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Studies on Lacrimal Gland Lipids in Essential Fatty Acid Deficiency[1] (40520)

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Extraorbital lacrimal glands, like the salivary glands, perform secretory function and are very similar ultrastructurally (1). Although there are a few studies on the lipid composition of Harderian gland, another exocrine eye gland, in rabbits (2, 3) and rats (4), there is a paucity of similar information on extraorbital lacrimal glands.

We have recently reported the effects of essential fatty acid (EFA) deficiency on the fatty acid composition of submandibular salivary glands (SMSG) in rats (5). It was found that, like other tissues, SMSG also undergo profound changes in their fatty acid patterns. These structural changes were associated with a decreased flow rate of pilocarpene-stimulated whole saliva. Because of the ultrastructural similarity of the salivary glands and the extraorbital lacrimal glands, and in view of recent reports (6, 7) in the literature indicating that nutrition may play an important role in influencing the flow rate and composition of tears, we have studied the effects of EFA-deficiency on the lipid composition of extraorbital lacrimal glands in rats.

Methods. Two groups of male, weanling, Sprague-Dawley rats[2] (10 per group) were fed *ad lib.* nutritionally adequate purified diets, one containing 7% corn oil (control group) and the other a fat-free diet (deficient group). The fat-free diet contained in percent: Casein (vitamin-free), 20.0; sucrose, 71.0; salt mix (Wesson), 4.0; cellulose, 4.0; vitamin mix,[3] 0.7 and choline chloride, 0.3. In the control diet, corn oil was added replacing 7% sucrose.

Body weights were measured twice a week. Half the rats from each group were killed by means of a guillotine at 8 weeks and the remaining half at 16 weeks of feeding the two diets. The extraorbital lacrimal glands were dissected out, weighed and homogenized with chloroform–methanol (2:1) for the extraction of lipids. Total lipid extracts were purified using Folch's method (8). Aliquots of washed lipid extracts were used for the analysis of total fatty acid composition and their concentrations using gas chromatography and internal standard of heptadecanoic acid. Lacrimal glands of rats fed for 8 weeks were analyzed only for their total fatty acid composition. Sixteen-week samples were analyzed for all the constituents described in the text.

An aliquot of total lipid extract was used for the separation of neutral and phospholipids by column chromatography over silicic acid (9). After thin layer chromatographic separation of free fatty acids (FFA) and triglyceride (TG) in neutral lipid fraction, and phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylserine + phosphatidylinositol (PS + PI) in the phospholipid fraction, the fatty acid composition of each of these lipid fractions was determined using gas chromatography (5). The concentrations of FFA and TG fractions were determined by gas chromatography after the addition of internal standards of heptadecanoic acid for FFA and triheptadecanoin for TG as described previously (10).

Total lipid phosphorus was determined by Bartlett's method (11) and free and total cholesterol by the method of Zak *et al.* (12).

All data were statistically analyzed using Student's *t* test.

Results. Rats fed EFA-deficient diet for 16 weeks had lower body weights as compared to the controls (383.6 ± 10.2 versus 503.6 ± 25.6 g). No significant differences were observed in lacrimal gland weights between the two groups. The gland weights were 125.0 ± 3.8 mg in the deficient group versus 142.0 ± 7.2 mg (± 1SE) in the control.

Total phospholipids were higher in the deficient group (33.3 ± 0.73 versus 29.9 ± 1.13 mg/g wet tissue), whereas the concentrations of free and total cholesterol and total fatty

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[2] Holtzman Co., Madison, Wisconsin.

[3] See Navia *et al.* J. Nutr. **97**, 133 (1969).

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acids were essentially the same in the two groups. Concentrations of FFA fractions were significantly lower ($P < 0.05$) in the deficient glands as compared to the controls (2.12 ± 0.15 versus 2.76 ± 0.21 mg/g wet tissue). The fatty acid composition of total lipids of plasma also showed a pattern characteristic of EFA-deficiency (5).

The fatty acid composition of total lipids of the lacrimal glands after 8 weeks of feeding the control and EFA-deficient diets is shown in Table I. This table contains some additional data obtained from rats in which EFA deficiency was induced by another means i.e., by feeding 7% hydrogenated coconut oil (HCO). The controls were fed 5% HCO + 2% corn oil. The results show that EFA deficiency, whether created by feeding a fat-free diet or a diet containing 7% HCO, induced similar changes in the fatty acid composition of the total lipids of the lacrimal glands.

