fatty acid. With further improvements in techniques, this method of identification of structure may come to be used with even greater confidence.

The values for ECL presented here may be applied to old data and to data from other laboratories without the use of a contemporary standard, provided that the same type of column packing was used in the experimental situation as was used in deriving the ECL value of the authentic standard, that other chromatographic parameters were approximately the same and that at least two peaks on the chromatogram are identifiable. Thus, for example, ECL values on EGS presented here were used to identify a component in a chromatogram run more than two years previously. On the chromatogram an unknown substance lay between 20:4ω6 and 22:6ω3 which were identifiable through knowledge of the history of the sample and an extensive experience with GLC analysis of tissue fatty acids. The log retention time values for 20:4ω6 and 22:6ω3 were measured from the old chromatogram, these were plotted against the ECL values taken from Table II and the straight line between them was constructed. The log retention time for the unknown was measured and the ECL value found from the straight line was equal to that of 22:3ω9 found in the table. Thus the tentative identification of the substance could be made. The value of this technique lies also in the elimination of the need to run standards frequently after familiarity with the GLC system and the series of samples allows quick identification of some of the major components.

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The donors listed in Table II provided samples of unusual fatty acids.

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The Fatty Acid Composition of the Lipids from Bovine and Porcine Reproductive Tissues[1]

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Abstract

Beef and pork testes, graafian follicles and the residual ovaries were extracted and the lipids from each were separated into lipid classes by thin-layer chromatography. The fatty acids from each class were analyzed as their methyl esters by gas-liquid chromatography. The lipids from the reproductive tissues were found to be relatively rich in polyunsaturated acids, many of which did not correspond to the more commonly encountered unsaturated acids. These less familiar acids were identified by comparing their chromatographic characteristics with standards of established composition.

The polyunsaturated acids of lipids of the reproductive tissues examined are predominantly of the linoleate family. Only in the phospholipids of ovarian tissues did the linolenate family of acids reach high proportions of the total polyunsaturates. Nine members of the linoleate family were identified in the lipids of reproductive tissues. Five higher metabolites of oleate were identified as normal components of these tissues. Diglycerides were found as a significant lipid class only in testis tissue. The diglycerides and cholesteryl esters of beef testis contain tetracosatetraenoic acid as major fatty acid. The triglycerides of reproducitve tissues are notably rich in polyunsaturated acids. In the study, 16 polyunsaturated acids were identified by ozonolysis-reduction and several others were tentatively identified by retention time data. Two acids, previously unreported, are 10,13,16-docosatrienoic acid and 9,12,15,18-tetracosatetraenoic acid.

Introduction

THE DEFICIENCY of essential fatty acids (EFA) is known to result in sterility in both the male and the female rat (1,2). The ovary and the testis which produce reproductive cells contain lipids which are especially rich in polyunsaturated acids (3). The content of polyunsaturated fatty acids (PUFA) in the the rat testis decreases as essential fatty acid deficiency sets in (4). Semen (5) and eggs (6) contain lipids rich in polyunsaturates, and the cause of the sterility of EFA-deficient animals may be the insufficiency of the polyunsaturates for the synthesis of the fertile reproductive cells.

The lipids of the testes, ovaries, ovarian follicles and corpora lutea have been studied by many investigators using classical methods of lipid biochemistry (7–13). These investigations produced information concerning the content of phospholipid, cholesterol, neutral fat and unsaponifiable matter in these tissues. In some the approximate fatty acid composition was given for the lipids, and evidence exists for the presence of highly unsaturated acids in lipids. However, precise information concerning the fatty acid composition of the several lipids present in these tissues gathered using the newer, more discriminating, techniques of

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TABLE I
Fatty Acid Composition of the Principal Lipids from Beef and Pork Testis Tissues

