HMG CoA reductase of intestinal mucosa and liver of the rat

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Abstract Methods were developed for the determination of HMG CoA (3-hydroxy-3-methylglutaryl CoA) reductase activity in subcellular fractions of intestinal mucosa and liver of Wistar strain rats. In the liver, reductase activity was located exclusively in the microsomal fraction. In the intestinal mucosa, activity was found in both mitochondrial and microsomal fractions of crypt cells but not of villi. The microsomal HMG CoA reductases of liver and intestinal mucosa had similar kinetic characteristics and pH optima. However, the activity of the hepatic enzyme differed with age and sex of the experimental animals while that of the intestinal crypt cells did not. Cholestyramine treatment enhanced the activity of the microsomal HMG CoA reductase in both liver and intestinal mucosa. Reductase activity of the intestinal crypt cells was elevated in both jejunum and ileum. The greatest stimulation, both relatively and absolutely, was observed in the distal half of the jejunum.

Supplementary key words regulation · cholesterol biosynthesis · cholestyramine · subcellular fractions · villi · crypt cells

The enzyme 3-hydroxy-3-methylglutaryl CoA reductase (mevalonate:NADP oxidoreductase [acylating CoA], EC 1.1.1.34) is considered to be the rate-limiting enzyme of cholesterol biosynthesis in liver and intestinal mucosa (1, 2). In earlier work hepatic HMG CoA reductase activity was usually studied indirectly by measurement of incorporation of acetate and mevalonate into cholesterol. These studies indicated that the enzyme was inhibited by cholesterol feeding, fasting, and bile acid administration and was stimulated by injection of thyroid hormone and certain detergents (3, 4). The activity has also been shown to undergo a striking diurnal variation which is abolished by cycloheximide and puromycin (5, 6). Recently, many of these results have been confirmed by the direct assay of microsomal HMG CoA reductase of rat liver (5, 7–9). The HMG CoA reductase activity of the intestinal mucosa has not been studied directly. However, the indirect procedure employed by Dietschy et al. indicated that there may be a difference in regulatory mechanisms between the hepatic and intestinal enzyme (10–12). The activity of the latter was reduced by dietary cholesterol and by fasting, but to a considerably smaller extent than the hepatic enzyme. For example, the conversion of acetate to cholesterol by ileal slices was reduced about 50% by a 48-hr fast and about 25% by 6 wk of cholesterol feeding. In the liver, dietary cholesterol produced a 99% inhibition, and fasting a 95% inhibition, of cholesterol biosynthesis. Biliary diversion led to a 3–5-fold enhancement of hepatic as well as intestinal cholesterol biosynthesis (2). However, on the basis of recent studies Weis and Dietschy (13) arrived at the conclusion that cholesterol biosynthesis (i.e., HMG CoA reductase activity) of the intestinal mucosa is largely controlled by the circulating bile acid pool while hepatic cholesterol biosynthesis is regulated by cholesterol undergoing enterolymphatic circulation. Some of these conclusions have recently been challenged by Hamprecht et al., who observed that bile acids reduced hepatic HMG CoA reductase activity in rats with lymphatic fistulas (in which cholesterol presumably did not reach the liver) (14, 15).

In order to examine the problem of the regulation of hepatic and intestinal HMG CoA reductase in greater detail, methods for the study of intestinal HMG CoA reductase were needed. This paper describes the properties

Abbreviations: HMG, 3-hydroxy-3-methylglutaric acid or 3-hydroxy-3-methylglutaryl; TLC, thin-layer chromatography; GLC, gas–liquid chromatography.

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of microsomal HMG CoA reductase of liver and intestinal mucosa *in the same animal* and illustrates the effect of cholestyramine administration on the activity of the two enzymes.

EXPERIMENTAL PROCEDURES

Animals and diets

Male rats of the Wistar strain, obtained from the Otisville Laboratories of the New York City Health Department, were used for most of the experiments. The stock diet consisted of ground Purina rat chow pellets, fed ad lib. In the experiments with cholestyramine-treated food, 5 g of the ion exchange resin was mixed thoroughly with 95 g of the stock diet. Cholestyramine (Questran, Mead Johnson & Co., Evansville, Ind.) is a quaternary ammonium anion exchange resin which binds bile salts at the pH of the small intestine.

Preparation of substrate

R,S-[3-^{14}C]HMG CoA was prepared from [3-^{14}C]-HMG and CoASH as described previously (16, 17). The specific radioactivity was 1371 dpm/nmole.

Preparation of microsomes

a) *Liver*. The rats were killed by cervical dislocation, and the livers were removed immediately and chilled on ice. All subsequent operations were carried out at 0–5°C. Each entire liver was extruded through a tissue press (Harvard Apparatus Co., Millis, Mass.). A 1-g aliquot was then homogenized in a loose-fitting Potter-Elvehjem homogenizer (0.5 mm radial clearance) with 9 ml of a solution containing sucrose, 300 mM, nicotinamide, 75 mM, neutralized EDTA, 2.5 mM, and neutralized reduced glutathione, 25 mM. The homogenate was centrifuged at 800 *g* for 10 min, and nuclei and cell debris were discarded. The supernatant solution was centrifuged at 6700 *g* for 10 min to sediment the mitochondrial fraction. The supernatant solution was centrifuged for 10 min at 9500 *g* and the precipitate was discarded. The microsomal fraction was then sedimented at 100,000 *g* for 60 min. When care was taken to preserve the integrity of the mitochondria, appreciable HMG CoA lyase (EC 4.1.3.4) activity was not detectable in the 9500 *g* supernatant solution. Consequently, the microsomes did not require washing for routine assays. The protein concentrations of the fractions were determined with Folin phenol reagent (18).

b) *Intestinal mucosa*. The small intestine was removed and washed with cold saline. All subsequent operations were carried out at 0–5°C. The intestine was cut along its entire length to expose the mucosa. Preparations of crypt cells and villi were made as described by Dietschy and Siperstein (10). Subcellular fractionations of these cell fractions were carried out as described above for the liver, with two exceptions. A 5% homogenate was prepared from the mucosal cell fractions. Secondly, after the first centrifugation of the crypt cell homogenate at 800 *g* for 10 min, a fluffy layer was found at the top of the tube which was removed with a Pasteur pipette and discarded.

