Metabolism of Phospholipids and the Characterization of Fatty Acids in Bovine Corpus Luteum

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1. Phosphatidylcholine was the predominant phospholipid in bovine corpora lutea; it accounted for about 50% of the total phospholipid phosphorus. Phosphatidylethanolamine (13%) and ethanolamine plasmalogen (8–9%) were the next two major components. 2. After incubation of the tissue with [32 P]orthophosphate the total radioactivity and specific radioactivity of phosphatidylinositol were higher than those of any other lipid. 3. Luteinizing hormone failed to increase significantly the incorporation of [32 P]orthophosphate into total phospholipids from luteal tissue slices, but did stimulate progesterone synthesis and lactate production. 4. The proportion of oleate (18:1) in the neutral lipids and phospholipids was higher than that of any other fatty acid. 5. The proportion of unsaturated fatty acid in the tissue lipids exceeded 60%, and almost half of this was polyunsaturated. Arachidonate (20:4), docosatetraenoate (22:4) and docosapentaenoate (22:5) were the principal polyunsaturated fatty acids. 6. After incubation of luteal tissue with [14 C]acetate, the greatest proportion of radioactivity in the fatty acids isolated from the total lipid fraction was in palmitate (16:0) and docosatetraenoate (22:4). Polyunsaturated fatty acids accounted for almost 50% of the 14 C radioactivity incorporated and this pattern was observed in phospholipids, triglycerides and free fatty acids.

The phospholipids of the male gonads of rat (Collins & Shotlander, 1961; Scott, Dawson & Rowlands, 1963; Davis, Bridges & Coniglio, 1966) and sheep (Scott & Setchell, 1968) have been examined, but, to our knowledge, no similar detailed studies have been made on the phospholipids of female gonads, including the corpus luteum. The present studies were therefore undertaken to provide information on the proportions of individual phospholipids in corpus luteum and to examine their metabolism (in the presence and absence of luteinizing hormone) by incubating the tissue with [32 P]P_i. In addition to characterizing the phospholipids, it was thought worth while to examine the fatty acids of the corpus luteum, as the tissue was not included by Holman & Hofstetter (1965) in their studies on the fatty acid composition of bovine reproductive organs. During these investigations it became apparent that this tissue had a high proportion of polyunsaturated fatty acids. In an attempt to understand their synthesis and metabolism, luteal tissue was incubated with [14 C]acetate and results of these experiments are also reported.

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METHODS

Incubation of tissues. Corpora lutea (estimated by weights and histological examinations to be at 9–13 days of the oestrous cycle) were acquired from non-pregnant dairy cows through an incision in the anterior vagina (Hansel & Seifort, 1968). For incubations, the tissue was placed in ice-cold Krebs–Ringer bicarbonate buffer, pH 7.4 (Umbreit, Burris & Staffuer, 1957), and transported to the laboratory. Where lipid analyses were made on unincubated corpora lutea, the tissue was immediately frozen on solid CO₂ before extraction. The outer capsule of the corpus luteum was dissected off and the gland was divided into four parts before being sliced. All tissue preparations were performed at 0–2° and the time taken before incubations commenced was less than 1 hr. Slices (100–140 mg.) were incubated at 38° for 2 hr. in Krebs–Ringer bicarbonate buffer, pH 7.4 (2 ml.) containing either 5 μ C of [32 P]P_i or 5 μ C of sodium [14 C]acetate/ml., together with unlabelled glucose (2 mg./ml.), by using the general procedures described by Scott, Jay & Freinkel (1966a). Bovine luteinizing hormone (NIH-LH-B₃, 1.02 U.S.P. units/mg. of protein) was obtained from the Endocrine Study Section of the National Institutes of Health (Bethesda, Md., U.S.A.) and was freshly dissolved in 0.9% NaCl (containing 0.1% albumin) for each experiment. When luteinizing hormone (0.05 or 0.2 μ g./vessel) was used in the experiments, it was

added to the flasks just before the commencement of incubation.

