STUDIES ON EASTERN EQUINE ENCEPHALOMYELITIS

I. Histopathology of the Nervous System in the Guinea Pig

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Plates 32 to 35

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The pathology of the nervous system in equine encephalomyelitis has been described for both the Western (1) and the Eastern (2) forms of the disease. Much attention has been paid to the intranuclear inclusion bodies, found in glial and mesodermal cells as well as in neurones. The intense inflammation, together with the necrosis of nerve cells, has been reported. It is the purpose of this paper to record in detail the neuropathologic features of the disease in the guinea pig, and to describe the histogenesis of the disease process. Only the nervous system findings are reported. Details of visceral pathology, described by Hurst (2), have been confirmed but no new observations added.

Material and Methods

In the experimental work a strain of the Eastern virus, isolated in the summer of 1937, was used. The virus was passed through guinea pigs, and the 2nd, 3rd, and 4th passages were used for subinoculations. It was considered especially important to keep the virus as close to its natural host as possible, for, as is well known, repeated passages through a new host may modify the characteristics of the virus. Guinea pigs were used exclusively, and were infected by a variety of routes, the principal methods being subcutaneously in the plantar tissues, intracerebrally, intraocularly, and intranasally. Animals were killed at various stages of the disease, the earliest being about 55 hours after inoculation, when symptoms were not yet manifest. The great majority of animals were perfused through the aorta with fixative before the brains were removed. Bouin's fluid, Heidenhain's Susa fixative, and 10 per cent formalin were the fixatives employed. Over 40 brains, all containing lesions, were sectioned serially in paraffin, at a thickness of 15μ. In most instances every 15th section was mounted and stained, but in

later cases, when the pathological findings had become thoroughly familiar, every 20th or even every 25th section was utilized. Phloxin- or eosin-methylene blue, hematoxylin and eosin, and thionin were used in different brains. Thionin was the most satisfactory. Some blocks, known to contain lesions, were sectioned serially, and every section mounted and stained. In this way the entire extent of the lesion was made available. In addition other brains, not cut serially, were examined in paraffin, frozen, or celloidin sections. Appropriate staining methods were used specifically for myelin, fat, and neurofibrils, as well as the usual cell stains.

OBSERVATIONS

Peripheral Inoculation.—Examination of the brain of an animal moribund from the disease shows a very extensive destructive process which may involve any or every part of the neuraxis. A great variety of pathologic changes may be observed. Since, however, the study of the late or end-stage teaches very little about the evolution or natural history of the disease, special attention has been paid to the very early stages. This term requires some definition. In an animal killed 55 to 60 hours after inoculation (which is 20 to 30 hours before the first symptoms become apparent), all lesions found may properly be called early. Such early lesions, however, vary greatly in intensity; that is, the observed abnormalities may be very slight or very marked. The damage is frequently very severe, but sharply limited and not extensive. In the later cases, killed after symptoms are well advanced, the difference from the earlier cases is largely quantitative; that is, lesions are more numerous rather than more intense.

From a study of a graded series of cases, a typical lesion may be described, occurring in both early and late cases. An example is illustrated in Fig. 1. There is a fairly well circumscribed inflammatory reaction involving a limited area of the cortex, tending to extend radially. In such a focus there are large numbers of polymorphonuclear leucocytes scattered through the tissue. In the molecular layer of the cortex the polymorphonuclear leucocytes are in general much fewer, and the infiltrating cells are composed chiefly of blood mononuclears and glial cells.

Such an isolated focus may range in size from about 1.5 mm. to less than 0.5 mm. That is, in serial sections 0.225 mm. distant, a collection of leucocytes may be present through as many as 8 successive sections, or may be visible in only one section, with sections on either side completely normal. The overwhelming majority of lesions, however, involve more than one section.

The leucocytes may vary greatly in number. Figs. 2 and 5, under higher

power, show average examples. In the more intense examples (as in Fig. 1), the center of the lesion shows a loss of all the nerve cells, with the destruction diminishing sharply towards the periphery. In less severe instances there may be practically no neuronal destruction, even though the leucocytes are no less numerous. In Fig. 5 the arrow points to a necrotic cell. In this, leucocytes may be seen (under the microscope) actually invading the cell body. The other neurones visible have normal nuclei and cell bodies, although this fact is not so well appreciated in the photograph as under the microscope where the focus may be altered.

The marked pyknosis is not the usual type of cell destruction in this disease. When neurones are damaged, the sequence of cell destruction is as follows: First apparent is a pallor of staining reaction, affecting both nucleus and cytoplasm, but cellular morphology remains completely unchanged. The cytoplasm gradually disintegrates, becoming more and more pale, and showing a foamy type of vacuolation and swelling reminiscent of the well known water-change artifact. The nucleus may be absolutely intact during this stage, even though the cytoplasm is disintegrating and the cell is surrounded by leucocytes. The cytoplasm may disappear except for a very fine peripheral rim, in the center of which is the pale nucleus. Forms may be seen where no cytoplasm is visible at all, while the characteristic nerve cell nucleus remains recognizable. In all these stages of cell degeneration the nucleus, though pale staining, shows a distinct membrane, within which is a pale granular reticulum. The nucleolus may or may not be well preserved. Finally even this disappears, and no trace is left of the nerve cell. This type of change affects especially the granule cells of the cerebral cortex. The pyramidal cells exhibit to a greater degree a clustering of leucocytes around them, with vacuolation and granular disintegration of cytoplasm and nucleus occurring simultaneously.

The criterion of a necrotic nerve cell lies not in the condition of the cytoplasm but in the state of the nucleus. Where the architecture of the nucleus is preserved, that is, where the nuclear membrane is intact, the nucleolar complex normal, and the oxy- and basichromatin masses preserved, the neurone cannot be called necrotic. Severe cytoplasmic changes may, indeed, indicate irreversible changes, with the probability that the cell will soon die. But nuclear morphology, and not the tinctorial reaction of the cytoplasm, is the ultimate standard of the condition of the neurone. When eosin is used, a bright red staining of the cytoplasm, indicative of necrosis, is invariably accompanied by characteristic nuclear changes which are unmistakable and readily appreciated with thionin, or any other nuclear stain.

Figs. 6 and 7 show small circumscribed foci of polymorphonuclear leucocytes in the Ammon's horn and the cerebellar cortex, respectively. In Fig. 6 it should be especially noted that the pyramidal cells are entirely normal.

These foci, which may be large or small, dense or sparse, in early cases appear almost exclusively in the gray matter and chiefly in the neo- or the olfactory cortex.

