

ENCEPHALOPATHY FOLLOWING INJECTIONS OF BONE MARROW EXTRACT

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PLATES 30 TO 32

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In lymph nodes affected by Hodgkin's disease, Gordon (1) demonstrated an agent which proved fatal to rabbits after intracerebral injection. A characteristic syndrome was produced in these animals, with incoordination, ataxia, and paralysis, usually leading to death. A large number of confirmatory studies, reviewed by McNaught (2), showed that most, although not all, cases of Hodgkin's disease could elicit this reaction. At first, interest centered around the problems of whether this procedure could be used as a biological test for Hodgkin's disease, and whether a virus was involved. Friedemann and Elkeles (3), and Friedemann (4), however, showed that a similar syndrome could be produced by injections of normal human bone marrow or leucocytes. The induced disease was not transmissible in series (4, 5) and was probably due to an enzyme. Evidence was brought forward by Turner, Jackson, and Parker (6), and by McNaught (2) to show that the reaction depended on the presence within the inoculum of sufficient eosinophils, approximately 2000 per cubic millimeter of suspension being necessary to induce the effect. The reaction was considered as restricted to primate eosinophils, while other blood cell types were without influence. Recently, however, Edward (7) has shown that mouse and chicken tissue could give the reaction, as well as pure suspensions of human neutrophil leucocytes.

The clinical picture caused by intracerebral injections into guinea pigs of monkey bone marrow extract is accompanied by definite brain pathology. This was first described in a previous communication (5). The cardinal reaction was a destruction of Purkinje cells of the cerebellum, together with chromatolysis of ganglion cells elsewhere, and a meningitis. Encephalitis, in the sense of parenchymatous inflammation of the brain, did not occur. These essential findings have been confirmed for the similar active agent derived from Hodgkin's disease lymph nodes, or human bone marrow or

leucocytes (2, 7). Various studies have been made on the properties of the active principle. These will not be discussed at the present time.

In the course of further researches on this pathogenic agent, certain additional pathologic observations were made which form the subject of the present communication. Relationships between the pathology induced by this non-infectious, non-transmissible agent and that caused by certain filtrable viruses also deserve mention. Studies on the intrinsic nature of the active principle are still in progress.

Material and Methods

For the most part experiments were performed with monkey bone marrow, which was prepared in accordance with the procedure already described (5). The steps involve extraction with acetone, suspension of the residue in glycerin, and precipitation from glycerin by absolute alcohol and ether. The precipitate was suspended in saline and injected into guinea pigs. Injections were usually intracerebral but in a few instances were made into the cisterna magna. In the course of numerous experiments histological observations were made on about 125 guinea pigs, with confirmatory observations in a few rabbits. In some instances the entire brain was sectioned serially, but for the most part sections through the cerebellum and brain stem were used, frequently with additional sections through other parts of the neuraxis.

Lymph nodes from one case of Hodgkin's disease and leucocytic cream from the blood of one patient with periarteritis nodosa were also tested for comparisons with monkey bone marrow.

Symptomatology

Guinea pigs injected with potent material show a characteristic and quite constant syndrome.

The earliest symptoms, appearing from 1 to 4 days after inoculation, are weakness and slight incoordination, shown best when the animal is placed on its back. Resumption of the upright position is attended with obvious faulty control over the hind legs, and, after three or four such trials, with dyspnea and diminution of exertion. In more advanced stages, the weakness and incoordination increase, always involving the hind legs more than the fore legs. A sick animal placed on its side at first makes vigorous efforts to right itself but apparently cannot execute purposeful movements correctly. In trying to regain the upright position the guinea pig may overexert and, having become upright, will fall over on the opposite side. After three or four tests the animal will lie on its side, pawing at the air with all four legs but unable to get up.

In time, which may vary from 4 to 16 days, the symptoms increase in severity; the animal remains down flat on its side, performing ineffectual rhythmic leg movements. Clonic convulsions have not been observed, but at times tonic spasms of an athetoid type may occur. Spontaneous rhythmic chewing motions, without salivation, may also be present. The animal may remain in this state for several days before death supervenes. Death usually occurs between the 5th and 12th day, but may take place as early as 2 or as late as 20 days after inoculation.

Partial or complete recovery may occur. Early symptoms of weakness may be present for 3 to 4 days, followed by complete regression. Such an animal may then be clinically indistinguishable from normal. Or a moderate degree of weakness and ataxia may persist unchanged for several weeks. True paralysis is extremely rare. In such unusual instances, the hind legs may be totally paralyzed and dragged passively in locomotion. Such an animal when anesthetized would not in the attendant struggles show any power of movement in the hind legs. Other animals with severe weakness on clinical observation invariably showed unsuspected strength in the hind legs in the struggles during anesthesia.

Pathology

The above symptomatology is associated with characteristic cerebellar damage with invariable constancy. Although the loss of Purkinje cells has already been described (5), many additional features are worthy of note.

Apart from the nervous system findings, the only complicating pathology is pneumonia, found in about a third of the animals allowed to go on until death. The pneumonic involvement is generally very slight in amount and is undoubtedly related to the final period when the animal lies prostrate for several days.