Fatty acid	ECL[a]	Beef Testis						Pork Testis					
		Total lipid %	Phospho-lipids %	Diglyc-erides %	FFA %	Triglyc-erides %	Chol. ester %	Total lipid %	Phospho-lipids %	Diglyc-erides %	FFA %	Triglyc-erides %	Chol. ester %
10:0	10.00	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	0	0	0	0	0	0
12:0	12.00	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	0.6	0	0	0	0	0.3
14:0	14.00	1.5	0.2	2.6	1.0	1.4	3.8	1.4	1.2	5.8	2.6	10.5	3.9
14:unknown	14.80	2.1	5.3	10.9	Tr.	0.8	Tr.	1.5	0.6	0	Tr.	0.9	Tr.
16:0	16.00	28.3	25.4	20.2	32.3	39.2	17.6	17.3	25.3	17.5	25.7	19.4	11.9
16:1ω7	[b]16.56	1.6	0.7	2.9	3.3	2.5	6.3	2.6	0.4	1.5	3.9	4.1	3.8
16:2ω4	17.32	0.9	0.7	Tr.	1.1	0.8	Tr.	Tr.	Tr.	Tr.	1.9	Tr.	Tr.
18:0	18.00	11.1	8.7	4.5	7.4	6.9	13.7	11.0	12.9	10.8	15.0	9.2	2.9
18:1ω9	[b]18.50	23.9	14.3	6.7	17.0	21.2	15.1	13.4	13.1	7.0	18.1	9.1	10.6
18:2ω6	[b]19.22	7.7	6.0	3.6	6.0	6.6	4.3	6.4	6.8	11.0	5.4	5.1	3.2
18:3ω6	19.78	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	1.7	Tr.	Tr.	Tr.
18:3ω3	[b]20.13	0.3	0.2	1.9	1.4	0.8	2.7	0.9	2.4	0.8	1.1	1.0	1.6
20:0	20.00												
20:1ω9	20.38	Tr.	0.3	Tr.	1.1	0.9	1.3	0.7	0	0	0	0	0
20:2ω9	20.95	0	0	0	0	0	0	Tr.	0	1.7	0	1.6	1.3
20:2ω6	[b]21.13	0.4	0.4	Tr.	1.4	1.3	Tr.	0.8	0.8	Tr.	1.4	Tr.	1.3
20:3ω9	[b]21.57	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	0
20:3ω6	[b]21.65	1.8	1.9	1.0	4.1	3.8	Tr.	4.7	3.8	2.8	3.8	3.4	4.6
20:4ω6	[b]22.25	12.1	20.3	5.2	8.4	3.3	2.9	15.5	11.3	4.2	13.4	2.1	3.6
20:5ω3	[b]22.92	1.5	Tr.	Tr.	Tr.	0.9	Tr.	Tr.	4.6	3.0	0	2.5	Tr.
22:3ω9	[b]23.17	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	0	0.7	0	1.1	0.7
22:3ω6	[b]23.38	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	0	2.2	0	2.1	0.8
22:4ω9	23.60	0	0	0	0	0	0	Tr.	0	6.6	Tr.	6.4	0
22:4ω6	[b]23.85	1.5	1.0	1.1	2.5	1.5	2.1	3.8	4.1	2.7	2.5	2.3	5.3
22:5ω6	[b]24.37	1.5	4.1	4.5	3.9	4.3	3.5	12.1	5.6	9.6	5.4	9.9	27.9
22:5ω3	[b]24.93	1.4	0.8	1.7	1.4	1.1	Tr.	Tr.	Tr.	6.2	0	3.3	0.7
22:6ω3	[b]25.40	2.6	8.0	8.6	6.9	1.9	Tr.	4.4	7.1	4.3	Tr.	6.0	7.4
24:4ω6	[b]25.73	Tr.	0.4	22.3	0.9	Tr.	26.9	1.4	0	0	0	0	2.4
unknown A	26.00	Tr.	Tr.	0	Tr.	Tr.	0	0	0	0	0	0	0
unknown B	26 37	Tr.	Tr.	Tr.	Tr.	Tr.	0	1.5	0	0	Tr.	0	5.7
unknown C	26.73	Tr.	Tr.	Tr.	Tr.	Tr.	0	0	0	0	0	0	0
unknown D	27.28	Tr.	Tr.	Tr.	Tr.	Tr.	0	0	0	0	0	0	0
Total PUFA		31.7	43.8	49.9	38.0	26.3	42.4	51.5	46.5	57.5	34.9	46.8	66.5
Total ω6		25.0	34.1	37.7	27.2	20.8	39.7	44.7	32.4	34.2	31.9	24.9	49.1
Total ω3		5.8	9.0	12.2	9.7	4.7	2.7	5.3	14.1	14.3	1.1	12.8	9.7
Total lipid in tissue: 2.5%								Total lipid in tissue: 3.8%					
Lipid class, % of total lipid		44.0	0.6	10.0	2.5	1.6		64.7	0.5	0.5	7.5	5.0	