Tissue fractionation procedures after incubation. After incubation, the tissues were divided into two sets, one for determination of progesterone and the other for lipid analyses.

Determination of progesterone. Progesterone in the tissue plus medium was determined as described by Hansel & Seifart (1968).

Extraction and fractionation of lipids. For lipid analyses, the tissue was removed from the medium and immediately frozen on solid CO₂. Frozen tissue was homogenized in 10 ml. of chloroform-methanol (2:1, v/v) and the extract filtered through glass wool. The filtrate was washed once with 0.2 vol. of water and three times more by using the procedures of Folch, Lees & Sloane-Stanley (1957). A sample of the total lipid fraction was assayed for radioactivity (Scott *et al.* 1966a).

Neutral lipids were separated from phospholipids by silicic acid column chromatography (Scott, Good & Ferguson, 1966b). Individual phospholipids were characterized by the procedures of Dawson, Hemington & Davenport (1962), and the resulting paper chromatograms were radioautographed (if the tissues were incubated with [³²P]P_i) and assayed for radioactivity as described by Scott *et al.* (1966a). Phosphorus was determined by the method of Fiske & Subbarow (1925). Phosphorus recoveries with these procedures were in the range 85–93%.

Thin-layer chromatography of lipids. After incubation of tissues with sodium [1-¹⁴C]acetate, the lipid extract was prepared as described above. One-tenth of the extract was applied to large glass plates (40 cm. × 20 cm. × 0.5 cm.) coated with 500 μ silica gel G (E. Merck A.-G., Darmstadt, Germany) by using the device of Scott & Beeston (1966). The plates were developed in one dimension with two solvent systems, namely di-isopropyl ether-acetic acid (45:2, v/v) for 45 min. and light petroleum (b.p. 60–80°)-diethyl ether-acetic acid (90:10:1, by vol.) for 90 min. The second solvent system moved approx. 30 cm. along the plate. The plates were radioautographed to localize the individual components (identified by lipid standards run on the same plates) and these were aspirated from the plate by using the apparatus of Goldrick & Hirsch (1963) for assay of radioactivity (Scott *et al.* 1966b) or for further characterization of the fatty acid components. Recovery of radioactivity was over 85%.

Preparation of fatty acid extracts. Neutral lipid and phospholipid fractions were saponified and fatty acids extracted by using the procedures described by Scott, Satchell & Bassett (1967). Where the lipids had been divided into individual classes by thin-layer chromatography, the areas of gel corresponding to phospholipids, triglycerides and free fatty acids were aspirated into test tubes containing 10 ml. of chloroform-methanol (1:1, v/v). The tubes were gently shaken for 60 min. at 37° to extract the lipid and then centrifuged to separate the gel. The extracts were evaporated to dryness and saponified as described by Scott *et al.* (1967). Methyl esters of the fatty acids were prepared by the technique of Schlenk & Gellerman (1960).

Gas-liquid chromatography of fatty acid methyl esters. Methyl esters of the fatty acids extracted from unincubated luteal tissue were separated and identified on a column of diethylene glycol succinate (17%, w/w) (Ward, Scott &

Dawson, 1964; Scott *et al.* 1967). Methyl esters of labelled fatty acids were separated at 197° on a 5 ft. × ¼ in. (internal diam.) column of diethylene glycol succinate (17%, w/w) on Chromosorb W (HMDS 60–80-mesh size) in an Aerograph 200 series instrument (Varian Enterprises, Crows Nest, Sydney, N.S.W., Australia). The chromatograph was fitted with a gas-stream splitter, which allowed 10% of the gas flow to pass directly into the flame detector while the remaining 90% was diverted into glass cartridges, as described by Karmen, Giuffrida & Bowman (1962). Radioactivity was assayed by counting the cartridges in a liquid-scintillation counter (Packard Instruments, La Grange, Ill., U.S.A.). Recoveries of radioactivity with the above procedures exceeded 80%. Methyl [1-¹⁴C]palmitate standards were used to check on recoveries and for calculating specific radioactivities of the individual fatty acids. Where further characterization of the isolated fatty acid methyl esters was necessary, these were collected into fine glass wool (Bennett & Coon, 1966).

