OVARIAN PHOSPHOLIPID COMPOSITION AND INCORPORATION OF [1-¹⁴C]ACETATE INTO THE PHOSPHOLIPID FATTY ACIDS OF OVARIES FROM NON-PREGNANT AND PREGNANT RABBITS

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Summary. Ovarian slices from non-pregnant and 6-day-pregnant rabbits were incubated with [1-¹⁴C]acetate. ¹⁴CO₂ was collected, and subsequently the amounts, specific radio-activities, fatty acid composition and fatty acid specific activities of the isolated phospholipids were determined.

Phosphatidyl choline was increased in amount in the ovary during pregnancy, and [1-¹⁴C]acetate incorporation into phosphatidyl choline and phosphatidyl inositol was also increased. ¹⁴CO₂ production was greater from ovaries of pregnant than non-pregnant rabbits.

The percentage of oleic acid in both phosphatidyl choline and phosphatidyl ethanolamine was higher in ovaries from the pregnant rabbits, and palmitic acid in phosphatidyl ethanolamine was slightly decreased during pregnancy. Of the major fatty acids of phosphatidyl choline, only palmitic acid showed an increased radio-acetate incorporation with pregnancy.

INTRODUCTION

Alterations in phospholipid concentration and metabolism appear to occur in several organs during pregnancy. In rats, as well as in humans, there appears to be a decreased level of plasma lysolecithin and increases in phosphatidyl choline and phosphatidyl ethanolamine with pregnancy (Vikrot, 1964, 1965). Serum and liver fatty acids also are changed during pregnancy, with the predominant effects being increases in palmitate and oleate, and decreases in stearate and arachidonate (McKay & Kaunitz, 1963).

Although the content and metabolism of cholesterol and total fatty acids in the ovary have been the subject of several investigations (Marion & Sell, 1963; Ryan & Short, 1965; Holman & Hofstetter, 1965), there have been only a few studies of ovarian phospholipids (Boyd, 1935; Bloor, Okey & Corner, 1930). Boyd (1935) studied the total phospholipids of the rabbit ovary during pregnancy and found a gradual increase in content up to the 14th to 16th days, after which the ovarian phospholipid concentration began to decrease. In the sow,

Bloor *et al.* (1930) found that high levels of ovarian phospholipids are attained at ovulation and continue at this high level throughout pregnancy.

The objectives of the present experiments were to determine the content of specific phospholipids and phospholipid fatty acids in ovaries from non-pregnant and pregnant rabbits, and to study the alterations in incorporation of [^{14}C]acetate into these phospholipids occurring during pregnancy.

MATERIALS AND METHODS

Sixteen female New Zealand white rabbits, 4 months old and fed a stock commercial diet (Purina) since weaning, were divided into two groups of eight each. One group was mated to induce pregnancy. After 6 days the rabbits were anaesthetized by intravenous injection of sodium pentobarbital. Uteri of the mated rabbits were examined and all found to be gravid. The ovaries from each animal were sliced at 0.4-mm intervals with a McIlwain Tissue Chopper and then placed in a flask with 10 ml of Tyrode's solution. After addition of 50 μC [1- ^{14}C]sodium acetate (Nuclear-Chicago, specific activity 29.0 mc/mm) to each, the flasks were incubated for 4 hr in an air atmosphere at 37.5° C in a Dubnoff metabolic shaking incubator. Carbon dioxide was collected in a centre well containing 20% NaOH. The flasks were then placed on ice, and after cooling to 0° C, the contents were centrifuged, washed twice with 20 ml 0.9% NaCl for 20 min each time and re-centrifuged. The tissues were then refrigerated overnight in 50 ml of 0.155 M unlabelled sodium acetate. The next day, after one additional washing with 0.9% NaCl and centrifugation, the total ovarian lipids were extracted in a VirTis 45 Homogenizer with two successive 50 ml portions of methylal/methanol, 4:1. The combined extracts were evaporated to dryness in a rotary vacuum evaporator and, under nitrogen, redissolved in chloroform, filtered and evaporated down to 0.5 ml. Silica gel H (Brinkmann) plates 0.5 mm thick were prepared by the method of Skipski, Peterson & Barclay (1964). After drying and heating at 110° C for 1 hr, the plates were placed in the developing mixture and the solvents allowed to rise to the top of the plates, in order to remove impurities. The plates were then reactivated for 1 hr at 110° C just before use. The total ovarian lipids were applied as a band across the plate and the phospholipids separated, using an ascending developing mixture of chloroform : methanol : acetic acid : water 25:15:4:2. The lysophosphatidyl choline, sphingomyelin, phosphatidyl choline, phosphatidyl inositol, phosphatidyl serine and phosphatidyl ethanolamine bands were collected with a vacuum zone extractor. Elution of the phospholipids from the silica gel was accomplished as previously described (Skipski *et al.*, 1964). Aliquots of the eluates from each fraction were analysed for phosphorus content (Zilversmit & Davis, 1950). Other aliquots were assayed for radio-activity using a PPO-POPOP in toluene scintillator and a Packard Model 3314 liquid scintillation spectrometer. Quenching was monitored by the channels ratio technique, and all counts corrected to 100% efficiency.

