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Altered Bile Acid Physiology during Lactation in the Rat[1] (41857)

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Abstract. There is evidence that the rate of bile acid secretion increases significantly during lactation in the rat. We show that this increase in secretion rate is accompanied by an expanded bile acid pool and that occasioning the enhancement of both pool size and secretion is an increase in bile acid synthesis. The hypothesis is advanced that maternal prolactin, promoted by suckling young, amplifies cholesterol-7α-hydroxylase, the rate-limiting enzyme in bile acid biosynthesis, and perhaps HMG-CoA reductase, the rate-limiting enzyme in cholesterogenesis.

Lactation in the rat is accompanied by a marked elevation in bile acid secretion (1, 2). The functional value of such a change may derive from at least two events. First, the nursing female exhibits approximately a threefold increase in food intake to meet the metabolic demands of milk production (3, 4). This probably intensifies the need for bile acids, since bile acids are important for keeping biliary cholesterol in solution and for facilitating intestinal lipid absorption. Second, the nursing rat emits a maternal pheromone which can be experimentally induced in the nulliparous female by feeding cholic acid (5). This pheromone induces preweanling young to approach the feces of the mother. The ensuing consumption of these feces by the pups has been shown to be essential for their growth and survival (6).

Yet despite the suggested importance of an increased bile acid secretory rate during lactation in the rat, the phenomenon has received relatively little attention. Accordingly, several basic questions remain unanswered. For example, is the increased secretory rate achieved by an increase in hepatic synthesis, or by an increase in the rate at which bile acids cycle through the gut and return to the liver? The latter might be expected insofar as the gut undergoes extensive hypertrophy and hyperplasia during lactation (7, 8). On the other hand, if increased synthesis underlies increased secretion, is the elevated synthesis accompanied by an expansion of the bile acid pool? Herein we show that the bile acid pool is indeed expanded and that the expansion is achieved by a stimulation of bile acid synthesis.

Material and Methods. Bile was collected from mature female Wistar rats that were born and reared in the authors' laboratory. They were either pregnant for 18 days or lactating for 1, 5, 14, or 21 days, respectively. For purposes of comparison, bile was also collected from nulliparous females and from females 14 days postlactation. A total of 28 animals was used. Each had unlimited access to water and to Teklad diet containing 4% fat. (Teklad Standard Diets, P.O. Box 4220, Madison, Wisc. 53711.)

While the animal was anesthetized with ketamine HCl (8–10 mg/100 g body wt), the common bile duct was cannulated with PE 10 tubing, and the cannula was externalized dorsally. In each case the cannulation was begun between 1000 and 1400 hr to control for diurnal variations in bile acid synthesis. The tubing was secured by a specially designed rodent jacket and tether (Alice King Chatham Medical Arts, 5043 Onaknoll Avenue, Los Angeles, Calif. 90043) and then threaded through a swivel. The swivel allowed normal movement and, for those females that were lactating, free access to nursing young. Bile flowed continuously into tared vials contained in a fraction collector programmed at 2-hr intervals for 24 hr.

Total biliary bile acids were assayed in each 2-hr fraction by an enzymatic spectrophoto-

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metric method employing hydroxysteroid dehydrogenase (Worthington STDHP). The method employed was an amalgam of those described by Talalay (9), Iwata and Yamasaki (10), and Javitt and Emerman (11). In brief, a 3-ml reaction volume was made to contain 0.058 *M* Na pyrophosphate buffer, pH 9.5; 0.172 *M* hydrazine hydrate; 0.417 m*M* NAD; 0.27 units hydroxysteroid dehydrogenase; and 0.01 ml rat bile. The standard curve was linear to 0.35 m*M* bile acids. We allowed the reaction 1 hr to ensure completion and computed bile acid molarity based on the extinction coefficient of NADH at 340 nm.