Hydrogenation and oxidation of unsaturated fatty acids. Polyunsaturated fatty acids (22:n) labelled with ¹⁴C were further characterized by the following micro-hydrogenation and oxidation procedures. The methyl esters, which were separated by gas-liquid chromatography and collected into glass wool, were extracted with chloroform-methanol (1:1, v/v). The organic solvents were removed *in vacuo*; the residue was dissolved in methanol (7 ml.) and hydrogenated overnight with platinum dioxide as catalyst. The hydrogenated fatty acid esters were re-examined by gas-liquid chromatography. Oxidation of the unsaturated fatty acids (initially separated and purified by gas-liquid chromatography) to locate the position of the double bonds was performed by the procedure of Rudloff (1956) as modified by Scheuerbrandt & Bloch (1962). The oxidation products were examined by gas-liquid chromatography with the column described above and an operating temperature of 150°.

Other analytical procedures. Glucose was determined with glucose oxidase by the procedure of Huggett & Nixon (1957). Lactate was determined enzymically with lactate dehydrogenase as described by Lundholm, Mohme-Lundholm & Vamos (1963).

Chemicals. Glycerol monostearate and glycerol dioleate were obtained from K & K Laboratories (Plainview, N.Y., U.S.A.), tristearin, cholesterol and palmitic acid were from British Drug Houses Ltd. (Poole, Dorset), cholesterol stearate was from Sigma Chemical Co. (St Louis, Mo., U.S.A.) and progesterone was from Rotary Tableting Inc. (Melbourne, Vic., Australia).

Standard mixtures of fatty acids [mixture E and mixture F containing 10:0, 12:0, 14:0, 16:0, 18:0, 20:0, 22:0, 24:0, 20:5 (Δ⁵), 22:5 (Δ⁴) and 22:6 (Δ⁴) acids, where Δ refers to the position of the first double bond nearest the carboxyl end of the molecule] were obtained from the National Institutes of Health (Bethesda, Md., U.S.A.).

The [³²P]P_i was obtained from the Australian Atomic Energy Commission (Lucas Heights, Sydney, N.S.W., Australia); sodium [1-¹⁴C]acetate, sodium [1-¹⁴C]palmitate and [¹⁴C]progesterone were from The Radiochemical Centre (Amersham, Bucks.).

RESULTS

Distribution and metabolism of individual phospholipids in corpus luteum. Despite the difference

Table 1. *Distribution and metabolism of individual phospholipids in bovine corpus luteum*

Corpora lutea (at 9–13 days of the oestrous cycle) were obtained from two different animals. The total phospholipid P contents were 1.24 and 0.76 mg. of P/g. of tissue for corpora lutea 1 (CL 1) and 2 (CL 2) respectively. Specific ^{32}P radioactivities were obtained by isolating individual phospholipids from luteal slices (400 mg.) that had been incubated for 2 hr. at 38° in Krebs–Ringer bicarbonate buffer, pH 7.4, containing $[^{32}\text{P}]\text{P}_i$ ($5\text{ }\mu\text{C}/\text{ml.}$) and glucose ($2\text{ mg.}/\text{ml.}$). N.D., Not determined.

Phospholipid	Distribution of P (% of total phospholipid P)		Specific radioactivity (counts/min./mg. of P)
	CL 1	CL 2	
Phosphatidylcholine	53.3	52.9	600
Phosphatidylethanolamine	13.6	13.5	4400
Phosphatidylinositol	6.4	5.5	233000
Phosphatidylserine	4.6	4.3	13000
Phosphatidic acid	0.8	1.5	41800
Cardiolipin	3.9	3.5	2800
Ethanolamine plasmalogen	8.5	9.2	1700
Choline plasmalogen	1.7	1.4	700
Sphingomyelin	6.0	7.0	800
Alkyl ether lipid	1.2	1.2	N.D.