Electron microscopy of subcellular fractions obtained from intestinal mucosa

The mitochondrial and microsomal pellets obtained after ultracentrifugation were fixed for 5 min in 2% glutaraldehyde (19), washed with 0.1 M phosphate buffer, pH 7.0, and postfixed in 1% osmium tetroxide for 2 hr (19, 20). The pellets were dehydrated in graded ethanols, and the cellulose nitrate tubes containing the pellets were dissolved in propylene oxide. After standing overnight in a mixture of propylene oxide–epoxy resin (1:1), the propylene oxide was removed by evaporation, and small fragments of the top, middle, and bottom of each pellet were dissected out. These fragments were properly oriented and embedded in epoxy resin (21) at 60°C for 48 hr. Sections were cut with an LKB microtome using diamond knives, doubly stained with uranyl acetate and lead citrate, and examined in a Philips 300 electron microscope. Electron micrographs were taken at original magnifications ranging from 11,000 to 27,000 and were further enlarged photographically, as indicated (Figs. 1 and 2).

Assay of HMG CoA reductase activity

Liver and intestine. The complete assay system was in a volume of 0.8 ml: phosphate buffer, pH 7.2, 100 mM; $MgCl_2$, 3 mM; NADP, 3 mM; glucose-6-phosphate, 10 mM; glucose-6-phosphate dehydrogenase (EC 1.1.1.49), 2.5 enzyme units; reduced glutathione, 50 mM; R,S-[3-^{14}C]HMG CoA, 0.2 mM; and microsomal protein, 0.2–0.5 mg. The complete system was placed in 15-ml culture tubes and shaken in air at 37°C for 30 min. At the end of this period, 3 mg of unlabeled mevalonolactone was added to each tube and the reaction was stopped immediately by the addition of 0.4 ml of 1 N H_2SO_4. The tubes were then shaken at 37°C for an additional 30 min to ensure lactonization of the biosynthetic mevalonate at pH < 1 (5). The tubes were cooled to room temperature, and 0.5 ml of absolute ethanol and 1 g of anhydrous Na_2SO_4 were added to each tube. The solutions were then extracted four times with 2 ml of peroxide-free ethyl ether, shaking for 5 min each time (Vortex mixer with shaker head). The combined ether extracts were dried over Na_2SO_4 and evaporated under a stream of air at 25°C. An aliquot from each tube, in acetone, was applied as a streak 3 cm long to commercially available silica gel G plates, layer thickness 0.25 mm (Brinkmann

