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LIPID COMPOSITION OF QUAKING MOUSE MYELIN: COMPARISON WITH NORMAL MOUSE MYELIN IN THE ADULT AND DURING DEVELOPMENT[1]

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Abstract—In the 'Quaking' mouse, a deficiency in the long chain fatty acid content of galactolipids has been shown to occur.

Myelin in the mutant has been compared to myelin in adult and in 12-day-old controls. We have shown that myelin is not only quantitatively reduced but also qualitatively modified, with a higher protein and a lower galactolipid content. Cerebrosides contain only a small amount of kerasin, lacking long chain nonhydroxylated fatty acids in comparison to both controls; the relative percentage of phrenosin is increased.

Although many similarities exist between adult Quaking myelin and myelin at 12 days, differences have been shown to occur which may be in relation to a genetic block at an earlier stage of development.

THE QUAKING mouse is a mutant which bears a defect in myelin formation of the central nervous system.

Described in 1964 by SIDMAN, DICKIE and APPEL, it is among the myelin deficient mutants, the only one which reaches adult age. The mutation could be related to a qualitative defect of oligodendroglial cells of the central nervous system (BERGER, 1970, 1971; WISNIEWSKI and MORELL, 1971). Brain is deficient in cerebrosides and sulphatides (SIDMAN *et al.*, 1964; BAUMANN, JACQUE, POLLET and HARPIN, 1967; HOGAN and JOSEPH, 1970) especially in those containing long chain fatty acids, non-hydroxylated, saturated and monounsaturated (BAUMANN, JACQUE, POLLET and HARPIN, 1968; JACQUE, HARPIN and BAUMANN, 1969). Long chain containing sphingomyelins are also reduced. A lack of myelin could be due to a catabolic defect: isolated myelin would be in low quantity but normally constituted. Another possibility would be that paucity of myelin is caused by a block in the sequential events of myelination involving long chain fatty acids; these compounds, necessary to myelin sphingolipids, normally increase tremendously at the onset of myelination (JACQUE, BOURRE, MORENO and BAUMANN, 1971) in controls. Although in this myelin-deficient mutant, the deficit may rely at "some control point in the complex series of cellular events which normally result in adequate myelination" (HAUSER, EICHBERG and JACOBS, 1971), fatty acids may play a key role as the rate of fatty acid synthesis is depressed in Quaking and not in Jimpy or MSD (KANDUTSCH and SAUCIER, 1972).

In order to see whether there is a similarity in myelin between Quaking and controls at an early stage of development, myelin was isolated in adult and 12-day-old controls and in Quaking adult mice. Preliminary results have already been reported (BAUMANN,

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HARPIN and BOURRE, 1970; JACQUE, LOUIS, BOURRE and BAUMANN, 1971; BAUMANN, BOURRE, JACQUE and POLLET, 1971). Studies on adult Quaking myelin have been performed by SINGH, SPRITZ and GEYER (1971), and, while this paper was being revised, by GREGSON and OXBERRY (1972).

MATERIALS AND METHODS

Mice. Controls consisted of heterozygote and homozygote littermates of Quaking mice, between 9 and 10 weeks of age for the adults and at 12 days at the beginning of myelination. The Quaking strain was provided in 1966 by the Jackson Laboratory and maintained since then in our laboratory. Brain removal was performed as described previously (BAUMANN *et al.*, 1968).

Myelin preparations. Myelin in the adult was isolated by a slight modification of the method of NORTON, PODUSLO and SUZUKI (1967). Three grams of homogenate of whole brain in 55 ml 0·32 M-sucrose was filtered through cheese cloth and layered over 15 ml of 0·85 M-sucrose of each tube of the SW 25·1 rotor of the Spinco model L2 50 preparative centrifuge. Centrifugation was allowed for 30 min at 25,000 rev./min. The myelin layer (5 ml) at the interface of the two solutions was removed with care and placed in three other tubes. Water was added (28 ml) and the well-mixed solution was again centrifuged for 15 min at 25,000 rev./min. The pellets were transferred into two tubes of the RC2 Sorvall SS 34 rotor and cold water added. The pellet was washed three times at 20,000 rev./min. It was resuspended in 55 ml 0·32 M-sucrose and relayered over 45 ml 0·85 M-sucrose. Centrifugation in the Spinco was performed as previously. The intermediate phase was washed with water five times, each time centrifuged at 20,000 rev./min. In several cases, the last washings were done on preparations that had been kept in the freezer after the second Spinco centrifugation. In the young animals, each preparation was done under similar conditions, except that on the same day two preparations were pooled before the second Spinco centrifugation, in order to increase the yield of myelin. Quaking myelin was prepared similarly. The myelin to be analysed was lyophilized after resuspension in water, and weighed before being put into solution.