Such non-cortical regions as the olfactory bulbs or basal ganglia, as well as the thalamus, are sometimes affected by similar circumscribed foci. These frequently show a relation to blood vessels but are in no sense perivascular.

Concomitant changes in the blood vessels are found. White blood cells, predominantly mononuclear elements, accumulate within the lumina. This fact is of especial significance when it is remembered that the vascular system is perfused before the brain is removed, and the vessels are empty of red blood cells. The leucocytes stick to the endothelium, undoubtedly through chemotaxis, and resist the washing-out action of fixing fluid pouring through the vessels. At the same time the endothelium is somewhat swollen and the adventitial cells hypertrophy markedly and proliferate, becoming, as well, strongly basophilic and hyperchromatic. Small spindle-shaped or moderately rounded forms predominate. The multiplication of adventitial cells is even more prominent than the collection of white blood cells in the perivascular space, that is, than the "round cell infiltration." If the focus is of any size the blood vessels invariably show this change. In examining the sections serially, the first sign of disorder will usually be this alteration in the blood vessels of a small area, observed for one or two sections without any parenchymatous reaction. Then for a section or two polymorphonuclears appear, and then the reaction disappears as further sections are examined.

The minimal lesion, that is, the least deviation from the normal that is recognizable as such, consists of this vascular change without any detectable alteration in the parenchyma. Figs. 3 and 4 illustrate this change. Fig. 3, under low power, is from the inferior colliculus. The affected blood vessels stand out vividly under low power. No change in the nerve cells or parenchyma is detectable. Fig. 4, from the caudate nucleus, and under higher magnification, shows more clearly the normal condition of the nerve cells. Exactly similar alterations are found in the cerebral cortex and other portions of the brain.

It must be emphasized that this change is not the "secondary" or "symptomatic" inflammation of Spielmeyer. It is a primary change, and the earliest reaction that has been observed. Only venules and arterioles are thus affected. Capillaries never showed demonstrable changes.

The regions where these early blood vessel changes are prominent, with or without a few polymorphonuclear leucocytes in the tissue, frequently show very fine inclusion bodies in the endothelium, vascular adventitia, and glial cells. Thionin, because of its metachromatic staining qualities, gives an adequate demonstration of such inclusions.

The more usual type of perivascular cuffing with lymphocytes and plasma cells also occurs in this disease, but chiefly in the more advanced cases. In the early lesions adventitial cell proliferation is the first reaction. In the more severe lesions, polymorphonuclear leucocytes are frequently found within the perivascular sheaths, a feature previously noted by Hurst. In advanced cases and even in intense lesions of early cases, such leucocytes have been seen infiltrating the walls of the blood vessels, so that the picture superficially resembles that of an arteritis.

Extravasation of red cells, sometimes restricted to the Virchow-Robin spaces, sometimes free in the tissue, is also of frequent occurrence. Hurst believed these to be artifacts subsequent to the trauma of removing the brain while the blood vessels were congested. But since they also occur when the brain has been perfused (under physiological pressures) and fixed before removal, such hemorrhages must be considered as antemortem findings.

Neuroglial reaction is prominent in this disease. Very early lesions may, atypically, show a glial mobilization in the affected region. Fig. 8 is a good example. In this field there is not a single polymorphonuclear leucocyte to be seen, although, several sections deeper into the lesion, a few make their appearance. Similar glial proliferation often occurs in the cerebellar cortex. Sections are available very similar to Fig. 7 but of about half the cell density, where no leucocytes are to be seen but where the proliferated cells are all glial. Diffuse glial increase is very frequently seen in the lamina zonalis of the cerebral cortex, where polymorphonuclear accumulations are the exception rather than the rule.

Some care must be exercised in designating a given nucleus as belonging to a glial cell. Among undoubted glial elements are fairly typical blood monocytes and large lymphocytes that on superficial examination may cause some confusion. These cells often occur in clusters. But similar types are often to be seen within the lumina of blood vessels or in the perivascular spaces.

The glial nodule (Fig. 12) is a somewhat different type of reaction. Such nodules were at one time thought to be peculiar to rabies, but are now known to occur in a variety of virus diseases. The typical closely packed cluster is not of frequent occurrence in the gray matter, and bears no necessary relation to the exudative changes. In the white matter, however, the nodule is a common finding, and is usually composed not only of glial cells, but also of blood mononuclears and even of some polymorphonuclears. Foci composed purely of polymorphonuclears do occur in the white matter, even in early cases, but are much less usual than the mixed focus or the pure glial nodule.

Although lesions in the gray matter may occur entirely at random, in the white matter the lesions are usually located where there is presumptive evidence of "nerve spread" of the virus. For example, with lesions occurring both in thalamic nuclei and in the appropriate cortical projection centers, the corresponding thalamic peduncle and the centrum ovale will often show the above mentioned type of lesion. Or when virus is introduced into the eye, and the optic centers are affected, the intermediate fiber tracts are also involved. In the white matter, however, the lesions are always discrete and discontinuous. In the gray matter, especially in the subcortical centers, an entire nucleus may be involved by the inflammatory process, but an entire fiber tract is never similarly affected. The foci are always spotty and frequently, though not always, in relation to blood vessels.

The foregoing description covers the very great majority of the lesions which occur after peripheral inoculation. They are obviously

inflammatory in nature, with mesodermal reaction (that is, intra- and perivascular or adventitial change) appearing first, with exudation the most prominent feature, and with demonstrable neuronal change clearly following and not preceding the onset of the process. More rarely, however, there are foci where severe degeneration or even total loss of nerve cells occurs in the absence of significant exudation. In the midst of normal cortical architecture there may be well delimited regions where the neurones show all degrees of severe damage. Yet very few polymorphonuclear leucocytes may be present, and the degree of neuronal damage is disproportionate to the degree of inflammatory reaction. In such instances the term "degenerative reaction" may be employed to express contrast with the other more usual change previously described. A more striking example is shown in Fig. 14, where whole regions of Purkinje cells are wiped out. Here too a few polymorphonuclear leucocytes are visible under higher power, but the contrast with, for example, Figs. 1, 6, and 7, which are outspokenly inflammatory, is obvious.

Intracerebral Inoculation.—This distribution between inflammatory and degenerative is not meant to be an absolute dichotomy of the "either . . . or" variety. There is a certain degree of overlap. The value of this distinction, however, is immediately apparent when we consider, instead of peripherally inoculated animals, those which have received virus directly into the brain substance.

