Circadian variation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in swine liver and ileum

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Abstract The temporal variation of HMG-CoA reductase activity in the liver and intestine of swine was investigated. The thin-layer chromatographic method widely used in the assay of the reductase was successfully applied to the porcine enzymes. Parallel circadian rhythms were demonstrated in both hepatic and ileal reductases from mash-fed animals. Peak activity occurred approximately 6 hr after feeding, 2.7-fold over the basal level in the liver, and 1.6-fold in the ileum. A milk-cholesterol diet caused a marked depression of both rhythms (90% in liver, 50% in ileum); however, the hourly variation in activity persisted in both organs. Cholestyramine was found to elevate hepatic activity (2.7-fold throughout the rhythm) without affecting that of the intestine. Clofibrate had no effect on either enzyme at any time during the cycle despite a 34% reduction in serum cholesterol concentrations.—**Rogers, D. H., D. N. Kim, K. T. Lee, J. M. Reiner, and W. A. Thomas.** Circadian variation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in swine liver and ileum. *J. Lipid Res.* 1981. **22:** 811–819.

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The principal enzymatic site of control of cholesterol biosynthesis in mammalian tissues is considered to be the reduction of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to mevalonic acid (1). This reaction is catalyzed by the enzyme, HMG-CoA reductase (mevalonate: NADP oxidoreductase (acylating CoA), EC 1.1.1.34) and appears to be the rate-limiting step in the pathway under most physiological conditions.

Since the original report of the circadian variability of sterol synthesis and HMG-CoA reductase activity in mouse liver in 1969 (2), this phenomenon has been demonstrated conclusively only in two other species, the rat (3–5), and the hamster (6). In addition to that in the liver, a parallel rhythm has been observed in the small intestine of both rats (5, 7) and hamsters (6).

The present study was designed to examine the temporal variation in HMG-CoA reductase activity in the liver and ileum of mash-fed swine and to determine the effects of a milk-cholesterol diet and of the drugs, clofibrate and cholestyramine, on the cycle.

MATERIALS AND METHODS

Materials

The commercial sources of all chemicals were listed previously (8).

Animals

Male Yorkshire swine, approximately 8–10 weeks old were housed individually in slat-bottomed cages located in temperature-controlled rooms maintained at 25°C and lighted at all times. For most experiments, the animals were fed 630 g of commercial mash (Agway, Syracuse, NY) daily at 0900. All swine were fed mash on this schedule for at least 2 weeks prior to any experiments to ensure acclimatization to the facilities.

Diets and drugs

The compositions of the mash and milk-cholesterol diets were as described elsewhere (9). All additives and drugs were mixed with approximately 100 g of the respective diets and water, and the remainder of the diet was given following consumption of this portion. The swine consumed all of the diets under strict observation.

Preparation of microsomes

Preliminary investigations not described here indicated that the HMG-CoA reductase activities in the samples of liver and ileum routinely taken from each animal represented the enzyme activities of the whole organs.

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A.

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Liver

Hepatic microsomes were isolated as described previously (8) from a 10% (w/v) homogenate in a buffer consisting of 250 mM sucrose, 10 mM EDTA, and 10 mM reduced glutathione, at pH 7.2.

Ileum

Approximately 10 cm of ileum was taken 30 cm from the ileocecal junction and placed in normal saline at 4°C. The section was opened longitudinally and thoroughly rinsed to remove any fecal matter. With the mucosal surface facing upward, the external muscle layer was removed with a pair of blunt forceps. The remaining tissue, which included the mucosa, submucosa, and lymphoid regions, was used as a source of microsomes. Aside from the preparation of a 20% (w/v) homogenate, the remainder of the isolation procedure was identical to that described for liver.

Protein fractions from both tissues were measured as detailed elsewhere (8).

