THE LIMITED NEUROTROPIC CHARACTER OF THE ENCEPHALITIS VIRUS (ST. LOUIS TYPE) IN SUSCEPTIBLE MICE

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Plates 32 to 34

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The finding of a small experimental animal, the mouse, susceptible to the St. Louis encephalitis virus (1, 2) has provided a means of studying quantitatively a human type of central nervous system disease. The highly susceptible mice we have used are uniform in their reactions to the virus and following nasal instillation, regularly contract encephalitis. Preliminary tests on the infectivity and distribution of virus injected by different routes have been reported (3). The present paper describes in detail the limited neurotropism of the virus, its invasiveness following nasal instillation, its transmission from nose to brain by the olfactory route, and its establishment in the brain several days prior to the onset of clinical disease.

Predilection of Virus for Nervous Tissue (Neurotropism)

Results of Injecting Virus Intracerebrally.—Experiment 1. Jan. 22, 1934.— Twenty-five Swiss mice were injected intracerebrally with 0.03 cc. of mouse brain virus, Strain 3, diluted 1 to 50 in hormone broth. Thereafter, at intervals from 10 minutes to 4 days following injection, thirteen mice were tested for content of virus in brain, blood, and spleen, and twelve were reserved as controls. Mice to be tested were etherized, bled from the heart, and their brains and spleens removed and emulsified with alundum. The materials were then prepared in serial tenfold dilutions and 0.03 cc. of each was injected intracerebrally into two Swiss mice. The titre of virus in test material was taken as the highest dilution killing at least one of the two injected mice. This dilution was then expressed as numbers of intracerebral lethal doses. For example, the brain of a Swiss mouse dying of experimental encephalitis is fatal when injected into mice in doses of 0.03 cc. of a 10^{-7} dilution, which is 3×10^{-9}, or roughly 10^{-9}. Hence the brain content of virus is said to be 10^{9} intracerebral lethal doses for susceptible mice.

The results of this experiment are given in Table I. The original inoculum of about 10^7 intracerebral lethal doses was fatal to the controls on the 3rd and 4th days. Brain content of virus 10 minutes after injection was 10^6 and blood content at the same time, 10^4 doses. At 5 hours, the brain titre dropped to 10^3 doses and then rose rapidly to a maximum not measured in this test. Blood became non-infective

TABLE I

Brain, Blood, and Spleen Content of Encephalitis Virus in Susceptible Mice Following Intracerebral Injection of 10^7 Lethal Doses

Mouse No.	Time interval injection to test	Content of virus. 0.03 cc. of each dilution to two mice								
		Brain					Blood			Spleen
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Undiluted	10^{-1}	10^{-2}	10^{-1}
1	10 min.	5,* 5	5, 5	6, 7	11		5, 6	5, 5	8, 8	N.T.
2	20 "	5, 5	5, 6	6, 7		7	4, 4	5, 6	7, 9	"
3	1 hr.	D, 6	6, 8	8	6		5, 5	5, 7		"
4	3 hrs.	5, 6	6, 7	7, 12			5, 7			"
5	5 "	D, 6								"
6	7 "	6, 7		6						7
7	9½ "	5, 5	6, 8	7, 9						
8	10 "	6, 6	5, 5					N.T.	N.T.	
9	1 day	4, 6	5, 6	5, 5	5, 9	8		"	"	5, 5
10	2 days	D, 4	4, 4	4, 4	5, 5	7, 7	D, 7	"	"	5, 7
11	2 "	4, 4	4, 4	4, 4	4, 5	5, 6	6, 7	"	"	7, 7
12	3 "	4, 4	4, 5	5, 6	6, 6	5, 5	6	"	"	
13	3 "	4, 5	4, 6	6, 6	6, 7	5, 7	7	"	"	

* = duration of life of mouse in days.

Blank spaces = mice remained well 21 days.

D = mouse died immediately following injections.

N.T. = dilution not tested.

at 5 hours but at 2 and 3 days contained virus in 10^2 titre. Spleens contained virus in accordance with expectation from the blood findings.

Tests were made on susceptible mice injected with smaller amounts of virus, 10^5 and 10^3 lethal doses, with similar results. The brain titre of virus increased after a lag to 10^8 or 10^9, while the blood was positive only immediately following injection and preceding death. Brain and cords of these mice showed characteristic lesions; other organs appeared normal (2). Apparently, therefore, virus injected

intracerebrally into susceptible mice exhibits a predilection for nervous tissue.

Results of Injecting Virus Intraperitoneally or Subcutaneously.—Virus injected intraperitoneally or subcutaneously rapidly invaded the blood stream and survived in the spleen and yet did not harm the animal unless overwhelming doses were used or the brain was injured.

TABLE II

Brain, Blood, and Spleen Content of Encephalitis Virus in Susceptible Mice Following Intraperitoneal Injection of 10^7 Intracerebral Lethal Doses

Mouse No.	Time interval injection to test	Content of virus. 0.03 cc. of each dilution to two mice					
		Blood			Brain		Spleen
		Undiluted	10^{-1}	10^{-2}	10^{-1}	10^{-2}	10^{-1}
1	10 min.	4,* 4	5, 6	6, 6	5		
2	20 "	4, 4	4, 5	6, 6	7		
3	1 hr.	5, 5	4, 6	7, 7	6, 8		
4	3 hrs.	4, 5	6, 6	6, 8			
5	5 "	7, 7					
6	7 "	10					5, 6
7	9 "						6, 8
8	1 day		N.T.	N.T.		N.T.	5, 5
9	1 "		"	"		"	5, 7
10	2 days	5, 5	"	"		"	6, 7
11	2 "	8	"	"		"	6, 6
12	3 "		"	"		"	5, 5
13	3 "	6, 8	"	"	7, 8	"	5, 6
14	4 "	6, 7	"	"		"	5, 6
15	4 "		"	"		"	6, 6
16	5 "	8	"	"	8	"	
17	5 "		"	"		"	
18	15 "						5
19	15 "						

* = duration of life of mouse in days.
Blank spaces = mice remained well 21 days.
D = mouse died immediately following injections.
N.T. = dilution not tested.

