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OCCURRENCE OF ALKANES IN BRAIN MYELIN. COMPARISON BETWEEN NORMAL AND QUAKING MOUSE

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Abstract—Alkanes, a new class of neurolipid, were found in mouse brain, the level being reduced in the Quaking mutant. These hydrocarbons are concentrated in myelin; minor amounts being found in microsomes, mitochondria and synaptosomes. The average recovery is 7.1 μg/mg in normal myelin, 2.2 in the Quaking myelin. The distribution pattern of these alkanes was determined by gas liquid chromatography and was found to differ in normal and Quaking myelin; the hydrocarbons consist mainly of *n*-alkanes ranging from C_{21} to C_{32} with even and odd aliphatic chains.

ALKANES are found in trace amounts in mammalian liver (NAGY *et al.*, 1969; SCHLUNEGGER, 1972), heart (BANDURSKI & NAGY, 1975) and together with some lipoproteins (SKIPSKI *et al.*, 1967), but they are hardly detectable in brain (NICHOLAS & BOMBAUGH, 1965). However alkanes are widely distributed in nature, as they are an important part of the wax coating of plants (KOLATTUKUDY, 1974, 1976; CASSAGNE, 1972; EGHATON & HAMILTON, 1967), insects (SOLIDAY *et al.*, 1974; JACKSON & BAKER, 1970), and even mammalian skin (MACKENNA, 1952). In yeast, alkanes are strictly located in the plasma membrane (BLANCHARDIE, 1976); we have previously demonstrated that they are synthesized from very long chain (in the C_{20}–C_{30} range) fatty acids, in yeast (BLANCHARDIE, 1976) as well as in higher plants (CASSAGNE & LESSIRE, 1974, 1975, 1976).

The myelin sheath contains a large amount of very long chain fatty acids which are diminished in the Quaking mouse (SINGH *et al.*, 1971; BAUMANN *et al.*, 1973), a mutant which bears a defect in CNS myelin formation (SIDMAN *et al.*, 1964). One aspect of this leucodystrophy is a reduction in the microsomal synthesis of very long chain fatty acids, either saturated (BOURRE *et al.*, 1973*a*, 1975) or monounsaturated (BOURRE *et al.*, 1976). However the mitochondrial synthesis is normal in the mutant, but this organelle is not involved in elaboration of myelin fatty acids (BOURRE *et al.*, 1977).

Thus brain myelin from normal and mutant mice may be used to study the possible relationship between very long chain fatty acids and alkanes, and the role of these apolar aliphatic chains in the membrane structure.

MATERIAL AND METHODS

Myelin isolation. Myelin from adult animals (15 normal and 45 mutants for one experiment) was prepared according to NORTON & PODUSLO (1973), with modifications when using mutant animals. Briefly, the entire brains were removed and homogenized with a Potter homogenizer in 19 vol (w/v) of 0.32 M-sucrose by using 10 strokes of the pestle. Eighteen millilitres of this homogenate was layered over 15 ml of 0.85 M-sucrose and centrifuged at 75,000 *g* for 30 min (Spinco L 5 65 ultracentrifuge using an SW 27 rotor). The layers of crude myelin at the interface of the two sucrose solutions were collected with a Pasteur pipette, suspended in water (to give a 3-fold dilution) and centrifuged for 15 min under the same conditions. At this step, 4 layers from Quaking brains were combined. The pellets were dispersed in water by homogenization (two strokes) and centrifuged at 12,500 *g* for 15 min in a Sorvall R C 5

TABLE 1. ALKANE CONTENT IN NORMAL AND QUAKING MUTANT MICE

	Normal	Quaking
Brain (μg/mg)	0.65 ± 0.07	0.27 ± 0.03
Dry myelin (μg/mg)	7.1 ± 0.6	2.2 ± 0.3
Brain myelin (μg/brain)	49.0	1.3
Myelin yield (mg/brain)	6.9 ± 0.5	0.58 ± 0.1

Values ± S.D. For normal myelin 5 assays were performed, 3 for Quaking myelin.