The remainder of the fractions was then hydrolysed and methylated (Morin, Bernick, Mead & Alfin-Slater, 1962) and the fatty acid composition of each phosphatidyl choline and phosphatidyl ethanolamine fraction was determined

by gas-liquid chromatography in a Barber-Colman Model 10 Gas Chromatograph using diethylene glycol succinate on Gaschrom P, 70-80 mesh, at 187° C with an argon pressure of 24 lb. The fatty acids were identified by comparison of retention times to those of standards (obtained from the National Institutes of Health and Calbiochem) and by graphic representation of retention times. The areas under the peaks were estimated by triangulation. Only the major fatty acids (16:0, 16:1, 18:0, 18:1, 18:2 and 20:4) have been included in the tabulations. Other fatty acids, identified in amounts either too small or having too long a retention time to measure and collect accurately, were 14:0, 18:3, 20:1, 20:3(8,11,14), 22:4, 22:5 and 22:6. Aldehydes of sixteen and eighteen carbon atoms were also identified as minor constituents in most of the fractions. After weighing the total fatty acid methyl esters, the individual methyl esters were collected from the gas-chromatography effluent in anthracene cartridges using a Packard Gas Chromatography Fraction Collector equipped with a tight rubber seal. Average recovery of fatty acids was 88%. Specific activities of each fatty acid methyl ester were calculated as described by Evans & Norcia (1964). Of the fatty acids, only 16:0, 18:0 and 18:1 had sufficiently high radio-activity to determine accurately, and so only these are included in the tabulations.

The radio-active CO₂ evolved was determined by precipitating an aliquot of the NaOH centre well solution with 10% BaCl₂. The light precipitate of BaCO₃ which formed was trapped by filtration as a very thin layer on a disc of Whatman No. 42 filter paper and then the paper with the precipitate counted in the PPO-POPOP scintillator as described above.

The probabilities (*P*) that apparent differences in the data were due to chance were calculated by the *t*-test, and only those differences where *P* < 0.01 have been considered significant.

RESULTS AND DISCUSSION

The amounts of the individual phospholipid fractions in the ovaries from non-pregnant and pregnant rabbits are indicated in Table 1. The order of concentration of phospholipids was phosphatidyl choline > phosphatidyl ethanolamine > phosphatidyl serine > sphingomyelin > phosphatidyl inositol = lysophosphatidyl choline. The relative proportions of these phospholipids are in general

TABLE 1
AMOUNTS OF INDIVIDUAL PHOSPHOLIPIDS IN RABBIT
OVARIES*

	<i>Rabbit group</i>	
	<i>Non-pregnant</i>	<i>Pregnant</i>
Lysophosphatidyl choline	1.5 ± 1.6	2.3 ± 1.9
Sphingomyelin	2.3 ± 0.3	2.9 ± 1.7
Phosphatidyl choline	17.5 ± 2.1	23.0 ± 3.6
Phosphatidyl inositol	2.1 ± 2.7	1.5 ± 0.7
Phosphatidyl serine	6.8 ± 3.2	7.4 ± 2.6
Phosphatidyl ethanolamine	14.1 ± 1.8	12.8 ± 3.5

* mg phospholipid/g dry weight ovary ± standard deviation.

similar to those found in the liver and other organs (Youngs & Cornatzer, 1963; Morin, 1965, 1967a).