Experiment 2. Jan. 29, 1934.—Thirty Swiss mice were injected intraperitoneally with 0.5 cc. of virus diluted 1 to 50. At intervals thereafter, from 10 minutes to 15 days, nineteen mice were sacrificed and examined for content of virus in blood, brain, and spleen, and eleven were reserved as controls. Materials for testing were obtained, prepared, and injected as described in Experiment 1.

The results of this experiment are summarized in Table II. The original dose injected intraperitoneally per mouse was equivalent to 10^7 intracerebral lethal doses. One of the eleven controls died of encephalitis on the 6th day following the injection; the others, together with the nineteen test animals, remained well. The first animal was sacrificed 10 minutes after injection and virus was found in its blood in large amounts, 10^4 titre. Similar quantities were present in the blood of mice at 20 minutes, 1, and 3 hours. At 5 and 7 hours, and irregularly thereafter, the undiluted blood contained virus. The titre of virus in the brain 10, 20, and 60 minutes following injection was 10^3 but negative thereafter, save in single mice, on the 3rd and 5th days respectively. Virus was present in the spleen in 10^3 quantities when first tested 7 hours after injection and regularly through the 4th day, and in one mouse on the 15th day.

Tests with different doses of virus and different strains of susceptible mice gave essentially the same results except that if the injected dose was smaller or the strain of mice employed was slightly more resistant, the resulting titres of virus in blood and brain were correspondingly less. Virus content of liver, lung, and kidney was found to be negligible. The spleen, however, contained virus over periods as long as 30 days. Similar results were obtained when the virus was injected subcutaneously.

According to these experiments, virus circulating in the blood vessels of the brains of intraperitoneally injected mice is relatively incapable of causing encephalitis and yet when injected directly into brain tissue through the dura is extremely pathogenic (Experiment 1). It appears likely, therefore, that the conditioning factor is trauma of the brain. This supposition was tested by injecting mice intraperitoneally with virus and following it by a subdural injection of sterile starch, according to the method of the yellow fever protection test (4).

Experiment 3. Mar. 12, 1935.—Dilutions of virus from 10^{-2} to 10^{-6} were each injected intraperitoneally in 0.5 cc. quantities into ten Swiss mice. After 60 minutes, five of the mice given each dilution of virus received an intracerebral injection of 0.03 cc. of a 2 per cent sterile starch solution and the remainder served as controls. At the same time the intracerebral virulence of the virus suspension was determined.

The results of the test are shown in Table III. The intracerebral titre of the virus was as usual, 3×10^9. Mice receiving intraperitoneally the largest dose of virus, 0.5 cc. of 10^{-2} dilution, 10^7 intracerebral lethal doses, died of encephalitis regardless of the presence or absence of cerebral trauma. Of those receiving the 10^{-3} and 10^{-4} dilution of virus, 10^6 and 10^5 intracerebral lethal doses, only animals subsequently traumatized developed encephalitis and died. One of

TABLE III

Effect of Intracerebral Trauma on the Pathogenicity of Encephalitis Virus Injected Intraperitoneally in Susceptible Mice

Five mice injected intraperitoneally 0.5 cc. in dilutions	Presence of trauma	Duration of life	Mortality
		days	*per cent*
10^{-2}	+	5, 5, 6, 6, 6	100
10^{-2}	0	5, 5, 6, 6, 11	100
10^{-3}	+	5, 6, 6, 6, 6	100
10^{-3}	0	Remained well	0
10^{-4}	+	6, 6, 6, 7, 12	100
10^{-4}	0	Remained well	0
10^{-5}	+	6	20
10^{-5}	0	Remained well	0
10^{-6}	+	" "	0
10^{-6}	0	" "	0

Intracerebral virulence of virus

	0.03 cc. subdurally in dilutions			
	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Duration of life, *days*	4, 4, 4, 4	4, 4, 4, 5, 5, 6	5, 5, 6, 6, 6, 7	6, 7, 7, 8
Mortality rate, *per cent*	100	100	100	100

five mice receiving the 10^{-5} dilution of virus plus trauma died; the remainder, together with the five controls, were unharmed. All mice given the 10^{-6} dilution, 10^3 intracerebral lethal doses, remained well. The experiment shows that virus in the blood stream not ordinarily infectious for the intact brain becomes so when the brain is traumatized.

Results of Instilling Virus into the Nose.—A number of neurotropic encephalitis-producing viruses are infectious for mice by the nasal

route,—for example, louping ill, yellow fever, and equine encephalomyelitis. It was not surprising to learn, therefore, that the St. Louis virus was similar in this respect. Its ready penetration from nose to brain and its rapid multiplication in the brain are illustrated in the following experiment.

TABLE IV

Brain and Spleen Content of Encephalitis Virus in Susceptible Mice Following Nasal Instillation of 10^5 Intracerebral Lethal Doses

Mouse No.	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								
1	1			N.T.	N.T.	N.T.	N.T.	N.T.	
2	1			"	"	"	"	"	
3	2	5,* 5	6, 6	11	"	"	"	"	7, 7
4	2	5, 5	6, 10		"	"	"	"	5, 7
5	3	5, 5	6, 6	D, 6	9	"	"	"	6, 6
6	3	6, 6	5, 6	8, 9				"	6, 7
7	4	N.T.	5, 5	5, 5	5, 5	7, 7	8	"	5, 5
8	4	"	5, 7	8, 8				"	
9	5	"	4, 6	4, 6	6, 6	6, 6	6, 6	"	5, 6
10	5	"	4, 6	4, 6	6, 6	6, 6	6, 6	"	5, 7
11	6	"	N.T.	5, 5	5, 6	5, 5	5, 6	6, 6	5
12	6	"	"	5, 5	5, 5	5, 6	5, 5	6, 6	6, 6
13	7	"	"	5, 5	5, 5	5, 5	6, 6	6	5, 6
14	7	"	"	5, 7	8, 8	8, 8			8, 8

* = duration of life of mouse in days.
Blank spaces = mice remained well 21 days.
D = mouse died immediately following injections.
N.T. = dilution not tested.