Results on fatty acid composition of total lipids, FFA and TG fractions obtained at 16 weeks are shown in Table II. In each of the three lipids, the levels of 16:1 and 18:1 were significantly higher whereas those of 18:2 and 20:4 were lower in the lacrimal glands of EFA-deficient rats as compared to those fed the EFA-supplemented control diet. 5,8,11-eicosatrienoic acid (20:3 ω9) was present only in the EFA-deficient group and not in the control group. Dihomo-γ-linolenic acid (20:3 ω6), an intermediate in the synthesis of arachidonic acid from linoleic acid, was present only in the control groups and not in the deficient groups. TG fractions from both groups contained very little of C-20 fatty acids.

The fatty acid composition of total lipids of lacrimal glands of rats fed for 8 weeks was essentially similar to that observed after 16 weeks of feeding except that the levels of 18:2 in the gland were reduced even further (from 1.9% to 0.6%) when the deficient diet was fed for 16 weeks.

The fatty acid composition data for PC, PE and PI + PS fractions are presented in Table III. The lacrimal glands from the EFA-deficient rats showed similar modifications of their fatty acid patterns as seen in case of total lipids, FFA and TG fractions, i.e. an increase in the levels of 16:1 and 18:1, a decrease in 18:2 and 20:4, and an accumulation of 20:3 ω9. In addition to these changes, the levels of 16:0 in PC and PE and those of 18:0 in PE and PS + PI fractions were significantly lower in lacrimal glands of rats fed the EFA-deficient diet as compared with those fed the control diet.

Discussion. EFA deficiency in young rats is characterized by reduced weight gain, dermal lesions and, biochemically, by increased triene to tetraene ratio in plasma and tissue lipids. In the present study, by the sixteenth

TABLE I. FATTY ACID COMPOSITION OF TOTAL LIPIDS IN LACRIMAL GLANDS OF RATS FED EFA-DEFICIENT AND CONTROL DIETS FOR 8 WEEKS.

Fatty acid	Deficient (0% Fat)	Control (7% Corn Oil)	Deficient[c] (7% HCO)[b]	Control[c] (5% HCO[b] + 2% Corn Oil)
14:0	1.2 ± 0.08	1.2 ± 0.03	1.5 ± 0.05	1.3 ± 0.07
iso 16:0	0.2 ± 0.06	0.4 ± 0.05	0.4 ± 0.08	0.9 ± 0.27
16:0	28.7 ± 0.99	32.9 ± 0.70	28.6 ± 0.87	30.0 ± 0.66
16:1	21.6 ± 1.07[a]	6.8 ± 1.03	22.0 ± 1.10[a]	12.2 ± 1.18
18:0	8.4 ± 0.24	10.5 ± 0.44	8.8 ± 0.23	10.9 ± 0.40
18:1	25.7 ± 1.45[a]	14.7 ± 1.10	25.1 ± 0.87[a]	17.8 ± 0.96
18:2	1.9 ± 0.17[a]	15.8 ± 0.87	2.0 ± 0.12[a]	8.4 ± 0.74
20:0	—	0.4	—	0.3 ± 0.05
20:1	1.3 ± 0.13	0.9 ± 0.04	1.2 ± 0.20	1.4 ± 0.26
20:2	0.8 ± 0.07	—	0.7 ± 0.04	—
20:3, ω9	6.8 ± 0.36	—	6.0 ± 0.45	—
20:3, ω6	—	2.9 ± 0.30	—	1.7 ± 0.24
20:4	3.3 ± 0.21[a]	11.6 ± 0.60	3.0 ± 0.14[a]	11.5 ± 0.69
20:5	0.4	—	0.3	—

[a] Significantly different from respective control values ($P < 0.001$).
[b] HCO-Hydrogenated Coconut Oil.
[c] The strain, age and sex of rats and the basal diet composition was similar to that described under the methods section.
Values are means ± SEM of five rats/group.

TABLE II. FATTY ACID COMPOSITION OF TOTAL LIPIDS, TRIGLYCERIDES AND FREE FATTY ACIDS IN LACRIMAL GLANDS FROM CONTROL AND ESSENTIAL FATTY ACIDS DEFICIENT RATS.

