Lipid metabolism in regressing rat corpora lutea of pregnancy

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Abstract Lipid accumulation is a hallmark of corpus luteum regression and we characterized lipids stored in rat corpora lutea of pregnancy between days 21 and 24 post coitum, the period of luteolysis. A 10-fold rise in lutein triglyceride concentrations occurred between days 21 and 24, which represented the major alteration in luteal lipid metabolism during luteolysis, coinciding with the appearance of numerous lipid droplets in the luteal cells. The fatty acid composition of luteal triglycerides was altered between days 20 and 23 in a pattern consistent with the influx of blood triglyceride fatty acids. No evidence for increased de novo fatty acid synthesis by luteal tissue between days 21 and 24 post coitum was obtained using in vitro methods. [^{14}C]Acetate incorporation into lipids declined between days 16 and 24 and label was preferentially introduced into long chain polyunsaturated fatty acids. Rates of tritium incorporation into fatty acids from tritiated water were relatively low. [^{14}C]Glucose was predominantly incorporated into the glycerol moiety of luteal lipids and incorporation of label into this fraction was augmented between days 21 and 23 post coitum. The incorporation of [^{14}C]palmitate into triglycerides of luteal tissue during in vitro incubation also increased between days 21 and 23. In vitro lactate production, [^{14}C]glucose oxidation, and [^{14}C]-palmitate oxidation to $^{14}CO_2$ increased significantly during this time. We conclude that specific biochemical alterations occur in regressing corpora facilitating uptake and storage of blood glyceride fatty acids as triglycerides. The physiological significance of this triglyceride accumulation remains to be elucidated.

Supplementary key words Triglycerides · sterol esters · glucose metabolism · fatty acid metabolism · prostaglandin $E_{2\alpha}$

The accumulation of lipid droplets in the cytoplasm of lutein cells is a consistent feature of luteal regression in many species including laboratory rodents (1), domestic animals (2), and primates (3). Indeed, the histological finding of lipid vacuoles in luteal cells has been accepted by many investigators as an indicator of luteal demise. Both histochemical and direct biochemical analyses of luteal tissue have demonstrated high concentrations of sterol esters and neutral fat during luteolysis (4–7). These findings are well documented, but there have been few attempts to carefully correlate alterations in luteal lipid levels with the metabolic changes occurring during regression of the corpora. Although some authors have assumed that increasing lipid stores are directly related to declining steroidogenesis (i.e., accumulation of steroid precursors), this hypothesis has yet to be proven.

In the rat, the corpora lutea are the primary source of progesterone throughout gestation. The luteal potential for progesterone secretion declines immediately prior to term (luteolysis) and parturition occurs during day 23 post coitum (8). Histological and ultrastructural studies reveal a marked accumulation of lipid droplets in the luteal cell cytoplasm between days 21 and 23 post coitum.[4]

In order to analyze the process of lipid storage during luteolysis, the lipids accumulating in regressing rat corpora lutea of pregnancy were characterized, including their fatty acid compositions. The effect of exogenous prostaglandin $F_{2\alpha}$, an established luteolytic agent, on luteal lipid levels was also studied. Furthermore, we investigated some parameters of luteal lipid synthesis including metabolism of labeled acetate, glucose, and palmitate employing an in vitro incubation system. Our data demonstrate a striking increase in triglyceride storage during regression of rat corpora lutea of pregnancy. The in vitro studies reveal that certain specific metabolic alterations take place in the corpora during luteolysis that facilitate the storage of fatty acids as triglycerides.

MATERIALS AND METHODS

Animals and treatments

Pregnant Sprague-Dawley rats (150–180 g in weight at mating) were obtained from Charles River

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Breeding Labs, Wilmington, MA. The day of the vaginal sperm plug is designated as day 1 post coitum. Animals were quartered in a controlled environment of 14 hr of light and 10 hr of darkness with Purina lab chow and water available ad libitum.

Prostaglandin $F_{2\alpha}$, obtained as the tromethamine salt (mol wt 476.6) was dissolved in 0.9% saline and injected intramuscularly in doses of 500 μg, twice daily (10:00 and 18:00 hr) for 2 consecutive days (days 14 and 15 post coitum). Blood for lipid analysis was collected in heparinized syringes by heart puncture while the animals were under light ether anesthesia. Plasma was separated by centrifugation and stored at −20°C until analyzed. Animals were killed by cervical dislocation and the corpora lutea were quickly dissected from the ovaries. At necropsy, the condition of uterine fetuses was assessed.

Extraction of lipids

Corpora lutea dissected from one pair of ovaries (approximately 40–80 mg) or obtained after incubations were extracted with 3 ml of chloroform–methanol 2:1 (v/v) by homogenization with a glass homogenizer. The extracts were filtered through glass wool and the solvent was evaporated under nitrogen. The extracts from all in vitro studies were washed three times with 0.3 volumes of 0.05 M KCl. Triglycerides were extracted from 1 ml samples of plasma by adding 4 ml of 2-propanol–3N H_2SO_4 40:1 (v/v), 2 ml of water, and 5 ml of hexane followed by vigorous shaking for 1 min. The hexane phase was removed and taken for triglyceride analysis.

Thin-layer chromatography

Thin-layer chromatography was carried out on plates coated with silica gel G 250 μm in thickness. The plates were prerun in diethyl ether and then activated at 110°C for 30 min. After application of samples, plates were developed to 7 cm with hexane–diethyl ether–acetic acid 60:40:1 (v/v/v), dried and redeveloped to 15 cm with the same solvents in a ratio of 90:10:1 (9). Lipid fractions were localized by either exposing the plates to I_2 vapors or by spraying with 0.05% Rhodamine 6G in 95% ethanol. Lipid classes were identified by comparison with the mobilities of known standards.

Gas–liquid chromatography

Gas–liquid chromatography of fatty acid methyl esters was carried out on a Varian Aerograph Model 2100 gas–liquid chromatograph (Varian Assoc., Palo Alto, CA) equipped with flame ionization detectors and a 10:1 splitter–collector (10). A 6 ft × 4 mm ID glass U-column packed with 10% EGSS-X on 100–120 mesh Gas Chrom P was employed for all analyses with injector and column temperatures maintained at 250°C and 195°C, respectively. Identification of the various peaks was made on the basis of plots of log retention times vs. carbon number, relative retention times reported in the literature, and comparison with retention times of fatty acid methyl esters of known structure. The mass response was determined by triangulation of the peaks.

Quantitation of lipids

Triglycerides isolated from tissue and plasma extracts were quantitated by the charring technique of Kritchevsky et al. (9). Tripalmitin was used as the standard. Following thin-layer chromatography of lipid extracts, the sterols were eluted from the silica gel with 5 ml of chloroform–methanol 2:1 (v/v) and aliquots were taken for sterol determination by the method of Zlatkis, Zak, and Boyle (11). Recovery of added [^{3}H]cholesterol from the chloroform–methanol extracts after thin-layer chromatography averaged 92% and the values reported have not been corrected for the losses.

Sterol esters were also isolated by thin-layer chromatography and quantitated by the charring method of Kritchevsky et al. (9) using cholesteryl palmitate as a standard. Chloroform–methanol extracts of tissue were dissolved in 100 μl of chloroform and aliquots were taken for phospholipid analyses by the method of Raheja et al. (12) with dipalmitoyl phosphatidylcholine as a standard.

