Hepatic lipid metabolism. Age-related changes in triglyceride metabolism

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Abstract Age-related changes in hepatic triglyceride formation have been described in developing rats. Triglyceride formation was measured in vitro in the presence of [^{14}C]glycerol-3-phosphate, palmitate, ATP, CoA, and Mg^{2+} by using liver homogenates and microsomal fractions derived from various age groups of animals. Triglyceride formation was most active in one-day-old rats and then decreased with age. The increase in triglyceride formation following birth was prevented by the administration of puromycin or by denying suckling. In addition, changes in plasma and hepatic triglyceride concentrations, were also determined as functions of age. These studies suggest that the age of the animal significantly influences triglyceride metabolism.

Supplementary key words *sn*-glycerol-3-phosphate acyltransferase · cytochrome *c* reductase · glucose-6-phosphatase · plasma · palmitoyl CoA

Nutritionally, the early life of the laboratory rat is characterized by two changes. The first is physiologically defined when the high carbohydrate content of transplacental food is replaced by the high fat content of the milk diet after birth. The second change is less strictly defined and occurs at weaning when the milk diet of suckling is replaced by the laboratory diet which has a high carbohydrate and low fat content. Hormonal changes are also occurring during development, so the developing rat provides a natural system for the study of hormonal and dietary regulation of glycerolipid metabolism.

Previously, many workers have taken advantage of this system to study fatty acid synthesis (1–5), fatty acid oxidation (6, 7), ketosis (8), cholesterol synthesis (9), and gluconeogenesis (10, 11). Although it has been reported that triglyceride synthesis in adult rat liver is subject to dietary and hormonal regulation (12–15), the developing rat has been rarely used to study hepatic triglyceride formation.

In 1967, Ballard and Hanson (2) reported that the fetal rat liver has the ability to synthesize triglyceride de novo. Recently, Sinclair (16) reported that triglyceride concentration is very low in newborn rat liver and that it increases rapidly after birth. The rise in hepatic triglyceride concentration after birth may be due to increased synthesis, decreased degradation, impairment of its release into circulation, or the increased flux of chylomicron remnants into the liver because of high dietary intake of milk fat. In the present investigation, triglyceride formation from *sn*-glycerol-3-phosphate and a palmitoyl CoA-generating system was measured in liver homogenates and microsomal fractions derived from various age groups of animals to determine the influence of age on hepatic triglyceride formation.

MATERIALS AND METHODS

All reagents were of A grade quality. ATP, coenzyme A, dithiothreitol, cytochrome *c*, glucose-6-phosphate, palmitoyl CoA (85% pure), and puromycin-HCl were purchased from Sigma Chemicals, St. Louis, MO. The *sn*-[1-3^{14}C]glycerol-3-phosphate was purchased from ICN Chemicals and Radioisotope Division, Irvine, CA. [1-^{14}C]Palmitoyl CoA and [1-^{14}C]palmitate were purchased from New England Nuclear Corporation, Boston, MA. Fatty acid-poor albumin (fraction V, Pentex) was purchased from Miles Laboratories, Inc., Kankakee, IL. Other reagents were purchased from the sources reported previously (17).

Animals

Fetal and neonatal rats were of the Sprague-Dawley strain. Pregnant rats were purchased from Flow Laboratories, Dublin, VA. Birth dates and time of weaning (21 days of age) were recorded carefully. After weaning all rats received Purina Chow diet, Ralston Purina, St. Louis, MO. The animal colony was maintained in a temperature-controlled room with a 12 hour on, 12 hour off light cycle. For developmental studies,

Abbreviation: BSA, bovine serum albumin.

animals from both sexes were selected at random. Animals were killed by decapitation and blood was collected in heparinized tubes. In some studies blood was pooled from five or six animals (in fetal and newborn animals) to obtain sufficient plasma for triglyceride determination. All animals were killed between 9 and 11 AM.