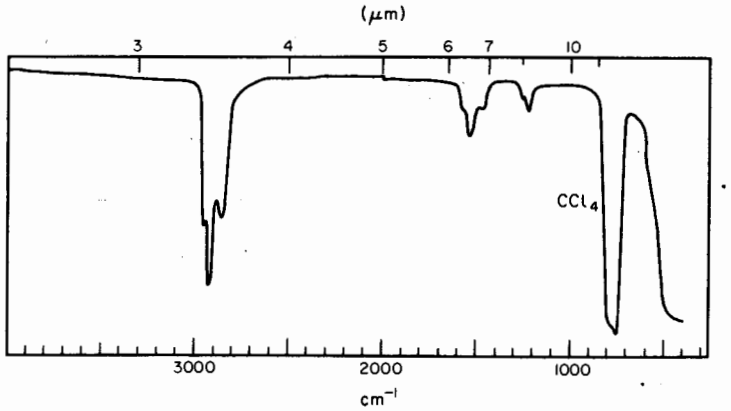

FIG. 1. IR Spectrum of brain myelin alkanes.

centrifuge. The supernatants were discarded and the loosely packed pellets again dispersed in water and re-centrifuged. The myelin pellets were homogenized in 0.32 M-sucrose (2 strokes) and the suspension layered over 0.85 M-sucrose and centrifuged for 30 min at 75,000 *g*. The pure myelin at the interface was removed with a Pasteur pipette, diluted with water and centrifuged for 15 min at 75,000 *g*. To remove most of the sucrose, myelin was washed 3 times with water and centrifuged at 15,500 *g* for 15 min. The pellets were finally lyophilized and weighed. The purity has been checked by electron microscopy and marker enzymes (BAUMANN *et al.*, 1973). Moreover the myelin from normal and mutant animals was not contaminated with microsomes, mitochondria and cytosol, as measured by marker enzymes (BOURRE *et al.*, 1973*b*), i.e. glucose-6 phosphatase, malate dehydrogenase, mono amino oxidase and glucose-6 phosphate dehydrogenase. Microsomes (BOURRE *et al.*, 1973*b*), mitochondria (PATURNEAU-JOUAS *et al.*, 1976) and synaptosomes (BOURRE *et al.*, 1977) were prepared as described.

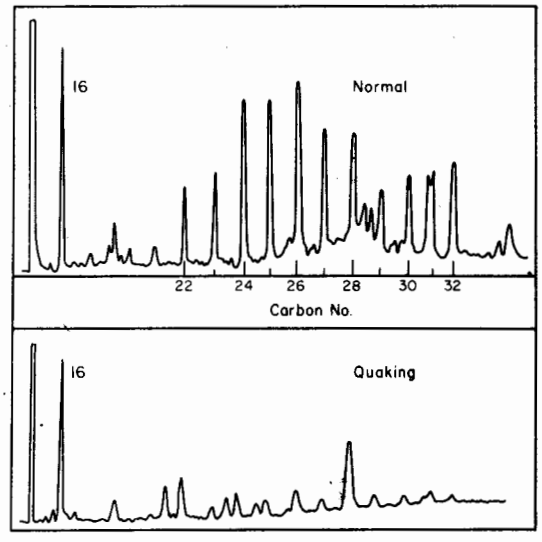

FIG. 2. Distribution of brain myelin alkanes in normal and Quaking myelin. 16: is the peak of hexadecane added as an external standard. The peak at 28 carbon atoms correspond also to squalene.

Alkane analysis. The solvents used in this study were freshly redistilled ($CHCl_3$, MeOH, spectrograde hexane). All the glassware was carefully washed, boiled with redistilled methanol, and washed with spectrograde hexane. In each case a complete blank run was done, including all the reagents and solvents without myelin, in order to detect any laboratory contamination. Alkanes were never detected in these conditions, and corrections for contamination were therefore not necessary.

Dry myelin (5–30 mg) plus hexadecane (60 μg) as internal standard were saponified with 5 M-KOH (0.5 ml) at 70°C for 1 h. The non-saponifiable matter including alkanes was extracted with 3 × 2 ml spectrograde hexane. The hexane solution was washed with water, dried with Na_2SO_4 and concentrated to 1 ml. This solution was applied to a glass column (30 cm × 3 mm) containing alumina (activity degree III), which had been prewashed with 30 ml of spectrograde hexane and eluted with 30 ml of spectrograde hexane. The eluate was evaporated to 100 μl under N_2 and the alkane solution was kept frozen, for GLC analysis and IR determination.

