Fate of linoleic, arachidonic, and docosa-7,10,13,16-tetraenoic acids in rat testicles

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Abstract A comparative study was made on the fate of linoleic, arachidonic, and docosa-7,10,13,16-tetraenoic acids in various subcellular fractions of liver and testis from rats of different ages. It was demonstrated that testicular microsomes can desaturate and elongate linoleic and arachidonic acids in a manner similar to liver microsomes, and that testicular mitochondria can convert docosa-7,10,13,16-tetraenoic acid to arachidonic acid. Testicular or liver microsomes actively desaturate linoleic acid to γ-linolenic acid and eicosa-8,11,14-trienoic acid to arachidonic acid. However, it was impossible to measure in vitro any direct conversion of adrenic acid (22:4 [n − 6]) to docosapentaenoic acid (22:5 [n − 6]) by either liver or testicular microsomes. Docosa-7,10,13,16-tetraenoic acid is incorporated preferentially into the triglyceride fraction of total testis, mitochondria, and microsomes, while linoleic and arachidonic acids are incorporated more into phospholipids. The capacity of testicular microsomes, but not of liver microsomes, to synthesize polyunsaturated fatty acids declines with age. It is proposed that the synthesis of acids of the linoleic family proceeds in two stages, a rapid one in which arachidonic acid is made and a second, slower, one in which C_{22} and C_{24} acids are synthesized. In addition, there appears to be a cycle between microsomes and mitochondria that acts to conserve essential polyunsaturated C_{20} and C_{22} fatty acids by means of synthesis and partial degradation, respectively. This cycle would restrict the loss of essential fatty acids and might be of importance for the supply of arachidonic acid in testis under specific requirements and especially in older animals.

Supplementary key words eicosa-8,11,14-trienoic acid · docosa-4,7,10,13,16-pentaenoic acid · fatty acid synthesis · fatty acid elongation · desaturation

Abbreviations: TLC, thin-layer chromatography; fatty acids are designated as number of carbon atoms:number of double bonds.

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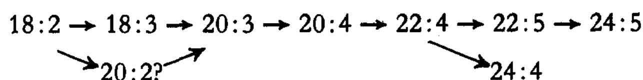
Polyunsaturated fatty acids of the linoleic series (n − 6) seem to play an important role in rat testis; there is marked testicular atrophy in rats that are deficient in essential fatty acids (1–6). Rat testicular lipids contain not only linoleic and arachidonic acids but also considerable amounts of docosa-4,7,10,13,16-pentaenoic acid and even C_{24} fatty acids (1–4, 7). The fact that docosapentaenoic (n − 6) acid is rather abundant in testis but appears in small amounts or is absent in other tissues led us to consider the possibility that this acid plays a specific essential role in that organ.

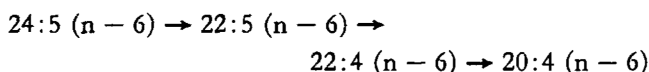
The general outline of the biosynthesis of docosapentaenoic (n − 6) and tetracosapentaenoic (n − 6) acids in testis was developed from studies by Davis and Coniglio (8), Bridges and Coniglio (9, 10), and Nakamura and Privett (11, 12), using direct intratesticular injection of labeled linoleic, arachidonic, and docosapentaenoic acids, or labeled trilinoleate. Peluffo, Ayala, and Brenner (4) also used direct intratesticular injection, and they demonstrated that desaturation of linoleic acid to γ-linolenic acid takes place in the microsomes. All of these studies have led to the conclusion that the synthesis of docosapentaenoic and tetracosapentaenoic acid from linoleic acid occurs in the testis as follows:



Nevertheless, there could be other alternative pathways in which 20:2 (n − 6) or 20:3 (n − 6) are elongated to C_{22} acids without intermediate formation of arachidonic acid. In any event, the synthesis of docosa-4,7,10,13,16-pentaenoic acid proceeds by oxidative desaturation of docosa-7,10,13,16-tetraenoic acid.

The increase of docosapentaenoic (n − 6) acid during testicular development (1, 3) led us to consider the existence of a relationship between this acid and spermatogenesis. Nevertheless, Peluffo et al. (4) have demonstrated that the capacity of the microsomes to desaturate linoleic acid to γ-linolenic acid is greatest prior to the active maturation process and that it diminishes with aging.

Bridges and Coniglio (10) demonstrated that rat testis can convert docosapentaenoic (n − 6) acid to arachidonic acid. Stoffel et al. (13) found that in liver the conversion of C_{22} polyunsaturated fatty acids to C_{20} acids takes place in the inner membranes of mitochondria. In in vivo experiments, Sprecher (14) demonstrated the conversion of docosatetraenoic (n − 6) acid to arachidonic acid in liver. Furthermore, Kunau and Couzens (15) observed that docosapentaenoic (n − 6), docosatetraenoic (n − 6), and tetracosapentaenoic (n − 6) acids are converted to arachidonic acid in liver. These reactions are called retroconversion reactions and the pathway would be then:



$$24{:}5\ (n-6) \rightarrow 22{:}5\ (n-6) \rightarrow 22{:}4\ (n-6) \rightarrow 20{:}4\ (n-6)$$

These results led us to consider that docosatetraenoic (n − 6) acid might play an important role in the testis because it can either be formed from arachidonic acid and converted to docosapentaenoic acid or be formed from docosapentaenoic acid and converted to arachidonic acid. For this reason, a study was made on the fate of labeled linoleic, arachidonic, and docosatetraenoic acids in rat testis in vivo and in vitro.