[a] See reference 23.
[b] Indicates these structures deduced from ozonolysis and reduction. Other unsaturated structures deduced by interpretation of equivalent chain length data. See accompanying report by Hofstetter and Holman (23).

GLC and TLC has not been previously available.

These considerations prompted a reinvestigation of the lipids of reproductive tissues, using thin-layer chromatography (TLC) and gas-liquid chromatography (GLC). Beef and pork testes and ovaries were chosen as tissues for investigation because of their ready availability. The lipids were fractionated into the general lipid classes to learn whether specific fatty acids were characteristic for certain of them. No attempt was made to further subdivide the phospholipids in this study. From one of the tissues, many of the individual fatty acids were isolated by liquid-liquid chromatography (14) and preparative GLC (15). The individual fatty acids were then identified by ozonolysis followed by reduction and GLC analysis of the fragments.

Concurrently, with our study, Peifer and Ahluwalia (16) have also investigated the effects of hypothyroidism and exogenous polyunsaturated acids on the fatty acid patterns of specific lipid esters of the testes and seminal vesicles of the rat and the lipid patterns of corpus luteum of the cow during different stages of its gestation period. Their studies have revealed that the reproductive tissue lipid patterns are susceptible to both hormonal imbalances and alterations in compositions by exogenous fats.

Experimental

Testes and ovaries were obtained fresh from the packing house. The ovaries were dissected immediately to yield the developing graafian follicles and the residual ovary. The lipids of the fresh tissues were extracted promptly with chloroform:methanol, 2:1, in a tissue blendor according to the method of Folch et al. (17). After evaporation of most of the solvent, the lipids were extracted with petrolether (Skelly F) and were spotted on thin-layer plates. The preparative plates were developed with petroleum ether:ether:formic acid, 80:20:1 which separated the lipids into classes. The streaks of separated lipids were made visible by spraying with 0.2% ethanolic 2′,7′-dichlorofluorescein and viewing under ultraviolet light (18). The separated zones were scraped from the plates, and the lipids were converted to methyl esters by transesterification with methanolic HCl. The methyl esters were analyzed by GLC using a Beckman GC-2A gas chromatograph equipped with a hydrogen flame detector. The column was 1.2 m by 4 mm packed with 20% ethylene glycol succinate (EGS) on Gaschrom P, 80–100 mesh. The carrier gas was helium at 60–80 ml/min and the column was operated at 180–200C.

In order to identify the many unknown acids encountered in the several lipid classes, it was necessary to conduct a detailed study of the fatty acids isolated from one of the tissues. To this end, the lipids from 350 g of beef testis tissue were extracted by the procedure described above, and the total lipid was saponified by 30 volumes of 10% methanolic KOH under nitrogen. The soaps were converted to free fatty acids by 1N sulfuric acid. The free acids were made up to 12% solution in anhydrous acetone and the saturated acids were removed by crystallization at −40C. The unsaturated portion was converted to methyl esters with methanolic HCl and the resulting con of polyunsaturated methyl esters was fractionated on a silicone-celite (1:1, w/w) column 150 cm long and 2.5 cm internal diam (14). The column was eluted successively with acetonitrile:water, 70:30, 75:25 and 80:20. This yielded four major fractions (2, 1 and 1 fractions, respectively) whose composition, determined by analytical GLC on the EGS column was principally as follows:

Fraction A consisted of 10:0, 20:5, 22:6 followed by unknown A.

