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Triacylglycerols and Glycerophospholipids in Ovaries from Maturing and Superovulated Immature Rats

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Abstract

The concentration and composition of triacylglycerols and glycerophospholipids in the rat ovary were measured during the development of follicles to corpora lutea. Changes occurring during normal development, as the rats aged from 20 to 41 days, were compared with those following treatment of 20-day-old rats with gonadotrophin to stimulate superovulation.

In the ovaries of immature superovulated rats, where many follicles developed into corpora lutea in synchrony, the glycerophospholipids increased in concentration throughout growth and luteinization. At the same time the total amount of triacylglycerol rose 10-fold but only increased in concentration during the 24 h after administration of pregnant mare's serum gonadotrophin. In both classes of lipid the proportion of polyunsaturated fatty acids rose throughout. Six days after administration of gonadotrophin 20% of the fatty acid in glycerophospholipid was arachidonic acid (20 : 4), while the amount of 22 : 5 acid had risen from 3 to 17% in the triacylglycerols.

During normal aging, changes in the developing follicles were masked by the number of other cell types present. In the whole ovary the glycerophospholipids did not change between 20 and 41 days, but the triacylglycerol concentration rose. However, there was a marked similarity between the concentrations of both lipid classes in corpora lutea dissected out of the 41-day-old ovary and those of the superovulated rats. The changes in proportion of the polyunsaturated fatty acids were also the same during normal and stimulated development of the corpora lutea. In the day-41 corpus luteum the phospholipid contained 22% arachidonic acid (20 : 4), and 20% of the fatty acids in the triacylglycerol was 22 : 5.

The changes in composition of fatty acids suggests that when a follicle begins to develop there is transfer of polyunsaturated fatty acids from cholesterol esters in the interstitial tissue to the triacylglycerols.

Introduction

Because cholesterol is the precursor of the steroid sex hormones (Claesson 1954), many studies on the free and esterified cholesterol content of the ovary at different stages of functional development have been reported (Bloor *et al.* 1930; Boyd 1935; Weinhouse and Brewer 1942; Armstrong 1968; Strauss *et al.* 1977; Tuckey and Stevenson 1979). Holman and Hofstetter (1965) showed that triacylglycerols and glycerophospholipids are also major components of the bovine and porcine ovary. They reported large differences between the follicles and the residual ovary from which the follicles had been removed with respect to the concentrations and fatty acid compositions of these lipids, and that they contained a high proportion of polyunsaturated fatty acids. The glycerophospholipid content of the ovary appears to

be related to the physiological activity of the gland (Bloor *et al.* 1930; Boyd 1935; Weinhouse and Brewer 1942), the highest concentrations being present during periods of maximal secretion of steroid hormones. The fatty acid composition of glycerophospholipids also changes with physiological activity: it differs in the ovaries of pregnant and non-pregnant rabbits (Morin 1968). Triacylglycerols accumulate in the corpus luteum during its regression (Weinhouse and Brewer 1942; Strauss *et al.* 1977), when steroid secretion declines, and the changes in fatty acid composition at this time suggest that fatty acids from plasma may be incorporated into the triacylglycerols of the ovary (Strauss *et al.* 1977).

Although there are these several reports on the triacylglycerol and glycerophospholipids in the ovary, data from a systematic study on the changes which occur in these lipids during development of the immature ovary to an organ with full secretory ability is not available. This paper presents such a study.

Materials and Methods

Glycerol tri[1-^{14}C]oleate (54 Ci/mol) was obtained from The Radiochemical Centre, Sydney, N.S.W., and methylheptadecanoate and dipalmitoyl phosphatidyl choline from Sigma Chemical Co., St Louis, Mo., U.S.A. The source of other chemicals and the purification of radiochemicals has been described previously (Tuckey and Stevenson 1979).

