The formation of long and very long chain (VLC) n-6 polyunsaturated fatty acids (PUFA) in isolated rat seminiferous tubules was investigated by following the metabolism of three 1-14C-labeled n-6 tetraenoic fatty acids (20:4, 24:4, and 32:4) and [U-14C]acetate. In contrast to [14C]32:4, which was poorly incorporated and altered, [14C]20:4 and [14C]24:4 were efficiently taken up by the tubules, esterified into lipids, elongated to VLCPUFA, and desaturated to pentaenoic fatty acids; the rate of [14C]24:4 desaturation to [14C]24:5 was notably high. The main products with [14C]acetate as precursor were labeled saturates and VLCPUFA, most of the label in tetraenoic and pentaenoic acids appearing in 24:4 and 24:5, respectively. These two C24 polyenes, connected by Δ6 desaturation, may play a central role in n-6 PUFA metabolism, in their capacity as potential precursors of longer polyenes via elongation and of shorter ones, such as 22:5n-6, via retroconversion. Triacylglycerols, rich in C22 and C24 polyenes, incorporated the greatest amounts of both [14C]acetate-derived and exogenous 14C-PUFA, suggesting that this lipid class is involved in the traffic and metabolism of testicular PUFA. The detection of a series of unusual odd-chain tetraenoic and pentaenoic fatty acids, also labeled with [U-14C]acetate, suggests that a PUFA chain shortening mechanism occurs in testis involving α- in addition to β-oxidation. We speculate that α-oxidation plays a role in the retroconversion of PUFA.

Docosapentaenoic (22:5n-6)¹ and arachidonic (20:4n-6) acids are known to be abundant polyunsaturated fatty acids (PUFA) in the lipids of the male reproductive tract in many mammalian species. In rat testis, where these two fatty acids are major PUFA constituents, n-6 pentaenoic and tetraenoic fatty acids with chain lengths in excess of 22 carbons, such as 24:5n-6 and 24:4n-6, have been isolated and characterized as normal products of the elongation and desaturation of 20:4n-6 (1-3), in turn known to derive from the essential fatty acid 18:2n-6. Also in rat testis, Grogan (4) characterized n-6 tetraenes and pentaenes with very long chains (VLC), such as 28:4n-6 and 30:5n-6, which were labeled with 14C 2 days after intratesticular injections of [1-14C]20:4n-6. Work from our own laboratories has shown that n-6 tetraenoic and pentaenoic as well as n-3 pentaenoic and hexaenoic fatty acids with chain lengths from 24 to 34 carbons (VLCPUFA) occur in mammals. They have been characterized in the spermatozoa of a variety of species including man (5) and in the retina photoreceptor cells of several vertebrates (6, 7). Whereas the VLCPUFA of retina are specifically concentrated in phosphatidycholine (PC) (7), those of spermatozoa are important components of sphingomyelin (SM) (8). Recent reports show that in several mammals spermatozoa and testis SM also contains 2-hydroxy derivatives of n-3 and n-6 VLCPUFA, those of the n-6 series predominating in rat (9).

Much is still to be learned on the biochemistry of VLCPUFA and their derivatives. In the present work, the metabolism of these polyenes has been studied in isolated seminiferous tubules from rat testis using three 1-14C-labeled n-6 tetraenoic fatty acids and [U-14C]acetate as precursors. Our purpose was to compare how tetraenoic fatty acids of different chain lengths, 20:4n-6, 24:4n-6, and 32:4n-6, are processed in the testicular tissue and to determine whether seminiferous tubules are able to produce labeled n-6 tetraenes and pentaenes from [14C]acetate, in the manner very long (n-3) pentaenes and hexaenes were shown to be synthesized in mammalian retina (10). During the course of our research into this specific area, we have come up with interesting findings on more general aspects of PUFA metabolism. We report results which are relevant to the mechanism of Δ4 desaturation of n-6 PUFA, suggest the involvement of triacylglycerols in PUFA metabolism, and show the occurrence of unusual odd chain tetraenoic and pentaenoic fatty acids which reveal the existence of non-conventional pathways in PUFA metabolism.
MATERIALS AND METHODS

[1-14C]Arachidonic acid (50–60 Ci/mol) and [U-14C]acetate acid, sodium salt (50–60 Ci/mol), were from Amerham International, Amersham, United Kingdom. [1-14C]Tetracatatetraenoic acid (24:4n-6) (50–60 Ci/mol) was prepared as described previously (11). [1-14C] Dotriscatatetraenoic acid (32:4n-6) (50–60 Ci/mol) was synthesized according to the procedure of Johnson (12). The fatty acids were purified using the chromatographic procedures described below.