(Shorthand notation: Compounds or isomeric mixtures without reference to position of double bonds are represented by numbers indicating chain length and numbers of double bonds, for example, 16:1 and 18:2 for octadecenoic acid and octadecadienoic acid, respec-

TABLE II

Fatty Acid Composition of the Principal Lipids from Beef and Pork Graafian Follicles

Fatty acid	ECL[a]	Beef Graafian Follicle					Pork Graafian Follicle				
		Total lipid %	Phospho-lipids %	FFA %	Triglyc-erides %	Chol. ester %	Total lipid %	Phospho-lipids %	FFA %	Triglyc-erides %	Chol. ester %
14:0	14.00	1.4	2.6	1.3	1.4	2.4	0.2	0.4	2.9	1.3	1.1
14:unknown	14.80	0	0	0	0	0	1.3	0	1.0	Tr.	Tr.
14:unknown	15.32	3.6	17.7	0.6	Tr.	10.5	Tr.	0.1	Tr.	9.7	4.2
16:0	16.00	9.6	29.3	10.4	9.9	15.4	7.6	17.5	27.2	24.1	15.1
16:1ω7	16.56	4.2	8.2	3.0	3.0	2.8	1.5	1.3	4.4	6.6	4.5
16:2ω4	17.32	Tr.	Tr.	1.5	Tr.	Tr.	2.3	Tr.	Tr.	Tr.	Tr.
18:0	18.00	10.7	9.6	12.0	9.5	4.6	10.5	18.0	17.8	8.2	5.9
18:1ω9	18.50	10.3	8.7	11.5	11.9	8.1	10.7	13.0	21.4	28.2	23.6
18:2ω6	19.22	11.6	4.9	14.6	14.6	11.9	8.3	7.9	6.4	8.2	8.7
18:3ω6	19.78	0.5	0.8	0.4	0.4	0.3	0.4	0.7	0.4	0.1	0.2
18:3ω3	20.13	0.9	1.3	1.1	1.4	0.7	0.6	2.8	1.2	1.3	1.1
20:0	20.00										
20:1ω9	20.38	0	0	0	0	0	Tr.	Tr.	Tr.	Tr.	Tr.
20:2ω9	20.95	0	0	0	0	0	Tr.	0	Tr.	Tr.	0.3
20:2ω6	21.13	5.5	1.3	6.3	7.1	3.4	1.8	1.0	2.2	0.6	0.9
20:3ω9	21.57	0	0	0	0	0	Tr.	0.5	0.6	0.3	0.9
20:3ω6	21.65	5.5	2.7	5.7	6.2	4.7	2.2	2.7	1.0	0.5	3.3
20:4ω6	22.25	13.8	2.7	13.2	9.8	11.0	24.7	13.2	2.8	4.6	11.3
20:5ω3	22.92	Tr.	2.2	Tr.	0.3	Tr.	0.3	6.2	0.4	0.1	0.5
22:3ω9	23.17	0	0	0	0	0	Tr.	0	0.1	0.3	0.6
22:3ω6	23.38	0.4	Tr.	0.7	0.6	0.5	Tr.	Tr.	0.5	0.2	0.8
22:4ω6	23.85	14.6	4.4	12.7	17.3	16.1	21.1	5.6	1.5	3.1	9.1
22:5ω6	24.37	0.8	Tr.	0.6	0.8	0.6	1.4	0.9	1.0	0.3	0.6
22:5ω3	24.93	4.2	3.5	3.5	3.1	1.6	3.0	1.7	0.2	0.7	0.5
22:6ω3	25.40	Tr.	Tr.	0	Tr.	Tr.	0.9	3.9	1.1	0.4	0.9
24:4ω6	25.73	1.5	Tr.	1.0	Tr.	Tr.	0.8	0	6.0	0.9	6.1
unknown	26.27	1.0	0	0	1.3	5.4	0.5	0	Tr.	Tr.	Tr.
Total PUFA		60.3	23.8	61.3	61.6	56.2	68.3	49.5	25.4	21.6	45.8
Total ω6		54.2	16.8	55.2	56.8	53.9	60.7	37.4	21.8	18.5	41.0
Total ω3		5.1	7.0	4.6	4.8	2.3	4.8	11.6	2.9	2.5	3.0
Total Lipid in tissue: 11.6%							Total lipid in tissue: 8.1%				
Lipid class, % of total lipid			24.0	8.5	26.5	22.6		29.7	3.9	20.5	22.2