Incubation conditions

In vitro incubations were carried out in Krebs-Ringer bicarbonate buffer (13), pH 7.4, containing 5.5 mM glucose and equilibrated with 95% O_2–5% CO_2. Corpora lutea, quickly isolated from the ovaries, were placed in a gassed medium at room temperature until a sufficient quantity of tissue was collected. Incubations were initiated by placing 5–10 corpora into 25-ml flasks that contained 4–10 ml of medium and that had been preincubated at 37°C under 95% O_2–5% CO_2. Labeled compounds were usually added to give 0.1 μCi/ml of incubation fluid. After addition of the tissue, the flasks were gassed for 30 sec, stoppered, and placed in a shaking incubator at 37°C. Incubations were routinely carried out for 2 hr and were terminated by the injection of 1 ml of 70% perchloric acid through the self-sealing stopper. When $^{14}CO_2$ was collected, 0.2 ml of 1M Hyamine hydroxide (Packard, Downers Grover, IL) was injected into a suspended center well (Kontes, Vineland, N. J.) at the end of the incubation and the flasks were shaken for an additional hr at 37°C to

insure complete collection of $^{14}CO_2$ before the center wells were placed in vials for liquid scintillation counting. Flasks containing all ingredients except tissue or tissue previously exposed to perchloric acid were processed along with sample flasks as controls so experimental data could be appropriately corrected. Following termination of the incubations, tissue was removed from the flasks, blotted dry and weighed, and then tissue and medium were stored at −20°C until analyzed.

To test the validity of studying intact corpora lutea in the in vitro system described above, a comparison was made between intact corpora lutea and quartered and minced luteal tissue with respect to [U-^{14}C]glucose oxidation to $^{14}CO_2$. Corpora lutea removed on day 18 post coitum were apportioned to each of the three groups and incubated for 2 hr in the presence of labeled substrate. Prior to the addition of the Hyamine and perchloric acid, all the flasks were opened and the intact and quartered corpora lutea were quickly removed, minced with a fine scissors, and replaced in the flasks. This was done to keep the conditions for acid-induced termination of the incubation constant. After collection of the $^{14}CO_2$, the minced tissues were collected and digested with 1N KOH at 55°C and the protein contents were determined.

The oxygen utilization by pools of corpora lutea was determined under conditions essentially identical to those used in the metabolic flux studies. Oxygen consumption was monitored continuously using a Yellow Springs Instrument (Yellow Springs, OH) oxygen probe as described by Flint and Denton (14). Eight to twelve corpora lutea were placed in 3 ml of Krebs-Ringer bicarbonate buffer, containing 5.5 mM glucose, which had been equilibrated with 95% O_2–5% CO_2. The chamber of the apparatus was maintained at 37°C. The rate of oxygen utilization was linear for periods of 10–15 min, but regassing of the medium for 5–10 min allowed utilization rates similar to those obtained on the initial reading to be resumed. By regassing the medium at 10 min intervals, constant rates of oxygen consumption were found for 2 hr periods. After determination of the oxygen utilization, corpora lutea were placed in 7% perchloric acid for 1 hr, blotted dry, and weighed.

Protein determinations

The protein contents of corpora lutea that had been digested in 1N KOH overnight at 55°C were determined by the method of Lowry et al. (15) with bovine serum albumin as a standard.

Lactate

For lactate determinations, the incubation medium was neutralized with a saturated solution of Na_2CO_3 and lactate was determined by enzymatic assay with lactate dehydrogenase in the presence of hydrazine as a trapping agent (16).

Decarboxylation studies

The decarboxylation of long chain fatty acid methyl esters was carried out according to the method of Brady, Bradley, and Trams (17). Radiolabeled samples collected off the stream-splitter of the gas chromatograph were placed in tubes and the solvent was evaporated under nitrogen. Fifty mg of sodium azide was then added and the tubes were stoppered with self-sealing rubber caps with suspended center wells. Reactions were initiated by addition of 0.5 ml of a mixture of 1 part fuming H_2SO_4 and 3 parts concentrated H_2SO_4 into the main compartments and 0.2 ml of Hyamine into the center wells. The tubes were then heated at 70°C for 1 hr. The Hyamine-containing center wells and residues remaining in the tubes were then taken for liquid scintillation counting. Controls containing the methyl esters of [1-^{14}C]-fatty acids and tubes without added sodium azide were run simultaneously.

Liquid scintillation counting

Liquid scintillation counting was carried out with a Packard Tri-Carb scintillation counter Model 3385 with a counting fluid composed of toluene–methyl Cellusolve 5:4 (v/v) with added Spectrofluor (Amersham/Searle, Arlington Heights, IL). Quench corrections were made with the use of an external standard.

Labeled compounds

[U-^{14}C]Glucose (3 mCi/mmol), [U-^{14}C]acetic acid (57 mCi/mmol), [1-^{14}C]oleic acid (58.9 mCi/mmol) and [1-^{14}C]palmitic acid (57.9 mCi/mmol) were purchased from Amersham/Searle. ^3H_2O (1 Ci/ml) was obtained from New England Nuclear, Boston, MA.

All labeled lipids were analyzed for purity by thin-layer chromatography. The purity of fatty acids was further analyzed by radio–gas–liquid chromatography of the fatty acid methyl esters. The purity of these substances was always greater than 98%.

Fatty acids were complexed to fat-poor bovine serum albumin (Nutritional Biochemical Co., Cleveland, OH) by a modification of the method of Donabedian and Karmen (18). Solutions were prepared to yield a molar ratio of fatty acid to albumin

of 2.25 for [1-^{14}C]palmitate, assuming a mol wt of 66,000 for bovine serum albumin. The fatty acid concentrations of these solutions were assayed following extraction by the method of Duncombe (19).

Statistics

Means were compared for statistical differences by Student's *t* test.

RESULTS

Free sterol levels in corpora lutea remained relatively constant at approximately 3 mg/g wet wt of tissue between days 16 and 24 post coitum **(Fig. 1).** Luteal sterol ester concentrations were about threefold higher than free sterol levels on day 16. Between days 16 and 20 the levels of sterol ester doubled and thereafter rose slightly (Fig. 1). The mean sterol ester concentrations in corpora removed on days 20–24 were significantly greater than the concentrations measured on day 16 ($P < 0.01$).

Between days 20 and 23 post coitum, luteal phospholipid concentrations declined and on day 23 phospholipid levels were significantly lower ($P < 0.02$) than those observed on day 20 (**Fig. 2**). There was a striking change in the concentrations of luteal triacylglycerols, which were present in low levels between days 16 and 21 and increased more than 10-fold between days 21 and 24 post coitum (Fig. 2). The mean triglyceride concentration on day 22 was significantly greater than the level measured on day 21, with a P value <0.02, and the difference in triglyceride concentrations between days 21 and 23 had a greater level of significance ($P < 0.001$). The increase in luteal triglyceride stores coincided with

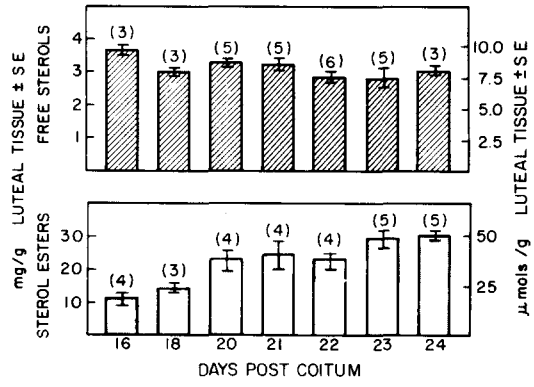

Fig. 1. Free and esterified sterol concentrations in corpora lutea of pregnancy. Bars represent the means ± SE with the number of observations indicated in parentheses.

