References and Notes

1. I. Harary and B. Farley, *Exp. Cell Res.* **29**, 451 (1963).
2. I. Harary, A. Fujimoto, R. McCarl, B. Farley, *Fed. Proc.* **23**, 427 (1964); I. Harary, R. McCarl, B. Farley, in preparation.
3. V. Oyama and H. Eagle, *Proc. Soc. Exp. Biol. Med.* **91**, 305 (1956).
4. Progress report on Pennsylvania Agricultural Station Project No. 1188. Authorized for publication on 1 September 1965 as paper No. 3057 in the journal series of the Pennsylvania Agricultural Experiment Station.

22 October 1965

Myelin Membrane: A Molecular Abnormality

Abstract. *Myelin was isolated from cerebral white matter from a patient who had died of metachromatic leukodystrophy, and its lipid composition was analyzed. Although the lipid content was nearly normal, the myelin contained a three- to fourfold excess of cerebroside sulfate and a threefold deficiency of cerebroside compared to normal myelin. The deficiency of cerebroside and the excess of cerebroside sulfate may account for defective myelination in this disease.*

Metachromatic leukodystrophy is an inborn error of metabolism characterized by faulty myelin formation. Cerebroside sulfate accumulates in the nervous system and in the viscera (*1*). Large round metachromatically stained bodies comprised of this lipid are present within glial cells in the central nervous system and within Schwann cells in the peripheral nervous system. In addition, a marked deficiency of cerebrosides has been found in white matter (*2, 3*), especially of those cerebrosides which contain very-long-chain fatty acids (19 to 26 carbon atoms) (*3*).

The relation of the cerebroside sulfate accumulation or the cerebroside deficiency, or both, to defective myelination in metachromatic leukodystrophy (MLD) is obscure. To shed more light upon this relation, the question whether the accumulation of cerebroside sulfate and the deficiency of cerebroside are also present in myelin in this disease needs to be answered. In partial answer we now report the analyses of myelin isolated from cerebral white matter from a boy, age 6 years, who expired from late infantile MLD.

White matter was dissected from frontal and occipital lobes and myelin was isolated from the white matter (*4*). The yield of myelin (4 mg) was 1/200 of that from the brain of a normal deceased child of a similar age. Examination by light microscopy showed that the myelin fraction was comprised of elongated tubules which were doughnut shaped on cross section. Electron microscopic examination showed that the myelin preparation was comprised of smooth-surfaced membranes, layered in lamellar fashion, many of which were wound in spiral fashion like native myelin. Granular material was also seen in the myelin preparation, but this was present in small proportions compared to the membranous material.

The myelin preparation was extracted with a mixture of chloroform and methanol (2 : 1) to obtain its constituent lipids. Similar to normal myelin (*4, 5*) the MLD myelin fraction was completely soluble in this solvent. The lipids could be obtained free of nonlipid residue by evaporating the myelin extract to dryness, drying the extract for a 24 hour period, and extracting again the dried residue with the chloroform-methanol (2 : 1) mixture (*5*). With this procedure, 76 percent of the dried extract became soluble in the chloroform-methanol. The lipids obtained from MLD myelin were then analyzed by chromatography on paper impregnated with silicic acid (*6*) for a comparison with normal myelin. The MLD myelin contained an excess of cerebroside sulfate and a deficiency of cerebroside compared to normal myelin.

The amounts of individual classes of lipids in the MLD myelin extract were then determined by x-ray fluorescence spectroscopy for analysis of sulfur and phosphorus (*7*), cholesterol determination (*8*), and colorimetric analysis by the anthrone method (*9*) for the determination of total galacto-lipids (predominantly cerebroside plus cerebroside sulfate). The lipids obtained from whole white matter from the patient's brain were also studied by column chromatographic procedures (*5, 6*).

The MLD myelin contained nearly the same lipid content as normal myelin but its composition was abnormal (Table 1). There was a three- to fourfold excess of cerebroside sulfate and a threefold deficiency of cerebroside compared to normal; in fact, the myelin values and the white matter values were nearly identical. The other lipids were present in proportions that were somewhat closer to normal.