[a] See reference (23).

tively. The "ω-notation" indicates by a number following the ω the position of the first double bond counting from the terminal methyl group. The "ω-notation" implies that all double bonds are *cis* and methylene-interrupted unless specifically stated. For example, natural linoleic acid is 18:2ω6.)

Fraction B consisted of 12:0, 14:1, 16:2, 18:3, 20:4, 22:5 and unknown B.

Fraction C consisted of 14:0, 16:1, 18:2, 20:3, 22:4 and unknown C.

Fraction D contained 16:0, 18:1, 20:2, 22:3 and 24:4.

Each of the four fractions was subjected to preparative GLC on a β-cyclodextrin acetate column (15). The preparative GLC was performed on a Beckman GC-2A equipped with a thermal conductivity detector using helium as carrier gas at 120–150 ml/min. The column was 3 m by 7.5 mm packed with 20% β-cyclodextrin acetate on Chromosorb R 30–60 mesh. The column was operated at 234C and the maximum sample size was 600 mg. Insofar as was possible, all individual components were collected. Each was analyzed for purity on the EGS column, hydrogenated and its chain length determined by GLC, and each was also subjected to ozonolysis to determine the positions of the double bonds. The hydrogenation was performed under 40 psi in methanol using platinum dioxide catalyst, and the chain length was determined by GLC on EGS. The ozonolysis and reduction of the ozonides was performed according to Privett and Nickell (19) and the aldehydic proximal and terminal fragments including the malondialdehyde were identified by GLC on a column 4 m by 4 mm packed with 30% silicone SE-30 on Chromosorb P, 100–120 mesh. The instrument was a Beckman GC-2A operated at 90C and 145C using helium at a flow rate of 60–80 ml/min. Those substances whose structures were so identified were used as standards for the identification of the polyunsaturated acids in the several lipid classes in the various tissues. Quantification of the chromatograms was done by triangulation and the results reported as area percent. The analytical data presented in Tables 1, 2 and 3 are from analyses performed on the EGS column. Amounts less than approx 0.05% are indicated in the tables by trace (tr).

The relative proportions of the lipid classes were estimated by quantitative gravimetric TLC and identified by authentic standards. The lipid fractions were scraped from the plates, extracted and weighed. In the case of diglycerides, which in this study showed unexpected compositions, the content was so small as to preclude identification by any means other than TLC. The cholesterol contents have been deleted from the tables but can be deduced by difference.

Results and Discussion

The lipids of reproductive tissues have been confirmed to be relatively rich sources of polyunsaturated acids. The number of individual polyunsaturated fatty acids present in their lipids is likewise rather high. A distinct segregation of certain acids in the lipid classes is evident from an inspection of Tables 1, 2 and 3. The presence of 18:2ω6, 18:3ω6, 20:2ω6, 20:3ω6, 20:4ω6, 22:3ω6, 22:4ω6, 22:5ω6 and 24:4ω6 in testis tissue constitutes our first observation of all the postulated metabolic products of linoleate in one tissue. They are likewise all detectable in pork testis and in both beef and pork ovarian tissues. Together they constitute approx 25% and 45% of the total fatty acids in beef testis lipid and pork testis lipid, respectively, whereas the ω3 family of acids are less than 6% of the total fatty acids in both cases. In beef and pork ovaries the combined ω6 acids of the total lipids amounted to 44% and 56%, respectively, vs. 5% and 3% ω3 acids. In the total lipids of the beef and pork graafian follicles these values were 54% and 61% ω6 acids, vs. 5% and 5% ω3 acids, respectively. The relatively low proportions of ω3 acids in the lipids suggests that these, known to have less biological activity than the ω6 acids, probably do not play a large role in the lipids of reproductive tissue in these species. The phospholipids of ovary and graafian follicle seem to be the exceptions to this generality. In these instances the ω3 group of acids is a significantly larger proportion of the total PUFA. The presence of very large amts of the metabolites of linoleate suggests that

