

lipase activity<sup>6</sup>, may render large amounts of normal milk unpalatable when admixed prior to processing for consumption.

More systematic investigations are in progress.

For the permission to use the gas liquid chromatography apparatus at the Department of Physiology I wish to thank Karl Halse M.S.

This investigation was supported financially by the *Norwegian Milk Producers National Association* and the *Norwegian Agricultural Research Council*.

1. Velle, W. *Undersøkelser over naturlig forekommende østrogener hos drøvtyggere og gris*, Thesis, Oslo 1958.
2. Velle, W. *Personal communication*.
3. Frankel, E. N. and Tarassuk, N. P. *J. Dairy Sci.* 38 (1955) 751.
4. James, A. T. and Martin, A. J. P. *Biochem. J.* 50 (1952) 679.
5. Garm, O. *A Study of Bovine Nymphomania*. Thesis. *Acta endocr. (Kbh.)* (1949) Suppl. 3.
6. Anonymus, *Md. Agr. Expt. Sta. Sixty-fifth Ann. Rpt.* (1951-1952) 43; Cit. from Herrington, B. L. *J. Dairy Sci.* 37 (1954) 775.

Received February 22, 1960.

## The Fatty Acid Composition of Cerebrospinal Fluid Lipids

ROLF BLOMSTRAND

*Swedish Medical Research Council, Unit for Biochemical Research Related to Atherosclerosis, University of Lund, Lund, Sweden*

The study of the fatty acid composition of the different lipids of human cerebrospinal fluid (CSF) has not previously been possible due to the lack of suitable analytical methods which could be applied to the small amounts of lipid available. The CSF contains only 1-1.5 mg of total lipids<sup>1</sup> per 100 ml and ultramicrochemical techniques have to be applied to normal and abnormal fluids if neurochemical correlations are to be made. Through the use of the technique described below it has been possible to identify the fatty acid pattern of cholesterol esters, glycerides and phospholipids of normal human CSF.

Pooled normal CSF (140 ml) obtained from individuals without neurological symptoms was used for the analysis. The CSF was immediately centrifuged after lumbar puncture, the cell-free supernate was carefully removed and aliquots taken for determination of total protein. The rest of it was kept at -20°C until analyzed. All specimens used had normal white cell count and normal protein concentrations. The pooled CSF was concentrated by dialysis in a collodium bag under reduced pressure according to Mies<sup>2</sup> and extracted with 20 volumes of chloroform:methanol 2:1 and washed with water<sup>3</sup>. The total lipids (1.8 mg) were separated on a silicic acid column<sup>4</sup> (0.2 g) into cholesterol esters, glycerides + free fatty acids and phospholipids. The different lipid fractions were hydrolyzed and the fatty acids were isolated after saponification and acidification. The fatty acid composition was defined by gas-liquid chromatography according to James and Martin<sup>5</sup> using an Argon Pye chromatograph with an ionization chamber as detector. Because of the complexity of the fatty acid pattern, it was necessary to chromatograph the mixed methyl esters on two different stationary phases, a polar polyester (LHC-R-296)<sup>6</sup> and silicone oil. In addition chromatograms were obtained on the mixed esters after complete hydrogenation in order to obtain an accurate chain-length analysis. The analytical procedures used otherwise have been described earlier.<sup>7</sup>

The determination of the fatty acid composition of the different lipid components of human CSF is summarized in Table 1. For comparison the fatty acid composition of the different lipid classes of normal serum is also included in the table. Three characteristic fatty acid patterns are found in the cholesterol esters, glycerides + free fatty acids and phospholipids of human CSF. The fatty acid composition of the CSF resembles the serum lipids, but there are certain differences. Especially remarkable is the very low concentration of linoleic acid in the cholesterol esters of CSF compared with that of the serum cholesterol esters. Another interesting finding is the rather high concentration of palmitoleic acid in the glycerides + free fatty acid fraction and in the cholesterol esters. In the group C<sub>20-22</sub> several peaks were found among the phospholipid fatty acids. Significant amounts of a fatty acid with 24 carbon atoms were also found in the phospholipids of the CSF. Work is in progress to identify these fatty

*Table 1.* The fatty acid composition of the different lipid classes of human cerebrospinal fluid (CSF) compared with those of serum. The total lipids were fractionated on silicic acid and the fatty acids were determined with the aid of gas-liquid chromatography. Values are expressed as percentage of total fatty acid methyl esters.

Fatty acid	Percentage of total fatty acids in respective lipids classes						
	Cholesterol ester F.A.		Glyceride + free F.A.		Phospholipid F.A.		
	C.S.F.	Serum	C.S.F.	Serum	C.S.F.	Serum	
<i>Saturated</i>							
Myristic	14:0	9.7	1.7	8.2	3.5	6.1	1.5
Pentadecanoic	15:0	7.1	1.5	4.9	1.6	4.7	1.2
Palmitic	16:0	22.1	14.8	33.8	24.1	32.5	29.3
Heptadecanoic	17:0	1.6	—	0.9	—	1.6	—
Stearic	18:0	5.3	2.3	10.6	6.4	11.0	16.8
<i>Monounsaturated</i>							
Palmitoleic	16:1	18.1	5.2	9.7	7.4	—	4.0
Oleic	18:1	15.4	24.2	20.9	36.4	20.1	16.2
<i>Diunsaturated</i>							
Linoleic	18:2	6.2	44.7	3.3	7.8	12.5	18.8
<i>Polyunsaturated</i>							
Linolenic	18:3	—	0.3	2.1	2.1	1.3	0.5
Arachidonic	20:4	1.6	2.3	—	—	2.3	5.7
	20—22?	2.8	1.2	2.8	1.9	3.4	3.6
	24?					1.6	—

The fatty acids are also designated by a dual symbol giving chain length and number of double bonds. The sum of each column is less than 100 % because traces of unusual fatty acids detected were omitted from the final tabulation. — indicates trace amounts.

acids. Preliminary investigations on the composition of the total fatty acids of CSF with neurologic disease indicate that abnormal fatty acid patterns may be obtained<sup>8</sup>.

This work is part of investigations supported by the Swedish Medical Research Council.

1. Tourtelotte, W. W., *Neurology* **9** (1959) 375.
2. Mies, H. J. *Klin Wochschr.* **31** (1953) 159.
3. Folch, J. and Lee, J. *J. Biol. Chem.* **191** (1951) 833.

4. Borgström, B. *Acta Physiol. Scand.* **25** (1952) 101.
5. James, A. T. and Martin, A. J. P. *Biochem. J.* **63** (1956) 144.
6. Orr, C. and Callen, J. E. *J. Am. Chem. Soc.* **80** (1958) 249.
7. Blomstrand, R. and Dahlbäck, O. *Acta Soc. Med. Upsaliensis* **114** (1959) 177.
8. Blomstrand, R., Dencker, S. J. and Swahn, B. *Kungl. Fysiogr. Sällsk., Lund. Förh. To be published.*

Received April 9, 1960.