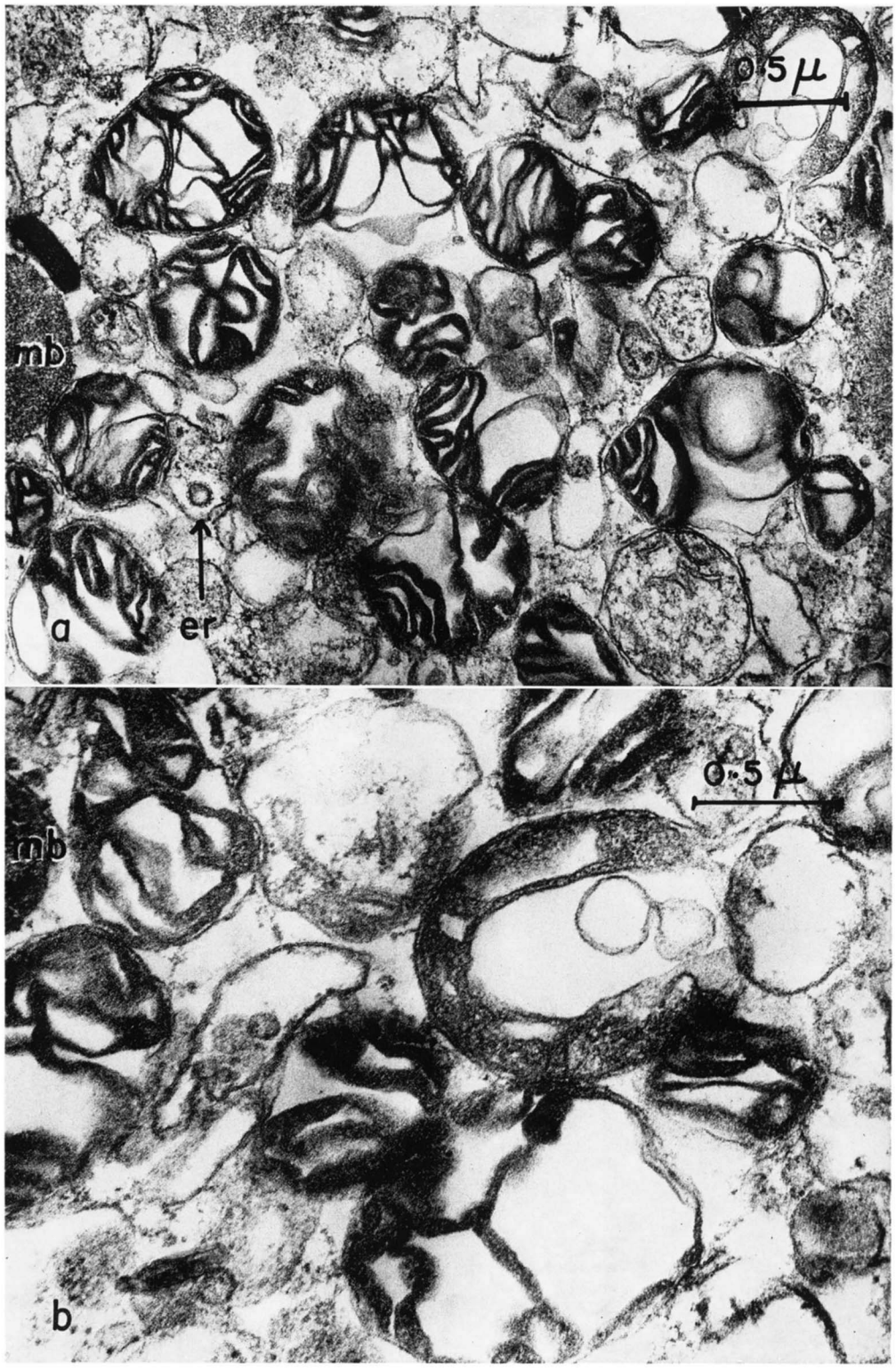

FIG. 1. Typical sections through the center of the mitochondrial pellet. The mitochondria display a condensed conformation and the inner membranes are irregularly folded. Inclusions of granular microbodies (*mb*) are noted. A vesicular derivative of endoplasmic reticulum (*er*) is shown in *a*. Additional membrane vesicles (not shown here) were observed near the top of the mitochondrial pellet. Magnification: *a*, × 33,000; *b*, × 55,000.

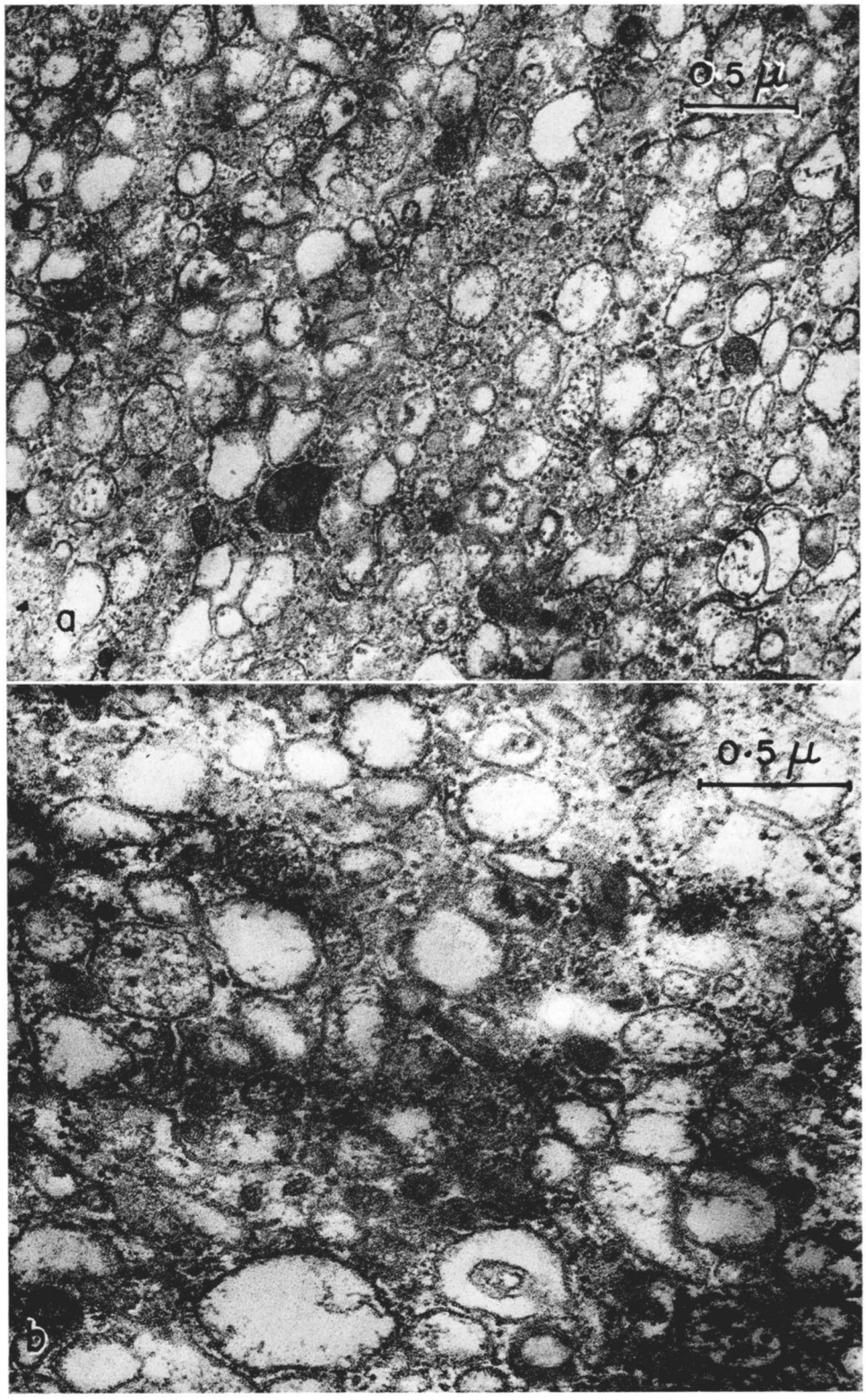

FIG. 2. Typical sections through the center of the microsomal pellet. Both plates show some membrane vesicles studded with ribosomes: There were no identifiable mitochondrial structures. *a*, × 33,000; *b*, × 55,000.