Preparation of homogenates and microsomes

Livers were removed, blotted free of blood, and weighed. They were homogenized in a Teflon glass homogenizer with four volumes of Medium A (0.25 M sucrose, 1 mM dithiothreitol, 1 mM EDTA, and 1 mM Tris, pH 7.5). In some experiments, the homogenates were further fractionated to isolate microsomes by the method of Pollack and Duck-Chong (18). The microsomal pellet was washed once by suspending it in Medium A and resedimenting at 105,000 *g* for 15 min to remove entrapped particle-free supernate. The washed microsomal pellet was resuspended in Medium A to assay glucose-6-phosphatase (19), cytochrome *c* reductase (20), *sn*-glycerol-3-phosphate acyltransferase (12), palmitoyl CoA synthetase (21), and palmitoyl CoA hydrolase (22).

Enzyme assays

Palmitoyl CoA synthetase (E.C. 6.2.1.3) from liver microsomes was measured according to Lloyd-Davis and Brindley (21). The reaction mixture (0.25 ml) contained 25 mM Tris, pH 7.5, 5 mM dithiothreitol, 55 μM CoA, 2.5 mM ATP, 2.5 mM $MgCl_2$, 0.8 mM potassium palmitate (0.84 μCi/μmol), and 6.0 mg of fatty acid-poor albumin/ml. In initial studies, the linearity of palmitoyl CoA formation with time and protein concentration was determined. With 5–20 μg of microsomal protein, palmitoyl CoA formation was linear for 5–7 min. In the standard assay, the reaction was continued for 5 min and terminated by the addition of 1 ml of isopropanol–heptane–1 N H_2SO_4 40:10:1 (by vol), 0.35 ml of H_2O, and 0.6 ml of heptane. Remaining palmitate was extracted six times with 0.6 ml of heptane and an aliquot of the aqueous extract containing palmitoyl CoA (200 μl) was dissolved in Aquasol 2 (New England Nuclear) and assayed for radioactivity. The ^{14}C radioactivity obtained in the absence of microsomal protein was subtracted as a blank.

Palmitoyl CoA hydrolase (E.C. 3.1.2.2) from microsomes and cytosol was assayed according to Lamb, Hill, and Fallon (22). The formation of [^{14}C]palmitate from [^{14}C]palmitoyl CoA was taken as a measure of enzyme activity.

sn-Glycerol-3-phosphate acyltransferase (E.C. 2.3.1.15) was measured in the presence of palmitate, ATP, CoA, and $MgCl_2$ by using either the homogenate or the microsomal fraction. In a final volume of 0.7 ml, the reaction mixture contained 17.5 mM Tris, pH 7.5, 49 mM KCl, 0.84 mM *sn*-glycerol-3-phosphate with 0.1 μCi of [^{14}C]-*sn*-glycerol-3-phosphate, 0.7 mM dithiothreitol, 0.039 mM CoA, 3.55 ml of ATP, and 1.4 mM NH_4-palmitate complexed with 1.25 mg of fatty acid-poor albumin. After 30 min of incubation at 37°C, the reactions were terminated by the addition of 3 ml of chloroform–methanol–1 N HCl 2:1:0.025. The radioactive lipids formed were extracted as described by van den Bosch and Vagelos (23) and dried under N_2. The dry lipids were dissolved in 0.5 ml of benzene and stored at −30°C. Samples (0.1 ml) were applied to silica gel G plates impregnated with 0.1 M borate and neutral lipids were separated with a solvent system of hexane–ether–acetic acid 73:25:2 (by vol) (24). Phospholipids were separated with chloroform–methanol–acetone–acetic acid–water 100:20:40:20:10 (by vol) on silica gel HR plates slurried in 10 mM sodium carbonate (25). The different classes of lipids were identified by authentic standards. Various lipids were visualized by exposure to iodine. After sublimation of iodine, appropriate areas from the plates were scraped directly into scintillation vials containing 10 ml of Liquifluor in toluene and the radioactivity was determined in a Beckman LS 250 scintillation counter.