Criteria of purity. Several criteria were used to test the purity of the myelin fraction.

1. Electron microscopy was performed on the last centrifugation pellet fixed in 2·5% glutaraldehyde and 2% osmic acid. Ultrafine specimens were stained with uranyl acetate and lead citrate before examination under the Hitachi HU II A ultramicroscope (Fig. 1).

2. 2′3′-Cyclic-AMP-3′-phosphohydrolase activity was tested by the technique of OLAFSON, DRUMMOND and LEE (1969) slightly modified. The incubation media contained 120 μg/ml *E. Coli* alkaline phosphatase (Sigma, 25 U/mg), sodium deoxycholate 0·1%, 0·04 M-tris–HCl buffer, pH 7·5, 2′3′-cyclic-AMP, 0·8 μmol/ml. The final incubation media of 0·25 ml contained 25–50 μl of 1% homogenate and 25–50 μl of myelin layer after the second gradient (1–5 dilution for adult control myelin and undiluted for Quaking adult and 12-day-old control brain myelin). Enzyme reaction was stopped after 10 min incubation with 2 ml 8% TCA. Inorganic phosphate determination was performed according to BARTLETT (1959). Under our conditions, results were expressed in μmol of phosphate hydrolysed/mg protein, or /g wet wt./h.

3. As indicated by NORTON (1971), myelin protein analysis is a good criterion for myelin purity. SDS acrylamide gels prepared according to MORRIS, LOUIS and SHOOTER (1971) were performed as well as acrylamide gels containing phenol–urea–acetic acid (TAKAYAMA, MCLENNAN, TZAGOLOFF and STONER, 1966). Results have been published (JACQUE, LOUIS, GUEDE and BAUMANN, 1972) and are in agreement with those obtained by GREENFIELD, NORTON and MORELL (1971) who used a continuous CsCl gradient as final step.

Lipid analysis. Techniques used for quantitative lipid and proteolipid protein determinations as well as those used for qualitative lipid analysis on silica gel have been described previously (BAUMANN *et al.*, 1968). After selective alkaline methanolysis of total lipids (JACQUE *et al.*, 1969) pure sphingolipids were obtained by column chromatography: for 40–100 mg lipids, columns of 11 mm int. diam. were used containing 4 g Unisil (100–200 mesh); 75 ml chloroform eluted cholesterol and free fatty acids; 125 ml chloroform–methanol (95:5, v/v) cerebrosides; 140 ml, 80:20 (v/v) sulphatides and 75 ml methanol, sphingomyelin. Glucocerebrosides and galactocerebrosides were separated on borate silica gel plates as described by KEAN (1966).

Cerebrosides were methylated according to MORRISON and SMITH (1964) and fatty acid methylesters were gas chromatographed under conditions described previously (JACQUE *et al.*, 1969) either directly or after separation by thin-layer chromatography between hydroxylated and nonhydroxylated fatty acids.

Lipid analysis and cerebroside purifications were performed each time on at least 120 mg lyophilized myelin which represented 20 mice for adult controls and a minimum of 170 mice for Quaking adults and 12-day-old controls.

