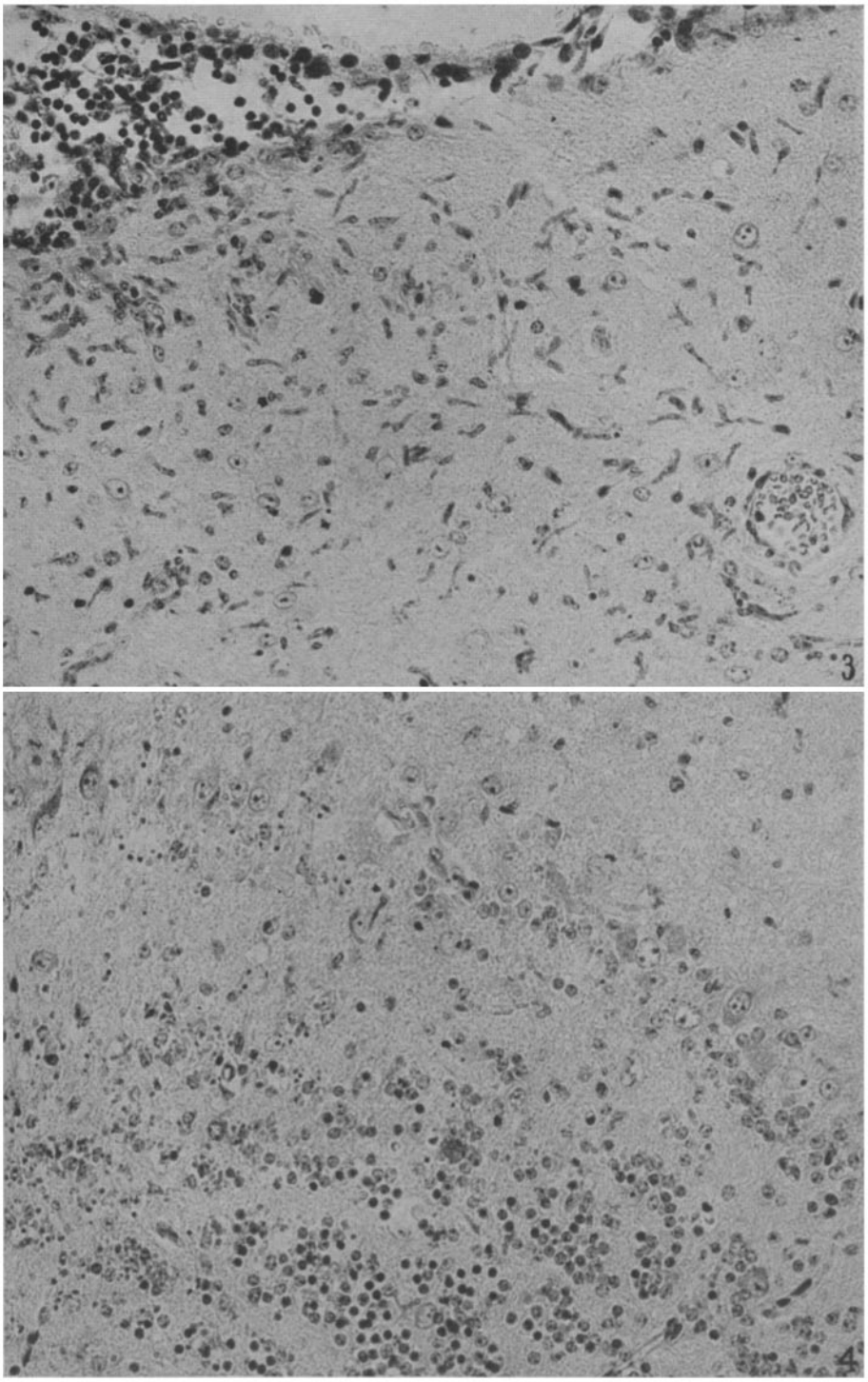
FIG. 5. 36 days. Caudate nuclei. This lesion was conspicuous in the greater percentage of mice examined between the 30th and 50th days. Mononuclear cells are collected in the perivascular spaces and in neighboring foci. These foci also contain an excess of glial cells and occasionally a necrotic nerve cell. × 300.