Batches of 80 female Wistar albino rats aged 20–42 days were used in this study. Some batches were allowed to mature naturally. Others were superovulated by subcutaneous injection of 50 i.u. of pregnant mare serum gonadotrophin when they were 20 days old, followed by 25 i.u. human chorionic gonadotrophin 54 h later. Groups of 10 or more treated or untreated rats were killed by cervical dislocation; ovaries in each group were pooled, cleaned and homogenized in 5 vol. 0·25 M sucrose. Lipids were extracted and the fatty acid compositions of triacylglycerols and glycerophospholipids were determined by gas chromatography of the methyl esters as described previously (Tuckey and Stevenson 1979). The probable bond configuration of each unsaturated ester is given. A known amount of methylheptadecanoate was added to the fatty acid methyl esters to allow quantitation of each lipid. In order to monitor losses of triacylglycerols throughout the procedure, tracer amounts of glycerol tri[1-^{14}C]oleate were added prior to extraction of the lipid. Recoveries were usually in the range 85–90% and results were corrected for the observed losses. Glycerophospholipid concentrations were not corrected for procedural losses because radiolabelled tracer was not available to us. Standard dipalmitoyl phosphatidyl choline gave a recovery of 85·4±2·3% (mean ± standard deviation, $n = 7$). The molar concentration of glycerophospholipids was calculated assuming 2 moles of fatty acid per mole of glycerophospholipid.

Results

The Maturing Rat

During the period of natural maturation of the rats, between 20 and 41 days, the triacylglycerols of the whole ovary increased in concentration while that of the glycerophospholipid remained relatively constant (Table 1). The rats commenced their first oestrous cycle between days 34 and 41 (determined by visual inspection of the ovaries). The resulting corpora lutea from ovaries of the 41-day-old rats had a higher glycerophospholipid and a lower triacylglycerol concentration than the remainder of the ovary from which the corpora lutea had been dissected. Between 20 and 34 days (i.e. before the first cycle) the fatty acid composition of the triacylglycerols was characterized by an 11% decrease in the proportion of saturated fatty acids and an increase of 8% in 16 : 1, 18 : 1 and 20 : 1 acids (Table 2) suggesting that the fatty acids in the ovary were being elongated and desaturated. From days 34 to 41 increases in the proportion of 20 : 4, 20 : 5, 22 : 4 and 22 : 5 acids were observed. These changes were largely due to the corpora lutea present on day 41

(Table 2), the composition of which was markedly different to the rest of the ovary. The fatty acid composition of the ovarian glycerophospholipids changed very little between 20 and 34 days of maturation (Table 3), but between days 34 and 41 the proportion of 18 : 1 and 20 : 3 acids decreased while the 20 : 4, 20 : 5 and 22 : 5 acids increased. These changes were again attributable to the development of corpora lutea in the ovaries by day 41.

Table 1. Concentration of triacylglycerols and glycerophospholipids in ovaries from maturing rats

For the 41-day-old rats, concentrations were measured in the whole left ovary of each rat and in corpora lutea dissected from the right ovaries (designated 41CL). Data are mean ± standard deviation ($n = 3$) except 41CL where values are from a single determination with tissue pooled from 20 rats

Age (days)	Triacylglycerol (μmol/g tissue)	Glycerophospholipid (μmol/g tissue)	Age (days)	Triacylglycerol (μmol/g tissue)	Glycerophospholipid (μmol/g tissue)
20	8·8±0·5	15·8±0·3	41	15·7±0	17·7±0·1
27	11·9±0·6	17·7±1·2	41 CL	10·5	22·5
34	14·8±0·4	15·6±1·8			

Table 2. Fatty acid composition of triacylglycerols in ovaries from maturing rats

The fatty acid composition was measured in both whole ovaries and corpora lutea (41CL) of 41-day-old rats as described in Table 1. Data are mean ± standard deviation ($n = 3$) except 41CL where values are from a single determination. Fatty acids consistently less than 1% of the total have been omitted. Trace, less than 0·5% of the total. Probable bond configurations of unsaturated fatty acids are given in parentheses

