sterols is concerned. The solubility of cholesterol in coconut oil is decreased by the presence of certain dicarboxylic acids and methyl substituted dicarboxylic acids, as well as by imidazole. Active acids thus far discovered in order of decreasing activity are the following: 5,5-dimethylazelaic acid; 4,4-dimethylpimelic acid; 2,2-dimethylglutaric acid; pimelic acid; methylmalonic acid; glutaric acid; 2,2'-dimethylsuccinic acid; 2-methylpimelic acid; 3,3-dimethylglutaric acid; 2,2-dimethylsuccinic acid; and 2-methylsuccinic acid. Approximately 100 miscellaneous compounds, including many other dicarboxylic acids and imidazole derivatives, are not associated with any decrease in the solubility of cholesterol in coconut oil. Free fatty acids in relatively small amounts increase the solubility of cholesterol in coconut oil.

The effect of the active dicarboxylic acids on the solubility of cholesterol is accomplished by the formation of an insoluble crystalline clathrate of cholesterol and dicarboxylic acid. In the case of pimelic acid this clathrate involves cholesterol and pimelic acid in equimolar amounts.

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Lipid Composition and Synthesis in Rat Liver During Pregnancy and the Puerperium.* (28952)

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The composition(1-4) and synthesis(5,6) of liver lipids has been reported for male and female rats, but a paucity of data exists for pregnant and puerperal animals. Some reports(7-9) compare the total liver lipids in non-pregnant and pregnant rats, but they do not relate the concentration of cholesterol, triglycerides, or phospholipids present, or describe these lipids in the puerperium. Most investigations in lipid synthesis during pregnancy are concerned with the origin of fetal lipids(5,10) and tend to neglect the metabolism of the maternal liver. Though data do exist showing hepatic lipogenesis in pregnancy(11-13) they are not in agreement.

This report describes the composition and synthesis of rat liver lipids during pregnancy and the early puerperium, and presents further evidence of the metabolic changes that influence the hyperlipidemia of pregnancy.

Materials and methods. Pregnant albino rats of the Sprague-Dawley strain (Holtzman Co., Madison, Wis.) were obtained 2 days after being sperm positive. Non-pregnant control and sperm positive rats were shipped at the same time so both groups would be subjected to the same conditions during transportation. When the animals were received, they were randomized, placed in cages, and subjected to a 15 hour day and 9 hour night period by use of artificial lighting in order to reduce the differences in the amount of food consumed. Rats were fed a rat diet†

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† Ingredients of the diet were as follows: non-fat powdered milk, 11.1%; dried brewer's yeast, 4.5%; salt, 1.5%; soy bean oil meal, 15%; ground yellow corn, 10%; 2nd clear wheat flour, 37.5%; wheat bran, 1.5%; alfalfa meal, 2%; fish meal, 10%; calcium carbonate, 0.5%; animal fat containing antioxidant, 5.9%; and vit. A and D supplement.

(Dietrich and Gambrill, Inc., Frederick, Md.) and watered *ad libitum*. Analysis of the diet showed it contained not less than 24% crude protein and not less than 5% crude fat.

Lipid composition. Fasted non-pregnant and pregnant (17th gestation day) rats were selected at random and sacrificed on the same day while puerperal rats from whom the young were weaned at birth were sacrificed on the second postpartum day. The animals were anesthetized by an intraperitoneal injection of sodium pentobarbital and bled from the aorta according to Lushbough and Moline(14). The liver was removed, blotted between filter paper, weighed, wrapped in Parafilm and immediately placed in a freezer at —20°C until analyses could be made. After denaturing the protein by immersion in boiling water, the lipids were extracted from 0.5-1.0 g of liver according to the procedure of Folch *et al*(15). An aliquot of the lipid extract was resolved on a silicic acid column(16) into two fractions. One fraction contained free and esterified cholesterol and triglycerides, while the other fraction consisted of phospholipids; total cholesterol(17), triglycerides(18), and phospholipid phosphorus(19) were determined.

Lipogenesis. Incorporation of acetate-1-C^{14} by rat liver slices into unsaponifiable substances and fatty acids was determined on fed animals weighing approximately 200-225 g. Pregnant rats were between 17-19 days' gestation, while puerperal animals as described above were taken on the second postpartum day. All groups were sacrificed by decapitation, the livers immediately removed, and slices prepared on a Stadie-Riggs microtome. The tissue was incubated in Krebs-Henseleit bicarbonate medium containing sodium acetate-1-C^{14} (specific activity 200-300 $\times$ 10^2 cpm/μmole), according to Felts *et al*(6), except 25 ml Erlenmeyer flasks were used. The incubation flasks were gassed 10 minutes with a 95% O_2–5% CO_2 mixture, stoppered, and incubated 3 hours at 37.5°C on a Dubnoff shaker set at 120 strokes/min. At the end of the incubation period, 1 ml of 30% aqueous KOH was added to each flask except the control flask which had KOH added at the beginning of the incubation period, and the tissue was digested on the shaker for an additional 30 min. To each flask was added 5 ml of 95% ethanol, a glass marble placed on top, and the contents hydrolyzed on a boiling water bath for 4-5 hours. The flask contents were acidified with 10 N H_2SO_4, filtered through a plug of glass wool into a separatory funnel and extracted with a 20 ml portion of diethyl ether after adding 10 ml of distilled water. The extraction was repeated 2 times and each time the incubation flask was rinsed. The ether extracts were collected, washed with distilled water until neutral, exaporated to dryness under nitrogen at reduced pressure, and dried overnight in a vacuum oven at 60°C. The lipids were dissolved in 5 ml of diethyl ether and the cholesterol containing fraction and total fatty acids separated(20). Radioactivity measurements were made in a Packard Tri-Carb liquid scintillation counter.

