Circadian distribution of bile acid in the enterohepatic circulatory system in hamsters

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Abstract The distribution of bile acid in the enterohepatic circulatory system at different times of the day was determined in 48 hamsters exposed to a rigid light schedule (6 AM to 6 PM) and fed, ad libitum, for 4 weeks. In each portion of the enterohepatic circulatory system, the relative amount of bile acid was determined 24 hours after an intraperitoneal administration of [^{3}H]taurocholic acid by comparing the radioactivity recovered from that portion with the total radioactivity remaining in the entire system. A circadian fluctuation of the relative bile acid content (percent of total) was observed in serum, liver, gallbladder, and intestinal contents. The patterns of such rhythmic change varied in various segments of the intestinal tract but correlated well with the time sequence of the movement of bowel content. Rhythms in the serum and liver were intimately related to the intestinal absorption of bile acid. Due to its small capacity, the gallbladder played only a minor role in the regulation of such a rhythm.

Supplementary key words gallbladder · [^{3}H]taurocholic acid · intestinal contents

Persistent daily fluctuations occur in many physiological variables in living organisms (1). In rats, a striking circadian rhythm of hepatic bile acid synthetic activity occurs, with a peak at 8 PM and a nadir between 8 AM and noon (2, 3). This rhythm is associated with the change in activity of the rate-limiting enzyme in bile acid biosynthesis, cholesterol-7α-hydroxylase (2, 3). Biliary excretion of bile acid also undergoes a daily cyclic variation in rats with chronic biliary drainage (4). The amount of bile acid in the serum, liver, and intestinal content of rats also fluctuates with time (5). The patterns of such rhythmic changes vary in various segments of the intestinal tract but correlate with the time sequence of bowel content movement and bile acid absorption. We believe that the circadian variation of the amount of bile acid in rat liver is an important factor in the regulation of hepatic bile acid synthesis (5).

Circadian fluctuations of the variables described above for rats have not been studied in animals with gallbladders. Because of the cyclic storage and discharge of bile by the gallbladder, a greater circadian fluctuation in the distribution of bile acid in the enterohepatic circulatory system might be expected in those species with gallbladders, e.g., hamsters, than in those without, e.g., rats. The cyclic variation of hepatic bile acid synthesis could also be exaggerated because of the presence of a gallbladder. In man (6–10), a difference occurs in the bile acid and lipid compositions of bile obtained during fasting and during feeding, and also before and after cholecystectomy. The present study examines circadian fluctuation of the distribution of bile acid in the enterohepatic circulatory system in hamsters and evaluates its magnitude.

MATERIALS AND METHODS

Forty-eight adult male golden hamsters (*Mesocricetus auratus*), initially weighing 104 ± 9 g (mean ± SD), were housed four animals in each cage. The windowless animal room was artificially illuminated from 6 AM to 6 PM. Animals were fed a commercial hamster chow (Wayne Lab-Blox, Allied Mills, Inc., Chicago, Ill.) ad libitum. The hamsters usually slept during the light period and started feeding soon after the onset of darkness. Average daily food consumption was 7 g per animal.

After 4 weeks of exposure to the fixed lighting schedule, the animals were divided randomly into twelve groups of four animals each. The hamsters in each group were scheduled to be given randomly labeled tauro[^{3}H]cholic acid at a fixed hour of the day (but not necessarily on the same day). The tauro-[^{3}H]cholic acid (sp act 3.39 Ci/mmole; radiochemical purity greater than 99%) was obtained from New England Nuclear Corp., Boston, Mass. It was purified by thin-layer chromatography on silica gel G in butanol–acetic acid–water 10:1:1 (v/v/v). The tauro-[^{3}H]cholic acid was eluted with ethanol and the solution was evaporated to dryness. The residue was dissolved in normal saline to a final concentration of 10 μCi per 0.3 ml. Each animal was lightly anesthetized with ether and injected intraperitoneally with 10 μCi of tauro[^{3}H]cholic acid in 0.3 ml of

normal saline. The animals were then housed in individual cages in the same room and fed and watered as before.

Twenty-four hours after the administration of tritiated taurocholic acid, the animals were killed under ether anesthesia by exsanguination through a puncture in the abdominal aorta. Usually 2–3 ml of blood was obtained, which was the source of serum for analysis of radioactivity. The gallbladder was separated from the liver by dissection and the entire liver was removed. The small intestine was divided equally into proximal and distal halves. The small intestine, cecum, and colon were opened longitudinally and their contents collected in separate preweighed containers. Wet weights of the liver, kidneys, luminal contents and walls of the proximal and distal small intestine, cecum, and colon were obtained.