Male Porton rats aged 6 weeks were killed by CO2 asphyxiation and the testes rapidly excised. Seminiferous tubules were obtained from the testes essentially as described in Refs. 13 and 14, except that the medium used was Dulbecco’s modified Eagle’s medium (DMEM), high glucose (25 mm), containing glutamine and sodium pyruvate (GIBCO-BRL), to which 50 and 100 mg/liter of penicillin and streptomycin, respectively, and 3.7 g/liter of sodium bicarbonate had been added. Gently decapsulated testes were incubated in standard plastic culture flasks for 15 min at 30 °C in 10 ml of the above medium containing collagenase (1 mg/ml), using an agitation of 50 cycles/min. The flask caps were pierced with syringe needles to allow oxygen in- and out-flow of gases during incubation. The gas phase consisted of a water-saturated medical air atmosphere (5% CO2). The incubations with the enzyme were followed by two successive washings, each with 10 ml of fresh DMEM medium. The long stretches of seminiferous tubules obtained were separated by decantation from the cells released from the testicular interstitium, pre-dominantly composed of blood cells, macrophages, and Leydig cells (13, 14). The tubules thus prepared were incubated for 1 or 2 h with the radioactive precursors in 10 ml of DMEM containing the 1-14C-labeled fatty acids (1 µCi/sample dissolved in 100 µl of ethanol), or for 1–4 h with [U-14C]acetate (62 µCi/sample dissolved in water). Each sample contained the tubules from 10 ml of medium in 10 ml of DMEM (530 ± 37 µg of total lipid phosphorus/flask). Incubations proceeded at 35 °C, under the mentioned gas atmosphere, with an agitation of 60 cycles/min. The effluent gas was bubbled into KOH to trap the [14CO2] produced.

After incubation, tubules were separated from incubation media by brief centrifugation. In the case of [1-14C]acetate, the collected tubules were resuspended in fresh DMEM and centrifuged again to reduce water-soluble metabolites present in the samples before lipid extraction. Lipid extracts from tubules or media were prepared and partitioned into organic and aqueous phases according to the procedures of Folch et al. (15). After TLC, the lipids were resolved on magnesium acetate-impregnated silica gel plates (Merck). 2 M NaCl was used instead of water to trap the fatty acids not containing phosphorus. Capillary GC-MS was used to confirm the identification of components of the FAME fractions separated by agerontage TLC essentially as described in previous work (5, 8). HPLC of the fractions was performed using acetonitrile as solvent (0.5 ml/min, room temperature) and a glass-lined 0.4 × 25-cm stainless steel column packed with 5-µm particle size octadecylsila (SGE, Victoria, Australia). Fatty acids were detected by their desorption at 192 nm (22) and cohersed for radioactivity measurements, this procedure yielding similar 14C distributions to reverse-phase TLC. Radioactivity was measured in glass vials by standard liquid scintillation counting techniques. When lipids were in the presence of silicon gel, water was added, the vial contents were thoroughly mixed with the scintillation mixture, and the support was sucked into a vial before measurement. In the case of FAME, silver as AgCl and then the mixture. The support was octadecylsila, no water was added to the system.

RESULTS

Metabolic Transformations of [1-14C]20:4n-6, 24:4n-6, and 32:4n-6—When incubated with similar amounts of these fatty acids (~1 µCi, ~1.7 µM), rat seminiferous tubules took up from the medium more of the total [1-14C]20:4 than of the [1-14C]24:4 initially provided, and much more of these two than of the [1-14C]32:4 (90.5, 76.7, and 28.5%, respectively, percentages which were the same after 1 and 2 h of incubation). Most of the label remaining in the media could be accounted for by fatty acids not utilized by the tissue (28.4 < 24:4 < 32:4), but a fraction was (22.4%) recovered as labeled water-soluble metabolites. In lipid extracts prepared both from media and tubules, this water-soluble fraction was the largest for [1-14C] 24:4 (Fig. 1A). Of the radioactivity present in tubules, most of that from [1-14C]24:4 and [1-14C]24:4 was esterified into lipids, whereas most of that from [1-14C]32:4 was remained as free fatty acid.

