STUDIES ON EASTERN EQUINE ENCEPHALOMYELITIS

II. Pathogenesis of the Disease in the Guinea Pig

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Plate 40

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In the first paper of this series (1), the histopathology of equine encephalomyelitis in the guinea pig was described and discussed in detail. The virus injected peripherally readily invades the blood stream and then infects the central nervous system. The mode of entrance of the virus into the nervous system from an injection site at the periphery is not clear.

Hurst (2), studying the guinea pig, believed that the local peripheral nerves are not involved in the pathogenesis. He suggested that virus is deposited from the blood on the olfactory mucosa, whence it travels to the subdural space by means of the perineural lymphatics. The recent work of Yoffey and Drinker (3) makes such an explanation improbable. Hurst also suggested the passage of virus from the blood across the endothelium of the cerebral blood vessels (in his phrase, a "growth through" the hematoencephalic barrier).

Sabin and Olitsky (4) believed that, in contrast to the behavior in mice, the virus in the guinea pig passes directly across the vascular endothelium, since in this animal the lesions bear a definite relation to blood vessels. With the Western strain of the virus, Larsell, Haring, and Meyer (5) similarly suggested a direct passage across the blood vessels, also on the basis of the perivascular nature of the lesions. The validity of this evidence will be treated in the discussion.

Because of the lack of unequivocal data, the problem was reinvestigated. Topographical analysis of early cases seemed the most promising method. This method depends on the assumption that the earliest localization of the virus will produce the first lesions. Although, as is well known, virus may be present without causing tissue damage, an area with a demonstrable typical lesion presumably has harbored the virus for a longer period than any area without lesions.

By charting all the lesions in a brain the earliest localizations can thus be determined.

Method

Since topographical analysis would be valueless when the encephalitis is fully developed, it was essential to secure very early cases for study. To ensure the presence of lesions before the brains were serially sectioned, a method of vital staining was used, modified from that of McClellan and Goodpasture (6). The following procedure was used in guinea pigs. 1 to 2 hours before the animal is to be sacrificed, 2 to 3 cc. of 2 per cent trypan blue are injected intravenously. It is usually desirable to administer an additional 1 cc. subcutaneously the day previously. The intravenous injection usually prostrates the animal which, after 1 to 2 hours, is killed with chloroform. The nervous system is then perfused with formalin through the aorta under physiological pressure. When the brain is removed, lesions are clearly outlined in blue against the colorless background. The entire success of the method depends on securing a good perfusion. Otherwise even the normal portions of the brain may be colored, due to dye contained within the blood vessels.

With this method, animals were killed at various intervals after inoculation. The nerve tissue was imbedded in paraffin and sectioned serially at 15 microns. Every 15th or 20th section was mounted and stained.

Two strains of virus were used. One was isolated in 1937, and the 2nd, 3rd, and 4th subinoculations in guinea pigs were employed. The 2nd was isolated in 1938, and the 1st guinea pig subinoculation was used. The results of the two strains were indistinguishable.

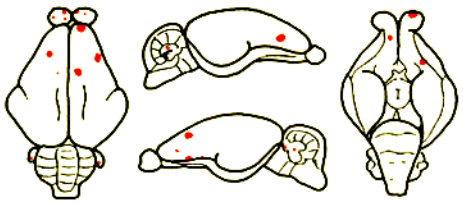
OBSERVATIONS

Observations on 9 selected animals, none of which showed any clinical signs of disease at the time of sacrifice, are presented in detail. Even a cursory survey of the material shows that, with a constant mode of inoculation, the lesions are never the same in any two cases. In the accompanying figures are charted the lesions which appear on the surface. All those which are hidden from view in the interior of the brain are described in the accompanying text. Summaries are presented in Table I and should be consulted in conjunction with the charts. For a detailed description of the anatomical structures mentioned below, reference may be made to standard works on the nervous system of rodents (7).