Experiment 4. Dec. 28, 1934.—Brain virus was prepared and diluted 1 to 100 in the routine manner. 0.03 cc. was then dropped through a 0.25 cc. syringe with blunt needle into the nasal orifices of each of twenty Swiss mice. This quantity is equivalent to 10^7 intracerebral lethal doses and about 10^2 intranasal lethal doses. At daily intervals thereafter for 7 days, two mice were sacrificed and their brains and spleens tested for the presence of virus according to the technique described in Experiment 1. Six mice were set aside as controls. The results of the experiment are given in Table IV.

The controls died of encephalitis on the 7th and 8th days. On the 1st day after nasal instillation, brains of the two test mice showed no virus. On the 2nd day, however, the brain titres of virus in the two tested mice were 10^5 and 10^4 respectively. On the 3rd day the brain titres were 10^6 and 10^5, on the 4th day, 10^8 and 10^5, on the 5th day, 10^8, 6th day, 10^9, and 7th day, 10^9 and 10^7. The spleens contained virus on the 2nd day and consistently thereafter.

Five additional experiments of the same sort were made demonstrating the presence of virus in the brain 48 hours after nasal inoculation, its rapid multiplication there to a titre of 10^9 in 6 days, and furthermore its presence in the spleen after 48 hours. The experiments also included nineteen futile attempts to recover virus from the entire emulsified brain 5 minutes to 24 hours after nasal instillation of virus. In addition, unsuccessful searches for virus in the undiluted blood were made on twenty-seven mice 5 minutes to 6 days after inoculation. Seventeen bleedings were made on seven mice within 19 minutes and on seventeen additional mice within 90 minutes of inoculation. In two or three instances the blood killed one of two injected mice after prolonged incubation periods or protected mice against subsequent intracerebral injection. Spleens, however, contained virus regularly from the 2nd to 7th days after nasal instillation of virus.

The above experiments demonstrate the predilection of the encephalitis virus for the central nervous system. Its tendency to localize, multiply, and form lesions almost exclusively in the brain and cord following intracerebral or intranasal injections, together with its inability, when injected directly or indirectly into the blood stream, to gain a foothold there in the absence of cerebral trauma, bespeaks its neurotropism. And yet the virus does find its way to the spleen following intranasal instillation, presumably *via* the blood, and survives there a surprisingly long period of time. So marked was this property that experiments were made to determine whether the virus could actually multiply in the spleen.

Experiment 5. Mar. 4, 1935.—Mouse brain virus was prepared, diluted 1 to 10, and injected in 0.5 cc. quantities intraperitoneally into three Swiss mice. 24 hours later the mice were sacrificed, spleens removed, emulsified, and taken up in about 1.8 cc. of broth. 0.5 cc. of this spleen emulsion was then injected intraperitoneally into each of three mice and 0.03 cc. intracerebrally into two mice.

The intraperitoneally injected mice were sacrificed at 24 hours and their spleens removed and prepared as above. Again the spleen emulsion was injected intraperitoneally into three mice. This procedure was repeated forty-four times at intervals of 24 to 48 hours with accompanying intracerebral injections into two mice after the 1st, 2nd, 5th, 10th, 16th, 21st, 24th, 27th, 32nd, 38th, and 44th passages to determine whether the spleen emulsions still contained active virus. The 44th passage was made Apr. 29, 1935, 56 days after the 1st passage.

The virus remained active after 44 spleen passages in this test and after 32 passages in a similar experiment. All spleen emulsions tested intracerebrally in mice gave rise to encephalitis fatal on the 6th to 8th days. The initial intraperitoneal inoculum of 10^8 intracerebral doses was probably diluted by each passage due to failure of spleens to take up all injected virus and to the difficulty of reinjecting the entire amounts of prepared spleen emulsion. It seems likely, therefore, that not only prolonged survival but actual multiplication of virus took place in the spleens of susceptible mice.

Infective Route of Virus from Nose to Brain

By what route does virus instilled into the noses of susceptible mice travel to the brain and set up a fatal encephalitis? Not by the blood stream, though minimal amounts probably reach the circulation from the nasal mucosa. For it has been shown that virus in the blood does not readily induce encephalitis in the absence of brain injury. A more likely possibility is that virus extends directly from nasal mucosa to brain by way of the olfactory tract, either axis cylinders or perineural spaces.

If the direct extension idea is correct, one would find virus and lesions following intranasal instillation, not scattered irregularly throughout the brain at any given time, but first in the olfactory bulbs and later in the brain proper. Evidence of this orderly progression of virus and lesions is given in the following experiments.

The first tests dealt with regional distribution of virus in brain and cord.

Experiment 6. June 4, 1935.—0.03 cc. of mouse brain virus diluted 1 to 100 was dropped into the nares of twelve Swiss mice. At daily intervals thereafter mice were sacrificed and tested for the presence of virus in the olfactory bulbs and piriform area, the remainder of the brain, and the cord. The olfactory bulb and piriform tissue was obtained by placing the ventral surface of the brain uppermost

and cutting from posterior to anterior to remove the piriform area and olfactory bulbs. The remainder of the brain was tested separately. The spinal cord was tested *in toto*. The various tissues were triturated in a mortar with alundum and diluted 1 to 10 with broth. Each emulsion was then injected intracerebrally into two Swiss mice to detect the presence of active virus.