The liver and each segment of the intestinal tract were cut into small pieces and homogenized in ethanol in a VirTis tissue homogenizer. Five 1-hr extractions were then made by refluxing with ethanol on a boiling water bath. The serum, gallbladder, and intestinal contents were extracted in the same way, but without homogenization. Extracts were adjusted to a suitable final volume by either dilution or concentration and the radioactivity was determined on a suitable aliquot using a liquid scintillation spectrometer with PPO (2,5-diphenyloxazole)–bis-MSB(*O*-methylstyrylbenzene)–toluene as scintillation solution (New England Nuclear Corp., Boston, Mass.). Quenching, if present, was corrected by further dilution or by the channels ratio method (11).

In order to make sure that the extraction of radioactivity was complete and that the extracted radioactive materials were indeed cholic acid and its metabolites, the ethanol extracts and residual tissues of three liver specimens were further tested. When the residual tissues were subjected to alkaline hydrolysis (KOH–ethanol), the hydrolysates contained negligible amounts of radioactivity. Thin-layer chromatography on silica gel G in butanol–acetic acid–water 10:1:1 (v/v/v) was applied to the ethanol extracts; the radioactivity was confined to bands with the same R_f values as taurine- and glycine-conjugated cholic acid and deoxycholic acid.

RESULTS

Variation in weight

The body weight of the animals at the time they were killed (122 ± 10 g, mean ± SD) and the wet weights of liver (4.11 ± 0.41 g), walls of the proximal and distal small intestine (0.85 ± 0.11 g and 0.67 ± 0.09 g), and colon (0.68 ± 0.12 g) were quite stable and showed no significant consistent fluctuation during a 24-hr period. The mean lengths of the small intestine, cecum, and colon were 40.6 ± 1.6, 5.7 ± 0.6, and 37.8 ± 2.5 cm, respectively, and were not different among various groups of animals. The mean plasma volume, calculated on the assumption of 40 ml/kg body wt (12), was 4.88 ± 0.41 ml.

The contents of various segments of the intestinal tract, on the other hand, exhibited persistent daily variations as displayed in **Fig. 1.** The existence of a circadian rhythm in the amount of luminal contents in a particular portion of the intestinal tract is shown by a statistically significant difference between nadir and peak values, and by a smooth transition between them. The food residue in the proximal small intestine increased gradually from 0.03 g between noon and 4 PM to a peak of 0.24 g between 10 PM and midnight. The nadir of the content of the distal small intestine occurred at 6 PM and the peak at 6 AM. The contents of both cecum and colon, which exceeded that of the small intestine at all times, also exhibited a circadian fluctuation. For the cecum the nadir was at 8 PM to midnight, with a peak at noon; for the colon the nadir was from 10 PM to 8 AM and the peak was at 2 PM.

Distribution of bile acid in the enterohepatic circulatory system

Since the absolute quantity of bile acid was not determined, the distribution of bile acid in various portions of the enterohepatic circulatory system was

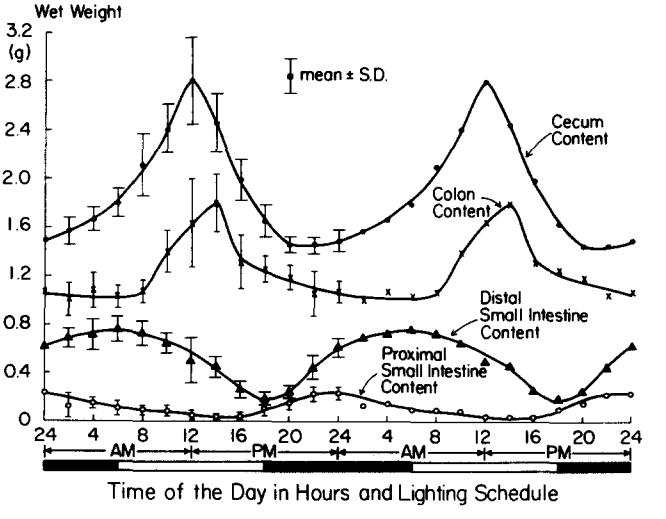

Fig. 1. Daily fluctuations of the wet weights of luminal contents in various segments of the intestinal tract. Each point on the first cycle is the mean ± SD for four animals killed at the same hour of the day but not necessarily on the same day. The values for the second 24-hr cycle are the same as those in the first cycle without the standard deviations.