Results and Discussion. We constructed a "washout curve" (12) for each animal by plotting bile acid secretion (μmole/g liver/2 hr) *vs* time. Using the criteria of Mok *et al.* (12) we calculated: (a) *pool size* as the total amount of bile acids obtained from the time of cannulation until the time bile acid secretion reached a low point *minus* the amount due to basal synthesis; (b) *basal synthesis* rate as the mass of bile acids contained in the 2-hr fraction falling at the low point of the washout curve, the point, that is, at which all recycling bile acids have been removed; (c) *secretion rate* per 24 hr as micromoles of bile acids obtained in the first 2-hr fraction multiplied by 12; and, finally, (d) *circulation frequency* as the ratio of secretion rate to pool size.

These parameters are illustrated in Fig. 1 for nulliparous, 18-day pregnant, lactating, and postlactating rats, respectively. As seen in Panel A, basal synthesis rate was elevated significantly by Day 5 of lactation relative to that in both nulliparous and pregnant females. This heightened synthesis was also in evidence on Days 14 and 21 of lactation. Two weeks postlactation, synthesis was again at prelactational levels.

Panel B shows that bile acid secretion was also elevated during lactation, as previously reported (1, 2). However, the secretion rate, unlike the synthesis rate, was not heightened significantly until Day 14. Thereafter secretion followed the same course as synthesis, remaining high on Day 21 of lactation and then declining to nulliparous and pregnant levels 2 weeks after lactation.

As seen in Panel C, bile acid pool size was also elevated significantly, but not until Day 21 of lactation, that is not until after the ele-

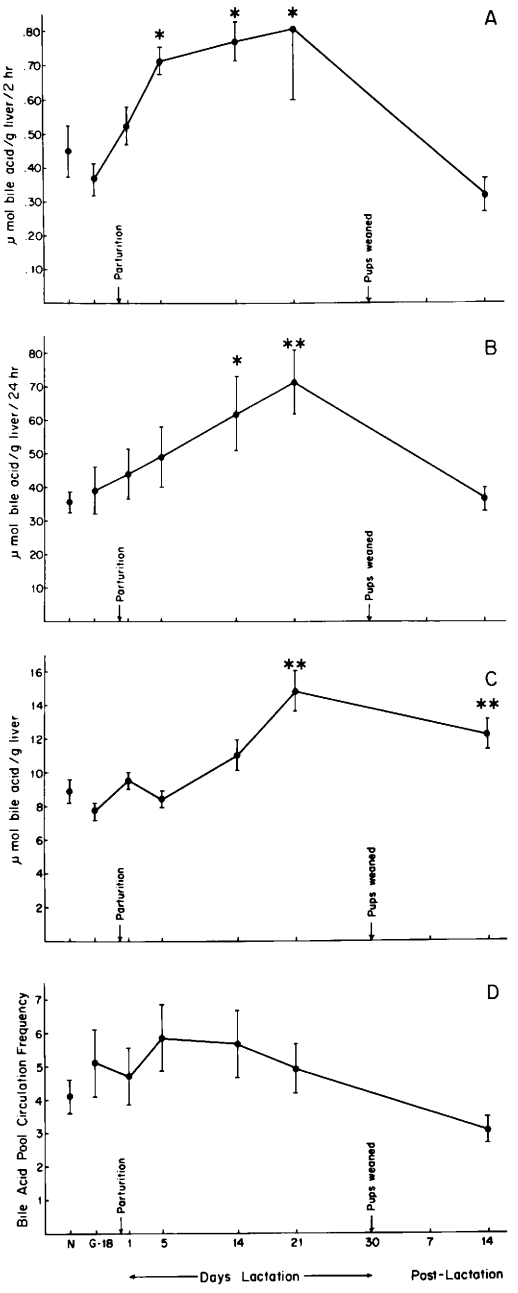

FIG. 1. Changes in basal bile acid hepatic synthesis rate (A), 24-hr bile acid secretion rate (B), bile acid pool size (C), and bile acid pool circulation frequency (D) over time for nulliparous (N), 18-day gestation (G-18), lactating, and postlactating rats. The bars represent the standard error of the mean with four animals per group. The data were analyzed using an SPSS program for one-way ANOVA (21) *a priori* comparison of all groups to nulliparous control, and the Student–Newman–Keuls post-hoc ranking (22). * $P \leq 0.05$; ** $P \leq 0.01$.

vation of synthesis and secretion rates. Pool size subsequently decreased, although at 2 weeks postlactation it was still above nulliparous and gestational levels. This may reflect the lag-time needed for pool size, once expanded, to return to baseline. It would be of interest to monitor pool size at points later than 2 weeks postlactation.

