3-Hydroxy-3-methylglutaryl coenzyme A reductase activity in the human gastrointestinal tract

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Abstract Activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (EC 1.1.1.34) was measured in intestinal mucosa of the human gastrointestinal tract. Activity was highest in gastric mucosa (18.2 pmol per mg per min) and there was a constant low level in the small bowel and colon (approximately 10 pmol per mg per min). Phosphorylation/dephosphorylation modulation of intestinal reductase activity was demonstrated in normal mucosa. Expressed jejunal reductase activity was significantly higher in celiac sprue mucosa and mucosa from defunctionalized intestine of jejunoileal bypass patients. Enzyme activity also increased during 24-hr mucosal organ culture in the absence of exogenous cholesterol. Addition to the culture medium of pure cholesterol or 25-hydroxycholesterol dissolved in a small volume of ethanol suppressed the culture-induced increase to 86 ± 3% and 69 ± 5% of paired controls, respectively. This evidence suggests that a moderate degree of feedback regulation of intestinal cholesterol synthesis by luminal sterol occurs in man. Mucosal HMG-CoA reductase activity was also measured in patients with hyperlipoproteinemia. Patients with either predominant hypercholesterolemia or predominant hypertriglyceridemia lipid profiles had "normal" expressed reductase activity, but feedback regulation by free cholesterol could not be demonstrated in either group under these conditions.—**Gebhard, R. L., B. G. Stone, and W. F. Prigge.** 3-Hydroxy-3-methylglutaryl coenzyme A reductase activity in the human gastrointestinal tract. *J. Lipid Res.* 1985. **26:** 47–53.

Supplementary key words HMG-CoA reductase • intestinal cholesterol synthesis • hyperlipoproteinemia

Mucosa of the gastrointestinal tract is responsible for cholesterol absorption and is an active site of cholesterol synthesis (1, 2). Measurements of cholesterol synthesis activity in various species show intestine to be second to liver in most species and the predominant organ in some circumstances (3). Synthesis occurs in epithelial crypt cells, probably in large measure for local use in making cell membranes of these rapidly replicating cells (4, 5). Synthesis also occurs in villous cells and is incorporated into circulating lipoproteins, possibly to stabilize chylomicrons and other lipoproteins during triglyceride absorption (6, 7). In intestinal mucosa, as in other tissues, the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is rate-determining in cholesterol synthesis and its activity correlates with synthesis rate (8, 9). In previous work, we have shown that the activity of this enzyme in intestine is subject to feedback regulation by cholesterol (9).

In addition to absorption and synthesis of cholesterol, intestinal mucosa is also involved in other aspects of cholesterol metabolism. Cholesterol esterification is an active process via the enzyme acyl coenzyme A:cholesterol acyltransferase (ACAT) (10). Apoproteins are synthesized within intestinal epithelial cells; apoB, A-I, and A-II have been identified (11–14). Finally, intracellular assembly and release of chylomicrons, nascent high density lipoprotein, and an intestinal form of very low density lipoprotein occur in intestinal mucosa (15, 16).

Most previous studies of intestinal cholesterol synthesis and metabolism have been done in the rat or dog; only one study has measured the rate of synthesis in human intestine (17). This study reported the rate of cholesterol synthesis from [^{14}C]acetate to be higher in ileum than jejunum and did not find regulation by cholesterol feeding. However, measurement of cholesterogenesis by acetate incorporation is known to be complicated by variability in acetate pool size (18). Therefore, we have measured activity of HMG-CoA reductase as a reflection of cholesterol synthesis in gastrointestinal mucosa of normal subjects, patients with celiac sprue, and patients with hyperlipidemia. Feedback regulation of reductase activity is assessed using cultured human mucosa.

MATERIALS AND METHODS

Tissue

Informed consent for mucosal biopsy was obtained from normal volunteers, subjects undergoing upper GI

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low density lipoprotein; JIB, jejunoileal bypass.