It is known that fetal rat liver contains excessive deposits of glycogen and that its breakdown occurs after birth (26). This could expand the endogenous pool of glycerophosphate in newborn rat liver and increase total glycerophosphate in the incubation mixture if there is no compartmentalization of substrate. Although excess substrate is added to the usual incubation mixture, the additional contribution by endogenous substrate was determined in some experiments by direct measure of hepatic glycerophosphate at different phases of development. Immediately after exposure of the peritoneal cavity, liquid nitrogen was poured on liver in situ. Livers were promptly removed and kept frozen at −40°C until use. The frozen liver was weighed and extracted with perchloric acid. After neutralization with 5 M potassium carbonate, the deproteinized samples were used to measure glycerophosphate concentration spectrophotometrically as described by Hohorst (27). In other experiments, liver homogenates were subjected to extensive dialysis against several volumes of Medium A to remove endogenous glycerophosphate prior to assay. Liver homogenates from fetal, newborn, or one-day-old rats were used for the dialysis experiments.

Hepatic and plasma triglyceride concentrations were determined according to Haux and Natelson

(28). Protein was determined by the procedure of Lowry et al. (29) with bovine crystalline albumin as standard.

Statistical comparisons were made by Students' *t* test. Differences between means were considered insignificant if $P > 0.05$.

RESULTS AND DISCUSSION

Changes in plasma and liver triglycerides

Fig. 1 shows the changes in hepatic and plasma triglyceride concentration as a function of age. In agreement with the previous results (16), hepatic triglyceride concentration was found to be low in newborn rats and increased rapidly within the first 24 hr after birth. This level then decreased to adult values by the tenth day of age. The decrease in the hepatic triglyceride concentration reported here at day 10 does not agree with the results of Sinclair (16), who reported the highest concentration of triglyceride in neonatal rats at this age. The explanation of this discrepancy is not presently clear.

The plasma triglyceride concentration was low in the newborn rat. It rose rapidly to reach a peak value by 9 days of age with no further changes until 21 days. After weaning, the plasma triglyceride concentration began to decrease gradually so that the adult animals had only 40% of the triglyceride concentration found in 21-day-old rats. The increase in plasma triglyceride concentration coincided with a decrease in hepatic triglyceride concentration in 9-day-old rats, suggesting that the decrease in hepatic triglyceride concentration may result from secretion of triglyceride into plasma. The decrease in plasma triglyceride concentration after 21 days of age may be related to the dietary changes accompanying weaning.

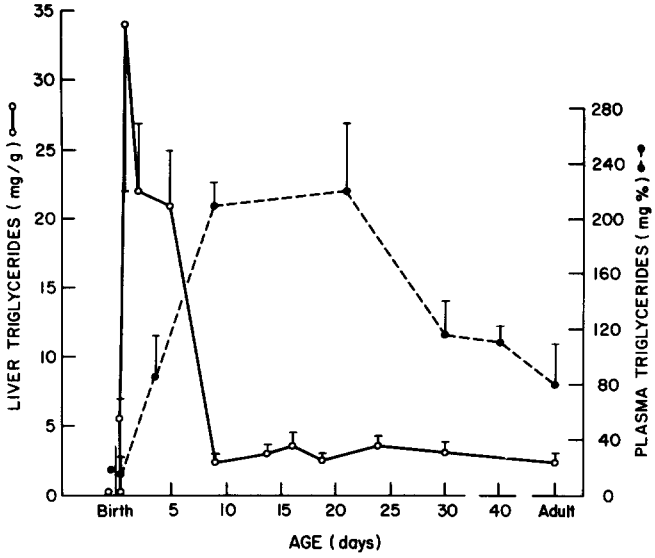

Fig. 1. Changes in hepatic and plasma triglyceride concentration as a function of age. Each value is mean ± SD from 3–6 animals. In fetal and newborn animals, plasma from 4–6 animals was pooled.

