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THE LIPID COMPOSITION OF RAT BRAIN AGGREGATING CELL CULTURES DURING DEVELOPMENT

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SUMMARY

The lipid and fatty acid composition of rat brain was studied during its development both in vivo and in an aggregating cell culture system. Although the amount of lipid present in the cultures was very low, the increase in glycolipid content corresponded closely to the period of intense myelin formation. Very long chain fatty acids (hydroxylated and unsubstituted) were present in 41-day cultures. In comparsion to the in vivo situation, myelination was delayed in vitro and, after 40 days in culture, cholesterol esters were 5-fold higher than in vivo, indicating that demyelination was occurring.

Tissue and cell culture systems are becoming of widespread use for the analysis of neurobiological problems. Recently, morphological and biochemical [6] studies indicated that aggregating cell cultures could be a useful model to study myelination in vitro. The present investigation was undertaken to further characterize this in vitro system, by comparing the lipid and fatty acid composition of rat brain aggregates with that of rat brains of similar postnatal ages.

The methods used for the isolation and culture of fetal brain cells have been reported previously [4]. For each analysis, 4 flasks of aggregate cultures were washed in a modified Puck's solution, homogenized in water and lyophilized [6].

Lipid extracts were made as previously described [9]. The lyophilized samples were suspended in chloroform—methanol—water (70 : 30 : 4, v/v) and extracted after sonication. The residue was pelleted after centrifugation and the extract was evaporated to dryness. Lipids were dissolved in chloroform—methanol (2 : 1, v/v) and a Folch partition was performed [3]. Lipids were separated on thin layer chromatography (TLC) plates coated with silica gel HPTLC 60 F_{254}

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(Merck Co. Darmstadt, G.F.R.). The eluting solvent was chloroform—methanol—water (70 : 30 : 4, v/v). Two-dimensional TLC was also used [1,9]. For cerebroside and sulfatide analysis, the eluting solvent was chloroform—acetone— methanol—acetic acid—water (50 : 20 : 10 : 10 : 5, v/v); the spots were detected in iodine vapor, scraped and eluted 3 times with chloroform—methanol (2 : 1, v/v). Lipids were eventually detected by staining with molybdenum reagent for phospholipids, α-naphtol for glycolipids, antimony trichloride for sterols and potassium dichromate for all lipids. Lipids were quantified as previously described [1,9], and gluco- and galacto-cerebrosides and sulfatides were measured by gas liquid chromatography (GLC) as trifluoroacetate derivatives. The lipids fraction was methylated with 14% boron trifluoride in methanol. A preparative TLC separated the unsubstituted fatty acids from the other fatty acids (solvent: 20% ether in hexane). Analysis of fatty acid methylesters was performed by GLC on a 3% SE 30 column with temperature programming at 2°C/min between 150°C and 285°C. Fatty acids were identified by retention time and equivalent chain length and chromatography with standards. Aggregating cell cultures were labelled for 16 h in a medium containing [^{35}S] sodium sulfate [6]. After incubation, the aggregates were washed and homogenized in water. Aliquots (10 μl) were removed for protein assay [5] and the rest was lyophilized. Lipids were extracted [3] and lipid aliquots were used for sulfatide colorimetric determination and radioactivity counting as previously reported [6].

The amount of lipid present in the cultures was very low (Table I) when compared to brains of the same age animals. After 41 days in culture, the ratio lipid/protein was 0.29 instead of 0.42 (in vivo) reflecting the low amount of myelin present in the aggregates. The cholesterol content was nearly constant during development in vitro (Table I). Cholesterol esters at 41 days represented 3% of the total cholesterol, whereas in vivo only 0.6% is esterified under normal conditions, indicating that demyelination was occurring after 40 days in culture. The cholesterol ester concentration increased about 3 times between 18 and 41 days in vitro. Phospholipids were decreasing slightly during development but multiple two-dimensional TLC did not show any major alteration.

TABLE I

LIPID COMPOSITION OF RAT BRAIN AGGREGATES

Lipids	Days in culture			
	4	18	29	41
Total lipids[a]	17	19	20	21
Cholesterol[b]	24 ± 3	24 ± 2	23 ± 3	24 ± 3
Phospholipids[b]	69 ± 6	68 ± 5	60 ± 4	61 ± 7
Glycolipids[b]	0.3 ± 0.1	0.7 ± 0.1	1.0 ± 0.2	2.7 ± 0.3

[a] mg of total lipids after Folch partition in percentage of lyophilized aggregate homogenate.
[b] mg in percentage of total lipids, means ± S.D. from 3 separate determinations.

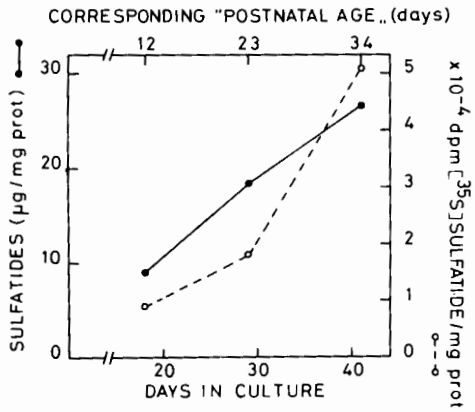

Fig. 1. Sulfatide content and synthesis in rat brain aggregates. Sulfatide content of two separate experiments measured in duplicates, •——•. Specific radioactivity in the same experiments is expressed as dpm/mg of protein, ○— — —○.

Glycolipids (cerebrosides and sulfatides) showed a marked increase during in vitro development (Table I). While barely detectable after 4 days in culture, they reached 2.7% of the total lipid extract at 41 days. The amount of sulfatides tripled in culture between days 18 and 40: the incorporation of radioactive sulfate showed a substnatial increase between days 30 and 40 (Fig. 1), indicating a very fast turnover in the older cultures. Cerebrosides and sulfatides are major myelin lipids [2,8] and have been shown to increase during myelination [2,7]. In aggregating cell cultures the increase in glycolipid content corresponded closely to the period of intense myelin formation. The percentage of glucosphingolipids, 0.5% of the lipid extract, is important and indicates the extra-myelinic lipid synthesis. Monogalactosyldiglyceride and sulfomonogalactosyldiglyceride were not detected under our in vitro conditions. Approximately 50% of the glycosphingolipids contained hydroxylated fatty acids. The analysis of the fatty acid pattern showed that appreciable amounts of very long chain fatty acids (hydroxylated and unsubstituted) were present in cultures after 41 days, while these compounds were barely detectable after 4 days.

These results demonstrate that rat brain aggregating cell cultures synthesize typical myelin lipids and confirm our previous morphological observations [6], which showed that myelination in aggregating cell cultures is depressed relative to that in vivo. The concentration of glycolipids in 40 day aggregates is comparable to 12—13-day-old rat brains (Bourre, unpublished). It appear that myelin lipid biosynthesis is starting nearly normally, but slows down, resulting in a decreased myelin content and delayed growth and maturation of the myelin sheath. Although one should bear in mind the limitations associated with all cell and tissue culture systems, the present data indicate that rotating cell aggregates of mechanically dissociated fetal rat brain can provide a useful system in the study of myelin formation.

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