Table 2. *Effect of luteinizing hormone on $[^{32}\text{P}]\text{P}_i$ incorporation into total phospholipids, progesterone content, glucose uptake and lactate production by slices of bovine corpus luteum*

Control vessels contained about 100 mg. of tissue and were incubated for 2 hr. at 38° in Krebs–Ringer bicarbonate buffer, pH 7.4, containing glucose ($2\text{ mg.}/\text{ml.}$) and $[^{32}\text{P}]\text{P}_i$ ($5\text{ }\mu\text{C}/\text{ml.}$). Luteinizing hormone (0.05 or $0.2\text{ }\mu\text{g.}/\text{vessel}$) was added before the commencement of incubation. Results are given as means $\pm$ S.D. of six determinations. Values in **bold** type indicate significant differences from the control values (t test $P < 0.05$).

	Control	Luteinizing hormone	
		($0.05\text{ }\mu\text{g.}/\text{vessel}$)	($0.2\text{ }\mu\text{g.}/\text{vessel}$)
Total lipid radioactivity (counts/min./g. wet wt. of tissue)	19260 ± 8394	24272 ± 13127	21114 ± 10814
Phospholipid specific radioactivity (counts/min./mg. of P)	24314 ± 14736	30963 ± 21320	25697 ± 15080
Progesterone content ($\mu\text{g.}/\text{g.}$ wet wt. of tissue)	112.4 ± 25.3	144.9 ± 48.8	151.0 ± 33.9
Glucose uptake ($\text{mg.}/\text{g.}$ wet wt. of tissue)	5.82 ± 0.95	6.21 ± 1.86	6.03 ± 1.69
Lactate content ($\text{mg.}/\text{g.}$ wet wt. of tissue)	2.09 ± 0.65	2.50 ± 0.82	2.51 ± 0.86

in the total amounts of phospholipid in the corpora lutea obtained from two animals, the proportions of the individual phospholipids were very similar (Table 1). Phosphatidylcholine was the major lipid and represented more than 50% of the total phospholipid phosphorus; phosphatidylethanolamine was the next most abundant component, followed by ethanolamine plasmalogen, which accounted for 8–9% of the total phospholipid. Most of the other known phospholipids were also detected in the corpus luteum (Table 1).

After a 2 hr. incubation of corpus luteum with $[^{32}\text{P}]\text{P}_i$, phosphatidylinositol accounted for more than 55% of the radioactivity incorporated into phospholipid (though it represented only 6% of the phospholipid phosphorus) and its specific ^{32}P radioactivity (counts/min./mg. of P) was above that of any other component, including phosphatidic acid (Table 1). The specific radioactivity of phos-

phatidylethanolamine was greater than that of phosphatidylcholine, and the specific radioactivity of ethanolamine plasmalogen was higher than that of the choline form.

Effects of luteinizing hormone on the metabolism of the corpus luteum in vitro. Progesterone synthesis by the tissue was significantly increased by both concentrations of the hormone (Table 2). However, luteinizing hormone did not increase glucose uptake by the tissue, even though it increased the amount of lactate produced during the incubation. Moreover, the incorporation of $[^{32}\text{P}]\text{P}_i$ into total phospholipid was only slightly increased by luteinizing hormone ($0.05\text{ }\mu\text{g.}/\text{vessel}$), but because of the large variability between animals the difference was not statistically significant. The higher concentration of luteinizing hormone ($0.2\text{ }\mu\text{g.}/\text{vessel}$) also failed to increase the labelling of the phospholipids. Further analysis of the individual phospholipids revealed

Table 3. *Effects of luteinizing hormone in vitro on the distribution of ^{32}P radioactivity from bovine corpus luteum*

Details of experimental procedure are discussed in Table 2. Results are given as means $\pm$ s.d. of six determinations. No significant difference (*t* test) was observed between the groups.