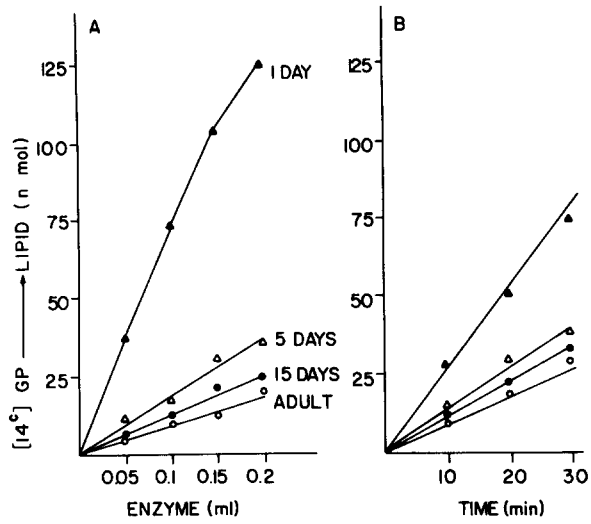

Fig. 2. Glycerolipid formation from [^{14}C]glycerol-3-phosphate, palmitate, ATP, CoA, and Mg^{2+} by liver homogenates as a function of protein concentration (*A*) and as a function of time (*B*). In Figure *A*, total lipid (phospholipids and neutral lipids) formation is expressed as nmol of [^{14}C]glycerol-3-phosphate incorporated into lipid for 30 min. In Figure *B*, 0.1–0.2 ml of homogenate was used to study glycerolipid formation as a function of time. Individual classes of lipids were separated and identified on TLC and the results are reported in Table 1. Liver homogenates derived from 1 day, 5 days, 15 days, and adult rats contained 14.2, 15.9, and 20.8 mg protein/ml, respectively.

Glycerolipid formation by liver homogenates

The assay system developed previously to study hepatic glycerolipid formation in adult rats (15) was also satisfactory for measuring the esterification rates in various enzyme preparations from newborn and neonatal rats as evidenced by the linearity of reaction with time (30 min) and protein (0.5–3 mg of homogenate and 0.1–0.3 mg of microsomal protein, **Fig. 2**).

In the absence of bovine serum albumin, glycerolipid formation was apparent and was slightly stimulated further in the presence of albumin. The concentration of albumin that gave optimal reaction rates in various enzyme preparations differed from that needed to bind the added fatty acid completely. Studies by Spector, John, and Fletcher (30) reveal that one mole of bovine serum albumin binds 6 moles of fatty acids at strong binding sites and over 60 moles at comparatively weak binding sites. On the basis of this observation, over 10 mg BSA/ml would be required to bind the amount of palmitate used in our assay

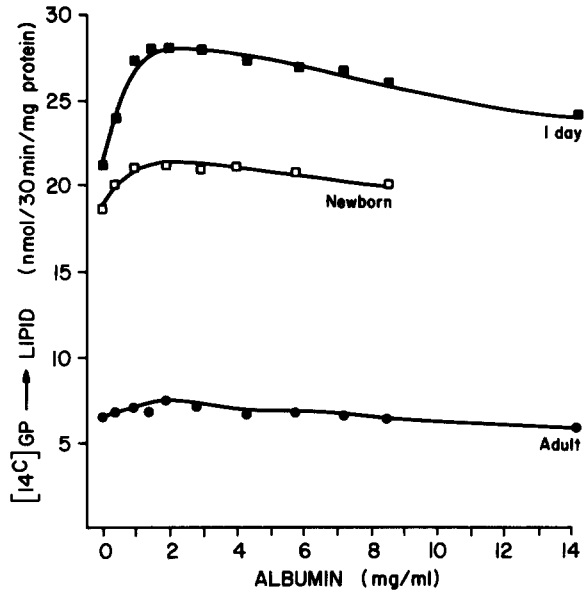

Fig. 3. Effect of albumin on glycerolipid formation. Assays were conducted in duplicate by using liver homogenates derived from newborn, 1-day-old, and adult rats with variable concentration of bovine serum albumin.

system. However, albumin in excess of 5 mg/ml was inhibitory for glycerolipid formation (**Fig. 3**). Therefore, the concentration of albumin that gave optimal reaction rates in various enzyme preparations (1.8 mg/ml or 1.25 mg/assay) was used. Similar to that reported here for liver, the inhibition of adipose glycerolipid formation by albumin was noted previously by Christie, Hunter, and Vernon (31). In fact, these workers did not observe any albumin requirement for incorporation of palmitate into various glycerolipids, including triglycerides, by the adipose tissue homogenates.

