Studies on fat digestion, absorption, and transport in the suckling rat. IV. In vivo rates of triacylglycerol secretion by intestine and liver[1]

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Abstract The rate of triacylglycerol entry into plasma in 14- to 15-day-old suckling rats was measured using the Triton WR1339 method. When the pups were left with the mother and allowed to nurse continually, the rate was 31.8 ± 2.3 μmol/hr per rat. If the pups were removed from the mother with full stomachs and kept in a warm box at 30–32°C, the rate was 12.5 ± 1.5 μmol/hr per rat. The rate of triacylglycerol output from the intestine measured in lymph duct-cannulated animals (kept in a warm box at 30–32°C) was 14.9 ± 2.7 μmol/hr per rat. Triton treatment of lymph duct-cannulated animals caused only a small increase in plasma triacylglycerol concentration (<2 μmol/hr per rat). These data suggest that nearly 100% of plasma triacylglycerol arises from intestinal input in the suckling rat.—**Frost, S. C., W. A. Clark, and M. A. Wells.** Studies on fat digestion, absorption, and transport in the suckling rat. IV. In vivo rates of triacylglycerol secretion by intestine and liver. *J. Lipid Res.* 1983. **24:** 899–903.

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The suckling rat is a useful model system for studying the utilization of large amounts of dietary fat because of the high fat (70% of calories) content of rat milk (1, 2). A 14- to 15-day-old suckling rat consumes about 4.5 g of fat/day per 100 g body weight (3), compared to about 0.5 g of fat/day per 100 g body weight for an adult rat on a normal chow diet (4). In the adult rat, on the normal low fat diet, plasma triacylglycerols (TG) arise primarily (about 80%) from VLDL secreted by the liver (5–11). When adult rats are fed a diet containing 70% of calories as fat, the intestine contributes about 85% of plasma TG (5, 6). This is due to increased chylomicron TG output from the intestine and decreased VLDL TG output from the liver (5, 6).

Previous studies from this laboratory have shown that when long chain fatty acid oxidation is inhibited in the suckling rat liver, a marked fatty liver develops (12). Thus, within 18 hr after treatment with a single dose of tetradecylglycidic acid, a carnitine acyl transferase inhibitor (13), the TG content of liver increased from about 10 μmol/g to about 80 μmol/g, without a significant increase in plasma TG (12). In contrast, an acute study with this compound stimulated very low density lipoprotein (VLDL) TG secretion fivefold from perfused adult liver (14). These data imply that the neonatal liver has a limited capacity to secrete TG.

The apparent reduced TG secretion from the neonatal liver might be an effect of the high fat content of the milk diet. In order to further investigate this problem we have measured the rate of TG secretion into plasma using Triton WR1339, which blocks TG-rich lipoprotein clearance from plasma (15–17). We have carried out these experiments in control and lymph duct-cannulated animals in order to measure the relative rates of TG secretion by the liver and intestine in the suckling rat.

METHODS

Sprague-Dawley rats (Hilltop Lab Animals, Chatsworth, CA) were maintained in our breeding colony as previously described (2). Litters were culled to ten pups at postnatal day 2. All experiments utilized 14- to 15-day-old pups with an average body weight of 35.0 ± 1.2 g. All experiments were begun between 9:00 and 10:00 AM, at which time the pups had full stomachs. All experiments were initiated within 30 min of removal of pups from the nest.

Cannulation of the mesenteric lymph duct was carried out under a dissecting microscope using essentially the technique described by Warshaw for adult rats (18).

Abbreviations: TG, triacylglycerol; VLDL, very low density lipoprotein.

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Pups were anesthetized by intramuscular injection of 50 μg/g body weight of Ketaset (ketamine hydrochloride, 100 mg/ml, Veterinary Products Bristol Laboratories, Syracuse, NY). A polyethylene cannula (PE-10, Clay Adams, Parsippany, NJ) filled with a heparin solution (1000 units/ml) was inserted 1–2 mm into the main mesenteric lymph duct. After positioning the cannula so that lymph flow occurred, it was held in place with cyanoacrylate glue. Accessory ducts were cut and sealed with glue. Approximately 1 ml of physiological saline was introduced into the abdominal cavity and the incision closed with 7.5-mm metal wound clips (Roboz Surgical Instruments, Inc., Washington, DC). The incision was irrigated with a small amount of a lidocaine solution (20 mg/ml) and the pup was placed in a restraining cage in a warm box at 30–32°C. After recovery from the anesthesia (about 45 min), lymph was collected into chilled, tared, tubes in hourly intervals. In order to maintain fluid balance, about 0.2 ml of physiological saline was placed in the pup's mouth every 30 min, using a cotton-tipped applicator. Only animals that showed constant lymph flow for 2 hr were included in the experiments. The surgical procedure required 15–20 min and about 60% of the cannulations were successful.

