INFLUENCE OF AGE ON SUSCEPTIBILITY OF MICE TO ST. LOUIS ENCEPHALITIS VIRUS AND ON THE DISTRIBUTION OF LESIONS*

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Plate 4

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Evidence has accumulated that young mice are more susceptible than older ones to many neurotropic viruses if they are inoculated by a peripheral route. The most significant decline in susceptibility apparently occurs as mice pass 2 weeks of age, but there is uncertainty concerning the factors which contribute to this age difference. Andervont (1) showed that 2-week-old mice were less resistant than adults to intracutaneous inoculation of herpetic virus into the abdominal skin and Theiler (2) demonstrated the greater susceptibility of young mice to the intraperitoneal inoculation of the virus of yellow fever.

Several explanations have been advanced to account for the greater susceptibility of young mice to peripherally inoculated virus. Sabin and Olitsky (3, 4) suggested that the myoneural junction or specialized nerve endings might impede the progress of the virus in adult mice inoculated intramuscularly with the virus of vesicular stomatitis. To explain the difference in susceptibility when the same virus was introduced by the olfactory route they offered the explanation that a central barrier preventing the passage of the virus existed in adult mice between the primary olfactory centers of the telencephalon and the remainder of the brain. King (5), studying Eastern equine encephalomyelitis, believed it probable that the increased resistance of older mice to peripheral inoculation could be explained by a change in the brain tissue itself such as might be brought about by increased cell maturity,—myelination, change in water content, and in reaction to specific and non-specific injury. Following intramuscular inoculation of the virus of Western equine encephalomyelitis Sabin and Olitsky (6, 7) observed in mice 2 weeks of age, a high incidence of symptoms of encephalitis, with lesions indicative of invasion along the olfactory or other pathways without evidence of either diffuse hemato-encephalitic spread or progression by way of the local nerves. On the other hand, a high incidence of symptoms attributable to ascending infection was observed in mice 3 weeks of age. At one month of age and beyond, many mice were entirely resistant following intramuscular inoculation.

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They attributed the difference between the first two groups to the greater permeability of certain blood vessels of the young animal for the virus, and the difference between the latter two groups to changes in the muscle or specialized nerve endings.

A difference in invasive capacity between fresh and fixed viruses, emphasized by the studies of King (5) and Casals (8), may also play a rôle in determining the greater susceptibility of young animals. Other factors of possible significance in this respect are the ability of a virus to multiply in the blood (Hurst (9), equine encephalomyelitis, monkey, and guinea pig) or other tissues of the body, and the rate at which antibodies are produced (Morgan (10), Eastern equine encephalomyelitis, mice) at different ages.

The influence of age on the susceptibility of mice inoculated intraperitoneally with the virus of St. Louis encephalitis has been determined in the present investigation. The explanation for the difference in susceptibility between young and old animals has been sought in a study of the distribution of lesions in mice between 2 and 8 weeks of age killed at intervals following intraperitoneal inoculation. The distribution of the lesions has been compared with that which followed intranasal inoculation in adult mice. Special attention has been directed to two possibilities: (1) that evidence of a direct hematogenous invasion of the C.N.S. might be found in young mice; (2) that young mice inoculated intraperitoneally, in contrast to older mice, might show a distribution of lesions coinciding with that found when the olfactory route of inoculation was used.

Material and Methods

The Hubbard Strain of St. Louis encephalitis virus, isolated in 1937, was used in this study. At present the virus has a constant infective titer as great as 10^{-7} when measured by intracerebral inoculation of mice 2 months of age. On some occasions the infective titer so measured reaches 10^{-8}. When these experiments were begun, the virus had been carried through approximately 100 successive passages in the mouse brain. The material for injection was prepared in a uniform manner for all experiments. Brains removed from mice in the convulsive stages of encephalitis were kept in the freezing unit of the refrigerator ($-15°$ to $-20°$C.) for 1 to 2 days. Brains, shown to be bacteriologically sterile, were ground without abrasive and suspended in sufficient nutrient broth (pH 7.4) to make a 10 per cent suspension. Following centrifugation at 200 R.P.M. for 2 minutes the supernatant fluid was drawn off and used to make further tenfold dilutions in nutrient broth. For a given route of inoculation the same quantity of fluid inoculum was used irrespective of the size of the mouse.