Glycerolipid formation was detected in both fetal and newborn rat livers (**Table 1**). Following birth, glycerolipid formation increased sharply to reach a peak value at one day of age. This was followed by a decline in the rates of glycerolipid formation. Glycerolipid formation increased again at day 15 and declined after weaning to reach an adult level by 25 days of age.

Direct measure of endogenous concentration of glycerophosphate indicated that developmental changes

TABLE 1. Glycerolipid formation by liver homogenates as a function of age

No. of Animals and Age	Glycerolipid Formation[a]	Percent Distribution of Glycerol-3-Phosphate into			Ratio NL/PL[e]	Glycerophosphate μmol/g
		Phospholipid[d]	Diglyceride	Triglyceride		
(11) Fetus (20 days)	13.84 ± 1.30[b] (14.94 ± 1.40)[c]	24 ± 3.3	22 ± 2.6	54 ± 8.8	3.27 ± 1.30	0.33 ± 0.13 (5)
(10) 0–5 hours	8.39 ± 1.29 (9.06 ± 1.4)	22 ± 9.5	23 ± 4.4	55 ± 16.5	3.54 ± 1.45	0.31 ± 0.09 (7)
(13) 1 day	25.57 ± 3.32 (26.59 ± 3.5)	30 ± 11.0	25 ± 4.8	45 ± 8.2	2.89 ± 2.06	0.16 ± 0.03 (4)
(3) 3 days	10.79 ± 1.93	41 ± 5.1	25 ± 2.8	34 ± 8.6	1.42 ± 0.2	
(3) 6 days	10.57 ± 2.94	36 ± 4.6	26 ± 8.3	37 ± 5.3	1.74 ± 0.15	
(5) 10 days	6.73 ± 1.79 (7.26 ± 1.9)	39 ± 15.8	26 ± 11.0	33 ± 11.0	1.63 ± 0.68	0.31 ± 0.07 (3)
(3) 15 days	17.99 ± 4.69	43 ± 11.0	28 ± 6.8	29 ± 6.7	1.33 ± 0.04	
(4) 21 days	13.46 ± 2.99 (15.61 ± 3.5)	60 ± 8.0	22 ± 8.7	16 ± 4.9	0.62 ± 0.14	0.67 ± 0.11 (4)
(4) 25 days	6.25 ± 1.05	59 ± 15.0	22 ± 11.0	19 ± 1.7	0.74 ± 0.10	
(4) 46 days	5.92 ± 0.28	67 ± 7.7	19 ± 1.0	14 ± 3.5	0.46 ± 0.08	
(3) 80 days (adult)	5.65 ± 0.71 (7.06 ± 0.88)	67 ± 8.0	20 ± 2.4	13 ± 2.8	0.49 ± 0.06	1.06 ± 0.14 (7)

[a] nmol of glycerol-3-phosphate incorporated into lipid/30 min per mg protein.

[b] Each value represents mean ± SD of assays conducted in duplicate using liver homogenates from the number of rats given in parentheses.

[c] In some experiments, endogenous concentration of glycerol-3-phosphate was measured to determine the dilution of the added substrate and the rates of glycerolipid formation corrected for this dilution are reported in parentheses along with the uncorrected rates to indicate that the endogenous glycerophosphate did not significantly influence the observed glycerolipid formation under different phases of development.

[d] Phospholipid was mainly comprised of phosphatidate with lesser amounts of lysophosphatidate. No incorporation of [^{14}C]glycerol-3-phosphate into 3-*sn*-phosphatidylcholine and 3-*sn*-phosphatidylethanolamine was noted.

[e] NL, sum of di- and triglyceride; PL, phospholipid.