TABLE 1.—COMPOSITION OF MYELIN IN QUAKING AND CONTROL MOUSE BRAIN

Group	Lyophilized myelin yield (mg/g wet wt.)	Proteins (mg/100 mg lyophilized myelin)	Cholesterol (mg/100 mg lyophilized myelin)	Phospholipids (mg/100 mg lyophilized myelin)	Galactolipids (mg/100 mg lyophilized myelin)	Cholesterol / Phospholipids (Molar ratios)	Galactolipids / Phospholipids (Molar ratios)
12-day-old controls	1·6 ± 0·2	26 ± 1·3	18 ± 0·6	37 ± 1·1	19 ± 1·9	0·92	0·48
Adult controls	17 ± 1	21 ± 1·0	16 ± 0·6	38 ± 1·1	24 ± 2·4	0·84	0·58
Quaking adults	1·7 ± 0·2	33 ± 1·6	17 ± 0·6	37 ± 1·1	11 ± 1·1	0·93	0·28

The results for each group were obtained from pools of myelin preparations from the brains of 75–100 12-day-old or Quaking mice or from 25–40 adult mice. Each determination was performed at least twice. The values are the means ± the deviation corresponding to the accuracy of the method.

RESULTS

Myelin was obtained in low quantity in Quaking adults and in 12-day-old control mice and represented 10 per cent of the dry weight recovered in adult controls (Table 1). Electron micrographs showed that myelin preparations were devoid of mitochondrial and microsomal contaminants (Fig. 1). As noted by GREGSON and OXBERRY (1972) micrographs performed on water-shocked myelin form far fewer lamellae in the mutant compared to the adult controls. At 12 days in controls, the picture is close to the one observed in Quaking.

Specific activities and recoveries of 2'3'-cyclic-AMP-3'-phosphohydrolase are presented in Table 2. In comparison to adult control homogenates, 60 per cent enzyme

TABLE 2.—2',3'-CYCLIC-AMP-3'-PHOSPHOHYDROLASE ACTIVITY IN BRAIN HOMOGENATE AND MYELIN PREPARATIONS

	Homogenate		Myelin	
	(μmol/g brain/h)	(μmol/mg protein/h)	(μmol/g brain/h)	(μmol/mg protein/h)
Adult controls	1132	11·8	747	139
Quaking adults	750	3·5	14·6	42
12 days controls	238	3·2	24·4	31·6

activity was detected in Quaking adult and 20 per cent at 12 days in controls. Also, the amount of enzyme recovered in the myelin fraction was much less than in adult controls. Nevertheless the increase in specific activity was between 8- and 12-fold for each type of preparation.

Results concerning the composition of myelin are summarized in Table 1. Quantitative determination of the lipid content in controls showed very similar proportions of cholesterol, phospholipids and galactolipids to those observed by ENG and NOBLE (1966), NORTON *et al.* (1967) in the rat, and by HORROCKS (1968) in mice. In Quaking cholesterol to phospholipid ratios were very similar to controls. The differences observed in mutant myelin concern essentially the amount of the galactolipid fraction which increased with age in controls. In Quaking adults, the galactolipid to phospholipid ratio was considerably lower than in developing control myelin. Our results are similar to those observed by SINGH *et al.* (1971) in adult controls and mutants, and different from those obtained by GREGSON and OXBERRY (1972) whose values are lower for phospholipids and higher for galactolipids. The relative proportion of proteins to lipids was higher in Quaking adults than in both controls. A high value for protein content in Quaking myelin has also been reported by SINGH *et al.* (1971) although preparations were done differently on mice of a different age group (42–60 days instead of 56–70 days).

Thin-layer chromatography of myelin lipids has been performed. Cholesterol esters are not present in myelin of all three groups. Ganglioside changes were observed in myelin during its development (SUZUKI, PODUSLO and NORTON, 1967); nevertheless essentially GM_1 but also Gd_1a predominated in myelin fraction from adult and 12-day-control myelin. In Quaking, the ganglioside fraction contained higher amounts of Gd_1b and Gt_1 than controls. The cerebroside pattern was also markedly different