	Total lipids		Triglycerides		Free fatty acids	
Fatty acid	Deficient	Control	Deficient	Control	Deficient	Control
14:0	0.7 ± 0.16	0.5 ± 0.03	2.2 ± 0.07	1.9 ± 0.10	0.9 ± 0.12	0.6 ± 0.07
ISO 16:0	1.1 ± 0.15	1.4 ± 0.14	—	—	—	—
16:0	28.1 ± 0.69	28.1 ± 0.46	35.2 ± 0.60	32.5 ± 1.18	26.1 ± 1.06	24.6 ± 1.15
16:1	17.0 ± 0.42[a]	4.1 ± 0.18	11.6 ± 0.21[a]	7.6 ± 0.32	17.1 ± 0.40[a]	4.2 ± 0.26
18:0	8.9 ± 0.26	10.9 ± 0.40	5.3 ± 0.15	5.3 ± 0.24	10.3 ± 0.44	11.2 ± 0.32
18:1	31.0 ± 0.92[a]	17.1 ± 0.86	45.2 ± 0.92[a]	29.8 ± 0.26	36.4 ± 0.97[a]	16.3 ± 0.66
18:2	0.6 ± 0.08[a]	17.6 ± 1.24	—	21.4 ± 1.06	1.4 ± 0.13[a]	15.8 ± 0.91
20:1	3.4 ± 1.77	1.2 ± 0.13	0.4	0.5 ± 0.16	2.5 ± 0.94	2.0 ± 0.66
20:2	0.5 ± 0.09	0.2	—	—	—	—
20:3 ω9	6.4 ± 0.43	—	—	—	5.3 ± 1.10	—
20:3 ω6	—	3.0 ± 0.38	—	—	—	4.0 ± 0.35
20:4	2.8 ± 0.17[a]	15.6 ± 1.14	—	1.1 ± 0.20	—	19.0 ± 1.68
20:5	—	—	—	—	—	2.0 ± 0.19

[a] Significantly different from respective control values ($P < 0.001$).
Values are means ± SEM of five rats.

TABLE III. FATTY ACID COMPOSITION OF THE MAJOR PHOSPHOLIPIDS IN LACRIMAL GLANDS FROM CONTROL AND ESSENTIAL FATTY ACIDS DEFICIENT RATS.

	Phosphatidylcholine		Phosphatidylethanolamine		Phosphatidylserine + Phosphatidylinositol	
Fatty acid	Deficient	Control	Deficient	Control	Deficient	Control
14:0	1.0 ± 0.09	0.6 ± 0.12	0.3 ± 0.03	0.1 ± 0.04	0.6 ± 0.08	0.5 ± 0.18
16:0	34.5 ± 0.62[a]	40.0 ± 0.68	12.4 ± 0.49[b]	14.5 ± 0.36	23.6 ± 1.45	24.0 ± 1.30
16:1	14.3 ± 0.59[a]	2.5 ± 0.24	16.1 ± 0.89[a]	3.6 ± 0.17	9.0 ± 0.37[a]	1.6 ± 0.04
18:0	12.0 ± 0.67	15.5 ± 1.75	11.4 ± 0.24[a]	16.5 ± 0.45	36.3 ± 1.56[b]	45.2 ± 1.16
18:1	24.9 ± 1.43[a]	9.7 ± 0.39	28.1 ± 1.82[a]	13.2 ± 0.53	22.5 ± 1.56[a]	14.0 ± 0.65
18:2	1.2 ± 0.09[a]	12.6 ± 0.76	1.4 ± 0.07[a]	9.6 ± 0.82	0.8 ± 0.12[a]	5.0 ± 0.73
20:1	—	—	0.8 ± 0.29	0.5 ± 0.06	—	—
20:2	—	—	1.3 ± 0.14	0.5 ± 0.12	—	—
20:3 ω9	9.6 ± 1.04	—	17.7 ± 0.99	—	6.8 ± 0.58	—
20:3 ω6	—	3.3 ± 0.15	—	2.5 ± 0.25	—	2.6 ± 0.71
20:4	2.0 ± 0.26[a]	15.0 ± 0.73	9.9 ± 0.28[a]	32.0 ± 1.08	—	6.8 ± 0.62
22:5	—	—	—	2.7 ± 0.27	—	—
22:6	—	—	—	3.1 ± 0.19	—	—

[a] Significantly different from respective control values ($P < 0.001$).
[b] Significantly different from respective control values ($P < 0.01$).
Values are means ± SEM of five rats.

week of feeding the EFA-deficient diet, the rats showed markedly reduced weight gains and high triene to tetraene ratio in plasma total lipids (5), thus indicating that the rats were frankly deficient in EFA.

Increase in total phospholipid content of lacrimal gland in EFA-deficient rats which we have observed in our study is consistent with a similar increase reported in kidney and plasma (13) but at variance with the results obtained in liver (14), lung (15) and brain (16).