Fatty acid	Fatty acid content (g/100 g total fatty acids extracted)				
	Day 20	Day 27	Day 34	Day 41	Day 41CL
12 : 0	2·63±0·24	1·25±0·41	Trace	Trace	Trace
14 : 0	6·12±0·02	3·80±0·16	2·45±0·31	1·67±0·18	0·45
16 : 0	27·42±0·99	26·09±0·41	24·65±0·29	22·65±0·05	11·49
16 : 1 (9)	1·93±0·08	2·78±0·09	3·56±0·38	4·11±0·32	1·18
18 : 0	9·82±0·17	8·47±0·14	8·50±0·23	8·51±0·09	13·76
18 : 1 (9)	26·44±0·39	31·87±0·18	31·12±0·36	29·55±0·01	21·69
18 : 2 (9,12)	11·21±0·11	14·75±0·08	15·25±0·09	12·63±0·04	10·12
20 : 1 (11)	1·79±0·11	2·07±0·06	2·61±0·15	1·62±0·03	1·47
20 : 3 (8,11,14)	0·97±0	0·66±0·04	0·87±0·26	0·57±0·02	0·90
20 : 4 (5,8,11,14)	2·57±0·03	1·25±0·10	1·74±0·15	2·40±0·08	5·60
20 : 5 (5,8,11,14,17)	0·65±0·10	0·81±0·04	1·19±0·15	2·39±0·01	3·25
22 : 4 (7,10,13,16)	1·02±0·03	0·62±0·08	1·01±0·05	1·83±0·15	5·48
22 : 5 (7,10,13,16,19)	3·09±0·03	2·16±0·19	2·93±0·27	7·73±0·15	20·43
22 : 6 (4,7,10,13,16,19)	4·34±0·11	3·42±0·21	4·12±0·27	4·34±0·02	4·18
Total polyunsaturated fatty acids	23·9	23·7	27·1	31·9	50·0

The Superovulated Immature Rat

(i) *Concentration of triacylglycerols and glycerophospholipids*

The ovaries of 20-day-old rats consist largely of interstitial tissue, where most of the lipid is located, and of immature follicles (Rennels 1951). Administration of pregnant mare serum gonadotrophin to the rats causes superovulation. Previously we showed that after 50 i.u. hormone the cells of the follicles divide and the ovary

increases in weight from about 9 mg before treatment to about 80 mg 4 days later (Klinken and Stevenson 1977). The injection of the rats with human choriogonadotrophin 54 h after the pregnant mare serum gonadotrophin causes synchronous luteinization of the follicles which begins on the fourth day of the superovulation and causes a further increase in weight of the ovary to about 90 mg by the sixth day. The luteinization is accompanied by a large increase in the rate of progesterone production by the ovary (Klinken and Stevenson 1977). One day after administration of pregnant mare serum gonadotrophin there is a marked decrease in concentration of cholesterol fatty acid esters (Tuckey and Stevenson 1979); in the present study we observed increases in both the triacylglycerol and glycerophospholipid concentrations

Table 3. Fatty acid composition of glycerophospholipids in ovaries from maturing rats

The fatty acid composition was measured in both whole ovaries and corpora lutea (41CL) of 41-day-old rats as described in Table 1. Data are mean ± standard deviation ($n = 3$) except 41CL where values are from a single determination. Fatty acids consistently less than 1% of the total have been omitted. Trace, less than 0·5% of the total