TABLE 2. Effect of puromycin on hepatic triglyceride formation

No. of Experiment and Treatment	Glycerolipid Formation	% Distribution of Glycerophosphate into			Cytochrome *c* Reductase	Glucose-6-Phosphatase	Liver Triglyceride
		Phospholipid	Diglyceride	Triglyceride			
	nmol/30 min/mg protein				*nmol/min/mg protein*		*mg/g*
1 Control (3)	24.05 ± 5.29[a]	14 ± 1.7	30 ± 9	56 ± 11			36.81 ± 6.97
Puromycin (5)[b]	14.87 ± 4.13	18 ± 1.5	21 ± 4	59 ± 23			31.93 ± 9.21
	$P < 0.05$						
2 Control (5)	57.10 ± 5.32	37 ± 9	30 ± 4	33 ± 9	30.4 ± 2.1	137 ± 31.0	
Puromycin (3)[b]	22.7 ± 4.20	64 ± 24	25 ± 4	11 ± 3	19.3 ± 1.15	56 ± 15.8	
	$P < 0.01$			$P < 0.05$	$P < 0.05$	$P < 0.01$	
3 Puromycin addition							
(none)	20.4 ± 0.96[c]	26 ± 2	29 ± 2	45 ± 3			
+ 100 μg puromycin	21.04 ± 0.31	25 ± 1	31 ± 2	44 ± 2			
+ 200 μg puromycin	21.78 ± 0.75	26 ± 2	31 ± 2	43 ± 3			
+ 300 μg puromycin	22.62 ± 0.55	24 ± 2	33 ± 1	43 ± 1			

[a] Mean ± SD of assays conducted in duplicate or triplicate using liver homogenates (experiment 1) and liver microsomes (experiment 2) from the number of rats referred to in parentheses.

[b] In experiments 1 and 2, puromycin was dissolved in 0.9% saline and pH was adjusted to 7.4 with dilute NaOH for the intraperitoneal injections. Control rats received saline. In experiment 3, puromycin was dissolved in 0.05 M Tris, pH 7.5, to study the effect of puromycin addition in the assay system. In experiments 1 and 2, newborn rats were injected repeatedly with 100 μg of puromycin at 2, 4, and 10 hr after birth. Thus, each rat received 300 μg of puromycin. Animals were killed 24 hr after birth. One part of the liver was used to determine triglyceride concentration and the other was used to prepare homogenates and microsomal fractions. In experiment 3, liver homogenates from 18-hr-old rats were used.

in the rates of glycerolipid formation as measured in vitro could not result from variation in the glycerophosphate pool. When the endogenous glycerophosphate content and added exogenous substrate were considered in calculations of the rate of glycerolipid synthesis, no significant effect was noted (Table 1). This is related to the relatively large excess of exogenous substrate added to the incubation mixture. Furthermore, extensive dialysis of liver homogenates prior to assay to remove excess endogenous glycerophosphate had no significant effect on the rate of glycerolipid formation (data not reported). Therefore, changes in glycerolipid formation measured at various phases of development were not the result of variations in the endogenous concentration of glycerophosphate.

The rise in glycerolipid formation after birth coincided with the increase of several hepatic enzymes, including cytochrome *c* reductase and glucose-6-phosphatase (32). It has been noted previously that administration of puromycin blocks the rise in cytochrome *c* reductase and glucose-6-phosphatase at this time due to inhibition of protein synthesis (32). Therefore, the effect of puromycin administration on the rate of glycerolipid formation was investigated. Administration of puromycin to newborn rats in a dose that produces inhibition of protein synthesis prevented the early rise in glycerolipid formation (**Table 2**). Because deprivation of food intake in the newborn rats also prevents an increase in the rate of glycerolipid formation (**Table 3**), an examination of

TABLE 3. Effect of suckling on glycerolipid formation

Age and Group	Treatment	Glycerolipid Formation	% Distribution of Glycerophosphate into			Liver Triglyceride
			Phospholipid	Diglyceride	Triglyceride	
		nmol/30 min/mg protein				*mg/g*
Newborn (A)	None	10.55 ± 0.02[a]	20 ± 5	23 ± 2	57 ± 6	0.085 ± 0.02
16 hours (B)	Suckled	13.85 ± 1.72	20 ± 6	18 ± 4	62 ± 16	18.66[c] ± 6.8
16 hours (C)	Denied suckling	6.81[b] ± 1.50	25 ± 1	20 ± 1	55 ± 4	nd[d]

[a] Mean ± SD of assays conducted in duplicate using liver homogenates from three rats.