With all three precursors, but particularly with 24:4, a large proportion of the esterified label was accounted for by non-polar lipids (Fig. 1B). 14CO2 was also produced in all three cases (Fig. 1C), and the amounts were the largest with [1-14C] 24:4 as precursor.

The incorporation and distribution among lipids of the three polyenes was similar after 1 and 2 h of incubation, indicating that a plateau of incorporation had been reached by 1 h (Table I). Most of the label esterified in neutral lipids was concentrated in triglycerides (TG). These were in fact the most highly labeled lipids in all three cases, especially taking into consideration that they amounted to only 15.0 ± 0.6 mol/100 mol of lipid phosphorus. Another highly labeled non-polar lipid was a minor fraction tentatively identified as 1-O-alkyl, 2,3-diacylglycerols (0.6 ± 0.1 mol/100 mol of lipid phosphorus). The proportion of 14C in diacylglycerols (DG) differed with the fatty acid, showing that the utilization of these intermediates for the synthesis of other lipids was more active the shorter the fatty acid (20.4 > 24:4 > 32:4).

Phosphatidylcholine (PC), the major lipid constituent (44.5 ± 1.2 mol/100 mol of total lipid phosphorus), was the most highly labeled phospholipid, followed by phosphatidylinositol (PI), phosphatidylinosine (PS), or ethanolamine glycerophospholipid (EGP), depending on whether the substrate was [1-14C] 20:4, [1-14C]24:4, or [1-14C]32:4, respectively. The proportion of PI and PS was similar, at around 7 mol/100 mol of lipid P,
Distribution of esterified label (%)  
| Precursor                                      | [1-14C]20:4n-6 | [1-14C]24:4n-6 | [1-14C]32:4n-6 | [U-14C]acetate |
|-----------------------------------------------|----------------|----------------|----------------|----------------|
| Phosphatidylcholine                           | 15.6           | 17.7           | 19.5           | 22.6           |
| Plasmalogenylcholine                          | 24.5           | 25.5           | 26.9           | 23.1           |
| Phosphatidylethanolamine                      | 6.7            | 7.5            | 8.7            | 9.2            |
| Plasmalogenylammine                           | 0.4            | 0.7            | 1.0            | 1.2            |
| Phosphatidylserine                            | 0.7            | 1.0            | 1.1            | 1.3            |
| Phosphatidic acid                             | 0.9            | 1.3            | 1.5            | 1.7            |
| Lyso phosphatidylcholine                      | 0.1            | 0.1            | 0.1            | 0.1            |
| Diphosphatidylglycerol                        | 0.9            | 1.1            | 1.3            | 1.5            |
| Sphingomyelin                                 | 0.1            | 0.1            | 0.1            | 0.1            |
| Diaclylglycerol                               | 0.3            | 0.3            | 0.3            | 0.3            |
| Triacylglycerol                               | 4.3            | 4.3            | 4.3            | 4.3            |
| Ether-linked triglycerides                    | 0.1            | 0.1            | 0.1            | 0.1            |

and their fatty acid composition was consistent with the preferential distribution of 14C. PI was the lipid with the highest proportion of 20:4 and the lowest of 24:4 (24 and 0.7% of PI fatty acids), while PS contained 12 and 4%, respectively, of these two components. Although EGP was the second lipid constituent (29.3 ± 0.9 mol/100 mol of lipid P), the turnover of its tetraenoic PUFA was not high; only with [14C]20:4 was plasmalogenylammine (30% of the EGP) significantly labeled. The incorporation of [1-14C]32:4 was poor in all lipid classes of seminiferous tubules, this being consistent with the fact that 32:4n-6 was virtually undetectable as a component. Under similar incubation conditions, rat retinas, which have small but measurable amounts of 32:4n-6 in PC (6), exhibited a relatively higher level of [14C]32:4 incorporation than did seminiferous tubules (not shown).