Assay of HMG-CoA reductase activity

HMG-CoA reductase activity of hepatic and ileal microsomes was assayed as described previously (8). Briefly, 0.1–0.5 mg of microsomal protein was incubated for 30 min in the presence of 3 mM NADP, 10 mM glucose-6-phsophate, 3 units/ml of glucose-6-phosphate dehydrogenase (EC 1.1.1.49), 50 mM reduced glutathione, 100 mM potassium phosphate, pH 7.4, and 160 μM R, S-[3-^{14}C]HMG-CoA (sp act 0.53 Ci/mol). Following addition of acid, the radioactive product was extracted into ether and isolated by thin-layer chromatography by the method of Shefer et al. (10). Values were corrected by subtraction of zero-time controls, obtained by denaturing the microsomes with heat (100°C for 10 min) or acid (6 N H_2SO_4) prior to assay, or by eliminating the NADPH-generating system. These were consistently less than 10% of the lowest activities measured. All values reported represent the mean of duplicate assays. HMG-CoA reductase activity is expressed as picomoles of mevalonate formed per min per mg protein.

TABLE 1. Identification of biosynthetic mevalonolactone

Solvent System[a]	R_f (MVL)[b]	Specific Activity[c] Liver	Specific Activity[c] Ileum
Benzene–acetone 1:1[d]	0.50	758 ± 28	191 ± 18
Benzene–acetone 1:1	0.50	801 ± 30	167 ± 9
n-Butanol	0.62	770 ± 26	163 ± 12
n-Butanol–propionic acid–water 10:5:2	0.55	740 ± 31	165 ± 11

[a] Thin-layer chromatographic solvent systems used to isolate biosynthetic mevalonolactone on silica gel G plates.
[b] Relative migration of authentic mevalonolactone in relation to the solvent front (1.00).
[c] Specific radioactivity of the mevalonolactone isolated in each system as measured by gas–liquid chromatography (13) and liquid scintillation counting (dpm/μmol). Each value is the mean of triplicate determinations ± SEM.
[d] Initial isolation.

Assay of glucose-6-phosphatase activity

Glucose-6-phosphatase activity was measured by the method of Collipp, Chen, and Halle (11), using the method of Chen, Toribara, and Warner (12) for the determination of released orthophosphate.

RESULTS

Validity of the assay system

Standard assays of swine hepatic and ileal HMG-CoA reductase were scaled up 10-fold (10 ml assay volume); the mevalonolactone was isolated on preparative thin-layer plates developed in benzene–acetone 1:1. The lactone was eluted from the silica gel with acetone, dried under nitrogen, and dissolved in chloroform. Aliquots of this solution were then subjected to thin-layer chromatography in three solvent systems (13) and the specific radioactivity of the material migrating as mevalonolactone was measured by gas–liquid chromatography (10) and liquid scintillation counting. The identity and radioactive purity of the biosynthetic mevalonolactone was established for HMG-CoA reductase assays of both liver and ileum by co-chromatography with authentic material. The radioactive product migrated as a single band and the specific radioactivity remained constant throughout each procedure (**Table 1**).

Subcellular localization of hepatic and illeal HMG-CoA reductase

Samples of liver and mucosa were homogenized as indicated above and then filtered through nylon mesh to remove undisrupted material. The remainder of the microsomal isolation was as detailed elsewhere (8). The pellets that sedimented during the two low-speed centrifugations were pooled and resuspended in the homogenization buffer. Aliquots of the filtered homogenates, low-speed pellet resuspensions, microsome resuspensions, and 100,000 *g* supernatant fractions were taken for protein, glucose-6-phosphate activity, and HMG-CoA reductase activity determinations. The homogenization procedure used in these experiments appeared to preclude the disruption of the mitochondria and the release of their associated HMG-CoA cleavage activity. Attempts to

demonstrate HMG-CoA lyase activity in any of the fractions by the method of Clinkenbeard et al. (14), were unsuccessful.

Utilizing glucose-6-phosphatase as a marker for the microsomal membranes (15, 16), it was apparent that about 40% of the microsomes sedimented during the low-speed centrifugations of both homogenates (**Table 2**). HMG-CoA reductase activity was most prevalent in the microsomal fractions and its distribution closely paralleled that of glucose-6-phosphatase, suggesting localization to the microsomes in both tissues. With this in mind, microsomes obtained from liver and ileum were used throughout these studies as the source of reductase.

Properties of the HMG-CoA reductase assay system

The production of mevalonolactone by the hepatic and ileal reductases was linear with time through a 60-min incubation (**Fig. 1**) and with respect to microsomal protein over a range of 30 to 600 μg during a 30-min incubation (**Fig. 2**).