Blood Vessel Changes.—An unusual change in blood vessels at the surface of the brain is observed in about half the cases. The fundamental change is a hyalinization of the wall, beginning immediately underneath the endothelium. The hyaline layer stains bright red with eosin, and also stains red with the Mallory acid fuchsin-aniline blue stain. The elastic tissue laminae are not entirely destroyed. The Weigert elastic tissue stain (Fig. 3) shows that a much thinned, sometimes fragmented, elastic membrane persists, even in the presence of considerable hyaline. On the other hand, the Verhoeff elastic tissue stain sometimes shows a reaction of elacin in the hyaline layer. In the hyaline there may be nuclear fragments, embedded as in a matrix.

A blood vessel may be affected in its entire cross section or in only part of its wall (Fig. 2). Or, a branch vessel may be affected while the parent stem remains normal.

Different types of this reaction may occur (Figs. 1 and 2). A thin hyaline layer may be observed between an intact endothelium and a normal appearing muscularis. Or, there may be intense endothelial proliferation leading to virtual blockade of the lumen. In later stages the entire wall of the blood vessel becomes hyalinized, with more or less reaction of the endothelium. Accompanying the hyalinization there is generally a vigorous inflammatory reaction involving the adventitia. Occasional leucocytes penetrate the hyaline layer.

The blood vessels over the entire base of the brain are especially involved by the process when it occurs. To a lesser extent the vessels on the surface of the cerebellum may be affected. Involvement of vessels within the parenchyma, even when just entering or leaving, has never been observed. Nor has this change been seen in any organ but the brain.

Complete occlusion of the blood vessels by the proliferative process is very rare and then occurs only in small arterioles. In the large vessels, even with much endothelial proliferation, red blood cells are present in the patent lumen. Furthermore, in spite of the severe necrosis of the vascular walls, rupture or hemorrhage has never been seen.

In reference to the negative findings it must be remembered that an entire blood vessel need not be involved. Vascular disturbance may be present in other vessels which do not appear in the sections examined. From the large amount of material available it can be said with confidence that the above changes occur in a large percentage of injected animals, but not in all. There is thus no necessary relationship between vascular hyalinization and other pathological findings.

Purkinje Cells and Inclusion Bodies.—Although the general features of Purkinje cell loss have already been described, new observations have been made concerning chiefly the nuclear alterations which are the first sign of disturbance.

The normal nucleus may be briefly described. Within the nuclear membrane, and embedded in the oxyphilic nucleoplasm network, the nucleolus consists of a central core with two or three attached accessory bodies. With the Mann stain, the central core is red, the accessory bodies blue. With a metachromatic stain such as thionin or toluidine blue, the central core is light blue or lavender, the satellite bodies a dark blue. The oxyphilic network stains a light blue. With hematoxylin and eosin, the accessory bodies stain a deep blue, while the central body appears sometimes pinkish, sometimes a pale greenish blue. The Giemsa stain shows the central body as lilac or slightly pink, in contrast to the pure deep blue of the accessory bodies. With phloxin-methylene blue, there is generally no color differentiation, so that the entire nucleolar complex is a deep blue, in contrast to the pinkish strands of the nucleoplasm. A normal nucleolus is illustrated in Fig. 5. The color contrast between central and accessory bodies is distinct. Such contrast may be brought out with any of the above stains, although phloxin-methylene blue is least satisfactory for this purpose. The preparation illustrated was stained with toluidine blue, which gives a good differentiation for black and white reproduction. There are slight variations in this typical structure. Sometimes the entire nucleolar complex may exist in duplicate; sometimes there are additional oxyphilic or basophilic masses embedded in the oxyphilic network. With phloxin-methylene blue, inclusion bodies in the nucleus stain red, in contrast to the nucleolus. With toluidine blue the inclusion bodies are similar in tone to the central body of the nucleolus, and unmistakably different from basophilic accessory bodies.

After injection of a bone marrow extract the first change occurs in the nucleus, where nucleoplasm tends to disappear and the nuclear contents are clumped together in the form of numerous different sized conglomerate bodies, largely basophilic, but sometimes brilliantly acidophilic. A low power view of an early change is shown in Fig. 6. These changes are readily observable with any of the stains mentioned above. A phloxin-methylene blue preparation is illustrated because this stain is considered standard for the demonstration of inclusion bodies. It will be observed that the cytoplasm is essentially normal, and that there is a complete absence of any interstitial or tissue reaction. Abnormal acidophilic bodies are present but are not very evident because of the low magnification and the photographic filter used.

An example of inclusion bodies is shown in Fig. 7. The arrows indicate the acidophilic bodies which are brilliantly red. They range up to about 3 microns in diameter and always have sharp, precise contours. Halos are not observed. The acidophilic bodies are always in conjunction with one or more basophilic bodies, equally round and with sharp margins. In Fig. 7, the cytoplasm is somewhat chromatolytic and shows poor photographic contrast from the remaining tissue, but nevertheless the cell is only mildly damaged. When the cell becomes necrotic, nucleus and cytoplasm each becomes homo-

geneous, the latter brilliantly acidophilic, the former generally basophilic. Usually the entire cell eventually disappears, leaving an empty tissue space.