[b] $P < 0.05$, between B and C.

[c] $P < 0.01$, between A and C.

[d] nd = not detected.

A litter comprised of nine animals was used. Within an hour after birth, three animals were killed (A). One part of the liver was used to prepare homogenates and the other was used to measure triglyceride concentration. From the remaining animals, three were taken away from the mother and placed in the incubator at 34°C under humidified conditions (C), and the other three were allowed suckling (B). Sixteen hours after birth, these animals were killed and livers were used to measure hepatic triglyceride concentration and glycerolipid formation from *sn*-glycerol-3-phosphate and a palmitoyl CoA generating system as described in Methods.

the stomach contents was performed. This confirmed the presence of milk in the puromycin-treated rats and excluded fasting as a factor in these results. Direct addition of puromycin to the assay system did not cause inhibition of glycerolipid formation (Table 2). Therefore, these results suggest that the rise in hepatic glycerolipid formation after birth may be related to increased synthesis of new enzyme protein(s) rather than activation of preformed enzyme(s).

Although puromycin administration decreased the rate of glycerolipid formation, it did not inhibit the accumulation of hepatic triglycerides (Table 3). Therefore, besides increased synthesis of triglycerides, other mechanisms including severe impairment in transport of newly synthesized triglycerides into circulation, increased influx of chylomicron remnants into liver, or decreased degradation of newly synthesized triglycerides by triglyceride lipase (33) may be responsible for the generation of fatty livers under this condition. Puromycin is known to produce fatty livers in adult rats because of inhibition of lipoprotein synthesis and it is likely that this also occurs in the neonatal animals (34).

Deprivation of milk intake in the newborn rats completely abolished the accumulation of hepatic triglycerides (Table 3). Liver homogenates from these animals also showed a diminished capacity for triglyceride formation. These results suggest that intake of milk may be responsible for the increased capacity for hepatic triglyceride formation in the newborn rat. The mechanism of this effect is not clear; however, changes in substrates or hormones could be responsible. Since nonesterified fatty acid content in both the serum and liver increased as a result of milk intake in the newborn rats (35), the nonesterified fatty acids may be responsible for the induction of esterifying enzymes synthesis, possibly by "substrate induction" (36).

Liver homogenates derived from fetal, newborn, and one-day-old rats synthesized more triglyceride than phospholipid (largely phosphatidate) using *sn*-glycerol-3-phosphate and a palmitoyl CoA generating system. As the rats grew older, the rate of phospholipid formation was increased with a concomitant decrease in triglyceride formation (Table 1). These changes in the product formation (ratio of neutral lipids to phospholipids) are consistent with the decrease in phosphatidate phosphohydrolase activity in the liver homogenates as a function of age. Thus, these results are in agreement with the well-documented role of phosphatidate phosphohydrolase in the regulation of hepatic triglyceride formation (37–39).

Changes in microsomal enzymes

Because liver homogenates prepared from newborn and neonatal rats showed an increased capacity for triglyceride synthesis, further studies of the individual enzyme reactions involved in triglyceride formation were performed (**Table 4**). In agreement with findings in liver homogenates, the microsomal fractions from one-day-old rats showed the highest activity for *sn*-glycerol-3-phosphate acyltransferase. Adult rat liver microsomes showed significantly lower activity of this enzyme. In contrast to homogenates, microsomal fractions prepared from rats of various ages primarily formed phosphatidate with lesser amounts of neutral lipids. This difference in product between homogenates and microsomal fractions may be related to the absence of soluble phosphatidate phosphohydrolase in the latter preparation. It was suggested previously that soluble phosphatidate phosphohydrolase plays an important role in hepatic triglyceride synthesis (12, 37, 39).