Fatty acid	Fatty acid content (g/100 g total fatty acids extracted)				
	Day 20	Day 27	Day 34	Day 41	Day 41CL
14 : 0	2·18±0·09	1·80±0·27	1·70±0·38	Trace	Trace
16 : 0	19·23±0·32	19·13±1·60	17·22±0·48	17·57±0·21	19·55
16 : 1 (9)	1·73±0·06	1·56±0·13	1·42±0·19	Trace	Trace
18 : 0	17·26±0·33	18·92±1·53	18·82±0·34	20·16±0·37	18·39
18 : 1 (9)	20·01±0·73	18·37±0·41	19·19±0·40	16·03±0·23	14·74
18 : 2 (9,12)	9·04±0·29	8·89±0·19	10·24±0·14	10·01±0·21	9·41
20 : 3 (8,11,14)	3·49±0·06	4·09±0·06	4·00±0·41	2·62±0·09	0·85
20 : 4 (5,8,11,14)	17·08±0·06	17·30±0·27	18·86±0·23	21·75±0·58	22·84
20 : 5 (5,8,11,14,17)	1·84±0·04	1·90±0·09	1·94±0·35	3·71±0·25	4·25
22 : 4 (7,10,13,16)	1·64±0·35	2·06±0·07	2·05±0·18	1·87±0·01	2·47
22 : 5 (7,10,13,16,19)	2·16±0·05	2·28±0·09	2·27±0·14	3·87±0·03	5·31
22 : 6 (4,7,10,13,16,19)	4·34±0·14	3·70±0·10	2·29±0·36	2·41±0·06	2·19
Total polyunsaturated fatty acids	39·6	40·2	41·6	46·2	47·3

of the ovary. Table 4 shows that glycerophospholipid concentration remained constant between days 1 and 4 of superovulation, then increased with luteinization. After a transitory decrease on the second day of superovulation, the triacylglycerol concentration remained constant until the sixth day when it again decreased. As a result of the marked increase in weight of the ovary between days 0 and 6 of superovulation there were large increases in the total amounts of glycerophospholipid and triacylglycerol in the ovary over this period. For example, the amount of triacylglycerol increased from 71 nmol on day 0 to 764 nmol on day 6. Earlier work demonstrated that some of the cholesteryl esters in the immature ovary appear to be hydrolysed in the 24 h following administration of pregnant mare serum gonadotrophin to rats (Tuckey and Stevenson 1979), releasing free fatty acids which might be re-esterified in these other lipid classes.

Five days after the initiation of superovulation the ovary consisted almost entirely of corpora lutea. The concentration of glyceryl lipids in the corpus luteum of the 41-day-old untreated rat (Table 1) and these corpora lutea of superovulated rats (Table 4) were very similar. For example, the triacylglycerol concentration in the

ovaries 6 days after superovulation was 8·3 μmol/g tissue, while that in the corpora lutea of 41-day-old rats was 10·5 μmol/g tissue; the glycerophospholipid concentrations were 26·8 and 22·5 μmol/g tissue respectively. This glycerophospholipid level is close to the value reported by Schuler *et al.* (1978) for ovaries from rats 10 or 11 days after superovulation.

(ii) *Fatty acid composition of triacylglycerols and glycerophospholipids*

In the first 24 h of superovulation there was a large increase in the proportion of 22 : 5 and 22 : 6 acids in the triacylglycerols (Table 5). These are the major fatty acids hydrolysed from the cholesteryl esters during this period (Tuckey and Stevenson 1979). The proportion of 22 : 5 acid in the triacylglycerols continued to increase on subsequent days of superovulation while the 22 : 6 acid increased until day 3 and then decreased marginally in proportion. The percentage of 20 : 4 and 22 : 4 acids also increased between days 1 and 6 of superovulation while 12 : 0, 14 : 0 and 16 : 0

Table 4. Concentration of triacylglycerols and glycerophospholipids in ovaries from superovulated immature rats after treatment with pregnant mare serum gonadotrophin

Data are mean ± standard deviation ($n = 3$)