TABLE III

Fatty Acid Composition of the Principal Lipids from Beef and Pork Ovarian Tissue

Fatty acid	ECL[a]	Beef Ovary					Pork Ovary				
		Total lipid %	Phospho-lipids %	FFA %	Triglyc-erides %	Chol. ester %	Total lipid %	Phospho-lipids %	FFA %	Triglyc-erides %	Chol. ester %
12:0	12.00	0.4	Tr.	Tr.	Tr.	Tr.	0	Tr.	0	0	0
14:0	14.00	1.5	1.2	1.9	1.8	4.5	0.1	0.3	0.8	0.4	0.7
14:unknown...	15.13	2.3	0.5	1.5	0.8	Tr.	0.1	Tr.	Tr.	0	1.9
16:0	16.00	14.6	14.8	11.1	10.1	15.3	11.4	17.8	13.4	15.7	8.2
16:1ω7	16.56	2.5	1.9	5.7	6.0	7.3	1.1	1.3	1.3	1.6	2.3
16:2ω4	17.32	Tr.	Tr.	Tr.	Tr.	Tr.	1.0	Tr.	1.5	Tr.	Tr.
18:0	18.00	14.1	15.3	13.7	9.4	5.8	13.0	16.0	13.8	13.1	6.9
18:1ω9	18.50	15.3	10.3	14.5	15.3	12.4	14.2	10.9	15.7	20.3	21.6
18:2ω6	19.22	16.5	12.1	13.9	17.3	19.1	13.4	10.2	12.5	14.9	12.6
18:3ω6	19.78	Tr.	0.5	0.8	1.9	1.0	0.2	0.5	0.6	Tr.	0.8
18:3ω3	20.13	1.3	1.8	2.7	2.4	2.2	0.7	1.0	1.0	1.4	1.4
20:0	20.00										
20:2ω9	20.95	0	Tr.	0	0	0	Tr.	Tr.	0	0	Tr.
20:2ω6	21.13	3.1	2.4	6.1	6.9	2.7	1.4	0.8	1.7	1.6	2.1
20:3ω9	21.57	0	Tr.	0	0	0	Tr.	0.9	Tr.	Tr.	Tr.
20:3ω6	21.65	3.7	3.1	4.9	5.7	3.1	1.9	1.8	2.0	1.7	3.5
20:4ω6	22.25	13.4	13.0	11.1	7.8	6.9	26.2	22.0	19.7	12.8	17.0
20:5ω3	22.92	Tr.	3.9	0.4	Tr.	Tr.	0.2	1.5	Tr.	0	Tr.
22:3ω9	23.17	Tr.	0	Tr.	Tr.	0	0	0	0	0	0
22:3ω6	23.38	Tr.	0	Tr.	Tr.	0	0	0	0	0	0
22:4ω9	23.60	Tr.	1.5	Tr.	0	0	Tr.	1.7	0	0	0
22:4ω6	23.85	6.4	5.3	6.8	9.9	10.8	12.3	6.4	13.3	15.0	15.2
22:5ω6	24.37	Tr.	0.8	0.3	Tr.	Tr.	0.8	0.9	0.5	Tr.	1.0
22:5ω3	24.93	3.9	8.0	4.1	4.0	3.2	1.5	2.3	1.4	1.4	1.3
22:6ω3	25.40	Tr.	0	Tr.	0	0	0.8	3.6	0.8	Tr.	0
24:4ω6	25.73	1.2	0	0.4	0.8	5.8	Tr.	0	0	0	3.4
Total PUFA		49.5	52.4	51.5	56.7	54.8	60.4	53.6	55.0	48.8	58.3
Total ω6		44.3	37.2	44.3	50.3	49.4	56.2	42.6	50.3	46.0	55.6
Total ω3		5.2	13.7	7.2	6.4	5.4	3.2	8.4	3.2	2.8	2.7
Total lipid in tissue: 11.4%							Total lipid in tissue: 6.3%				
Lipid class, % of total lipid			56.5	9.8	19.3	3.0		69.9	2.9	15.5	4.3

[a] See reference (23).

these have function in the reproductive process. If one-fourth to one-half of the fatty acids of the lipids of testis and ovary are of one family of fatty acids, it is not surprising that dietary deficiency of the precursory member of this family of acids should induce sterility in experimental animals (20).