Both enzymes showed broad pH optima between 7.0 and 8.2. NADPH was required as a donor of reducing equivalents, NADH being inactive. Maximal activity was found in the presence of high concentrations of thiol reagents, such as glutathione (10 mM), cysteine (10 mM), or dithiothreitol (5 mM). Apparent K_m values for S-HMG-CoA were estimated from data obtained by the methods of Langdon and Counsell (17). The kinetic constants for the hepatic and ileal microsomal reductases were 0.5 and 0.7 μM, respectively. Recognizing the possibility of interference by other NADPH-requiring enzymes in the microsomes, estimates of the apparent K_m values for NADPH were similarly obtained; 410 μM for the liver and 420 μM for the ileum.

TABLE 2. Localization of HMG-CoA reductase and glucose-6-phosphatase activities in subcellular fractions of swine liver and ileal mucosa

	Protein		G-6-Pase		HMG-CoA Reductase	
	mg	%[b]	U/mg[c]	%	U/mg[d]	%
Liver						
H[a]	750		114		104	
P	165	22	151	36	194	46
M	98	13	360	51	342	48
S	480	65	19	13	9	6
Recovery		99		81		90
Ileum						
H	1327		19		22	
P	451	38	21	44	32	40
M	199	17	45	41	82	45
S	527	45	6	15	10	15
Recovery		89		86		123

[a] Tissue fractions: H, filtered homogenate; P, combined low-speed pellets; M, microsomal pellet; S, 100,000 *g* supernatant fraction.

[b] Relative percent distribution of total recovered in fractions P. M. and S. Actual recoveries relative to the homogenate are shown below each column.

[c] Glucose-6-phosphatase specific activity (nmol/min/mg protein).

[d] HMG-CoA reductase specific activity (pmol/min/mg protein).

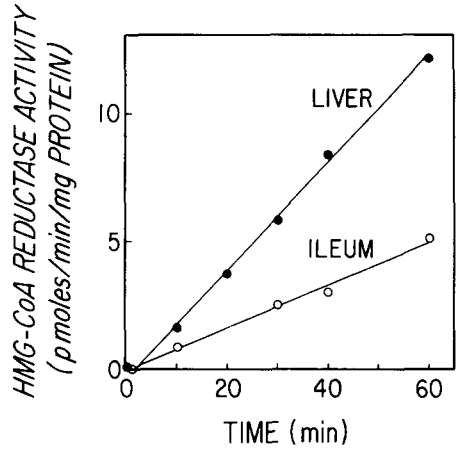

Fig. 1. Dependence of hepatic (● —— ●) and ileal (○ —— ○) HMG-CoA reductase activity on incubation time. Standard assay conditions, except that incubation time was varied from 0 to 60 min. Each point is the mean of duplicate assays.

Circadian rhythms in hepatic and ileal HMG-CoA Reductase Activity

In order to eliminate light cycling as a determinant of any rhythms observed in the swine enzyme ac-

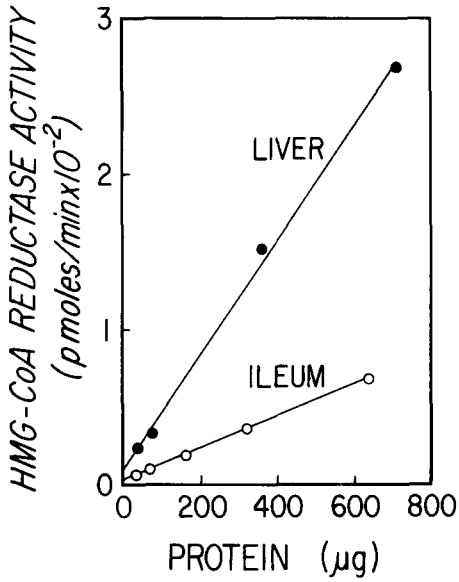

Fig. 2. Dependence of hepatic (● —— ●) and ileal (○ —— ○) HMG-CoA reductase activity on the amount of microsomal protein. Standard assay conditions, with microsomal protein varied over the indicated ranges. Each point is the mean of duplicate assays.

tivities, the rooms in which the animals were housed were maintained under constant illumination. Following the standard 2-week adjustment period, during which mash was fed once daily at 0900, experimental animals were placed on their respective regimens for an additional 2 weeks. All experiments were terminated after 2 weeks on a diet. Food and drugs were administered together as a single daily 0900 meal. Weight gain of all animals during the experimental period was not significantly different from mash-fed controls. Unless stated otherwise, the animals were routinely sacrificed in groups at 0900, 1400, 1700, and 2400 hrs.