Triton WR1339 (Sigma Chemical Co, St. Louis, MO, 100 mg/ml in physiological saline) was injected intraperitoneally (4.7 μg Triton/g body weight) (19). After injection, animals were either returned to their mother or maintained in a warm box at 30–32°C.

Blood was collected from ether-anesthetized animals via aortic puncture into EDTA-containing tubes. Plasma was prepared and analyzed for TG as previously described (12). The output of lymph was determined by weight assuming a density of 1.0 g/ml. Lymph lipids were extracted as previously described (2) and TG content was measured as for plasma. Statistical comparisons were made using Student's *t*-test.

RESULTS

The purpose of these experiments was to evaluate the rate of TG secretion by liver and intestine under conditions that approximate as closely as possible the normal fed-state of the suckling rat. The experimental design is similar to that used in several adult studies (5–11) and relies on the observation that Triton WR1339 inhibits lipoprotein lipase and therefore prevents clearance of TG-rich lipoproteins from plasma. The experiment involves two parts. First, the rate of plasma TG increase after Triton treatment of intact animals is measured. This gives the total rate of secretion of TG into plasma by both liver and intestine. Second, the rate of plasma TG increase after Triton treatment of lymph duct-cannulated animals is measured. This gives the rate of TG secretion by the liver. An estimation of the rate of TG secretion from the intestine is obtained from the rate of TG secretion into lymph. Alternatively, the rate of TG secretion by the intestine can be obtained as the difference between the first and second experiments.

With suckling animals it is possible to carry out the first experiment in the normal fed-state, i.e., continual nursing by the mother. However, it is not possible to carry out the second experiment under these conditions, since the mother will remove the cannula. Therefore we have first determined the total rate of TG entry into plasma in the presence of the mother. We repeated this measurement in the absence of the mother to provide data for comparison with lymph duct-cannulated animals, and used these data to determine the relative rates of liver and intestinal TG secretion.

The effect of Triton WR1339 on plasma TG concentration is given in **Table 1.** Whether the pups were left with the mother or not, there was a rapid rise in plasma TG levels between 2 and 4 hr after detergent administration. When the pups were left with the mother, the rise was more rapid and the final concentration attained after 6 hr was greater (36.8 vs 15.4 mM). We defined the rate of TG entry into plasma as the change in TG concentration that occurred between 2 and 3 hr after administration of Triton. It can be seen from the data in Table 1 that this corresponds to the initial rapid increase in TG concentration. Based on this definition, the TG entry rate observed in pups with their mother was 16.8 ± 1.2 μmol/hr per ml plasma and 6.6 ± 0.8 μmol/hr per ml plasma in the absence of the mother. The plasma volume of 13- to 15-day-old rats is 5.4 ml/100 g body weight (20). Thus, for our animals, the rate of TG entry into plasma is 31.8 ± 2.3 μmol/hr per rat in the presence of the mother, a condition that allows continual nursing, and 12.5 ± 1.5 μmol/hr per rat in the absence of the mother, a condition in which only the milk present in the stomach at the time of removal from the mother can be digested and absorbed.

Adult rats under anesthesia have a lower rate of plasma TG turnover than conscious animals (21). Since the second experiment, lymph duct cannulation, required anesthetization, we determined whether this treatment affected any of the parameters of TG secretion in the suckling rat. In one experiment five pups were anesthetized with ketamine and kept in a warm box for 3 hr after recovery. The plasma TG concentration of these pups, 1.04 ± 0.15 mM, was not significantly different from the value for five littermates that

TABLE 1. Effect of Triton WR1339 on plasma triacyglycerol (TG) levels in the suckling rat[a]

	Plasma TG (mM)			
	With Mother		Without Mother	
Time	Control	Experimental	Control	Experimental
hr				
0	1.28 ± 0.11 (8)		1.26 ± 0.26 (6)	
1		1.21 ± 0.21 (3)		1.04 ± 0.15 (3)
2		3.88 ± 0.91 (6)	1.15 ± 0.33 (4)	2.56 ± 0.65 (6)[b]
3		20.7 ± 1.2 (6)		9.16 ± 1.31 (6)
4		31.8 ± 2.4 (4)	1.23 ± 0.31 (4)	13.1 ± 1.4 (5)[c]
6	1.36 ± 0.22 (4)	36.8 ± 3.6 (4)[c]	0.95 ± 0.8 (4)	15.4 ± 2.6 (3)[c]