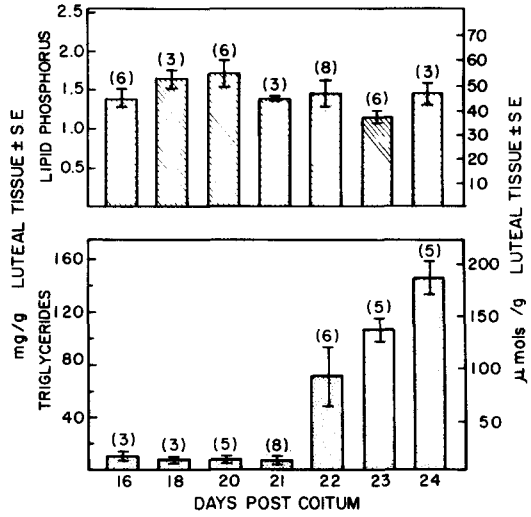

Fig. 2. Lipid phosphorus and triglyceride concentrations in corpora lutea of pregnancy. Bars represent the means ± SE with the number of observations indicated in parentheses.

the marked accumulation of lipid droplets in the lutein cell cytoplasm. Although wet weight and protein content per gland (**Fig. 3**) both declined significantly between days 18 and 24, the increase in triglycerides remained as striking when the data were considered in terms of changes in lipid content per corpus luteum between days 21 and 24 post coitum. It should be pointed out that triglyceride storage did not occur in the liver while lipid built up in the corpora lutea, indicating that the accumulation of triglycerides in lutein cells was a selective process. On days 20 and 23 post coitum, liver triglyceride concentrations were 6.26 ± 0.86 (N = 6) and 6.87 ± 0.60 (N = 5) mg/g wet wt ± SE, respectively.

The fatty acid compositions of the luteal phospholipids, triglycerides, and sterol esters were analyzed in samples obtained on days 20 and 23 post coitum and each of these classes of lipids was found to have a characteristic fatty acid distribution (Tables 1–3). In luteal phospholipids the principal species present were palmitate, stearate, and fatty acids whose methyl esters had retention times similar to arachidonate **(Table 1)**. There were no marked differences in the fatty acid composition of phopholipid fractions obtained on days 20 and 23, although there were small but significant reductions in 16:1,2 and 22:4 (docosatetraenoate) and a small increase in 22:5 (docosapentaenoate). The fatty acid compositions of luteal triglycerides and sterol esters underwent more notable alterations between days 20 and 23 post coitum (Tables 2 and 3). There were increases in the weight percentages of oleic, linoleic, and arachidonic

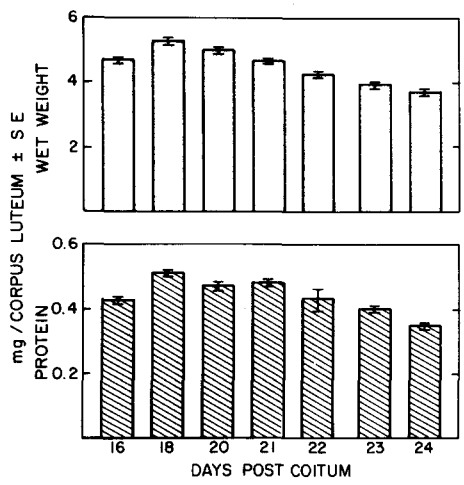

Fig. 3. Weight and protein content of corpora lutea of pregnancy. Bars represent the means ± SE of at least six determinations.

TABLE 2. Fatty acid in luteal triglycerides

Fatty Acid	Day 20	Day 23	*P* Value
	% by weight ± SE		
14:0	2.41 ± 0.89	0.93 ± 0.20	NS[a]
14:1	0.97 ± 0.27	0.22 ± 0.02	<0.05
16:0	20.79 ± 3.00	24.36 ± 1.42	NS
16:1, 2	1.50 ± 0.32	2.45 ± 1.02	NS
18:0	29.96 ± 3.88	19.81 ± 1.12	<0.05
18:1	13.14 ± 0.60	19.23 ± 0.78	<0.005
18:2	6.74 ± 1.54	11.62 ± 0.42	<0.02
20:0	1.47 ± 0.19	0.52 ± 0.003	<0.05
18:3, 20:1	3.32 ± 0.33	2.13 ± 0.15	<0.02
20:2	1.22 ± 0.04	0.85 ± 0.08	<0.02
22:0	0.41 ± 0.10	0.61 ± 0.11	NS
20:3, 20:4, 22:1	4.68 ± 0.57	8.53 ± 0.44	<0.005
22:4	9.20 ± 1.58	5.99 ± 0.55	NS
22:5	1.99 ± 0.08	1.37 ± 0.15	<0.025
22:6	1.65 ± 0.68	1.76 ± 0.32	NS
	N = 3	N = 4	

[a] Not significant, $P > 0.05$, by Student's t test.

acids in the triglycerides, while the weight percentages of stearic acid and several fatty acids present in smaller quantities declined (**Table 2**). In general, the fatty acid composition of the sterol esters tended to change between days 20 and 23 post coitum in a pattern similar to that described for the triglycerides. However, the higher contents of docosatetraenoic (22:4) and docosahexaenoic (22:6) acids and the lower contents of palmitate and oleate distinguished the sterol ester fatty acid patterns from those of triglyceride fractions (**Table 3**).

In the rat, a pronounced lipemia occurs near the end of gestation but the blood lipid levels are reduced by the time of parturition (20). The lipemia results primarily from an elevation in triglycerides due to several factors, including increased maternal caloric intake, enhanced fatty acid mobilization from fat, and diminished uptake of triglycerides by adipose tissue (20–22). The decline in blood tirglyceride levels prior to term is thought to result, at least in part, from increased uptake of triglyceride fatty acids by the mammary glands. These observations suggested that the accumulation of triglycerides in corpora lutea between days 21 and 24 post coitum could also result from increased uptake of blood triglyceride fatty acids. When blood triglyceride levels were analyzed in rats in the present study, plasma triglyceride concentrations were found to be 264 ± 10 (N = 3), 348 ± 17 (N = 3), 88 ± 7 (N = 4) and 64 ± 19 (N = 5) mg/dl of plasma ± SE on days 20, 21, 22, and 23, respectively. The marked decline in plasma triglyceride concentrations coincided with initiation of triglyceride storage in the luteal tissue (Fig. 2).[5]

Since significant alterations in fatty acid composition of luteal triglycerides are observed during lipid storage (Table 2), the composition of plasma trigly-

TABLE 1. Fatty acid in luteal phospholipids

Fatty Acid	Day 20	Day 23	*P* Value[c]
	% by weight ± SE		
14:0[a]	ND[b]	ND	
14:1	0.99 ± 0.16	1.81 ± 0.21	
16:0	19.59 ± 0.48	19.20 ± 1.55	
16:1, 2	1.82 ± 0.46	3.13 ± 0.21	<0.05
18:0	24.43 ± 0.53	22.15 ± 0.89	
18:1	7.25 ± 0.93	8.69 ± 0.85	
18:2	7.10 ± 0.53	8.03 ± 0.63	
18:3, 20:1	0.26 ± 0.06	0.36 ± 0.07	
20:2	0.38 ± 0.03	0.37 ± 0.08	
22:0	1.25 ± 0.004	1.00 ± 0.15	
20:3, 20:4, 22:1	27.32 ± 0.28	25.61 ± 2.41	
20:5, 24	0.88 ± 0.11	1.29 ± 0.14	
22:4	4.87 ± 0.18	4.00 ± 0.20	<0.02
22:5	1.28 ± 0.14	0.93 ± 0.03	
22:6	2.65 ± 0.30	3.60 ± 0.13	<0.05
	N = 5	N = 4	

[a] Number of carbon atoms:number of double bonds.
[b] Not detected.
[c] Differences are not significant, $P > 0.05$, unless otherwise noted.