For the susceptibility studies, groups of mice of different ages were usually inoculated in the same experiment. When this was not possible because of the difficulty of having on hand a sufficient number of mice of all the required ages, the titer of the virus was confirmed by the intracerebral inoculation of mice approximately 2 months of age. Swiss mice obtained from a single source were used with the following exception. Part of the mice in the 2 weeks age group were white mice of the "Old Buffalo" strain. However, the latter strain and the Swiss mice which we have used

have shown the same degree of susceptibility to the virus of St. Louis encephalitis when inoculated intracerebrally.

For the anatomical studies the virus was instilled intranasally and inoculated intraperitoneally. Usually the mice of a single experiment were killed at spaced intervals preceding the time at which it might be expected that clinical signs of the disease would develop. Several mice of each group were kept until symptoms had appeared. The brains were fixed in a mixture of aqueous corrosive sublimate and absolute alcohol, embedded in paraffin, and sectioned serially in a frontal plane at 20 μ. Every third slide of each series was stained with cresyl violet, the maximum distance separating stained slides being 0.4 mm. With few exceptions representative levels of the spinal cords were cut in all mice of the series inoculated intraperitoneally. Other cords were preserved and cut when it appeared desirable.

164 brains were obtained, 25 from mice 6 to 8 weeks of age instilled intranasally with 10^{-3} or 10^{-4} dilution of virus and 139 from mice 2 to 8 weeks of age inoculated intraperitoneally with 10^{-1} and higher dilutions of virus.

Susceptibility to Intracerebral, Intranasal, and Intraperitoneal Inoculation of Virus

Intracerebral Inoculation.—In each of two experiments groups of 4 mice, 3 weeks, 6 weeks, and 15 months of age were inoculated intracerebrally with 0.03 cc. of tenfold dilutions of virus in broth. In a 3rd experiment 2 groups of mice 8 weeks and 10 months of age were inoculated with corresponding dilutions of virus. Finally two groups of mice, 2 weeks and 3 months of age, were inoculated in a similar manner. As shown in Table I, mice of all ages were uniformly infected when they received 10^{-7} dilution of virus intracerebrally. When the titer of the virus reached 10^{-8} approximately the same number of the mice died in all groups.

Intranasal Inoculation.—Groups of 8 mice, 3 weeks, 6 weeks, and 15 weeks of age were inoculated intranasally with 0.03 cc. of dilutions of virus in broth. Again no difference in the response of the various age groups could be distinguished (Table II). At all ages at least a part of the mice died following intranasal instillation of the 10^{-4} dilution of the virus while only an occasional mouse in any age group showed evidence of infection following instillation of the 10^{-5} dilution. The same results were obtained in other experiments using these three age groups, and also in one experiment in which a group of mice 10 months of age was used.

Intraperitoneal Inoculation.—In contrast to the intracerebral and intranasal routes, the age of the mice influenced the results when the virus was inoculated intraperitoneally, (Table III). Tenfold dilutions of the virus in broth were injected in 0.25 cc. amounts by this route. When groups of mice 3, 6, and 15 weeks of age were used, most of the mice in the oldest age group remained well following the inoculation of a 10^{-1} dilution of the virus. Of mice 6 weeks of age, a slightly greater number succumbed to this amount of virus but only an

occasional mouse died following the inoculation of the 10^{-2} dilution. On the other hand all mice 3 weeks of age succumbed when inoculated intraperitoneally

TABLE I

Susceptibility of Mice of Different Ages to the Virus of St. Louis Encephalitis Inoculated Intracerebrally

Age of mice	Dilutions of virus		
	10^{-6}	10^{-7}	20^{-8}
3 wks.	(1)* 5‡ 5 5	4 4 5 5	4 4 5 5
6 wks.	(1) 4 5 6	4 4 4 5	4 5 5 5
15 wks.	4 4 4 5	4 4 5 5	4 4 5 5
3 wks.	4 4 4 5	4 5 5 5	5 S§ S S
6 wks.	4 5 5 6	4 5 5 6	5 S S S
15 wks.	(1) (1) 4 5	5 5 5 6	5 5 S S
8 wks.		5 5 6	7 S S
10 mos.		5 6 6 6 6 9	9 12 18 S S S
14 days	———	4 4 5 5	4 4 6 S
8 wks.	4 4 5 5	5 5 5 5	5 6 S S

* ( ) = death from unknown cause.

‡ Numbers refer to day of death following inoculation.

§ S = survival of mouse.