Table V gives the results of the experiment. The three controls died on the 7th and 8th days. Two mice tested 24 hours and one tested 48 hours after nasal instillation showed virus in the bulb and

TABLE V

Content of Encephalitis Virus in Central Nervous System Following Nasal Instillation of 10^5 Lethal Doses

Mouse No.	Time interval injection to test	Presence of virus. 0.03 cc. of dilution 10^{-1} to two mice		
		Olfactory area*	Remainder of brain	Cord
	days			
1	1	6**		
2	1	6, 6		
3	2	7, 8		
4	2	4, 5	5	
5	3	5, 7	5, 5	
6	3	5, 5	5, 6	
7	4	5, 6	4, 5	6, 6
8	5	4, 4	4, 6	6, 6
9	7	N.T.	4, 5	5, 5

* Ventral portion of piriform lobes plus olfactory bulbs.
** = duration of life of mouse in days.
Blank spaces = mice remained well 21 days.
D = mouse died immediately following injections.
N.T. = dilution not tested.

piriform tissue but none in the remainder of the brain or in the cord. The second mouse tested at 48 hours, and the two tested on the 3rd day contained virus in bulbs and piriform tissue and in the remainder of the brain but not in the cord. On the 4th, 5th, and 7th days, virus was found in the three regions tested, olfactory bulb and piriform area, remainder of the brain, and the cord.

Experiment 7. June 20, 1935.—A similar test was made with tissue from olfactory bulbs alone, piriform area, and remainder of the brain. The olfactory bulbs were severed close to the brain and the piriform areas removed as in the

previous experiment. A total of ten mice were sacrificed and tested at 4, 5, 6, 24, 25, 28, 30, and 48 hours. Four additional mice were reserved as controls.

The results of this experiment are summarized in Table VI. The four controls died between the 7th and 9th days Virus was not found until 25 hours after instillation, when it was present only in the olfactory bulbs. At 28 hours it was present in olfactory bulbs and piriform area; in another mouse, at 30 hours, only in the bulbs. Likewise in one mouse at 48 hours, virus was found both in the bulbs and

TABLE VI

Content of Encephalitis Virus in Olfactory Bulbs and Brain Following Nasal Instillation of 10^5 Lethal Doses

Mouse No.	Time interval injection to test	Presence of virus, 0.03 cc. of dilution 10^{-1} to two mice		
		Olfactory bulbs	Piriform area	Remainder of brain
	hrs.			
1	4			
2	5			
3	6			
4	6			
5	24			
6	25	7*		
7	28	6, 7	7	
8	30	6, 7		
9	48	4, 5	6, 7	
10	48	5, 5		

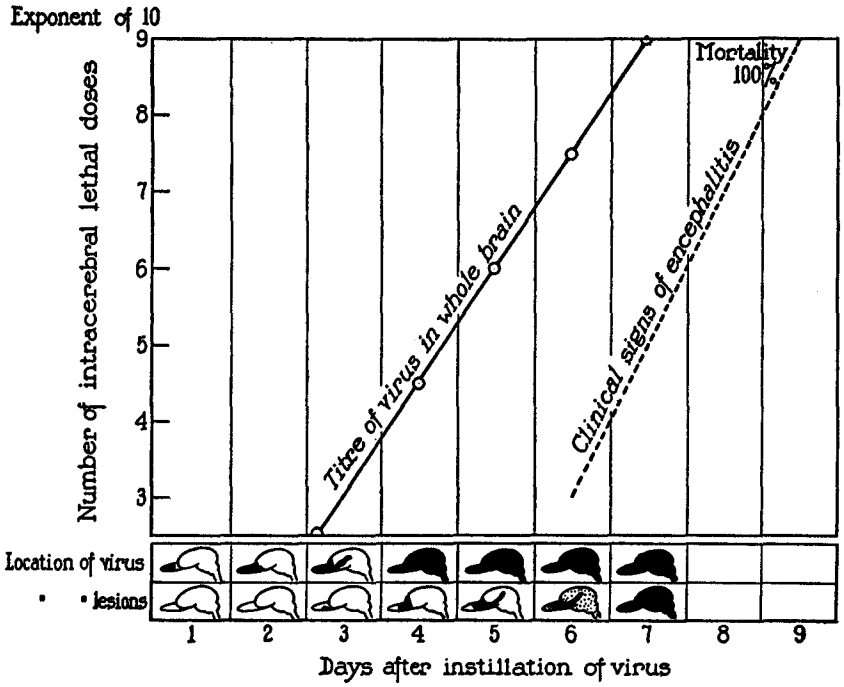
* = duration of life of mouse in days.
Blank spaces = mice remained well 21 days.
D = mouse died immediately following injections.
N.T. = dilution not tested.

piriform area; in the other, however, only in the bulbs. The remainder of the brain contained no virus for 48 hours following nasal instillation.

Taken together, the tests show that virus is demonstrable at 24 hours in the olfactory bulbs but not in the brain and the cord, that at 48 hours it is abundant in the bulbs and present occasionally in the anterior piriform area of the brain. At 3 days, however, it is present in the remainder of the brain, and at 4 days has reached the spinal cord.

The route by which virus travels from nose to brain was studied

further by histological methods. Mice given virus intranasally were sacrificed in batches at daily intervals thereafter and their olfactory bulbs, brains, and cords sectioned and stained to determine the time, location, and nature of the initial lesion and its subsequent advance.



TEXT-FIG. 1. Time relation between onset of encephalitis in the nasally infected susceptible mouse and the appearance of tissue alterations and virus in the brain.

