DEMYELINATING DISEASES WITH SPECIAL EMPHASIS ON THE
METACHROMATIC TYPE (POSTNATAL AND EARLY INFANTILE)†

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It is the intent of the symposium committee that this presentation be limited to a discussion of diffuse sclerosis, the most common group of demyelinating diseases in infancy. Pathologically, diffuse sclerosis is characterized by the presence of broad zones of degeneration affecting major portions of the central white matter of the cerebrum. In gross sections these areas may show a gray discoloration, may be retracted beneath the adjacent surfaces, and, when the process is severe, the tissues may become liquified and cysts result. In other instances, the degeneration is not evident on gross examination. The process tends to involve the deeper portions of the white matter often sparing the arcuate fibers. The preservation of the arcuate fibers, frequently considered characteristic of diffuse sclerosis, may be observed in other processes as well, such as edema. The cerebral cortex and the basal ganglia are not affected. The cerebellar white matter and the tissues of the brain stem may be involved in some cases. In the brain stem the lesions may take the form of sharply circumscribed plaques like those seen in multiple sclerosis.

Microscopically, the most striking change is the loss of the myelin sheaths in the affected parts, and this may be complete. This myelin loss usually affects broad uniform areas. In some instances there is a concentric lamellation whereby rings of preserved myelin alternate with rings of myelin destruction (1), while in other cases one observes a relative preservation of the myelin in perivascular sites (2, 3). In contrast to the profound myelin loss, the axons are affected to a lesser degree so that preserved axons, often in considerable numbers, may be demonstrated in the zones of complete demyelinization. In lesions which extend into the gray matter, such as those in the brain stem, the myelin is affected but the neurons are not. The relative preservation of axons and of neurons is characteristic of the other forms of demyelinating disease as well. The glial reaction in this process is quite variable. One may observe a proliferation of phagocytic cells, mainly of microglial origin, and this is often very marked, but may be minimal or absent. The large, often multinucleated “epithelioid” cells seen in some cases are probably of microglial origin as well. The astrocytes also tend to enlarge and multiply to a variable degree. In some instances the reactive astrocytes are numerous and assume bizarre and giant forms, so that a relationship to neoplastic states has been suggested (4). These reactive astrocytes do not infiltrate into the cortex and the resemblance to a neoplasm is probably fortuitous. The oligodendroglia vary in their reactions, at times appearing unaffected, more often

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† Presented at the Meeting of the American Association of Neuropathologists, Atlantic City, N. J., on June 16, 1958.
decreasing in number or disappearing completely (5). The oligodendroglia are susceptible to injury of almost any sort and their loss in any degenerated area would be anticipated. In some instances a loss of oligodendroglia has been described in areas in which demyelinization had not as yet occurred (6, 7).

All of these elements which comprise the tissue reactions in diffuse sclerosis are variable so that very heterogenous pathologic patterns may result. One may find that the altered tissues are markedly rarefied, that large numbers of phagocytic cells predominate, that large “epithelioid” cells may be present, that the tissues are almost totally destroyed and replaced by a cyst, or that a spongy state prevails. Such greatly varying lesions may be found in different portions of the degenerated areas within the same case, as well as in different cases within this group. Since the etiology and pathogenesis are obscure, these heterogenous tissue changes must serve as the pathological basis for determining which cases constitute this entity, if it be an entity, and for their subclassification. The resulting difficulties are readily apparent in the lack of agreement concerning these aspects (4, 8–12).

In recent years there has been considerable interest in the possibility that at least some cases of diffuse sclerosis might represent the effect of a specific abnormality of lipid metabolism. In a sense almost any pathologic change may be assumed to represent the morphologic aspect of a process with chemical and physical aspects as well. In this group to which the term “leuкоencephalopathy” is often applied, the more specific metabolic character of the process is indicated by the presence in the tissues of abnormal materials not generally noted in other circumstances. Some of these materials are metachromatic.