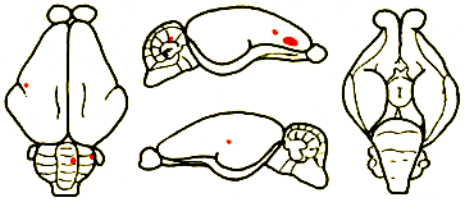
No. 1. Inoculation into both hind pads. Sacrificed after 58 hours. Every 15th section mounted and stained.—Both olfactory bulbs contain a few small discrete

TABLE I

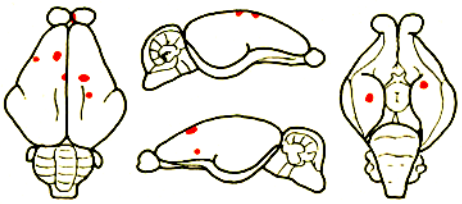
Guinea pig No.	Route of inoculation	Killed (time after inoculation)	Summary of lesions
		hrs.	
1	Hind pads	58	Small discrete lesions scattered over the frontal part of the brain, involving the olfactory and neocortical portions about equally. Similar lesions present in the cerebellum. Subcortical centers intact
2	" "	$57\frac{3}{4}$	Scattered lesions present in neocortex and cerebellar cortex, as well as in a few unrelated subcortical centers
3	" "	$58\frac{3}{4}$	Scattered lesions involving portions of the olfactory brain and unrelated areas in the neocortex. Subcortical lesions situated only in the intermediate auditory centers; lowest, primary auditory nuclei normal
4	" "	$62\frac{1}{4}$	Cerebral lesions restricted to extensive involvement of the olfactory regions, without affecting the hippocampus. Isolated lesions in the cerebellar cortex
5	" "	$58\frac{1}{2}$	Inflammatory involvement primarily of the lower and higher visual centers of both sides, more severe on the left. Most of the thalamus, including the non-visual portions, affected, as well as certain auditory centers. Parts of the olfactory pathways, as well as the basal ganglia, also injured
6	Intranasal	67	Widespread lesions in lower olfactory connections; but circumscribed, unrelated lesions also present in neocortical areas, the caudate nucleus, the cerebellum, and the mid-brain
7	Intraocular (right)	66	Involvement of right anterior olfactory regions, and of scattered neocortical areas. Thalamus and subcortical centers intact
8	" "	58	Involvement of entire optic pathway of both sides, from the chiasm to the cortex, the left side more than the right. Lesions extend to contiguous areas which are functionally independent. Isolated lesions present in the left claustrum and pons, regions independent of each other and of the affected portions elsewhere. Certain intermediate acoustic centers affected
9	" "	$58\frac{1}{2}$	A few scattered lesions in the intermediate olfactory centers and in the neocortex. Olfactory bulbs normal. Lower visual centers corresponding to the inoculated eye show very early involvement, but of much less intensity than the cortex



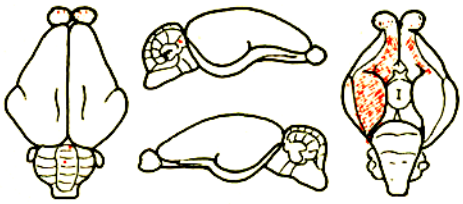
TEXT-FIG. 1. Guinea pig 1.



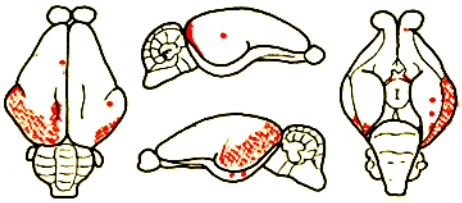
TEXT-FIG. 2. Guinea pig 2.



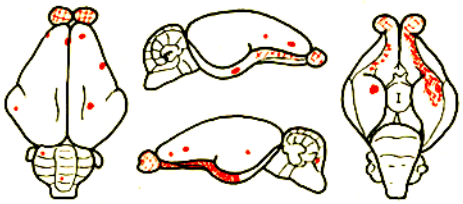
TEXT-FIG. 3. Guinea pig 3.



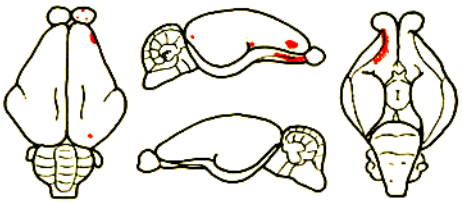
TEXT-FIG. 4. Guinea pig 4.



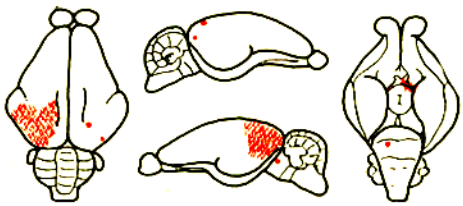
TEXT-FIG. 5. Guinea pig 5.