MATERIALS AND METHODS

Radiochemicals

All-*cis*-[1-^{14}C]linoleic acid, 56.0 mCi/mmole, 99% pure, was from the Radiochemical Centre, Amersham, England. [1-^{14}C]Arachidonic acid, 52.5 mCi/mmole, 99% pure, was purchased from Applied Science Laboratories, State College, Pa. All-*cis*-[2-^{14}C]eicosa-8,11,14-trienoic acid, 55.0 mCi/mmole, 98% pure, was from New England Nuclear, Boston, Mass. All-*cis*-[^{3}H]-docosa-7,10,13,16-tetraenoic acid, 3.06 mCi/mmole, 63% pure, containing less than 3% *trans* isomer, was synthesized by Kunau, Lehmann, and Gross (16). Unlabeled linoleic and arachidonic acids, 99% pure, were purchased from the Hormel Institute, Austin, Minn. Unlabeled all-*cis*-docosa-4,7,10,13,16-pentaenoic acid, 92% pure, was synthesized by Kunau et al. (16). Malonyl CoA was purchased from P-L Biochemicals, Milwaukee, Wis. Other cofactors were from Boehringer, Mannheim.

Animals

Male Wistar rats fed Purina chow ad lib. were used. However, when 3-wk-old animals were needed, they were killed immediately after weaning. All the animals were killed by decapitation at the same time (8:00 a.m.) to avoid circadian variations (17).

Separation of microsomes

The homogenizing solution used for liver and testis was that described by Peluffo et al. (4). However, three volumes of solution were used per volume of liver, while the ratio for testis was 6:1. Microsomes from liver and testis were separated as described previously (4) by differential centrifugation in the cold at 105,000 *g* for 1 hr. The microsomal protein concentration was determined by the procedure of Gornall, Bardawill, and Daud (18).

Measurement of the desaturation and/or elongation of [1-^{14}C]linoleic and [1-^{14}C]arachidonic acids in liver and testis

3-, 5-, and 6-wk-old animals were used. Three pools of 9 animals each were used for the 6-wk-old group, whereas two pools of 9 animals each and one pool of 21 animals were used for the 5- and 3-wk-old groups, respectively. Individual measurements were made in duplicate for each pool and the mean values were calculated. 2 mg or 4 mg of liver or testis microsomal protein, respectively, was incubated with 10 nmoles of [1-^{14}C]linoleic acid or 6 nmoles of [1-^{14}C]arachidonic acid for 2 hr at 37°C. The incubation mixture used to measure the oxidative desaturation contained, in 3 ml of 0.15 M KCl and 0.25 M sucrose, 4 μmoles ATP, 2.5 μmoles NADH, 15 μmoles $MgCl_2$, 4.5 μmoles glutathione, 1 μmole nicotinamide, 125 μmoles NaF, 125 μmoles phosphate buffer (pH 7), and 0.2 μmole CoA (Table 1). In this case, the incubation was performed under air. The oxidative desaturation of eicosa-8,11,14-trienoic acid was investigated by incubation of 3.3 nmoles of 2-^{14}C-labeled 20:3 (n − 6) acid with 1.3 mg of liver or testis microsomal protein from 5-wk-old rats for 20 min at 37°C under air (Table 2). 1 ml of the same incubation mixture was used to investigate the elongation and desaturation of linoleic acid and arachidonic acid, except that 0.05 μmole of malonyl CoA was added and the incubation lasted 2 hr (Table 3).

The complete medium supplemented with 0.05 μmole of malonyl CoA and 3 μmoles of KCN (19) but without CoA was used for elongation studies. For these experiments incubations were performed under argon (Table 4).

In all cases the acid was added in propylene glycol solution and the incubation was stopped by the addition

of 10% KOH in alcohol. The fatty acids were extracted and converted to methyl esters as previously described (20), and the distribution of radioactivity between substrate and product was measured by gas–liquid radiochromatography in a Pye apparatus with a proportional counter (20). The fatty acids were always identified by their retention times and by comparison with authentic standards.

Measurement of the oxidative desaturation of docosa-7,10,13,16-tetraenoic acid

In these experiments, liver and testis microsomes from 5- and 6-wk-old animals were used. They were incubated with either [1-^{14}C]linoleic or [^{3}H]docosa-7,10,13,16-tetraenoic acid. 4 mg of microsomal protein was incubated with 48 nmoles of the labeled acids for 20 min at 37°C in air. The incubation mixture contained in a total volume of 1 ml of 0.15 KCl and 0.25 M sucrose: 4 μmoles ATP, 2.5 μmoles NADH, 15 μmoles $MgCl_2$, 1.5 μmoles glutathione, 0.3 μmole nicotinamide, 41.7 μmoles NaF, 41.7 μmoles phosphate buffer (pH 7), and 0.1 μmole CoA. In additional experiments, 10 and 20 mg of liver or testis microsomal protein from 5-wk-old rats were incubated with 25 and 50 nmoles of the labeled acid, respectively, for 20 min and for 2 hr in a total volume of 3 ml. The cofactor concentrations were those described in the first experiment for the oxidative desaturation of linoleic acid.