The damage is at first solely to the Purkinje cells. Silver stains for axis cylinders show that around the empty spaces from which cells have disappeared the basket and the climbing fibers still persist. In Fig. 4 arrows point to two empty baskets from which the Purkinje cells have disappeared. Of interest is the alteration of the *boutons terminaux* or synaptic endings. In Fig. 4 are visible the club-like formations and exaggerated balls and loops characteristic of altered synaptic endings. These are present around the persisting cell shown and in the empty baskets as well. A similar hypertrophy of synaptic endings has been described in rabies encephalitis. At later stages the basket, climbing, and parallel fibers disappear as the tissue damage becomes more intense.

The distribution of the Purkinje cell damage is primarily around the periphery of the cerebellum. This is best shown in mid-sagittal sections where the cells all around the periphery show injury while those in the depths of the sulci are intact except in severe cases where they too suffer. This is shown in Fig. 11 where, however, loss of granule cells occurs in addition to Purkinje cell loss.

In the later stages there is considerable glial proliferation, which will be considered under another heading. Mention here may be made of certain changes in the ectodermal glia in the Purkinje cell layer. The nuclei of these cells undergo hydropic change wherein the center of the nucleus becomes clear and margination of chromatin occurs. Very frequently within such glial cells there are well marked acidophilic inclusions (Fig. 9), similar in appearance to those in neurones, but smaller. Changes in the nuclei of glial cells generally occur a few days later than the nuclear change of the Purkinje cells.

Granule Cells.—For the most part the granule cells of the cerebellum are intact. Occasionally, however, there may be short stretches of acute necrosis wherein the nuclei are reduced to pycnotic masses but with a fairly sharp line of demarcation from normal tissue. There is no leucocytic and usually only a slight glial reaction in such areas. These regions of granule cell loss are frequently, although not always, in relation to partial softening, considered below.

Fig. 11 shows in sagittal section an unusually severe instance of granule cell loss. The damage is restricted to the periphery of the cerebellum, and the injured areas are clearly marked off from the normal granular layer. In some areas there is an intense reaction, an example of which is shown under higher power in Fig. 13. But in other areas there is the same degree of granule cell loss although quite without reaction. The damage to the granular layer is invariably associated with Purkinje cell loss, as if the destructive process found the Purkinje cells most susceptible and then, in occasional instances, went on to attack the granule cells.

Inclusion bodies have not been seen in injured granule cells, nor have they ever been reported in these cells in any other condition.

Hippocampal Necrosis.—In confirming the original pathologic studies (5), Edward (7) made the additional observation that necrosis of hippocampal cells may also occur. The present series furnishes abundant evidence of this change, which is illustrated in Fig. 10. In about 25 per cent of the cases examined there was a symmetrical loss of pyramidal cells, while the granule cells of the fascia dentata were always intact.

Since the inoculum was injected intracerebrally, the needle track was frequently in relation to the hippocampus. The damage from the injection is always unilateral and consists of a small localized area of necrosis involving all tissue elements indiscriminately,

with hemorrhage, gitter cell mobilization, and vascular response. The hippocampal necrosis, on the other hand, selectively involves the pyramidal cells and is always bilateral, usually with exquisite symmetry. The reaction to the selective cell loss is totally different from that of the injection site.

Reaction and Repair.—In the present studies the reaction to injury is almost exclusively glial. In the cerebellum a typical response to the Purkinje cell loss is illustrated in Fig. 8. The Purkinje cells have entirely disappeared, although occasional hypertrophied glial cells appear under low power to resemble neurones. The microglia have been mobilized in the form of rod cells, oriented perpendicular to the surface of the tissue. A few cells show short thick processes, but there are no gitter cells and no transitional forms. The blood vessels show no reaction, and there is an absence of inflammatory cells, either perivascular or free in the tissue.

The time factor has been adequately controlled, since cases of from 2 to 40 days after injection have been studied. The presence of white blood cells is so rare as to be entirely negligible. Karyorrhectic and fragmented nuclei are occasionally seen in severe early cases. Some of these may have stemmed from leucocytes, but intact leucocytes are found only very rarely. It cannot be denied that leucocytes may be present in the tissue, but their occurrence is so rare as to be entirely negligible. Even a rare leucocyte does not affect the conclusion that the reaction is non-inflammatory, in contrast to what is found, for example, in virus encephalitis.

In a small percentage of cases there is a quite different reaction, which may be designated as partial softening. In the low power Fig. 11 is seen extensive neuronal damage with scattered areas of very intense cellular reaction. Such an area is shown under higher power in Fig. 13. There is an increase of small blood vessels, with an intense hypertrophy and proliferation of endothelial and adventitial cells. The latter round off and become phagocytes free in the tissue. There is a great increase of mononuclear cells in the tissue, all tending to become compound granular corpuscles, with irregular foamy cytoplasm, much vacuolated. In spite of the mesenchymal response, reticulin nets free in the tissue could not be demonstrated by specific stains.

This type of reaction, quite characteristic of incomplete softenings in the nervous system, is usually in relation to a damaged blood vessel on the surface of the cerebellum. Frequently a hyalinized, partially occluded blood vessel is present in the meninges between two lobules, while the above reaction radiates from the blood vessel as a center, involving the molecular layers of contiguous lobules. However, such injured blood vessels cannot always be demonstrated. The reaction may occur where surface blood vessels appear normal. In such cases it is possible that vascular damage has occurred some distance from the particular section examined. It must be mentioned that damaged blood vessels may be seen on the surface of the cerebellum without provoking this reaction of softening. The reaction tends to occur in rather fulminating cases.