The fatty acid compositions of the various lipids in beef testis and in pork testis do not yield identical patterns. In beef testis lipid, phospholipids are 44% and cholesteryl esters are 1.6%, whereas in pork testis phospholipids are 65% and cholesteryl esters 5%. Triglycerides are a larger proportion of pork testis lipid than of beef testis lipid. Some polyunsaturated acids exist as traces in the testis lipid of one species and occur as significant proportions of the lipid in other species. Thus, 18:3ω6 was detectable in measurable amt in the diglycerides of pork testis but only as traces in beef testis diglycerides, and 20:3ω6 occurs as a trace in beef testis cholesteryl esters, but as 4.6% of cholesteryl esters of pork testis. These two substances, shown to be intermediates in the metabolism of linoleate by Mead and Howton (21,22) using radioactive tracer techniques, were undetected in the tissue lipids they studied. In testes and ovaries these substances occur as measurable components of lipids. In the same family of fatty acids 22:3ω6 occurs in traces in beef testis lipids but in measurable amts in diglycerides, triglycerides and cholesteryl esters of pork testis. The next higher metabolites, 22:4ω6 and 22:5ω6, are present in significant amts in both species, but are more abundant in the lipids of pork testis. The longest chain metabolite of this family of acids detected in this study, 24:4ω6, occurs in all lipid classes of beef testis and to the extent of 22.3% and 26.9% of the acids of diglycerides and cholesteryl esters, respectively. This previously undescribed acid occurs as the largest single component of both diglycerides and cholesteryl esters of beef testes.

Several higher metabolites of the oleate family have been detected in testis and ovary lipids. Beef testis lipids contain measurable amts of 20:1ω9, and it occurs in traces in pork ovarian follicle lipids. The next higher metabolite, 20:2ω9, was not detected in beef testis lipids but occurs in diglycerides, triglycerides and cholesteryl esters of pork testis. Traces of 20:3ω9 are present in all the lipids of testis of both species, and occur as measurable components of the lipids of pork graafian follicle. The metabolite 22:3ω9 is found in many of the lipids studied here, but occurs in highest amts in the lipids of pork reproductive tissues. The highest metabolite of this family thus far detected, 22:4ω9, occurs in surprisingly high concn, greater than 6%, in diglycerides and triglycerides of pork testis and in phospholipids of pork and beef ovaries. The occurrence of so many higher metabolites of oleate in such high concn points out that these are normal constituents of tissues of normal animals, and are not formed only in EFA deficiency. In EFA deficiency they merely increase in proportion.

Diglycerides were found in significant amts only in testis tissue. The presence of this lipid together with free fatty acids suggests lipolysis prior to extraction. However, in this study there is no consistent relationship between the fatty acid compositions of phospholipids or triglycerides and their possible hydrolysis products, diglycerides and free fatty acids. For example, 24:4ω6 occurs as major component of diglycerides and cholesteryl esters in beef testis, but occurs in very much lower concn in free fatty acids, triglycerides and phospholipids. In the same tissue, the unknown acid having an equivalent chain length of 15.35 is particularly concd in the diglycerides. In pork testis 18:3ω6 occurs in diglycerides more abundantly than in any other lipid, and 20:2ω9 occurs as a distinct component of diglycerides, triglycerides and cholesteryl esters, but not of phospholipids or free fatty acids. On the other hand, 22:4ω9 occurs as a large component of diglycerides and triglycerides of pork testis, but is absent in the other lipid classes of that tissue. Thus, the diglycerides could not have arisen from either triglycerides or phospholipids except by an extremely fatty acid-specific lipase or phospholipase. Therefore, it appears that simple nonspecific lipolysis cannot account for the diglycerides. Moreover, it appears that diglycerides, triglycerides and perhaps free fatty acids should not be considered single metabolic pools, but that each class may involve

more than one metabolic pool, the distinction being on the basis of fatty acids in each.