The incubations were performed as described previously (4), and the methyl esters of the fatty acids were prepared as above. The distribution of radioactivity between linoleic and γ-linolenic acids was also measured by gas–liquid radiochromatography. However, the conversion of 22:4 to 22:5 was estimated by two procedures, due to its low specific radioactivity. In the first method, unlabeled 22:4 (n − 6) and 22:5 (n − 6) methyl esters were added to the total methyl esters and were chromatographed at 180°C in a Pye gas chromatograph with an ionization detector. The column was packed with 10% diethylene glycol succinate in Chromosorb W (80–100 mesh). The effluents were collected every 3 min in vials containing 10 ml of scintillation solution (4 g 2,5-diphenyloxazole and 100 mg 1,4-bis-2-[5-phenyloxazolyl]-benzene per liter in toluene). The radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer with a counting efficiency of 33% for ^{3}H.

In the second procedure, the same carrier methyl esters (22:4 [n − 6] and 22:5 [n − 6]) were added, and the individual esters in the mixture were separated by TLC on silica gel HF impregnated with 10% $AgNO_3$ in ethyl ether–petroleum ether 90:10 (v/v). The spots were visualized with 2,7-dichlorofluorescein. 0.5-cm segments of silica gel were scraped off and added to vials containing the same scintillation solution. The samples were then counted. Recovery of radioactivity from the plate was greater than 80%.

Measurement of the conversion of docosa-7,10,13,16-tetraenoic acid to arachidonic acid

6-wk-old rats were used. The liver and testis mitochondria were separated by the procedure of Schneider (21), modified by Bygrave (22), and the proteins were determined by the method of Gornall et al. (18).

0.5 μmole of [^{3}H]docosa-7,10,13,16-tetraenoic acid (ammonium salt) was incubated with 10 mg of mitochondrial protein for 60 min at 37°C. The total volume was 3.4 ml and contained 10 μmoles ATP, 1 μmole CoA, 1 μmole NAD, 10 μmoles $MgCl_2$, 500 μmoles phosphate buffer (pH 7.4), and 10 μmoles carnitine. The reaction was stopped by the addition of 10% KOH in alcohol, and the fatty acids were extracted and methylated as described previously (20). The distribution of label was measured by gas–liquid radiochromatography. Radioactivity was detected only in arachidonate and docosatetraenoate (n − 6) and identified by comparison with standards of arachidonate and docosatetraenoate (n − 6).

Incorporation of [^{3}H]docosatetraenoic (n − 6) acid into mitochondrial lipids

In another experiment in which 22:4 (n − 6) was incubated with mitochondria, the reactions were stopped by the addition of 20 vol of chloroform–methanol 2:1 (v/v) containing 1% 0.25 N HCl. The lipids were extracted by the procedure of Folch, Lees, and Sloane Stanley (23) and partitioned with 0.37% KCl instead of water. The lipids were then separated by TLC on silica gel G on 0.20 × 0.40 cm plates which were developed sequentially with two solvent mixtures. Polar lipids were separated by development in chloroform–methanol–water 65:25:4 (v/v/v) until the solvent front reached a height of 20 cm. The plate was then dried and the neutral lipids were separated immediately with petroleum ether–ethyl ether–acetic acid 80:20:1 (v/v/v). The spots that corresponded to lecithin and triglycerides were identified by comparison with standards. They were scraped off and esterified directly by heating with 3 N HCl in methanol for 3 hr at 56°C (20). The methyl esters were extracted, and the distribution of radioactivity between 20:4 (n−6) and 22:4 (n − 6) was measured by gas–liquid radiochromatography.

In other samples, lipids were separated by TLC and the spots were visualized with iodine vapors. The appropriate areas were scraped off and the lipids were directly esterified with 3 N HCl in methanol. In this case, the methyl esters were extracted and radioactivity

TABLE 1. In vitro oxidative desaturation of linoleic acid to γ-linolenic acid by testis and liver microsomes from rats of different ages[a]

Age	Testis	Liver
wk	*% conversion*[b]	
3	33.0 (32.8–33.4)	24.4 (22.7–26.5)
5	12.7 (12.4–13.0)	31.2 (29.5–32.0)
6	10.1 (8.0–12.3)	22.7 (21.1–25.2)

[a] 10 nmoles of [1-^{14}C]linoleic acid was incubated for 2 hr at 37°C with microsomes in air.
[b] Results are means and ranges (in parentheses) of values of at least two samples.

TABLE 2. Oxidative desaturation of [1-^{14}C]eicosa-8,11,14-trienoic acid to arachidonic acid and [1-^{14}C]linoleic to γ-linolenic acid by the microsomes of testis and liver[a]

	Testis	Liver
	% conversion[b]	
20:3 (n − 6) →20:4 (n − 6)	12.1 (11.9–12.2)	58.2 (52.5–63.8)
18:2 (n − 6) →18:3 (n − 6)	16.9 (16.0–18.1)	21.7 (19.3–24.4)

[a] 3.3 nmoles of the labeled acids was incubated with 1.3 mg of microsomal protein for 20 min at 37°C in air in a total volume of 1 ml.
[b] Results are means and ranges (in parentheses) of values of at least two samples. Lots of two animals each were used.

was counted as described above. By this procedure, the incorporation of labeled acids into the lipids was measured. The recovery on the plates was greater than 80%.