Flask contents	Distribution of radioactivity (%)				
	Phosphatidyl-choline	Phosphatidyl-ethanolamine	Phosphatidyl-inositol	Phosphatidyl-serine	Phosphatidic acid
Control	22.7 $\pm$ 9.9	3.7 $\pm$ 2.5	53.3 $\pm$ 12.2	0.2 $\pm$ 0.1	3.7 $\pm$ 1.9
Luteinizing hormone (0.05 $\mu\text{g.}/\text{vessel}$)	20.4 $\pm$ 11.8	5.3 $\pm$ 2.1	52.2 $\pm$ 14.4	0.5 $\pm$ 0.3	4.1 $\pm$ 2.2
(0.2 $\mu\text{g.}/\text{vessel}$)	25.2 $\pm$ 13.3	4.8 $\pm$ 1.7	52.8 $\pm$ 13.3	0.2 $\pm$ 0.1	3.8 $\pm$ 0.4

Table 4. *Fatty acid composition of lipids extracted from corpus luteum*

Fatty acid notation in this and subsequent Tables is that used by Ahrens *et al.* (1959). Values are expressed as percentage compositions (w/w) of long-chain fatty acids isolated from lipid fractions. Corpora lutea 1 (CL 1) and 2 (CL 2) were obtained from two different animals. —, Not detected.

Fatty acid	Fatty acid composition (% w/w)			
	Neutral lipids		Phospholipids	
	CL 1	CL 2	CL 1	CL 2
12:0	Trace	0.2	Trace	Trace
12:1	Trace	Trace	Trace	—
14:0	0.8	1.3	0.3	0.7
14:1	0.5	Trace	Trace	0.2
15:0	0.5	0.8	0.4	0.7
16:0	11.9	14.0	16.8	15.5
16:1	5.0	4.0	2.8	4.9
17:0	1.6	1.5	1.2	1.6
17:1	1.1	0.6	0.7	0.7
18:0	9.7	9.0	12.0	6.9
18:1	27.0	22.1	18.6	21.7
18:2	11.3	12.5	14.6	11.1
18:3	1.9	2.3	1.3	1.1
20:0	0.8	1.0	—	0.4
20:1	1.9	0.6	0.4	0.4
20:2	3.0	1.5	1.6	0.7
20:3	1.7	2.3	2.7	3.6
20:4	7.2	9.0	13.8	16.4
X_1	0.9	1.2	0.5	1.6
X_2	0.6	1.9	1.5	2.2
22:4	4.4	5.8	3.5	2.4
22:5	7.5	8.3	7.0	7.1
22:6	Trace	Trace	Trace	Trace

that luteinizing hormone (at both concentrations) had no effect on the distribution of ^{32}P radioactivity (Table 3). Phosphatidylinositol accounted for approx. 50% of the total radioactivity, and over 82% of the radioactivity was labile to mild alkaline methanolysis (Dawson *et al.* 1962) (Table 3).

Fatty acid composition of corpus luteum. The proportion of oleate (18:1) in both the neutral lipid and phospholipid fractions was higher than that of any other fatty acid (Table 4). The propor-

tion of linoleate (18:2) was almost the same as that of palmitate (16:0) in the two lipid classes. The proportion of arachidonate (20:4) was higher in the phospholipids than in the neutral lipids. The amount of unsaturated acids in both lipid fractions exceeded 60% of the total fatty acids isolated from the tissue. Docosapentaenoate (22:5) and docosatetraenoate (22:4) were the main C_{22} acids and only traces of docosahexaenoate (22:6) were detected. The two components referred to as X_1

and X_2 are further discussed in the following section.