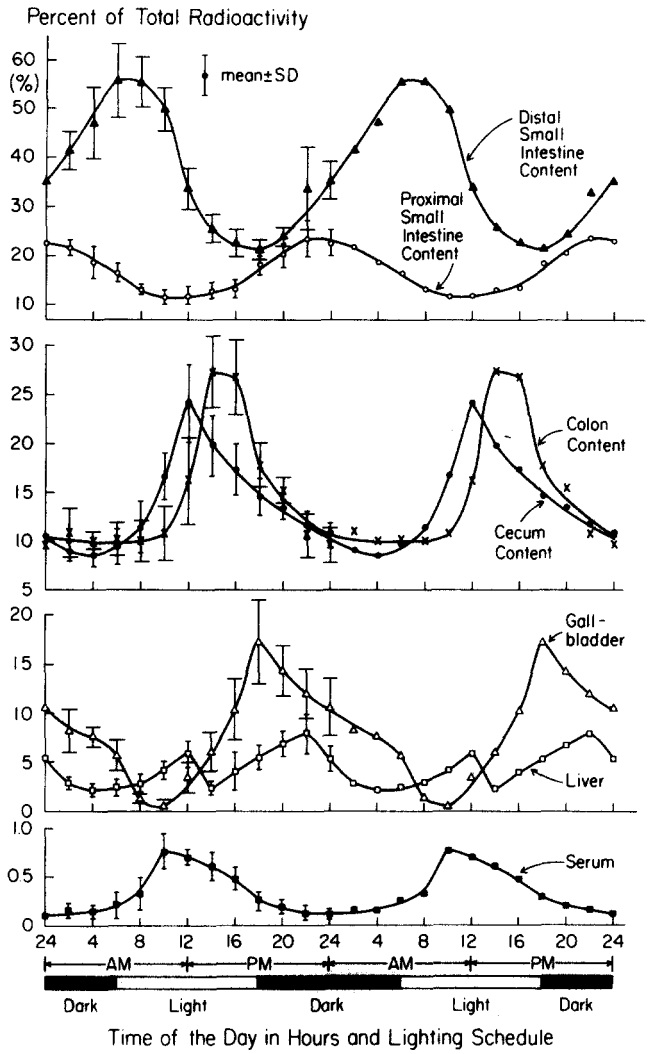

Fig. 2. Daily fluctuations patterns of the percent distribution of bile acid in serum, gallbladder, liver, and luminal contents of various segments of the intestinal tract. Each point on the first cycle is the mean ± SD for four animals killed at the same hour of the day but not necessarily on the same day. The values for the second 24-hour cycle are the same as those in the first cycle without the standard deviations.

expressed as radioactivity recovered relative to total radioactivity in the entire system at the time the hamsters were killed. The radioactivity present in the wall of the intestinal tract was 6.56% of the total radioactivity, with 3.65 ± 0.70% (mean ± SD) in the proximal small intestine, 2.24 ± 0.51% in the distal small intestine, 0.42 ± 0.10% in the cecum, and 0.25 ± 0.05% in the colon. No definite daily fluctuation was observed.

On the other hand, the relative amounts of bile acid in the gallbladder, serum, liver, and in the contents of various segments of the intestinal tract exhibited a circadian variation (**Fig. 2**). The maximum relative bile acid content of the gallbladder (17.2 ± 4.5%) occurred at the end of the fasting period, immediately before the onset of darkness. As soon as the hamsters started feeding, the bile acid content of the gallbladder decreased gradually and steadily with a further decline at 6 AM. A nadir value of 0.6 ± 0.5% occurred at 10 PM. The relative bile acid content of the liver had two peaks during a 24-hr period: a smaller peak (5.98 ± 1.30%) at noon and a larger peak (6.96 ± 1.89%) at 10 PM. The nadirs were at 4 AM and 2 PM.

The relative luminal bile acid content in the proximal small intestine started to increase 2 hr before the onset of darkness and reached a peak value of 23.3 ± 3.6% at 10 PM. It then decreased gradually to reach a low value of 11.5 ± 1.4% at 10 AM. The distal small intestine contained the largest amount of bile acid at all times; its peak (55.7 ± 7.7%) and nadir (21.0 ± 2.0%) occurred 8 hr after the occurrence of the corresponding peak and nadir of the proximal small intestine.