TABLE II

Susceptibility of Mice of Different Ages to the Virus of St. Louis Encephalitis Inoculated Intranasally

Age of mice	Dilutions of virus		
	10^{-3}	10^{-4}	10^{-5}
3 wks.	5 6 7 S	5 6 6 6	S S S S
6 wks.	5 5 6 7	6 7 S	S S S S
15 wks.	5 6 6 7	6 6 6 7	7 S S S
3 wks.	6 6 7 7 7 7 9 S	(2) 6 7 8 8 10 S S	———
6 wks.	5 6 6 6 7 9 10 10	7 S S S S S	———
15 wks.	5 6 7 8 10 S S S	7 7 7 9 9 9 S S	———
3 wks.	6 6 7 7	6 S S S	S S S S
6 wks.	6 6 6 7	6 6 7 S	8 S S S
15 wks.	6 6 7 S	6 7 7 S	7 S S S
10 mos.	6 6 7 7 7 7	7 7 8 8 S S	7 S S S S S

with dilutions of 10^{-1} and 10^{-2} of the virus, and an occasional mouse receiving either the 10^{-3} or 10^{-4} dilution, died. In comparison with the mice 3 weeks

of age, those 2 weeks of age were much more susceptible to the virus when it was inoculated intraperitoneally, succumbing to dilutions of the virus as great as 10^{-6}.

In summary these results indicate that no significant age difference in susceptibility is apparent in mice inoculated intracerebrally or intranasally, whereas mice inoculated intraperitoneally show increasing resistance with age, the most striking difference appearing between the 2nd and the 3rd week.

TABLE III

Susceptibility of Mice of Different Ages to the Intraperitoneal Inoculation of the Virus of St. Louis Encephalitis

Age of mice	Dilutions of virus					
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
3 wks.	5 5 5 5	5 6 6 7	8 15 S S	9 S S S	—	—
6 wks.	5 6 S S	S S S S	S S S S	—	—	—
15 wks.	S S S S	S S S S	S S S S	—	—	—
3 wks.	4 4 5 5 5 5 7 S	4 5 5 5 6 6 6 6	5 8 8 S S S S S S	—	—	—
6 wks.	5 6 6 10 S S S S	5 7 S S S S S	—	—	—	—
15 wks.	5 6 10 S S S S S	—	—	—	—	—
2 wks.	4 5 5 5 5	5 6 6 7 8	6 7 8 8 S	8 8 9 9 S	8 8 9 9 S	6 8 10 10 S
3 mos.	6 6 S S	S S S S	S S S S	—	—	—
2 wks.	—	—	5 6 6 6	7 8 8 8	6 8 10 S	8 8 11 S
2 wks.	—	4 4 4 5 5	4 4 4 5 6	7 9 10 11 12	8 8 13 S S	7 8 8 16 S

Distribution of Lesions

Webster and Fite (11, 12) and Smadel and Moore (13) described the histological changes produced by the virus of St. Louis encephalitis in mice inoculated intracerebrally; they emphasized the subpial and perivascular accumulations of mononuclear cells, foci of microglial proliferation about vessels, and beneath the pial membrane and necrosis of neurons. Webster and Clow (14) proved that virus instilled intranasally invaded the brain by the olfactory route. The presence of the virus in the olfactory bulbs was demonstrable 48 hours before the occurrence of the earliest lesions in that site.

The lesions which we have studied are, for the most part, the perivascular accumulations of cells and the mesodermal-glial response. Bodian and Howe (15, 16), studying experimental poliomyelitis in the monkey, demonstrated the feasibility of utilizing these lesions to follow the invasion of the C.N.S. by neurotropic viruses. After intranasal instillation of the virus of poliomyelitis, monkeys killed during the preparalytic stage of the disease showed lesions in the primary olfactory regions, and in the hypothalamic nuclei, the tegmentum of the mid-brain and the reticular formation of the hind-brain,—centers con-

nected with the olfactory region by the olfactotegmental pathway. Certain centers not on this primary preferential pathway such as the amygdaloid nuclei, paraolfactory and septal areas, pyriform cortex, and midline and dorsomedial thalamic nuclei, were also involved. In the late paralytic stage this pattern of distribution was not obscured but lesions were also found associated with the pathway between the motor cortex and the lower centers. In the late paralytic stage of the ascending infections, which followed inoculations into the sciatic nerve, peritoneal cavity or spinal cord, the distributions of lesions were similar to those observed following intranasal instillations, but as a rule lesions were more abundant in the reticular formation and tegmentum and diminished suddenly rostral to the region of Forel's fields. Lesions were absent from the olfactory bulbs, most of the other olfactory centers and the midline thalamic nuclei.