If the virus is injected intracerebrally, quite a different picture results. Clinically, the course of the disease is more rapid; with the dosage used, the virus placed in the brain substance causes death in 3 to 4 days, whereas a peripheral inoculation of comparable dosage requires 4 to 8 days for exitus.

The site of the inoculation into the brain is readily found in serial section. One of the most striking facts is that there is no special reaction, and no trace of virus encephalitis, around the needle track. Histologically the locus of injection does not differ at all from a needle wound with a bland non-specific injection mass. Polymorphonuclear leucocytes are not found, but only the familiar and quite characteristic glial reaction attendant on any needle puncture.

Yet the virus, introduced without causing especial damage, produces encephalitis throughout the entire brain. There is very widespread

but relatively mild vascular and perivascular reaction, similar to that described for the peripheral type of inoculation but with much greater prominence of true round cell perivascular infiltration. However, after peripheral inoculation even very early cases show a much more severe focal reaction than is met with in intracerebral inoculation. Furthermore, in the latter cases, the perivascular hemorrhages are rare.

Although there are abundant polymorphonuclear leucocytes scattered through the tissue, they are more diffuse and rarely form such compact foci as with peripheral inoculation. The leucocytes appear singly or in small groups, and the dense accumulations, as seen in Fig. 1, for example, are most unusual in intracerebral injections.[1]

The constant and characteristic feature of intracerebral inoculation is the degenerative change mentioned above. That is, there is striking disproportion between the destruction of nerve cells and any inflammatory reaction that may be present. Fig. 13 shows a portion of the hippocampus with a stretch of the pyramidal cells simply wiped out. In this particular picture, a higher power examination shows a few leucocytes, but instances are available where there are none. Fig. 13 should be compared with Fig. 6, of the hippocampus after a peripheral inoculation. In the one there is a small focus of leucocytes, with intact neurones; in the other the destruction of ganglion cells is clearly the primary feature, the scattering of leucocytes secondary.

This type of involvement of the hippocampus is absolutely constant with intracerebral inoculation (it appears in all of 7 cases sectioned serially). Frequently the damage is exquisitely symmetrical, as shown by Fig. 9, where the exact segments destroyed on one side are equally destroyed on the other. (The inoculation, in this case, was entirely in the right hemisphere, as shown by serial section, and the needle track did not penetrate the ventricle.) Rarely, a very slight degree of this type of hippocampal damage is seen after pad inoculation, but only in cases of long duration, never in early cases.

Another type of destruction of nerve cells is seen in Fig. 10. The fundamental change may be seen at a glance. Whole massive areas of neurones are wiped out with scarcely a trace remaining. The glial cells suffer relatively little, and are present in essentially normal numbers. Leucocytes are scattered lightly through the tissue, without forming definite foci, but are no more frequent in areas where the neurones have vanished than where they remain intact.

These areas of cell loss are essentially random in distribution. The pyramidal cells are somewhat more severely affected, for, between the arrows, the external and internal granular layers are seen persisting rather feebly, although the pyram-

[1] After intracerebral inoculation, severe involvement of the inferior olive is a quite constant exception to this statement.

idal cells of the 3rd and 5th layers are gone. At other times, however, this distinction does not obtain. The illustration here recorded is a particularly severe and widespread example. In other areas, and in other brains, the cell loss may be more patchy, wiping out an irregular area of neurones, but leaving the remainder of the lamination intact. Such *Verödungsherden* are frequently perivascular, and resemble the type of cell loss occasionally found in vascular disease or in some types of cerebral ischemia.

A further characteristic of the intracerebral inoculation is the severe degeneration found in the large multipolar ganglion cells of the brain stem, rarely found after peripheral inoculation with a long duration. Fig. 16, taken from the red nucleus, illustrates the type of change which corresponds to the "severe cell disease" (*schwere Zellerkrankung*) of Spielmeyer. The nuclei of the neurones remain intact, although they are somewhat eccentric; the cell bodies, though somewhat rounded, show relatively little change in contour. But the Nissl granules show profound changes, being reduced in some instances to a powder, with pale staining reaction, in other instances being only partially broken up and disarranged. The simple, relatively benign cell change of acute swelling with chromatolysis is not observed in this disease. The severe cell disease is much more ominous.

Especially to be noted is the fact that there is no inflammatory reaction around these affected neurones. They are being destroyed under the influence of the disease process. Yet, instead of the direct inflammatory change, seen in the earlier figures, the virus action is indirect or secondary. This point will be treated further in the discussion. Fig. 16 should be compared with Fig. 15 which is taken from an animal inoculated in the pads. Here the neurone is intact, as may be seen by comparison with other cells of the same nucleus (facial nucleus). Yet there is vigorous glial mobilization. On the other hand, in Fig. 16 the opposite obtains.

In many of the affected large ganglion cells of the brain stem, and even in others which show but little morphological change, intranuclear inclusions are sometimes found. These are more prominent in the intracerebral than in the pad inoculations. This aspect is adequately described by Hurst.

Changes in the Spinal Fluid Pathway.—The involvement of the spinal fluid pathway deserves mention.

In early cases after peripheral inoculation there are scattered patches of meningeal exudate, chiefly with mononuclear cells. But almost invariably these areas are directly overlying a cortical focus of parenchymatous involvement. In favorable sections a cuffed blood vessel may be seen emptying into the subarachnoid space, and leucocytes packed along the sheath of this vessel are continuous with those in the pial meshwork. Occasionally, however, a small area of meningitis, mononuclear in type, may occur entirely independent of any parenchymal damage. Such independent meningeal foci, usually at the base of the brain, are not necessarily due to direct attack by virus discharged into the cerebrospinal

fluid. They may be non-specific in nature, although the alternative of virus etiology cannot be ruled out.

With intracerebral inoculation, wherein the virus comes into immediate contact with the subarachnoid fluid and its pathway, meningeal reaction is insignificant. Because of the presence of intranuclear inclusion bodies in cells of the pia-arachnoid, the susceptibility of meningeal cells to the virus is considered established. Yet the virus, which may produce such an intense reaction in brain tissue when injected peripherally, produces very little reaction when it comes into direct and immediate contact with the meninges through intracerebral inoculation. The reason for this peculiar behavior is not clear, but the fact is worth recording.