Bile acid content in the lumen of cecum and colon followed one another closely. The peak value occurred at noon in cecum (24.0 ± 3.8%) and 2 PM in colon (27.1 ± 3.6%). The nadir value of 8.5 ± 1.2% was attained at 4 AM for cecum and a low value of approximately 10% was maintained in the colon during the later part of the dark period and early part of the light period.

The relative amount of bile acid in the serum ranged from a peak value of 0.76 ± 0.17% at 10 AM to a nadir value of 0.11 ± 0.05% at midnight. The increase from nadir to peak was rapid whereas the decrease was more gradual.

DISCUSSION

The primary function of the gallbladder is to concentrate and store bile during fasting. The capacity of the hamster gallbladder probably does not exceed 0.03 ml because the mean weight of gallbladders plus bile inside was only 0.038 ± 0.013 g. The maximum bile acid content of the gallbladder was only 17.2 ± 4.5% of the total bile acid in the enterohepatic circulatory system (Fig. 2). While prolonged fasting might increase the amount of bile acid stored in the gallbladder, 77–95% of the bile acid was present in the intestinal tract. This was consonant with the observation that the intestinal tract always contained some food residue, which could retain a considerable amount of bile acid. The gallbladder in hamsters seems, therefore, to play a minor role in bile storage and to contribute to a very limited extent to the circadian fluctuation of bile acid in other portions of

the enterohepatic circulatory system. This conclusion is supported by the observation that despite the lack of a gallbladder in rats, the amount of bile acid in various portions of the enterohepatic circulatory system undergoes a circadian fluctuation (5).

Consistent with the distal small intestine being the major site of bile acid absorption (13), the continuous filling of the gallbladder with bile acid from 10 AM to 6 PM corresponded to the rapid decrease of luminal bile acid content in the distal small intestine. After the onset of darkness, when the hamster started to eat, the bile acid in the gallbladder decreased gradually. After the slow discharge of the gallbladder bile, the luminal bile acid of the proximal small intestine gradually increased. Actually the luminal bile acid of the proximal small intestine started to increase 2 hr earlier than the discharge of gallbladder bile. This suggests that the hepatic bile indeed bypassed the gallbladder and drained directly into the intestine, at least during this time.

An inverse relationship between the relative luminal bile acid content occurs in the proximal and distal segments of the small intestine. The decrease in the proximal segment was accompanied by an increase in the distal segment. The decrease in the distal small intestine, on the other hand, was accompanied by a sharp increase of the bile acid content in the cecum. The latter, in turn, was followed by an increase in the colon, but with a 2 hr delay (Fig. 2). This correlates exactly with the change in the amount of food residue in various segments of the intestinal tract (Fig. 1). Therefore, the patterns of the rhythmic change of bile acid content in various segments of the intestinal tract were intimately related to the time sequence of the movement of the bowel content.

A similar phenomenon was observed in rats (5). Although the amount of bile acid in various portions of the enterohepatic circulatory system fluctuates with time in rats, about 98% of the total bile acid remained at all times in the lumen (92.5%) and wall (5.5%) of the intestinal tract. The intestinal lumen of the rat may be considered to function as a reservoir for bile acid.

Fluctuation of bile acid content in the serum reflected the intestinal absorption of bile acid. A sharp increase of serum bile acid content was accompanied by a sharp decrease of bile acid content in the distal small intestine, which is the major site of bile acid absorption (13).

There were two peaks in the daily fluctuation of hepatic bile acid content. The smaller peak at noon probably was related to the intestinal absorption of bile acid, for it corresponded to the rapidly decreasing phase of the luminal bile acid content in the distal small intestine. The higher peak at 10 PM could be the net result of bile acid absorption from the proximal small intestine, cecum, and colon. The fact that this second peak was higher than the first peak suggested a greater retention of labeled bile acid in the liver during this interval. Retention might be due to the decreased amount of bile acid in other parts of the enterohepatic circulatory system or to an increase in newly synthesized bile acid in the liver, which would compete with the labeled bile acid for excretion. This can be answered by the demonstration of a circadian rhythm of hepatic bile acid synthesis in hamsters and the evaluation of its relationship to hepatic bile acid content.

It is clear from the above discussion that the circadian fluctuation of bile acid distribution reflects the eating pattern of the hamster rather than the "light on, light off" schedule of the experimental design. Furthermore, dietary fiber has been strongly implicated in binding bile acid in the intestinal tract and making it unavailable for absorption (14, 15). The feeding schedule and the quantity and quality of dietary fiber should affect the pattern of such a circadian phenomenon. This is reserved for further study.

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