In one series of experiments, the liver slices were removed, extracted as previously described(15), and the lipids separated into 3 fractions(21). Each fraction was hydrolyzed and the esterified fatty acids found in cholesterol esters, triglycerides, and phospholipids recovered.

Results are shown as μmoles of acetate incorporated/g liver, and are calculated from the specific activity of the added sodium acetate-1-C^{14} and the total counts incorporated into each fraction. Tests of significance were determined by the "t" test.

Results. The lipid composition of rat liver expressed as mg/g liver for non-pregnant, pregnant, and puerperal animals is shown in Table I. The results show that concentrations of cholesterol and lipid phosphorus were similar in each group, and a statistical comparison of the group means was not significantly different. However, the puerperal levels of the liver triglycerides were significantly higher than those found in either the pregnant or non-pregnant rats ($P<.01$); a comparison of the latter 2 groups showed no difference. When liver lipids for these groups were determined on a total liver basis and expressed as mg/100 g body weight to correct for differences in liver weight attributable to body weight, the gross lipid composi-

TABLE I. Lipid Composition of Liver from Non-Pregnant, Pregnant and Puerperal Rats.

Group	No. of rats	Body wt (g)	Liver wt (g)	Cholesterol (mg/g)	Triglycerides (mg/g)	Lipid phosphorus (mg/g)
Non-pregnant	11	160	6.1	3.95 ± .99*	7.16 ± 2.89	1.86 ± .39
Pregnant	12	248	8.7	3.87 ± 1.36	5.34 ± 1.69	1.74 ± .49
Puerperal	5	213	9.2	4.06 ± .70	13.51 ± 4.22	1.58 ± .34

* Mean ± standard deviation.

tion remained essentially unchanged except for puerperal triglycerides. Hence, levels found were 15.10, 13.26, and 17.54 mg for total cholesterol; 27.30, 18.73, and 58.35 mg for triglycerides; and 7.09, 6.11 and 6.83 mg for phospholipid phosphorus per 100 g body weight for the non-pregnant, pregnant, and puerperal groups, respectively. Results calculated by both methods showed the puerperal triglyceride concentrations were about twice those found in livers from non-pregnant and pregnant rats. The effect of pregnancy on lipogenesis as measured by incorporation of sodium acetate-1-C^{14} by rat liver is shown in Table II. Increased synthesis was found for both the unsaponifiable fraction and fatty acids during pregnancy that was significantly greater ($P<.01$) than observed in non-pregnant and puerperal rats. Incorporation of acetate-1-C^{14} in the unsaponifiable fraction was increased 5 times over that found in non-pregnant rats and over 22 times that recorded for puerperal animals. Similarly, fatty acid synthesis in pregnant animals showed increases of 26 and 39 times that recorded for non-pregnant and puerperal animals, respectively. These increases were highly significant ($P<.001$). No differences were found in the synthesis of the unsaponifiable fraction or fatty acids from liver tissue of non-pregnant and puerperal rats.

TABLE II. Incorporation of Sodium Acetate-1-C^{14} into Unsaponifiable Substances and Fatty Acids by Liver Slices from Non-Pregnant, Pregnant, and Puerperal Rats.

Group	No. of rats	Unsaponifiable substances (μmoles/g)	Fatty-acids (μmoles/g)
Non-pregnant	8	.65 ± .59*	1.59 ± 1.35†
Pregnant	8	3.32 ± 1.50	41.99 ± 12.99
Puerperal	4	.15 ± .11	1.07 ± .57

* Mean ± standard deviation.
† 6 rats.

Since the synthesis of fatty acids by liver was increased in pregnancy, it was desirable to know whether differences existed in the incorporation of acetate-1-C^{14} into the fatty acids of cholesterol ester, triglycerides, and phospholipids. Table III shows no difference in the acetate-1-C^{14} incorporated in the fatty acids of triglycerides and phospholipids, while fatty acids esterified to cholesterol represented a much lower amount. Average levels of incorporation obtained for total acids by this method (33.08 ± 12.05 μmoles/g) agreed well with those obtained for total hydrolysates (41.99 ± 12.99 μmoles/g in Table II) and the percent incorporation of acetate-1-C^{14} into each class of esterified fatty acid was 1.51, 43.80, and 56.10 for cholesterol esters, triglycerides, and phospholipids, respectively.