An interesting reaction is observed in the ependyma, in 20 to 25 per cent of cases. Fig. 17 illustrates the phenomenon, which has been found only in the lateral ventricles and may be either unilateral or bilateral. The ependymal cells have proliferated and have been thrown into folds, whorls, and small acini. Between the ependyma and the free surface of the ventricle there appear large numbers of interlacing spindle-shaped cells, apparently glial in nature. Leucocytes may or may not be present in the affected areas. This type of change has been observed as early as 58 hours after inoculation and may occur after either peripheral or intracerebral inoculation.

In Fig. 17, which comes from an advanced case, very widespread and intense inflammatory changes are observable. Superficial to the affected ependyma is a large dense focus of polymorphonuclear leucocytes, while elsewhere, in the cortex, centrum ovale, hippocampus, thalamus, and thalamic peduncle, various types of inflammation are visible. But there is no necessary relationship between the parenchymal inflammation and the ependymal change. The one may occur entirely without the other.

The ependymitis here illustrated and described is considered to be part of the pathology of the disease, although its fundamental nature is obscure. Conceivably it is not at all related to the virus action, but is merely fortuitous. However, this type of alteration has not been observed in this laboratory in guinea pigs not infected with equine encephalomyelitis.

The presence of leucocytes or red blood cells free in the ventricles or infiltrating the choroid plexuses, is a variable finding. In general, the longer the disease has progressed, the more such cells are found. Very definitely, however, the presence of inflammatory cells in the ventricles is not related to the involvement of the ependyma by an inflammatory focus. That is, probably the leucocytes do not invade the ventricular cavity from the side of the brain. One case serves as a vivid, but not the only, illustration. In an area of ependymitis a large focus of polymorphonuclear leucocytes extends from the white matter, through the ependyma and the spindle cells, to abut on the ventricular cavity. But the ventricle is free of leucocytes. In many instances, the presence of cells in the ventricular fluid appears to be roughly proportional to the degree of inflammatory involvement of the choroid plexuses, but this relationship is not absolute.

Myelin.—Myelin is not primarily affected by the disease process.

The guinea pig does not show spontaneous demyelination, nor have plaques of demyelination ever been produced experimentally as has been done, for example, with the cat, the dog, or the monkey. In equine encephalomyelitis, appropriate stains show that a considerable degree of inflammation may be present and yet the myelin sheaths are unaltered. However, areas do occur where the parenchyma is destroyed, and in such foci all tissue elements are severely injured. Myelinated fibers are no exception, and in areas of necrosis are seen to be lacking. The axis cylinders are more resistant to destruction than are the myelin sheaths, and in areas where the latter have completely disappeared, fragments of axis cylinders are still demonstrable by silver impregnations.

These areas of necrosis, however, are not productive of neutral fat. The inflammatory and *abbau* cells never include gitter cells, and specific fat stains have consistently failed to demonstrate free neutral fat in lesions. To eliminate the possibility of technical error, sections of canine disseminated encephalomyelitis were stained at the same time with affected guinea pig sections. The former readily showed free fat, the latter did not. This peculiarity of reaction of the guinea pig brain to the virus is more striking when mechanical injury is considered. For example, in intracerebral inoculation, the needle track may show numerous gitter cells. Thermal injury, performed in certain experiments to be published elsewhere, also showed an abundance of the compound granular corpuscles. But such cells have invariably been lacking in areas of necrosis produced by the virus, even though myelin has been destroyed.

One unusual type of myelin damage has been observed. In Fig. 11 the fiber tracts of the callosal radiation bordering the ventricle show a high degree of rarefaction, appearing to involve the axis cylinders as well as the myelin. The fibers have disappeared without a trace. There is a moderate diffuse infiltration with polymorphonuclear leucocytes and lymphocytes, but here too gitter cells are not present. The fibers of the centrum ovale, however, are quite normal. This type of damage is quite rare, but has been seen following both intracerebral and peripheral inoculation.

DISCUSSION

Throughout the foregoing description, emphasis has been placed on two different types of lesions. In the one the inflammatory reaction (that is, the exudation of leucocytes, and the alteration and proliferation in the mesodermal vascular adventitia) is prominent. In the early examples, neuronal damage may be entirely non-existent; or, later, neurones may show varying degrees of damage more or less proportional to the degree of exudation. In the second type, neuronal degeneration or destruction is marked, while the inflammatory changes are insignificant or even entirely lacking. These

two types, however, are not mutually exclusive, yet for the sake of convenience the reactions may be called inflammatory and degenerative, respectively.

Following peripheral inoculation of the virus, in the overwhelming majority of lesions, the inflammatory reaction appears in scattered cerebral foci. In the earliest detectable cases, alterations are visible only in the lumen and adventitia of the blood vessels, with the nerve cells completely intact. From this beginning, graded transitions to the most profound inflammatory lesions may easily be traced. The degenerative reaction, that is, destruction with relatively insignificant amount of exudation, sometimes occurs but is not prominent. Following intracerebral inoculation, on the other hand, there is profound nerve cell destruction, while the inflammatory changes are, with few exceptions, slight.

Hurst (2), in his study of the histopathology of this disease, stated that the lesions were substantially the same in the two routes of infection, although they were more intense in the animals inoculated peripherally. This he attributed to the longer duration of the disease in such cases, with longer time for the changes to develop. Similarly, Syverton (3), when the present data were presented before the American Association of Pathologists and Bacteriologists, raised the question whether the difference between the two types might not be explained on the time factor.

This is clearly not the case. Animals killed 58 hours after subcutaneous injection in the pad may show inflammatory changes of extreme severity. Here the virus must first have multiplied locally in the leg, then been transferred to the brain. The virus has thus been present in the brain for much less than the 58 hours. On the other hand, direct injection into the brain, with sacrifice 67 to 78 hours later, has given much more time for the virus to act. Yet the lesions are quite different. Since the time factor cannot be invoked as the explanation of the difference, we are forced to conclude that we are dealing with two types of reactions that are perhaps fundamentally distinct.

There exists a certain amount of confusion around the problem of necrosis of nerve cells, and the question of whether neuronal damage precedes or follows the inflammatory reaction. The simple generalization of a picture of events,

stated and implied in many writings of Hurst and coworkers, might be given as follows: Neurotropic viruses, as obligatory intracellular parasites, invade the neurone (usually by way of the axis cylinder) and by primary and direct attack cause its destruction. If the animal be killed at a suitable stage (4) the ruling picture will be acute necrosis of ganglion cells. But the animal may survive longer than this initial moment, "and then, as in all lesions accompanied by necrosis of tissue, polymorphonuclear leucocytes migrate swiftly into the damaged areas" (5). "While, therefore, degeneration in nerve cells and perhaps in glial cells is clearly primary, and at an early stage in some localities of the nervous system may occur in the absence of any trace of inflammation in the mesodermal structure, this latter phenomenon is an integral part of the later histological picture." It is in accordance with this view that the distinction between intracerebral and peripheral inoculation is minimized, the former being taken to represent the acute stage, before inflammation has developed, wherein necrosis of cells is the ruling picture, while the latter represents the late stage.