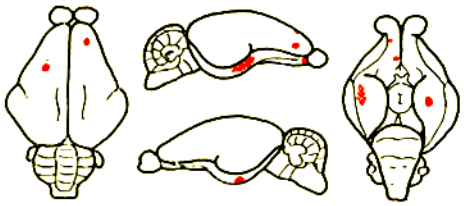
TEXT-FIG. 6. Guinea pig 6.



TEXT-FIG. 7. Guinea pig 7.



TEXT-FIG. 8. Guinea pig 8.



TEXT-FIG. 9. Guinea pig 9.

lesions. In the remainder of the olfactory brain there are only two other lesions, one in the anterior olfactory nucleus on the right, the other astride the rhinal fissure on the left, involving the neo- and olfactory cortex about equally on either side of the fissure. There are other lesions in the neocortex, as indicated in the chart, affecting the orbital, precentral, postcentral, and insular areas. There are no concealed lesions; the hippocampus, basal ganglia, thalamus, midbrain, pons, and medulla are entirely normal, as is the spinal cord. In the cerebellum there are a few sharp foci, two of which, in the flocculi, show exquisite bilateral symmetry.

No. 2. Inoculation into both hind pads. Sacrificed after 57¾ hours. Every 15th section mounted and stained.—No part of the olfactory system is involved. In the neocortex lesions are present in the right postcentral area, involving the insular cortex to some extent, and the right parietal region. There is a small focus of low intensity in the left temporal cortex. Two isolated lesions are present in the cerebellar cortex. Several lesions are present in lower centers, not indicated in the chart. There is bilateral ependymitis with, in one area, a collection of polymorphonuclear leucocytes impinging on the ventricle. The left caudate nucleus also contains a small but intense focus of inflammation. The right medial geniculate body and the right inferior colliculus contain early lesions, but the acoustic tubercles and intermediate centers are entirely normal. No other lesions are present in any portion of the brain or cord. In the meninges are two small areas, one in the left temporal cortex and one at the base at the level of the posterior thalamus, where there is some mononuclear infiltration, slight in both degree and extent. These meningeal areas are not related to regions of parenchymatous inflammation.

No. 3. Inoculation into both hind pads. Animals sacrificed 58¾ hours later. Every 15th section mounted and stained.—In the entire olfactory system there are only three lesions, one in the medial portion of the right bulb and one in the entorhinal region of either side. The intermediate centers are intact, as is the hippocampus. Most of the lesions are situated in the neocortex where the left precentral and parietal, and the limbic or infraradiar areas are involved. On the right the lesions affect the parietal and the most anterior portion of the striate areas. Hidden from view are early lesions in the right inferior colliculus and right medial geniculate body. No other portion of the thalamus shows any injury. The lateral geniculate, sending fibers to the striate cortex, and the anterior nuclei, which send afferents to the limbic area, are intact. The medullary acoustic centers, along with the entire medulla, cerebellum, and cord, are normal.

No. 4. Inoculation into both hind pads. Sacrificed after 62¼ hours. Every 20th section mounted and stained.—The olfactory bulbs of both sides contain numerous minute foci of polymorphonuclear leucocytes. There is in addition much more extravasation of blood than is usually seen. The olfactory centers at the base of the brain are affected in very widespread fashion. On the right practically the entire extent is involved by innumerable small foci, sometimes dicrete, sometimes confluent, of varying degrees of intensity. On the left the process is similar but essentially limited to the anterior pyriform cortex, sparing the tuberculum

olfactorium. The process extends inward to a slight extent, to include the amygdala and the nucleus accumbens. Although the presubiculum is damaged, the involvement does not include the hippocampus. For the most part the inflammatory changes are sharply limited dorsally by the rhinal fissure, but in a few areas there is a very slight extension into the adjacent neocortex. The only other sign of encephalitis in the entire brain is the presence of three isolated discrete foci of inflammation in the cerebellar cortex. Thalamus, midbrain, pons, and medulla are intact.

No. 5. Inoculation into both hind pads. Sacrificed after 58½ hours. Every 20th section mounted and stained.—The olfactory bulbs are intact. There is a minute lesion in the left olfactory crus, in the dorsal part of the left anterior olfactory nucleus adjacent to the orbital cortex. There are also two discrete foci of destruction in the left entorhinal cortex. Apart from a single isolated focus in the left precentral area, the other neocortical lesions are restricted to the striate, temporal, and occipital areas. On the right there is a single circumscribed lesion in the visual area, and a more diffuse region of injury more posteriorly, which overflows into the inferior temporal cortex and the subiculum. On the left, however, the damage is quite extensive, involving the greater part of the visual and posterior temporal cortex, and also overflowing into the subiculum. It will be noticed that the chiasm is intact, but the optic tracts exhibit small discrete lesions, more on the left.