In view of the large number of animals required for such studies, these experiments were carried out over a period of several months. The data for the reference circadian rhythms represent a composite of all control swine (single daily mash feeding) assayed during these investigations. At least two control animals were included at various times in every experiment to ensure the validity of comparisons to the standard rhythms.

Reference circadian rhythms

Fig. 3 illustrates the circadian variations in HMG-CoA reductase activity of microsomes from the liver and ileum of swine fed a single daily meal of mash at 0900. These curves represent the reference data to which all other experimental groups were compared.

The hepatic enzyme exhibited a sharp increase in activity between 0900 and 1400 to 1500, peaking 2.7-fold over the basal level ($P < 0.001$). This was followed by a rapid decrease in activity by 1700 to a point approximately 50% above that at 0900. Thereafter, activity gradually returned to the 0900 nadir. A small secondary peak between 2100 and 2400 was suggested by the data, but the activities between 1700 and 0500 were not significantly different.

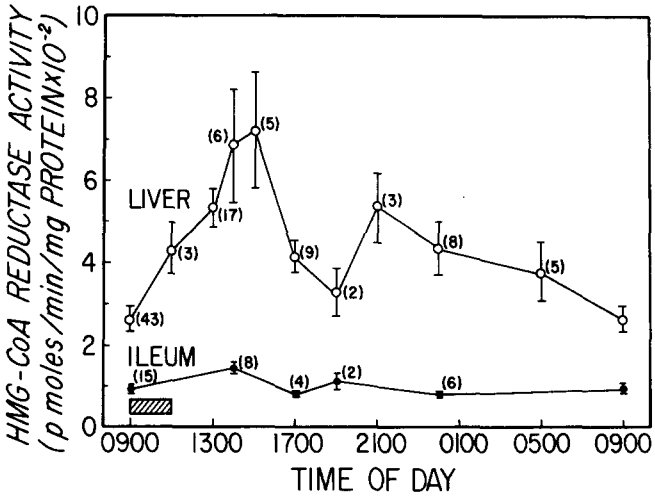

Fig. 3. Circadian variation in hepatic (○ —— ○) and ileal (● —— ●) HMG-CoA reductase activity, mean ± SEM, in swine fed a single daily meal of mash at 0900. Food was consumed during the period designated by the shaded bar. Number of swine for each determination shown in parentheses.

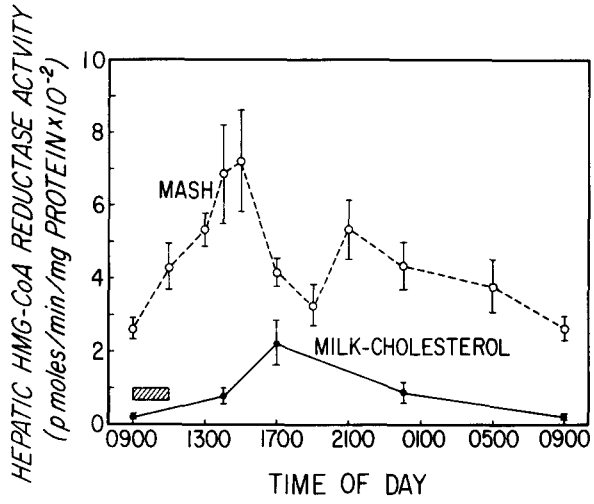

Fig. 4. Circadian variation in hepatic HMG-CoA reductase activity, mean ± SEM, (● —— ●) in swine fed a single daily meal of milk-cholesterol diet. Numbers of swine at each time were 0900, 8; 1400, 4; 1700, 4; 2400, 4. Data from single mash feeding (○ – – ○, Fig. 3.) shown for comparison.

