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Effect of Cholesterol Feeding on Circadian Rhythm of Hepatic and Intestinal Cholesterol Biosynthesis in Hamsters[1] (39018)

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A circadian rhythm of hepatic cholesterol synthesis was first demonstrated in mice by Kandutsch and Saucier (1) and subsequently in rats by a number of investigators (2–12). This rhythmic variation has been found to be associated with changes in the concentration of the rate-limiting enzyme in cholesterol biosynthesis, namely β-hydroxy-β-methylglutaryl coenzyme A (HMG CoA) reductase (3, 5, 8–11).

In rats having unrestricted access to food, the hepatic cholesterol synthetic activity is maximal at midnight and minimal at noon (2, 4, 5, 8, 10), concomitant with the fluctuation of hepatic synthetic activity of bile acids (7, 12). However, the rhythm is more intimately related to the ingestion of food than to the lighting of the environment (4, 9, 10, 12). Furthermore, the amplitude of such rhythm can be magnified by dietary corn oil or cholestyramine (9), diminished by fasting (2), or ingestion of cholesterol (4) or cholic acid (2), and abolished completely by adrenalectomy (6). The intestine is another organ besides liver very active in cholesterol synthesis which also exhibits a circadian rhythm similar to that of the liver (10, 11).

Such an interesting and important phenomenon has thus far been demonstrated only in mice (1) and rats (2–12) but also might be present in other species of animals and even in human beings. The present study demonstrated in hamsters a similar circadian rhythm of hepatic and intestinal cholesterol synthetic activity and evaluated the effect of cholesterol feeding on such rhythmic changes.

Materials and methods. Animals and diets. Forty-eight adult male golden hamsters (Mesocricetus auratus), weighing 90–110 g at the beginning of the experiment, were housed in 12 community cages with four animals in each cage. The windowless animal room was artificially illuminated with light on at 6 AM and off at 6 PM. Half of the animals were fed a commercial hamster chow (Wayne, Lab-Blox, Allied Mills, Inc., Chicago, Ill.) mixed with 2% of corn oil. These animals and diet will be referred to as the control animals and diet, respectively. The control diet contained 0.063% of cholesterol (13). The other half of the animals were given the same diet supplemented with 2% of crystalline cholesterol which was dissolved in heated corn oil (2% of the diet) and mixed thoroughly with the pellets. The preweighed chow was given to the animals at 6 PM each day and any residual food was removed at 8 AM the next morning and weighed. The average food consumption was 7.0 ± 1.0 g (mean ± SD) per animal and showed no apparent difference between the control animals and those fed cholesterol diet. All animals had free access to water at any time.

In vivo incorporation of acetate-1-^{14}C into cholesterol. Sodium acetate-1-^{14}C with a specific activity (sp act) of 21.5 mCi/m*M* was obtained from Mallinckrodt Co., St. Louis, Missouri and purified by silica gel thin layer chromatography with a solvent system composed of ethanol:ammonium hydroxide:water (80:12:16). The eluate was dissolved in 0.85% saline to give a final concentration of 66.6 μCi per 0.3 ml

Three weeks after the aforementioned lighting and feeding treatment, the 24 animals in each group were divided equally into six subgroups scheduled to be given acetate-1-^{14}C at mignight, 4 AM, 8 AM, noon, 4 PM, and 8 PM, respectively. At the scheduled

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time, the animals were lightly anesthetized with ether and each received intraperitoneally 66.6 μCi of acetate-1-^{14}C in 0.3 ml of normal saline. The animals usually recovered from anesthesia immediately. Exactly 30 min later, the animals were killed by exsanguination. The gastrointestinal tract, entire liver, and two kidneys were immediately removed and placed in ice-cold saline The gastrointestinal tract was opened longitudinally and its contents were removed thoroughly under a stream of cold saline. It was then separated into stomach, small intestine, cecum and colon. After cleaning all organs were blotted dry and their weights were obtained. The animals were sacrificed over a period of 4 days.

Chemical analyses. The liver, stomach, small intestine, cecum, colon, and kidneys were cut into very small pieces and their lipid contents were extracted three times with Folch solution (chloroform:methanol = 2:1) and the water soluble contaminants were removed by the Folch wash (14). Their total and free cholesterol contents and that of the serum were determined by the method of Sperry and Webb (15).