[5] It should be noted that only a fraction of the triglyceride fatty acids cleared from the circulation between days 21 and 23 could be deposited in the corpora because of their relatively small mass (~50–60 mg/animal). The majority of the blood glyceride fatty acids are probably taken up by the mammary glands. If one assumes that the blood volume of the pregnant rat near term is 16 ml (22) and assumes a total luteal wt of 50 mg per animal, the increase in lutein triglyceride content between days 21 and 23 post coitum could account for approximately 10% of the fall in blood triglyceride during this time.

cerides was examined to determine whether changes seen in the luteal glycerides were consistent with an influx of plasma triglyceride fatty acids. There were significant differences between the plasma and the luteal triglyceride fatty acid patterns (**Tables 2 and 4**), including higher weight percentages of oleic and linoleic acids and a lower content of stearic acid in the plasma triglycerides. Between days 20 and 23 post coitum, the fatty acid composition of the plasma triacylglycerols altered; the decline in the weight percentage of linoleic acid and the rise in the weight percentage of arachidonic acid were two prominent changes. Comparing the fatty acid compositions displayed on days 20–23, it would appear that, with the exception of arachidonate, most of the changes in the luteal fatty acid pattern during this time could have resulted from the uptake of plasma triglyceride fatty acids (e.g., the rise in oleic and linoleic acid levels and the decline in the weight percentage of stearic acid).

We next examined whether triglyceride storage could be induced in functional corpora lutea by an established luteolytic agent, prostaglandin $F_{2\alpha}$ (23), at a time when blood triglyceride levels were not markedly elevated. In rats given intramuscular injections of prostaglandin on days 14 and 15 post coitum when plasma triglyceride levels were 80–100 mg/dl, lipid levels had increased in the corpora lutea by the time of killing on day 16. Both sterol ester and triglyceride concentrations were elevated in the corpora of the prostaglandin-treated rats while free sterol levels were unaltered (**Table 5**). Luteal weights were only slightly reduced by prostaglandin treatment. That prostaglandin treatment caused only a slightly greater than twofold increase in luteal triglyceride concentrations, in contrast to the more than 10-fold rise at term, appears to be related to the much lower blood triglyceride levels on days 14–16 post coitum (i.e., less triglyceride fatty acid is available for uptake into the corpora).

To explore the biochemical events occurring in the corpora lutea that lead to the accumulation of lipid during luteolysis, especially triglyceride, an in vitro incubation system was established so certain parameters of lipid metabolism could be assessed. To establish that isolated whole rat corpora lutea of pregnancy could be effectively studied using the in vitro incubation system described in the Methods section, rates of [U-^{14}C]glucose oxidation by intact

TABLE 3. Fatty acid in luteal sterol esters

Fatty Acid	Day 20	Day 23	*P* Value[a]
	% by weight ± SE		
14:0	1.56 ± 0.66	2.06 ± 0.92	
14:1	0.40 ± 0.04	0.26 ± 0.17	
16:0	12.07 ± 1.37	16.04 ± 2.62	
16:1, 2	1.38 ± 0.13	1.88 ± 0.17	
18:0	22.17 ± 2.14	14.70 ± 2.25	
18:1	12.77 ± 1.12	16.57 ± 2.14	
18:2	3.33 ± 0.18	4.48 ± 0.44	
20:0	2.25 ± 0.35	1.86 ± 0.23	
18:3, 20:1	5.61 ± 0.70	3.27 ± 0.38	<0.05
20:2	3.31 ± 0.13	1.27 ± 0.14	<0.001
22:0	1.39 ± 0.11	1.33 ± 0.16	
20:3, 20:4, 22:1	4.48 ± 0.34	10.54 ± 1.28	<0.01
20:5, 24	0.72 ± 0.06	0.53 ± 0.24	
22:4	17.02 ± 1.49	15.21 ± 3.08	
X[b]	0.49 ± 0.28	0.83 ± 0.50	
22:5	5.06 ± 1.47	2.40 ± 0.29	
22:6	5.84 ± 1.37	6.74 ± 1.51	
	N = 4	N = 4	

[a] Not significant, $P > 0.05$, unless otherwise noted.
[b] Unidentified peak.

TABLE 4. Fatty acid in plasma triglycerides

Fatty Acids	Day 20	Day 21	Day 22	Day 23
		% by weight ± SE		
14:0	0.95 ± 0.15	0.99 ± 0.19	1.06 ± 0.07	1.41 ± 0.17
14:1	0.53 ± 0.08	0.32 ± 0.04	0.44 ± 0.03	0.55 ± 0.13
16:0	28.18 ± 2.14	22.49 ± 0.98	25.68 ± 1.54	25.66 ± 0.87
16:1, 2	4.53 ± 0.84	3.07 ± 0.81	2.61 ± 0.28	2.97 ± 0.23
18:0	5.14 ± 0.15	3.85 ± 0.32	4.20 ± 0.64	6.42 ± 0.67
18:1	27.83 ± 1.55	26.11 ± 1.81	26.57 ± 1.08	23.16 ± 1.39
18:2	24.88 ± 0.80	27.51 ± 2.59	23.82 ± 0.88	18.24 ± 1.38
18:3, 20:1	2.15 ± 0.20	2.61 ± 0.32	2.48 ± 0.25	2.24 ± 0.49
20:2	0.74 ± 0.14	0.82 ± 0.11	0.48 ± 0.02	0.75 ± 0.17
22:0	0.62 ± 0.21	1.33 ± 0.00	0.77 ± 0.17	1.50 ± 0.26
20:3, 20:4, 22:1	1.06 ± 0.02	2.99 ± 0.41	4.19 ± 0.61	5.66 ± 0.73
20:5, 24	0.67 ± 0.10	0.79 ± 0.10	1.80 ± 0.24	1.75 ± 0.23
22:4	0.49 ± 0.06	1.10 ± 0.10	0.75 ± 0.12	1.42 ± 0.17
22:5	0.08 ± 0.06	1.58 ± 0.27	1.69 ± 0.29	2.33 ± 0.45
22:6	1.68 ± 0.15	4.38 ± 0.24	3.50 ± 0.64	4.87 ± 0.18
	N = 3	N = 3	N = 3	N = 3

TABLE 5. Effect of prostaglandin $F_{2\alpha}$ treatment on luteal lipids

	Vehicle-Treated	Prostaglandin $F_{2\alpha}$	*P* Value
	mg/g wet weight tissue ± SE		
Free sterols	4.24 ± 0.12	4.90 ± 0.34	NS[a]
Sterol esters	15.50 ± 0.50	21.33 ± 1.30	<0.005
Triglycerides	9.73 ± 1.06	22.03 ± 2.52	<0.005
Luteal wt (mg)	3.88 ± 0.14	3.56 ± 0.38	NS
	N = 5	N = 5	

Rats received 500 μg of the tromethamine salt of prostaglandin $F_{2\alpha}$ or the saline vehicle by intramuscular injection, twice daily, on days 14 and 15 post coitum. Animals were killed approximately 16 hr following the last injection on day 16.