A somewhat different reaction is tissue vacuolation affecting the molecular layer. There is a spongy rarefaction of the tissue, with a true *état criblé*, but with little or no cell proliferation or cellular reaction. On occasions this change seems to be related to blood vessel damage, but much less constantly than the softening reaction described above.

It is of great significance that although the hyalinization of blood vessels may occur over the entire base of the brain, the reactions of softening and of the *état criblé* are observed only in the cerebellum. There are no random disseminated areas of softening as

are found after experimental embolism, for example. Random areas of tissue damage might reasonably be expected but do not occur.

In the hippocampus the destruction of pyramidal cells is occasionally attended by distinct, though mild, inflammatory signs. A few polymorphonuclear leucocytes may rarely be detected among the necrotic cell debris, and a mild perivascular reaction has been observed in addition to the usual glial response. In one instance the hippocampus showed, in bilaterally symmetrical fashion, some proliferation of blood vessels and of glia comparable to Fig. 13 but much milder.

Meningeal reaction has been described previously. In occasional instances the blood vessels dipping into the brain from the surface may show some slight cuffing with mononuclear cells, rarely with a few polymorphonuclear leucocytes. This is a meningo-encephalitis, clearly secondary to meningeal involvement, and is in no sense indicative of a primary brain inflammation or encephalitis.

In one case an ependymitis of the lateral ventricle was observed similar to what is frequently found in equine encephalomyelitis (8). The ependymal epithelium proliferates into whorls and acini, and there is a growth of spindle-shaped cells at the ventricular surface.

In the previous paper mention was made of widespread chromatolysis of ganglion cells throughout the brain stem. In the present large series this change, though abundantly present, was not so constant as previously observed. The change is readily reversible; for in late, partially recovered stages, examined 25 to 40 days after injection, restorative changes in the ganglion cells are evident. The Nissl substance may not be altogether normal, but may show irregular clumping, comparable to the restitution of chromatolytic neurones after axonal section.

In the white matter of the cerebellar folia there may be seen in the more severe instances a vigorous glial activity indicative of secondary degeneration. This would clearly be the result of the loss of cerebellar cortical tissue. Less frequently there is intense glial reaction in the pons and adjacent white matter. Fig. 12 shows such a region. The microglia are rounded up to form compound granular cells. At the same time pontine nuclei can be seen with well preserved ganglion cells, although some show chromatolysis. Such a reaction is clearly distinct from the softening and general tissue destruction of Fig. 13. In the pons, the tissue as a whole is well preserved, although there is a selective myelin damage. Such a reaction can in no way be ascribed to vascular occlusion.

A somewhat similar reaction is occasionally seen in the spinal cord. In animals with severe paresis of the hind legs, a correspondingly severe damage to the anterior horn cells might be expected. Surprisingly, in such animals, the anterior horn cells are in general very well preserved. Rarely a few neurones may be lost, amidst neuronophagic rosettes. Neurones sometimes show a persisting acute swelling. But the nerve cell damage seems hardly enough to account for the symptoms. In such cases there is generally seen a peripheral rim of myelin damage, shown by glial activity. The microglia are progressively altered, but gitter cells are not so numerous as in Fig. 12.

The pathogenesis of this spinal cord change is obscure. Secondary changes in the spinocerebellar tracts might be expected, but the reaction is not restricted to these tracts. Instead, it is diffuse around the periphery of the cord.

Cisternal Inoculation.—Injection of the active agent into the cisterna magna resulted

in the characteristic syndrome. Control inoculations into the muscles of the neck are totally without effect. Only a small amount of inoculum can be introduced into the cistern, and the disease course is less acute, but the characteristic pathology occurs. The most significant fact is that the direct contact of inoculum and surface of the brain evokes no local destruction. The contact does not result in surface damage to the tissue. There is no special meningeal reaction, and the blood vessel changes may or may not be present. The injury is selectively to the Purkinje cells, as after intracerebral injection. The molecular layer of the cerebellum shows no special effect of the immediate contact. In the previous paper a reaction was described in one instance which was attributed to destructive surface action of the agent. This interpretation is now seen to be erroneous. No essential difference exists in regard to cerebellar pathology if the agent is placed within the brain tissue of the hemispheres or placed in the spinal fluid around the cerebellum.

DISCUSSION

Pathogenesis.—The rôle of the injured blood vessels in the total pathologic picture is not altogether clear. It does not seem probable to the author that the vascular damage is the cause of the Purkinje cell loss. In the present condition the damage to blood vessels is inconstant in incidence, distribution, and intensity. Although the site of predilection is the base of the brain, damaged vessels have been seen on the convexity of the hemispheres, in the intrahemispheric fissure, and on the surface of the cerebellum. At the base, the affected blood vessels may be in relation to the medulla, pons, midbrain, or hypothalamus. Damaged vessels within the parenchyma have not been observed. Sometimes the damage is minimal, consisting only of a thin hyaline membrane beneath the endothelium, but with the lumen unaltered. At other times the injury is severe.