Before reacting with color reagent, the cholesterol digitonide precipitate of each sample was washed first with ethanol:acetone (1:1), then acetone:ether (1:2), and followed by two washes with ether. Another aliquot from each specimen was used for determination of the radioactivity of free cholesterol. This aliquot was treated in the same way as the aliquot for colorimetry except that pyridine, instead of color reagent, was added to the dry washed cholesterol digitonide which was then transferred to scintillation vials and evaporated to dryness. The radioactivity was counted in PPO-POPOP toluene scintillation fluid in a Beckman liquid scintillation spectrometer.

Results. Effect of cholesterol feeding on body and organ weights. Within the same group of animals, either the control or the cholesterol-fed, the body and organ weights of the six subgroups were not different from each other and were pooled together (Table I). Each animal gained an average of 0.5–0.7 g/day during the experimental period. The cholesterol-fed hamsters seemed to have slightly heavier body and organ weights than the control animals but statistically such differences were not significant except the liver. The livers of the cholesterol-fed hamsters were grossly fatty and 12.8% heavier than that of the controls. The increase in weight was apparently due to the accumulation of lipids.

TABLE I. BODY AND ORGAN WEIGHTS OF THE HAMSTERS ON CONTROL DIET AND THOSE ON 2% CHOLESTEROL DIET.

Organ[a]	Hamsters on control diet (N = 24)[b]	Hamsters on 2% cholesterol diet (N = 24)[b]	Comparison P value
Liver	4.17±0.81[c]	5.35±0.84	<0.001
Stomach	0.76±0.08	0.76±0.07	>0.1
Small intestine	1.84±0.31	1.99±0.47	>0.1
Cecum	0.62±0.10	0.65±0.20	>0.1
Colon	0.75±0.14	0.81±0.17	>0.1
Kidney	0.77±0.09	0.82±0.08	>0.1
Body weight	114.3±16.5	120.9±15.9	>0.1

[a] Entire organ, such as whole liver, two kidneys.
[b] Number of animals in the group.
[c] Mean ± SD in grams wet weight.

Effect of cholesterol feeding on serum and tissue cholesterol contents. The total and free cholesterol contents of the serum and all tissues tested except the stomach were significantly higher in the cholesterol-fed hamsters than in the controls (Table II). The free and esterified cholesterol were increased proportionally in serum, cecum, colon, and kidney. Therefore, the percentage of free cholesterol in these organs was the same as those in the control hamsters.

The normal small intestine contained very little esterified cholesterol; however, after cholesterol feeding for 3 weeks the esterified cholesterol was increased to a greater extent than the free cholesterol. The percentage of free cholesterol was decreased from 97.1% in the controls to 87.3% in the cholesterol-fed animals (Table II). Such tendency was greatly exaggerated in the liver. The total cholesterol content of this organ in the cholesterol-fed animals was 8.4 times that of the controls. The increase was primarily due to the tremendous elevation of its esteri-

TABLE II. THE TOTAL AND FREE CHOLESTEROL CONTENTS OF THE SERUM AND VARIOUS ORGANS OF THE HAMSTERS ON CONTROL DIET AND THOSE ON 2% CHOLESTEROL DIET.

Organ	Hamsters on control Diet (N =24)[a]			Hamsters on 2% cholesterol diet(N = 24)		
	Total cholesterol	Free cholesterol	% Free	Total cholesterol	Free cholesterol	% Free
Liver	1.78 ± 0.35[b]	1.45 ± 0.28	81.5%	14.94 ± 5.57[d]	2.44 ± 1.04[d]	16.3%
Stomach	2.65 ± 0.14	2.59 ± 0.17	97.7%	2.61 ± 0.15	2.60 ± 0.11	99.6%
Small intestine	1.72 ± 0.34	1.67 ± 0.34	97.1%	2.37 ± 0.37[d]	2.07 ± 0.29	87.3%
Cecum	1.86 ± 0.33	1.72 ± 0.27	92.5%	2.21 ± 0.21[d]	2.01 ± 0.13[d]	91.0%
Colon	2.00 ± 0.30	1.80 ± 0.29	90.0%	2.24 ± 0.19[d]	2.03 ± 0.15[d]	90.6%
Kidney	3.43 ± 0.27	3.30 ± 0.29	96.3%	4.00 ± 0.26[d]	3.85 ± 0.19[d]	96.2%
Serum	88 ± 15[c]	18 ± 6	20.4%	167 ± 52[d]	33 ± 18[d]	19.8%

[a] Number of animals.
[b] Mean ± SD in mg cholesterol/g wet tissue.
[c] Mean ± SD in mg/100 ml serum.
[d] Significant difference from the control by t test ($P < 0.01$).

fied cholesterol content (38-fold), whereas the free cholesterol content was only increased to 1.7 times that of the controls (Table II).