With the foregoing viewpoint we find it impossible to agree entirely, for the following reasons.

1. It is erroneous to maintain that exudation of polymorphonuclear leucocytes is the natural reaction to necrosis of nerve cells *per se*. In cases of non-infectious etiology, as well as in certain cases of non-virus infections, many instances may be found of selective neuronal necrosis without exudation of polymorphonuclear leucocytes. A few of the most striking examples are: destruction of the hippocampal pyramidal cells, especially in the Sommer's sector; selective destruction of the Purkinje cells of the cerebellar cortex in many toxic conditions (6, 7); and the *Verödungsherden* of the cerebral cortex, chiefly ischemic in origin. Glial reaction may be present, and even perivascular round cell accumulation, but not polymorphonuclear leucocytes.

2. Recent research (8–10) has strengthened the older idea that polymorphonuclear leucocyte exudation represents a specific chemotactic response to specific agents. These agents are probably multiple, and either exogenous or endogenous in nature. It is conceivable, though unlikely, that virus particles are in themselves intrinsically chemotactic. It is more likely that virus action on the tissue produces intermediate products which are chemotactic and responsible for the exudation.

3. The earliest demonstrable changes are the mesodermal reactions described previously, which are antecedent to the passage of leucocytes into the tissue. Later, abundant examples are available that polymorphonuclear leucocytes invade the parenchyma in the absence of neuronal necrosis.

4. The presence of frank inflammatory changes with little or no neuronal loss, and the presence of extensive neuronal destruction with little or no inflammatory reaction, indicate that two distinct factors (or chains of factors) are operative. The time factor, once it is actually measured in hours, is seen to be irrelevant.

The contention that degeneration (neuronal destruction) is primary, while the inflammatory reaction is secondary, is not in conformity with the present data. Rather would these two elements seem to be coequal in importance, sometimes

acting together, sometimes separately. With peripheral inoculation the inflammatory component predominates; with intracerebral inoculation, the degenerative.

It is here maintained that the factors responsible for the inflammatory and degenerative components are qualitatively and not merely quantitatively different. Thus, it is claimed that on a basis of simply "more" or "less," Fig. 1 could never develop from Fig. 10, nor Figs. 6 and 7 from 13 and 14, respectively. Qualitative differences must enter in.

In a tentative fashion the following hypothesis may be suggested. To a certain extent the destruction of nerve cells is a non-specific effect of the disease. That is, although the virus is the inciting agent, it is not the primary and direct causative agent. Rather, its effect is produced by certain intermediate steps which may be common to a wide variety of conditions producing a similar end-result. For example, the symmetrical necrosis of the Ammon's horn (Fig. 9), although in this instance caused by the virus, is quite similar to the picture found in epilepsy, general paresis, or insulin shock. Perhaps some common factor mediates the destruction, this common factor being in turn produced by a variety of conditions. Likewise, the destruction of the Purkinje cells (Fig. 14) is similar to that found in other non-virus diseases. Where many widely dissimilar conditions produce a more or less common effect, none of the conditions in question is the primary and direct cause of the result. More likely, all these dissimilar conditions have some factor in common, acting on tissue which is especially vulnerable.

There is no intention of setting up here a rigid scheme of dichotomy. The factors at work in disease are multiple and overlapping. It is necessary to point out, however, that destruction of tissue is not a unitary concept. If elements are destroyed by one mode, one type of histological reaction will result; another mode will produce a different picture. The presence or absence of polymorphonuclear leucocytes, of neutral fat, of gitter cells, or lymphocytes, all indicate differences in the mode of action, referable, probably, to different intermediate steps. Just how any virus works is at present unknown. In part it may act primarily on nerve cells; in large part, however, the primary action is interstitial. Or, in part, some of its apparent action on nerve cells may be secondary and indirect, due to special vulnerability of these elements to intermediary factors resulting from virus activity.

It is impossible, in the present state of our knowledge, to disentangle these threads without too many groundless hypotheses. It must suffice, at present, to lodge a protest against any view of pathogenesis which is too schematic or oversimplified.

SUMMARY

The action of the virus of equine encephalomyelitis in the guinea pig brain has been studied, and various histological changes have been described in detail. After peripheral inoculation (as in the pad) the earliest detectable pathologic change in the nervous system is the accumulation of leucocytes within the lumen of blood vessels, and the proliferation of the vascular adventitia. This precedes the appearance of any significant perivascular cuffing, and may or may not be accompanied by a few polymorphonuclear leucocytes in the tissue.

The typical lesion is a fairly well circumscribed focus of polymorphonuclear leucocytes accompanying the blood vessel changes described above. The leucocytes may be numerous or sparse, and may or may not be accompanied by neuronal destruction.

In early cases, before the onset of symptoms, such circumscribed lesions appear in small number irregularly scattered through the gray matter. The neo- and olfactory cortices are the principal sites of predilection, although basal ganglia, thalamus, cerebellum, and lower olfactory centers may also be involved. The hippocampus is much less affected than other parts of the brain.

A rough distinction is made between inflammatory and degenerative lesions, a distinction which depends on the relationship between the neuronal destruction and the exudative changes in any given site. These two types are described, and their significance is discussed.

After intracerebral inoculation, the inflammatory changes are much less marked than after peripheral inoculation. This is due not to insufficient time for the development of lesions but to a different type of pathological process.

Following intracerebral inoculation, there is primary destruction of neurones, involving especially the hippocampus, and also large areas of the neo-cortex. This change, similar to ischemic necrosis, is regarded in part as a non-specific reaction of especially vulnerable tissue.

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EXPLANATION OF PLATES

All figures are unretouched photographs of sections 15μ thick.

