Influence of dietary cholesterol, saturated and unsaturated lipid on 3-hydroxy-3-methylglutaryl CoA reductase activity in rabbit intestine and liver

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Abstract To characterize further the behavior of the rate-limiting enzyme of cholesterol biosynthesis in animal species, we studied the kinetic properties and the influence of dietary lipid on intestinal and hepatic HMG-CoA reductase activity in the rabbit. In intestinal crypt and villous cells isolated by a dual buffer technique, the K_M value was 4.2 and 4.6 μM, respectively for DL-HMG-CoA. The specific activity of HMG-CoA reductase in the jejunum was 0.86 nmol/mg per hr, and evenly distributed between crypt and villous cells. By contrast, reductase activity was considerably lower in the ileum: in villous cells it was 0.40 nmol/mg per hr, and in crypt cells only 0.26 nmol/mg per hr. Liver microsomes had a K_M value of 3.0 μM, while the reductase activity averaged 2 nmol/mg per hr. An unexpected finding was the uneven distribution of HMG-CoA reductase in the various lobes of the liver in the single animal. The addition of 1% cholesterol to the diet for 48 hours was followed by an average decline of 73% ($P < 0.005$) of HMG-CoA reductase activity in villous and crypt cells of the jejunum. In the ileum, the decrease was less marked (38%, $P < 0.01$). Whereas the addition of 5% corn oil to a 1% cholesterol diet did not have an additional suppressant effect on intestinal reductase, the addition of 5% coconut oil to 1% cholesterol caused further decrease of HMG-CoA reductase in jejunum and ileum ($P < 0.05$). The 1% cholesterol diet resulted in a 25% decrease of hepatic reductase after 24 hours, whereas after 6 days, the enzyme activity was reduced by 90% of normal. Both 5% corn oil or 5% coconut oil, in addition to 1% cholesterol, further suppressed hepatic reductase activity. The weight of the experimental evidence presented in these studies suggests that cholesterol has a major regulatory effect on both intestinal and hepatic reductase in the rabbit.—**Stange, E. F., M. Alavi, A. Schneider, H. Ditschuneit, and J. R. Poley.** Influence of dietary cholesterol, saturated and unsaturated lipid on 3-hydroxy-3-methylglutaryl CoA reductase activity in rabbit intestine and liver. *J. Lipid Res.* 1981. **22:** 47–56.

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Although the capacity of the intestine to synthesize and secrete cholesterol is second only to that of the liver (1), all mechanisms controlling these processes in small bowel mucosa have not yet been fully characterized.

In both liver and intestine, cholesterol biosynthesis is controlled by the rate-limiting enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase, HMGR; EC 1.1.1.34). Kinetics and other properties of HMGR in intestine and liver of rodents are comparable (2), and diurnal rhythm of HMGR activity exists in the rat (3, 4) and hamster (5). However, in contrast to hepatic reductase, intestinal HMGR is influenced neither by age nor sex, nor localized in the same cell organelles (2, 6).

The rate of synthesis of hepatic cholesterol is inversely related to the amount of cholesterol in the diet. The suppressor signal for hepatic reductase is the cholesterol carried in chylomicron remnants (7). Although cholesterol feeding results in inhibition of intestinal reductase in hamsters (5) and guinea pigs (8, 9), it has little effect in dogs (10), and little (11) or no effect (12) in rats. The question remains unanswered, whether the inhibitory action on HMGR in the responding species is primarily due to cholesterol, or to a secondary rise of bile acid synthesis. This occurs in the cholesterol-fed guinea pig (13).

Dietschy (11) showed that bile decreased intestinal cholesterogenesis in biliary-diverted and cholestyramine-treated animals. He suggested that bile acids were effective in suppressing intestinal cholesterol

Abbreviations: HMG-CoA: 3-hydroxy-3-methylglutaryl-coenzyme A; HMGR, HMG-CoA reductase; TLC, thin-layer chromatography.

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synthesis. However, it could not be excluded that bile acids caused this effect by promoting micellar dispersion and thus facilitating the absorption of cholesterol. More recently, Shefer et al. (12) demonstrated that bile acids were only able to influence an already stimulated cholesterol synthesis, but were ineffective at the basal level of activity. They concluded that, in the rat, bile acids and cholesterol play a role in the regulation of intestinal HMGR.

Thus far, most of the work done to localize intestinal reductase has utilized the "differential scraping" technique to prepare crypt and villous-enriched cell fractions. However, Muroya, Sodhi, and Gould (14) have demonstrated that scraping of mucosa severely affected reductase activity, particularly in villi. This interference with HMGR activity was thought to be due to a trypsin-like protease (15), since it could be suppressed by trypsin inhibition.