The variation in the reductase activity of ileal microsomes closely paralleled that of the hepatic enzyme, although the rhythm was much less pronounced. The ileal rhythm is shown on the same scale in Fig. 3 as the hepatic cycle for comparative purposes. A better representation of the details of the intestinal enzyme variation may be observed in the later illustrations on an expanded scale (e.g., Fig. 5).

Ileal HMG-CoA reductase activity rose from a 0900 nadir to peak 1.6-fold at 1400 ($P < 0.01$). Basal levels were reached by 1700, and activity remained there for the rest of the cycle.

Effect of milk-cholesterol diet

After feeding a milk-cholesterol diet, hepatic HMG-CoA reductase activity was greatly reduced thoughout the circadian cycle (**Fig. 4**). Basal (0900) and 1400 levels were decreased by 90% ($P < 0.01$). A threefold increase in activity occurred between these two times, but a more striking peak, ninefold over the 0900 level, was observed at 1700. Thereafter, activity slowly returned to the nadir.

The ileal enzyme rhythm exhibited a similar alteration (**Fig. 5**). Activities at 0900 and 1400 were reduced to 50% of the mash-fed levels ($P < 0.02$), but a

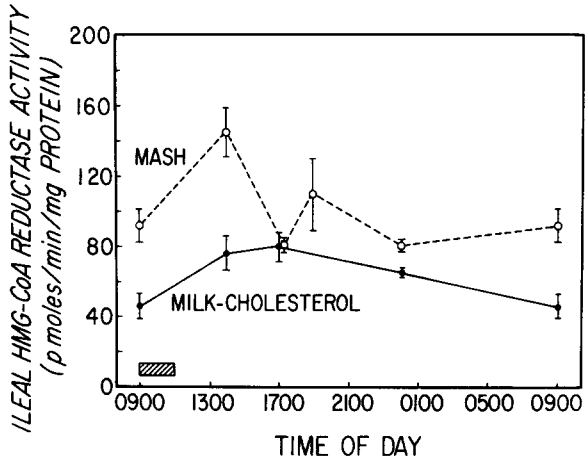

Fig. 5. Circadian variation in ileal HMG-CoA reductase activity, mean ± SEM, (● —— ●) in swine fed a single daily meal of milk-cholesterol diet. Four swine were used for each determination. Data from single mash feeding (○ -- ○, Fig. 3) shown for comparison.

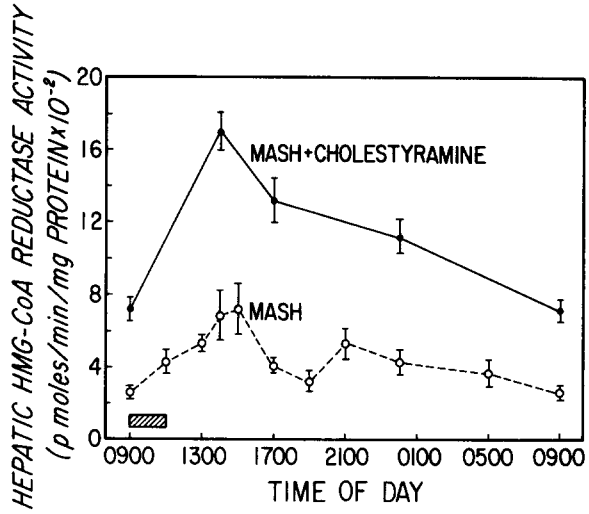

Fig. 6. Circadian variation in hepatic HMG-CoA reductase activity, mean ± SEM, (● —— ●) in swine fed a single daily meal of mash containing 12 g cholestyramine. Numbers of swine at each time were 0900, 5; 1400, 6; 1700, 5; 2400, 5. Data from single mash feeding (○ -- ○, Fig. 3) shown for comparison.

normal 1.7-fold increase between those times occurred. The activity remained at this level at 1700, so that no significant difference was observed between the mash and milk-cholesterol-fed animals at that time.

Effect of cholestyramine

Addition of cholestyramine to a single daily mash feeding resulted in substantial increases (2.7-fold, $P < 0.001$) in HMG-CoA reductase activity of swine liver at all times assayed during the cycle (**Fig. 6**). The overall shape of the rhythm was unchanged.