The amount of phosphatidyl choline was slightly higher in the ovaries from pregnant rabbits than those from the non-pregnant group. This is consistent with the earlier work of Boyd (1935) who demonstrated an increase in total

TABLE 2
SPECIFIC RADIO-ACTIVITIES OF INDIVIDUAL PHOSPHOLIPIDS OF RABBIT OVARIES INCUBATED WITH $[1-^{14}\text{C}]$ ACETATE*

	Rabbit group	
	Non-pregnant	Pregnant
Lysophosphatidyl choline	3612 $\pm$ 1078	2674 $\pm$ 1274
Sphingomyelin	3206 $\pm$ 658	3010 $\pm$ 1064
Phosphatidyl choline	4760 $\pm$ 1540	8680 $\pm$ 2352
Phosphatidyl inositol	4642 $\pm$ 1750	9438 $\pm$ 2730
Phosphatidyl serine	8582 $\pm$ 1274	8260 $\pm$ 2086
Phosphatidyl ethanolamine	3962 $\pm$ 1246	5586 $\pm$ 1760

* dpm/mg phospholipid $\pm$ standard deviation.

phospholipids of the rabbit ovary during pregnancy, and suggests that this increase is due primarily to an increase in phosphatidyl choline.

The specific activities of the individual ovarian phospholipids after incubation with $[1-^{14}\text{C}]$ acetate are indicated in Table 2, and the amounts of radio-acetate in μmoles incorporated into each phospholipid are given in Table 3.

TABLE 3
AMOUNTS OF $[1-^{14}\text{C}]$ ACETATE INCORPORATED INTO INDIVIDUAL PHOSPHOLIPIDS OF RABBIT OVARIES*

	Rabbit group	
	Non-pregnant	Pregnant
Lysophosphatidyl choline	84 $\pm$ 25	95 $\pm$ 45
Sphingomyelin	114 $\pm$ 23	135 $\pm$ 47
Phosphatidyl choline	1293 $\pm$ 418	3101 $\pm$ 840
Phosphatidyl inositol	151 $\pm$ 57	220 $\pm$ 63
Phosphatidyl serine	906 $\pm$ 134	949 $\pm$ 239
Phosphatidyl ethanolamine	867 $\pm$ 272	1110 $\pm$ 349

* μmoles $[1-^{14}\text{C}]$ acetate incorporated/g dry weight of ovary $\pm$ standard deviation.

There appeared to be less variation in the degrees of incorporation into the different phospholipids than is found in the liver, testes and aortas (Morin, 1967a, b, 1968), where phosphatidyl choline was far more active than the other phospholipids. Pregnancy seemed to increase significantly the specific activities of phosphatidyl choline and phosphatidyl inositol. This increase was not due to increased availability of the substrate because of decreased oxidation, as

indicated by the higher production of $^{14}\text{CO}_2$ by the ovaries from the pregnant rabbits ($116,300 \pm 8345$ disintegrations/min/mg dry ovary) as compared to $75,560 \pm 8040$ from the ovaries of the non-pregnant group.

The fatty acid compositions of the two highest concentration phospholipids, phosphatidyl choline and phosphatidyl ethanolamine, are indicated in Table 4. In the same group, aortic phosphatidyl choline showed higher percentages of palmitic and linoleic acids and lower stearic and arachidonic acids than did phosphatidyl ethanolamine. These percentages of fatty acids in the ovary are similar to the relative proportions of fatty acids found on comparison of phosphatidyl choline with phosphatidyl ethanolamine in other organs (Morin, 1965, 1967a, b, 1968). The percentages of oleic acid in both phosphatidyl choline and phosphatidyl ethanolamine were higher in the pregnant than in the non-pregnant state. In addition, the percentage of palmitic acid in phosphatidyl