Merchant and Heller (6) demonstrated that preparation of intestinal cell fractions by a dual buffer system resulted in intact and excellent recovery of both crypt and villous cell populations. Hence, we decided to utilize this technique for a re-investigation of some aspects of HMGR regulation in both cell fractions. We chose the rabbit as an animal model because synthesis and pool size of the main bile acid, deoxycholic acid, is largely unaffected by dietary cholesterol and other lipid (16). Lastly, we wanted to study the effect of dietary lipid on hepatic reductase, as well the distribution of HMGR in the various lobes of rabbit liver. In pilot experiments, we were unable to obtain reproducible results when only random samples of liver were used for measurements of HMGR activity.

EXPERIMENTAL METHODS

Animals and diets

Male New Zealand white rabbits weighing 1.8–2.2 kg were maintained in wire-bottomed cages. The lighting period lasted from 7 AM to 7 PM. The animals were divided into five groups, and all received their respective diets and water ad libitum, as previously described (17). Group I animals were fed commercial "fat-free" rabbit chow (George Plange, Soest, GFR), with a fat content of less than 1% (by weight). The animals of group II received an additional 1% cholesterol for 2 days. Group III was fed the 1% cholesterol diet for 14 days. Group IV received 5% corn oil, in addition to 1% cholesterol, and group V, 5% coconut oil, also in addition to 1% cholesterol. The latter dietary fat supplements were fed for 48 hours only. The animals were killed at 9 AM by exsanguination under light ether anesthesia.

Materials

DL-3-Hydroxy-3-methyl[3-^{14}C]glutaryl CoA (10 mCi/mmol), DL-[5-^{3}H]mevalonic acid dibenzylethylenediamine salt (5 Ci/mmol), and [^{3}H]thymidine were obtained from New England Nuclear Co., Dreieichenhain, GFR. Unlabeled DL-HMG-CoA was from Sigma, Munich, GFR. Other chemicals were purchased from the following sources: mevalonic acid lactone, bovine serum albumin, and dithiothreitol from Serva, Heidelberg, GFR; glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and NADP from Boehringer, Mannheim, GFR; disodium-EDTA, Folin reagent, and silica gel 60 G from E. Merck, Darmstadt, GFR; DEAE paper discs (DE-81, 1.6 cm) from Whatman, Clifton, NJ and trypsin inhibitor from soy bean, from Serva, Heidelberg, GFR, with an activity of 35 I.U./mg.

Preparation of subcellular fractions

The liver was dissected into the five lobes in ice-cold homogenization medium consisting of 0.1 M sucrose, 0.05 M KCL, 0.04 M KH_2PO_4, and 0.03 M disodium-EDTA, adjusted to pH 7.2. Each liver lobe was homogenized separately by ten strokes in a Potter-Elvehjem tissue homogenizer with a loose-fit pestle. The homogenate was then centrifuged at 30,000 *g* (Sorvall Superspeed RC-2-B) for 20 min at 4°C. The supernatant was re-centrifuged at 105,000 *g* (Beckman L 265 B) for 60 min to sediment the microsomes. The microsomal fraction was resuspended in the homogenization medium to obtain the appropriate protein concentration. Protein was determined by the method of Lowry et al. (18).

To fractionate intestinal mucosal homogenates, the nuclear fraction was sedimented at 160 *g*, mitochondria at 6,700 *g* and, finally, microsomes at 105,000 *g*. The supernate of the last fraction was considered cytosol.

Isolation of intestinal crypt and villous cells

Villous and crypt cells were obtained by the dual buffer technique (19), as modified by Merchant and Heller (6). Segments of 20-cm length of proximal jejunum and distal ileum were excised and rinsed thoroughly with ice-cold saline. After cleansing, the gut loops were closed at one end and distended with Buffer A containing 1.5 mM KCL, 96 mM NaCl, 27 mM sodium citrate, 8 mM KH_2PO_4, and 5.6 mM Na_2HPO_4, adjusted to pH 7.3. After closure at both ends, the loops were immersed in 0.9% saline and incubated at 37°C in a shaking water bath for 15 min. Thereafter, Buffer A was decanted and the segments

filled with Buffer B, containing 0.137 mM NaCl, 2.69 mM KCL, 8.1 mM Na_2HPO_4, 1.47 mM KH_2PO_4, 1.5 mM EDTA, and 0.5 mM dithiothreitol at pH 7.4.

After a series of timed experiments, we adopted a 40-min incubation period in Buffer B for the preparation of enriched villous cells, as indicated by marker enzyme activities, e.g., alkaline phosphatase and thymidine kinase (see below). The intestinal loops were then carefully flushed with 0.9% saline to remove any residual cells from the lumen and incubated another 20 min for the isolation of enriched crypt cells. Any mechanical manipulation of the tissue was avoided so that the loops containing the cells in Buffer B were cautiously decanted and the cells were pelleted in plastic tubes at 500 *g*. Finally, they were washed three times in homogenization buffer. Cell viability was checked by staining with 0.5% trypan blue solution. Cell fractions with at least 95% exclusion of the dye were homogenized in hypotonic medium (1.5 mM Tris, 10 mM $MgCl_2$, pH 7.4) with a tight fitting pestle.