These analyses indicate that central nervous system myelin is chemically abnormal in MLD. It is not possible to state that all the myelin in the central nervous system is abnormally constituted, since it is not known what fraction of the total myelin was isolated. However, the fact that the MLD myelin we obtained had a lipid composition very close to that of the white matter from which it was isolated suggests that there was little tendency toward the preservation of a normal-myelin lipid composition in this patient. It is also not possible to state whether myelin in MLD is abnormally constituted at the time of its initial synthesis. The patient studied here was in the late stages of his disease, and it is necessary to learn more about myelin at earlier ages to decide this point.

Mention should also be made of the deficiency of long-chain sphingolipids (those containing fatty acids with more than 18 carbon atoms) in MLD myelin. It was postulated (*3*) that a deficiency of long-chain sphingolipids may lead to the formation of unstable myelin in MLD since these molecules are thought to act in the stabilization of the myelin bimolecular lipid leaflet (*10, 11*). Unfortunately, it was not possible to determine the fatty acid composition of the sphingolipids in the MLD myelin we isolated because of insufficient quantity. The fatty acid compositions of cerebroside and sphingomyelin from white matter of the present patient, but not that of cerebroside sulfate, were shifted toward short-

Table 1. Analysis of myelin and white matter from metachromatic leucodystrophy. GP, glycerophosphatides. All values except total lipid are expressed as percent of the total lipid.

Component	Normal myelin*	MLD white matter	MLD myelin
Total lipid (% of dry weight)	78–81	47.7	76.0
Cholesterol	24.4	17.8	17.0
Total phospholipids	47.6	53.3	55.0
Ethanolamine GP	16.2	19.3	
Serine GP	6.0	7.5	
Choline GP	13.3	15.7	
Sphingomyelin	5.6	4.0	
Cerebroside	19.5	6.5	6.7
Cerebroside sulfate	5.6	20.4	20.2
Ceramide	1.3	2.0	
Uncharacterized†	6.5	6.8	

* Average of four humans aged 10 months, 6 years, 9 years, and 55 years (*5*). † Includes inositol glycerophosphatides as major components and smaller proportions of free fatty acids, gangliosides, and other phosphatides.

er chain lengths, similar to the two other patients we have studied (*3*). Recent information on the fatty acid composition of MLD myelin sphingolipids from a 10-year-old patient has been obtained by Norton and co-workers (*12*) who found that cerebroside and cerebroside sulfate contained normal proportions of long-chain fatty acids, but that sphingomyelin was deficient in them. Based on this evidence, the overall deficiency of long-chain sphingolipids in MLD myelin is smaller than originally anticipated. The possibility still remains that myelination does not proceed until sufficient quantities of long-chain sphingolipids are present, and that a deficiency of these molecules in white matter in MLD results in an impairment of myelin synthesis (*3, 11*).

Equally significant, however, are the excessive proportions of cerebroside sulfate in MLD myelin. The presence of excessive proportions of this sulfated galactolipid in the myelin membrane will lead to a preponderance of electronegative sulfate groups at the outer surface of the myelin bimolecular lipid leaflet. Unless these electronegative groups are balanced by cationic groups, such as the amino groups of the structural protein of myelin, or by other cations (calcium, potassium, and other), the surface charge of the myelin lipoprotein will be more electronegative than normal. Thus, the surface charge, the ionic environment, and, consequently, the degree of hydration of the myelin lipoprotein may be abnormal in MLD. These abnormalities in charge and hydration may alter the configuration of the structural protein of myelin in MLD; lead to an increased permeability of the myelin membrane, especially for cations; and impair the packing of adjacent myelin lamellae, owing to mutual charge repulsion or excessive hydration effects, or both (*13*). These molecular defects may contribute to the pathogenesis by impairing the synthesis of the myelin membrane, in turn giving rise to a myelin deficiency; hastening the breakdown of myelin due to instabilities in its molecular makeup; and resulting in a slowing of nerve impulse conduction (*14*), due both to deficient quantities of myelin and to the presence of abnormally constituted myelin which is an inefficient insulator. The chemical abnormalities of MLD myelin may also partially explain the ultrastructural morphologic abnormalities of myelin—especially its loose packing and apparent degradation—seen on electron microscopic examination of peripheral nerves in this disease (*15*). Despite the speculative nature of these conjectures, the disclosure of a molecular abnormality of the myelin membrane in a disorder of myelination indicates that other diseases in which myelination is defective may also involve the formation of chemically abnormal myelin.

