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Long Chain Fatty Acid Formation: Key Step in Myelination studied in Mutant Mice

MYELIN is the most stable membrane known and the fact that it contains ten times as much long chain sphingolipid as any other known membrane structure has led to the suggestion that the long chain fatty acids (> 18 carbon atoms) in these sphingolipids are concerned in the stability of myelin[1,2]. The finding[3,4] of a myelin deficient mouse mutant whose galactolipids and sphingomyelin lack long chain fatty acids led us to investigate whether the elaboration of long chain sphingolipid molecules is a key step in myelin formation. The comparison of histological and biochemical events related to myelination in normal and myelin deficient mutant mice supports the hypothesis that in the events leading to adult myelin, the appearance of fatty acids with 24 carbon atoms follows the appearance of cerebrosides and sulphatides in myelin.

The two myelin deficient mutant mice, "Quaking" and "Jimpy"[4], manifest symptoms at the time of myelin formation, around the tenth day after birth. Although they have the same apparent phenotype, characterized by an abnormal gait, tremor and seizures, they have a different genotype; the Quaking mutation is autosomal and recessive; the Jimpy mutation is sex linked, *X* linked, implying that genes located on at least two chromosomes are involved in myelin metabolism and control. Unlike Quaking mice, Jimpy mutants do not reach adulthood; their disease is more severe and histological and biochemical data relate this to an earlier stage corresponding to myelination. Glial cells normally multiply after birth and reach maximum density just before the onset of myelination. Second, they extend processes along the axonal fibres, so forming a myelinating glia constituted of oligodendrocytes whose function is analogous to that of the Schwann cell in the peripheral nervous system. In the Jimpy mouse there is a lack of myelinating glia[5]; by contrast, the normal types of neuroglial cells are present in the Quaking mouse[6], but the glial cells may be qualitatively deficient, for vacuoles and inclusions have been demonstrated inside their cytoplasm[7].

The biochemistry of myelination is still rather obscure. Galactolipids are the only lipids characteristic of brain white matter[8]. Experiments with radioactive isotopes have shown that cerebrosides and sulphatides are synthesized at birth[9–11]; galactolipids, especially cerebrosides, are only detectable in a significant amount at the onset of myelination[12] and possibly are of key importance

in this process[9]: the other lipid components of myelin have been shown to occur in other membranes and are already present in large quantities at birth. In the Jimpy mouse, the cerebroside and sulphatide content is negligible[13], which correlates with a very early impairment in the process of myelin formation. By contrast, cerebrosides and sulphatides are formed in the Quaking mouse although they are deficient in long chain fatty acids with 24 carbon atoms.

Myelin was isolated from the Quaking mouse to study whether myelin was only deficient in quantity or whether its composition was abnormal in relation to a maturation process involving the appearance of long chain fatty acids in sphingolipid molecules. We compared Quaking adult myelin with that of apparently normal adult litter mates using the method of Norton *et al.*[14]. We also isolated by the same method myelin from 12 day old apparently normal mice. The proportions of proteolipid to lipid showed little variation between young and adult stages. The values obtained for the molar ratios of phospholipids, cholesterol and galactolipids were similar to those observed by Norton *et al.*[14] and Eng *et al.*[15] for the rat, with a molar ratio of galactolipids to phospholipids of 0·28 at 12 days and 0·64 at 3 months. Comparison of the fatty acid pattern in 12 day old myelin lipids with that of adult brain myelin lipids confirmed the increase with age of stearic and oleic acids[16]; moreover, the proportion of long chain fatty acids (with 24 carbon atoms) was 10–11 per cent of the total fatty acids in adult normal myelin (Table 1). By contrast, the fatty acids of 12 day old myelin included only small amounts of long chain fatty acids (Table 1), although cerebrosides and sulphatides were already present. In the Quaking mouse, the lipid composition and protein content were closely related to the values observed in the early days of myelination, there being a great deficiency in long chain fatty acids (Table 1).

Table 1. FATTY ACID COMPOSITION (PER CENT) OF BRAIN MYELIN LIPIDS

	Adult normal	12 day old normal	"Quaking" adult
$C_{14:0}$*	0·30	1·40	0·30
$C_{16:0}$	12·64	27·43	24·82
$C_{18:0}+C_{18:1}$	45·87	35·15	43·50
$C_{20:2}$ (?)	5·85	11·32	8·43
$C_{20:1}$	7·13	—	0·50
$C_{22:0}$	5·98	12·79	10·50
$C_{22:0}$	2·01	0·31	0·86
$C_{22:0}$ h	4·50	2·14	1·08
$C_{24:1}$	7·09	2·04	0·66
$C_{24:0}$	3·69	1·27	0·78

Gas chromatography of fatty acid methyl esters of brain myelin lipids on 10 per cent 'SE 52' column. Temperature, 225° C. Flow rate, 25 ml. nitrogen/min. The results are the average of three analyses. The normal mice correspond to apparently normal homozygote and heterozygote littermates of Quaking mice.

* Fatty acids are identified by the number of carbon atoms and number of double bonds (for example $C_{18:0}$=stearic acid); h indicates the presence of a 2-hydroxy-group.

It seems therefore that in the normal maturation process leading to adult myelin, the appearance of fatty acids with 24 carbon atoms follows the appearance of cerebrosides and sulphatides. A deficiency in long chain sphingolipid molecules as observed in the Quaking mouse may lead either to cessation of myelination or to the formation of unstable myelin[2] and may explain the loose configuration of early myelin[17]. The key step (blocked in the Quaking mutation) may be the formation of these long chain fatty acids or their association with a cerebroside precursor through the action of chain length specific enzymes. Myelin membrane proteins may also be concerned: they are known in other membranes to determine the association of lipids containing specific fatty acids[18].

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