[a] Experimental animals were injected intraperitoneally with 4.7 μg of Triton/g body weight and controls were injected with a comparable volume of saline. The animals were either returned to the mother or kept in a warm box at 30–32°C. The results are expressed as mean ± standard deviation with the number of animals in parentheses.
[b] *P*, experimental vs control <0.01.
[c] *P*, experimental vs control <0.001.

had been kept in the warm box without prior anesthesia, 1.23 ± 0.31 mM. In another experiment, 12 pups were anesthetized with ketamine and after recovery they were injected with Triton WR1339 and then kept in the warm box. Six pups were killed 2 hr and six were killed 3 hr after detergent administration. The change in plasma TG concentration between 2 and 3 hr after Triton administration gave a TG entry rate of 6.7 ± 1.0 μmol/hr per ml plasma, which was not significantly different than the TG entry rate calculated from the data in Table 1 for pups away from their mother. Based on these data, there does not appear to be a significant effect of prior anesthetization with ketamine on either the rate of entry or the steady state level of plasma TG in the suckling rat. Therefore a direct comparison between data obtained with or without ketamine anesthesia is possible.

Table 2 presents data on lymphatic output from mesenteric lymph duct-cannulated pups. It should be noted that administration of Triton WR1339 had no significant effect on lymph flow or TG ouput. Triton has also been shown not to affect these parameters in adults (11). Lymph flow (123.7 ± 35.9 μl/hr), lymph TG concentration (128.6 ± 34.9 mM), and TG output (14.9 ± 2.7 μmol/hr) remained constant for 2 hr. During the third hour lymph flow decreased significantly, although the TG concentration did not. The rate of TG output in lymph, 14.9 ± 2.7 μmol/hr per rat, was not significantly

TABLE 2. Lymph flow and triacylglycerol (TG) content and output in suckling rats[a]

	Time		
	1 hr	2 hr	3 hr
Lymph flow (μl/hr)			
− Triton	142.5 ± 36.3[b,c]	124.3 ± 26.1[b,c]	46.7 ± 32.0[b,d]
+ Triton	120.2 ± 45.4[b,c]	107.7 ± 34.5[b,c]	53.0 ± 21.9[b,d]
Lymph TG (mM)			
− Triton	119.5 ± 31.3[b,c]	127.6 ± 24.3[b,c]	148.3 ± 65.0[b,e]
+ Triton	127.8 ± 44.9[b,c]	138.9 ± 41.9[b,c]	131.5 ± 60.2[b,e]
Lymph TG output (μmol/hr)			
− Triton	16.3 ± 3.9[b,c]	15.4 ± 1.0[b,c]	6.9 ± 3.8[b,d]
+ Triton	13.9 ± 2.6[b,c]	13.0 ± 1.0[b,c]	6.2 ± 1.6[b,d]

[a] Mesenteric lymph was collected in hourly intervals after recovery from anesthesia. Some animals were injected with Triton WR1339 (4.7 μg/g body weight) after recovery from anesthesia. Results are expressed as mean ± standard deviation. In each case the number of animals used was six.
[b] No significant effect of Triton.
[c] No significant difference between first and second hour samples.
[d] *P*, third vs second hour samples <0.001.
[e] No significant difference between second and third hour samples.

different from the total rate of TG entry into plasma, 12.5 ± 1.5 μmol/hr per rat, determined from Triton administration to rats kept in a warm box. These data suggest that most, if not all, plasma TG arise from intestinal input in the suckling rat.

This conclusion is further supported by data on the effect of Triton WR1339 on plasma TG concentration in lymph duct-cannulated pups. In pups not treated with detergent, plasma TG levels fell to 0.27 ± 0.10 mM (n = 6) 3 hr after lymph duct cannulation. When Triton was administered immediately after recovery from anesthesia to lymph duct-cannulated pups, the plasma TG concentration was 0.75 ± 0.23 mM (n = 6) after 3 hr, and the plasma TG levels were significantly higher in detergent-treated pups (P = 0.02). However, the TG concentration observed 3 hr after Triton administration in lymph duct-cannulated animals was less than 10% of the concentration observed in animals without lymph duct cannulation (Table 1). Based on these data one can estimate that the liver could contribute at most 5–10% of the TG entering the plasma. The increase in plasma TG observed after detergent treatment of lymph duct-cannulated animals could also be due to incomplete cannulation. Considering the large number of small accessory ducts we observe in the suckling rat, this explanation is quite reasonable. Therefore we conclude that 90–100% of plasma TG originates from the intestine in the suckling rat.