Assays

HMGR was determined according to Shapiro et al. (20), with minor modifications. The incubation solution contained 1.0 M potassium phosphate buffer, 1.0 M dithiothreitol, 10 mg/ml serum albumin, 0.5 M glucose-6-phosphate, 0.1 M NADP, 1 M EDTA, and 1 mg/ml glucose-6-phosphate dehydrogenase in a total volume of 0.18 ml, to which 0.07 ml of the respective protein solution was added. After a preincubation period of 5 min, the reaction was started by the addition of 20 nmol DL-[3-^{14}C]HMG-CoA in 0.05 ml. The reaction was linear for 30 min, both for liver microsomes and crypt and villous cells homogenates, and was proportional to the amount of protein up to 0.5 mg and 1.0 mg, respectively.

Under these conditions the enzyme was saturated with substrate. After an incubation period of 20 min in liver microsomes and 30 min in mucosal cell homogenates at 37°C, the reaction was terminated by the addition of 0.1 ml 12N H_2SO_4. Finally, mevalonolactone and HMG were added to give a concentration of 1.0 M and 0.1 M, respectively, in a volume of 0.1 ml. DL-[5-^{3}H]Mevalonic acid (50,000 cpm/assay) was used as internal standard, and was consistently recovered at rates exceeding 92%.

The incubation tubes were then transferred to a water bath at 50°C. After 15 min, they were put on ice to precipitate the protein and centrifuged at 3,000 *g*. Clear supernatant (0.15 ml) was applied to activated silica 60G glass plates (20 × 20 cm) that were developed for 1 hr in benzene–acetone 1:1 (v/v). The region of R_f 0.5–0.7 was removed with a razor blade and scraped into an Instagel scintillation mixture. Scintillation spectrometry was performed in a Nuclear Chicago Counter. In pilot experiments, carried out to compare trypsin inhibition of villous reductase in scraped mucosa versus dual buffer crypt and villous isolates, trypsin inhibitor in a concentration of 0.2 mg/ml increased the HMGR about 3-fold in villous homogenate of scraped mucosa. However, no such effect could be observed on reductase of cells isolated by the dual buffer technique.

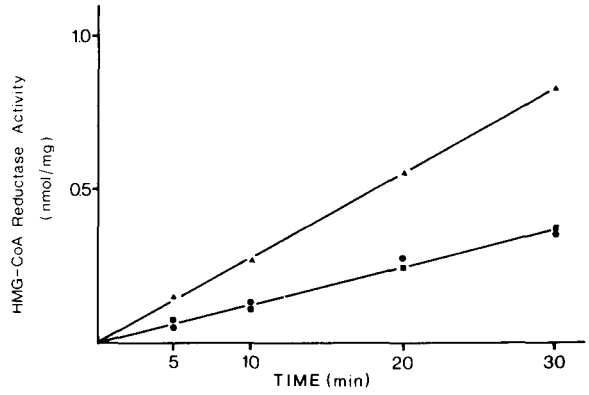

Fig. 1. Kinetic studies in rabbit liver microsomes (▲) and crypt (■) and villous cell (●) homogenate of jejunum. Both were isolated at 9 AM. Each point represents the mean of replicate determinations. The amount of protein incubated was 0.5 mg.

Alkaline phosphatase and cholesterol (21) were assayed with Boehringer-Mannheim enzyme kits. Thymidine kinase was determined as described by Klemperer and Hayes (22), with the exception that the product was isolated using DEAE-impregnated paper discs (Whatman DE-81) according to the method of Breitman (23). Statistical analyses were performed by the Student *t*-test.

RESULTS

Enzyme kinetics of rabbit HMGR

The reaction rate of HMGR in liver microsomes and mucosal cell homogenates was linear with time during an incubation period of 30 min (**Fig. 1**). Similar linearity was observed over ranges of protein concentration from 0 to 0.5 mg in liver microsomes, and from 0 to 1.0 mg in mucosal cell homogenates (**Fig. 2**). Substrate saturation was achieved with an HMG-CoA concentration above 70 μM, which corresponded to a K_M value of 3.0 μM in liver microsomes. In mucosal cell homogenates of jejunum, isolated by the dual buffer technique (**Fig. 3**), the K_M value was 4.2 and 4.6 μM in crypt and villous cells, respectively. Similar kinetic data were obtained in ileal cells, the respective K_M values being 4.5 and 4.3 μM, respectively.

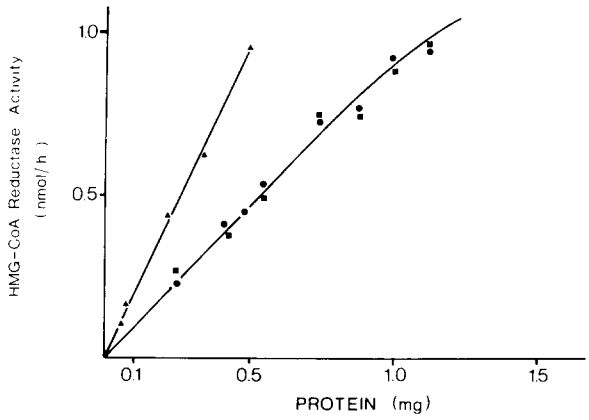

Fig. 2. Kinetic studies in rabbit liver microsomes (▲) and crypt (●) and villous cell (■) homogenate of jejunum. Incubations were with different amounts of protein for 30 min.