In contrast to the inconstancy of the vascular injury, the characteristic Purkinje cell loss is absolutely constant and invariably present. As a further contrast, the parenchymal damage is selective and localized, the blood vessel changes are scattered and diffuse. Although damaged blood vessels are sometimes seen on the surface of the cerebellum, similarly injured vessels elsewhere are not accompanied by signs of tissue alterations. Were the fundamental pathology vascular in origin, parenchymal damage elsewhere in the brain, especially in relation to the great vessels at the base, should reasonably be expected. This does not occur.

In the cerebellum mention has been made of small areas of softening which differ markedly from the essential and characteristic changes. The areas of softening are generally wedge-shaped, from the surface down, and involve the molecular layer primarily, usually in immediate relation to a severely injured blood vessel. The histological appearance is identical with that found in softenings in other conditions, and is beyond reasonable

doubt attributable to vascular occlusion. In contrast, the essential injury to the Purkinje cells affects this layer primarily, leaving the molecular layer abutting the surface quite undamaged by any primary destructive process. The type of damage and the type of response are scarcely compatible with what is known of the effects of vascular occlusion.

The distribution of Purkinje cell injury in early or mild instances is of considerable interest and can be appreciated only in sagittal sections. It is then seen that only that part of a folium which is directly in contact and parallel with the free surface of the subarachnoid space is affected. The sides of the folium, facing the buried fissures, and perpendicular to the free surface, are invariably unaffected in mild cases. A reference to Fig. 11, which was photographed from a severe case, shows nevertheless how the free surface is injured, while the surfaces buried in the convolitional fissures are relatively spared. This is even more striking in mild cases and in those not complicated by the infrequent granule cell loss illustrated in Fig. 11. The presence of injury at the free surface primarily, with extension into the fissures only in severe cases, suggests that the responsible active agent penetrates the tissue from the subarachnoid space. The fissures, of course, also communicate with the subarachnoid space. But any agent which gains access to the spinal fluid space spreads primarily over the convexity and acts there first and most intensely.

As shown by experiments wherein the active noxious agent was introduced directly into the cisterna magna, there is no contact reaction between the agent and the brain. Surface contact between agent and nerve tissue does not cause any special *in situ* response. This is strikingly similar to the effects of subarachnoid injections of equine encephalomyelitis virus mentioned elsewhere. The bone marrow agent apparently penetrates the free surface without leaving a trace and exerts its activity on the Purkinje cell layer well beneath the surface. At the same time there is a selective vulnerability of these elements, for tissue elements beneath other surfaces in contact with spinal fluid, such as the basal cisterns or the ventricles or the convexity of the cerebrum, do not show any reaction of significance.

As a tentative hypothesis it may be suggested that the active principle contained in monkey bone marrow when introduced into the cranial cavity enters the cerebrospinal fluid which is the agent of transport. From the spinal fluid the active principle penetrates the cerebellum from the free surface and exerts its activity on the Purkinje cell layer beneath the surface. A specific vulnerability of these cells must be postulated, since the characteristic reaction does not appear in other cells, such as those of the neocortex which, however, are specially sensitive to other types of injury.

The active principle may also damage surface blood vessels with which it comes in contact, but the vascular changes and the Purkinje cell damage are essentially independent reactions. Changes in the blood vessels are responsible for occasional small areas of softening, but this is a purely incidental reaction.

The necrosis of the pyramidal cells of the hippocampus appears to be a reaction of a different category. This inconstant damage to the hippocampus, so similar to that found in equine encephalomyelitis in the guinea pig (8), or in such varied conditions as cyanide poisoning (9), narcosis (10), or insulin shock (11), is probably a secondary reaction dependent on intermediate steps of which we are ignorant.

Inclusion Bodies.—The term inclusion body is purely morphological and descriptive, implying nothing about the causative agent. Such eosinophilic bodies within the nucleus have been described in a wide variety of virus diseases, as well as in naturally occurring diseases of presumably non-virus origin. In addition they are observed in reactions to certain inocula and even in supposedly normal tissues. The significance of these inclusions is not known. The whole subject has been admirably reviewed by Findlay (12).

In the present instance two facts stand out. In the first place, these inclusions, especially those in the neurones, are definitely a stage in the necrobiosis of the cell. The Purkinje cells which exhibit such inclusions are precisely those which later become necrotic. Secondly, from the morphological aspect, the inclusions are quite indistinguishable from those which have been observed and described especially in equine encephalomyelitis (13) and poliomyelitis (14). In both of these latter diseases the neurones so affected show other signs of injury or damage. At the same time the nuclear changes antedate the vigorous inflammatory reaction so characteristic of these virus encephalitides. In these diseases the occurrence of nuclear alterations before inflammation, has been considered an argument for the primacy of virus attack on the cell as the first stage in pathogenesis.

In the disorder caused by bone marrow injection, the neurones are clearly the first to be affected. Although the noxious agent is probably a protein, there is no evidence of multiplication. The morphological parallelism with the above named virus diseases suggests a parallelism in mode of action. At the present moment the hypothesis that seems most reasonable for the action of bone marrow extract is an interference with cellular oxidation, accomplished not through gross interference with blood supply, but through

chemical action in the immediate environment of the cell. An interference with respiratory ferments, as suggested by Meyer (10) in another connection, is conceivable. Regardless of the validity of this speculation it is worth pointing out that in the nervous system the sequence of intranuclear inclusions regularly followed by cell necrosis has hitherto been observed only in some forms of virus disease. The present example of a similar sequence produced by a non-viral agent is the first of its kind.