Discussion. The liver triglyceride concentration shown in Table I for non-pregnant rats was comparable to that reported in the literature(2) but values for total cholesterol and phospholipid phosphorus appeared to be high(3,4). This discrepancy in the concentrations of cholesterol and phospholipid phosphorus may be due to the effects of fasting or the sex of the animal(22-24). The point of interest is that pregnancy does not produce changes in the lipid composition of liver that differ significantly from non-pregnant rats, and agrees with other observations (7-9) where total lipids were determined. Though the puerperal liver levels of cholesterol and phospholipid phosphorus were similar to those in non-pregnant and pregnant rats, the triglycerides were significantly higher and represented about twice those concentrations found in the other groups. The occurrence of significant increases in liver lipids during the puerperium was indicated by Natale and Hufner(25) who demonstrated

TABLE III. Incorporation of Sodium Acetate-1-C^{14} into Fatty Acids of Cholesterol Esters, Triglycerides and Phospholipids by Liver Slices from Pregnant Rats.

Rat No.	Total (μmoles/g)	Cholesterol esters (μmoles/g)	Triglycerides (μmoles/g)	Phospholipids (μmoles/g)
1	52.75	.10	24.54	28.11
2	28.16	.33	9.58	18.25
3	38.04	.09	16.32	20.39
4	27.79	1.94	12.25	13.60
5	22.27	.06	9.77	12.44
X $\pm$ SD*	33.08 $\pm$ 12.05	.50 $\pm$ .81	14.49 $\pm$ 5.92	18.56 $\pm$ 6.10
% incorporation	100	1.51	43.80	56.10

* Mean $\pm$ standard deviation.

a fatty metamorphosis of the liver in a puerperal woman whose death was apparently due to extra-hepatic causes.

Whether the increase observed in puerperal liver triglyceride levels was due to a decrease in turnover rate of phospholipids, protein synthesis or increased uptake of plasma lipids is not known, but each would result in augmented liver triglyceride levels, and the possibility of triglyceride accumulation due to one or all of these factors is not unlikely. That a rate limiting factor resulting in decreased lipid mobilization from the liver was the predominant factor in augmented levels of triglycerides in the puerperium is supported by the evidence presented on lipogenesis in Table II which shows that fatty acid synthesis was increased 20-fold over that found in non-pregnant and puerperal animals and that no triglyceride accumulation occurred. Since protein synthesis and phospholipid turnover are known to increase in pregnancy and subside in the puerperium(26), the decreased mobilization of liver triglycerides is possibly due to decreased lipoprotein formation which requires both of these entities (27). This coupled with the observation that lipogenesis (Table II) and plasma lipids(28) return rapidly to prepregnant levels in the rat makes it appear that increased liver triglycerides may also be due to increased uptake of plasma lipids by the liver. Additional evidence to support this view of increased uptake is that triglycerides are the plasma component which show the largest increase during pregnancy and the greatest decrease in the puerperium(29). It appears then, that triglyceride accumulation in the liver may be due to at least 2 factors; lowered mobilization from the liver because of decreased protein synthesis and phospholipid turnover, and increased uptake of plasma lipids by the liver. That lactation may be a factor cannot be ignored as it was recently shown in studies on lipoprotein lipase that weaning decreased the removal of lipoprotein triglycerides from plasma lipids(30). Weaning may thus subject the liver to greater loads of plasma lipids and cause an increase in liver triglycerides.

The results showing increased lipogenesis during pregnancy by liver slices (Table II) are in agreement with previous reports showing enhanced lipid synthesis in the presence of acetate(12) and pyruvate(13), but are in conflict with the data of Popjak(11) who demonstrated that fatty acid synthesis by liver slices from the pregnant rabbit was lowered. Finally, pregnancy did not appear preferentially to divert fatty acids into triglycerides or phospholipids as similar magnitudes of acetate incorporation were observed (Table III) in both of these lipid classes. This is in agreement with the data found in non-pregnant animals(5,24).

Though pregnancy has been considered diabetogenic in regard to impaired carbohydrate utilization, insulin resistance, and increased plasma lipid levels(31), the capacity of the liver to synthesize fatty acids as shown in these experiments was not impaired as in diabetic animals(3). It has been postulated that the hyperlipidemia during pregnancy is due to decreased uptake and increased release of non-esterified fatty acids due to relative insulin insufficiency in extra-hepatic tissue(29), but it is apparent from these ex-

periments that it may also be due in part to increased hepatic lipid synthesis.

Summary. The composition and incorporation of acetate-1-C^{14} into liver lipids of non-pregnant, pregnant, and puerperal rats were determined. Results show that total cholesterol and phospholipid phosphorus in rat liver during pregnancy and the puerperium do not differ from those found in non-pregnant animals. However, the triglycerides from livers of puerperal animals were significantly higher than those found in either non-pregnant or pregnant rats. Incorporation of acetate-1-C^{14} into fatty acids and unsaponifiable substances by liver slices from pregnant rats was significantly greater than that observed for non-pregnant and puerperal rats. No differences were observed in the μmoles of acetate incorporated into the fatty acids of triglycerides and phospholipids. Possible factors related to the puerperal increase of triglycerides in the liver are discussed.

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