Hidden from view there is rather widespread damage to the telencephalon and diencephalon. Thus, the left caudate nucleus contains a small focal collection of polymorphonuclear leucocytes. The left putamen and globus pallidus and the amygdala more posteriorly also contain early lesions. In the thalamus there are inflammatory changes involving the anterior group, the lateral, and the ventral nuclei, on the left. The medial as well as the lateral geniculate bodies of both sides are severely injured, as well as the pretectal regions and the posterior nuclei. There is a much milder involvement of the medial nuclei of the thalamus, the subthalamus, and portions of the hypothalamus. The changes in the superior colliculi are well marked; those in the inferior colliculi are very mild, with the right somewhat greater than the left.

The white matter of the hemispheres, including the thalamic peduncles, shows very numerous glial nodules, and some cuffing of the blood vessels. The cerebellum, pons, and medulla, as well as the hippocampus, are normal. There are no changes in the medullary acoustic centers. The spinal cord is normal.

No. 6. Intranasal instillation, both nostrils. Sacrificed at 67 hours. Every 20th section mounted and stained.—The maximum involvement affects the basal olfactory centers. The bulbs are the seat of numerous small lesions, which, on the left, continue backward in a rather diffuse fashion, including the anterior olfactory nucleus and pyriform cortex. The tuberculum olfactorium is bilaterally spared. The lesions diminish in intensity posteriorly and disappear in the position marked. On the left the process is much less severe, only the anterior pyriform cortex being affected. More posteriorly is an isolated lesion as marked. The

hippocampus is entirely spared. In the neocortex there are numerous discrete circumscribed lesions situated in diverse architectonic areas—precentral, postcentral, parietal, insular, temporal, and striate. The cerebellar cortex also displays two isolated lesions.

Hidden from view there is a mild degree of ependymitis of the left lateral ventricle. The left caudate nucleus contains a small inflammatory focus. The right lateral geniculate body, which sends fibers to the striate cortex, is intact; but the right superior colliculus, which merely receives from the cortex without sending, shows very early changes. Both inferior colliculi show extensive but very early involvement, the right somewhat more than the left. But the medial geniculate bodies, as well as all the medullary acoustic centers, are completely normal.

No. 7. Intraocular inoculation into the right eye. Sacrificed after 66 hours. Every 20th section mounted and stained.—The right olfactory bulb shows a few small scattered lesions. On the same side the anterior olfactory nucleus and the anterior pyriform lobe show well marked inflammatory changes. In the neopallium there are three discrete lesions, one in the right postcentral cortex, and one in the right temporal cortex. There is in addition a small focus of neuronal degeneration in the right occipital cortex. The thalamus and other subcortical centers are entirely normal.

No. 8. Intraocular inoculation into the right eye. Animal sacrificed 58 hours later. Every 20th section mounted and stained.—The olfactory bulbs and entire anterior portion of the brain, including neocortex, olfactory cortex, and anterior basal ganglia, are entirely free of lesions. The first lesions are seen in the optic chiasm and optic tracts, where the left is more severely affected than the right, although both contain glial and leucocytic foci. The hypothalamus and olfactory regions adjacent to the ascending optic tract on the left show well marked inflammatory reaction. The surface lesions are restricted to the striate and occipital cortex on the left, where the involvement is widespread. On the right, there are only two discrete focal lesions in the striate cortex. In addition there are seen lesions in the left midbrain (nucleus of the lateral lemniscus) and in the right pons, both reaching the surface of the brain. Hidden from view are lesions in both lateral geniculate bodies and both superior colliculi, the left being more severely damaged than the right. Both medial geniculates are involved, but the inferior colliculi are intact. The damage to the midbrain and posterior thalamus is not restricted to the nuclei mentioned, but tends to extend medially in a somewhat diffuse fashion. There is an isolated circumscribed lesion in the left claustrum at the level of the infundibulum. The medulla, cerebellum, and cord are entirely normal. The medullary acoustic centers show no change.