Because of the small size in the mouse brain references to cytoarchitectonic designations of many well known nuclei have been avoided where possible and the observations have been reported in the conventionally used gross descriptive terms. However, it has been convenient to divide the pyriform cortex into retrobulbar, prepyriform, and periamygdalar regions, and to mention the more caudally situated entorhine region from which the hippocampus receives abundant olfactory afferent fibers. The site and extent of each of these regions is given in the cytoarchitectonic atlas of Rose (17). In the present study, the dorsal thalamus has been divided into anterior, medial, and lateral areas. The medial area includes the midline as well as the medial nuclear groups; the lateral area contains the cortical relay nuclei. The anterior nuclei are not mentioned because they appear in relatively few sections. Considerations based on the blood supply of the thalamus of the rodent (Schlesinger, 18) indicate that further subdivision is inadvisable. The nuclear configuration of the tegmentum of the mid-brain and the reticular gray of the hind-brain is described and illustrated by Craigie (19).

Intranasal Series.—Four groups totalling 35 mice 6 to 8 weeks of age received 10^{-3} or 10^{-4} dilutions of virus instilled nasally. The time of appearance of the clinical symptoms varied among the different groups with no constant relation to the dilution of the virus employed. Histologically it was found convenient to divide the series into early and late stages based upon the extent of invasion of the C.N.S.

In the early stage, lesions were localized primarily to the basal olfactory territory of the telencephalon and to centers associated with the olfactotegmental pathway leading from that territory to the mid- and hind-brains. Five of the 24 brains removed before clinical signs of disease developed showed such localized lesions, 11 were entirely negative, and in the remainder the lesions were more widely distributed.

Webster and Clow (14) recorded perivascular accumulations of lymphocytes in the olfactory bulbs as early as the 3rd day following intranasal instillation of the virus. We have confirmed this observation in one brain that was otherwise negative. In 4 other brains, obtained from animals killed upon the 4th and 5th days following

inoculation there was more extensive invasion of the C.N.S. Accumulations of cells were prominent about many vessels in the olfactory bulbs, and occasional foci of mesodermal-glial proliferation were observed. The retrobulbar and prepyriform regions showed similar lesions. The periamygdalar region was involved to a lesser degree, but occasionally slight proliferation of microglia in its plexiform layer was also found. At this time lesions also appeared in the tubercula olfactoria, septa, and ventral parts of the caudate nuclei. The latter appeared to have been invaded from the tubercula olfactoria and pyriform territory. Lesions were observed in the preoptic areas in all of these brains but, in 2 of the 4, none were encountered caudal to this level. In the other 2 brains lesions were also observed in the hypothalamus, the medial areas of the dorsal thalamus, tegmentum of the mid-brain, and the dorsal part of the reticular gray at the rostral end of the hind-brain. In addition, there was usually a slight involvement of the frontopolar, frontal interhemispheric, and temporal parts of the cortex which closely adjoin the basal olfactory territory.

An outstanding feature of the early stage was the constancy with which the lesions upon one side were more severe and abundant than upon the other. This difference was most obvious in the pyriform territories and tubercula olfactoria, but also appeared in the caudate nuclei, dorsal thalamus, and tegmentum. Differences between the two sides of the brain were also encountered in the intraperitoneal series; otherwise this result could be attributed to the amount of virus which entered one nostril as compared with that entering the other.

The lesions in the late stage were no longer confined to the primary olfactory centers and those associated with the pathway leading to the tegmentum and reticular formation. This more widespread distribution characterized the brains obtained from animals killed at the onset of clinical signs but was also observed in some of the brains obtained in the preclinical period. In the pyriform territory (Figs. 1 and 2) and tubercula olfactoria the mesodermal-glial response was always more severe than that observed during the early stage; this response in the plexiform layer of the pyriform cortex usually diminished in intensity from the retrobulbar toward the entorhine region. In 2 specimens of the late stage, widespread necrosis of nerve cells had occurred throughout the pyriform territory (Fig. 3) but this was not accompanied by an unusually severe mesodermal-glial reaction. Lesions were also abundant elsewhere in the fore-brain, although parts of the neocortex or one hippocampus sometimes escaped involvement. Lesions of the mid-brain occurred in the central gray and tectum as well as in the tegmentum. Lesions of the hind-brain were either localized to the dorsal part of the reticular gray as observed in specimens in the early stage, or were scattered throughout the reticular formation to the caudal end of the medulla.

Summary.—Following intranasal instillation an early stage of distribution of lesions can be recognized in which the primary olfactory centers and those associated with the olfactotegmental pathway are principally involved.