Time after treatment (days)	Triacyl-glycerol (μmol/g tissue)	Glycerophospho-lipids (μmol/g tissue)	Time after treatment (days)	Triacyl-glycerol (μmol/g tissue)	Glycerophospho-lipids (μmol/g tissue)
0	8·8 ± 0·4	15·8 ± 0·3	4	10·7 ± 0·6	19·3 ± 0·5
1	11·9 ± 0·9	19·8 ± 0	5	10·9 ± 0·5	24·6 ± 0·3
2	8·7 ± 0·4	20·1 ± 0·1	6	8·3 ± 0·5	26·8 ± 0·6
3	10·8 ± 0·6	19·4 ± 0·4			

acids decreased. Again the fatty acid composition of the triacylglycerols in the ovary 5–6 days after superovulation was similar to the composition noted for corpora lutea from 41-day-old untreated rats (Table 2). The total percentages of polyunsaturated fatty acids in the triacylglycerols more than doubled between days 0 and 6 of superovulation, and increased markedly during normal luteal maturation (Tables 2 and 5).

The fatty acid composition of the glycerophospholipids in the superovulated rats (Table 6) was remarkably constant in view of the large changes which occurred in the cholesteryl esters (Tuckey and Stevenson 1979) and triacylglycerols (Table 5). The most prominent change (Table 6) was in the proportion of 20 : 4 acid which decreased following administration of pregnant mare serum gonadotrophin to the immature rats and increased after the second day of superovulation. Jonsson *et al.* (1975) have also reported an increase in the content of 20 : 4 acid in the phospholipids of immature ovaries 3 days after treatment of the rats with pregnant mare serum gonadotrophin. Although the changes were slight the percentage of polyunsaturated fatty acids in the glycerophospholipids did increase throughout the period of superovulation (Table 6).

Discussion

Use of the whole cycling ovary for studying the biochemistry of follicular development has many inherent difficulties in that it is always composed of a number of different cell types, each with its unique intermediary metabolism: e.g. the thecal

Table 5. Composition of triacylglycerols in ovaries from superovulated immature rats

Data are mean ± standard deviation ($n = 3$). Fatty acids consistently less than 1% of the total have been omitted. Trace, less than 0·5% of the total

Fatty acid	Fatty acid content (g/100 g total fatty acids extracted)						
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
12 : 0	2·63±0·24	1·33±0·41	Trace	Trace	Trace	Trace	Trace
14 : 0	6·12±0·02	3·24±0·16	2·05±0·25	1·54±0·08	1·47±0·35	1·11±0·14	0·88±0·26
16 : 0	27·42±0·99	22·31±0·30	22·36±0·47	19·90±0·32	17·09±0·19	14·37±0·46	11·61±0·55
16 : 1 (9)	1·93±0·08	1·75±0·05	1·81±0·07	1·75±0·02	1·92±0·04	1·36±0·04	0·93±0·05
18 : 0	9·82±0·12	11·78±0·06	11·15±0·29	10·59±0·11	9·56±0·29	9·87±0·08	11·50±0·22
18 : 1 (9)	26·44±0·39	20·06±0·53	21·36±0·38	20·47±0·30	23·57±0·46	22·87±0·37	22·19±0·28
18 : 2 (9,12)	11·21±0·11	11·64±0·24	10·54±0·27	10·68±0·10	11·19±0·07	11·61±0·25	11·98±0·23
20 : 1 (11)	1·79±0·11	2·05±0·02	1·94±0·05	1·81±0·02	2·07±0·07	2·05±0·06	2·20±0·05
20 : 3 (8,11,14)	0·97±0·00	1·05±0·11	1·18±0·07	1·15±0·09	0·99±0·18	0·71±0·04	0·76±0·01
20 : 4 (5,8,11,14)	2·57±0·04	2·49±0·25	3·09±0·10	3·86±0·04	3·37±0·14	3·69±0·12	4·26±0·07
20 : 5 (5,8,11,14,17)	0·65±0·10	1·71±0·29	1·61±0·21	2·08±0·32	1·90±0·22	1·78±0·05	1·09±0·24
22 : 4 (7,10,13,16)	1·02±0·03	1·91±0·14	2·03±0·08	2·92±0·16	3·58±0·20	4·8 ±0·24	6·90±0·14
22 : 5 (7,10,13,16,19)	3·09±0·03	8·93±0·18	10·36±0·56	11·71±0·18	13·55±0·38	16·11±0·54	17·45±0·84
22 : 6 (4,7,10,13,16,19)	4·34±0·08	9·75±0·18	10·54±0·65	11·54±0·13	9·74±0·30	9·67±0·27	8·25±0·67
Total polyunsaturated fatty acids	23·9	37·5	39·3	43·9	44·3	48·4	50·7