Unlike the enhancement of hepatic activity, the circadian variation in the ileal reductase was unaffected by cholestramine treatment (**Fig. 7**).

Effect of clofibrate

Administration of clofibrate to mash-fed swine (2 g daily) had no effect on the HMG-CoA reductase activities of either liver or ileum, nor on their rhythmic variations, despite a significant (34%) reduction in serum cholesterol concentrations.

DISCUSSION

The thin-layer chromatographic method widely used in the assay of HMG-CoA reductase from various sources has been successfully applied to the swine hepatic and ileal enzymes. Optimal assay conditions and the kinetic constants observed for the swine reductases were essentially the same as those reported for the rat and other species (1).

An attempt was made to approximate the degree of association of HMG-CoA reductase activity with the microsomal fractions of swine liver and ileal mucosa. In the rat liver, the reductase is present exclusively in the microsomes (1, 10), while the ileal enzyme is reported to be distributed among several fractions (10, 18). The swine reductases were found in both the low-speed pellets, containing nuclei and mitochondria, and in the microsomal pellets, the highest specific activity being associated with the latter fraction (Table 2). The phenomenon of rapidly sediment-

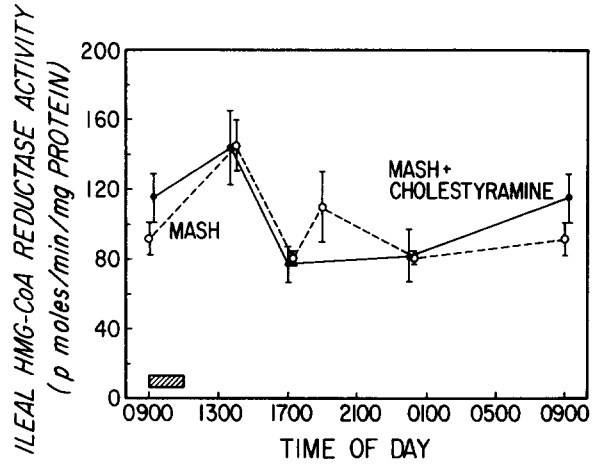

Fig. 7. Circadian variation in ileal HMG-CoA reductase activity, mean ± SEM, (● —— ●) in swine fed a single daily meal of mash containing 12 g cholestyramine. Numbers of swine at each time were as in Fig. 4. Data from single mash feeding (○ -- ○, Fig. 3) shown for comparison.

ing microsomal membranes associated with the mitochondrial pellet and containing a substantial fraction of the total homogenate glucose-6-phosphatase activity has been reported for both liver (15) and intestine (16). It seems likely, in view of the parallel distribution of the activities of glucose-6-phosphatase and HMG-CoA reductase, that the swine reductases are in fact located in the membranes of the endoplasmic reticulum of both liver and ileal mucosa. However, since the low speed pellets were heavily contaminated with microsomal membranes, the possibility of the presence of the reductase in other subcellular organelles cannot be eliminated.

The swine reductases were subject to rhythmic variations in activity similar to those observed in the rat (7, 19) and hamster (6), although the relative amplitudes were smaller. This is believed to be the first demonstration of a circadian rhythm in HMG-CoA reductase activity in a mammal other than a rodent. Ho et al. (20) have presented data suggesting that such a phenomenon may also exist in dogs fed after fasting.

The circadian variation in rat liver HMG-CoA reductase activity has been firmly associated with rhythmic changes in the rate of synthesis of new enzyme, rather than alteration of existing reductase (1–3). Since it was not the purpose of the present experiments to delineate the mechanism of the circadian rhythms in swine reductase activity, no definitive statements along this line may be made.

Huber et al. (21) have shown that the rhythm in hepatic HMG-CoA reductase activity persists in rats exposed to continuous light or darkness, and in surgically blinded animals. In those studies, shifts in the time of peak activity were associated with changes in the time of most rapid feeding. The present experiments suggest that the circadian variations in the swine hepatic and ileal reductases were also independent of light–dark cycling since the animals were constantly exposed to light.

Numerous investigators have stressed the importance of the act of feeding as a modulator of metabolic rhythms (22, 23). The timing of the hepatic peak in HMG-CoA reductase activity may be shifted by altering the light–dark period in ad libitum-fed rats or by feeding them discrete meals irrespective of the lighting pattern (1, 4, 24).