Metachromatic materials are those which are stained by selected dyes a color distinctly different from that of the dye itself. With toluidine blue, perhaps the dye most commonly employed for this purpose, most materials are stained blue, but the metachromatic materials appear violet or pink. In some mesenchymal tissues metachromatic deposits indicate the presence of mucopolysaccharides, and these retain their metachromasia when stained sections are passed through alcohol and xylol (13). In central nervous tissues the metachromasia exhibited when stained paraffin sections are viewed in aqueous media is generally lost when these sections are passed through alcohol and xylol. The metachromasia in frozen sections is sometimes lost, sometimes preserved in this circumstance. These materials are presumably not mucopolysaccharides. Perhaps the only chemical information gained by demonstrating their metachromasia is that they are composed of large molecules with many anionic groups (13).

The metachromatic deposits noted in diffuse sclerosis generally appear as clusters of granules up to 3 microns in diameter, the clusters ranging up to 50 microns in diameter. These may lie free in the tissues (fig. 1) or be so concentrated in a sharply circumscribed discrete mass that they seem to lie within phagocytic cells (fig. 2). There is much evidence to suggest that this metachromatic material is not a single substance, but a mixture of related substances differing in many histochemical properties. When one compares the staining and solubility properties of the materials in various cases in which these properties
Fig. 1. Diffuse sclerosis. The deposits of metachromatic materials, basophilic in this case, lie free in the tissues. Hematoxylin and eosin stain; X 200.

Fig. 2. Diffuse sclerosis. The deposits of metachromatic materials, eosinophilic in this case, tend to be concentrated as if lying within phagocytic cells. Hematoxylin and eosin stain; X 600.
were adequately studied, it almost appears that the materials are not identical in any two cases (5). For example the materials have been described as basophilic (fig. 1) or eosinophilic (fig. 2) with hematoxylin and eosin stains, as blue or tan with phosphotungstic acid hematoxylin stains, as red or blue with the Mallory stains, and as unstained, pink or rose with the Sudan stains. The material may not be homogeneous even within the same case. In one group of brains some of the metachromatic materials stained blue and some red with the Mallory method (6, 7). In another instance (5) very little of the abnormal materials were stained by the usual myelin stains, much by hematoxylin and toluidine blue, and additional materials were disclosed by Sudan black, a lipid soluble dye, the acid fast method, which generally stains lipoproteins, and the phosphomolybdic acid technique (14) thought to be specific for choline groups such as may be derived from sphingomyelin. In this case (5) other histochemical studies revealed positive staining with the periodic acid Schiff method (13), with the acid hematein technique (15), and the alloxan-Schiff procedure (16). These suggest respectively the presence of carbohydrate groups, as in the cerebrosides, the presence of phospholipids, as in sphingomyelin and phosphatidyl serine, and a protein moiety. These histochemical findings are like those expected if these materials were related to myelin, but do not prove such relationship. Some histochemical findings in other cases have been summarized (5), and these are variable. Biochemical studies (17, 18) have also demonstrated variable chemical alterations in this group of diseases. It must not be assumed that all metachromatic materials are related to the demyelinating diseases. One of the earliest references to the presence of metachromatic materials in the brain dealt with a patient with schizophrenia, and this observation was offered as a finding specific for this disease (19). Corpora amylacea are metachromatic (20), and so are the calcific deposits noted in an oligodendroglioma (21). Some reports have described the presence of metachromatic materials in neurons (6, 7), in other organs, such as the liver, and kidney (6, 7, 22, 23), and in urinary sediments (24), in cases in which metachromatic materials were present in the white matter. Although these materials may have been formed in the brain and transported to the viscera by the blood stream (23), it has also been suggested that a generalized metabolic defect like that in the lipoidoses may be operative (22). If these metachromatic materials were all the same, these concepts would have considerable merit. It should be evident that the common property of metachromasia is not sufficient in itself to establish this identity. On the other hand, the occurrence of diffuse degenerative changes with demyelination in the white matter in cases of infantile amaurotic family idiocy does suggest a link between diffuse sclerosis and a known metabolic disorder of the lipoidosis type. The demyelination in this disease has been attributed to the axonal loss secondary to the neuronal destruction (25, 26), but some investigators are not convinced of this (27, 28). We have just had an opportunity of studying one such case, and found that axons were demonstrable in the demyelinated white matter in appreciable numbers. These presumably arose from the cortical neurons, many of which were intact, although containing an abnormal lipid in their
cytoplasm. This abnormal lipid showed a disturbing tendency to be stained by myelin stains (Mahon) (fig. 3) and some of it was metachromatic. Further studies are contemplated.