Customarily, phospholipids are considered to be rich sources of highly unsaturated fatty acids, and triglycerides of animal origin are considered to be not especially rich in PUFA. However, the triglycerides of some of the reproductive tissues are notably rich in PUFA. Although beef testis triglycerides contain only 26% PUFA, pork testis triglycerides contain almost 47%. Beef graafian follicle triglycerides contain 61% PUFA whereas pork graafian follicle triglycerides contain only 21%. Beef and pork ovary triglycerides contain 57% and 49% PUFA, respectively. In all six instances, the major portion of the PUFA are of the ω6, the linoleate family.

In the course of this investigation, a number of PUFA have been identified. For 16 the identification is based upon ozonolysis-reduction of the isolated substances. For several others the identification has been by means of retention time data obtained in GLC analysis. The means of identification, and the data for a large body of individual PUFA are given in an accompanying publication (23). A number of acids still unidentified by these means were detected and are characterized in the tables by equivalent chain length. Of the several acids identified, two, to our knowledge, have not been reported before, 10,13,16-Docosatrienoic acid (22:3ω6) and 9,12,15,18-tetracosatetraenoic acid (24:4ω6) were isolated from beef testis lipid, the structures determined via ozonolysis, and characterized by retention time data on GLC analysis.

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Alkaline Methylene Blue Method for Determination of Anionic Surfactants and for Amine Oxides in Detergents

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Abstract

The concn of anionic surfactants in alkaline media has been determined by modification of the methylene blue titration. The method is based on the oxidation of methylene blue chloride to dimethylthionoline, a red dye, in the presence of chloroform and sodium hydroxide. This method in conjunction with the acid methylene blue titration, also may be used to determine the amt of amine oxides in formulated products. For concns of less than 100 ppm a spectrophotometric method was used.

Introduction

ANIONIC SURFACTANTS in alkaline solutions are used industrially in applications such as textile processing, metal cleaning, bottle washing, and fruit and vegetable peeling. In order to maintain the concn of surfactant in the cleaning bath, a quick method of analysis is desirable. The total anionic surfactant historically has been determined by titration with quaternary surfactant using methylene blue as an indicator (2), in an acidic system. Longwell and Maniece (4) reported that with aqueous solutions about pH 7 the chloroform layer turned various shades of pink and blue interfering with the end point. To destroy these colors successive extractions with buffered salt and neutral methylene blue indicator (1,4) were required. A spectrophotometric technique using methyl green (5) as the indicator also required the surfactant solution to be acidic. A titrimetric method using bromocresol green (3) as an indicator for the measurement of anionic surfactants in alkaline media has been published.

An investigation of methods for determining the amt of anionic surfactant in highly alkaline systems led to a modification of the methylene blue titration. The method proposed has been extended to include an analysis for amine oxides in formulated products, based on the cationic nature of amine oxides at low pH and nonionic nature at high pH. Concn ranges investigated titrimetrically were 10 to 0.01% actives. Spectrophotometrically the procedure appears sensitive to 10 ppm.

Experimental

Reagents

Methylene Blue Indicator. Dissolve 0.03 g methylene blue chloride (methylthionine chloride) in 50 ml water. Add 6.6 ml 96% sulfuric acid and mix. Add 20.0 g sodium sulfate (anhydrous) and dissolve. Add water to make up to 1 liter.

Hyamine 1622 Solution (ca. 0.0040 N). Dissolve 1.814 g Hyamine 1622, di-isobutyl phenoxy ethoxy ethyl dimethylbenzyl ammonium chloride (Rohm and Haas), in water and dilute to 1.0 liter. Standardize

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