In vivo demonstration of the circadian rhythm of hepatic and intestinal cholesterol biosynthesis. The intraperitoneally administered acetate-1-^{14}C was believed to be absorbed rapidly into the circulation and reached various organs and tissues in the body. A small portion would be incorporated into cholesterol molecules. There are several possible ways for measurement and expression of cholesterol synthetic activity of a particular organ concerned, such as cholesterol radioactivity of the entire organ, per gram of tissue, or per mg of cholesterol in the tissue. Since cholesterol is first synthesized in the free form, and only to a very limited extent the newly synthesized free cholesterol will be esterified immediately, we believe that the cholesterol synthetic activity of various organs will be best expressed as the specific activity of free cholesterol in the organs, especially for the purpose of comparison among various organs and various groups of animals, although it cannot be considered as the exact absolute value of the synthetic activity.

A remarkable circadian rhythm of cholesterol synthetic activity expressed as sp act of free cholesterol was demonstrated in the liver, small intestine, and kidney of the control hamsters, but not in their stomach, cecum, and colon (Table III and Fig. 1). In the liver, the nadir was at some time between 4 PM and 8 PM, probably shortly after darkness when the food was given. The sp act increased rapidly thereafter to reach a peak at midnight. From the shape of the curve shown in Fig. 1 the true peak might be higher and occur sometime between midnight and 2 AM. The amplitude of such rhythm in the liver was tremendous. The sp act at nadir obtained by extrapolation was about 3000 dpm/mg that was only 5.4% of the peak sp act at midnight.

The cholesterol synthetic activity of the small intestine also exhibited a similar circadian rhythm with the peak at midnight and a gradual decline to a baseline activity at 8 AM. The baseline activity was maintained from 8 AM to 6 PM (Table III and Fig. 1). The radioactivity detected in the kidney was generally very low as compared with that of all other organs tested (Table III). Although it exhibited a circadian rhythm with the peak at midnight and the nadir at 8 PM, the low radioactivity and its delayed uprise (2–4 hr behind that of liver and small intestine) suggested that such change of the sp act in the kidney might be secondary to the change in the blood sp act and could not represent true fluctuation of the de novo cholesterol synthetic activity in the kidney.

Effect of cholesterol feeding on the circadian rhythm of hepatic and intestinal cholesterol synthesis. After feeding the 2% cholesterol diet for 3 weeks, the ability of the liver to convert acetate to cholesterol was

TABLE III. SPECIFIC ACTIVITY OF FREE CHOLESTEROL IN VARIOUS ORGANS OF THE HAMSTERS ON CONTROL DIET AND THOSE ON 2% CHOLESTEROL DIET GIVEN 66.6 μCi OF ACETATE-1-^{14}C AT 4 HOUR INTERVALS AND SACRIFICED 30 MINUTES LATER.

Organ	Time of day (hr)					
	0	4	8	12	16	20
Hamsters on control diet (4 animals)						
Liver	55128 ±16006[a]	32025 ±8755	15606 ±5046	8834 ±2066	3896 ±955	8862 ±1720
Stomach	7090 ±2581	8036 ±1635	8344 ±1111	9112 ±2691	7533 ±2025	9013 ±1807
Small intestine	42891 ±7654	31508 ±6683	24005 ±5751	22983 ±8963	22086 ±6591	27095 ±9655
Cecum	6643 ±2809	4780 ±1644	4421 ±2668	6053 ±2108	5186 ±1808	6424 ±2611
Colon	5627 ±1674	4841 ±1377	5596 ±1188	6944 ±2360	4193 ±2764	4603 ± 904
Kidney	2699 ±1243	1895 ± 766	723 ± 397	593 ± 173	638 ± 209	301 ± 49
Hamsters on 2% cholesterol diet (4 animals)						
Liver	1620 ± 476[b]	1872 ± 629[b]	1253 ± 730[b]	2063 ± 852[b]	1692 ± 309[b]	1290 ± 609[b]
Stomach	6793 ±2526	8293 ±2690	7818 ±1914	6951 ±2430	8818 ±3062	6998 ±2235
Small intestine	27794 ±6390[b]	22166 ±4860	18222 ±7633	18610 ±6108	16908 ±5906	19805 ±5818
Cecum	4713 ±2548	5541 ±2089	3988 ±1980	4257 ±2389	5269 ±2644	4981 ±2125
Colon	5076 ±1757	4159 ±1523	4508 ±2108	3699 ±1579	4950 ±2625	3889 ±1685
Kidney	463 ± 160[b]	389 ± 98[b]	526 ± 219	374 ± 62	412 ± 149	450 ± 203