Validation of the product [^{14}C]mevalonic acid formed by HMGR

Homogenate from rabbit intestinal epithelial cells converts ^{14}C-labeled HMG-CoA to a ^{14}C-labeled product which co-migrates with [^{3}H]mevalonolactone during chromatography in a neutral TLC system (**Fig. 4A**) and with [^{3}H]mevalonolactone in an acidic TLC system (Fig. 4D).

The formation of this product requires the presence of NADPH. Less than 2% of the ^{14}C-dpm in this region occurred in the absence of the NADPH-generating system. Also after the conversion of the ^{14}C-labeled product and [^{3}H]mevalonolactone into mevalonic acid (Fig. 4B) or into a mixture of mevalonate and mevalonamide (Fig. 4C), and its rechromatography in an alkaline TLC system, as described by Young and Rodwell (24), there was a coincidence of the ^{14}C- and ^{3}H-profiles. We conclude from these data, that the ^{14}C-labeled product is [^{14}C]mevalonic acid formed by HMG-CoA reductase.

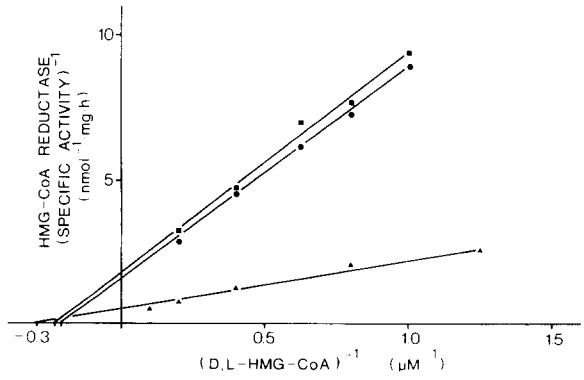

Fig. 3. Kinetic studies in rabbit liver microsomes (▲) and crypt (●) and villous cell (■) homogenate of jejunum. The assay utilized 0.5 mg protein in a volume of 1 ml, incubation was for 2.5 min at different concentrations of DL-HMG-CoA (27). Under these conditions, substrate utilization amounted to less than 5.2% at the lowest concentrations. Data points represent means of three animals.

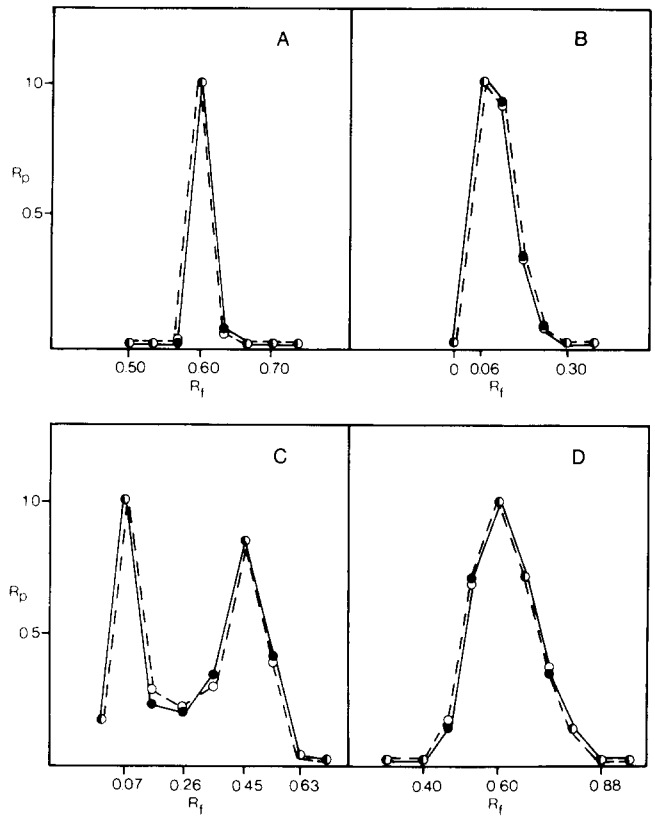

Fig. 4. Validation of the product of ^{14}C-labeled HMG-CoA obtained by incubation with mixed crypt and villous cell homogenates from jejunum and ileum under standard assay conditions. (Identical results were obtained from hepatic microsomes using TLC.) Panel A: Chromatography of [^{3}H]mevalonolactone (●) and the ^{14}C-labeled product (○) on silica gel TLC with the usual solvent (benzene–acetone 1:1 (v/v). Radioactivity of 1-cm sections are depicted relative to peak activity (R_p). Panel B: R_f 0.5–0.7 from panel A was eluted with acetone and methanol, dried under N_2, and incubated at 37°C for 15 min in 1.0 N NaOH. Subsequent chromatography was on a cellulose sheet developed in butanol–ammonia–water 20:1:1 (v/v/v). Panel C: Identical with panel B, except that the R_f 0.5–0.7 eluate was treated with concentrated ammonia to form mevalonamide and subsequently chromatographed. Panel D: Identical with panel B, except that the R_f 0.5–0.7 eluate was incubated with 2 N H_2SO_4 and chromatographed on silica gel TLC with butanol–propionic acid–water 10:4:1 (v/v/v).