TABLE 4
PERCENTAGES OF FATTY ACID METHYL ESTERS IN PHOSPHATIDYL
CHOLINE AND PHOSPHATIDYL ETHANOLAMINE OF RABBIT
OVARIES*

Fatty acid	Phosphatidyl choline		Phosphatidyl ethanolamine	
	Non-pregnant rabbits	Pregnant rabbits	Non-pregnant rabbits	Pregnant rabbits
16:0	36.2 ± 5.6	35.3 ± 6.5	12.4 ± 0.5	9.1 ± 2.1
16:1	2.0 ± 0.5	1.9 ± 0.7	1.6 ± 0.8	1.3 ± 0.4
18:0	14.3 ± 3.5	12.7 ± 1.5	29.6 ± 4.2	25.2 ± 2.8
18:1	19.4 ± 4.8	28.1 ± 3.7	24.7 ± 0.7	30.1 ± 2.5
18:2	9.1 ± 2.9	6.5 ± 1.5	5.5 ± 0.6	5.8 ± 1.6
20:4	19.1 ± 0.8	15.5 ± 4.8	26.3 ± 2.4	28.3 ± 4.1

* Percentage of each fatty acid methyl ester $\pm$ standard deviation.

ethanolamine was slightly lower in ovaries from the pregnant rabbits. An increased proportion of oleate was also found by McKay & Kaunitz (1963) in the total lipids of the serum and liver of rats during pregnancy. The increased palmitate and decreased stearate and arachidonate observed by the latter investigators, however, were not found in the ovaries of pregnant rabbits in the present study.

In Table 5 are shown the specific activities and amounts of $[1-^{14}\text{C}]$ acetate incorporated into palmitic, stearic and oleic acids of phosphatidyl choline and phosphatidyl ethanolamine. In the same group, the degree of incorporation was palmitic > stearic > oleic acid. In other organs, such as the liver, testes and aorta, palmitic acid is also found to be the most highly labelled after administration or incubation with $[1-^{14}\text{C}]$ acetate (Morin, 1965, 1967a, b, 1968). The predominant effect of pregnancy seemed to be an increase in the specific activity of palmitic acid of phosphatidyl choline.

Since oestrogenic hormones have been demonstrated to increase the concentration and synthesis of phosphatidyl choline and other phospholipids in the liver and uterus (Ranney & Weiss, 1958; Aizawa & Mueller, 1961; Hagopian

& Robinson, 1965), it is possible that the increased oestrogen levels produced by the ovary during pregnancy stimulate the synthesis of ovarian phosphatidyl choline and phosphatidyl inositol. It has been suggested in another study that oestrogens may increase the hepatic production of stearic and arachidonic-containing species of phospholipids (Lyman, Tinoco, Bouchard, Sheehan,

TABLE 5
SPECIFIC ACTIVITIES AND AMOUNTS OF [1-¹⁴C]ACETATE INCORPORATED INTO INDIVIDUAL FATTY ACID METHYL ESTERS OF PHOSPHATIDYL CHOLINE AND PHOSPHATIDYL ETHANOLAMINE IN OVARIES FROM NON-PREGNANT AND PREGNANT RABBITS

Fatty acid	Phosphatidyl choline		Phosphatidyl ethanolamine	
	Non-pregnant rabbits	Pregnant rabbits	Non-pregnant rabbits	Pregnant rabbits
16:0 S.A.*	1715 ± 246	3009 ± 760	1757 ± 833	1652 ± 526
μmoles†	169 ± 24	379 ± 96	48 ± 23	30 ± 10
18:0 S.A.	1050 ± 279	1187 ± 360	678 ± 146	676 ± 235
μmoles	41 ± 11	54 ± 16	44 ± 9	34 ± 12
18:1 S.A.	297 ± 203	465 ± 192	372 ± 189	378 ± 231
μmoles	16 ± 11	41 ± 19	20 ± 10	23 ± 14

* dpm/mg fatty acid methyl ester ± standard deviation.

† μmoles [1-¹⁴C]acetate incorporated/g dry weight of ovary ± standard deviation.

Ostwald & Miljanich, 1967). Since, in the present experiments, it appeared that the pregnancy ovary increased its content of oleic acid and increased the incorporation of radioacetate into palmitic acid, factors other than oestrogens, such as increased progesterone and/or gonadotrophic hormones, may have been acting on the ovary to produce the observed changes.

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