Most reports concerning these metachromatic materials and their relationship to myelin assume that the metachromatic materials represent intermediate stages in myelin destruction (4, 8, 10, 11, 12). Brain and Greenfield (6, 7), Norman (22), and possibly others have suggested that the basic defect lies in faulty myelin formation, rendering the myelin unduly susceptible to destruction. Some years ago, I was privileged to present before this society, data concerning a case of diffuse sclerosis which tended to support this thesis (5). In this instance, clinical signs of the cerebral disorder were evident shortly after the 6 weeks premature birth of an infant, and these persisted until death 6 weeks later. A severe degeneration limited to the cerebral white matter was evident, at the margins of which moderate quantities of metachromatic materials were noted. It is known that at term very little myelin is present in the central white matter of the newborn infant and even less may be anticipated 6 weeks earlier (29), when this disease had become manifest. It is unlikely that the metachromatic materials represent an intermediate stage in myelin destruction in this circumstance in which little if any myelin was present to be destroyed.
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It is pertinent to note that some of the lipids of which myelin is thought to be composed are present in the central white matter at birth in higher concentrations than in the gray (30), although myelin itself may not be demonstrable. It is possible that these represent intermediate stages in myelin formation which have not as yet achieved that physico-chemical structure which permits the usual myelin staining. It may also be noted that radioisotope studies have demonstrated that the lipids of the nervous system are not static, but are continuously being synthesized in situ at a replacement rate of approximately 10 per cent a week in the rat (31). It would follow that even the disappearance of myelin from sites in which it had been deposited need not be interpreted as myelin destruction in an active sense. If no new myelin were being formed, myelin might disappear as a result of its normal catabolism.

SUMMARY

It is evident that the cases of diffuse sclerosis form a heterogenous group; in some of these cases metachromatic materials may be observed. These metachromatic materials are not a single substance but a group of substances with properties which vary from case to case, and even within the same case. It is possible that in some instances they represent an abnormal intermediate in myelin formation. Metachromatic materials may also be observed in the brain in circumstances not obviously related to diffuse sclerosis or demyelinization. The pathogenesis and metabolic relationships of diffuse sclerosis remain to be clarified.

REFERENCES

DISCUSSION

DR. E. P. RICHARDSON, JR.: I feel very much honored to be asked to discuss Dr. Feigin's excellent review of this subject, a topic which is provocative and of great interest to all those who have come in contact with it. We are grateful, indeed, to Dr. Feigin for his own original contributions in this field, characterized as they are by the thoughtful application of histochemical techniques along with the more usual neuropathologic methods, as well as having provided for us the sensible working classification of the leukoencephalopathies of childhood.

Dr. Feigin has most wisely pointed out to us that the phenomenon of metachromasia, which is strikingly observed in some of these cases, is not one of any specific chemical significance, and that we should not fall into the error of over-rating its importance, particularly with regard to using it as a basis for classification of a disease entity.

It, nevertheless, remains true that while the normal myelin sheaths themselves can readily be shown to be metachromatic when properly stained and mounted, and while some metachromatically-staining materials may be found within the cytoplasm of phagocytic cells in the early stages in every sort of destructive lesion in myelin, this material ordinarily is not present in any large amounts and does not by any means predominate. Instead, the majority of the material in macrophages is not metachromatic and, with such dyes as Sudan III and Oil red 0, it gives the bright red staining that is typical of neutral fats.

Since the original papers of Scholz (Ztschr. f. d. ges. Neurol. u. Psychiat., 99: 651, 1925) and Bielschowsky and Henneberg (J. Neurol. u. Psychol., 38: 131, 1928), it has been realized that there is a form of familial diffuse symmetrical demyelinative cerebral sclerosis in which the breakdown products of myelin do not stain the expected bright red color of the usual fat stains. Norman's publication (Brain, 70: 234, 1947) made us aware that an intense metachromasia of such breakdown products could be demonstrated if frozen sections were used and the material did not come into contact with fat solvents. More recently, Von Hirsch and Peiffer in Munich (Arch. f. Psychiat., 194: 88, 1955) showed that intensely metachromatically staining myelin breakdown products could be demonstrated in material from previously published cases of the disorder that Scholz, Bielschowsky and Henneberg and others described, by using frozen sections, even though metachromasia had not been demonstrated by the earlier investigators.