In vitro incorporation of [1-^{14}C]linoleic, [1-^{14}C]arachidonic, and [^{3}H]docosatetraenoic (n − 6) acids into microsomal lipids of liver and testis

4 mg of liver or testis microsomal protein from 6-wk-old rats was incubated at 37°C with 69 nmoles of labeled 18:2 (n − 6), 20:4 (n − 6), or 22:4 (n − 6); the cofactors were those used when measuring the desaturation of 22:4 (n − 6) acid. In some experiments, 0.67 μmole of lysolecithin (Nutritional Biochemicals Corp., Cleveland, Ohio) was included in the incubation solution. After 20 min, the incubation was stopped by the addition of chloroform–methanol 2:1 (v/v) containing 1% 0.25 N HCl, and lipids were extracted (23). The lipids were separated by TLC on silica gel G with chloroform–methanol–water 65:25:4 (v/v/v). In this case, the lipids, visualized with iodine vapor, were extracted by the procedure of Arvidson (24), and the radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer, having an efficiency of 92% for ^{14}C and 33% for ^{3}H. The recovery of lipid from the plates was greater than 85%.

In vivo incorporation of [1-^{14}C]linoleic, [1-^{14}C]arachidonic, and [^{3}H]docosatetraenoic (n − 6) acids into rat testis

3-month-old rats were injected directly in one testicle, by the procedure used by Bridges and Coniglio (9), with 0.12 μmole of labeled 18:2 (n − 6), 20:4 (n − 6), or 22:4 (n − 6) per 100 g body weight. The acids were injected as ammonium salts dissolved in 50 μl of 0.1 M phosphate buffer (pH 7.4) containing 4 mg of albumin. The animals were killed 1 hr later, and testicles and livers were immediately excised. The lipids of each organ were quantitatively extracted (23) and dissolved in petroleum ether, and the radioactivity of an aliquot was measured. Lipids from the injected testis were separated by TLC, using the procedure described for mitochondria, and the distribution of radioactivity was measured. The recovery of radioactivity from the plates was greater than 80%.

RESULTS

Effect of age on the microsomal capacity of testis and liver to elongate and desaturate linoleic, arachidonic, and docosatetraenoic (n − 6) acids

Hepatic and testicular microsomes from 3-, 5-, and 6-wk-old rats were incubated in air in the presence of NADH, ATP, $MgCl_2$, and CoA with labeled linoleic acid. The results (Table 1) show a high and exclusive conversion to γ-linolenic acid in both testis and liver. However, whereas the oxidative desaturation for liver was the same at all ages, a decline in activity was found for the testis from 3 wk on. This agrees with previous results of Peluffo et al. (4) and may be related to an aging of the organ after an active period in some way related to maturation.

The microsomes of liver and testis of 5-wk-old rats were also very active in desaturating eicosa-8,11,14-trienoic acid to arachidonic acid (Table 2). But both 5-desaturation and 6-desaturation were more active in liver than in testis at this age.

In subsequent experiments, a comparative study was carried out to determine the capacity of testis and liver microsomes to desaturate linoleic acid to γ-linolenic acid and docosa-7,10,13,16-tetraenoic acid to docosa-4,7,10,13,16-pentaenoic acid. Whereas linoleic acid was actively desaturated to γ-linolenic acid by the 6-desaturase of liver and testis, no measurable conversion of docosa-7,10,13,16-tetraenoic acid to docosa-4,7,10,-13,16-pentaenoic acid was found using the same microsomes. Similar results were also obtained in a 2-hr incubation of microsomes from 6-wk-old rats maintained, after weaning, on a fat-deficient diet to decrease the content of n − 6 acids. These findings suggest that the 4-desaturation of fatty acids was not operative under

TABLE 3. In vitro desaturation and elongation of [1-^{14}C]linoleic and [1-^{14}C]arachidonic acids by liver and testicular microsomes of rats of different ages[a]

Labeled Fatty Acids	Testicle, Age (wk) 3		5	6		Liver, Age (wk) 3		5		6	
	18:2	20:4	18:2	18:2	20:4	18:2	20:4	18:2	20:4	18:2	20:4
18:2	72.5 (72.3–72.7)		81.1 (80.2–81.6)	89.6 (84.6–93.7)		74.8 (74.3–75.4)		70.6 (70.3–70.8)		73.8 (72.4–74.5)	
18:3	4.9 (4.6–5.2)		4.6 (4.6–4.8)	3.5 (1.3–5.2)		7.1 (7.0–7.2)		8.5 (8.4–8.9)		5.0 (4.7–5.4)	
20:2	5.2 (5.0–5.3)		2.9 (2.2–3.5)	1.3 (0.7–1.8)		0.8 (0.6–1.0)		1.0 (0.9–1.1)		0.8 (0.6–1.0)	
20:3	8.4 (8.1–8.6)		5.1 (4.8–5.8)	2.2 (1.8–2.8)		2.5 (2.3–2.7)		2.1 (2.0–2.2)		3.2 (2.9–3.9)	
20:4	6.2 (6.0–6.5)	90.9 (90.8–91.0)	5.1 (4.0–6.0)	2.0 (0.4–3.6)	96.0 (95.9–96.1)	10.7 (10.5–10.9)	88.9 (88.8–88.9)	17.3 (17.0–17.4)	93.1 (93.0–93.2)	16.6 (15.7–17.0)	95.7 (95.6–95.8)
22:2	tr		0.4 (0.4–0.5)	0.8 (0.5–1.2)		0.4 (0.3–0.5)				0.1 (0.1–0.2)	
22:4		6.6 (6.3–6.8)			4.0 (3.8–4.1)	tr	9.5 (9.1–9.9)		6.9 (6.8–7.0)		4.3 (4.2–4.4)
22:5	1.7 (1.6–1.9)		0.8 (0.8–0.9)	0.6 (0.5–0.8)		2.4 (2.0–2.8)		0.5 (0.5–0.6)		0.5 (0.4–0.5)	
X[b]		2.5 (2.4–2.5)					1.6 (1.3–1.9)				
24:4	1.1 (0.9–1.3)	tr				1.3 (1.1–1.5)					