Instruments, Westbury, N.Y.). The solvent system for TLC was acetone–benzene 1:1 (v/v) (9). The plates were air-dried and sprayed with a 0.2% (w/v) solution of 2′,7′-dichlorofluorescein in methanol. The position of the mevalonolactone band was marked under near ultraviolet illumination (366 nm). The following R_F values were observed: mevalonolactone, 0.7; HMG, 0.0; cholesterol and fatty acids, 0.9–0.95. The mevalonolactone band was removed from the plate by means of a suction device and its radioactivity was determined in a liquid scintillation counter (Beckman LS-200B), with suitable corrections for background and quenching. In each experiment a standard sample of R,S-[2-^{14}C]-mevalonolactone was carried through the entire procedure to correct for losses during extraction and TLC. On the average, recoveries ranged from 80 to 90%, and the results were corrected accordingly. Since the specific radioactivity of the substrate was known, the radioactivity data could be expressed in terms of nmoles of HMG CoA converted to mevalonolactone. All enzyme assays were carried out on two or three samples of tissue per animal.

GLC

In the identification experiments (see Results section), the biosynthetic mevalonolactone was quantitated by GLC, using 180 cm × 4 mm glass columns packed with 3% QF-1 at a column temperature of 160°C. Retention times of mevalonolactone averaged 3.7 min.

RESULTS

Identification of biosynthetic mevalonolactone

a) From incubation experiments with the microsomal fraction of mucosal crypt cells. The assay system for HMG CoA reductase described in the Experimental Procedures section was scaled up about 10-fold, and the labeled mevalonolactone formed (plus unlabeled carrier) was extracted and separated by TLC. The standard procedure was followed except that preparative TLC plates (layer thickness 1.5–2 mm) were used. The mevalonolactone band was eluted from the silica gel with acetone, and the specific radioactivity of the lactone was determined by scintillation counting and GLC. The labeled mevalonolactone was then subjected to column partition chromatography, with 0.1 N HCl as the stationary phase and $CHCl_3$ as the mobile phase (22). The specific radioactivity of the biosynthetic mevalonolactone remained constant throughout the mevalonolactone band, 157 ± 3 (mean ± SE) dpm/μmole (Fig. 3*A*). All column fractions containing mevalonolactone were combined and reacted with diphenylmethylamine to give the crystalline derivative [3-^{14}C]mevalonoaminodiphenylmethane (23). The specific radioactivity of the derivative remained

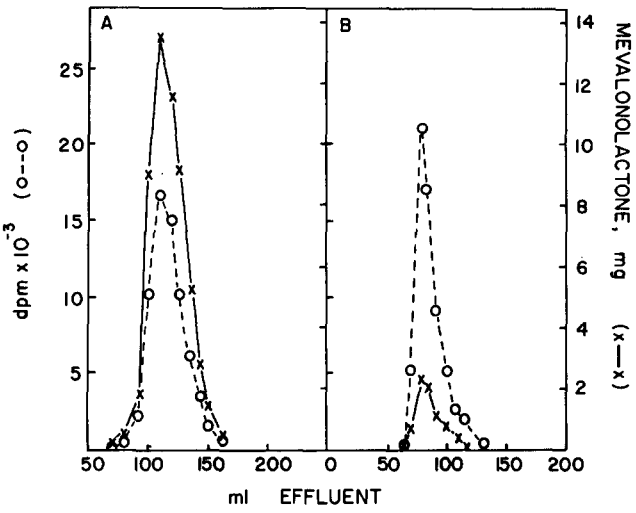

FIG. 3. Column partition chromatography of biosynthetic [3-^{14}C]mevalonolactone obtained by large-scale incubation of microsomes with [3-^{14}C]HMG CoA followed by preparative TLC. Partition columns, 30 × 1 cm, were packed with 0.1 N HCl on Chromosorb W as the stationary phase and were eluted with $CHCl_3$. Column fractions were assayed for mevalonolactone by GLC and for ^{14}C by scintillation counting. Biosynthetic mevalonolactone was obtained from intestinal crypt cell microsomes (*A*) and liver microsomes (*B*). Average specific activity of mevalonolactone (dpm/μmole) from (*A*) and (*B*) was 157 ± 3 SEM and 873 ± 9 SEM, respectively. O- - -O, dpm × 10^{-3}; ×——×, mevalonolactone, mg.

constant during two recrystallizations from benzene–hexane mixtures. These data are summarized in Table 1.

b) From incubation experiments with liver microsomes. The identity and radioactive purity of [3-^{14}C]mevalonolactone obtained from large-scale incubation of [3-^{14}C]-HMG CoA with liver microsomes were confirmed in an analogous manner (Fig. 3*B* and Table 1).