The analysis of the alkanes was done by GLC (Intersmat 14 C 120 OFL) on a 10% SE 30 column programmed from 130 to 310°C at 4°C/min or from 190 to 310°C at 2°C/min. The identification was done by comparison of their retention times with those of known standards. The relative amounts were estimated by calculation of the peak areas as compared to that of the internal standard, assuming a similar response of the detector for all the alkanes. Each experiment was performed at least 3 times. IR Spectra were determined with a Perkin Elmer 457 Spectrophotometer. The solvent of the myelin alkane solution was evaporated to dryness in order to avoid any contamination of the alkane Spectra by hexane; the remaining myelin alkanes were dissolved in Spectrograde CCl_4 and layered at the top of 2 μl extrocells made of AgCl (extrocells Beckman), then centrifuged at 2000 rev./min to permit the sample to penetrate into the cell. The IR Spectrum of the blank run presented only the bands due to CCl_4.

RESULTS AND DISCUSSION

Table 1 shows that brain contained alkanes, the content being reduced in the Quaking mutant. Alkanes were easily detected in myelin, from normal or mutant mice (in greater concentration than in

TABLE 2. ALKANE PATTERN IN NORMAL AND QUAKING MYELIN

Carbon atoms	Dry myelin (μg/mg) N	Qk	Dry myelin (nmol/mg) N	Qk
19	0.18	—	0.6	—
20	0.19	t	0.7	t
21	0.26	0.07	0.9	0.23
22	0.56	0.26	1.8	0.83
23	0.47	0.03	1.5	0.09
24	0.72	0.13	2.1	0.42
25	0.77	0.13	2.2	0.37
26	0.61	0.16	1.8	0.44
27	0.44	0.12	1.1	0.31
28 + sq	0.43	0.39	1.1	0.98
n.i	0.24	—	0.5	—
29	0.29	0.07	0.7	0.17
30	0.17	0.07	0.4	0.16
31	0.5	0.08	1.1	0.18
32	0.17	0.06	0.4	0.13

t: trace amount; sq: squalene; N: normal control littermate; Qk: Quaking animals. Mean values of 15 chromatograms.

whole brain). The amount in normal myelin was very high (7.1 mg/g) as compared to that found in beef heart (0.032 mg/g) (BANDURSKI & NAGY, 1975) or in beef liver (0.04 mg/g) (NAGY *et al.*, 1969). Moreover Quaking myelin contained 3 times less alkane than normal. Since myelin recovery was 6.9 mg/normal brain and 0.58 mg in the Quaking mouse brain, the alkane content in the Quaking brain is drastically reduced.

Infrared spectra showed the presence of $-CH_2$ and $-CH_3$ groups, but no other functional group was detected (Fig. 1). Figure 2 shows typical alkane distribution patterns from both 'normal' and 'Quaking' brain myelin. In the normal myelin the presence of all the alkanes ranging from C_{21} to C_{32} were present, while the Quaking myelin exhibited a different distribution pattern. Table 2 gives the amounts of the identified alkanes in both 'normal' and 'Quaking' myelin expressed as μg/mg dry myelin or as nmol/mg dry myelin. In the normal myelin approximately equal amounts of both even and odd chains in the C_{20}–C_{32} range with a maximum near the C_{25} alkanes were present.

Only trace amounts of alkanes were found in other subcellular particles: 0.5 μg/mg in microsomes, 0.4 μg/g in mitochondria and 0.13 μg/g in synaptosomes.

The alkane content in the normal myelin is at least 3 times higher than in the Quaking myelin. Since the Quaking mouse has a much lower level of very long chain fatty acids, the results presented here suggest a close relationship between very long chain fatty acids and alkanes, as those two families are diminished to the same degree in the mutant. This also occurred in higher plants (KOLATTUKUDY, 1975, 1976; CASSAGNE, 1972; CASSAGNE & LESSIRE, 1974) and in yeast (BLANCHARDIE, 1976). The study of this relationship is now in progress in our laboratories. An exogenous (nutritional) origin of alkane is not excluded as brain saturated fatty acids come partly from blood (GOZLAN-DEVILLIÈRRE *et al.*, 1976) and are then incorporated into myelin. Alkanes probably increase the organization of the myelin lipids, and thus enhance the stability and the rigidity of this membrane (*in vitro*, the effect of alkanes on micelle organization is well known).

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