[a] Not significant, $P > 0.05$.

corpora were compared with rates displayed by quartered and minced lutein tissue. Highest rates of substrate oxidation were obtained with intact corpora lutea (436 ± 37 cpm in $^{14}CO_2$/mg protein per 2 hr ± SE, N = 3) and no differences were seen when whole and quartered corpora lutea (410 ± 4 cpm, N = 3) were compared. Minced luteal tissue oxidized the least substrate (305 ± 22 cpm, N = 3).

Rates of oxygen consumption by whole corpora lutea were also examined. Pools of corpora lutea removed from rats on day 18 post coitum displayed an oxygen utilization of 47.4 ± 2.2 nmol/mg tissue per hr (mean ± SE, N = 4) while rates were significantly higher ($P < 0.01$) when the corpora lutea were obtained on day 23 (103.8 ± 3.6 nmol/mg tissue per hr, N = 3). These values are quite similar to those reported by Flint and Denton (14) using a comparable incubation system with thinly sliced superovulated rat ovarian tissue.

[U-^{14}C]Acetate incorporation into luteal lipids

To evaluate the importance of lipid biosynthesis during glyceride accumulation in luteal tissue between days 21 and 24 post coitum, the incorporation of [^{14}C]acetate into luteal lipids was first examined. During a 2 hr incubation of isolated corpora lutea in the presence of 1 mM [U-^{14}C]acetate, label was incorporated into total tissue lipids at a linear rate. Since the addition of 5.5 mM glucose enhanced by 2.5-fold the incorporation of [U-^{14}C]acetate into lipids without altering $^{14}CO_2$ production, glucose was routinely included in the incubation medium.

Incorporation of [U-^{14}C]acetate into total tissue lipids was highest by corpora lutea removed on day 16 post coitum and thereafter fell to low levels by day 24 (**Fig. 4**). Oxidation of [U-^{14}C]acetate to $^{14}CO_2$ also declined significantly between days 21 and 23, but appeared to rebound by day 24.

When the chloroform–methanol extracts of corpora lutea incubated for 2 hr in the presence of [U-^{14}C]acetate were analyzed by thin-layer chromatography, the majority of the radioactivity was localized in the triglyceride, phospholipid, and free fatty acid fractions. Representative distributions are displayed in **Fig. 5** and it can be seen that the pattern of labeling is not markedly altered between days 18 and 23 post coitum, except that slightly less label is incorporated into phospholipids on days 21 and 23. On transesterification of the lipid extracts in methanolic HCl, 90% of the recovered radioactivity was found in the fatty acid methyl ester fractions.

[U-^{14}C]Acetate incorporation into fatty acids

To ascertain whether label incorporated into the fatty acids represented de novo lipid synthesis or elongation of preexisting fatty acyl chains, the fatty acyl moieties synthesized in the presence of [U-^{14}C]-acetate were analyzed by radio–gas–liquid chromatography. Corpora lutea removed on day 18 post coitum incorporated label primarily into long chain unsaturated fatty acids, the majority of radioactivity being associated with docosatetraenoic acid (22:4). Less than 15% of the recovered label was found in myristic and palmitic acids (**Table 6**).

A similar labeling pattern was obtained when corpora lutea removed on day 23 post coitum were studied, although there was less label incorporated into docosatetraenoic acid and slightly greater radioactivity in the myristic and palmitic acid peaks.

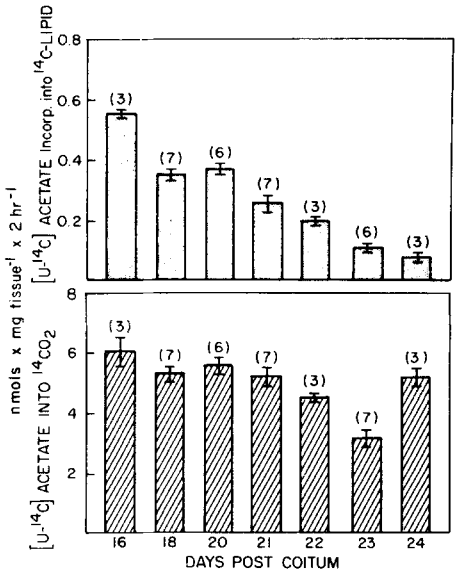

Fig. 4. Rates of conversion of [U-^{14}C]acetate to total tissue lipids and $^{14}CO_2$. Corpora lutea were incubated for 2 hr in Krebs-Ringer buffer containing 1 mM [U-^{14}C]acetate and 5.5 mM glucose. Radioactivity trapped as $^{14}CO_2$ and present in the chloroform–methanol extracts was determined. Bars represent means ± SE with the number of observations indicated in parentheses.

The labeling distributions presented in Table 6, with the predominance of radioactivity in long chain polyunsaturates, strongly suggests that chain elongation is the pathway by which [U-^{14}C]acetate is incorporated into fatty acids by luteal tissue. To further susbstantiate the primacy of elongation and desaturation over de novo fatty acid synthesis in this system, the docosatetraenoic acid peaks obtained from several incubations were degraded to determine the percentage of radioactivity in the carboxyl carbons. Thirty-nine percent of the recovered label was found in the carboxyl carbons of the docosatetraeonic acid samples (**Table 7**), while 98% of the label was in the carboxyl carbons of [1-^{14}C]oleic acid methyl esters, which were run simultaneously as controls. The presence of sodium azide was essential for the selective decarboxylation of C_1 since only 0.69% of the label from [1-^{14}C]oleic acid methyl ester was recovered as $^{14}CO_2$ in its absence. If the docosatetraenoic acid had been synthesized de novo, the label would be distributed uniformly throughout the chains and only 4–5% of the radioactivity would have been recovered from carboxyl carbons. However, the presence of values nine-fold greater than this is consistent with chain elongation of unsaturated fatty acids containing 20 carbons.

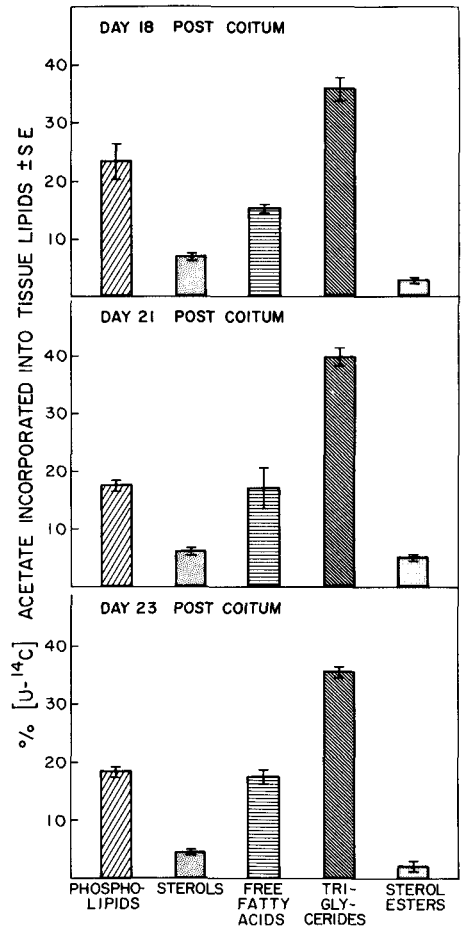

Fig. 5. Distribution of radioactivity in chloroform–methanol extracts of corpora lutea incubated in the presence of [U-^{14}C]-acetate. Corpora lutea were incubated for 2 hr in Krebs-Ringer buffer containing 1 mM [U-^{14}C]acetate and 5.5 mM glucose. Chloroform–methanol extracts of tissue were subjected to thin-layer chromatography and radioactivity recovered in the various lipid fractions was determined. Bars represent means ± SE of at least three determinations.