Intraperitoneal Series.—Groups of mice of different ages (2, 3, 4, and 6 to 8 weeks) were inoculated intraperitoneally with different concentrations of virus (Table IV) and killed, usually in groups of 3, at half day intervals from 2 days following inoculation until clinical signs of disease appeared. Significant enough differences in distri-

butions of lesions did not appear between the brains of the 3, 4, and 6 to 8 week age groups to warrant separate presentation. Therefore they are reported as a unit followed by the findings in 2-week-old mice.

All brains and spinal cords of mice in the 3 to 8 week age group were negative until 3 days after inoculation. When 10^{-1} dilution of virus was used, a limited distribution of lesions was found in at least 1 of each group of 3 brains obtained at that time; at intermediate periods (3½ to 5 days) an increasing number of brains contained lesions, and at 5 to 6 days a majority of them. With 10^{-2} and higher dilutions of virus a large majority of the brains including all those observed at the 3 day period were negative. Lesions did not appear until later and, compared with corresponding periods where 10^{-1} dilution of virus was used, the distribution of lesions was frequently quite limited.

In 2 specimens of the 3 to 8 weeks age group convincing evidence was obtained that ascending invasion of the C.N.S. occurs after intraperitoneal inoculation. Le-

TABLE IV

Number of Mice of Different Ages Inoculated Intraperitoneally with Different Concentrations of Virus

Age	Concentration of virus		
	10^{-1}	10^{-2}	Higher dilution
wks.			
2	11	14	10
3	11		26
4	20	23	—
6–8	24	—	—

sions were found in the spinal cord and scattered through the reticular formation of the medulla. In one brain, the lesions disappeared in the hind-brain at the level of the nucleus of the VIIth nerve; in the other, the tegmentum of the mid-brain was also involved and a group of lesions appeared in one lateral thalamic area and in the corresponding parietotemporal area of the neocortex. The lesions in the lateral thalamic area also extended toward the parafascicular nucleus which is situated in the medial thalamic area.

The majority of infected brains obtained at early and intermediate periods (10^{-1} dilution of virus) showed lesions which were scattered and mild in the pyriform territories, absent or very slight in the entorhine regions, hippocampi, and olfactory bulbs. The spinal cords were not invariably involved but lesions always appeared in the reticular formation at the caudal end of the hind-brain. Rostrally in the hind-brain, they were usually confined to the dorsal reticular gray. Lesions appeared in the tegmentum, central gray, and frequently in the tectum of the mid-brain and in the lateral and medial areas of the dorsal thalamus. The hypothalamus, preoptic areas (Fig. 5), and tubercula olfactoria were also involved. In the latter region lesions were sometimes severe in contrast to the mild lesions usually observed in the pyriform territories. A variable part of the neocortex was frequently involved.

In other specimens obtained at the late periods, the spinal cords were always involved and the pyriform territories frequently showed extensive mesodermal-glial proliferation as well as perivascular accumulations of lymphocytes. Two of these brains showed widespread necrosis of nerve cells in the pyriform, periamygdalar and entorhine regions of one side. Lesions always appeared in the hippocampi, entorhine regions, and olfactory bulbs, and in the plexiform layer of the bulbs extensive proliferation of mesodermal-glia has been observed.

Finally in another small but significant group of brains belonging to the 3 to 8 weeks age group, and not restricted to the younger of these mice, the distribution of lesions indicated that invasion had occurred by the olfactory route. The lesions in these brains were more advanced and abundant in the olfactory bulbs (Fig. 4) and pyriform territories as compared with those observed further caudally; the spinal cords were almost always negative.

An isolated cluster of lesions widely separated from an obvious portal of entry occurred in only one brain (4-week-old, 10^{-2} dilution of virus, 4th day). This was observed in the interior of the cerebellum.

In 2-week-old mice receiving a 10^{-1} dilution of virus, lesions were distributed throughout the C.N.S. as early as 2½ days following inoculation. It was impossible to distinguish the portal of entry but the distribution of the lesions did not differ from those observed in older mice in the late stage in such a way as to suggest that the virus had involved the C.N.S. by the hematogenous route. Using 10^{-2} dilution of virus the lesions did not appear until 3 days following inoculation but likewise involved the entire C.N.S. although they appeared more scattered than when the 10^{-1} dilution of virus was used. Using still higher dilutions of virus the first appearance of lesions was retarded and a limited distribution similar to that obtained in the mice of the 3 to 8 weeks age group was obtained. One mouse killed on the 7th day following inoculation with a 10^{-4} dilution of virus showed a limited distribution of lesions indicating that the virus had entered the brain by the olfactory portal. In another mouse which showed a flaccid paralysis upon the 7th day (10^{-5} dilution of virus), a typical ascending distribution of lesions occurred in which the fore-brain was completely negative.