[a] 10 nmoles [1-^{14}C]linoleic acid and 6 nmoles of [1-^{14}C]arachidonic acid were incubated for 2 hr with microsomal proteins of testicle or liver (4 mg or 2 mg, respectively) as described in the text. Results are means and ranges (in parentheses) of values of the percentage distribution of radioactivity in the fatty acids of at least two samples.
[b] Not identified.

the conditions of the experiment or had a very low activity that was not measurable.

In spite of the failure to demonstrate the direct desaturation of docosatetraenoic (n − 6) acid by liver and testis microsomes, it was possible to observe that microsomes of both organs were able to convert labeled linoleic acid to docosapentaenoic (n − 6) acid in the presence of malonyl CoA, NADH, ATP, and CoA. The results (Table 3) show that after a 2-hr incubation there was some labeling of docosapentaenoic (n − 6) acid and somewhat more ^{14}C in 18:3, 20:2, 20:3, and 20:4. Some ^{14}C was also found in 22:2 and 24:4. Therefore, microsomes from both organs were able to desaturate and elongate linoleic acid by similar routes. Liver synthesized a higher proportion of arachidonic acid than testis, which showed a significant decline in synthesis with aging.

In the incubation of labeled arachidonic acid, both liver and testis microsomes were able to elongate arachidonic acid to 22:4 (n − 6), but desaturation to 22:5 (n − 6) was not observed (Table 3). This would corroborate the absence or a very low activity of the 4-desaturation of 22:4 (n − 6) (Table 1).

Microsomes of liver and testis were also incubated under strictly elongating conditions with labeled linoleic or arachidonic acid (Table 4). In these experiments, both types of microsomes actively elongated linoleic acid to 20:2 (n − 6) acid, and labeling was also found in the higher homologs 22:2 and 24:2. Arachidonic acid was elongated to 22:4, and the younger animals also synthesized 24:4. Except for this latter observation, no important decline of the elongating activity was found for either liver or testis.

These results emphasize two facts: *1*) both liver and testis microsomes elongate and desaturate linoleic acid to the polyunsaturated acids of 22 and 24 carbons, but the in vitro activity of the 4-desaturase to desaturate 22:4 (n − 6) acid is very low; and *2*) there is a decline in the synthesis of polyunsaturated fatty acids with aging that is due to a decrease primarily in microsomal desaturation and, to a lesser extent, elongation. This decline is clearly shown in the testis but not in the liver.

In vitro incorporation of linoleic, arachidonic, and docosatetraenoic acids into microsomal lipids

Data on the incorporation of labeled linoleic, arachidonic, and docosatetraenoic (n − 6) acids into microsomal lipids of testis and liver after a 20 min incubation at 37°C appear in Tables 5 and 6, respectively. All fatty acids were incorporated mainly into phosphatidylcholine and neutral lipids, and liver was more active than testis. However, incorporation of 22:4 (n − 6) was remarkably less than that of linoleic and arachidonic acids. In addition, it was preferentially incorporated

TABLE 4. In vitro elongation of linoleic and arachidonic acids by liver and testicular microsomes of rats of different ages[a]

Labeled Fatty Acids	Testicle Age (wk) 3 18:2	Testicle 3 20:4	Testicle 5 18:2	Testicle 5 20:4	Testicle 6 18:2	Testicle 6 20:4	Liver Age (wk) 3 18:2	Liver 3 20:4	Liver 5 18:2	Liver 5 20:4	Liver 6 18:2	Liver 6 20:4
18:2	85.7 (84.0–87.5)		86.2 (86.0–86.4)		91.2 (90.9–91.7)		75.3 (74.4–76.3)		72.2 (72.1–72.2)		74.0 (74.0–74.1)	
20:2	12.0 (10.3–13.6)		12.0 (11.9–12.1)		7.4 (7.0–7.7)		20.7 (19.7–20.7)		27.1 (27.1–27.2)		24.7 (24.4–24.8)	
20:4		96.0 (95.5–96.2)		93.4 (93.3–93.5)		94.3 (94.1–94.5)		84.0 (83.8–84.2)		93.1 (93.0–93.2)		91.3 (91.2–91.4)
22:2	1.2 (1.0–1.4)		1.2 (0.9–1.3)		0.8 (0.6–0.9)		2.1 (1.8–2.2)		0.7 (0.6–0.7)		0.6 (0.5–0.8)	
22:4		2.1 (2.0–2.5)		6.6 (6.3–6.9)		5.7 (5.5–5.9)		10.5 (10.1–10.9)		6.9 (6.6–7.2)		8.7 (8.5–8.9)
24:2	1.1 (0.9–1.3)		0.6 (0.6–0.6)		0.6 (0.5–0.6)		1.9 (1.9–2.0)		tr		0.7 (0.6–0.7)	
24:4		1.9 (1.7–2.1)						5.5 (5.3–5.7)				

[a] 10 nmoles of [1-^{14}C]linoleic acid and 6 nmoles of [1-^{14}C]arachidonic acid were incubated for 2 hr with 4 and 2 mg of microsomal protein of testis and liver, respectively, as described in the text. Results are means and ranges (in parentheses) of values of the percentage distribution of radioactivity in the fatty acids of at least two samples.