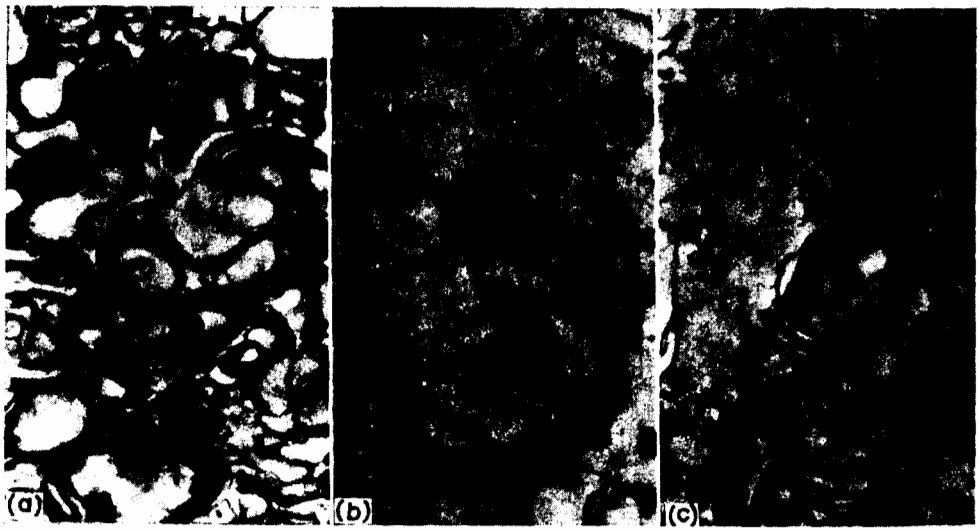

FIG. 1.—Myelin fraction magnification ×25,500. (a) Adult control (b) 12-day-old control (c) Quaking adult. We are grateful to Dr. B. BERGER for these electronmicrographs.

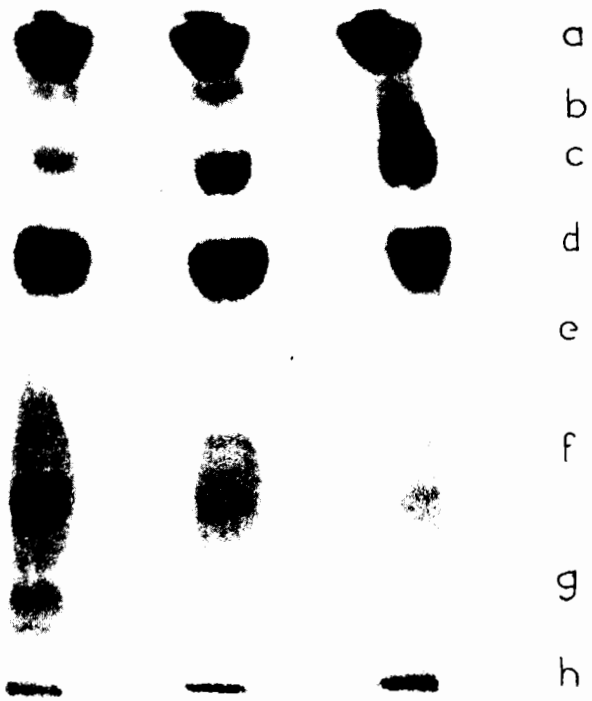

FIG. 2. Thin-layer chromatography on silica gel H: solvent chloroform methanol H_2O (70:30:4, by vol.); staining Mangold's sulphochromic reagent (MANGOLD, 1961). From left to right lipid extracts of 12-day-old control, Quaking adult and adult control. a: cholesterol, b: kerasin, c: phrenosin, d: phosphatidyl ethanolamine, e: sulphatides, f: lecithin phosphatidyl inositol and serine, g: sphingomyelin, h: gangliosides.

in the three groups (Fig. 2). As reported by JACQUE *et al.* (1971) and GREGSON and OXBERRY (1972), mutant myelin contained much less of the fast running cerebroside fraction, with nonhydroxylated fatty acids (kerasin). This was not found by SINGH *et al.* (1971). In developing myelin, α-hydroxylated cerebrosides (phrenosin) were markedly higher than in the adult. In Quaking mice, the quantity of phrenosin was greater than in both control groups. This is well indicated by fatty acid analysis of total cerebrosides (Table 3). In all cases, myelin cerebrosides consisted of galactocerebrosides; no glucocerebrosides could be detected on borate-impregnated thin-layer plates.