Summary.—The results in all age groups indicate that the entire C.N.S. may be invaded by a virus which reaches it through the spinal cord and that this method of invasion probably occurs in the majority of mice inoculated intraperitoneally. However, a small but significant number of animals, irrespective of age, give decisive evidence of invasion of the C.N.S. by the olfactory route. Limited distributions of lesions do not occur in mice 2 weeks of age following intraperitoneal inoculation of 10^{-1} or 10^{-2} dilutions of virus; nevertheless they do occur when the virus is inoculated in higher dilutions.

DISCUSSION AND SUMMARY

The greatest change in the susceptibility of mice to the virus of St. Louis encephalitis inoculated intraperitoneally occurs between the 2nd and 3rd weeks of life. To investigate the mechanism of the influence of age on sus-

ceptibility, mice of different ages (2, 3, 4, and 6 to 8 weeks) were killed at intervals following intraperitoneal inoculation and the distribution of the lesions in the C.N.S. was compared with that which resulted from infections via the nasal portal in mice 6 to 8 weeks of age.

Following intranasal instillation of the virus, lesions first appeared in the olfactory bulbs and advanced through the pyriform areas and the other fore-brain olfactory centers. Before the entorhine region and hippocampus were involved, lesions had already appeared in centers associated directly or indirectly with the olfactotegmental tract. The remainder of the C.N.S. was more or less extensively involved in mice showing clinical signs of the disease.

In mice 2 weeks of age inoculated intraperitoneally with 10^{-1} dilution of virus, lesions appeared throughout the C.N.S. as early as 2½ days subsequent to inoculation; the virus spread so rapidly that the portal of entry could not be detected. The majority of the mice of the same age receiving 10^{-2} to 10^{-5} dilutions of virus and of the older mice receiving 10^{-1} dilution of virus showed intermediate stages in the progression of lesions through the C.N.S. In the latter group this pointed to an ascending involvement in which the virus reached the fore-brain olfactory centers in retrograde fashion; the preoptic areas and tubercula olfactoria were extensively involved before the pyriform areas and olfactory bulbs. In another small but significant group of intraperitoneally inoculated mice representing all ages, lesions of restricted distribution occurred in the C.N.S. which appeared in all respects identical with those which followed infection by the nasal route.

The significant difference in the susceptibility to infection between mice 2 weeks of age inoculated intraperitoneally and those 3 weeks of age or older inoculated by the same route is not readily explained by changes in the permeability of capillaries to the virus. It has been pointed out that in the 2-week-old mice (10^{-1} dilution of virus) the whole C.N.S. was involved as early as 2½ days following intraperitoneal inoculation. However, the distribution of lesions did not differ materially from those observed when the C.N.S. was completely involved in older mice killed at longer periods following inoculation. Therefore, the conclusion cannot be drawn that virus reaches the C.N.S. of the 2-week-old mice directly through the blood steam while it reaches that of the mice 3 weeks of age and older by axonal pathways leading from the portal of entry. Our data strongly support the view that contamination of the nasal mucous membrane and subsequent invasion of the C.N.S. by the olfactory route occurs not uncommonly in mice inoculated by peripheral routes (Sabin and Olitsky, 3), but we have observed no age difference in this respect for the virus of St. Louis encephalitis.

The C.N.S. of the mouse during the first 2 weeks of life is still undergoing rapid changes leading to a complete neuronal and glial differentiation. In a critical discussion of the tectogenetic principle of cortical development Lorente

de Nó (20) has presented evidence concerning the stage of development attained by the entorhine cortex at intervals preceding and after birth. He observed that the migration of glial cells from the ependymal layer through the cortical plate began shortly before and was not finished until several days after birth; at 5 days glial cells had already entered the cortex and presented a very mature form, but other cells of the ependymal layer were still commencing their migration. Other evidence demonstrated that different kinds of neurones in the entorhine cortex could be recognized in their embryonic form at 12 hours subsequent to birth; at 5 days differentiation was more advanced in the deeper cortical layers but was still incomplete throughout. Definite information was not given concerning the age at which the glial and neuronal elements of the whole cortex become completely developed, but presumably it is considerably later than 5 days.