No. 9. Intraocular inoculation into the right eye. Sacrificed after 58½ hours. Every 20th section mounted and stained.—The olfactory bulbs are free of lesions although there are discrete scattered lesions more posteriorly in the rhinencephalon, namely, small early foci in the right anterior pyriform cortex and the right tuberculum olfactorium. More posteriorly there is a well circumscribed

focus in the left entorhinal cortex and a more diffuse area of involvement in the comparable region on the right side. In the neocortex there are lesions in the right precentral and insular areas, and also in the right postcentral and parietal areas. In the subcortical centers, hidden from view, the left lateral geniculate and the left anterior colliculus show extremely early but very definite lesions. The remainder of the thalamus is entirely intact, as is the hippocampus, as well as all other portions of the brain.

General Features

It is of interest to observe whether virus lesions are isolated or whether they tend to involve systems. Of the 9 cases presented, in 4 there is definite system involvement, although of different sense modalities: the olfactory pathway in Nos. 4 and 6, and the visual pathway in Nos. 5 and 8. It is noteworthy that different routes of inoculation were utilized. In line with this, it is seen that the same route of inoculation can, in different instances, produce totally different distribution of lesions (as No. 8 contrasted with No. 9, both with intraocular injections; or, with pad inoculation, No. 4 contrasted with No. 2). On the other hand, as already mentioned, different modes of inoculation can produce substantially the same result.

Apart from the occasional system involvement, there should be noted the frequency of discrete scattered lesions in the cortex, of the hemispheres, the cerebellum, and the olfactory brain. The virus appears to attack the brain not only with reference to nerve paths, but also independently of known system connections.

It is of further interest that although the cortex is the site of predilection, subcortical centers may also be affected at times. Thus, various regions in the basal ganglia, thalamus, hypothalamus, and midbrain show damage in one or more instances. It is important to realize that no antipathy exists between the subcortical regions and the virus.

Evidence for Nerve Transmission

The inference that the nerve pathway is implicated in pathogenesis depends for its force on two requirements. First, the number of lesions in the brain must be small. Where the entire nervous system is a mass of inflamed tissue, no differential significance can be attached to the involvement of any particular part. Second, the nerve centers

implicated must be connected by simple and direct nerve paths. A scheme whereby a circuitous nerve connection is imagined, involving several intermediate stations and tracts of doubtful existence, is ingenious but not convincing. The force of the "nerve path transmission," theory is proportional to the directness of the connecting pathway, and inversely proportional to the total number of lesions.

The best example of the importance of nerve connections in the spread of the virus is offered by No. 8. Here inoculation was into the eye, and the lesions involve, in a quite selective manner, the entire optic pathway up to the cortex. Other unrelated lesions will be discussed below. In No. 5, with virus injected into the pads, there is a strikingly similar distribution of lesions. In this latter case, the virus carried by the blood attacked some part of the optic pathway, and then spread out along the nerve paths of the visual system.

The utilization of existing nerve paths in the spread of virus within the brain is shown by the olfactory connections. Virus instilled into the nose (No. 6) affected the olfactory bulbs, the pyriform lobes, and entorhinal cortex, which are connected with the bulbs by the lateral olfactory tract. The other lesions will be discussed below. In No. 4, a pad inoculation, the bulbs and pyriform cortex are affected in a manner very similar to the previous case. Similarly in No. 7, the right olfactory bulb and the right anterior pyriform cortex both contain lesions. Here too the obvious link between the two involved regions is the nerve pathway.

These examples suffice to show that in certain instances, where there are relatively few lesions in the entire brain, anatomically related nerve centers may be involved in a quite selective and striking fashion. There are two possibilities: either pure coincidence has occurred, or the nerve connections have had some part in the spread of the virus. The latter choice alone seems plausible in the instances cited.

Evidence for Spread by Direct Contiguity

When two contiguous portions of the nervous system are involved, the relationship may be one of simultaneity and independence; or, as in the preceding section, a spread from one region to the other by means of nerve connections; or, third, direct extension from one area

to the other, as by passive transport of virus particles in the interstitial fluid.

It is very difficult to rule out possible nerve spread, for the short nerve connections of different regions of the brain are incredibly numerous. But in No. 3 a crucial example is presented, namely, the involvement of adjacent regions where nerve connections are known not to exist. The optic tract on the left is severely damaged, and the contiguous hypothalamus, medially, and amygdala, laterally, also show inflammatory changes. Neuronal relationship between the tract and these adjacent regions does not exist. The alternative of spread by direct extension appears overwhelmingly probable.

Many other probable examples exist in the foregoing protocols, but this mode of spread once established does not need elaboration.