TABLE 5. Distribution of radioactivity in lipid fractions after incubation of testicular microsomes with [1-^{14}C]linoleic, [1-^{14}C]arachidonic, and [^{3}H]docosatetraenoic (n − 6) acids

Lipid Class	Incubated Acid					
	18:2		20:4		22:4	
	Without LPC[a]	With LPC	Without LPC	With LPC	Without LPC	With LPC
	% of total radioactivity[b]					
PC[a]	19.3[c]	58.2	14.4	48.7	4.5	15.3
	(17.8–20.3)	(57.7–58.8)	(11.1–17.7)	(47.2–50.2)	(3.1–6.0)	(12.4–18.2)
PE + PS	2.1	2.3	3.3	2.6	0.5	0.5
	(1.6–2.8)	(2.1–2.6)	(1.9–4.8)	(2.5–2.8)	(0.3–0.7)	(0.5–0.6)
FFA	59.3	33.5	45.6	43.5	85.1	79.6
	(57.9–59.9)	(32.6–34.4)	(44.3–46.9)	(42.4–44.1)	(82.8–87.4)	(77.1–82.2)
NL	14.9	2.6	22.5	2.4	7.3	2.3
	(13.1–17.3)	(2.4–2.9)	(20.5–24.5)	(2.4–2.5)	(7.3–7.4)	(2.3–2.3)

[a] LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; FFA, free fatty acids; NL, neutral lipids (including triglycerides and cholesteryl esters).
[b] Some radioactivity was in other unidentified fractions.
[c] Means and ranges (in parentheses) of values of at least two samples.

into neutral lipids. The addition of lysolecithin to the incubation medium drastically increased the incorporation of all the acids into phosphatidylcholine in both organs. This increase was at the expense of the free fatty acids and neutral lipids.

The presence of a relatively high amount of radioactive free fatty acid after a 20-min incubation suggests that there was adequate substrate to be desaturated. Therefore, the low activity of the 4-desaturase cannot be ascribed to a reduced amount of 22:4 (n − 6) available for desaturation, due to a high incorporation into lipids.

In vivo incorporation of linoleic, arachidonic, and docosatetraenoic acids into rat testis

We felt that in vivo studies might shed light on reasons for the differences found in the in vitro incorporation of linoleic, arachidonic, and docosatetraenoic (n − 6) acids into testicular lipids. As previously demonstrated (9, 10), the direct intratesticular injection of the ammonium salts of the labeled linoleic, arachidonic, and docosatetraenoic acids, complexed with albumin, allows a study of the incorporation of the acids into lipids of the injected organ. The possibility that the present results might have been due to a testicular–hepatic circulation is eliminated by the very low incorporation of any of the acids in the noninjected testicle or in liver (Table 7). Distribution of radioactivity among testicular lipids is detailed in Table 8. 1 hr after injection, incorporation was appreciable in most cases. However, as found in the in vitro experiments, 22:4 (n − 6) was incorporated less than the other acids. The predominant incorporation of linoleic and arachidonic acids into phospholipids and of docosatetraenoic acid into triglycerides is clearly

TABLE 6. Distribution of radioactivity in lipid fractions after incubation of liver microsomes with [1-^{14}C]linoleic, [1-^{14}C]arachidonic, and [^{3}H]docosatetraenoic (n − 6) acids

Lipid Class	Incubated Acid					
	18:2		20:4		22:4	
	Without LPC[a]	With LPC	Without LPC	With LPC	Without LPC	With LPC
	% of total radioactivity[b]					
PC[a]	29.5[c]	79.8	24.2	68.4	4.0	67.5
	(25.9–33.1)	(78.1–83.2)	(24.2–24.3)	(64.7–72.2)	(2.8–5.2)	(61.0–74.1)
PE + PS	4.0	0.7	7.4	1.6	1.0	0.6
	(2.9–5.1)	(0.3–1.2)	(7.4–7.4)	(1.5–1.8)	(0.9–1.1)	(0.6–0.7)
FFA	24.6	12.0	39.0	16.6	67.7	26.5
	(19.6–29.3)	(10.4–13.5)	(39.0–39.0)	(16.3–17.0)	(60.0–75.5)	(20.2–32.6)
NL	28.2	1.0	19.3	1.8	12.3	2.3
	(27.1–29.3)	(0.8–1.2)	(19.1–19.4)	(1.7–1.9)	(10.6–14.0)	(2.2–2.5)

[a] LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; FFA, free fatty acids; NL, neutral lipids (including triglycerides and cholesteryl esters).
[b] Some radioactivity was in other unidentified fractions.
[c] Means and ranges (in parentheses) of values of at least two samples.

shown. These results corroborate the in vitro experiments and point out that 22:4 (n − 6) is stored principally in the triglycerides of the testicular cells.