The modifications undergone by [14C]20:4, 24:4, and 32:4 were studied in FAME prepared from seminiferous tubule total lipid, first separated into groups by degree of unsaturation (Table IIA) and then resolved according to chain length (Table IIIB). Prior to this separation it had been observed that most of the radioactivity was in the three cases in regular fatty acids (>99.8%); however negligible, the conversion into 2-hydroxy-fatty acid derivatives was relatively more active for [14C]32:4 than for [14C]20:4 and [14C]24:4 (0.17, 0.03, and 0.02%, respectively). Desaturation to pentaenoic fatty acids affected each substrate differently, involving more of the total [14C]24:4 than [14C]20:4 and leaving [14C]32:4 unaffected. A considerable proportion of the [14C]20:4 was elongated to produce 22:4, 24:4, and even longer tetraenes; on average, such elongations were more active than the total production of pentaenoic fatty acids. With [1-14C]24:4 as precursor, desaturation to [14C]24:5 was considerably more active than elongation to [14C]26:4; very long labeled pentaenes such as 26:5 and 28:5 were produced, most likely by further elongations of the newly synthesized [14C]24:5 and [14C]26:5. In addition to these changes, label from the three tetraenoic precursors was
A characteristic of adsorption chromatography that, for a given un-
supports. For $^{14}$C\:32:4, the longer the chain, the faster the migration on silica
fatty acid.

inevitable lagging of the highly labeled but "short" $^{14}$C\:20:4, respon-
products reutilized, as was evident from the formation of $^{14}$C\:22:5.

The desaturation and the formation of $^{14}$C\:02 and water-soluble
lipid extracts were converted to fatty acid methyl esters. These were
lipid extracts were converted to fatty acid methyl esters. These were

**TABLE II**

| Precursor | [\(^{14}\)C]\:20:4 | [\(^{14}\)C]\:24:4 | [\(^{14}\)C]\:32:4 |
|-----------|----------------|----------------|----------------|
| Saturates | 14:0 | 15:0 | 16:0 | 17:0 | 18:0 |
| Monoenes | 11.8 | 10.8 | 3.6 | 3.5 | 3.7 |
| Dienes | 0.2 | 0.2 | 4.0 | 4.4 | 4.8 |
| Trienes | 0.2 | 0.4 | 4.2 | 4.4 | 4.8 |
| Tetraines | 5.9 | 6.4 | 12.4 | 12.6 | 12.8 |
| Pentaines | 0.5 | 0.5 | 3.4 | 3.8 | 4.0 |
| Hexaines | 0.1 | 0.1 | 0.5 | 0.5 | 0.5 |

**TABLE III**

| Precursor | [\(^{14}\)C]\:20:4 | [\(^{14}\)C]\:24:4 | [\(^{14}\)C]\:32:4 |
|-----------|----------------|----------------|----------------|
| Saturates | 14:0 | 15:0 | 16:0 | 17:0 | 18:0 |
| Monoenes | 11.8 | 10.8 | 3.6 | 3.5 | 3.7 |
| Dienes | 0.2 | 0.2 | 4.0 | 4.4 | 4.8 |
| Trienes | 0.2 | 0.4 | 4.2 | 4.4 | 4.8 |
| Tetraines | 5.9 | 6.4 | 12.4 | 12.6 | 12.8 |
| Pentaines | 0.5 | 0.5 | 3.4 | 3.8 | 4.0 |
| Hexaines | 0.1 | 0.1 | 0.5 | 0.5 | 0.5 |


**Long and Very Long Chain Polyenoic Fatty Acids**

\(^{14}\)C in fatty acids, only a small percentage (0.19 ± 0.02%) was in 2-hydroxy derivatives. As much as 75% of the label in fatty acids was in saturated components, the rest being mostly concentrated in fatty acids with 1-6 double bonds.

The incorporation of $\text{[U-\(^{14}\)C]acetate}$ into lipids increased continuously between 1 and 4 h of incubation, the rate tending to reach a plateau between 3 and 4 h. A large proportion of the esterified label was in non-polar lipids. As with $\text{[U-\(^{14}\)C]acetate}$ the ether-linked TG were highly labeled in proportion to their mass.

Despite the increasing $\text{^{14}C}$ incorporation over 4 h of incubation, the percentage distribution of label among lipid fatty acid types remained remarkably constant, as indicated by the relatively small deviations in the data in Table III. There were, nevertheless, some significant differences among lipid classes in the proportions of saturated and polyenoic fatty acids incorporated into the various lipid classes. TG contributed most, not only to the actual amounts of radioactivity but also to the pattern of labeling observed in the total lipid. These lipids concentrated the largest share of the total saturated and polyenoic fatty acids synthesized from $\text{[14C]acetate}$ and were the constituents in which the percentage of label in polyenes was the largest.