During these procedures, the specific radioactivities of the biosynthetic mevalonolactone from both tissues re-

TABLE 1. Identification of biosynthetic mevalonolactone obtained from incubation of hepatic and intestinal crypt cell microsomes with [3-^{14}C]HMG CoA

	Crypt Cell Microsomes	Liver Microsomes
	dpm/μmole	
[3-^{14}C]Mevalonolactone from preparative TLC[a]	163	873
[3-^{14}C]Mevalonolactone from Celite column	157	898
After addition of carrier	157[b]	175
[3-^{14}C]Mevalonoaminodiphenylmethane 1st crystallization	170	172
2nd crystallization	169	178

[a] The biosynthetic [3-^{14}C]mevalonolactone obtained from large-scale incubation experiments of microsomes with [3-^{14}C]HMG CoA was separated from the incubation mixture by ether extraction followed by preparative TLC. Subsequent purification steps were carried out as described in the Results section.

[b] No carrier added.

mained constant within the precision of measurement (± 5%), thus confirming the identity and radioactive purity of the biosynthetic material.

Intracellular localization of HMG CoA reductase activity

A 10% homogenate of liver and a 5% homogenate of intestinal crypt cells were prepared from the tissues of a cholestyramine-treated rat. The homogenates were separated by ultracentrifugation into mitochrondria, microsomes, and final supernatant solution. In the liver, HMG CoA reductase activity was present predominantly in the microsomal fraction. In the intestinal crypt cells the enzyme appeared to be distributed between mitochondria and microsomes (Table 2).

Cellular localization of intestinal HMG CoA reductase

The intestinal mucosa was separated into villi and crypt cells by successive scrapings, and the cellular fractions were examined histologically to evaluate the efficiency of the scraping technique (10). Homogenates were prepared from the two cell populations and were fractionated ultracentrifugally as illustrated in Table 3. HMG CoA reductase activity of the villi fractions was very low, amounting to approximately 10% of the activities observed with the particulate fractions of crypt cells.

TABLE 2. Intracellular localization of HMG CoA reductase[a]

Fraction	HMG CoA Reductase Activity	
	Intestinal Crypts[b]	Liver
	nmoles/mg protein/min	
Nuclei-free homogenate	0.150	0.104
Mitochondria (unwashed)	0.302[c]	0.011
Mitochondria (1× washed)	0.306	
Mitochondria (2× washed)	0.290	
Microsomes (unwashed)	0.312	0.550
Microsomes (1× washed)	0.309	0.560
Microsomes[d] (2× washed)	0.303	0.542
Mitochondria + microsomes (2× washed)[e]	0.300	0.281[f]
Final supernatant solution	0.001	0.002

[a] Tissue fractions were prepared from livers and intestinal mucosa of male Wistar rats pretreated with 5% dietary cholestyramine for 7 days.

[b] Subcellular fractions were prepared from a piece 25 cm long of the terminal ileum.

[c] The formation of mevalonolactone by the mitochondrial fraction was established by reverse isotope dilution experiments of the type described in Fig. 3 and Table 1.

[d] 0.5 mg of protein.

[e] 0.25 mg of protein in each fraction.

[f] The specific activity of the intestinal microsomal enzyme was not decreased by the addition of intact intestinal mitochondria. This was to be expected, since microsomal and mitochondrial reductases had almost identical specific activities. In contrast, the hepatic mitochondrial enzyme had very little activity, so that the specific activity of the hepatic microsomal enzyme was lowered by addition of the inactive mitochondrial preparation. This does not indicate an inhibitory effect of the mitochondria.

TABLE 3. Distribution of HMG CoA reductase activity between intestinal crypts and villi[a]

Subcellular Fraction	Mevalonolactone Formed	
	Crypts	Villi
	nmoles/mg protein/min	
Nuclei-free supernatant solution	0.062 (0.052–0.068)[b]	0.004 (0.003–0.004)
Mitochondria	0.121 (0.101–0.145)	0.009 (0.008–0.013)
Microsomes	0.130 (0.125–0.163)	0.012 (0.009–0.015)
Final supernatant solution	0.002 (0.001–0.003)	0.001 (0.001–0.002)

[a] Each value represents the average of four experiments with rats on stock diet.

[b] Range.

Properties of the microsomal assay systems

The relationship between reaction rate and enzyme concentration is illustrated in Fig. 4. Proportionality was observed when the protein concentration ranged from 0 to 1 mg/ml for liver microsomes and from 0 to 2 mg/ml for crypt cell microsomes. In the standard assay system, containing 0.3–0.5 mg/ml of protein, the rate of reduction of HMG CoA was linear with time during a 45-min period (Fig. 5). The effect of substrate concentration on reaction rate is illustrated in Fig. 6. Under standard conditions the hepatic enzyme was saturated with substrate, 40 μM S-HMG CoA; the crypt microsomes required a substrate concentration of 20 μM for saturation. In the standard assay system a 70 μM concentration of S-HMG

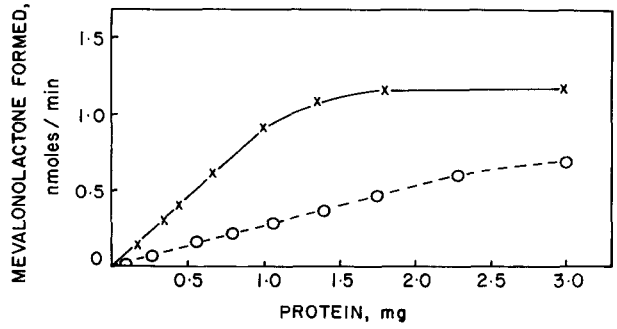

FIG. 4. Effect of increasing amounts of microsomes on the rate of reduction of HMG CoA (cholestyramine-treated rat). Standard assay conditions except for protein concentration. ×——×, liver microsomes; O- - - -O, intestinal crypt cell microsomes.