Two lots of mice were studied. The first consisted of thirty-two Swiss mice which received on Dec. 16, 1933, 0.03 cc. of a 10^{-2} brain virus suspension intranasally. Daily for 7 days following the instillation, four mice were sacrificed, their brains and cords removed, fixed in Zenker's acetic solution, and sectioned. The four remaining mice died of encephalitis on the 8th and 9th days. The second lot of sixteen mice given virus in June, 1935, was treated similarly. Sections of brain were taken at the levels of the lateral ventricles, Ammon's horn, and midcerebellum. The cord was examined at the cervical, thoracic, and lumbar levels. Furthermore, serial sections were made of the olfactory bulbs and portions of the brain anterior to the lateral ventricles. Ten consecutive sections each of 5 $\mu\mu$

thickness were alternately prepared and discarded to furnish more than 100 sections of the anterior olfactory region from each mouse. The tissue was stained by Giemsa's method and with eosin-methylene blue.

24 Hours after Nasal Instillation.—The virus is present in the olfactory bulbs (Text-fig. 1). No lesions were recognized, however, in the serial sections of olfactory bulbs and brains of the five mice examined at this time.

2 Days after Nasal Instillation.—The virus is abundant in the olfactory bulbs and is present in the piriform area of the brain proper. Again, serial sections of olfactory bulbs and brains of the five mice studied at this time appeared normal.

3 Days after Nasal Instillation.—The virus has reached a titre in the whole brain of 10^3 lethal doses. Of the five mice examined at this time, two appeared normal and three showed a definite and similar lesion. On the ventral aspect of the olfactory bulbs near the brain, one or two focal accumulations of round cells and an occasional polymorphonuclear leucocyte were noted in the Virchow-Robin spaces about the blood vessels in or beneath the pia (Fig. 1). In addition, the neighboring superficial blood vessels were congested and a few leucocytes were scattered about the bundles of non-medullated nerves.

4 Days after Nasal Instillation.—The virus content of the whole brain titres 10^4.

All five mice examined showed lesions similar in character but varying slightly in extent. The congestion of superficial blood vessels and perivascular exudate were more advanced than on the 3rd day, extending throughout the ventral posterior two-thirds of the olfactory bulbs. In one mouse the lesion had reached the ventral, medial regions of the piriform area (Figs. 2, 3). Likewise the scattered leucocytes about the olfactory nerve bundles were more conspicuous. In addition, a second type of lesion was noted in three mice, namely, hyperplasia of pial endothelium, extending in some instances only along the ventral posterior third of the olfactory bulbs, but in the mouse showing the most advanced changes continuing over the ventral, medial portion of the piriform lobes (Fig. 4).

5 Days after Nasal Instillation.—The virus has reached a titre in the whole brain of 10^6 lethal doses. The animals sacrificed at this date still appeared clinically healthy (Text-fig. 1). Two of four mice examined showed an extension of the exudative and hyperplastic lesions over the ventral surfaces of olfactory bulbs and piriform lobes. The remaining two mice showed an abatement of these lesions and the beginnings of the third and most conspicuous change, namely, nerve cell necrosis. First to show damage were the pyramidal nerve cells of the olfactory bulbs and piriform lobes. They were in various stages of necrosis. The cytoplasm of some was deeply eosin-staining; in others, it was eosin-staining and granular. Other cells showed a shrunken, eosin-staining cytoplasm and nucleus in various stages of pycnosis. Some cells were shrunken to a mere dot of a nucleus in a fragment of eosin-staining cytoplasm. Elsewhere the cells were entirely missing and the tissue took on a punched out appearance. Relatively little exudate was present nearby.

This nerve cell necrosis did not antedate, in so far as we could determine, the inflammatory lesion. Early nerve cell changes were searched for in the 3 day and

4 day material by the use of Goodpasture's carbol-anilin-fuchsin stain with no evidence of chromatolysis or alteration of Nissl substance. Glial cells were examined, isolated nerve cells, and occasional nerve cells in an inflamed area on the 3rd day, but the impression remained that inflammation preceded specific, obvious nerve cell involvement by 24 to 48 hours.

6 to 8 Days after Nasal Instillation.—The brain titre of virus reaches a maximum of 10^9 lethal doses. The animals all develop encephalitis and die. The exudative lesion was found throughout the brain and cord. Nerve cell necrosis was extensive and in each animal appeared to develop progressively along the olfactory tracts. In all cases, the pyramidal cells of the olfactory bulbs and piriform lobe were first affected and here the inflammatory and hyperplastic lesions were subsiding. In some the olfactory ganglion and tubercle were involved. In others the necrosis had extended to the central grey substance, anterior limbic area, and hypothalamus. Most characteristic on the 6th day, however, was the extension of necrosis to the pyramidal cells of Ammon's horn (Fig. 5). These large nerve cells, together with similar ones in the piriform lobe, were invariably affected. Finally, nerve cells of the thalamic region and even certain non-olfactory areas of the cortex became involved. Scattered nerve and glial cells everywhere, both in brain and cord, became necrotic. The basal ganglia, cerebellum and motor cells of the anterior horns and the posterior root ganglion cells appeared to be spared for the most part, but by the 8th day very little of the brain remained normal (Fig. 6).

In summary (Text-fig. 1), the first lesion occurred, following nasal instillation of virus, on the 3rd day and consisted of dilatation of subpial blood vessels with exudation of round cells and polymorphonuclear leucocytes on the ventral surface of the olfactory bulbs. On the 4th day, this lesion spread to the pia covering the ventral surfaces of the piriform lobes and a second alteration was noted in these areas, namely, hyperplasia of pial endothelium. Finally, on the 4th or 5th day, necrosis of pyramidal nerve cells in the olfactory bulbs and piriform areas appeared. On the 6th and 7th days, the nerve cell necrosis spread to Ammon's horn, hypothalamus, and thalamus. Other types of nerve cells scattered through brain and cord were involved and perivascular accumulations of round cells were widespread.