The distribution of acetate-derived $\text{^{14}C}$ among individual fatty acids separated by chain length (Table IV) was also relatively constant between 1 and 4 h of incubation. More than 80% of the total $\text{^{14}C}$ in saturates was in palmitate. The higher the degree of unsaturation, the larger the proportion of radioactivity in components having longer chains. Tetraines from C20 to C28 and pentaines from C28 to C30 all had some $\text{^{14}C}$ from acetate; albeit with meagre labeling, 30:4 and 32:5 were also detected (Table IV and Fig. 2). Of the total $\text{^{14}C}$ in tetraines and pentaines, the largest proportions were in 24:4 and 24:5, respectively. Comparing the distribution of radioactivity and the composition of the fractions (also shown in Table IV), it was apparent that the lowest relative specific radioactivities among tetranoi and pentaenoic fatty acids corresponded to the major components, 20:4 and 22:5, and the highest to the pairs 22:4/24:4 and 24:5/26:5, respectively.

**Very Long Polyenoic Fatty Acids of Rat Seminiferous Tubules**—In seminiferous tubule total lipid, 22:5n-6 accounted for 22%, and the sum of VLCPUFA for about 6% of the fatty acids. Almost 90% of this sum was made up of 24:4n-6 and 24:5n-6, and the rest of polyenes up to C24. None of the major glycerophospholipids contained significant amounts of VLCPUFA longer than 24:4 and 24:5. PUFA with chain lengths longer than C24 were found to be concentrated in specific neutral lipids and in sphingomyelin. Triacylglycerols were considerably rich in PUFAs, with nearly 5% 20:4, 5% 22:4, and 26% 22:5; almost 10% of TG fatty acids was accounted for by VLCPUFA, most (90%) of this being 24:4 and 24:5. The minor lipid in the band of ether-linked TG contained similar percentages of 20:4 and 22:4 to TG, had somewhat less 22:5 (22%), and was even richer in VLCPUFA. We were surprised to find that as much as 34% of the fatty acids of this lipid were VLCPUFA, of which 70% were the C24 polyenes, and the rest longer. Similarly unexpected was the fact that VLCPUFA accounted for as much as 33% of the acyl chains of seminiferous tubule cholesterol esters, this minor lipid class (0.5 ± 0.05 mol/100 mol lipid phosphorus)
being the richest in, and the main source of, very long chain pentaenoic fatty acids such as 26:5, 28:5, and 30:5 (5, 14, and 1%, respectively, of cholesteryl ester fatty acids). SM (4.9 ± 0.7 mol/100 mol of lipid P) was the lipid with the lowest percentage of C24 polyenes, but was the main source of 28:4n-6 (alone, 5.0 ± 0.5% of SM fatty acids) among other VLCPUFAs (total, ~7%). This phospholipid was also accountable for the 2-hydroxy derivatives of 28:4, 30:4, 30:5, and 32:5 shown in Fig. 2 (9).

**Odd Chain Polyenes—**Along with the regular even chain fatty acids, all fatty acid fractions of rat seminiferous tubules, from saturates to pentaneas, were found to contain a series of minor odd chain components (Table IV). We first observed the odd chain PUFA as 14C-FAME on the autoradiograms we employed to locate compounds labeled with 14C-acetate on reverse-phase TLC plates, which prompted us to examine them by HPLC (Fig. 2). By separating the acids first by degree of unsaturation and then by chain length, we were able to spot unusual odd chain n-6 tetraenoic and pentaenoic fatty acids of diverse chain lengths. Their identity was confirmed by capillary GC-MS as described for other PUFA (5, 8). In fact, some odd chain polyenes had previously been noticed along with the fatty acids of spermatozoas (5, 9). On reverse-phase columns, the odd chain tetraenas and pentaeas of seminiferous tubules conformed to the chromatographical behavior expected of a complete homologous series, covering being
the whole range of chain lengths from 21 to 31 carbon atoms (Fig. 2).

**DISCUSSION**

The results presented here show that rat seminiferous tubule cells are capable of synthesizing tetraenoic and pentaenoic fatty acids of the n-6 series covering a whole range of chain lengths up to 32 or more carbons. The use of [1-14C]24:4n-6 and [U-14C]acetate as precursors provides evidence that the 24-carbon polyenes, 24:4 and 24:5, play an important role in the formation of other polyenoic fatty acids of testis. The high level and fast turnover of [14C]24-carbon polyenes in triglycerides point to this lipid class as playing an active role in the metabolic transformations of polyenoic fatty acids. The occurrence and synthesis in situ of a complete family of odd number of carbons poses new questions and widens our understanding of naturally occurring PUFA and their metabolism in mammals.