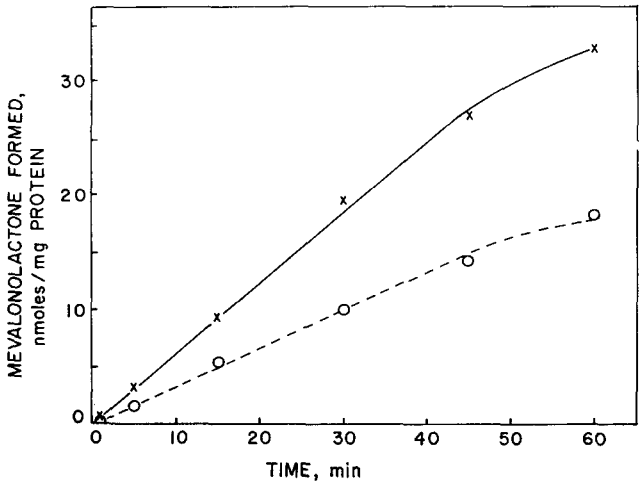

FIG. 5. Time course of enzymatic reduction of HMG CoA (cholestyramine-treated rat). Standard assay conditions except for incubation time. ×——×, liver microsomes; O- - -O, crypt cell microsomes.

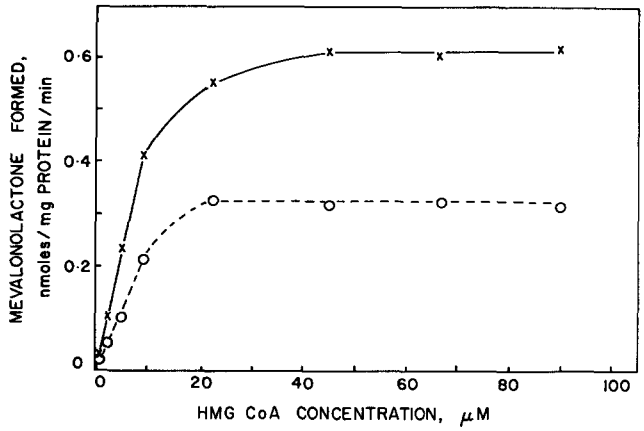

FIG. 6. Effect of substrate concentration on reduction of HMG CoA by liver and crypt cell microsomes (cholestyramine-treated rat). Standard assay conditions except for substrate concentration. ×——×, liver microsomes; O- - -O, crypt cell microsomes.

CoA was chosen to ensure saturation of the enzyme with substrate. Optimal pH values near pH 7.5 were observed with hepatic and mucosal enzymes (Fig. 7). Apparent K_m values obtained by Lineweaver-Burk double reciprocal plots were 6.25 × 10^{-6} moles/l for hepatic microsomes and 7.14 × 10^{-6} moles/l for crypt cell microsomes. Intestinal and hepatic HMG CoA reductase required NADPH as electron donor and was inactive with NADH (Table 4). The microsomal preparations required high concentrations of glutathione or other SH-protecting agents (cysteine, mercaptoethanol) for optimal activity (Table 4). The substrate, R,S-[3-^{14}C]-HMG CoA, as prepared for these studies generally contained 10–15% free [3-^{14}C]HMG and CoASH. Therefore, the standard assay system contained maximally

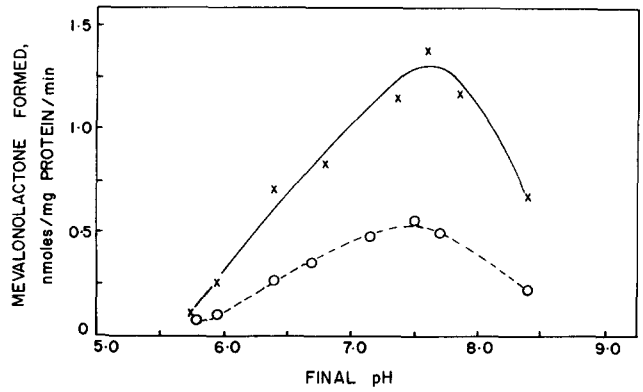

FIG. 7. Effect of pH on microsomal HMG CoA reductase activity (cholestyramine-treated rat). Standard assay conditions except for pH of buffer. Tris buffer was used for pH values above 7.6. ×——×, liver microsomes; O- - -O, crypt cell microsomes.

10 μM [3-^{14}C]HMG and CoASH. The data summarized in Table 5 suggest that at these relatively low concentrations the two impurities had little effect on the reported enzyme activities, since even much higher concentrations were not inhibitory.

HMG CoA reductase activity as a function of age and sex

In male rats, the specific activity of hepatic HMG CoA reductase was greatest in young animals and decreased with age (Table 6). Females exhibited a significantly higher activity than males of approximately the same age. HMG CoA reductase activity per liver was similar in all groups because the larger (older) animals had heavier livers. The specific activity of intestinal HMG CoA reductase was approximately equal in all four groups of rats.

TABLE 4. NADPH and glutathione requirements of microsomal HMG CoA reductase[a]

Cofactors Added	Mevalonolactone Formed	
	Intestinal Crypts	Liver
	nmoles/mg protein/min	
Complete system[b]	0.130	0.150
Complete system minus NADPH-generating system	0.002	0.001
Complete system minus NADPH-generating system plus NADPH (6 μmoles)	0.110	0.130
Complete system minus NADPH-generating system plus NADH (6 μmoles)	0.003	0.003
Complete system minus glutathione	0.008	0.005

[a] Normal rat, stock diet.
[b] Standard assay system.