and granulosa cells of the follicle, the interstitial tissues, the corpora lutea and corpora albicans. Treatment of immature rats with small concentrations of gonadotrophins, currently fashionable for physiological studies, does not overcome the problem of the multiple cell types. On the other hand, superovulation of immature rats with large amounts of pregnant mare serum gonadotrophin and, 54 h later, human choriogonadotrophin causes large numbers of follicles to develop simultaneously; 5 days after the beginning of treatment the ovaries consist almost entirely of corpora lutea. This synchronous development of the follicles to corpora lutea and the large increase in ovarian weight accompanying superovulation would seem to make this source of tissue ideal for studying the biochemistry of ovarian development (Klinken and Stevenson 1977). Our results on changes in lipid composition through development to steroidogenic competence supports this view: the changes in fatty acid composition of glycerophospholipids and triacylglycerols, as well as those in cholesteryl esters (Tuckey and Stevenson 1979), during stimulated luteinization are all similar to those found when corpora lutea develop normally in ovaries of untreated cycling rats. Furthermore, Rennels (1966) has shown by electron microscopy that luteal tissues from superovulated rats and rats treated with small doses of gonadotrophin are similar in appearance.

The increase in triacylglycerol concentration between days 0 and 1 of superovulation could be associated with the formation of lipid storage granules in the growing follicles. In the untreated immature ovary, lipids are stored in the interstitial tissue and there is no appreciable neutral lipid in the small follicles (Rennels 1951). Histochemical studies (R. C. Tuckey, P. M. Stevenson and N. D. Costa, unpublished data) show a decrease in concentration in the interstitial tissue and the appearance of neutral lipid in the thecal cells of these follicles 24 h after administration of gonadotrophin to the rats. Hydrolysis of cholesteryl esters appears to precede the movement of lipids from the interstitial tissues to the newly forming follicles, and our evidence suggests that at this time there is a transfer of fatty acids from the cholesteryl esters to the triacylglycerols: the large increase in percentage of 22 : 5 and 22 : 6 acids in the triacylglycerols corresponds with depletion of these two fatty acids from the cholesteryl esters (Tuckey and Stevenson 1979). However, the amount of free fatty acids released from the cholesteryl esters is not sufficient to account for the increase in fatty acids esterified to glycerol in this 24-h period. Therefore, fatty acids must be either synthesized, or absorbed from the plasma between days 0 and 6 of superovulation to cause the accumulation seen in the amounts of triacylglycerols and glycerophospholipids in the ovary throughout this period.

Our finding that the lipids of the ovary contain a high proportion of C_{22} polyunsaturated fatty acids agrees with that of Schuler *et al.* (1978). They showed that in superovulated rat the 22 : 4 acid was the predominant species. They did not specify whether this acid was of the $n-3$ or $n-6$ class. In our colony of rats, the fatty acids were predominently 22 : 5 and 22 : 6 and were of the $n-3$ class. Scott *et al.* (1968) and Strauss *et al.* (1977) have demonstrated that the ovary can cause the elongation and unsaturation of fatty acids. It is likely, therefore, that the C_{22} polyunsaturated fatty acids of the ovary are synthesized from the essential fatty acids of the diet. The fatty acid in the diet fed to our rats contains 30–40% unsaturated acids, more than half of which is 18 : 2 ($n-6$). The percentage of $n-3$ class fatty acids from which the 22 : 5 and 22 : 6 acids present in the ovary must be derived is only 9% and only 0·4% of all dietary fat is 22 : 5 *per se*.