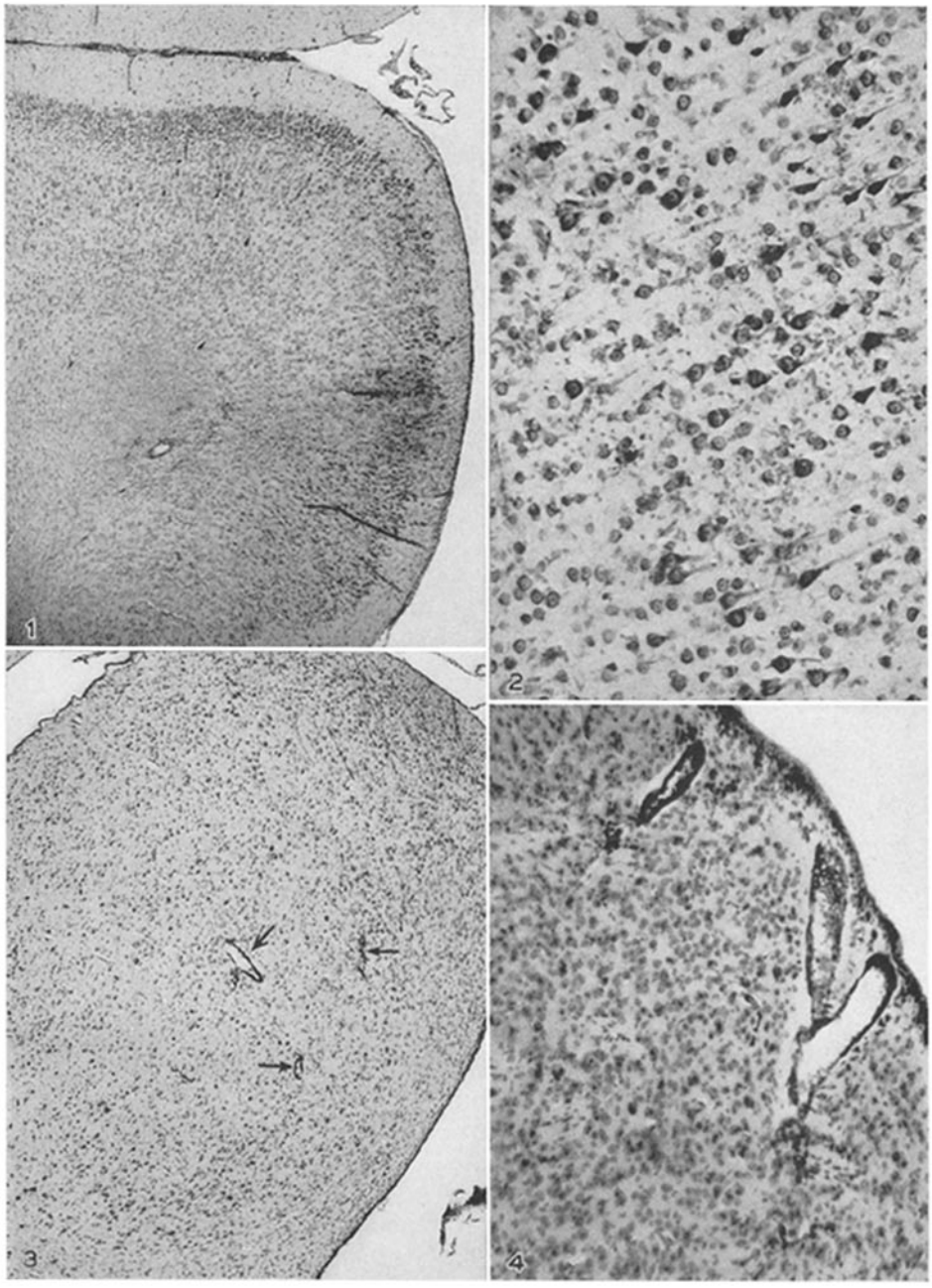
PLATE 32

FIG. 1. Typical encephalitic focus, with prominence of blood vessels, extensive leucocytic infiltration, and mild overlying meningeal reaction. The focus extends radially the width of the cortex. Pad inoculation. Thionin. × 32.

FIG. 2. Higher power photograph of a focus similar to Fig. 1. The abundant polymorphonuclear leucocytes stain rather weakly. The neuronal architecture is not disturbed, and the intact neurones are readily appreciated. Nuclei are perfectly normal. In the upper part of the figure are a few dark staining neurones, which, although hyperchromatic, are normal for this cortical area. Pad inoculation. Thionin. × 127.5.

FIG. 3. A field from the inferior colliculus, showing the minimal degree of detectable pathology. The three blood vessels indicated by arrows show proliferation of the vascular adventitia, but no round cell cuffing. Within the lumina, numerous white blood cells are adhering to the endothelium. There is no nerve cell destruction. Pad inoculation. Thionin. × 32.

FIG. 4. A similar lesion, from the caudate nucleus, bordering the ependyma. The nerve cells are entirely intact. Pad inoculation. Thionin. × 82.5.



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(King: Pathology of equine encephalomyelitis)

PLATE 33

FIG. 5. Another cortical focus, similar to Fig. 2. This section is a little more deeply stained. Neurones morphologically normal and with intact nuclei are present among the very dense collections of leucocytes. The arrow points to a single neurone which is being invaded by polymorphonuclear leucocytes. The dark staining cell above it is perfectly normal, for the nucleus can be clearly seen under the microscope by slightly altering the plane of focus. Pad inoculation. Thionin. × 151.

FIG. 6. Hippocampus, showing a circumscribed focus of polymorphonuclear leucocytes. The pyramidal cells are normal. Pad inoculation. Thionin. × 115.

FIG. 7. A similar type of lesion, but located in the molecular layer of the cerebellar cortex. There is moderate meningeal reaction in the sulcus overlying the lesion. Pad inoculation. Thionin. × 39.

FIG. 8. Early focus in the cerebral cortex. There is an increase in glial cells in radial streaks, indicated by arrows. No polymorphonuclear leucocytes are present in the tissue. There is a moderate degree of adventitial hypertrophy in the blood vessels. The neurones are normal. The dark streak at the surface of the brain represents not meningitis, but a thin layer of India ink applied to the tissue before section to indicate the right side of the brain. Pad inoculation. Thionin. × 50.