Incorporation of [1- 14 C]acetate into long-chain fatty acids isolated from lipids of corpus luteum. Palmitate (16:0) and docosatetraenoate (22:4) each represented about 20% of the 14 C radioactivity

incorporated into the fatty acids. The polyunsaturated fatty acids accounted for about 50% of the 14 C radioactivity incorporated (Table 5). In particular, the eicosaenoates (20:1, 20:2, 20:3 and 20:4) and the docosaenoates (22:4 and 22:5) accounted for approx. 21 and 26% respectively of the total 14 C radioactivity (Table 5). The identification of docosatetraenoate (22:4) and docosapentaenoate (22:5) was strengthened by hydrogenation and oxidation. Each fraction was hydrogenated and a mixture of 16:0, 18:0, 20:0, 22:0 and 24:0 acids was added. This was re-chromatographed and each saturated fatty acid was collected and assayed for radioactivity. The radioactivity coincided with 22:0 acid. After periodate-permanganate oxidation the products of each fatty acid fraction contained 14 C radioactivity that coincided with an added pimelate standard. These results, together with the relative retention times of the acids from the gas chromatograph, suggested that the most likely structure for the C_{22} acids was docosatetraenoate [22:4 ($\Delta^{7,10,13,16}$)] and docosapentaenoate [22:5 ($\Delta^{7,10,13,16,19}$)].

The specific 14 C radioactivity (counts/min./ μ g. of C) of myristate (14:0) was higher than that of any other component, though it represented only about 0.8% of the total fatty acid mass. Docosatetraenoate (22:4) had the next highest specific radioactivity and it was much greater than any of the other polyunsaturated fatty acids, including docosapentaenoate (22:5). The specific radioactivities of the 20:1, 20:2 and 20:3 acids were almost the same,

Table 5. *Incorporation of [1- 14 C]acetate into fatty acids extracted from total lipids of corpus luteum*

Corpus luteum (100 mg.) was incubated for 2 hr. at 38° in Krebs-Ringer bicarbonate buffer, pH 7.4, containing 5 μ C of [1- 14 C]acetate. Individual fatty acids were separated by gas-liquid chromatography.

Fatty acid	Distribution of radioactivity (%)	Specific radioactivity (counts/min./ μ g. of C)
14:0	7.1	1192
16:0	19.6	91
16:1	1.0	48
18:0	6.1	61
18:1	3.9	29
18:2	1.6	12
18:3	0.5	31
20:1	1.6	241
20:2	6.3	255
20:3	8.0	292
20:4	5.3	56
22:4	21.6	680
22:5	5.0	107
22:6	0.5	—
X_1	2.4	288
X_2	1.3	194

Table 6. *Incorporation of [1- 14 C]acetate into long-chain fatty acids extracted from the phospholipids, triglycerides and free fatty acids of corpus luteum*

Corpus luteum was incubated at 38° for 2 hr. in Krebs-Ringer bicarbonate buffer, pH 7.4, containing [1- 14 C]-acetate (5 μ C/ml.) and glucose (2 mg./ml.). Phospholipids, triglycerides and free fatty acids were separated by thin-layer chromatography.

Fatty acid	Distribution of radioactivity (%)		
	Phospholipids	Triglycerides	Free fatty acids
14:0	11.5	10.8	2.4
16:0	28.7	22.9	12.9
16:1	3.0	0.9	0.2
18:0	4.8	6.2	7.7
18:1	3.5	5.5	4.9
18:2	2.4	2.1	0.9
18:3	1.5	0.3	1.0
20:0	1.3	0.5	1.0
20:3	6.5	5.7	5.4
20:4	7.2	3.9	7.9
22:4	14.6	28.4	33.9
22:5	10.3	12.1	18.9
22:6	4.1	0.8	3.1
Total radioactivity (counts/min.) examined by gas-liquid chromatography	1646	5652	2214

and the specific radioactivity of each was above that of arachidonate (20:4). Excluding arachidonate (20:4), the specific radioactivities of the C₂₀ and C₂₂ polyunsaturated fatty acids were greater than those of all other identified components, with the exception of myristate (14:0). Moreover, the specific radioactivities of palmitate (16:0) and oleate (18:1) (which account for about 15 and 20% respectively of the mass) were very low in comparison with those of the above-mentioned components.