Evidence for Blood Stream Transmission

The virus, once it has attained the central nervous system, may at times spread along the nerve processes or axones, that is, by nerve transmission; or it may spread by direct extension. But how does the virus reach the central nervous system following a subcutaneous inoculation?

In the present study the evidence for passage directly from the blood stream into the brain is the converse of that used to demonstrate nerve transmission. The latter evidence depends for its force on the involvement of successive nerve levels connected by known anatomical pathways. Conversely, the isolated involvement of a given center, all of whose known nerve connections are normal, is presumptive evidence that the nerve pathway is not involved. Blood stream transmission is the only reasonable alternative.

It is readily seen that in the brains previously described the majority of lesions occurred independently of nerve pathways. A few obvious examples will suffice. In Nos. 5 and 9, there are isolated lesions in various parts of the rhinencephalon, but the olfactory bulbs, where all incoming olfactory fibers end, are entirely normal. In Nos. 2 and 3, there are lesions in the acoustic centers of the midbrain and thalamus, yet the acoustic tubercles, where the peripheral nerves end, are normal. Brain 6 has a lesion in the striate (visual) area, but the corresponding lateral geniculate body is intact. Similarly

No. 3 has a well marked focus in the left limbic (infraradiar) region, but the anterior nucleus of the thalamus, which is the afferent subcortical center, is unaffected. Brain 7 shows a massive focus of destruction in the postcentral (sensory) cortex, yet the entire thalamus is normal. Several brains show small foci in the cerebellar cortex, but the cerebellar connections are normal.

The frequency of discrete small lesions in the neocortex, together with the intact state of the subcortical centers, is readily apparent. The virus injected into the periphery appeared in the highest level of the nervous system without any evidence of having passed through the appropriate lower centers. To travel along nerve paths from the periphery to the cortex, certain primary and intermediate nuclei must be traversed. Severe lesions in the higher centers, with perfectly normal lower centers, would seem to exclude the utilization of nerve paths. The only reasonable alternative is that the virus attacked the cortex directly through the blood vessel wall.

The Rôle of the Spinal Fluid

Were the cerebrospinal fluid of significance in the spread of the virus through the nervous system, an entirely different histological picture would be called forth. It is known that this virus is capable of attacking the leptomeninges. Consequently a primary meningoencephalitis, with fairly uniform distribution of lesions at the margins of the spinal fluid pathway, would be expected. This has not been observed. Instead, sharply circumscribed focal lesions appear, not only on the surface of the brain, but also deep in the hemisphere and brain stem. There appears no way to harmonize these data with any possible rôle of the spinal fluid in the pathogenesis of this disease.

DISCUSSION

The importance of the blood in the pathogenesis of this disease is beyond question. The virus is known to circulate in high titer. We see further that after a constant mode of inoculation, as by pad injection, or intraocular inoculation, the distribution of lesions is inordinately varied. The blood stream is the only agent which could account for such vagaries.

This is of especial interest in relation to intraocular or intranasal

inoculation. Out of three cases of intraocular injection presented, in only one was there clearly an involvement of the optic system. Even though the virus may utilize nerve connections from the eye there is still an escape into the blood. Virus once in the blood acts essentially at random, regardless of the site of inoculation. In such instances as Nos. 6 and 8, the primary spread is related to the site of inoculation (nose and eye, respectively). But there are numerous totally unrelated lesions. These latter are attributable to secondary spread by way of the blood stream, and not by way of hypothetical nerve paths as yet undiscovered.

That the spread of viruses may be determined by existing nerve paths is an inference. This inference has been invoked by many authors in the study of different neurotropic viruses (8). In the present study of the guinea pig, the involvement of related nerve centers is sometimes extremely striking. For example, Nos. 4, 5, 6, and 8 show an involvement of successive neurones of a given system (olfactory or visual), with relatively little damage to the remainder of the brain. The probability is overwhelmingly high that this selective involvement was not merely due to chance: in some way the anatomical connections influenced the spread of the virus.

In certain other instances the probability of nerve spread is less strong—much less so. For example, in No. 3 there are a single lesion in the left olfactory bulb and single lesions in the posterior pyriform cortex, both right and left. There may be some causal connection between these three lesions; that is, the known nerve connections might be invoked as a connecting link. But there might, in this case, be a relationship merely of coincidence. If we compare the olfactory regions of Nos. 1 or 3 with those of Nos. 4 or 6, we see a marked difference. In the two latter instances, nerve spread seems definite; in the two former, it is problematic. Coincidence is equally or even more likely, especially in view of the spotty distribution of lesions elsewhere in the nervous system.