Total cholesterol in the lacrimal gland was not changed, nor the ratio of free to total cholesterol. Free to esterified cholesterol ratio in various tissues has been examined in EFA-deficient animals. A number of tissues such as liver (14, 17, 18), skin (19), adrenal (20) and ovary (21) show an increase in cholesterol esters (22).

A reduction in FFA levels in lacrimal glands of EFA-deficient rats as observed in the present study is consistent with our previous findings in SMSG (23). A possible

mechanism for reduced FFA levels in lacrimal glands in EFA-deficient animals may be related to lower prostaglandin levels. Although prostaglandin levels in the gland were not measured, in EFA deficiency, tissues are known to contain lower prostaglandin levels (24). A reduction in prostaglandins may in turn decrease cAMP mediated lipolysis thus resulting in lower FFA levels in lacrimal gland of EFA-deficient animals.

The fatty acid composition of total lipids, FFA, TG, PC, PE and PI + PS in lacrimal gland was substantially modified as a result of EFA deficiency. As previously observed in SMSG (5), the main changes in fatty acid composition consisted of increase in 16:1, 18:1, a decrease in 18:2 and 20:4 and an accumulation of 20:3 ω9 fatty acids in the deficient gland. This was true of total lipids as well as the other lipid fractions except TG which contained very little of C-20 fatty acids. Our results on the fatty acid composition of lacrimal glands in EFA deficiency are consistent with the findings of other investigators with several other tissues (25–31). Also, the changes in fatty acid composition of total lipids of the lacrimal gland induced by EFA-deficiency were similar to those observed in plasma (5).

The fatty acid composition of lacrimal gland lipids was similar to that reported for the SMSG (5). However in lacrimal gland of EFA-deficient rats, there were higher levels of 16:1 and lower levels of 18:1 in total lipids, FFA, PC and PE fractions. Since the values for these two fatty acids in the lacrimal gland lipids of the control rats were not significantly different from those of the SMSG lipids, it appears that EFA deficiency may induce somewhat different types of changes in the activity of various enzymes which are involved in the metabolism of monoenoic fatty acids in the two glands.

Changes in the fatty acid composition of lipids, especially structural lipids such as PC, PE and PI + PS which were observed in the lacrimal glands of EFA-deficient rats may alter the gland function. It is known that modifications in fatty acid composition of membrane phospholipids in various tissues are generally associated with functional changes such as transport and in the activity of various membrane-bound enzymes (32–34). There is some evidence that EFA deficiency is associated with alteration in renewal of photoreceptor membranes (35), the electrical response of the retina (36) and the development of the rat retina (37).

We have previously observed a reduction in flow rate of whole saliva in EFA-deficient rats (5). Since the changes in fatty acid composition of the extraorbital lacrimal gland as observed in the present study were similar to those previously found for the SMSG, it is quite likely that the flow rate and/or the composition of lacrimal secretion may also be altered in EFA deficiency. If so, this would have important clinical implications in view of the recent report (6) that in the tears of malnourished children, the levels of immunoglobulin A (IgA), lysozyme and amylase are significantly reduced. It has been suggested that increased susceptibility to infection of mucosal surfaces in malnourished children may be due to reduced concentrations of protective substances such as IgA and lysozyme in secretions. Whether the composition of lacrimal secretion is affected by EFA deficiency needs further investigation.

Summary. Two groups of young, male, weanling rats were fed purified diets, one containing 7% corn oil (control) and the other 0% fat (EFA-deficient). After sixteen weeks of feeding, rats were killed, their extraorbital lacrimal glands were extracted for lipids, and the lipid composition was studied. Total phospholipid concentration was higher whereas total cholesterol, free cholesterol and cholesterol to phospholipid ratios were the same in lacrimal glands of EFA-deficient rats as compared to the controls. Total fatty acid concentrations were the same in the two groups. However, free fatty acid levels were reduced in the EFA-deficient gland. The fatty acid composition of total lipids, free fatty acids, triglycerides, phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine + phosphatidylinositol fractions obtained from the lacrimal glands of EFA-deficient rats showed changes in their fatty acid patterns which are characteristic of EFA deficiency, i.e., an increase in 16:1 and 18:1, a decrease in 18:2 and 20:4 and an accumulation of 20:3 ω9 fatty acids.