[a] Mean ± SD in dpm/mg free cholesterol.
[b] Significant difference from the control by t test ($P < 0.05$).

remarkably suppressed and the phenomenon of circadian rhythm was totally abolished (Table III and Fig. 1). The average sp act was 1631 dpm/mg or only about half of the minimal value of the control animals. Since the hepatic free cholesterol content of the cholesterol-fed hamsters was 1.7 times that of the controls, the excessive amount of free cholesterol in the former would dilute their sp act. When this dilution factor was corrected, the hepatic cholesterol synthetic activity of the cholesterol-fed animals was almost identical to that of the baseline activity (at the nadir) of the control animals. In other words, approximately 95% of the hepatic cholesterol synthesis was suppressed by cholesterol feeding.

In the case of the small intestine, cholesterol feeding decreased the amplitude of the circadian rhythm but failed to abolish the phenomenon (Table III and Fig. 1). The mean sp act of the cholesterol-fed animals at any given time was lower than that of the controls, but only the sp act at midnight showed statistically significant difference. Even when the correction was made for the dilution of sp act due to the increase of free cholesterol content in the small intestines of the cholesterol-fed animals, their peak sp act was still only 83% that of the controls. Apparently there was a 17% suppression or reduction of peak cholesterol synthesis as a result of cholesterol feeding.

In the cholesterol-fed animals, the sp act of

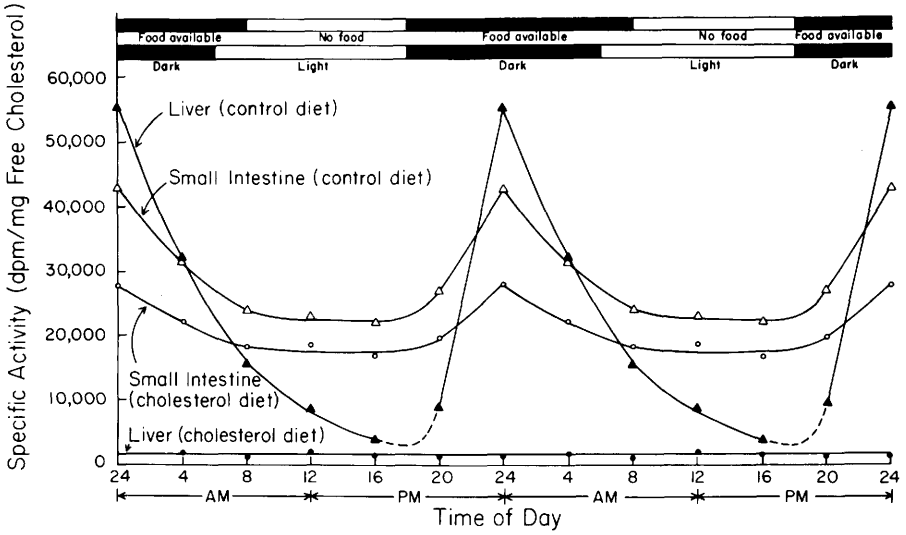

FIG. 1. Changes of the specific activity of free cholesterol in the liver and small intestine of the hamsters on control diet and those on 2% cholesterol diet given 66.6 μCi of acetate-1-^{14}C at 4-hr intervals and sacrificed 30 min later.

free cholesterol in the kidney remained low all the time, and those of the stomach, cecum, and colon were not significantly different from that of the control animals even after correction for the dilution factor due to increase of free cholesterol content in the former.

Discussion. The present study confirmed the feasibility and validity of the *in vivo* assay of cholesterol synthetic activity in individual organs employed previously by Edwards *et al.* (10). The quantitative values obtained by such *in vivo* studies would be closer to the true activity in a living organism than those obtained by an *in vitro* artificial system. However, one may argue that the organs having very low cholesterol synthesis activity might retain through the circulation some radioactively-labeled cholesterol synthesized in other organs during the period of *in vivo* incubation. It has been shown that most of the serum cholesterol is contributed by the liver and small intestine (16–18). Therefore, the contamination of the liver and small intestine with the labeled serum cholesterol synthesized in other organs could be considered as relatively insignificant. The kidney, on the other hand, had very low sp act but very large blood flow, and a large proportion of its radioactively-labeled cholesterol could have originated in the liver and small intestine and been carried to it through the blood flow.