TABLE 3.—MYELIN TOTAL CEREBROSIDE FATTY ACIDS (SATURATED, UNSATURATED AND α-HYDROXYLATED)*

Chain length	12-day-old controls (per cent)	Adult Quaking (per cent)	Adult controls (per cent)
16	1·8 ± 0·5	4·8 ± 1·1	2·8 ± 0·8
18	7 ± 0·2	7·2 ± 0·2	5·0 ± 1·1
20	3·6 ± 0·9	2·8 ± 0·8	2·7 ± 0·8
22	15·3 ± 0·9	5·1 ± 1·1	7·4 ± 0·3
23	1·7 ± 0·3	1·5 ± 0·3	2·1 ± 0·4
22 h + 24	50·9 ± 2·0	31·7 ± 2·3	44·6 ± 2·5
23 h	2·0 ± 0·5	12·5 ± 0·8	6·4 ± 0·2
24 h	15·6 ± 3·0	30·8 ± 2·1	28·0 ± 2·0

* The lower detection of α-hydroxylated fatty acids has not been taken into account. These results were from three different pools of myelin preparations: from 75–100 12-day-old control or adult Quaking mice or from 25 to 40 adult mice. Each chromatography was performed at least twice. The values are the means ± the deviation corresponding to the accuracy of the method. h, α-hydroxylated.

The fatty acid composition of brain myelin nonhydroxylated cerebrosides was determined (Table 4). The content in long chain fatty acids—over 18 carbon atoms—

TABLE 4.—NONHYDROXYLATED FATTY ACID COMPOSITION OF BRAIN MYELIN CEREBROSIDES

Chain length	12-day-old controls (per cent)	Adult Quaking (per cent)	Adult controls (per cent)
16:0 + 16:1	3·9 ± 0·6	6·9 ± 1·1	2·6 ± 0·4
18:0 + 18:1	12·6 ± 2·2	25·2 ± 4·4	5·3 ± 0·9
20:0 + 20:1	4·8 ± 2·4	1·7 ± 0·8	1·3 ± 0·6
22:0 + 22:1	25·6 ± 5·1	16·8 ± 3·2	12·3 ± 2·4
23:0 + 23:1	2·5 ± 0·7	7·5 ± 2·2	4·6 ± 0·9
24:0 + 24:1	49·9 ± 2·5	40·0 ± 1·9	71·5 ± 3·6

Conditions as in Table 3.

is close to 90 per cent in the adult. The sum of fatty acids with 20, 22 and 24 carbon atoms appears relatively constant in both controls and above 80 per cent, although, among the long chains, the proportion of C24 increased markedly during development. In Quaking mice, the amount of long chain fatty acids was lower than in young controls, with a relative increase of fatty acids with 18 carbon atoms. Nevertheless in

Quaking the quantity of long chain fatty acids with 23 carbon atoms was relatively high. Values in both adults are in agreement with SINGH *et al.* (1971), although in our case, among the long chain fatty acids, mono-unsaturated were not the only ones to be reduced.

α-Hydroxylated fatty acids in cerebrosides were only examined on one preparation of Quaking adult mice myelin, as these galactolipids did not seem to be proportionally reduced in the mutant: values obtained were: C18: 5·6 per cent, C20: 7 per cent, C22: 34·8 per cent, C23: 20·5 per cent, C24: 30·2 per cent, values close to those reported by SINGH *et al.* (1971), although monounsaturated fatty acids were not singled out in our data.