1. Chambaut-Guerin, A. M., Busson, S., Ovtracht, L.,

and Rossignol, B., J. Microsc. Biol. Cell. **27,** 5a (1976).
2. Rock, C. O., Fitzgerald, V., Rainey, Jr., W. T., and Snyder, F., Chem. Phys. Lipids **17,** 207 (1976).
3. Jost, U., Hoppe-Seyler's Z., Physiol. Chem. **355,** 422 (1974).
4. Murawski, U., and Jost, U., Chem. Phys. of Lipids **13,** 155 (1974).
5. Alam, S. Q., and Alam, B. S., J. Nutr. **108,** 1642 (1978).
6. McMurray, D. N., Rey, H., Cassaza, L. J., and Watson, R. R., Amer. J. Clin. Nutr. **30,** 1944 (1977).
7. Watson, R. R., Reyes, M. A., and McMurray, D. N., Proc. Soc. Exp. Biol. Med. **157,** 215 (1978).
8. Folch, J., Lees, M., and Sloane-Stanely, G. H., J. Biol. Chem. **226,** 497 (1957).
9. Rouser, G., Kritchevsky, G., and Yamamoto, A., *in* "Lipid chromatographic analysis" (G. V. Marinetti, ed.), Vol. 1, p. 118. Dekker, N. Y. (1967).
10. Alam, S. Q., and Alam, B. S., J. Dent. Res. **57,** 643 (1978).
11. Bartlett, G. R., J. Biol. Chem. **234,** 466 (1959).
12. Zak, B., Dickenman, R. C., White, E. G., Burnett, H., and Cherney, P. J., Amer. J. Clin. Pathol. **24,** 1307 (1954).
13. Privett, O. S., Blank, M. L., and Verdino, B., J. Nutr. **85,** 187 (1965).
14. Morton, R. A., and Horner, A. A., Biochem. J. **79,** 631 (1961).
15. Kyriakides, E. C., Beeler, D. A., Edmonds, R. H., and Balint, J. A., Biochim. Biophys. Acta. **431,** 399 (1976).
16. Odutuga, A. A., Biochim. Biophys. Acta. **487,** 1 (1977).
17. Alfin-Slater, R. B., J. Amer. Oil Chem. Soc. **34,** 574 (1957).
18. Alfin-Slater, R. B., Aftergood, L., Wells, A. F., and Deuel, H. J., Arch. Biochem. Biophys. **52,** 180 (1954).
19. Ziboh, V. A., and Hsia, S. L., J. Lipid Res. **13,** 458 (1972).
20. Walker, B. L., and Carney, J. A., Lipids **6,** 797 (1971).
21. Carney, J. A., and Walker, B. L., Comp. Biochem. Physiol. **41 B,** 137 (1972).
22. Krause, B. R., Alam, S. Q., and Hartman, A. D., Proc. Soc. Exp. Biol. Med. **157,** 297 (1978).
23. Alam, S. Q., and Alam, B. S., J. Dent. Res. **57,** in press.
24. Van Drop, D. A., Ann. N.Y. Acad. Sci. **180,** 181 (1971).
25. Mead, J. F., J. Biol. Chem. **227,** 1025 (1957).
26. Mohrhauer, H., and Holman, R. T., J. Lipid Res. **4,** 346 (1963).
27. Sand, D., Sen. N., and Schlink, H., J. Amer. Oil Chem. Soc. **42,** 511 (1965).
28. Walker, B. L., J. Nutr. **92,** 23 (1967).
29. Fulco, A., and Mead, J. F., J. Biol. Chem. **234,** 1411 (1959).
30. Holman, R. T., J. Nutr. **70,** 405 (1960).
31. Holman, R. T., Prog. Chem. Fats **9,** 279 (1968).
32. Walker, J. A., and Wheeler, K. P., Biochim. Biophys. Acta. **394,** 135 (1975).
33. Silbert, D. F., Cronan, J. E. Jr., Beacham, I. R., and Harder, M. E., Fed. Proc. **33,** 1725 (1974).
34. Solomonson, L. P., Liepkalns, V. A., and Spector, A. A., Biochemistry **15,** 892 (1976).
35. Landis, D. J., Dudley, P. A., and Anderson, R. E., Science **182,** 1144 (1973).
36. Benolken, R. M., Anderson, R. E., and Wheeler, T. G., Science **182,** 1253 (1973).
37. Anderson, R. E., Benolken, R. M., Jackson, M. B., and Maude, M. B., in "Advance in Experimental Biology and Medicine Series" (N. G. Brazen, R. R. Brenner and N. M. Guisto, eds.), Vol. 83, p. 547. Plenum Press, N.Y. (1977).

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