TABLE 5. Effect of added CoASH and HMG on microsomal HMG CoA reductase activity[a]

Additions	Mevalonolactone Formed	
	Intestinal Crypts	Liver
	nmoles/mg protein/min	
None	0.12	0.18
CoASH, 10 nM	0.11	0.20
CoASH, 20 nM	0.12	0.17
CoASH, 50 nM	0.11	0.18
CoASH, 100 nM	0.09	0.14
HMG, 10 nM	0.11	0.18
HMG, 20 nM	0.12	0.16
HMG, 50 nM	0.12	0.18
HMG, 100 nM	0.12	0.17
[3-^{14}C]HMG[a]	0.01	0.01
[3-^{14}C]HMG + CoASH[a]	0.01	0.01

[a] Standard assay system except in the experiments with [3-^{14}C]-HMG and [3-^{14}C]HMG + CoASH, where no substrate (HMG CoA) was added; normal rat, stock diet. Each experiment in this table was performed in triplicate.

paper requires the isolation and radioassay of the reaction product, mevalonolactone. The procedure is based upon published work, and involves deproteinization of the incubation mixture with sulfuric acid and simultaneous lactonization of biosynthetic mevalonic acid, ether extraction (5, 8), and TLC–radioassay (9) of the mevalonolactone. The reverse isotope dilution studies reported here prove that the assay is specific for mevalonolactone and that there is no interference by other labeled metabolites derived from [3-^{14}C]HMG CoA.

A number of procedures for the ultracentrifugal separation of subcellular fractions from the mucosa of rat intestine have been described (e.g., 24–26). In this species it has been difficult to obtain "clean" fractions, presumably because of the presence of mucus (27). Hübscher, West, and Brindley (28) examined subcellular fractions prepared from intestinal mucosa of rabbit, guinea pig, and cat and were able to obtain relatively

TABLE 6. Variation in microsomal HMG CoA reductase activity with age and sex of experimental animals[a]

Weight and Sex of Animals	No. of Animals	Weight of Liver	HMG CoA Reductase Activity			
			Intestinal Crypts		Liver	
g		*g*	*nmoles/mg protein/min*	*nmoles/section/min*[b]	*nmoles/mg protein/min*	*nmoles/total liver/min*
98, M (80–110)[c]	5	5.5 (5.0–5.7)	0.12 (0.11–0.14)	0.73	0.20 (0.18–0.25)	25
210, M (150–235)	8	11.2 (9.8–11.9)	0.13 (0.11–0.14)	0.70	0.11 (0.09–0.13)	33
290, M (275–330)	8	13.7 (12.9–14.5)	0.12 (0.10–0.13)	0.75	0.08 (0.06–0.09)	27
175, F (140–185)	4	7.5 (7.0–8.4)	0.13 (0.12–0.15)	0.63	0.19 (0.17–0.22)	36

[a] Normal rats on stock diet. Standard assay conditions; see section on Experimental Procedures. On the basis of available growth curves the ages of the animals were approximately as follows: males weighing 98 g were 30 days old, those weighing 210 g were 50 days old, and those weighing 290 g were 70 days old. The group of females weighing 175 g were 50 days of age and should therefore be compared with 210-g males.
[b] Entire small intestine was divided into four equal parts. Distal ileum was used for preparation of microsomes.
[c] Range.

Effect of cholestyramine treatment

HMG CoA reductase activity was enhanced in hepatic and mucosal microsomes of male Wistar rats fed 5% dietary cholestyramine for 7 days. The increase was approximately threefold in liver and twofold in the distal ileum (Table 7). In control rats fed the stock diet, the specific activity of HMG CoA reductase was highest in the distal ileum and lowest in the distal jejunum. Cholestyramine treatment enhanced HMG CoA reductase activity throughout the intestinal tract, and the largest relative increase was found in the distal jejunum (Fig. 8).

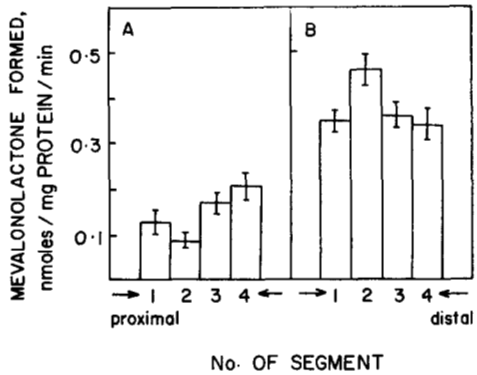

FIG. 8. Distribution of microsomal HMG CoA reductase activity along the intestinal tract. Standard assay conditions; *A*, normal rat; *B*, cholestyramine-treated rat. The intestinal tract was divided into four equal parts and the crypt cells from each quarter were assayed for microsomal HMG CoA reductase activity. The bars represent the means obtained with three rats, ± SEM.