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References and Notes

1. J. H. Austin, *Neurology* **10**, 470 (1960); H. Jatzkewitz, *Z. Physiol. Chem.* **311**, 279 (1958).
2. L. Svennerholm, in *Brain Lipids and Lipoproteins and the Leucodystrophies*, J. Folch-Pi and H. Bauer, Eds. (Elsevier, New York, 1963), p. 104.
3. J. S. O'Brien, *Biochem. Biophys. Res. Commun.* 15, 484 (1964).
4. L. A. Autilio, W. T. Norton, R. D. Terry, *J. Neurochem.* **11**, 17 (1964).
5. J. S. O'Brien and E. L. Sampson, *J. Lipid Res.* **6**, 537 (1965).
6. J. S. O'Brien, D. L. Fillerup, J. F. Mead, *ibid.* **5**, 339 (1964).
7. G. Alexander, *Anal. Chem.*, in press. We thank G. Alexander, Department of Nuclear Medicine, Univ. of California, Los Angeles, for performing these analyses.
8. L. L. Abell, B. B. Levy, B. B. Brodie, F. E. Kendall, *J. Biol. Chem.* **195**, 357 (1952).
9. N. S. Radin, F. B. Lavin, J. R. Brown, *ibid.* **217**, 789 (1955).
10. F. A. Vandenheuvel, *J. Am. Oil Chem. Soc.* **40**, 455 (1963).
11. J. S. O'Brien, *Science* **147**, 1099 (1965).
12. W. T. Norton and S. Podluso, personal communication. Data presented at Intern. Neurochem. Conf., Oxford, England, 25 to 30 July 1965.
13. Similar suggestions have been made by J. Austin, in *Ultrastructure and Metabolism of the Nervous System*, S. R. Korey, A. Pope, E. Robins, Eds. (Williams and Wilkins, Baltimore, 1962), p. 200.
14. J. W. Isler, A. Bischoff, E. Esslen, *Helv. Paediat. Acta* **18**, 107 (1963).
15. H. Cravioto, J. O'Brien, B. Landing, B. Finck, unpublished electron microscopic study of peripheral nerves of the present patient's sibling; H. F. DeWebster, *J. Neuropathol. Exptl. Neurol.* **21**, 534 (1962).
16. Supported by NIH grants HE 08429 and NB 04136.

1 September 1965

Hemoglobin J_{Korat} in Thais

Abstract. *Hemoglobin J_{Korat}, a "fast" hemoglobin with an anomaly in its beta chain different from the anomalies previously reported, was the major hemoglobin component in the blood of nine subjects among 1923 Thais from northeastern Thailand. After hemoglobin E, J_{Korat} is the second most frequent of the anomalous hemoglobins among Thais.*

A survey was made in 1962 among a group of normal Thai adults from northeastern Thailand to determine the distribution of haptoglobin types (*1*) and to compare it with the distribution of anomalous hemoglobins in the same population (*2*). Among 676 subjects tested, one individual had, in addition to the normal hemoglobin A, another exhibiting the increased anodal mobility characteristic of hemoglobin J (*3, 4*).

Subsequent studies among members of the individual's family who were living near Nakhornratchsima (Korat), in Korat province, northeastern Thailand, revealed an interesting group of individuals with the following combinations of hemoglobins: E, A+E, A+J, and J+E (*5*). Pending completion of our analytical studies, which should establish the exact nature of the structural anomaly, the "fast" hemoglobin from this family has been identified provisionally as J_{Korat} (*2, 5*).

A survey currently is in progress to determine the relative frequency of occurrence of J_{Korat} in northeastern Thailand. Preliminary results of the study suggest that heterozygotes for hemoglobin J_{Korat} are by no means rare.

Blood samples have been analyzed (*6*) from 1923 Thai adults; almost all the individuals originated from northeastern Thailand, and most of them are residents of Korat province. Hemolysates made from the blood clots (*7*) were analyzed electrophoretically by the vertical starch-gel method of Smithies (*8*); the tris-EDTA-borate buffer, *p*H 9, of Aronsson and Grönwall (*9*), at the lower concentrations described by Goldberg (*10*), was used in the analysis.

In contrast to the first survey, in which just one individual among 676 exhibited A+J hemoglobins, nine individuals, or 0.47 percent, of the 1923 were heterozygous for hemoglobin J. Among these nine subjects, six had A+J hemoglobins, two had J+E, and one had J along with an unidentified "slow" hemoglobin with a mobility slightly faster than E and approximately equal to that of D. For all nine subjects, visual inspection of