DISCUSSION

Great similarities have been observed between developing myelin and Quaking myelin, for the lipid and fatty acid pattern as well as for the protein pattern (GREENFIELD *et al.*, 1971). According to these authors, the proteolipid/basic protein ratio in Quaking myelin is closely related to that observed in 10-day-old controls. Lipid and fatty acid pattern in mutant myelin are not in contradiction with this fact. Cerebroside fatty acid pattern in total brain (JACQUE, BOURRE, MORENO and BAUMANN, 1971) indicate a cessation in the evolution of the fatty acid pattern in Quaking. Although, according to SINGH *et al.* (1971) there is a preferential decrease in monounsaturated C24 fatty acids, saturated long chain fatty acids are also reduced and their biosynthesis impaired (BOURRE, POLLET, DAUDU and BAUMANN, 1971). The ganglioside pattern is also consistent with a block at an early step in myelin formation. The presence of a 'myelin-like' fraction more abundant at 12 days and in the adult mutant, may not be excluded on the lipid data; however, the fraction isolated by AGRAWAL, BANIK, BONE, DAVISON, MITCHELL and SPOHN, 1970) is devoid of basic protein. GREENFIELD *et al.* (1971) and JACQUE *et al.* (1972) have shown that it is not the case in Quaking. The results observed are not inconsistent with the attribution of a key role to long chain sphingolipids in myelin maturation in Quaking (BAUMANN *et al.*, 1972). According to UZMAN and RUMLEY (1958) and others, myelination involves to a great extent the reorientation of preexisting lipids which is made possible by the appearance of long chain myelin sphingolipids.

Several facts remain puzzling. The abundance of a α-hydroxylated fatty acid has always been intriguing, as well as the relative importance of C23. JOSEPH, DRUSE, NEWELL and HOGAN (1972) suggest that C23 accumulation may be due to an increased degradation of long chain fatty acids in Quaking brain. In demyelination JATZKEWITZ and MEHL (1962) have shown that cerebrosides of the kerasin type are degraded more rapidly than those of the phrenosin type, with a preferential degradation of C24 fatty acids. MEAD and LEVIS (1962) have shown that the odd chains in brain can be formed not only by synthesis but also by degradation (α-oxidation) of the C20 to C26 fatty acids of the brain sphingolipids. Histological data (BERGER 1970, 1971; WISNIEWSKI and MORELL, 1971) and comparisons of function with age are more in favour of a block in maturation of the oligodendrocytes. Nevertheless pathological vacuoles and inclusions (BERGER, 1971) indicate that this phenomenon is not simple and that other reactions which may be secondary occur.

Among the facts to be discussed is the persistance of 60 per cent activity of 2′,3′-cyclic nucleotidase-3′-phosphohydrolase in homogenates also observed by OLAFSON

et al. (1969) and KURIHARA, NUSSBAUM and MANDEL (1970), although histological and biochemical data indicate that myelin is considerably more diminished. It is worth noting that although the myelin yield at 12 days is closely related to adult Quaking's, the amount of the 'enzyme marker' is considerably more reduced, indicating a 20 per cent level compared to the adult's thus confirming results also observed by these authors. Although myelination is comparable at 12 days and in Quaking adults, the two groups of animals are not at the same stage of growth of neuronal processes; BRAUN and BARCHI (1972) suggest that the enzyme may also be an axonal component.

REFERENCES

AGRAWAL H. C., BANIK N. L., BONE A. H., DAVISON A. N., MITCHELL R. F. and SPOHN M. (1970) *Biochem. J.* **120,** 635–642.

BAUMANN N., JACQUE C., POLLET S. and HARPIN M. L. (1967) *C.r. hebd. Séanc. Acad. Sci., Paris* **264** D, 2953–2956.

BAUMANN N., JACQUE C., POLLET S. and HARPIN M. L. (1968) *Eur. J. Biochem.* **4,** 340–344.

BAUMANN N. A., HARPIN M. L. and BOURRE J. M. (1970) *Nature, Lond.* **227,** 960–961.

BAUMANN N., BOURRE J. M., JACQUE C. and POLLET S. (1971) Ciba Foundation Symposium. *On lipids, malnutrition and the developing brain.* pp. 91–100. Elsevier, 1972.

BARTLETT G. R. (1959) *J. biol. Chem.* **234,** 466–468.

BERGER B. (1970) In *Les mutants pathologiques chez l'animal, leur intérêt pour la recherche biomédicale* (Edited by SABOURDY M.) pp. 91–98. CNRS Paris.

BERGER B. (1971) *Brain Res.* **25,** 35–53.