In addition to the acids identified (Table 5), two unknown components (X₁ and X₂) were observed. Though these accounted for only 2.4 and 1.3% respectively of the ¹⁴C radioactivity, their specific radioactivities were almost equal to those of the C₂₀ fatty acids. Components X₁ and X₂ had retention times of 5.55 and 6.43 (relative to that of 16:0 acid) on gas-liquid chromatography. After hydrogenation the retention time of component X₁ was decreased to 4.03 and that of component X₂ to 3.62; neither of these corresponded to a straight-chain saturated fatty acid.

The proportions of ¹⁴C radioactivity in the fatty acids isolated from the phospholipid, triglyceride and free fatty acid fractions were very similar for most components (Table 6); however, that in the phospholipid fraction was highest in palmitate (16:0), whereas in the triglyceride and free fatty acid fractions docosatetraenoate (22:4) contained the most radioactivity. The ¹⁴C radioactivity incorporated into docosapentaenoate (22:5) was highest in the free fatty acid fraction. In all three lipid fractions most of the ¹⁴C radioactivity was present in the polyunsaturated fatty acids.

DISCUSSION

The phospholipid pattern in bovine corpora lutea is very similar to that observed in the male gonads of a number of species, including the rat (Scott *et al.* 1963; Davis *et al.* 1966) and sheep (Scott & Setchell, 1968). It also resembles the pattern observed in bovine liver (Dawson *et al.* 1962), with the notable exception of ethanolamine plasmalogen, the proportion of which is greater in the gonadal tissue. Another similarity between the corpus luteum and the male gonad is in the pattern of [³²P]P_i incorporation into the individual phospholipids. In both situations the labelling of phosphatidylinositol is far above that of any other lipid component. Moreover, in both the corpus luteum and the testis (Scott & Setchell, 1968) the inclusion of exogenous pituitary hormone did not consistently alter the pattern of phospholipid metabolism. This result is in contrast with those obtained with many other endocrine organs [e.g. pancreas (Hokin & Hokin, 1958), adrenal (Hokin, Hokin, Saffran, Scholly & Zimmerman,

1958) and thyroid (Freinkel, 1957; Scott *et al.* 1966a)], where the rate of [³²P]P_i incorporation into phospholipids, and in particular phosphatidylinositol, is increased by hormonal supplementation. Thus it would seem that the rate of phospholipid turnover in these two reproductive tissues is already maximally stimulated, and perhaps the high labelling observed in phosphatidylinositol is indicative of this. On the other hand, it was possible to confirm the earlier observations (Armstrong, 1966) that luteinizing hormone did increase the biosynthesis of progesterone and also stimulate lactate formation.

The distribution of fatty acids in neutral lipids and phospholipids from bovine corpora lutea is very similar to that reported by Holman & Hofstetter (1965) for the fatty acid composition of ovarian lipids. The very high content of the polyunsaturated fatty acids in this reproductive tissue further exemplifies their essential role in maintaining normal gonadal function and this subject, together with their importance in other biological systems including membrane structure, enzyme function and transport of lipids, has been reviewed by Aaes-Jorgensen (1961).

The appearance of over 50% of the radioactivity in the polyunsaturated fatty acids extracted from the fatty acids after incubation of luteal tissue with [¹⁻¹⁴C]acetate is indicative of an active synthesis in this reproductive tissue. Moreover, the occurrence of large amounts of ¹⁴C radioactivity in the polyunsaturated fatty acids is not confined to the fatty acids extracted from the phospholipids, but is also present in the fatty acids isolated from the triglyceride and free fatty acid fractions. In fact, in these two lipid classes the proportion of radioactivity in the polyunsaturated fatty acids is even greater than that obtained in the phospholipids.