To our best knowledge, this is the first demonstration of a circadian rhythm of the hepatic and intestinal cholesterol synthetic activity in hamsters. The rhythm is quite similar to that observed in rats (2–12). Since hamsters usually eat at night and sleep during daytime, the restriction of the food to a rigid schedule from 6 PM to 8 AM in this study was not intended to alter their natural habits but only to control better the experimental condition. The sharp rise of the synthetic activity shortly after darkness when food was given indicated an intimate relationship between the activity and the darkness and/or the food intake. The ingestion of food might be the major triggering factor since change of the restricted feeding period from dark to light shifted the peak of activity from midnight to noon in rats (10).

From this study we may postulate that the food, when low in cholesterol, stimulates cholesterol synthesis in the intestinal mucosa during the process of absorption, and when the absorbed food subsequently reaches the liver, it stimulates the hepatic cholesterol synthesis. The increase in the synthetic activity is most likely a result of new enzyme synthesis, particularly HMG CoA reductase, as shown in rats (2, 8, 10). In the liver when

the amount and activity of this rate-limiting enzyme reaches a maximum, the liver ceases to synthesize any more enzyme, and the existing enzyme starts to decay and falls to a minimal value before the next stimulus for a new cycle. In the small intestine, the synthetic activity starts from a high baseline to the peak at midnight and returns to the baseline within 8 hr. The high baseline activity is maintained during most of the daytime. Factors other than food intake should also be considered. For instance, adrenalectomy abolished the circadian rhythm of hepatic cholesterol synthesis in rats (6), and the catecholamines in certain organs of the hamster also exhibited a rhythmic change (19).

It is well established that the hepatic, but not the extrahepatic cholesterol synthesis can be suppressed by dietary cholesterol intake (20). In this study we have observed that cholesterol feeding elevated serum cholesterol level, cholesterol content of the liver, and to a lesser extent that of the small intestine, cecum, colon, and kidney in hamsters. Such responses are similar to those in rabbits and prairie dogs (25). The hepatic cholesterol synthetic activity in cholesterol-fed hamsters was suppressed to a minimum or 5% of the peak activity of the control animals. Part of the activity measured by this *in vivo* technique might be actually the cholesterol newly synthesized in the intestine and carried through the circulation to the liver. Therefore, the degree of suppression could approach completion. Since the dietary cholesterol intake suppressed, instead of stimulated, the synthesis of new enzymes, no circadian rhythm could be demonstrated in the cholesterol-fed hamsters. In rats, although cholesterol feeding caused inhibition at all times, the diurnal effect was still present (4). Such discrepancy might be due to species difference.

Contrary to the liver, the circadian rhythm of the intestinal cholesterol synthetic activity persisted with lower amplitude after cholesterol feeding. It is widely believed that the intestinal cholesterol synthesis is controlled by bile acids rather than dietary cholesterol (22). In this study in hamsters, however, the discharge of gallbladder bile into the small intestine after feeding did not inhibit but stimulated the intestinal cholesterol synthesis. The stimulatory effect of feeding seemed to be stronger than the inhibitory activity of bile acids. Furthermore, a 17% reduction of the peak activity of the rhythm was found in hamsters after cholesterol feeding. A similar phenomenon with a 20% reduction has been shown in the proximal one-third segment of small intestine in rats (23). Therefore, the small intestine of both hamsters and rats is not totally unaffected by dietary cholesterol.

Summary. Forty-eight adult hamsters were divided equally into two groups fed a control diet and a 2% cholesterol diet, respectively, under a rigid lighting (6 PM–6 AM) and feeding (6 PM–8 AM) schedule for three weeks. The cholesterol synthetic activity of the liver, stomach, small intestine, cecum, colon and kidney was measured by *in vivo* conversion of acetate-1-^{14}C to cholesterol in four animals each time at 4 hour intervals. A remarkable circadian rhythm with the peak at midnight and the nadir at noon was found in the liver of the control hamsters, but was completely abolished in the cholesterol-fed animals since the activity was nearly totally suppressed at all times. The small intestine exhibited a similar rhythm with the peak at midnight but maintained a high baseline activity from 8 AM to 6 PM. Cholesterol feeding did not alter the baseline activity but reduced 17% of the peak activity. Other organs failed to show such a circadian rhythm.

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