TABLE 6. [U-^{14}C]Acetate incorporation into fatty acids by corpora lutea in vitro

Fatty Acid Peak	Day 18 post coitum	Day 23 post coitum
	% Recovered Radioactivity	
14:0	1.74 ± 0.15[a]	4.43 ± 0.90
14:1	0.52 ± 0.10	1.28 ± 0.56
16:0, 16:1	9.93 ± 3.98	13.58 ± 2.14
18:0	1.80 ± 0.32	2.90 ± 0.62
18:1	1.39 ± 0.37	4.56 ± 1.62
18:2	0.89 ± 0.16	3.33 ± 0.52
18:3, 20:1	1.98 ± 0.19	4.33 ± 1.10
20:2	1.02 ± 0.13	2.95 ± 0.34
22:0	1.66 ± 0.18	1.72 ± 0.23
20:3, 20:4, 22:1	2.10 ± 0.35	5.26 ± 2.28
20:5, 24	2.41 ± 0.28	6.02 ± 1.96
22:4	62.22 ± 4.98	45.05 ± 0.92
X[b]	5.86 ± 0.46	4.67 ± 0.46
22:5	2.19 ± 0.27	3.23 ± 0.24
22:6	3.97 ± 0.07	4.99 ± 0.93

Corpora lutea removed on days 18 or 23 post coitum were incubated for 2 hr in Krebs-Ringer buffer containing 0.5 mM [U-^{14}C]acetate and 5.5 mM glucose. The total chloroform–methanol extracts were transesterfied in methanolic HCl and the fatty acid methyl esters formed were subjected to gas–liquid chromatography. Aliquots of each peak were collected for liquid scintillation counting employing a 10:1 splitter–collector.

[a] Mean ± SE of three determinations.

[b] Unidentified peak.

[U-^{14}C]Glucose incorporation into luteal lipids

When in vitro studies were carried out employing [U-^{14}C]glucose as the substrate, incorporation of label into the tissue lipid extracts proceeded at a linear rate during 2-hr incubations. Lactate production also increased linearly with time, but the oxidation of the label to $^{14}CO_2$ only became linear after the first hr of incubation.[6]

[U-^{14}C]Glucose incorporation into tissue lipid extracts increased significantly ($P < 0.01$) between days 21 and 22 post coitum, with values on day 24 returning near to those observed with tissue removed on day 21 (**Fig. 6**). Glucose oxidation to $^{14}CO_2$ and lactate production also increased on days 21–22, declining on days 22–24. In these studies, incubations

[6] There is a lag in the yield of $^{14}CO_2$ during the first hour of in vitro incubation, but thereafter the yields are constant and a linear rate of [U-^{14}C]glucose oxidation is maintained for up to 4 hr.

TABLE 7. Decarboxylation of [^{14}C]docosatetraenoic acid synthesized by corpora lutea from [U-^{14}C]acetate

Sample Number	dpm in $^{14}CO_2$	dpm in Residue	% Radioactivity in C_1
1	573	916	38.6
2	1,075	1,586	40.5
3	1,130	1,874	37.7

Three separate experiments were carried out with [^{14}C]docosatetraenoic acid peaks collected by gas–liquid chromatography from extracts of luteal tissue incubated in the presence of 0.5 mM [U-^{14}C]acetate. The isolated [^{14}C]docosatetraenoic acid methyl esters were subjected to degredation to release the C_1 carbon.

were carried out for 0.5, 1, and 2 hr, but only the data from the 2 hr points have been presented since [U-^{14}C]glucose incorporation into tissue lipids and lactate release proceeded at a linear rate. When the rates of $^{14}CO_2$ production are corrected for the lag during the first hr of incubation by using the rates observed between the 1 and 2 hr time points, the same pattern of change presented in Fig. 6 is observed, although the rates of glucose oxidation are higher. The pattern of glucose metabolism observed in the present study, with lactate production exceeding glucose oxidation and less glucose carbon flowing into luteal lipid synthesis, is similar to results obtained with superovulated rat ovaries (14, 24).

A determination of the distribution of [U-^{14}C]glucose into the principal lipid classes revealed that over 80% of the label incorporated during a 2 hr incubation was found in glycerolipids. Between days 18 and 23 post coitum the incorporation of label into phospholipids declined, while incorporation into triglycerides increased (Fig. 7). On transesterification, however, less than 8% of the radioactivity in the total lipid extracts was associated with the fatty acid methyl esters. This demonstrates that [U-^{14}C]glucose is not a major precursor for luteal fatty acid

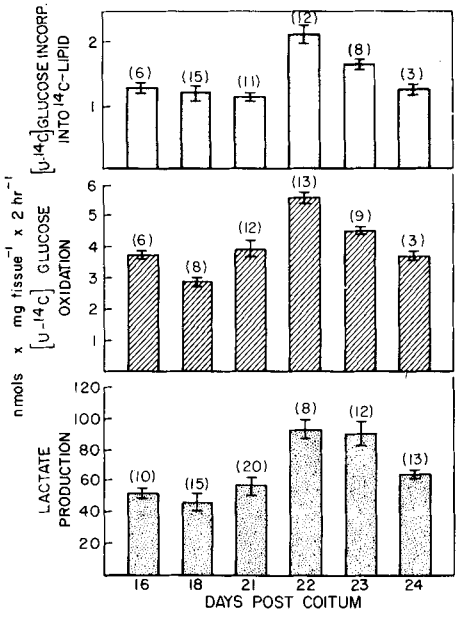

Fig. 6. In vitro [U-^{14}C]glucose metabolism by corpora lutea removed on days 16–24 post coitum. Corpora lutea removed on the days indicated were incubated for 2 hr in Krebs-Ringer buffer containing 5.5 mM [U-^{14}C]glucose. Yields of $^{14}CO_2$, lactate in the medium, and label in tissue lipid extracts were determined. Bars represent means ± SE with the number of observations noted in parentheses.