Since rats fed ad libitum normally begin eating near the onset of darkness and consume the bulk of their food during the dark period, a correlation between food intake and the cyclic rise in reductase activity has been suggested (4, 21, 24, 25). A similar connection has been made between feeding and the circadian rhythms in cholesterol biosynthesis in hamster liver and intestine (26). Evidence offered in opposition to this view has concerned the persistence of the rhythm, although progressively attenuated, during a 1- to 3-day fast (4, 19). However, it is likely that the cyclic rise is not effected by the ingestion of food per se, but by the anticipation of food intake, presumably modulated through neuroendocrine hormones (1, 4). Note that the timing of peak activity in relation to the start of feeding (5 to 6 hours later) was the same for the rat (1, 4, 24), hamster (26), and swine (Fig. 3) enzymes.

The high-fat milk–cholesterol diet used in these studies has been well characterized in regard to its effects on cholesterol metabolism in swine (9, 27, 28). As anticipated from those cholesterol balance studies, the hepatic HMG-CoA reductase activity was reduced throughout the day as compared to mash-fed animals (Fig. 4) and a similar depression of ileal enzyme activity was observed (Fig. 5). The apparent delay in attainment of peak reductase activity in both the liver and ileum during milk–cholesterol feeding may have been related to the slower gastric emptying time associated with a meal rich in fats.

It was not the aim of these experiments to determine the component or components of the milk–cholesterol diet that caused these changes; however a brief discussion of the differences between the diets and their possible effects seems warranted.

The relative lipid contents of the two diets is a major factor for consideration. The milk–cholesterol diet consisted of 54% fat on a caloric basis, from milk and peanut oil, and a daily intake of 1.7 g of cholesterol, while the mash diet contained approximately 10% fat, primarily from vegetable sources, and no cholesterol. Both elevated hepatic reductase activities (29) and sterol synthesis (30) and depressed cholesterogenesis (31) have been reported in rats following ingestion of various fats in the absence of cholesterol. Results concerning the degree of saturation of the ingested lipids have been similarily ambiguous (32, 33). The feeding of semipurified diets containing fats without cholesterol was found to decrease hepatic HMG-CoA reductase activities in both rats (33, 34) and swine (35) relative to stock mash diets. The addition of cholesterol to such diets caused a further depression of the reductase activity in these animals[3] (33, 36) and this potentiation appeared to be greater in rats fed unsaturated lipids (33).

Observations of rat intestinal sterol synthesis and HMG-CoA reductase activity have suggested a lack of effect of cholesterol feeding (37). However, cholesterol synthesis in the ileum of guinea pig (38) and

[3] Rogers, D. H., and D. N. Kim. Unpublished observations.

hamster (6) may be suppressed by such a diet and a decrease in the HMG-CoA reductase activity of cultured dog intestinal mucosa was observed when the tissue was exposed to exogenous cholesterol (39). Cholesterol-free semipurified diets have been found to reduce ileal HMG-CoA reductase activity in rats (40) and swine (35), an effect that may be specific for unsaturated fats in the rat. As with the liver enzyme, addition of cholesterol to a semipurified diet containing unsaturated lipids resulted in a further decrease in rat ileal activity, saturated fats being ineffective (40).

The nature of the fiber content of the two diets is also of primary importance in relation to cholesterol metabolism in swine, as we have recently discussed (35). Briefly, swine fed a semipurified diet, containing amounts of fat, protein, and carbohydrate similar to those in mash, exhibited greatly decreased hepatic and ileal HMG-CoA reductase activities when compared to animals fed mash (35). This effect seemed to be due mainly to a substantial reduction in the rate of fecal bile acid excretion which in turn could be explained by the low bile acid binding capacity of the cellulose in the semipurified diet compared to that of the natural fibers present in mash (35). Reiser et al. (34) similarly concluded that the elevated hepatic reductase activities observed in stock-fed rats relative to those in animals fed a semipurified diet were largely the result of differences in the type of nonnutritive fiber.