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endoscopy and colonoscopy, patients undergoing small bowel biopsy to evaluate celiac sprue, patients undergoing intestinal surgery (duodenal ulcer surgery, cecal carcinoma resection or jejunoileal bypass reconstruction), and hyperlipidemic patients who volunteered for jejunal biopsy with informed consent obtained.[1] All subjects were male, ages 23–65, and all were fasted overnight prior to morning biopsy. Endoscopic tissue was obtained by standard biopsy forceps passed through an Olympus endoscope; several intact specimens were utilized. Suction biopsy mucosa, using the Quinton-Rubin multipurpose biopsy tube from Quinton Instruments (Seattle, WA), or surgical mucosal specimens were trimmed at the muscularis mucosa prior to use in the studies. Surgical biopsy specimens of normal liver were available for study from two patients undergoing resection of renal cell carcinoma. Informed consent was obtained separately for this study which was approved by the Investigational Review Board. Reductase was measured in a microsomal fraction as previously described (19).

Materials

[3-^{14}C]HMG-CoA and DL-[5-^{3}H]mevalonic acid were obtained from New England Nuclear (Boston, MA). Cofactors, nonradioactive HMG-CoA, and other chemicals were obtained from Sigma Chemical (St. Louis, MO). Purified cholesterol (99% purity) and 25-hydroxycholesterol (97% purity) were obtained from Steraloids (Wilton, NH). Organ culture dishes were obtained from Falcon Plastics (Oxnard, CA) and Roswell Park Memorial Institute (RPMI) 1640 tissue culture medium was obtained from Difco (Detroit, MI).

Enzyme assay

HMG-CoA reductase was measured in whole mucosal homogenates by a modification of the method of Shapiro et al. (20), as previously reported (7). Tissue fragments were homogenized in a Dounce homogenizer with 0.1 M potassium phosphate buffer (pH 7.2) containing 0.2 M sucrose and 30 mM ethylenediaminetetraacetic acid. Twenty of the mucosal samples were additionally homogenized and assayed in the presence of either 50 mM NaF or 4 mM Mg^{2+} plus 2 mM ATP (insufficient tissue was available to measure fluoride effect on all samples). Each assay for HMG-CoA reductase contained 0.1–0.2 mg of mucosal protein and cofactors in a volume of 0.16 ml. Final cofactor concentrations were 25.5 mM glucose-6-phosphate, 1 U/ml of glucose-6-phosphate dehydrogenase, 3 mM NADP, 15 mM dithiothreitol, and 70 mM NaCl. Samples were preincubated at 37°C for 5 min prior to the addition of [^{14}C]HMG-CoA (31 μM; sp act, 12 nCi/nmol). Incubations were terminated after 15 min by addition of 0.025 ml of concentrated HCl followed by DL-[5-^{3}H]mevalonic acid [2 pmol (20,000 dpm/pmol)]. Mevalonate (R_f = 0.8–0.9) was separated from HMG-CoA (R_f = 0.0–0.1) by silica gel G chromatography in benzene–acetone 1:1 (7). Radioactivity was measured by liquid scintillation counting. Results were calculated as previously described (7). Control assays with enzyme denatured by 0.025 ml of concentrated HCl added prior to substrate, whether subsequently buffered to pH 7.2 with NaOH or not, showed essentially no [^{14}C]-mevalonate formation. Reductase activity was expressed as picomoles of mevalonate formed per milligram of whole mucosal protein per minute. Protein was determined by the method of Lowry et al. (21). The rate of mevalonate formation was directly proportional to the protein concentration in the range of 0.05–0.3 mg/assay and was linear for incubations up to 20 min. In standard assays, HMG-CoA and cofactor concentrations were chosen so that the reaction was zero order with respect to them. We have previously used these assay conditions to measure hepatic and intestinal reductase activity and have shown a reduction of expressed activity with the phosphatase inhibitor NaF (80% reduction in liver, 15–40% reduction in intestine) (19). Addition of purified alkaline phosphatase enzyme to the assay does not increase apparent mucosal activity. Thus the phosphate content of the incubation does not appear to strongly influence measured activity. Mucosal alkaline phosphatase activity was measured by a modification of the method of Bessey, Lowry, and Brock (22).

Organ culture

Mucosal organ culture was performed as previously described (7, 23). The wells were filled with approximately 0.7 ml of sterile tissue culture medium enriched with 10% delipidated fetal bovine serum, penicillin (6.2 mg/100 ml), streptomycin (13.5 mg/100 ml), neomycin (7.3 mg/100 ml), glutamine (30 mg/100 ml), glucose (366 mg/100 ml), and insulin (0.5 mg/100 ml). Three to five mucosal fragments of 1–2 mm diameter were floated on wire grids for 6, 24, or 48 hr. We have previously reported that human tissue remains viable in this culture system based on light and electron microscopic histology (23) and that canine mucosa is viable based on histology and 3-O-methyl glucose uptake (7). In some experiments, free cholesterol or 25-hydroxycholesterol were added to the medium in 10 μl of ethanol.