SUMMARY

Monkey bone marrow extract when injected intracerebrally into guinea pigs or rabbits produces a distinctive encephalopathy. The Purkinje cells are severely affected, especially those at the periphery of the cerebellum. Nuclear alterations first appear, with well marked intranuclear acidophilic inclusion bodies. Similar inclusions appear at a little later stage in the ectodermal glia. The affected cells later become necrotic and usually disappear. The reaction is essentially glial and non-inflammatory, and hence is called encephalopathy rather than encephalitis.

A hyaline necrosis of cerebral blood vessels, especially at the base of the brain, is described. Small areas of softening may appear in the cerebellum as a result, but this is considered a secondary process, independent of the Purkinje cell loss. There may also inconstantly be found a loss of cerebellar granule cells and a selective necrosis of the hippocampal pyramidal cells. Secondary and reparative reactions are described.

Similar changes are produced by extracts of lymph nodes from Hodgkin's disease, and by leucocytic cream of human blood.

A tentative explanation of the pathogenesis is suggested, and similarities to certain virus diseases are pointed out.

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EXPLANATION OF PLATES

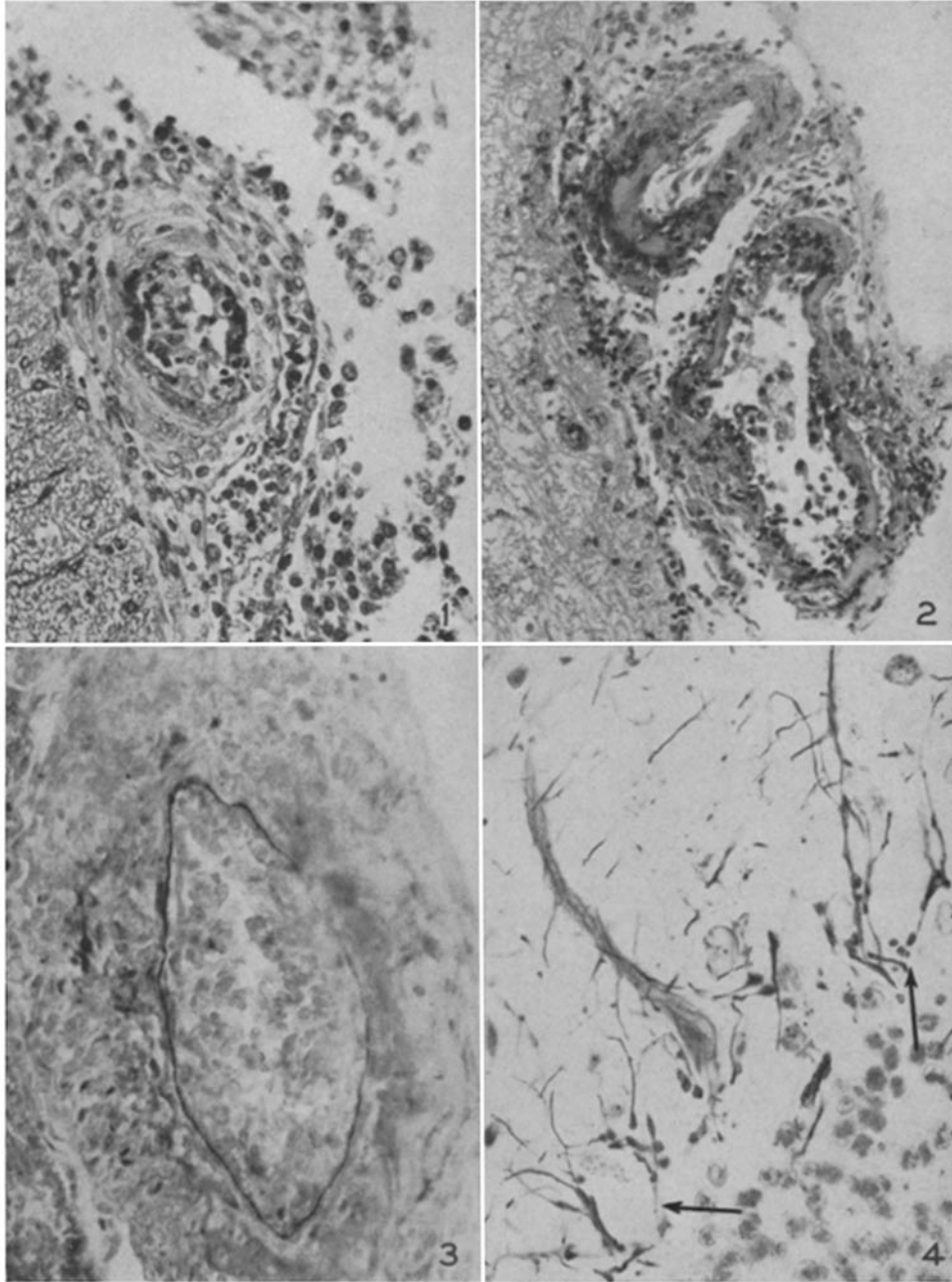
PLATE 30

FIG. 1. Blood vessel at base of brain showing the strongly eosinophilic hyaline layer (black in the photograph) under the endothelium. The endothelium has proliferated and partly occluded the lumen. The muscularis is intact, but there is a vigorous inflammatory reaction in the adventitia and meninges. Hematoxylin and eosin. $\times 266$.