On the other hand, in this series the majority of lesions in the brain, especially in the neocortex, do not bear any relationship to each other according to known anatomical connections. In such instances a nerve spread of virus seems overwhelmingly improbable. The alternative of direct spread from the blood stream is the only satisfactory explanation.

There is only one criterion of nerve spread, namely, the topographical relationship of affected areas. Certain authors (4, 5) have held that the perivascular nature of some lesions is an indication that the virus passed through the blood vessels. In the present series of cases, where several hundred lesions were studied, this distinction is found not to obtain. No histological difference could be observed between regions where the virus was presumably blood borne, as compared with regions where nerve transmission presumably occurred. Only the anatomical relations of the affected regions, compared with each other and with the remainder of the nervous system, furnish a ground for distinction.

It is thus seen that the virus of equine encephalomyelitis is bipotential. It enters the brain from the blood stream through the blood vessel wall. Once in the nervous system, it may or may not spread along preexisting nerve paths. No explanation can at present be offered why in one instance it does and in another does not.

A question which must be kept open is that of possible deposition of virus from the blood on the olfactory mucosa, with subsequent spread along the olfactory connections (Hurst (2), Sabin and Olitsky (4)). The mere presence of lesions somewhere in the olfactory brain is obviously not sufficient to establish this theory. However, a marked involvement of the olfactory bulbs is entirely consistent with the hypothesis. In the present series of cases, No. 4 might be interpreted in this sense if one so desired. In the present state of our knowledge, this theory must be held under reserved judgment. There can be no doubt that in reference to certain neurotropic viruses the nose and the olfactory pathway stand in a different category from most other sense modalities and other parts of the body. The reason is as yet obscure, and further studies in anatomy as well as pathology are necessary.

The findings in the guinea pig may be compared with the results obtained by Sabin and Olitsky (4) in young mice, which alone were found susceptible to peripheral injection. According to these authors, the virus may occasionally invade the central nervous system along the local peripheral nerves. More often, they believe, virus transported by the blood invades by means of the olfactory or auditory pathways, or possibly along the seventh nerve. "No evidence was found of a direct passage of virus across the blood vessels of the brain" (9).

In the guinea pig, occasional animals show primary involvement of

the olfactory pathway, and there is no way to rule out a prior deposition of virus on olfactory mucosa. But in no other case was there evidence of spread *via* the peripheral nerves; that is, in no other case were the primary receptive nuclei involved. The cases of spread along the optic nerve are not exceptions, since the optic nerve is not a peripheral nerve at all. With the possible exception of the olfactory nerve, the virus seems to have attacked the central nervous system directly, without the intermediation of the peripheral nerves. This attack is considered to take place by passage across the blood vessels of the brain.

SUMMARY

After inoculation with equine encephalomyelitis virus by various routes, guinea pigs were sacrificed at early stages, before symptoms were apparent. The brains were studied histologically, with serial sections; all lesions were noted, and subjected to topographical analysis. Nine cases are presented in detail.

With any given mode of inoculation the distribution of lesions varied very widely from one instance to another. In some cases, affected regions bore a striking and definite anatomical relationship to each other. These distributions can be explained only by the assumption that the anatomical pathways played some rôle in the spread of the virus. In other instances lesions were present in areas, the anatomical connections of which were entirely normal. Attention is called to the frequency of lesions in the neocortex, with intact subcortical centers. Such distribution is held to render nerve spread extremely improbable. The only satisfactory explanation of such random distributions is by direct passage of virus from the blood stream into the brain tissue. There is no histological difference between lesions which result from blood spread and those resulting from nerve spread.

CONCLUSIONS

In the guinea pig, the virus of Eastern equine encephalomyelitis, injected peripherally, invades the blood stream and passes directly from the blood stream into the brain. This seems to be the principal, though not necessarily the exclusive, mode of pathogenesis. Once in

the nervous system the further spread of the virus may occasionally be determined by anatomical connections.

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EXPLANATION OF PLATE 40

FIGS. 1 and 2. Two typical lesions, from guinea pig 1. Thionin. Fig. 1, × 43. Fig. 2, × 65.