DISCUSSION

The foregoing data bear on the classification and pathogenesis of the St. Louis encephalitis virus in susceptible mice. It is neurotropic according to general definition, in that it multiplies and forms lesions chiefly in the brain and attacks nerve cells directly. It invades the brain by the olfactory but not by the blood stream route. But it is not

strictly neurotropic in the sense of being nerve cell specific, in that it multiplies in the spleen.

Whether virus travels from nose to brain by nerve axons or perineural spaces is difficult to determine. Favoring nerve cell transmission is the evidence of general neurotropism of the virus, its multiplication in olfactory tissue, and its orderly progress by way of the olfactory tracts. When it comes to discovering the situation, progression, and character of central nervous system lesions following nasal instillation of virus, however, the data are less convincing, since lesions in a given region did not appear until 48 hours after virus was demonstrable. For example, the primary lesion was found in the olfactory bulbs on the 3rd day but virus, already present there 2 days previously, had extended to various regions of the brain proper. Situation and progression of lesions, therefore, cannot be taken to define precisely the site and progress of virus. The same difficulty obtains with respect to neurotropic louping ill (5) and yellow fever virus in the mouse (6) where considerable time elapses between the arrival of virus and the development of lesions. One further difficulty confronts us in testing the theory of axon transmission of this virus, namely, that inflammation was found definitely to antedate the nerve cell lesions. True, unseen alterations of nerve cells may have preceded the pouring out of leucocytes in the Virchow-Robin spaces, but the evidence at hand indicated without exception that at least 24 hours elapsed between the onset of exudation and nerve cell necrosis. On the basis of present knowledge, therefore, the question cannot be decided.

The time relation between the presence of virus and lesions in the brains of susceptible mice following nasal instillation and the onset of clinical encephalitis is noteworthy from the standpoint of therapy. 48 hours after nasal infection, 4 days before the first sign of disease, the virus is present in the brain; 2 days before disease, lesions are abundant; and finally, when the animal falls ill, virus in the brain has increased to a titre of 10 million intracerebral lethal doses. This fulminating type of infection in the susceptible host sharply limits the possibilities of specific or other forms of therapy.

CONCLUSIONS

1. St. Louis encephalitis virus injected intracerebrally into susceptible mice multiplies there to reach a titre of 10^9 intracerebral lethal

doses. It is found also in the blood in small amounts immediately following injection and preceding death.

2. Injected intraperitoneally or subcutaneously the virus circulates in the blood for several hours and survives in the spleen for days. It does not multiply in the brain and cause encephalitis, however, unless overwhelming doses are injected or the brain is traumatized.

3. Virus dropped into the nares is demonstrable in the olfactory bulbs at 24 hours, in the piriform lobes at 24 to 48 hours, in the remainder of the brain at 3 days, and in the spinal cord at 4 days. In the brain it reaches a titre of 10^9 in 6 days. Virus is not readily demonstrable in the blood but is present in the spleen after 48 hours.

4. Virus survives and is capable of multiplying in the spleen.

5. Lesions following nasal instillation of virus appear first in the olfactory bulbs on the 3rd day, in the piriform lobes on the 4th, and in Ammon's horn on the 5th day. The character of the lesions in order of their appearance is exudation of mononuclear cells about superficial blood vessels and in the pia, hyperplasia of the endothelium of the pia, and necrosis of nerve cells of the olfactory tract.

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EXPLANATION OF PLATES

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

PLATE 32

FIG. 1. 3 days. Olfactory bulbs. Ventral, medial, posterior surface. One of a few areas showing exudate of round cells and polymorphonuclear neutrophiles about the blood vessels in and just beneath the pia. A few leucocytes are at some distance from the blood vessels. × 675.

FIG. 2. 4 days. Piriform area. Ventral, medial surface. The pia is thickened. Round cells and a few polymorphonuclear cells are collected near the blood vessels in and beneath the pia and are scattered about the nearby superficial brain tissue. Superficial capillaries are distended. The deeper portions of the brain, including the nerve cells, appear normal. × 275.