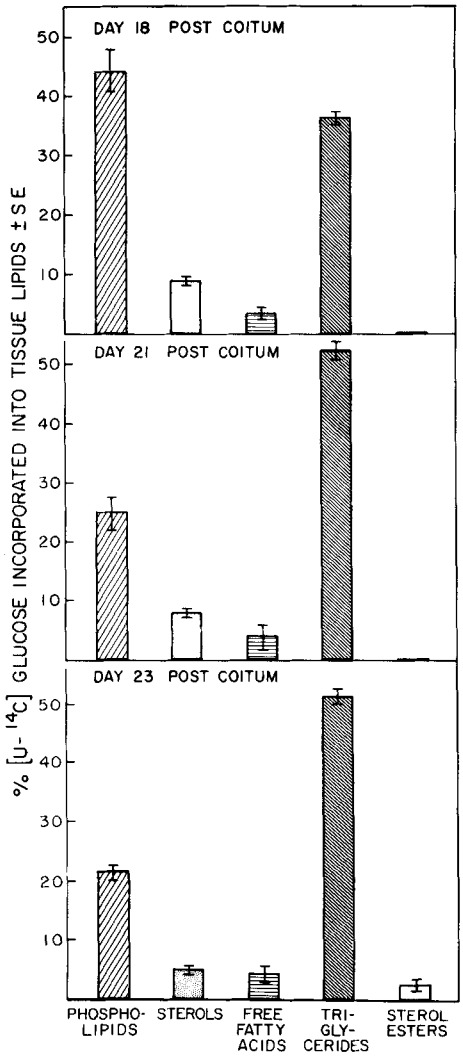

Fig. 7. Distribution of radioactivity in lipid extracts from corpora lutea incubated in the presence of [U-^{14}C]glucose. Corpora lutea were incubated in Krebs-Ringer buffer containing 5.5 mM [U-^{14}C]-glucose for 2 hr. The chloroform–methanol extracts of the tissue were subjected to thin-layer chromatography and radioactivity in the various lipid fractions was determined. Bars represent means ± SE of at least three determinations. Aliquots of the total lipid extracts were also transesterified in methanolic HCl and the hexane-extractable radioactivity and label remaining in the methanol phase were quantitated. 5.5 ± 0.9%, 7.8 ± 1.5%, and 3.6 ± 1.2% of the counts ± SE were recovered in the hexane phase on days 18, 21, and 23 post coitum, respectively.

synthesis in vitro, but it is a source of 3-glycerophosphate for phosphatides and glycerides.

Incorporation of ^3H_2O into luteal lipids

Diminishing lipogenesis during luteolysis is indicated by the marked reduction in in vitro incorporation of [U-^{14}C]acetate into tissue lipids (Fig. 4). However, use of ^{14}C-labeled precursors to assess rates of lipid synthesis is subject to limitation since the specific activity of the substrate can be appreciably diluted by endogenous compounds. The incorporation of ^{3}H from ^3H_2O into tissue lipids can be used to determine total rates of lipid synthesis, averting the problem of dilution by endogenous substrates if one assumes that the tissue water content is relatively stable (25). Attempts were made, therefore, to define changes in luteal lipogenesis between days 16 and 23 post coitum using this technique. Incorporation of tritium during incubation into luteal fatty acids was determined by subjecting the lipid extracts to methanolysis and quantitating the hexane-soluble radioactivity (fatty acid methyl esters). Rates of incorporation of tritium into luteal lipids did not change between days 16 and 21 post coitum, but there was a significant increase between days 21 and 23 ($P < 0.01$) (**Table 8**).

Metabolism of [1-^{14}C]palmitate

When corpora lutea removed on different days of gestation were incubated in the presence of a fixed concentration of [1-^{14}C]palmitate complexed to bovine serum albumin, rates of oxidation of the labeled fatty acid to $^{14}CO_2$ were found to be highest by corpora removed on day 18 post coitum, to decline between days 18 and 21 ($P < 0.05$), but then to increase between days 21 and 23 (**Fig. 8**). The latter increase is statistically significant at the $P < 0.02$ level. Incorporation of the label into luteal phospholipids declined between days 18 and 21 ($P < 0.02$), while labeling of the triglyceride fraction increased significantly between days 21 and 23 post coitum ($P < 0.001$) (Fig. 8). Rates of incorporation of label into the sterol esters and diglycerides remained unaltered during the time periods studied.

TABLE 8. Tritium incorporation into luteal lipids from ^3H_2O

Days Post Coitum	μg-atoms ^{3}H incorporated × g tissue^{-1} × 2 hr^{-1}	N
16	0.246 ± 0.006[a]	(3)
18	0.251 ± 0.046	(3)
21	0.264 ± 0.049	(3)
23	0.374 ± 0.019	(4)

Pools of luteal tissue were incubated in Krebs-Ringer buffer containing 5.5 mM glucose and ^3H_2O (1 mCi/ml). Incubations were carried out for 2 hr and chloroform–methanol extracts of the tissue prepared. The lipid extracts were subjected to transesterification in methanolic HCl and hexane-extractable radioactivity was quantitated by liquid scintillation counting.

[a] Mean ± SE.

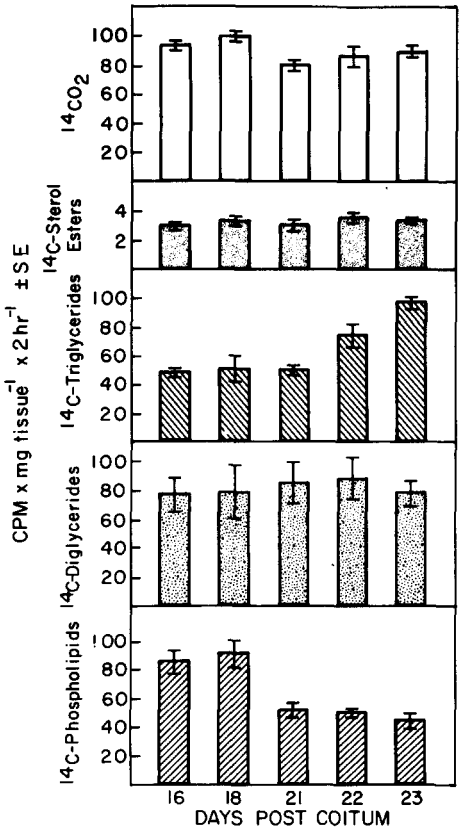

Fig. 8. In vitro metabolism of [1-^{14}C]palmitic acid by corpora lutea. Corpora lutea removed on the indicated days were incubated in Krebs-Ringer buffer containing 5.5 mM glucose and 75 μM [1-^{14}C]palmitic acid (2×10^6 cpm/μmol) complexed to fat-poor bovine serum albumin with a molar ratio of 2.25. Yields of $^{14}CO_2$ and incorporation of label into tissue lipids during a 2 hr incubation were determined. Bars represent the means ± SE for 4–9 determinations.

DISCUSSION

As in other mammals, luteolysis in the rat is attended by a significant accumulation of lipid in the luteal cells. In this species there appear to be two distinct phases of lipid storage in the aging corpus luteum of pregnancy: an increase in sterol ester concentrations between days 16 and 20 post coitum (Fig. 1) followed by a striking increase in triglyceride levels between days 21 and 24 (Fig. 2). It is the increase in triglyceride content that coincides with the appearance of large lipid droplets in the lutein cells and the loss of potential to form active progestogens. The following discussion deals predominantly

with the accumulation of triglyceride in the luteal tissue, but the reader should be cognizant that significant alterations in the metabolism of other lipid classes seem to occur in regressing corpora (e.g., alterations in sterol ester fatty acid composition and decreased phospholipid synthesis).

The triglyceride fatty acids stored in the corpora lutea between days 21 and 24 post coitum appear to be derived from the plasma triglyceride pool. Although we have no direct proof for this statement (e.g., kinetic studies of plasma triglyceride uptake), the following observations favor this interpretation: *1*) the fatty acid composition of luteal triglycerides changed between days 20 and 23 in a pattern consistent with the influx of plasma triglyceride fatty acids. *2*) The amount of triglyceride stored in the corpora lutea during regression seemed to be directly related to the blood triglyceride concentrations present at the onset of luteolysis. When luteolysis was induced by prostaglandin $F_{2\alpha}$ at a time when blood glyceride levels were relatively low, less triglyceride accumulated in the corpora than during luteal regression at term. *3*) No evidence for markedly enhanced de novo lipogenesis in luteal tissue during the period of lipid accumulation was obtained.