Distribution of marker enzymes in mucosal cell fractions

Separation of mucosal cells was achieved by the dual buffer technique described above. After 40 min of incubation in Buffer B, a sharp rise in thymidine kinase activity was observed, a crypt marker (**Fig. 5**). Simultaneously, alkaline phosphatase activity, a villous cell marker, declined rapidly. Consequently, we considered that the 0–40-min fraction contained en-

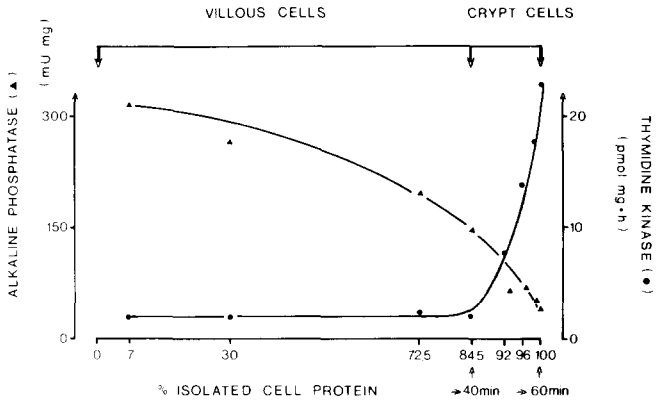

Fig. 5. Homogenates of intestinal epithelial cells (isolated by the dual buffer technique and homogenized in hypotonic medium) were incubated for different periods of time and assayed for alkaline phosphatase and thymidine kinase. Points represent means of replicate experiments. The 0–40-min fraction was designated "enriched villous cells" and the 40–60-min fraction was designated "enriched crypt cells".

riched villous cells, and that the 40–60 min fraction contained predominantly crypt cells. Cell recovery and viability of subsequent fractions decreased steadily. Approximately 85% of total recovered cell protein was found in the villous cell fraction, the remaining 15% in crypts.

The thymidine kinase specific activity was 7.5-fold higher in crypt cells than in villous cells, whereas alkaline phosphatase specific activity was 4.1-fold higher in villous cells. Both enzymes were measured regularly in all cell fractions.

TABLE 1. Distribution of HMG-CoA reductase in jejunal villous and crypt subcellular fractions

Fraction	Villi		Crypts	
	Specific Activity (nmol/mg/hr)	% (Total Activity)	Specific Activity (nmol/mg/hr)	% (Total Activity)
Homogenate	0.84	100	0.81	100
Nuclei	0.79	32	1.16	76
Mitochondria	0.71	15	1.40	15
Microsomes	0.23	0	0.26	0
Cytosol	0.09	2	0.40	0
Total recovery		49		91

Total activity was calculated from both protein concentrations and volume of respective cell fractions.

Distribution of HMGR in villous and crypt cells of jejunum and ileum

In subcellular fractions of jejunal mucosa, most reductase activity was found in the nuclear and mitochondrial fractions of both villous and crypt cells (**Table 1**). Negligible activity was detected in microsomes and cytosol. Expressed in percent of total activity, most of the HMGR activity was in the nuclear fraction, and some activity in mitochondria. Enzyme recovery from crypt cells was excellent, while that from villous cells was not as good. The reasons for this are not clear.

HMGR specific activities were identical in enriched villous and crypt cell fractions of jejunal mucosa (**Table 2**). However, in the ileum, the specific

TABLE 2. Effect of cholesterol, saturated and polyunsaturated fat on intestinal HMG-CoA reductase activity

Dietary Periods	N	Jejunum		Ileum	
		Villi	Crypt	Villi	Crypt
		(nmol/mg/hr)			
I. Normal diet	5	0.86[a] (0.22)	0.86[a] (0.33)	0.40[d] (0.18)	0.26[d] (0.08)
II. 1% Cholesterol for 2 days	5	0.22 (0.10)	0.24[b] (0.06)	0.22[e] (0.09)	0.18 (0.11)
III. 1% Cholesterol for 14 days	5	0.19 (0.08)	0.15 (0.03)	0.07 (0.02)	0.09 (0.01)
IV. 1% Cholesterol + 5% coconut oil for 2 days	5	0.15 (0.04)	0.11[c] (0.03)	0.12 (0.04)	0.13 (0.02)
V. 1% Cholesterol + 5% corn oil for 2 days	5	0.22 (0.08)	0.20 (0.07)	0.07 (0.04)	0.15 (0.07)

[a] $P < 0.005$ vs. II, III, IV and V.
[b] $P < 0.05$ vs. III and I.
[c] $P < 0.05$ vs. V.
[d] $P < 0.01$ vs. III, $P < 0.05$ vs. IV and V.
[e] $P < 0.05$ vs. IV and V, $P < 0.01$ vs. III.