FIG. 2. Two blood vessels at the base of the brain, with advanced hyaline changes involving the entire thickness of the vascular wall. In the upper blood vessel the change affects only half the circumference, the upper half being essentially normal. Hematoxylin and eosin. $\times 340$.

FIG. 3. Persisting elastic tissue lamina, in spite of advanced hyaline changes. The red blood cells in the lumen are not clearly marked off from the proliferated endothelium. Weigert's elastic tissue stain. $\times 181$.

FIG. 4. Cerebellar cortex, stained for neurofibrils. Two empty baskets are indicated by arrows. From them the Purkinje cells have disappeared. One severely injured Purkinje cell remains, in the center. Particularly to be noted are the club, ball, and loop formations, characteristic of degenerating *boutons terminaux* or synaptic endings. Bodian stain. $\times 545$.



Photographed by J. A. Carlile

(King: Encephalopathy after bone marrow extract injections)

PLATE 31

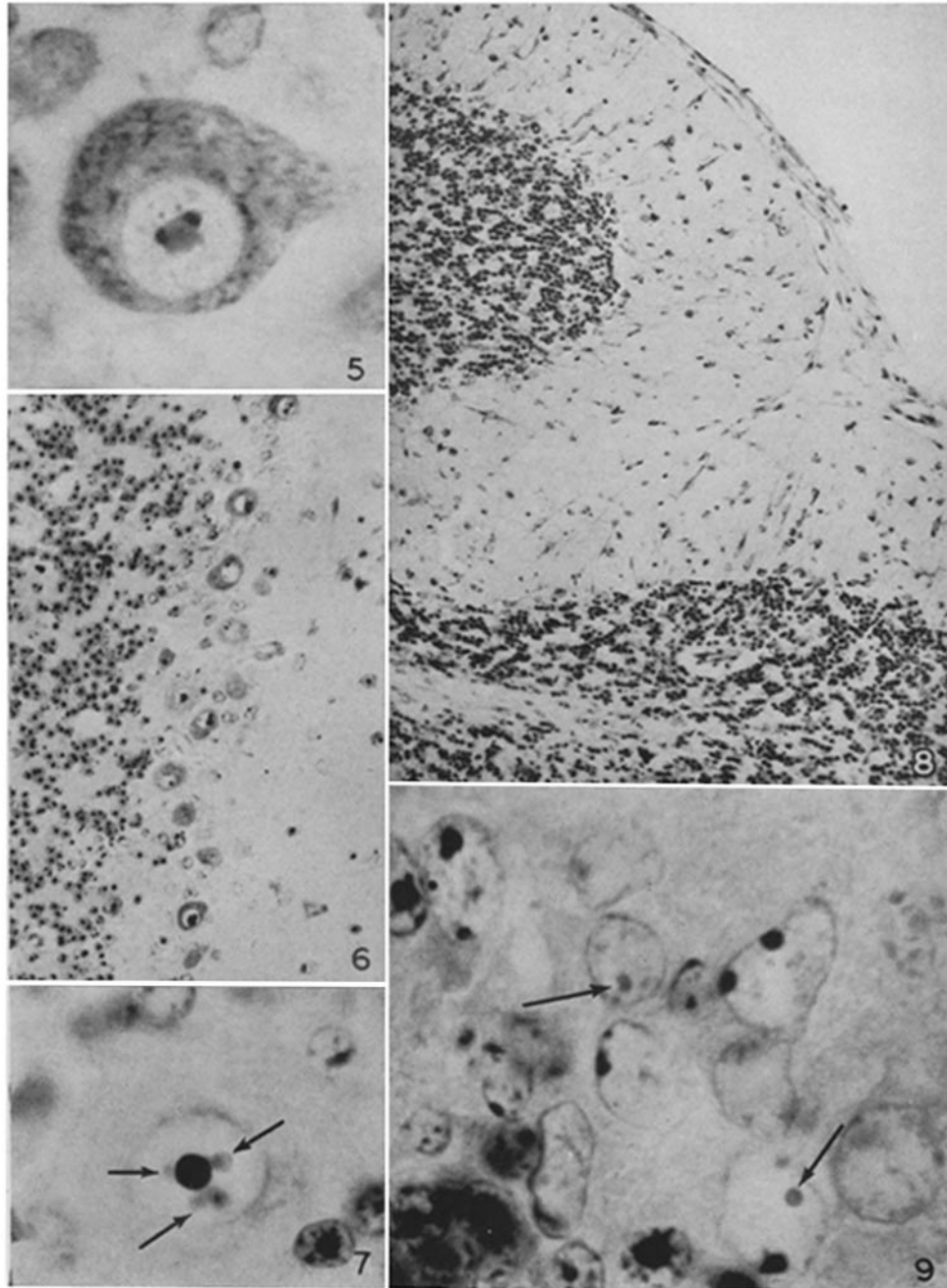
FIG. 5. Normal Purkinje cell, showing the constitution of the nucleolar complex. The central core is relatively acidophilic, the accessory bodies strongly basophilic. Consult text. Toluidine blue. $\times 1583$.