Since the specific radioactivities of the polyunsaturated fatty acids were above the specific radioactivities of the possible precursors, namely palmitoleate (16:1), oleate (18:1), linoleate (18:2) and linolenate (18:3), it is reasonable to assume that acetate was incorporated into the polyunsaturated acids by chain elongation and desaturation as suggested by Mead (1961). In addition, the distribution of ¹⁴C radioactivity in the individual polyunsaturated fatty acids (e.g. docosatetraenoate, 22:4) suggests that the rate of adding C₂ units to linoleate (18:2) is greater than that for linolenate (18:3) (Mead, 1961). Further studies on the metabolism of polyunsaturated fatty acids during different stages of the oestrous cycle may prove valuable in understanding the mechanisms associated with corpus luteum function.

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REFERENCES

- Aaes-Jorgensen, E. (1961). *Physiol. Rev.* **41**, 1.
- Ahrens, E. H., Insull, W., Hirsch, J., Stoffel, W., Petersen, M. L., Farquhar, J. W., Miller, T. & Thomasson, H. J. (1959). *Lancet*, i, 115.
- Armstrong, D. T. (1966). *J. Reprod. Fert. Suppl.* 1, p. 101.
- Bennett, M. & Coon, E. (1966). *J. Lipid Res.* **7**, 448.
- Collins, F. D. & Shotlander, V. L. (1961). *Biochem. J.* **79**, 316.
- Davis, J. T., Bridges, R. B. & Coniglio, J. G. (1966). *Biochem. J.* **98**, 342.
- Dawson, R. M. C., Hemington, N. & Davenport, J. B. (1962). *Biochem. J.* **84**, 497.
- Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
- Freinkel, N. (1957). *Endocrinology*, **61**, 448.
- Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). *J. biol. Chem.* **226**, 497.
- Goldrick, B. & Hirsch, J. (1963). *J. Lipid Res.* **4**, 483.
- Hansel, W. & Seifart, K. (1968). *J. Dairy Sci.* **50**, 1948.
- Hokin, L. E. & Hokin, M. R. (1958). *J. biol. Chem.* **233**, 805.
- Hokin, M. R., Hokin, L. E., Saffran, M., Scholly, A. V. & Zimmerman, B. U. (1958). *J. biol. Chem.* **233**, 811.
- Holman, R. T. & Hofstetter, H. H. (1965). *J. Amer. Oil Chem. Soc.* **42**, 540.
- Huggett, A. St G. & Nixon, D. A. (1957). *Lancet*, **273**, 368.
- Karmen, A., Giuffrida, L. & Bowman, R. L. (1962). *J. Lipid Res.* **3**, 44.
- Lundholm, L., Mohme-Lundholm, E. & Vamos, N. (1963). *Acta physiol. scand.* **58**, 243.
- Mead, J. F. (1961). *Fed. Proc.* **20**, 952.
- Rudloff, E. von (1956). *Canad. J. Chem.* **34**, 1413.
- Scheuerbrandt, G. & Bloch, K. (1962). *J. biol. Chem.* **237**, 2064.
- Schlenk, H. & Gellerman, J. L. (1960). *Analyt. Chem.* **32**, 1412.
- Scott, T. W. & Beeston, J. W. U. (1966). *J. Lipid Res.* **7**, 456.
- Scott, T. W., Dawson, R. M. C. & Rowlands, I. W. (1963). *Biochem. J.* **87**, 507.
- Scott, T. W., Good, B. F. & Ferguson, K. A. (1966b). *Endocrinology*, **79**, 949.
- Scott, T. W., Jay, S. M. & Freinkel, N. (1966a). *Endocrinology*, **79**, 591.
- Scott, T. W. & Setchell, B. P. (1968). *Biochem. J.* **107**, 273.
- Scott, T. W., Setchell, B. P. & Bassett, J. M. (1967). *Biochem. J.* **104**, 1040.
- Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1967). *Manometric Techniques*. Minneapolis: Burgess Publishing Co.
- Ward, P. F. V., Scott, T. W. & Dawson, R. M. C. (1964). *Biochem. J.* **92**, 60.