Villous and crypt cell fractions from jejunum and ileum. HMGR was assayed in fresh homogenate. Numbers represent means, with standard deviation in parentheses.

TABLE 3. Distribution of HMG-CoA reductase activity in the liver

	N	Liver Lobes R	L	M	PL	PS	Mean
		(nmol/mg/hr)					
I. Small rabbits (1.8–2.2 kg)	6	1.92 (0.23)	1.90 (0.50)	2.13 (0.54)	2.16 (0.63)	3.95[a] (0.17)	2.29 (0.42)
II. Large rabbits (3.5–4.0 kg)	6	0.68 (0.20)	0.97 (0.19)	0.79 (0.10)	1.11 (0.62)	0.69 (0.26)	0.85 (0.19)

[a] $P < 0.5$ vs. R, L, M, and PL.

Microsomal homogenates from the right (R), left (L), middle (M), large posterior (PL), and small posterior (PS) lobes.

enzyme activity in villous cells was only 47% of that of corresponding cells in the jejunum, and ileal crypt activity only 30% of that in jejunal crypt cells. If recovery of cell protein in the two mucosal fractions is considered, villous cells account for 85% of total mucosal enzyme activity in the jejunum and for 94% in the ileum.

Influence of dietary lipid on intestinal HMGR activity

Addition of 1% cholesterol to regular rabbit chow reduced the enzyme activity after 2 days by an average of 73% in jejunal mucosa (n = 5, Table 2). This suppression effect was similar in villi and crypts. In the ileum, enzyme activity decreased as well, but to a lesser extent. The average reduction was 45% in villous and 31% in crypt cells.

Feeding of 1% cholesterol for 14 days (n = 5) resulted in further decrease of reductase activity in jejunal and ileal mucosa. In villous cells of ileum, the degree of suppression of enzyme activity was now similar to that in the jejunum, whereas it was less pronounced in crypt cells.

Addition of 5% coconut oil to a 1% cholesterol diet (n = 5) decreased reductase activity in jejunal villous and crypt cells, to an extent comparable after cholesterol feeding for 14 days. This effect was not so pronounced in ileal mucosa. By contrast, the addition of 5% corn oil to 1% cholesterol (n = 5) did not have an additional suppressive effect on reductase in villous or in crypt cells of jejunal mucosa. However, it decreased reductase activity in villous cells of ileum comparable to the levels induced by 14 days of cholesterol feeding. Coconut oil had an additional effect on jejunal crypt cell reductase.

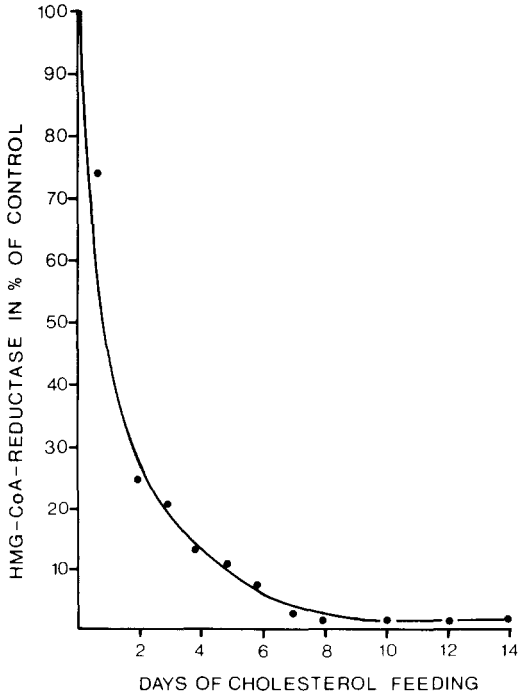

Fig. 6. HMGR in microsomes of rabbit liver in relation to days of supplementary feeding of 1% cholesterol.

Distribution of HMGR in the liver

In small rabbits, HMGR activity varied widely between the five liver lobes (n = 6, **Table 3**). Activity was highest in the small posterior lobe, followed by the large posterior and the middle lobes. However, the mean values of HMGR activity in all lobes approximated 2 nmol/mg/hr.

In the larger animals (n = 6), reductase activity was not as high and it was more evenly distributed between the lobes, with the exception of the large posterior lobe, which contained the highest activity.