The pattern of fatty acid labeling observed in this work suggests that the synthesis of very long tetraenes and pentaenes up to C52 occurs through sequential addition of two carbon units to pre-existing fatty acids, starting from 20:4n-6 and 22:5n-6, respectively, in analogy to the usual, malonyl CoA-dependent elongations (23) through which other even chain fatty acids are formed. This explains both the high rate of formation of the pairs 22:4/24:4 and 24:5/26:5 and the poor incorporation of [14C]acetate into the two major PUFA, 20:4 and 22:5, since the latter are the first in the series of naturally occurring (n-6) tetraenes and pentaenes, respectively. The results are consistent with previous work in retina, showing a low incorporation of [1-14C]acetate in 20:4n-6 and 22:5n-6 and in 20:5n-3 and 22:6n-3 (the two latter in turn being the first in the series of naturally occurring (n-3) pentaenes and hexaenes) and a relatively high specific radioactivity of the corresponding products of their elongation (10).

The finding that 24:5 contains a high proportion of the label from [1-14C]24:4 and that 24:4 and 24:5 are together the most highly labeled polyenes formed with [U-14C]acetate as precursor suggests that the C4 polyenes play a central role in the metabolism of testicular n-6 tetraenes and pentaenes: 24:4 and 24:5 are connected through a desaturation, and both may be potential sources of longer as well as shorter PUFA. The production of CO2 and labeled water-soluble metabolites, more active with [14C]24:4n-6 than with [14C]20:4, suggests that 24:4 and its main product, 24:5, may have been in part chain-shortened to 22:4 and 22:5, with loss of the 14C label.

$$\begin{align*}
18:2n-6 & \downarrow \Delta 6 \\
18:3n-6 & \rightarrow 20:3 \\
20:4 & \Rightarrow 22:4 \Rightarrow 24:4 \Rightarrow 26:4 \Rightarrow 28:4n-6 \\
\downarrow \Delta 5 & = 24:5 & \Rightarrow 26:5 & \Rightarrow 28:5 & \Rightarrow 30:5n-6
\end{align*}$$

(Eq. 1)

Whereas the presence of label in [14C]22:4 when the precursor was [14C]acetate is a clear indication of elongation of 20:4, the relatively low labeling of [14C]22:5 with the same precursor is consistent with the fact that a specific desaturation is required for the synthesis of the latter. The amount of label from [1-14C]acetate we observed in 22:5, however small, could have originated in (a) a direct desaturation of [14C]22:4 at Δ4, or (b) in the chain shortening of [14C]24:5, provided that the latter was labeled, at least, at C3. Although on the basis of the present results option (a) cannot be entirely ruled out, the evidence is on the whole against it: microsomes from rat testis have been shown *not* to produce such a desaturation using [1H]22:4 as substrate (24), and the very existence of this putative Δ4 desaturase activity has been not only questioned for some time, but has recently been disproven (25). As regards option (b), for [14C]22:5n-6 to have arisen via the chain shortening of [14C]24:5n-6, the latter would have to have been labeled, at least, at C5; in other words, it would first have to have been produced from a [14C]24:4 which had in turn evolved from the elongation of an already labeled 22:4. Such a special [14C]24:4 would have to be desaturated at Δ6.

In our experiments, both 24:4 and 24:5 were highly labeled with acetate, and we had evidence for a Δ6 desaturase acting on 24:4 from the synthesis of [14C]24:5 we had from [1-14C]acetate. This Δ6 desaturase could well be the same enzyme which is responsible for the conversion of 18:2n-6 to 18:3n-6, the first step in the synthesis of arachidonic acid and other n-6 PUFA.