Therefore, the hypothesis that the mature C.N.S. is a less favorable substrate for the growth of neurotropic viruses than the immature one must be considered as a possible explanation for the greater susceptbility of 2-week-old mice to virus inoculated intraperitoneally. Reviewing the results obtained following intraperitoneal inoculation with the 10^{-1} dilution of virus, it is apparent that only in mice 2 weeks of age did the virus spread so rapidly through the C.N.S. that lesions could be detected everywhere as early as 2½ days subsequent to inoculation. In certain mice of the 6 to 8 weeks age group a restricted distribution of lesions was encountered at 3 and 3½ days, but it was not until later that the C.N.S. became completely involved. Also favoring the point of view that tissue of the nervous system may vary in its ability to support the growth of the virus is the fact that certain centers such as the entorhine regions, hippocampi, and neocortex may escape involvement in the mice 3 to 8 weeks of age killed at the early periods following inoculation.

On the other hand, the results obtained with the higher dilutions of virus (10^{-4} and 10^{-5}) in 2-week-old mice indicate that whatever difference may exist between the resistance of the immature and the mature nervous substrate must be only relative. Protracted incubation periods can occur and quite restricted distributions of lesions remain as late as 7 to 9 days following the inoculation of small amounts of virus. This observation, considered with the equal susceptibility of young and old mice to virus inoculated intracerebrally or instilled intranasally, makes it seem unlikely that the greater susceptibility of young mice to virus inoculated intraperitoneally depends upon the immaturity of the tissue of the C.N.S.

All the observations point to the importance of the amount of virus reaching the C.N.S. following intraperitoneal inoculation. A possible explanation of the difference in susceptibility, as well as the early appearance of widespread lesions in the young animal receiving large amounts of virus, is that a greater amount of the virus inoculated intraperitoneally survives in the young animal

and reaches accessible portals of the C.N.S. The data are consistant with this explanation, as is the recent study of Morgan (21), who drew a parallel between the resistance of young vaccinated and adult normal mice to peripheral inoculation of the active virus of Eastern equine encephalomyelitis. The analogy drawn between young vaccinated mice and adults was based on the observation that, although susceptible to virus given by the intracerebral route, the young vaccinated animals, by the 4th day after the beginning of vaccination, resisted large doses of virus given by the intraperitoneal route. These results linked with the demonstration of the more rapid immune response of older animals to the virus of Eastern equine encephalomyelitis, as judged by the appearance of demonstrable neutralizing antibody (10), appear to offer an explanation for the greater resistance of older mice to the peripheral inoculation of active virus of Eastern equine encephalomyelitis.

The failure of some investigators (22) to demonstrate neutralizing antibody in the serum of mice immunized by the subcutaneous inoculation of active virus of St. Louis encephalitis until several weeks following the immunizing inoculation might seem to weaken the evidence for the immune response being involved in the resistance of mice to the peripheral inoculation of the virus of St. Louis encephalitis. However, recent observations by one of us (23) have shown that neutralizing antibody to the virus of St. Louis encephalitis can be demonstrated at least as early as one week (the earliest interval when tested) following the subcutaneous inoculation of mice over 3 weeks of age with active virus.

CONCLUSIONS

1. Young mice are more susceptible than older mice to the virus of St. Louis encephalitis inoculated intraperitoneally, but with virus inoculated intracerebrally or intranasally, there is no significant age difference in susceptibility. The greatest change in the resistance to the virus inoculated intraperitoneally occurs between the 2nd and 3rd weeks of life.

2. The distribution of the lesions of St. Louis encephalitis in the C.N.S. of young and of old animals following intraperitoneal inoculation indicates that the virus may reach the brain either by the ascending pathway from the spinal cord or by the olfactory pathway irrespective of the age of the animal. However the ascending pathway is most frequently concerned.

3. The distribution of lesions does not offer evidence that the virus enters the C.N.S. of young animals directly from the blood stream following intraperitoneal inoculation.

4. Although widespread lesions occur earlier in the C.N.S. of young mice than in that of older mice inoculated intraperitoneally with large doses of virus, this fact is not satisfactorily explained by assuming the more rapid increase of the virus in the C.N.S. of young animals, since the latter are not more susceptible to virus inoculated directly into the brain.

5. The observations can be explained by the hypothesis that a greater amount of virus survives and reaches the portals of the C.N.S. in young animals following intraperitoneal inoculation and that this is an important factor in the influence of age on susceptibility to the virus.