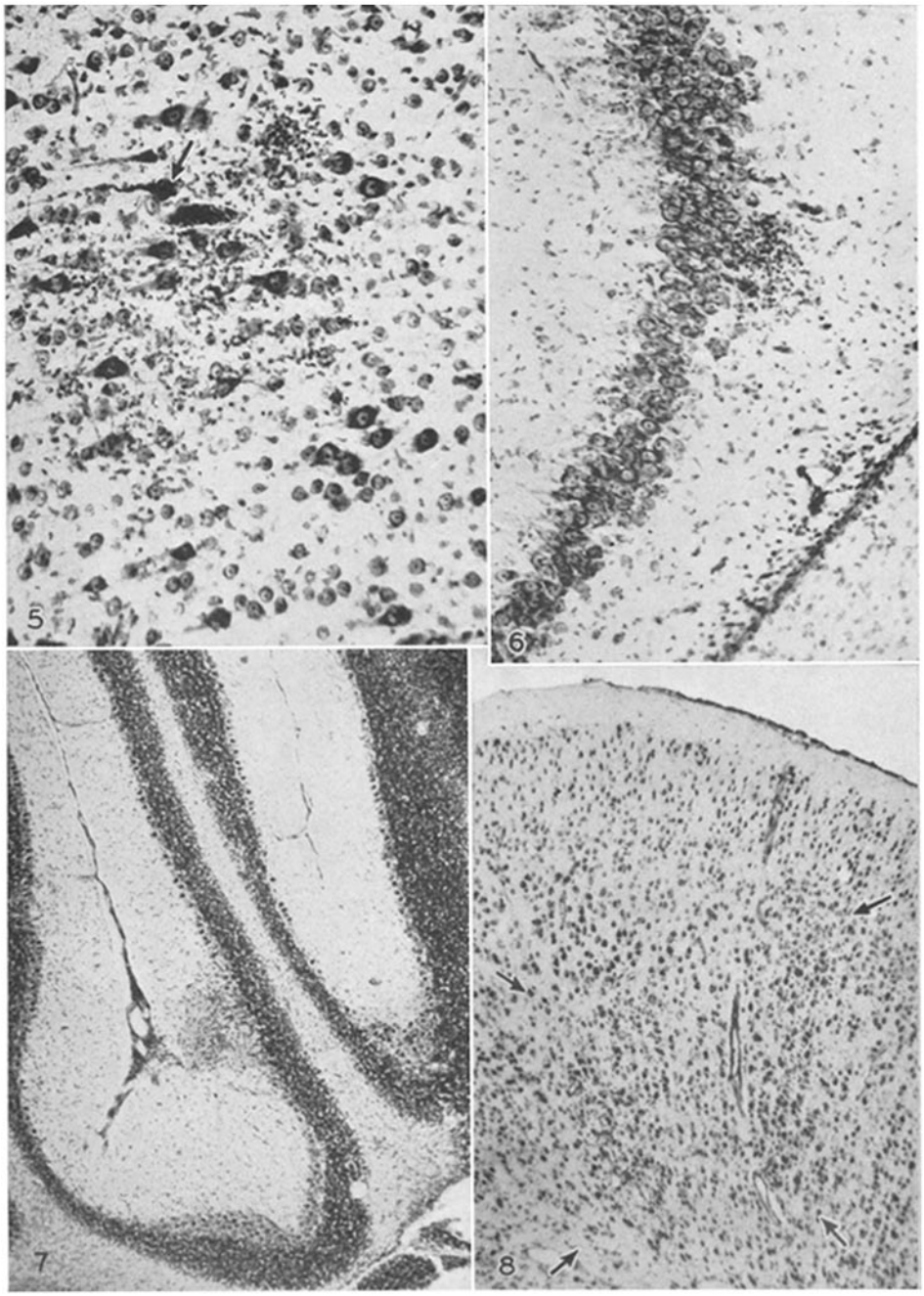

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PLATE 34

FIG. 9. Intracerebral inoculation. There is bilaterally symmetrical necrosis of the pyramidal cells of the hippocampus, indicated by arrows. There are a few polymorphonuclear leucocytes free in the neocortex, and a very minute degree of perivascular cuffing, neither of which is appreciable at this magnification. The contrast with Fig. 1 is readily apparent. Hematoxylin-eosin. × 7.

FIG. 10. Intracerebral inoculation. Massive areas of neuronal disappearance in the neocortex. The retrosplenial cortex, at the upper right, is normal. Between the arrows is an area where the 2nd and 4th layers of the cortex are better preserved than the 3rd, 5th, and 6th. At the left, however, all layers are equally affected. The dark line on the surface again represents India ink, and not meningitis. The absence of significant inflammatory changes should be noted. Thionin. × 15.

FIG. 11. Rarefaction of myelinated fibers of the callosal radiation. The caudate nucleus is in the upper right. The ependyma bordering the injured fibers is destroyed. In the rarefied area there is an increase in cells, but no gitter cells or compound granular corpuscles are present. Intracerebral inoculation. Hematoxylin-eosin. × 95.

FIG. 12. Isolated glial nodule in the neocortex, without inflammatory changes. Cortical architecture undisturbed. Pad inoculation. Thionin. × 54.