A number of other features of cases with these findings have been described in the past and have been so regularly encountered in our experience, direct or indirect, in seven cases, that we have been led to consider them of importance. One is that myelin at all levels, central and peripheral, may be affected, associated with the presence of considerable amounts of metachromatic material. This suggests a disorder of myelin, rather than one intrinsic to the brain as an organ.

Another prominent feature is distension of nerve cell bodies with material that stains identically to that found in the diseased white matter, giving at times an appearance of cell distorsion so great as to remind one of cases of amaurotic family idiocy. Principally affected are cells of the basal ganglia, nuclei of the cerebellum, brain stem, anterior horn, cells of the spinal cord and cells of the Clarke-Stilling column, whereas nerve cells of the cerebral and cerebellar cortex are extremely slightly, if at all, affected. Dr. David Cogan in the Howe Laboratory of Ophthalmology in Boston has recently been able to demonstrate for the first time similarly staining material in the large ganglion cells of the retina, and we have lately found it in the spinal root ganglion cells.

Another abnormality worthy of emphasis is the peculiar change in the oligodendrocytes which we have observed well in three cases and which has been remarked on by others, particularly the late Dr. Greenfield (Brain & Greenfield, Brain, 73: 291, 1950), who first called attention to it. This consists of disappearance, or a very unusual pale, blurred appearance of many of the oligodendrocytes in the white matter, even in places where the myelin, as shown in the usual stains, is relatively well preserved. Our own experience with
two recent cases indicates that this phenomenon, existing together with the other findings mentioned, need not be confined to sporadic cases with onset in infancy and death before the age of three, nor are the lesions necessarily more severe in fiber systems myelinated during the first few months, despite Dr. Greenfield's suggestion that there might be a separate entity with those characteristics.

These neuropathologic experiences have led me to believe, therefore, that one is justified in setting aside a group of cases of diffuse leukoencephalopathy, often affecting children and often familial, but at times appearing sporadically and in adults, in which there may be disease of myelin at all levels, with myelin breakdown products which stain in an unusual way including the striking display of metachromasia; in which certain nerve cells become filled with similarly staining material, and in which there may be an unusual degree of evidence of disease in oligodendrocytes in the white matter. This group of findings seems to stand up in observation after observation, despite other variations, such as whether or not mucicarmine-positive material is seen. These unusual materials, incidentally, are apparently always PAS-positive whenever this method has been performed.

As a remarkable associated finding, there is the frequently reported presence of similarly metachromatic material in kidney tubules, the nature of which is currently under active investigation by Dr. James Austin, who has been able to make the diagnosis of cases of this kind during life by examination of the urine.

I should like to show a very few lantern slides to supplement the interesting pictures Dr. Feigin has shown.

[Slide] This is a picture of cerebral white matter, showing the large amount of metachromatic breakdown products. This is the normal staining property of this dye, which is cresyl violet.

[Slide] This is a pair of anterior horn cells from the spinal cord, showing the presence, in the cytoplasm, of similarly staining material.

[Slide] This is a spinal ganglion showing in the cytoplasm of ganglion cells the presence of similar staining material, while in the interstices where the peripheral nerve fibers are running, some metachromatic material can likewise be demonstrated in the cytoplasm of phagocytes.

[Slide] This is a picture of a frozen section of the kidney, showing in convoluted tubules and in the loops of Henle the presence, in the epithelial cells, of similarly staining metachromatic material.

With regard to the biochemical background of this disorder, which is a most provocative aspect, judgment must be reserved. I get the impression that so far, histologic methods, rather than purely chemical ones, have been the most sensitive indicator for the detection of these cases. One is certainly led to the supposition that underlying this disorder is an endogenous or inborn deviation of processes involved in the synthesis or maintenance of myelin, and it may well be that the accumulation of the unusual breakdown products in the lesions represents substances, normally present in myelin, piling up in abnormal amounts, rather than a failure of the phagocytic cells, as has at times been postulated. The behavior of macrophages in an adult case of a similar leukoencephalopathy in adult life, in which damage to the white matter was artificially produced by a brain biopsy, may support this viewpoint.