Incubation was carried out at 37°C in a humidified

[1]University of Minnesota Investigational Review Board approval for this study was obtained April 13, 1977 and Veterans Administration Medical Center Board approval was obtained on December 19, 1979.

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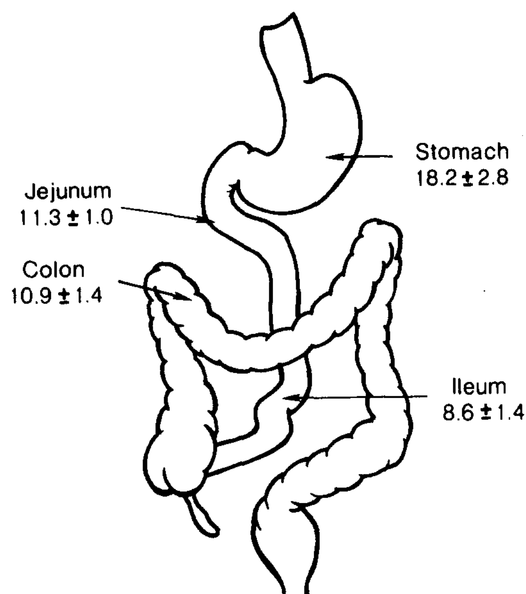

Fig. 1 Expressed mucosal HMG-CoA reductase activity (pmol of mevalonate formed per mg of mucosal protein per min) at various levels of the human gastrointestinal tract. Mucosal biopsies or samples obtained from a number of subjects were homogenized and assayed as described in Methods. Results are mean ± SE. Number of patients include: stomach, 12; jejunum, 52; ileum, 12; colon, 6. Activity is significantly higher in stomach compared to jejunum and ileum ($P < 0.01$). Values at other levels are not significantly different.

95% O_2, 5% CO_2 environment. At the end of the incubation periods, tissue was removed and rinsed in cold 0.15 M NaCl for homogenization and enzyme assay.

RESULTS

Basal activity

Expressed activity of HMG-CoA reductase in homogenates of normal mucosa from various levels of the gastrointestinal tract is shown in **Fig. 1**. The rank of activity, from highest to lowest, was stomach, jejunum, colon, and ileum. Activity in stomach was significantly higher than that of small intestine ($P < 0.01$) but not colon. The relationship of enzyme activity to substrate concentration is shown in **Fig. 2**. The intestinal enzyme K_m was calculated to be 2.2 μM, which is similar to reported values for the enzyme in other tissues of other species (24). In 20 experiments, mucosa was divided in half to be homogenized and assayed under standard conditions as well as with the addition of 50 mM NaF or, in 2 experiments, with 4 mM Mg^{2+} plus 2 mM ATP. Fluoride slightly inhibited apparent HMG-CoA reductase activity to 85% of control levels in normal mucosal while the addition of Mg^{2+}/ATP inhibited activity to 32% of control. This degree of NaF inhibition was the same for jejunal and ileal mucosa as well as for cultured mucosa.

Mucosal samples were also obtained from patients with abnormal intestinal tissue. **Table 1** compares HMG-CoA reductase activity in normal jejunal mucosa with activity in jejunal mucosa of patients with untreated celiac sprue. Sprue patients demonstrated malabsorption of fat and D-xylose, villous flattening with crypt hypertrophy on biopsy, and positive therapeutic response to gluten-free diet. Expressed reductase activity was significantly higher in sprue mucosa. Also shown in Table 1 is a comparison of HMG-CoA reductase activity in mucosa obtained from two separate intestinal segments of patients having jejunoileal bypass (JIB). JIB patients had undergone bypass surgery 6 months to 6 years previously and study tissue was obtained at the time of take-down of the bypass segments. Reductase activity was measured in mucosa from the bypassed jejunum and from the incontinuity ileum of the same patients. Bypass mucosa displayed shortened villi and crypts histologically. Expressed reductase activity was 69% higher in the bypassed tissue, significant by paired analysis.