The developmental pattern of microsomal palmitoyl CoA synthetase was similar to that reported previously

TABLE 4. Changes in microsomal enzymes as a function of age

Animal Age	Palmitoyl CoA Synthetase[a]	Palmitoyl CoA Hydrolase[a]	*sn*-Glycerol-3-P Acyltransferase[a]	Percent Distribution of Glycerophosphate into[c]			
				LPA	PA	DG	TG
1 day	12.53 ± 2.6 (3)[b]	8.5 ± 5.09 (4)	8.03 ± 2.3 (3)	10 ± 1.5	70 ± 2.8	10 ± 0.5	10 ± 0.6 (3)
3 days	18.64 ± 7.80 (3)	9.86 ± 0.5 (3)	4.73 ± 1.23 (8)	9 ± 3.5	81 ± 3.5	7 ± 3	3 ± 1.5 (3)
4–5 days	29.34 ± 3.45 (5)						
10–11 days	30.88 ± 6.40 (3)	24.9 ± 3.25 (11)	2.24 ± 0.53 (6)				
14–16 days	29.60 ± 3.47 (8)	19.81 ± 3.79 (13)	4.30 ± 0.25 (4)	8 ± 2.0	88 ± 5.0	2.5 ± 1.0	1.5 ± 0.6 (4)
22 days	27.61 ± 1.48 (5)	31.17 ± 4.66 (8)	1.98 ± 0.14 (6)				
30 days		46.44 ± 6.08 (6)	1.52 ± 0.25 (7)				
40 days	29.96 ± 3.36 (5)	53.84 ± 5.57 (4)	1.38 ± 0.42 (5)				
Adult (2–3 months)	35.0 ± 11.33 (4)	50.33 ± 7.50 (3)	0.95 ± 0.36 (3)	10 ± 3.0	88 ± 3.0	1.75 ± 0.4	0.25 ± 0.2 (3)

[a] nmol product formed/min/mg microsomal protein.
[b] Mean ± SD of assays conducted in duplicate using microsomal fractions from the number of rats referred to in parentheses.
[c] Reaction products from the *sn*-glycerol-3-phosphate acyltransferase assays were separated and identified by TLC. LPA, lysophosphatidate; PA, phosphatidate; DG, diglyceride; TG, triglyceride.

in whole homogenate (40) and mitochondrial fractions (7). Although adult rat liver microsomal fractions contained twice as much activity of palmitoyl CoA synthetase compared to newborn and neonatal rats, the rate of glycerolipid synthesis in these animals was lower than the corresponding fractions derived from one- to three-day-old rats. Thus, no correlation between the activities of palmitoyl CoA synthetase and *sn*-glycerol-3-phosphate acyltransferase was noted at various ages in the rat. Previously, Lloyd-Davis and Brindley (21) also failed to observe any correlation between the activities of palmitoyl CoA synthetase and *sn*-glycerol-3-phosphate acyltransferase in rat liver microsomes under conditions different from those reported here.

Microsomal palmitoyl CoA hydrolase activity was low in the newborn rats and activity increased with age. Adult rat liver microsomes were five times more active in hydrolyzing palmitoyl CoA than microsomal fraction derived from one- to three-day-old rats. This increased activity of palmitoyl CoA hydrolase would reduce the amount of palmitoyl CoA available for synthesis of glycerolipids in the adult rat. This may contribute to the lower capacity for lipid formation noted in adult rats. Palmitoyl CoA hydrolase activity was also detected in the cytosol. The developmental pattern of the cytosol enzyme was similar to that of the microsomal enzyme. However, the cytosol palmitoyl CoA hydrolase was significantly less active compared to microsomal enzyme. The typical values for the specific activity of cytosol palmitoyl CoA hydrolase in 5-day-old rats and adult rats were (mean ± SD) 5 ± 2 (4), and 12 ± 3 (4) nmol/min per mg protein, respectively.

The activities of the individual enzymes provide evidence that the entire pathway of esterification via *sn*-glycerol-3-phosphate is accelerated in fetal, newborn, and neonatal rat livers. Measurement of the endogenous glycerophosphate concentration suggests that these changes in hepatic triglyceride synthesis with age were related to changes in enzyme concentration rather than to variation in the pool size of glycerophosphate. Among the various enzymes measured, palmitoyl CoA hydrolase was most active and showed an inverse relationship with the activity of *sn*-glycerol-3-phosphate acyltransferase in various age groups of animals. It is concluded from these studies that birth is associated with increased triglyceride formation resulting from increased synthesis of esterifying enzymes.

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