BOURRE J. M., POLLET S., DAUDU O. and BAUMANN N. (1971) *Cr. hebd. Séanc. Acad. Sci., Paris* **273** D, 1534–1537.

BRAUN P. E. and BARCHI R. L. (1972) *Brain Res.* **40,** 437–444.

ENG L. F. and NOBLE E. P. (1968) *Lipids* **3,** 157–162.

GREENFIELD S., NORTON W. T. and MORELL P. (1971) *J. Neurochem.* **18,** 2119–2128.

GREGSON N. A. and OXBERRY J. M. (1972) *J. Neurochem.* **19,** 1065–1072.

HAUSER G., EICHBERG J. and JACOBS S. (1971) *Biochem. biophys. Res. Commun.* **43,** 1072–1080.

HOGAN E. L. and JOSEPH K. C. (1970) *J. Neurochem.* **17,** 1209–1214.

HORROCKS L. A. (1968) *J. Neurochem.* **15,** 483–488.

JACQUE C. M., HARPIN M. L. and BAUMANN N. A. (1969) *Eur. J. Biochem.* **11,** 218–224.

JACQUE C., BOURRE J. M., MORENO P. and BAUMANN N. (1970) *Cr. hebd. Séanc. Acad. Sci., Paris* **271** D, 798–801.

JACQUE C., BOURRE J. M., MORENO P. and BAUMANN N. (1971) *Biochimie* (1971) **53,** 1121–1124.

JACQUE C., LOUIS CH., BOURRE J. M. and BAUMANN N. (1971) *Proc. 3rd int. Meet. Int. Soc. for Neurochem.*, Budapest, p. 83.

JACQUE C. M., LOUIS C. F., GUEDE C. and BAUMANN N. (1972) *Cr. hebd. Séanc. Acad. Sci., Paris.* **274,** 126–129.

JATZKEWITZ H. and MEHL E. (1962) *Hoppe-Seyler's Z. physiol. Chem.* **329,** 264–277.

JOSEPH K. C., DRUSE M. J., NEWELL L. R. and HOGAN E. L. (1972) *J. Neurochem.* **19,** 307–312.

KANDUTSCH A. A. and SAUCIER S. E. (1972) *Biochim Biophys. Acta.* **260,** 26–34.

KEAN E. L. (1966) *J. Lipid Res.* **7,** 449–452.

KURIHARA T., NUSSBAUM J. L. and MANDEL P. (1970) *J. Neurochem.* **17,** 993–997.

MEAD J. F. and LEVIS G. M. (1963) *Biochem. biophys. Res. Commun.* **11,** 319–324.

MANGOLD H. K. (1961) *J. Am. Oil Chem. Soc.* **38,** 708.

MORRIS S. J., LOUIS C. F. and SHOOTER E. M. (1971) *Neurobiology.* **1,** 64–67.

MORRISON W. M. and SMITH L. R. (1964) *J. Lipid Res.* **5,** 600–608.

NORTON W. T., PODUSLO S. E. and SUZUKI K. (1967) *Proc. 1st int. Meet. International Society for Neurochemistry.* Strasbourg, p. 161.

NORTON W. T. (1971) *Adv. exp. Med. Biol.* **13,** 327–337.

OLAFSON R. W., DRUMMOND G. I. and LEE J. F. (1969) *Can. J. Biochem.* **47,** 961–966.

SIDMAN R. L., DICKIE M. M. and APPEL S. H. *Science, N.Y.* **144,** 309–310.

SINGH H., SPRITZ N. and GEYER B. (1971) *J. Lipid Res.* **12,** 473–481.

SUZUKI K., PODUSLO S. E. and NORTON W. T. (1967) *Biochim. biophys. Acta* **144,** 375–381.

TAKAYAMA K., MCLENNAN D. H., TZAGOLOFF A. and STONER C. J. (1966) *Archs Biochem. Biophys.* **114,** 223–230.

UZMAN L. L. and RUMLEY M. K. (1958) *J. Neurochem.* **3,** 170–184.

WISNIEWSKI H. and MORELL P. (1971) *Brain Res.* **29,** 63–73.