Finally, circulation frequency was not heightened during lactation, as illustrated in Panel D. The observed elevation in secretion rate, therefore, cannot be attributed to an increased rate of bile acid reabsorption.

Liver weight has been observed to increase in the lactating rat (13, 14). We also found liver weight as a fraction of body weight to be higher in each group of lactating females than in each group of nonlactating females—nulliparous, pregnant, and postlactational, respectively (see Table 1). Accordingly, the data presented in Fig. 1 are expressed as units per g liver rather than as units per 100 g body weight to eliminate the effects of liver size, as such, on bile acid physiology.

The results of the present experiment leave no doubt that profound changes occur in bile acid physiology during lactation in the rat. Pool size expands, and secretion and synthesis rates increase. Since the observed increase in synthesis rate preceded changes in pool size and secretion, it is reasonable to conclude that the elevation in bile acid synthesis was causal, occasioning the enhancement of both secretion rate and pool size. This conclusion, of course, raises the question of what stimulated bile acid synthesis.

Vonk *et al.* (15) presented data showing a positive correlation between biliary bile acid output and food intake during the circadian feeding cycle of the rat. However, they did not study the hyperphagic animal, nor did they study bile acid synthesis. We found that pregnancy did not enhance bile acid synthesis, even though pregnancy is associated with a 60% increase in food intake (3). It seems unlikely, therefore, that the heightened synthesis evinced by our lactating females was the result of the hyperphagia that typically accompanies lactation (3, 4).

We think that it is possible that the enhanced bile acid synthesis that we observed reflected an elevation in cholesterol-7α-hydroxylase. This hepatic enzyme is considered to be rate limiting in bile acid production, and it is enhanced by thyroxine and corticosterone, respectively (16, 17). Perhaps prolactin, which is increased in the lactating female by the suckling litter, also amplifies the enzyme. Such a hypothesis has not been tested.

It is also possible that prolactin amplifies 3-hydroxy-3-methyl glutaryl-CoA reductase (HMG-CoA reductase), the rate limiting enzyme in cholesterol biosynthesis. First, hepatic HMG-CoA reductase increases during lactation (18), as does the biliary output of cholesterol (1). And second, agents that stimulate cholesterol synthesis stimulate bile acid production as well (19, 20).

It would be of interest to study both cholesterol-7α-hydroxylase and HMG-CoA reductase to determine whether or not, in fact, their activity is affected by elevated blood levels of prolactin. It would also be of interest to determine whether or not lactation in other mammalian species, including the human, is accompanied by alterations in bile acid physiology similar to those that we observed in the rat.

TABLE I. RATIO OF LIVER WEIGHT TO BODY WEIGHT FOR NULLIPAROUS, 18-DAY GESTATION, LACTATING, AND POSTLACTATING RATS

Group	Liver weight[a] (g/100 g body wt)	Statistical[b] significance *P*
Nulliparous	3.13 ± 0.06	—
18-Day gestation	3.62 ± 0.18	NS
1 Day lactation	4.35 ± 0.34	<0.001
5 Days lactation	4.68 ± 0.20	<0.001
14 Days lactation	4.85 ± 0.26	<0.001
21 Days lactation	4.09 ± 0.20	<0.001
2 Weeks post-lactation	3.55 ± 0.15	NS

[a] Values are means ± standard errors.
[b] Statistical methods are described in Fig. 1.

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