Work by Voss et al. (25) has provided conclusive evidence that the controversy Δ4 desaturase is not required for the biosynthesis of 22:6n-3 from [14C]22:5n-3 and that an alternative pathway exists involving elongation of [14C]22:5n-3 to 24:5n-3, followed by the Δ6 desaturation of this 24:5n-3 to 26:5n-3 and then by the chain shortening of 24:6n-3 to 22:6n-3. This is consistent with the present results and interpretation, since such a mechanism is also likely to apply to n-6 PUFA. Furthermore, this indirect pathway would provide an
that odd chain PUFA are metabolic intermediates. A third desaturase activity possibly interconnecting α- and ω-tetraenoic and pentae-noic fatty acids could be that involved in the desaturation of \([14C]26:4\) to \([14C]26:5\). Although proven to be absent in liver (26), αΔ desaturase has been proposed as responsible for the conversion of \([14C]20:2\) to \([14C]20:3\) in rat testis (27). Even though such desaturation cannot be ruled out with the present data, the elongation of \([14C]24:5\) does appear to be more prominent quantitatively as a source of the available \([14C]26:5\).

Our data suggest that of all seminiferous tubule lipid classes, it is triglycerides which have a special role to play in PUFa metabolism. These lipids contain substantial proportions of PUFa and VLCPUFA, particularly the Cα tetra- and pentae-noic fatty acids, and concentrate the highest levels of \(14C\)-PUFA, whether exogenously provided or endogenously synthesized from \([14C]acetate\). These observations agree with earlier work showing that intratesticular injection of \(1\)-\(14C\)-labeled 20:4αα and 22:4αα results in the incorporation of a large proportion of the 20:4 into TG (1,24). Significant labeling of TG has also been observed in testicular slices incubated with \([14C]acetate\) (28). Far from constituting a mere "reservoir" for the accumulation of spare fatty acids, the triglycerides of seminiferous tubules appear to be involved actively in the metabolic transformation affecting PUFa. With both labeled PUFa and acetate as precursors, it is clearly apparent that most of the changes observed in the total lipid of seminiferous tubules take place in fatty acids that are esterified to TG. Whether PUFa are transformed metabolically and immediately transferred to TG, or whether intact TG are used directly as substrates for changes affecting their acyl chains remains to be elucidated and provides a challenging area for future research.

The presence of odd chain tetra- and pentae-noic PUFa in seminiferous tubule lipids raises several questions, of which one of the most intriguing relates to the possible metabolic origin of these components. Odd chain straight saturates and, especially in the stepwise addition of 2-carbon units to two even-carbon essential fatty acids, 18:2\(α\)-6 and 18:3\(α\)-3, or (b) during the \(β\)-oxidation of any of such even-carbon PUFa, since this would be bound to result only in even-carbon (n-2) homologues. Odd chain PUFa are most likely to arise (c) via oxidation of even carbon PUFa, 1-carbon unit at a time, converting each to its corresponding (n-1) homologue and releasing a 1-carbon unit molecule, such as CO\(_2\), in the process. We speculate that such \(α\)-oxidation is an important mechanism in the shortening of fatty acids and that odd chain PUFa are metabolic intermediates. The presence of label from \([14C]acetate\) in odd chain polyenes including 21:4 and 23:5 supports the view that, in addition to one step of \(β\)-oxidation, two steps of \(α\)-oxidation of \([14C]22:4\) and \([14C]24:5\) could provide an additional way of synthesizing 20:4 and 22:5, the major PUFa of rat testis. The formation of 20:4 by retroconversion of 22:5 was demonstrated long ago to occur in this tissue (31) after injection of \([14C]22:5\). Retroconversion of n-6 and \(-\)3 C\(_2\) PUFa has also been documented in a number of animal tissues and cells (32) using \(3\)-\(14C\)-labeled C\(_2\) PUFa. Although fatty acid retroconversion was not specifically addressed in the present work (a) the active production of \(14C\)-labeled \(14CO_2\) and water-soluble metabolites from \([1\)-\(14C\)]24:4; (b) the continuously increasing and parallel patterns of \(14C\)-fatty acid and \(14CO_2\) production during incubation with \([U\)-\(14C]acetate\); (c) the lack of time-dependent changes in the distribution of label among acetate-derived fatty acids from 1 to 4 h of incubation; and (d) the formation of labeled odd-chain PUFa, are consistent with the possibility of VLCPUFA retroconversion. In our view it is probable that PUFa undergo a continuous process of elongation, the resulting VLCPUFA being continuously chain shorted or retroconverted to maintain a concerted, balanced equilibrium in the proportion of PUFa and to enable cells to conserve fatty acids with metabolically costly double bonds. α-Oxidation could play a specific role in fatty acid metabolism, providing an alternative mechanism to \(β\)-oxidation for the formation of shorter fatty acids from longer ones, including the major C\(_{20}\) and C\(_{22}\) polyenes.

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