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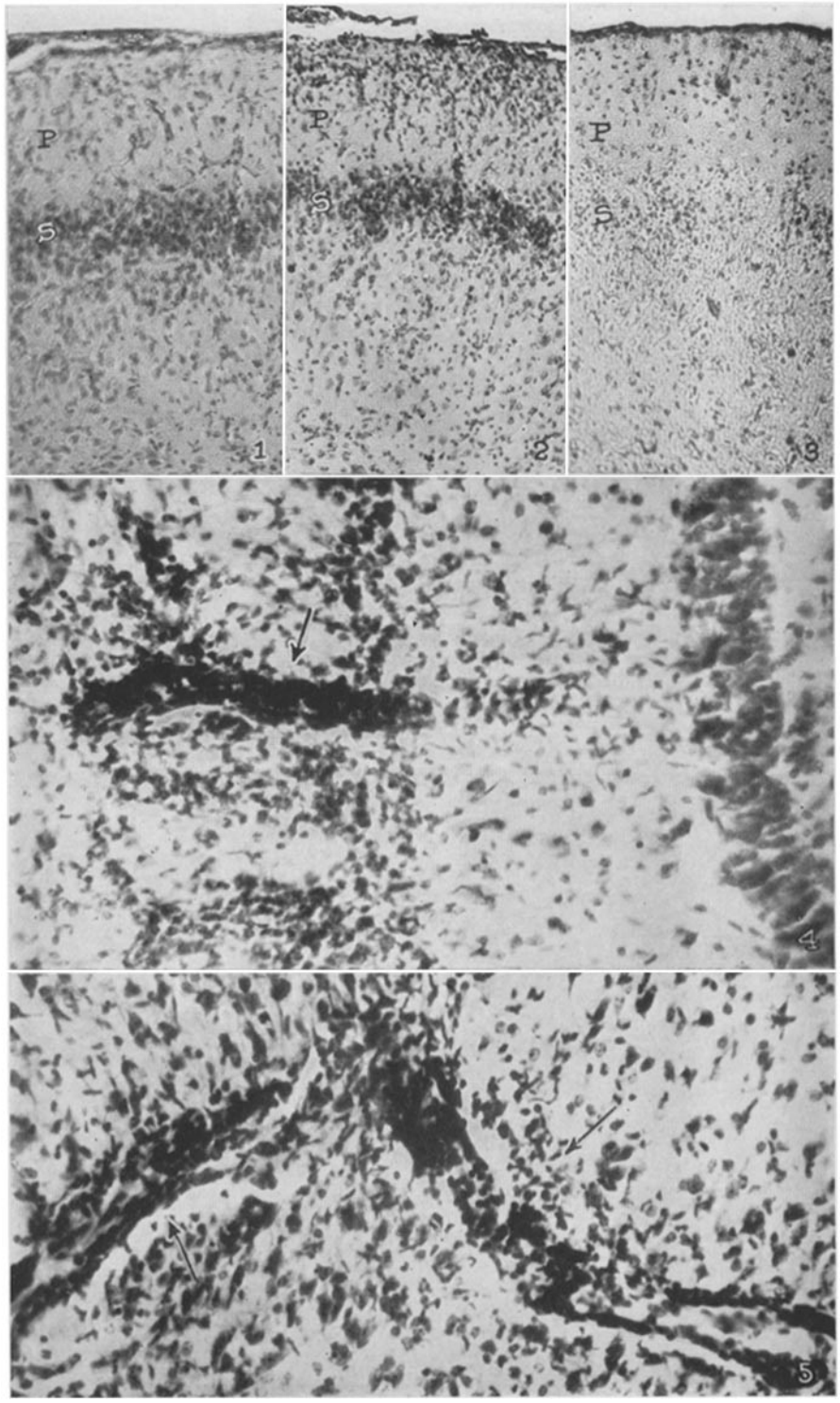
EXPLANATION OF PLATE 4

FIGS. 1 and 2. Subpial glial reaction in mice 6 weeks old receiving 10^{-3} dilution of virus by nasal instillation. From the cortex of the pyriform lobe. *P*, plexiform layer; *S*, layer of superficial pyramids. × 100.

FIG. 3. Same area as represented in preceding figures. Illustrates a massive necrosis of nerve cells that followed nasal instillation of virus in a 6-week-old mouse; layers of superficial (*S*) and deep pyramids have been destroyed. This reaction was also observed in animals infected by the peritoneal route. × 100.

FIG. 4. Olfactory bulb of a 3-week-old mouse that received 10^{-1} dilution of virus intraperitoneally and was killed 3½ days later. Note the accumulation of lymphocytes about penetrating vessels (arrow). No lesions were observed in this brain caudal to the preoptic area. Scattered vessels of the spinal cord were surrounded by accumulations of lymphocytes. × 250.

FIG. 5. Accumulation of lymphocytes (arrows) about vessels of the lateral preoptic area of a 6-week-old mouse that received 10^{-1} dilution of virus by the peritoneal route and was killed 4½ days thereafter. × 250.



(O'Leary *et al.*: St. Louis encephalitis virus)