Influence of dietary lipid on HMGR in the liver

Feeding a diet supplemented by 1% cholesterol decreased hepatic reductase by about 25% after 24 hr, and by about 90% after 6 days (**Fig. 6**). From the eighth day onward, hepatic reductase activity was virtually undetectable. This contrasts sharply with the situation in the small intestine (Table 2), where measurable activity was still present after 14 days on

TABLE 4. Effect of cholesterol, saturated and polyunsaturated fat on hepatic HMG-CoA reductase activity

	N	Mean	Liver Lobes R	L	M	PL	PS	Plasma Cholesterol mmol/l
			nmol/mg/hr					
I. Normal (1.8–2.2 kg)	6	2.29[a] (0.42)	1.92 (0.23)	1.90 (0.50)	2.13 (0.54)	2.16 (0.63)	3.95 (0.17)	1.92 (0.44)
II. 1% Cholesterol for 2 days	6	0.39[b] (0.15)	0.36 (0.09)	0.28 (0.10)	0.42 (0.19)	0.44 (0.20)	0.47 (0.23)	5.44 (1.61)
III. 1% Cholesterol for 14 days	6	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	
IV. 1% Cholesterol + 5% coconut oil for 2 days	6	0.19 (0.08)	0.19 (0.04)	0.19 (0.08)	0.17 (0.04)	0.19 (0.10)	0.21 (0.08)	5.83 (1.50)
V. 1% Cholesterol + 5% corn oil for 2 days	6	0.22 (0.10)	0.19 (0.05)	0.19 (0.06)	0.20 (0.06)	0.24 (0.11)	0.32 (0.29)	4.38 (2.16)

[a] $P < 0.005$ vs. II, III, IV, and V.
[b] $P < 0.05$ vs. IV and V.
Microsomal homogenates from the right (R), left (L), middle (M), large posterior (PL), and small posterior (PS) lobes. Numbers represent means, with standard deviation in parentheses.

the same diet. Addition of either 5% coconut or 5% corn oil to 1% cholesterol had a more pronounced effect on hepatic reductase after 2 days, than 1% cholesterol alone (n = 6, **Table 4**).

DISCUSSION

Intestinal HMGR

As in the rat (2), intestinal reductase in the rabbit displays similar kinetic behavior as the hepatic enzyme, as manifested by comparable K_M values. However, in contrast to hepatic reductase, intestinal HMGR was mainly localized in the mitochondrial and nuclear cell fractions, with only negligible activity in microsomes. Our findings are in agreement with the observation of Merchant and Heller (6) in rat intestine. They recovered about 5% of total intestinal reductase in microsomes, and considered this the result of mucus-induced cross-contamination between subcellular fractions.

As with Merchant and Heller (6) and Panini et al. (25), we therefore had to use the Weiser (19) cell homogenates as enzyme source. To obviate problems with enzymatic activities competing for the substrate HMG-CoA, we carefully validated the reaction product and preferred TLC for its isolation, as recommended by Ness and Moffler (26).

Since "HMG-CoA utilizing activity" leads to an overestimation of the apparent K_M value (26), the low values obtained in the crude intestinal cell homogenate and liver microsomes of the rabbit compare favorably with data obtained in rats (6, 27) and reasonably exclude any such interference.

The application of the dual buffer techniques, rather than the use of the gradual scraping technique, allows a more precise assessment of vertical distribution (villous versus crypt cells) of reductase activity in intestinal mucosa. The dual buffer technique is probably superior to scraping or other techniques for the isolation of villous and crypt cell fraction (6, 14). Sugano et al. (15) entertained that low HMGR activities in villous cells were caused by impairment of reductase by proteases liberated by scraping. Such inhibition was prevented by trypsin inhibitors. Therefore, we checked the possibility of whether or not reductase of cells isolated by the dual buffer technique was sensitive to proteases as well. However, like Panini et al. (25), we were unable to stimulate reductase activities in villous or crypt cells.

In the small intestine of the rabbit, HMGR activity predominates in the jejunum, and is considerably lower in the ileum. In both jejunal and ileal mucosa, most of the reductase activity is located in villous cells. As a matter of fact, villous cell reductase is about 6-fold higher than crypt reductase. This is in accordance with the work of Merchant and Heller (6), who demonstrated a similar distribution of HMGR in rat intestine.

Crypt reductase is believed to serve as provider of structural cholesterol for cell regeneration. By contrast, villous cell cholesterol is primarily used for lipoprotein synthesis. Both systems probably utilize exogenous and endogenous cholesterol to accomplish this task.

Little information is available in the accessible literature concerning factors regulating villous cell reductase. Due to methodological difficulties, most

authors concentrated on crypt cells (12) or used tissue slices of whole intestine (28). The results presented in this study provide suggestive evidence that dietary exogenous cholesterol exerts a feedback inhibition of HMGR activity in villous and crypt cells of jejunum and ileum. In a recent study in the rat, it was reported that both villous and crypt HMGR respond to the dietary state and to cholestyramine, which blocks the enterohepatic circulation of bile acids (25).