Conversion of docosatetraenoic (n − 6) acid to arachidonic acid

In order to have a more general picture of the fate of 22:4 (n − 6) acid in the testis, it was necessary to study its conversion to arachidonic acid. This was measured by incubating the acids with the mitochondria from the testis or liver for 60 min. The results of four experiments showed that in liver 14.8 ± 0.8% of labeled docosatetraenoic (n − 6) was converted to arachidonic acid, while testis mitochondria converted 2.6 ± 0.2% of the incubated 22:4 (n − 6).

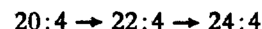
During the incubation, 32.7% and 39.6% of the incubated acid was incorporated into the mitochondrial lipids of testis and liver, respectively. However, whereas in testis 73.4% of the incorporated radioactivity was found in the triglycerides, only 6.3% was found in liver triglycerides.

The fatty acid analysis of liver and testis triglycerides by gas–liquid radiochromatography showed that both docosatetraenoic (n − 6) acid and arachidonic acid were incorporated (Table 9). It was also shown that relatively more arachidonic acid was incorporated into liver phosphatidylcholine than into liver triglycerides.

DISCUSSION

The general scheme of the biosynthesis of unsaturated fatty acids of the linoleic family in the testis, deduced from the work of Davis and Coniglio (8) and Nakamura and Privett (11), is confirmed by the present experiments. The data indicate that the biosynthesis occurs in testicular microsomes by elongation and desaturation reactions similar to those of liver. The results in Tables 3 and 4 show that when testis or liver microsomes are incubated with linoleic acid under conditions for elongation, the following sequence of reactions takes place:

Arachidonic acid is similarly elongated by the microsomes:



The microsomes of testis have an active 6-desaturase that desaturates linoleic acid to γ-linolenic acid (Table 1) and also a 5-desaturase that converts 20:3 (n − 6) to arachidonic acid (Table 2). However, there was a remarkably high degree of labeling of 20:2 and 20:3 in the testis compared with liver when the microsomes were incubated with linoleic acid under simultaneous elongating and desaturating conditions (Table 3). This difference is especially significant when the labeling of 20:2 and 20:3 is compared with arachidonic acid. A possible explanation for this difference is that the 5-desaturase of testis is less active than the similar enzyme of liver, whereas the elongating enzymes have the same or somewhat more activity. The results of Table 2 not only confirm that the 5-desaturase of liver is more active than the similar enzyme of testis, but also that the 5-desaturase of testis is a little less active than the 6-desaturase. In liver, the 5-desaturase is more active than

TABLE 7. Distribution of radioactivity in testis and liver 1 hr after the injection of labeled acids in one testicle

Fatty Acid Injected	Testis			% Recovery in Injected Testicle
	Injected	Noninjected	Liver	
	% of total radioactivity recovered			
18:2	91.1 ± 1.3	3.0 ± 0.7	5.4 ± 0.6	49.7 ± 1.8
20:4	95.6 ± 0.03	1.3 ± 0.4	3.1 ± 0.5	47.2 ± 1.5
22:4	88.6 ± 1.1	2.3 ± 0.3	8.8 ± 1.5	48.2 ± 4.2

Results are mean values of triplicate samples ± SE.

TABLE 8. Incorporation of [1-^{14}C]linoleic, [1-^{14}C]-arachidonic, and [^{3}H]docosatetraenoic (n − 6) acids into testicular lipids 1 hr after direct intratesticular injection

Lipid Class[a]	Injected Acids		
	18:2	20:4	22:4
	% of total radioactivity[b]		
Free fatty acid	3.8 (2.6–6.3)	9.1 (5.9–12.3)	14.2 (11.9–15.7)
Phosphatidylcholine	63.1 (61.9–64.9)	37.0 (30.1–44.0)	8.9 (3.9–12.0)
Phosphatidylserine + phosphatidylethanolamine	8.0 (7.4–8.9)	8.7 (8.3–9.3)	4.7 (4.0–5.5)
Triglycerides	17.6 (14.6–20.4)	26.2 (21.5–31.0)	60.5 (52.6–65.9)
Cholesteryl esters	0.3 (0.2–0.4)	1.4 (1.4–1.5)	2.2 (1.8–2.7)
Other fractions	7.2	17.6	9.5

[a] Lipids were separated by TLC as described in the text, and percentage distribution of labeling was measured.
[b] Means and ranges (in parentheses) of values of at least three samples.

TABLE 9. Distribution of radioactivity between docosatetraenoic (n − 6) and arachidonic acids in lecithin and triglycerides after incubation of mitochondria with [^{3}H]docosatetraenoic (n − 6) acid

Lipid Fractions	Testis		Liver	
	22:4	20:4	22:4	20:4
	% of radioactivity in tetraenoic acids			
Lecithin			78.6	21.3
Triglycerides	87.2	12.8	90.7	9.3

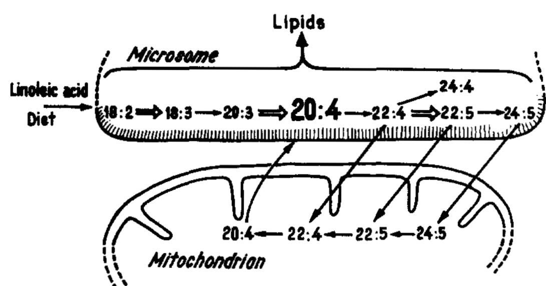


FIG. 1. Biosynthesis and retroconversion of acids of the linoleic series in testicles.

the 6-desaturase. The existence of very active 5-desaturases in liver has been reported by Castuma, Catalá, and Brenner (25) and by Ullman and Sprecher (26).