Rates of tritium incorporation from ^3H_2O into luteal fatty acids in vitro increased significantly between days 21 and 23 post coitum (Table 8). However, these rates were relatively low in comparison to those displayed by rat adipose tissue that incorporates approximately ten times more label when incubated in vitro under very similar conditions (25).

In contrast to the data obtained with ^3H_2O, studies on the in vitro incorporation of acetate into lipids suggested declining rates of luteal lipid synthesis between days 21 and 24 post coitum (Fig. 4). However, the [U-^{14}C]acetate data were not corrected for possible dilution of the label specific activity by endogenous compounds and this may account, in part, for the apparent discrepancy. The acetate experiments did reveal a most important aspect of luteal lipid metabolism: the pattern of [U-^{14}C]acetate incorporation into luteal fatty acids determined by radio–gas–liquid chromatography indicated that the corpora prefer to modify preexisting fatty acyl chains by elongation and desaturation rather than to synthesize them de novo (Tables 6 and 7). The corpus luteum of the rat is not unique in its propensity for chain elongation and desaturation, since bovine luteal tissue (26) and rat renal papillae (27) also incorporate [^{14}C]acetate primarily into polyunsaturated acids like docosatetraenoic acid during in vitro incubation. It should be noted that docosatetraenoic acid is a major component of the sterol ester fatty acid pool (Table 3).

In view of the apparent prominence of chain elongation in luteal fatty acid metabolism, one must be cautious in interpreting the results of the ^3H_2O experiments, since label could be incorporated into fatty acids either during de novo fatty acid synthesis or during chain elongation. A knowledge of the distribution of ^3H in the various fatty acid species might have shed some light on this problem, but we were unable to obtain lipid extracts with sufficiently high specific activities to perform reliable radio–gas–liquid chromatographic analyses. Based on our present studies, we suggest that the observed increased in vitro ^3H incorporation between days 21 and 23 post coitum is mostly a manifestation of increased chain elongation to provide long chain polyunsaturated fatty acids.

Several metabolic alterations occur in regressing corpora lutea that would facilitate the storage of preformed fatty acyl chains, presumably derived from the blood lipids, as triglycerides between days 21 and 24 post coitum. The in vitro incorporation of [^{14}C]acetate and palmitate into luteal phospholipids declines between days 18 and 21 (Figs. 5 and 8) so that between days 21 and 24 the pathways for phospholipid biosynthesis would draw less precursor away from the route for glyceride formation. Furthermore, glucose metabolism to form glycerolipid glycerol was noticeably enhanced between days 21 and 23 (Figs. 6 and 7), which may well have contributed to the observed increased efficiency of palmitate incorporation into luteal triglycerides during this time (Fig. 8).

In addition to the activation of biochemical pathways involved in triglyceride synthesis, we have demonstrated the stimulation of certain catabolic sequences during regression of the rat corpus luteum of pregnancy. [U-^{14}C]Glucose and [1-^{14}C]palmitate oxidation to $^{14}CO_2$ and lactate production increase between days 21 and 23 post coitum (Figs. 6 and 8). Since luteal free fatty acid concentrations increased between days 22 and 24, the [1-^{14}C]fatty acid pool was probably diluted at this time and the yields of $^{14}CO_2$ from [1-^{14}C]palmitic acid thus underestimate the rate of fatty acid oxidation (Fig. 8) (28). Furthermore, we observed greater in vitro oxygen consumption by corpora lutea removed on day 23 as compared to tissue harvested on day 18. These findings of enhanced catabolic activity during luteal regression speak against the possibility that lipid storage during luteolysis is a result of diminished cellular metabolism. Luteinizing hormone stimulates glucose metabolism in rat ovarian tissue (14, 24) and the increment in metabolic activity displayed by corpora lutea between days 21 and 23 post coitum could be the consequence

of rising blood levels of luteinizing hormone during this time (29).

The decline in [U-^{14}C]acetate oxidation to $^{14}CO_2$ between days 21 and 23 post coitum (Fig. 4) appears to contradict the observations that [1-^{14}C]palmitic acid and [U-^{14}C]glucose catabolism are enhanced. This discrepancy can be explained by the competition between fatty acids and acetate as substrates for mitochondrial oxidation; the increasing endogenous luteal free fatty acid pool depresses yields of $^{14}CO_2$ from [U-^{14}C]acetate (30). An explanation for the apparent rebound in rates of acetate oxidation on day 24 post coitum is not readily available.

The initial event in the process of luteal triglyceride storage may be an increase in lutein lipoprotein lipase activity. Glycerides are hydrolyzed within the capillary lumen by lipoprotein lipase to yield free fatty acids that cross the endothelial barrier. Lipoprotein lipase activity has been identified in mammalian ovarian tissue (31, 32) and in preliminary studies we have found a significant increase in rat luteal lipoprotein lipase activity between days 21 and 24 post coitum (31). The factors that might induce luteal lipoprotein lipase activation have not been explored, but our observations that exogenous prostaglandin $F_{2\alpha}$ causes luteal triglyceride storage would suggest that this agent might be one of the regulators.

The physiological significance of triglyceride accumulation during luteolysis is not obvious. However, it seems evident that specific changes occur in lutein metabolism during regression that can facilitate the uptake and storage of blood triglyceride fatty acids. Probably the simplest explanation for this observation is that the biochemical sequence for storage of blood glyceride fatty acids is activated by the hormonal milieu associated with luteolysis, perhaps by prostaglandin $F_{2\alpha}$ (8, 28).

It is doubtful that free fatty acids or triglyceride fatty acids are carbon sources for luteal steroidogenesis (33) so it is difficult to envision a direct relationship between the storage of this lipid fraction and declining progesterone synthesis during luteolysis. On theoretical grounds, there are several mechanisms by which triglyceride accumulation might interfere with luteal function. An increase in the intracellular concentrations of free fatty acids or long-chain acyl-CoA derivatives could occur during triglyceride storage. In fact, luteal free fatty acid levels are significantly elevated on days 23 and 24 post coitum (28). Both free fatty acids and long-chain acyl-CoA derivatives are potent enzyme inhibitors, acting either as allosteric effectors or as biological detergents (34). The increased stores of triglycerides containing unsaturated fatty acids may also leave the lutein cells vulnerable to damage via lipid peroxidation. Massive lipid collections in the luteal cells could mechanically damage organelles in the cytoplasmic compartment. Such a sequence is thought to take place in fatty degeneration of the liver and possibly in the genesis of artherosclerotic plaques (35), but this has not been reported in ultrastructural studies of regressing luteal tissue (1). An important role for triglycerides enriched with unsaturated fatty acids, particularly arachidonate and its precursors, may be to provide substrates for intraluteal prostaglandin synthesis. Prostaglandins produced within the lutein cells might participate in the process of luteolysis. The mechanisms by which prostaglandin induces luteal regression have not been fully elucidated. Several alterations in luteal steroid and sterol metabolism result from prostaglandin action, including increased catabolism of progesterone (23) and diminished activities of cholesterol ester synthetase and esterase (36). The latter may account for storage of sterol esters in luteal tissue following prostaglandin treatment.

This work was supported by Program Project Grant HD 6274-05, 5-T-05-GM-02046-03 and a Grant from the Ford Foundation. Prostaglandins were kindly provided by Dr. J. Pike of the Upjohn Company. J. F. S. is grateful to Drs. A. I. Winegrad, P. Spooner, S. Chernick and J. Blanchette-Mackie for helpful discussions during the course of this work.

Manuscript received 8 July 1976; accepted 16 November 1976.

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