Little is known about the effects of various proteins and carbohydrates on hepatic and ileal HMG-CoA reductase activities. The mash diet contained protein primarily from vegetable sources, especially corn, while casein was present in the milk–cholesterol mixture. Neither soy protein nor casein had any effect on the hepatic reductase activity in swine when added to mash (41), however, Reiser et al. (34) observed increased enzyme activities in rats fed a semipurified diet containing soy protein in place of casein. The mash contained 74% carbohydrate on a caloric basis, from vegetables, but the milk–cholesterol diet was composed of 26% lactose. In this regard, the substitution of lactose for sucrose in a diet fed to rats caused a decrease in hepatic cholesterol synthesis (42). In contrast, the replacement of dietary sucrose by starch had no effect on hepatic reductase activity (34).

In conclusion, it appears that any or all of the component parts of the mash and milk–cholesterol diets may be responsible for the differences in hepatic and ileal HMG-CoA reductase activities observed in swine. However, it seems likely that the cholesterol in the milk–cholesterol diet, particularly in conjunction with the unsaturated lipids in peanut oil, was a principal factor in causing the lowered reductase activities. The comparatively high enzyme activities associated with the mash diet appear to be related to the poor absorption of cholesterol and/or bile acids as a result of the type of fiber present and the low fat content (27, 35).

Cholestryamine greatly enhanced the overall rhythm in swine hepatic HMG-CoA reductase activity, supporting reports of higher rates of sterol synthesis in the liver of swine after such treatment (43). Elevated rates of hepatic sterol synthesis (43) and HMG-CoA reductase activity (7, 10) reported in the cholestyramine-treated rat have been attributed to the release of inhibition (or repression) of the reductase by bile acids and/or cholesterol (44) as a result of increased fecal acidic and neutral steroid excretion. Similar effects have been observed in rat intestine following cholestyramine administration (7, 10), and this was offered as evidence of the regulation of the ileal HMG-CoA reductase by bile acids (44). In contrast, no effect on the swine ileal reductase occurred (45, Fig. 5). Identical findings were reported for the guinea pig by Turley and West (38) and for the dog by Gans and Cater (46). While hepatic sterol synthesis was massively increased by cholestyramine feeding, that in the ileum was unaffected. This was thought to indicate that the intestinal sterol synthetic pathway was not regulated by bile acids in these animals. However, another explanation may be that the ileal enzyme is maximally active when a mash diet is fed so that further stimulation would be impossible (45).

As anticipated from our previous in vitro (8) and cholesterol balance studies (28) in swine, in which clofibrate exerted no suppression of hepatic or whole body cholesterol synthesis despite a reduction in serum cholesterol levels, the drug had no effect on hepatic or ileal HMG-CoA reductase activities at any time assayed. Gans and Cater (46) were similarly unable to show any effect by the drug on sterol synthesis in dog liver or ileum. These observations contrast with the decreases in whole body cholesterol synthesis and hepatic reductase activity exhibited in the rat (47), and reduced hepatic and intestinal cholesterogenesis in the hamster (48). However, Turley and Dietschy (49) failed to demonstrate any significant changes in cholesterol synthesis by the liver or in intestine of clofibrate treated rats. Recent reports suggested that HMG-CoA reductase in mouse L cells (50) and cholesterol synthesis in rat and monkey hepatocytes (51) may be inhibited by clofibrate exposure in vitro and sodium clofibrate effectively inhibited the rat and mouse liver reductases at 1.35 mM (52). However, in our laboratory, even 10 mM sodium clofibrate added

to standard swine hepatic reductase assays was without effect.

These observations seem to support the idea that a species difference in relation to the response of HMG-CoA reductase and cholesterol biosynthesis to clofibrate administration may exist between the rat and swine. The hypocholesterolemic effect in rats is believed to be partially due to a marked reduction in cholesterol synthesis imposed at the level of mevalonate production (47). In swine, however, the reduction of serum cholesterol levels may be the result of an increased neutral steroid excretion (28) that is not seen in the rat (47, 49). A mechanism similar to that proposed for swine may operate in humans. Reduced serum cholesterol concentrations and increased fecal excretion of neutral steroids have been shown in man (53), yet, Angelin et al. (54) could not demonstrate any changes in HMG-CoA reductase activity in human liver biopsies following clofibrate treatment.

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