Another indication of minimal TG secretion by the liver of the suckling rat is the observation that starvation for 18 hr completely abolishes Triton-induced hyperlipidemia. Control animals starved for 18 hr had plasma TG levels of 1.05 ± 0.73 mM (n = 6), and 3 hr after Triton administration to starved animals the level was not significantly different, 0.90 ± 0.53 mM (n = 6).

DISCUSSION

All the data presented in this paper are consistent with the hypothesis that in the suckling rat nearly 100% of plasma TG arises from intestinal input. Considering the high-fat diet of the neonate, it is not surprising that the bulk of plasma TG would arise from dietary sources. What was unexpected is the apparent lack of TG secretion by the liver of the suckling rat, since in adult rats consuming a comparable level of fat in the diet, there is still a measurable contribution of the liver to the plasma TG pool (5, 6). However, it should be noted that the apparent lack of TG secretion by the liver observed in this study would explain the massive fat accumulation observed when fatty acid oxidation in the suckling rat liver is blocked with tetradecylglycidic acid (12). Thus it appears that the liver of the suckling rat has the capacity to synthesize TG but not to secrete VLDL at an appreciable rate. Whether this lack of TG secretion is a peculiar feature of the neonatal liver or arises from the ultimate effect of a high-fat diet in suppressing VLDL secretion, as observed in adult liver, is unknown at present.

As noted earlier, we assumed that the rate of TG entry into plasma measured in the presence of the mother represents the physiologically relevant value, since the pups can continually suckle. It is therefore of interest to compare this value (31.8 μmol/hr per rat) to that which can be calculated from milk intake. A 14- to 15-day-old rat consumes about 9 ml milk/day (3), which, with a TG concentration of 140 mM (2), amounts to an input of about 3750 μeq of fatty acid/day. Of this fatty acid about 1310 μeq (35%) is medium chain length (C_{12} or less) and 2440 μeq (65%) is long chain length (greater than C_{12}) (2). Based on the TG entry rate into plasma determined in the presence of the mother and assuming that all TG originates from intestinal input, the TG output from the intestine would be 2300 μeq/day. Based on the fatty acid composition of lymph TG (85% long chain and 15% medium chain length fatty acids) (2), this would amount to 1950 μeq of long chain and 350 μeq of medium chain length fatty acid. In the case of the long chain fatty acids this corresponds to about 80% of that calculated from milk input, and for medium chain length fatty acids about 25%. Three points can be made from this calculation. *1*) Given that the TG entry rate into plasma estimated using Triton WR1339 is certainly an underestimate, since lipoprotein lipase is not completely inhibited (15–17), and that estimates of milk consumption are not very precise (3), it appears that the data obtained in this study can satisfactorily account for all the long chain fatty acid consumed by the suckling rat as being secreted in the form of lymphatic TG. *2*) It is known that a large proportion of medium chain length fatty acids are released from milk TG in the stomach of the suckling rat (22–24) and are readily absorbed into the portal venous system (23). The data presented here would suggest that about 75% of milk TG medium chain length fatty acids are absorbed via this route. *3*) The reasonable agreement between the rate of TG output from the intestine calculated from milk intake and from the rate of TG entry into plasma measured in the presence of the mother support our assumption that this latter value is the physiologically relevant number.

The data presented in this paper show two striking features of fat digestion and absorption in the suckling rat. First, the rate of TG secretion by the small intestine of the suckling rat is considerably higher than that found in adult rats (5–11, 25–27). Second, the rate of TG secretion by the liver of the suckling rat is considerably

lower than that found in adult rats (5–11). While one could suggest that the former results from the large amount of ingested fat, the reason(s) for the negligible secretion of TG by the suckling rat liver must await further investigation. The data also demonstrate the efficiency of the neonate in utilizing large quantities of TG, which can be appreciated from the following calculation. With a total plasma pool of 2.5 μmol and an input rate of 0.5 μmol/min, the entire plasma pool of TG must turnover in 5 min, and in a steady state situation, the half life of plasma TG is about 1.4 min.

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