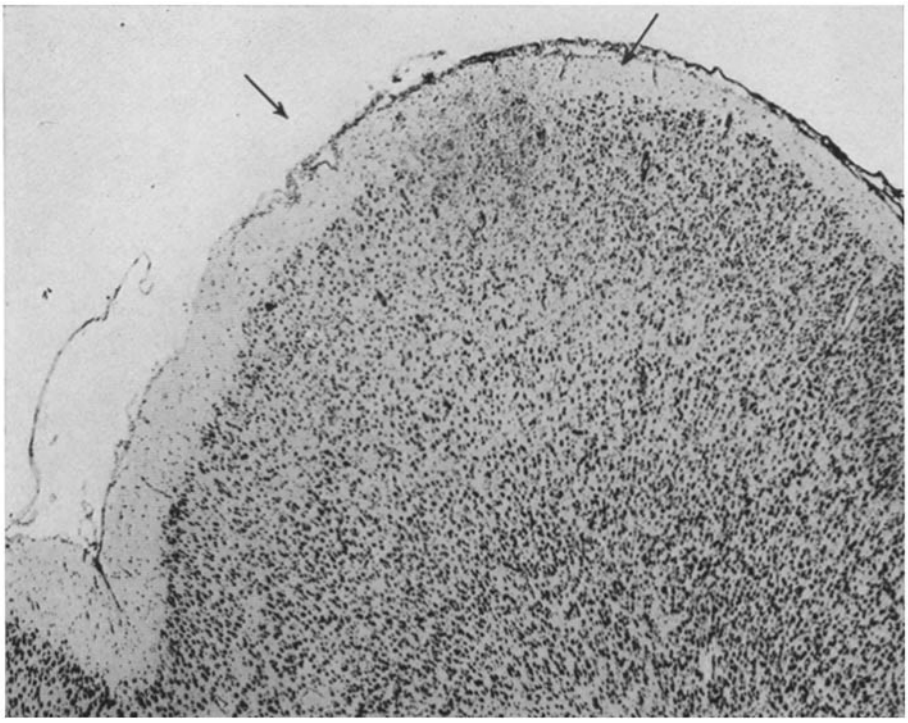

FIG. 1

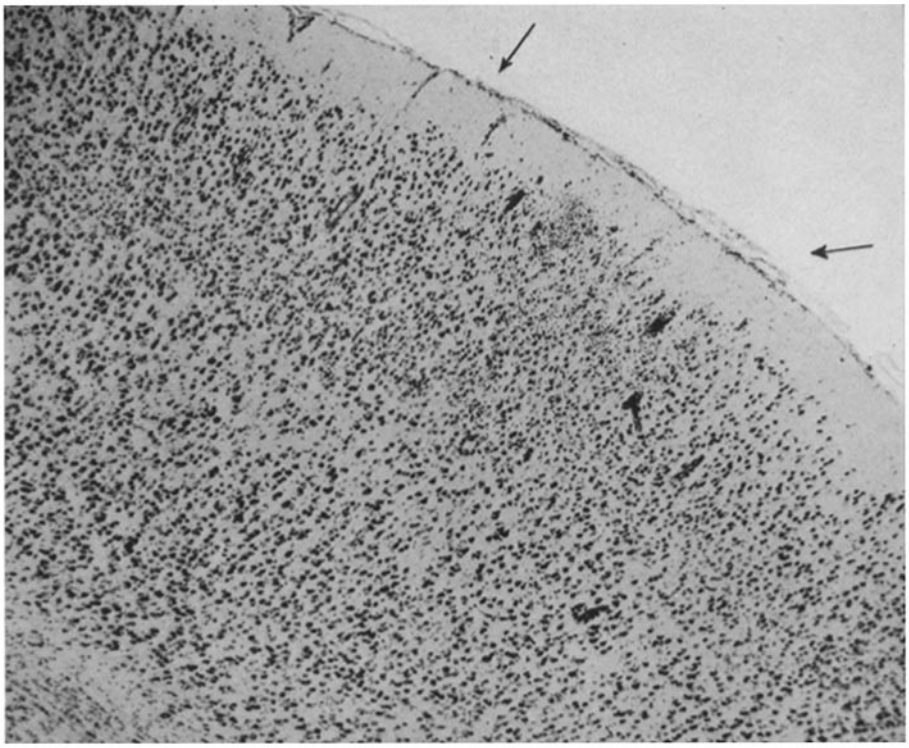

FIG. 2

Photographed by J. A. Carlile

(King: Eastern equine encephalomyelitis. II)