It is important to consider that 20:3 must not necessarily be all 8,11,14 isomer, the normal product of the elongation of γ-linolenic acid (octadeca-6,9,12-trienoic acid), but it may include a high proportion of eicosa-5,11,14-trienoic isomer. Ullman and Sprecher (27) have recently demonstrated that this latter acid can be synthesized from eicosa-11,14-dienoic acid in liver. The extremely high degree of labeling of 20:2 found in testis (Table 3) would also suggest this possibility. The aforementioned authors demonstrated not only that 20:2 (n − 6) is mainly desaturated to 5,11,14-20:3, but also that the trienoic acid is apparently not converted to arachidonic acid. Therefore, the high degree of labeling of 20:3 may also be due to the presence of the 5,11,14 isomer, which may represent a dead end in the series of reactions that occur with the acids of the linoleic family. Unfortunately, due to the small amount of available substrate, the structure of 20:3 was not determined to confirm this hypothesis.

The failure to measure the in vitro desaturation of docosatetraenoic (n − 6) acid to docosapentaenoic (n − 6) acid (Tables 1 and 3) may be attributed to a very low activity of the desaturating enzyme, not detected in the conditions of the experiment. It would be very difficult to consider the existence of any other logical series of reactions to synthesize docosapentaenoic (n − 6) acid bypassing 22:4 (n − 6) desaturation.

In conclusion, these experiments would suggest the existence of two main stages in the synthesis of acids of the linoleic family. The first, a very active one, would end in arachidonic acid, a major component of tissue phospholipids. The second stage would seem to be a slower one, apparently due in part to the low activity of 22:4 (n − 6) desaturase. This latter stage would include the biosynthesis of 22:5 (n − 6), a major component of the testicular lipids, and 24:5 (n − 6) from C_{20} acids. This conclusion is supported by the work of Nakamura and Privett (11) and Bridges and Coniglio (9), who showed that the injection of labeled linoleic acid into the testis evoked a rapid synthesis of arachidonic acid and only a slow linear synthesis of 22:5 (n − 6) acid.

Tables 1, 3, and 4, as well as previous experiments (4), reveal a progressive decline with age of the desaturation and of the desaturation and elongation reactions in the microsomes of the testis but not of liver. This decline would be consistent with a progressively smaller requirement of highly polyunsaturated acids for the building of the necessary structures of the testis formed before and during puberty. On the other hand, in the liver, continuous lipoprotein synthesis and regeneration of the tissue occur during the whole life span, and therefore a high activity is always necessary.

Concomitant with the decrease of the desaturating capacity of the microsomes of the testis with age, there is an increase in the proportion of docosapentaenoic acid (1–4). Therefore, in spite of the apparent importance of the acid in the testis maturation (6), the highest amount of 22:5 (n − 6) is found when the synthesis of the polyunsaturated fatty acids, including arachidonic acid, is lowest and the maturation of the testis is over. This accumulation of docosapentaenoic acid with age suggests another function of the acid.

Studies on the lipid composition of testis by Oshima and Carpenter (2) and by Carpenter (3) demonstrate that 22:5 (n − 6), 22:4 (n − 6), and polyunsaturated acids of 24 carbons appear in high concentrations in triglycerides, whereas the relative proportion of linoleic and arachidonic acids in these lipids is low. Thus, C_{22} and C_{24} polyunsaturated acids are found principally in lipids whose main function is storage, whereas linoleic and arachidonic acids are mainly found in the phospholipids. The results of Tables 5 and 8, which show the incorporation of linoleic, arachidonic, and docosatetraenoic (n − 6) acids into lipids of the testis, also show a relatively preferential incorporation of linoleic and arachidonic acids in the phospholipids, whereas docosatetraenoic acid is found mainly in the triglycerides.

These results suggest, therefore, that at least part of the increase of the C_{22} and C_{24} polyunsaturated acids with aging, especially in the triglycerides, is for storage of these acids. This reserve function becomes significant when one considers that: (*a*) 22:4 (n − 6), 22:5 (n − 6), and 24:5 (n − 6) are converted to arachidonic acid (10, 13–15); (*b*) arachidonic acid, but not linoleic acid, seems to play an important role in testis, for it prevents the testicular atrophy produced by diabetes (28); and (*c*) arachidonic acid is a precursor of prostaglandin E_2 (29). In addition, the physiological function of the testis is controlled by mechanisms completely different from those of liver. They must be independent of the general circadian variations (17), and at the same time must satisfy the needs for special substrates.

These results suggest the existence of a cycle between microsomes and mitochondria in which the microsomes

synthesize the polyunsaturated acids of 22 and 24 carbons from linoleic acid. These acids accumulate in the lipids, preferentially in the triglycerides, and the mitochondria can degrade them to arachidonic acid. This cycle, described in Fig. 1, might be very important. It would allow an economical recycling of the essential polyunsaturated fatty acids, and it could provide arachidonic acid locally without the need of synthesis from linoleic acid. Consequently, it could have an important function in spermatogenesis.

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