Since the rabbit does not respond to cholesterol feeding with an increase in bile acid synthesis (16), such as rat and guinea pig (11, 13), experimental evidence provided by the present study suggests that cholesterol, rather than bile acid, is the principal regulating agent in rabbit intestinal HMGR. A further decrease of intestinal reductase by coconut fat is consistent with the view that the effect of saturated fat on the absorption of dietary cholesterol is greater than that of unsaturated fat (29). This effect lends further support to the role of cholesterol as a feedback inhibitor. Bile acid kinetics in the rabbit remain uninfluenced by dietary fat as well (30).

In the rat, the effect on intestinal HMGR of dietary saturated and unsaturated fatty acids with cholesterol, suggests that the latter is the regulator of this enzyme (31). However, a direct comparison with our data in the rabbit is difficult, since no data are given on the effect of cholesterol alone.

Finally, our results, obtained in vivo, are in agreement with recent findings by Gebhard and Cooper (32), who demonstrated suppression of intestinal HMGR by exogenous cholesterol in an in vitro organ culture system of dog mucosa. Preliminary results obtained in our laboratory with rabbit mucosa organ cultures (33) confirm this observation.

During prolonged feeding of cholesterol, hepatic (see below) but not intestinal reductase is completely suppressed. The main site of residual cholesterol synthesis in the rabbit is now shifted to the intestine. Similar findings have been obtained in the rat, the guinea pig, and the squirrel monkey (9, 12, 34). It has been suggested (35, 36), that intestinal cholesterol incorporated in intestinal lipoproteins may contribute to the circulating pool. It has not been substantiated, however, whether or not cholesterol synthesis in enterocytes plays a role in experimental and, possibly, human (37) atherosclerosis.

Hepatic HMGR

Rabbit hepatic HMGR has a much lower activity than the corresponding enzyme in the rat, confirming recent results of Kovanen, Goldstein, and Brown (38). However, these authors did not comment on kinetic properties of hepatic reductase. The K_M values obtained in this study, e.g., 3.0 μM in the liver and 4.2 and 4.6 μM in the intestinal crypt and villous cells, respectively, are similar to corresponding tissues in the rat (6, 27). Surprisingly, we did not observe a diurnal rhythm with peak activities at 12 PM, contrasting the fluctuations reported in rats (3, 4) and hamsters (5). Our findings are preliminary, because our data do not represent nadir activities and we cannot exclude minor oscillations.

An interesting finding was the somewhat unhomogeneous distribution of HMGR in different liver lobes, particularly in the smaller animals. The uneven distribution of reductase in rabbit liver calls for either the homogenization of the whole liver or of each different liver lobe separately. A single lobe or piece is not representative of the whole organ and that, consequently, is conducive to errors in experimental work.

In comparison to the rat, where hepatic reductase activity is suppressed by 88% within 24 hr after dietary cholesterol supplementation (39), this decline is less precipitous in the rabbit. Cholesterol-induced hepatic reductase suppression comparable to that observed in rats takes 5 days in the rabbit, but it is complete after 8 days.

Addition of saturated or polyunsaturated fat to the cholesterol diets further suppressed hepatic reductase activity. These findings may be interpreted on the basis of the "cholesterol vehicle" function of these fats, which may augment intestinal absorption of cholesterol (29, 40). This is reflected in respective plasma cholesterol values.

Bochenek and Rodgers (41) showed that, in rats, unsaturated fat inhibited hepatic reductase more than saturated fat in the presence of high cholesterol diets. This effect correlated with a high sterol excretion in the saturated fat group, but such a mechanism has not been demonstrated in the rabbit. The rapid increase in plasma cholesterol level after initiation of a cholesterol diet probably reflects the appearance of cholesterol-rich lipoproteins of intestinal origin (42), which differ in structure (17, 43) and metabolism (44) from their normal counterparts. These lipoproteins of cholesterol-fed animals have a strong suppressor effect on hepatic reductase activity of cultured primary liver cells (45). Intestinal lipoproteins mediate a regulatory signal to hepatic reductase in the normal animal as well (12, 46–48). Hence, it seems reasonable to assume that a decrease of hepatic reductase can be effected by lipoprotein-cholesterol in the rabbit as well. Further, structural alterations of these particles, induced by saturated and polyunsaturated fat, may influence such an effect (17). It is of interest that dietary fat alone (without

cholesterol) such as corn oil (49) or other fats (50) increases rather than decreases hepatic cholesterol synthesis in the rat, possibly due to a higher lipoprotein secretion as suggested by Goh and Heimberg (51).

In summary, our data provide suggestive evidence that, in the rabbit, HMGR of enterocytes and hepatocytes has characteristic kinetic properties similar to the rat enzyme, and is sensitive to dietary cholesterol. The inhibitory effect of cholesterol was enhanced by additional saturated as well as unsaturated fat in hepatocytes, but only by saturated fat in enterocytes. After rabbits were fed a diet containing 1% cholesterol for 14 days, the hepatic cholesterogenesis was negligible, whereas the main site of synthesis shifted to intestinal mucosa. The major effective regulatory feedback inhibitor of rabbit intestinal and hepatic reductase is probably cholesterol itself.

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