FIG. 6. 74 days. Caudate nuclei. Similar lesions showing two affected nerve cells in the midst of mononuclear leucocytes collected near a blood vessel (lower left of figure) which is itself surrounded by round cells. × 550.



Photographed by Louis Schmidt

(Webster and Clow: Encephalitis in mice with high resistance)



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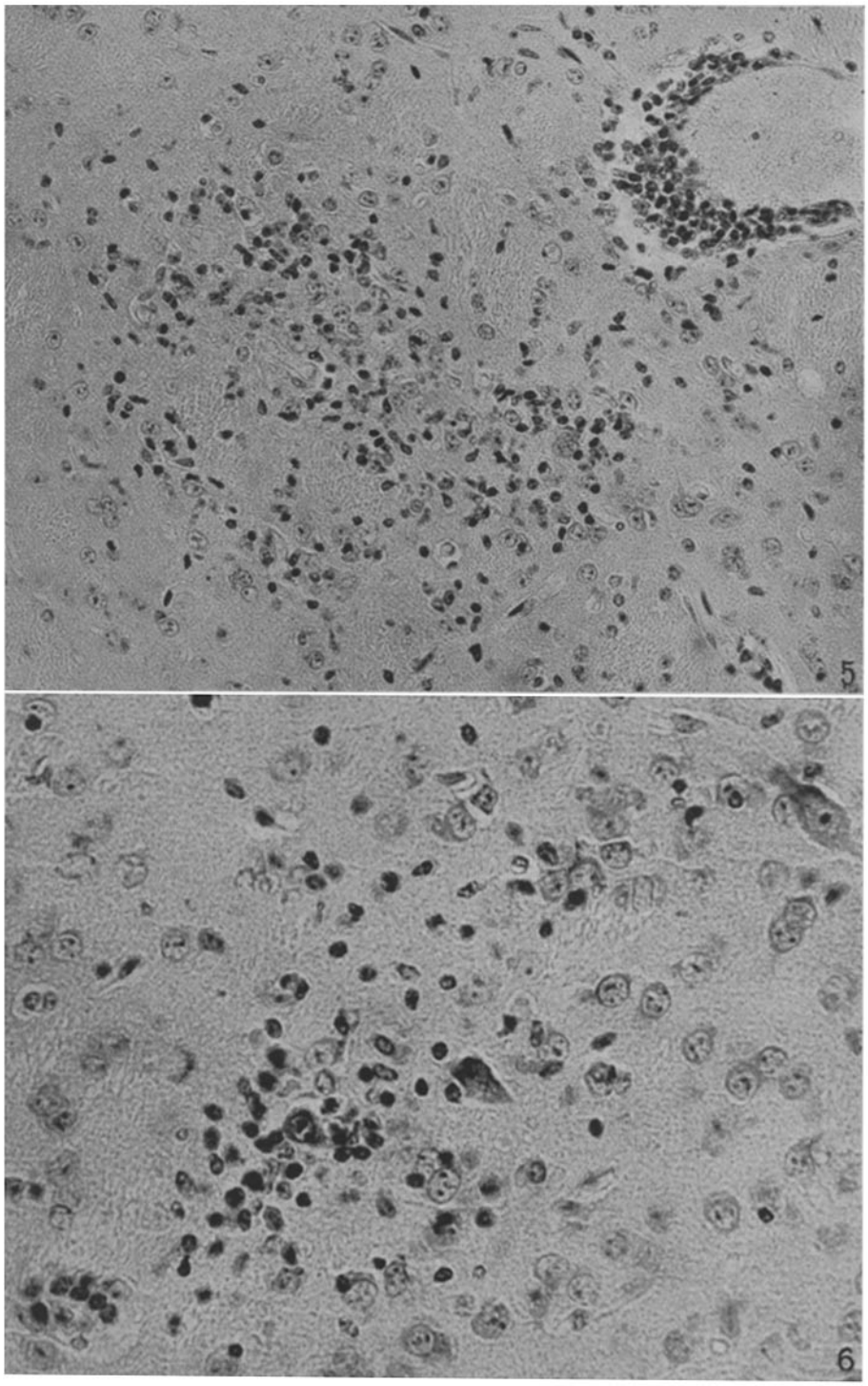

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