PLATE 33

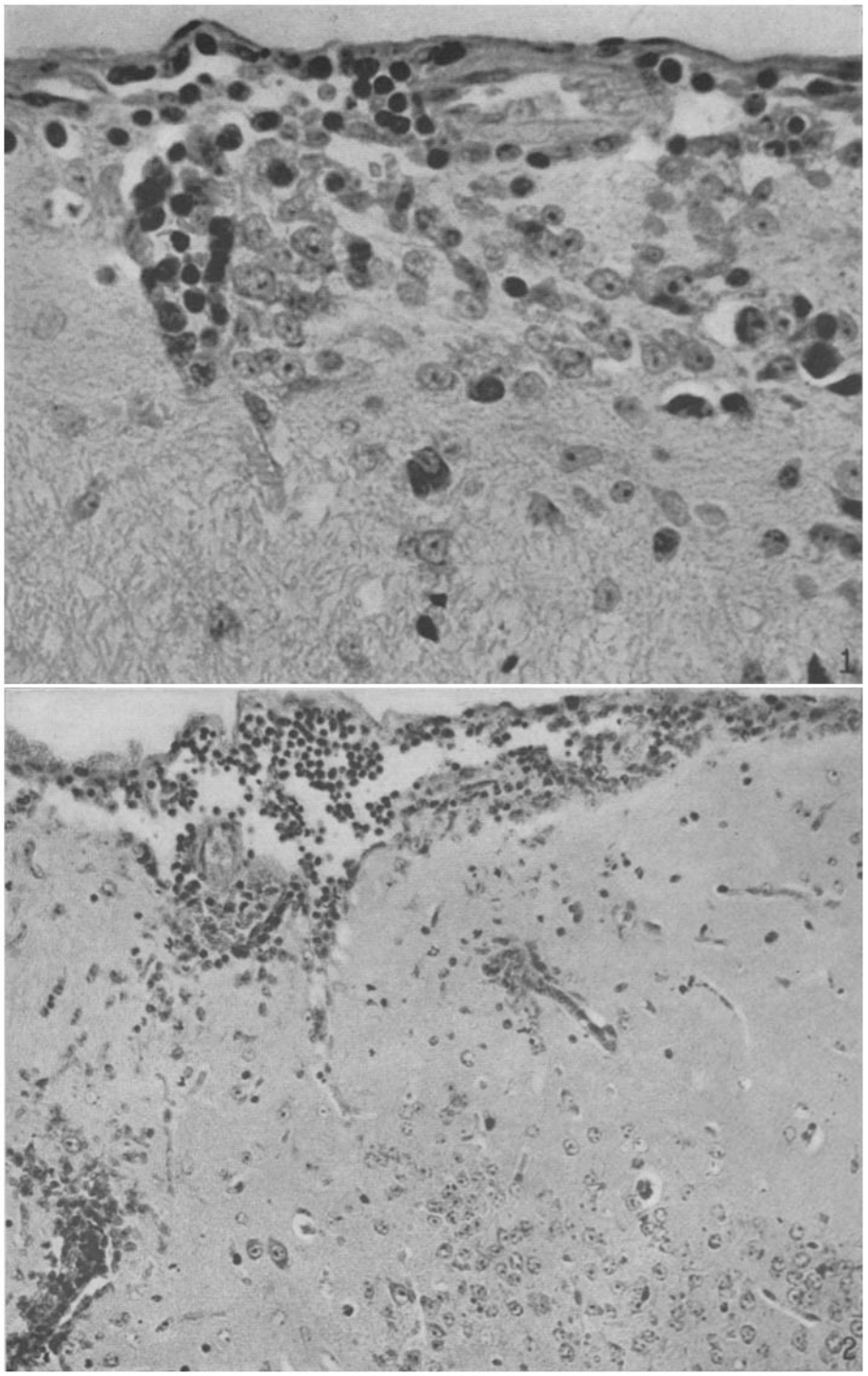
FIG. 3. 4 days. Same region. The changes described in Fig. 1 are shown in detail. × 675.

FIG. 4. 4 days. Same region. The pial endothelium is markedly hyperplastic and infiltrated with leucocytes from neighboring blood vessels. × 675.

PLATE 34

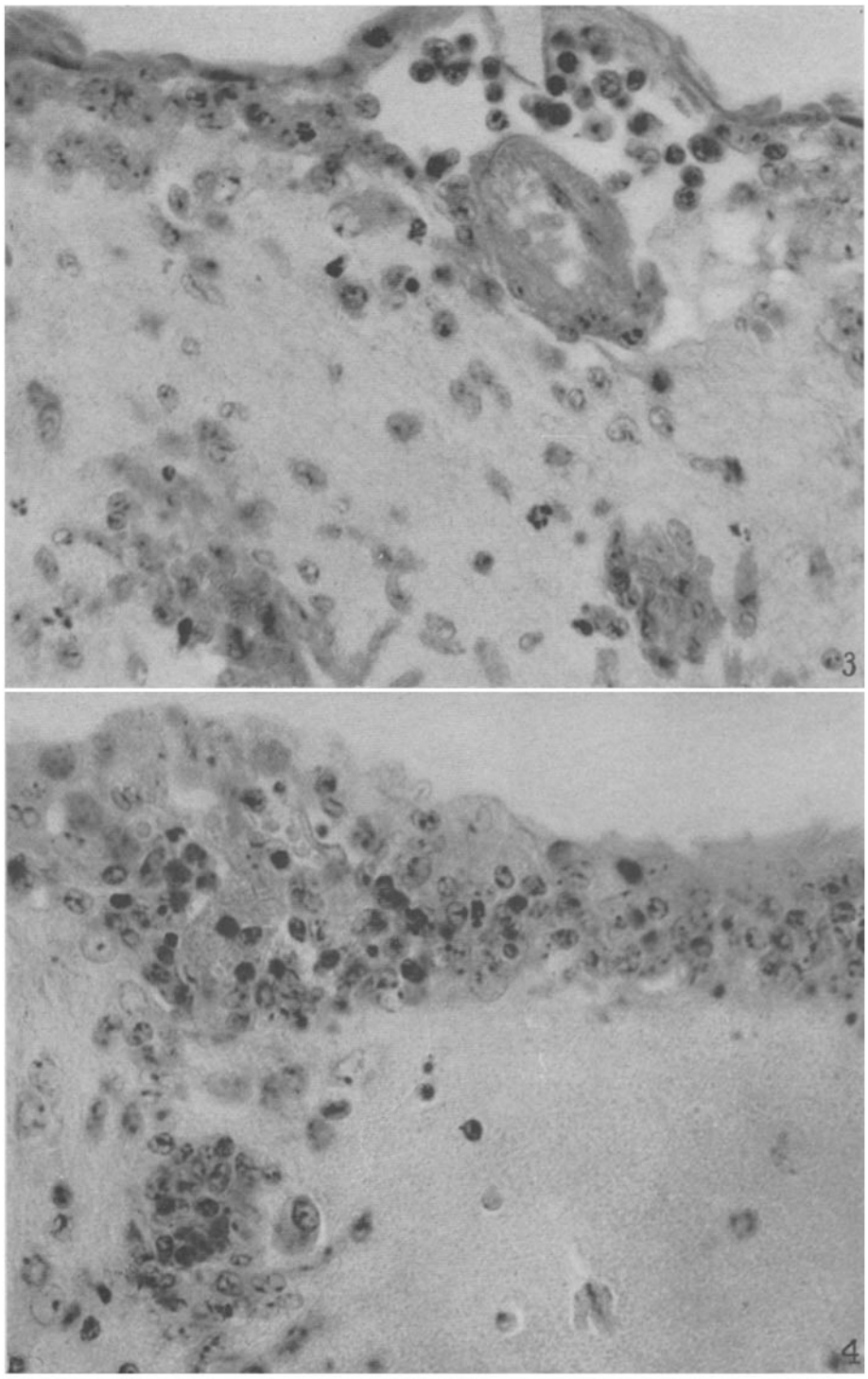
FIG. 5. 6 days. Ammon's horn. To the left of the figure, the nerve cells appear normal; to the right, they are in various stages of disintegration. The earliest alteration seems to be an increase in the affinity of the cytoplasm for eosin with a tendency to granulation. The cytoplasm of other cells still takes a deep eosin stain but is shrunken and surrounds a nucleus in various stages of pycnosis. Finally, the cell shrinks to a small, round, deep staining nucleus with little cytoplasm, and then seems to disappear, leaving the surrounding tissue vacuolated. An occasional nerve cell in the nearby matrix exhibits the same sort of degeneration.