FIG. 6. Early changes in the Purkinje cell layer, consisting exclusively of marked nuclear alterations. Phloxin-methylene blue. $\times 247$.

FIG. 7. Early change in affected Purkinje cell. Brilliantly acidophilic intranuclear inclusions are designated by arrows. Phloxin-methylene blue. $\times 1405$.

FIG. 8. Characteristically injured cerebellar cortex. All Purkinje cells have entirely disappeared. There is a vigorous glial reaction consisting chiefly of microglia many of which are changed into typical rod cells. There is no trace of inflammatory reaction. Toluidine blue. $\times 127$.

FIG. 9. Small but strongly acidophilic intranuclear inclusions, indicated by arrows, in ectodermal glial cells in the Purkinje cell layer. The nuclei show considerable hydropic change. Such inclusions are not found in microglia. At the lower left are a few granule cells. Phloxin-methylene blue. $\times 1725$.



Photographed by J. A. Carlile

(King: Encephalopathy after bone marrow extract injections)

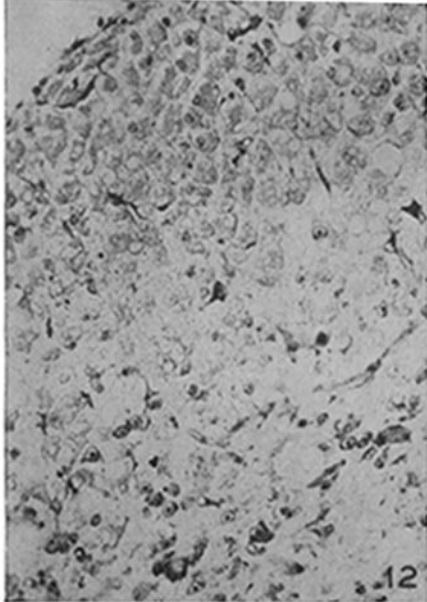
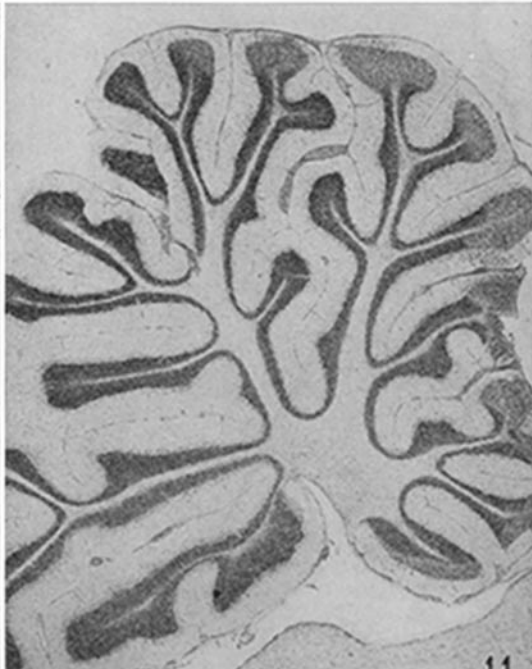
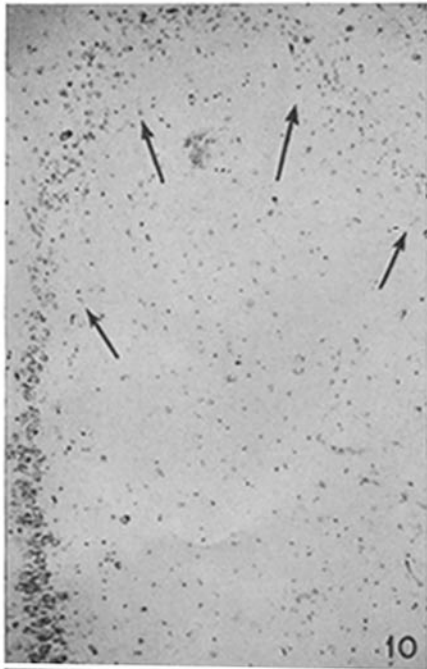
PLATE 32

FIG. 10. Pyramidal cell layer of the hippocampus. The portion of the cell band indicated by arrows is necrotic. Note the absence of reaction. Toluidine blue. $\times 71$.

FIG. 11. Sagittal section of cerebellum. This case is somewhat unusual in showing extensive granule cell destruction in addition to the invariable Purkinje cell loss. To be noted are the localization of injury around the periphery, especially at the free margins of the lobules; the scattered areas of intense reaction, one of which is shown in Fig. 13 under higher power; and the persistence of Purkinje cells in the deeper portions of the lobules, especially on the left side of the figure. Toluidine blue. $\times 12$.

FIG. 12. Area at the margin of the pons, showing intense glial activity with formation of compound granule cells. There is, however, no reaction of softening (as in Fig. 13) and the neurones at the lower part are preserved although with some signs of chromatolysis. Similar reactions are sometimes found in the spinal cord. Toluidine blue. $\times 192$.

FIG. 13. Area of softening, from the same slide as Fig. 11, but under higher magnification. Apart from the characteristic vascular and glial response, the necrosis of the granule cells should be noted. Normal granule cells on the left. Toluidine blue. $\times 100$.



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