DISCUSSION

The assay method for microsomal HMG CoA reductase in liver and intestinal mucosa described in this

TABLE 7. Effect of cholestyramine treatment on microsomal HMG CoA reductase

Treatment	No. of Animals	HMG CoA Reductase Activity	
		Intestinal Crypts[a]	Liver
		nmoles/mg protein/min	
Stock diet	8	0.145 (0.120–0.171)[b]	0.185 (0.160–0.210)
5% Cholestyramine, 7 days	6	0.330 (0.271–0.363)	0.580 (0.510–0.632)

[a] Entire small intestine was divided into four equal sections; crypt cells from distal ileum were used for preparation of microsomes. Standard assay conditions.
[b] Range.

pure preparations as determined by electron microscopy and the use of marker enzymes. These methods were apparently not applicable to rat intestine (28). In most of the studies reported so far the subcellular fractionation of villi or villi plus crypt cells was carried out. The present paper reports the ultracentrifugal separation of nuclei-free homogenates of villi and crypts. We attempted to avoid excessive cross-contamination of the subcellular fractions by centrifuging a dilute (5%, w/v) homogenate and by discarding an intermediate particulate fraction containing both mitochondria and microsomes sedimenting at 95,000 *g*-min. Like Hübscher et al. (28) we were unable to confirm the purity of the particulate fractions by the use of marker enzymes. The activity of glucose-6-phosphatase (EC 3.1.3.9), a microsomal marker, was too low to be useful. Studies in which NADPH:cytochrome C reductase (EC 1.6.99.1) was used as microsomal marker and succinate:cytochrome C reductase (EC 1.3.99.1) as mitochondrial marker were unsuccessful because in both cases less than 60% of the activity of the nuclei-free whole homogenate was recovered in the subcellular fractions.

The purity of the particulate subcellular fractions obtained in the present study was therefore determined by electron microscopy. Examination of the microsomal pellets disclosed negligible contamination by mitochondrial fragments (Fig. 2) and relatively little contamination of the mitochondrial fraction by microsomal membranes (Fig. 1). It seems possible, therefore, that the HMG CoA reductase activity of the mitochondrial fraction of intestinal crypt cells is not merely due to contamination of this fraction with microsomes. Additional support for this hypothesis is provided by the finding that the specific activity of the mitochondrial reductase is of the same order of magnitude as that of the microsomal enzyme and that this specific activity remains constant during repeated washings (Table 2).

In liver, HMG CoA reductase activity is present exclusively in the microsomal fraction; liver mitochondria (but not intestinal crypt cells) contain a soluble HMG CoA lyase which has a high affinity for HMG CoA and transforms it readily into acetoacetate and acetyl CoA. Thus, a hepatic, membrane-bound, mitochondrial HMG CoA reductase, if indeed it exists, would be difficult to detect in the presence of the active lyase which utilizes the same substrate. The lyase did not interfere with the assay of the microsomal HMG CoA reductase, since during the subcellular fractionation of rat liver care was taken to preserve the integrity of the mitochondria and presumably little lyase was present in the unwashed microsomal fraction. Moreover, the standard assay system contained an excess of substrate (to ensure saturation of the enzyme with HMG CoA) so that a relatively small leakage of lyase should not have affected the observed reductase activities. This was also apparent when the microsomal and mitochondrial fractions of rat liver were incubated in combination and HMG CoA reductase activity per milligram of microsomal protein remained unchanged (Table 2).

In the studies of HMG CoA reductase reported so far the labeled substrate was prepared either biosynthetically or by chemical synthesis. The biosynthetic substrate contained the biologically active form of HMG CoA plus methylglutaconyl CoA in a ratio of about 5:1 (8). During the chemical synthesis HMG anhydride is reacted with reduced CoA and a mixture of two diastereoisomers is obtained, of which only one is biologically active. At present it is assumed (as in the studies reported here) that the active and inactive isomers are present in equal proportions and that the unnatural isomer does not interfere in the enzyme assay.

Microsomal preparations of both liver and intestinal crypt cells were assayed for HMG CoA reductase activity. These crude enzyme preparations exhibited a number of similarities: they required NADPH or an NADPH-generating system as electron donor and were inactive with NADH. They required relatively high concentrations of glutathione to preserve maximal activity and had similar pH optima. The specific activities of the microsomal HMG CoA reductase in liver and in intestinal mucosa were approximately equal (although activity per total organ was 5–15 times greater in liver because of its greater mass). The apparent K_m values for the two microsomal activities were approximately equal. However, these similarities do not constitute proof that the hepatic and intestinal enzymes are identical. Studies with solubilized and highly purified enzyme preparations will be needed to furnish proof of identity or nonidentity. In any case it cannot be assumed that the enzymes are identical until the apparent differences between their control mechanisms are better understood.

The specific activity of the hepatic HMG CoA reductase decreased with the age of the animals, as re-

ported previously (5), and was greater in females than in males. In contrast, the specific activity of the mucosal reductase did not vary as a function of age or sex of the rats. These differences may be a reflection of different control mechanisms operative in the regulation of enzyme activity. If it is assumed that the activity of hepatic HMG CoA reductase is proportional to enterolymphatic cholesterol flux and independent of bile acid flux while the intestinal enzyme is unaffected by cholesterol and controlled by bile acid, the two enzymes would not be expected to show parallel changes in activity under all conditions. This type of reasoning would also serve to explain the stimulation of both enzymes by cholestyramine feeding; removal of part of the bile acid pool by adsorption on the anion exchange resin and by fecal excretion would not only reduce bile acid flux at the level of the intestinal mucosa but would also decrease cholesterol absorption, lymphatic cholesterol transport, and, presumably, hepatic cholesterol flux. It is not yet clear, however, why the jejunum, which presumably has a greater cholesterol flux and lower bile acid flux than the ileum, shows a greater relative response to cholestyramine administration.

The properties of the intestinal HMG CoA reductase reported here were predictable on the basis of the studies by Dietschy and his associates dealing with intestinal cholesterogenesis from acetate. The studies on cellular localization, variation in cholesterogenic activity along the small intestine, and the effect of cholestyramine reported here are entirely in accord with the work cited above. In addition, the effect of cholesterol feeding and fasting on intestinal HMG CoA reductase[1] was likewise predictable from Dietschy's studies.

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