FIG. 6. 7 days. Piriform area. Ventral, medial surface. The pial exudative and hyperplastic lesions are not conspicuous. The pyramidal nerve cells to the lower left of the figure are in various stages of necrosis. To the right, they are almost entirely absent, leaving vacuolated areas without inflammatory reaction. × 675.



Photographed by Louis Schmidt

(Webster and Clow: Neurotropic character of encephalitis virus)



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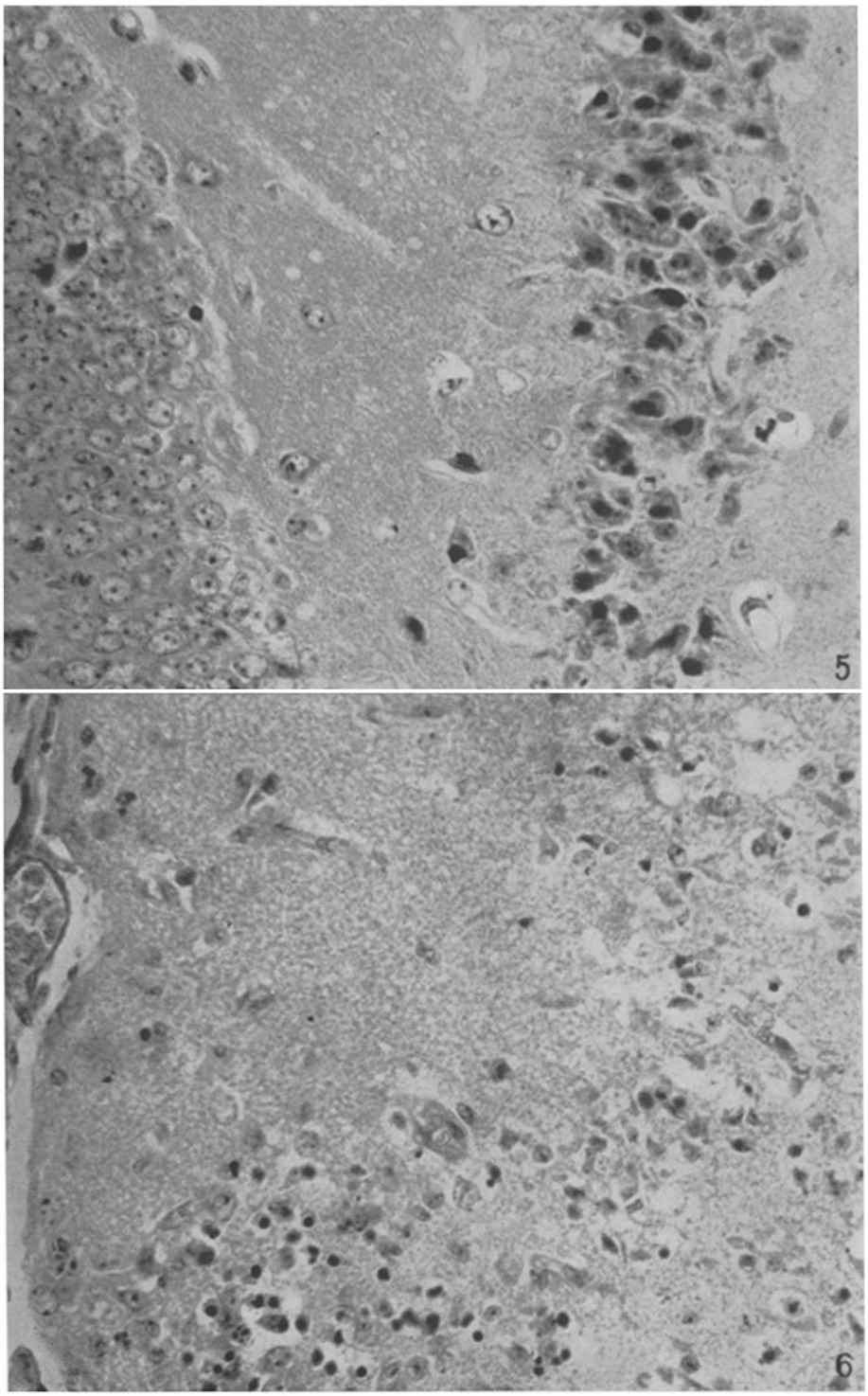
(Webster and Clow: Neurotropic character of encephalitis virus)



Photographed by Louis Schmidt

(Webster and Clow: Neurotropic character of encephalitis virus)