Table 6. Fatty acid composition of glycerophospholipids in ovaries of superovulated immature rats

Date are mean ± standard deviation ($n = 3$). Fatty acids consistently less than 1% of the total have been omitted

Fatty acid	Fatty acid content (g/100 g total fatty acids extracted)						
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
14 : 0	2·18±0·09	1·58±0·35	1·21±0·01	1·60±0·13	1·39±0·12	1·60±0·13	1·52±0·34
16 : 0	19·23±0·32	19·50±0·28	20·63±0·26	20·17±0·40	19·89±0·25	20·03±0·04	20·53±0·41
16 : 1 (9)	1·73±0·06	1·58±0·15	1·54±0·02	1·46±0·07	1·00±0·07	0·87±0·09	0·75±0·00
18 : 0	17·26±0·33	18·20±0·24	16·66±0·22	16·84±0·25	16·84±0·06	17·04±0·21	16·28±0·25
18 : 1 (9)	20·01±0·73	18·33±0·40	18·96±0·05	17·97±0·28	18·83±0·17	17·00±0·16	16·37±0·16
18 : 2 (9,12)	9·04±0·29	10·55±0·18	10·48±0·11	10·50±0·12	10·53±0·12	10·47±0·18	10·57±0·13
20 : 3 (8,11,14)	3·49±0·06	2·33±0·10	1·96±0·04	1·61±0·04	1·43±0·06	1·22±0·03	0·95±0·10
20 : 4 (5,8,11,14)	17·08±0·06	14·99±0·26	14·89±0·02	16·00±0·34	17·12±0·18	19·02±0·69	20·24±0·19
20 : 5 (5,8,11,14,17)	1·84±0·04	3·41±0·48	3·52±0·12	3·33±0·04	3·50±0·17	3·16±0·07	2·46±0·04
22 : 4 (7,10,13,16)	1·64±0·35	1·31±0·04	1·29±0·09	2·02±0·94	1·29±0·01	2·05±0·88	2·81±0·09
22 : 5 (7,10,13,16,19)	2·16±0·05	3·24±0·06	3·71±0·06	3·67±0·12	3·99±0·04	3·85±0·07	3·78±0·04
22 : 6 (4,7,10,13,16,19)	4·34±0·14	4·98±0·20	5·15±0·04	4·83±0·14	4·19±0·02	3·69±0·16	3·74±0·09
Total polyunsaturated fatty acids	39·6	40·8	41·0	42·0	42·1	43·5	44·6

Despite many reports of a high proportion of polyunsaturated fatty acids in the lipids of steroidogenic tissues no one to date has presented any firm evidence as to their role. The increase in the proportion of polyunsaturated fatty acids in the ovary accompanying both development of the follicles and luteinization suggests an involvement of these fatty acids in steroidogenesis. The high proportion of 20 : 4 (arachidonic) acid in the glycerophospholipids of the ovary may be obligatory for the increased synthesis of prostaglandins brought about by luteinizing hormone in both the preovulatory (rat) and luteal tissues (bovine) (Shemesh and Hansel 1975; Clark *et al.* 1976). Hong *et al.* (1979), working with transformed fibroblast cells in culture, have shown that the concentration of arachidonic acid in a cell can govern the amount of prostaglandin synthesized. Seybert *et al.* (1979) have demonstrated with studies on the reconstitution of purified adrenal cytochrome P450 with glycerophospholipid vesicles, that the fatty acid moiety of the glycerophospholipid influences the rate of cleavage of the side-chain of cholesterol. Their studies were not done with polyunsaturated fatty acids but their results suggest that increasing the degree of unsaturation of the fatty acids in the environment of the cytochrome P450 complex may increase the rate of cholesterol conversion to steroids.

It is possible that the polyunsaturated fatty acids are playing the non-specific but vital role of scavenging superoxide radicles generated during interaction between molecular oxygen and the cytochromes.

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