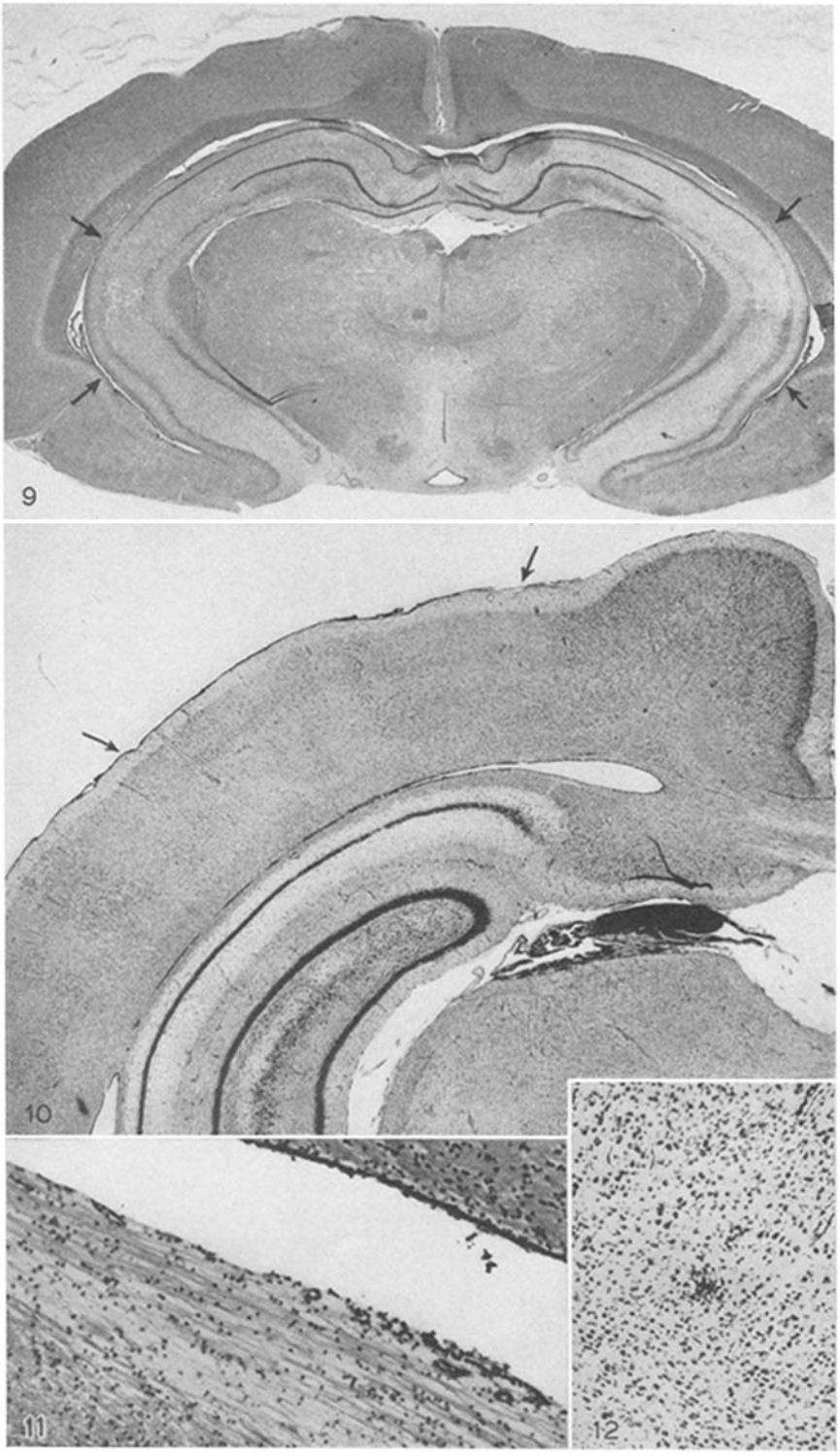

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PLATE 35

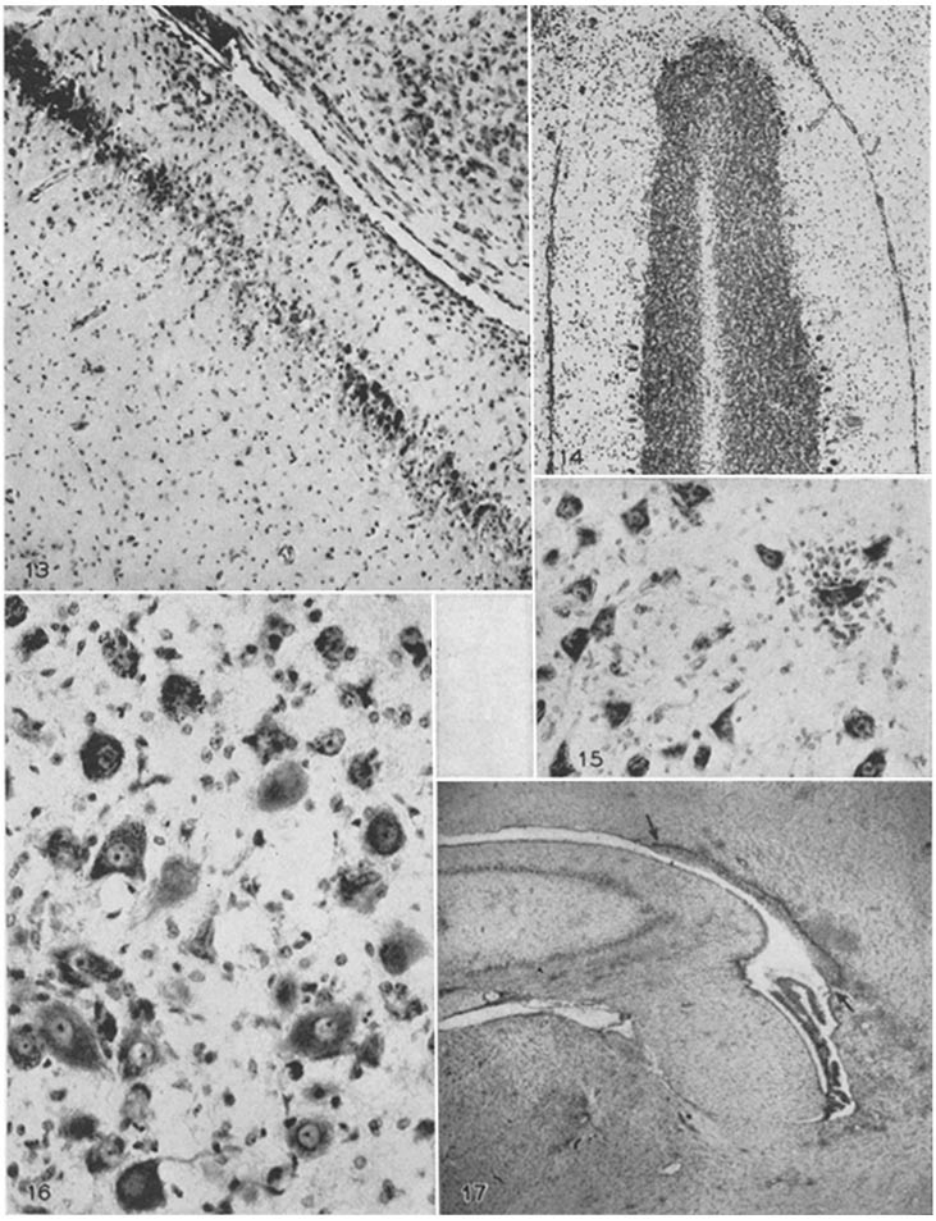
FIG. 13. Hippocampal necrosis following intracerebral inoculation. This picture should be compared with Fig. 6, showing the reaction after pad inoculation. Thionin. × 87.

FIG. 14. Cerebellar cortex, illustrating the destruction of Purkinje cells in the absence of significant inflammatory reaction. This should be compared with Fig. 7. Pad inoculation. Phloxin-methylene blue. × 40.

FIG. 15. Clustering of glial cells around a morphologically intact neurone from the facial nucleus. Pad inoculation. Phloxin-methylene blue. × 157.

FIG. 16. Severe degeneration of neurones in the red nucleus. Intracerebral inoculation. Thionin. × 227.

FIG. 17. Ependymitis of the lateral ventricle. In addition, intense inflammatory changes are present throughout. Pad inoculation. Phloxin-methylene blue. × 64.



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