Patients with normal mucosal histology but elevated blood lipid levels were also evaluated. Six jejunal biopsies were obtained from five patients with predominant hypercholesterolemia (one patient biopsied on two separate occasions) and six biopsies from six patients with predominant hypertriglyceridemia. **Table 2** expresses clinical data on these patients. Results, in Table 1, show that HMG-CoA reductase activity was slightly lower in hypercholesterolemic mucosa and slightly higher in hypertriglyceridemic mucosa. The values were not significantly different from control tissue activity.

Culture studies

Samples of normal jejunal mucosa were maintained in organ culture for 6 to 24 hr and, in some instances,

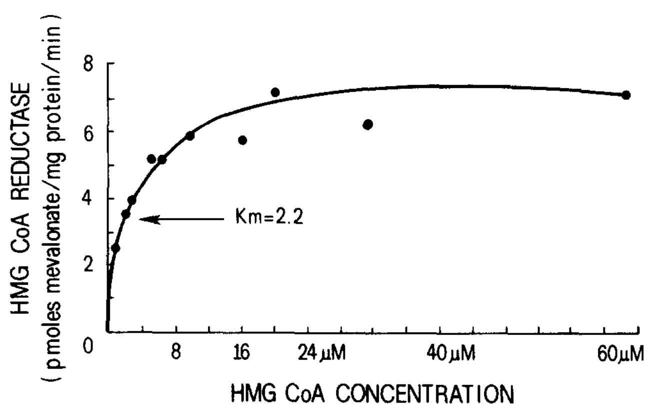

Fig. 2 Substrate concentration versus apparent enzyme activity. A sample of jejunal mucosa was homogenized and assayed for HMG-CoA reductase activity using varying concentrations of HMG-CoA substrate. A double reciprocal plot of these data indicates the apparent K_m of the intestinal enzyme to be 2.2 μM.

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TABLE 1. Basal intestinal HMG-CoA reductase activity

	HMG-CoA Reductase[a]	Subjects[b]	P
Normal jejunum	11.3 ± 1.0	52	
Celiac sprue jejunum	23.5 ± 2.4	6	0.001
Bypassed jejunum	12.0 ± 1.2	4	
Incontinuity ileum	7.0 ± 0.7	4	0.05
Normal jejunum	11.3 ± 1.0	52	
Hypercholesterolemic jejunum	8.2 ± 0.9	6	NS
Hypertriglyceridemic jejunum	14.1 ± 2.4	6	NS

[a]Basal activity of intestinal HMG-CoA reductase (pmol mevalonate formed per mg of homogenate protein per min) was measured and compared for three conditions: *1*) normal jejunal mucosa versus celiac sprue jejunal mucosa; *2*) mucosa from bypassed jejunal mucosa versus ileal mucosa in luminal continuity from the same JIB patients; and *3*) jejunal mucosa from normocholesterolemic subjects versus jejunal mucosa from two forms of hyperlipoproteinemia.

[b]The number of different subjects tested is shown, except that six samples were obtained from five hypercholesterolemic patients (one patient biopsied twice at 6-month interval).

up to 48 hr. During the culture period, activity of HMG-CoA reductase increased as shown in **Fig. 3**. The reason for increasing enzyme activity during culture is uncertain, but activity of other enzymes such as alkaline phosphatase also increases (9, 23). Absence of available cholesterol for absorption may be the stimulus which causes mucosa to increase reductase activity and endogenous synthesis of cholesterol. This explanation is given credence by the results shown in **Fig. 4**. In these experiments, fragments of mucosa from the same samples were simultaneously cultured in plain media and in media containing added cholesterol or 25-hydroxycholesterol. A modest, but significant, inhibition of stimulated total HMG-CoA reductase activity occurred in the tissue cultured with sterol.

Jejunal mucosa from eight patients with hyperlipidemia was also cultured for 24 hr in the presence and absence of cholesterol. The number of cultures was

TABLE 2. Clinical data[a]

	Age	Cholesterol	Triglyceride
	yr	*mg/dl*	
Hypercholesterol lipid profile			
Patient #1 (biopsied twice)	23	391	150
2	43	341	122
3	29	362	80
4	62	385	126
5	26	490	96
Hypertriglyceride lipid profile			
Patient #A	53	440	3270
B	62	325	505
C	55	431	608
D	64	375	477
E	52	650	1200
F	58	347	520

[a]Laboratory values at time of small bowel biopsy in eleven hyperlipoproteinemic subjects studied.

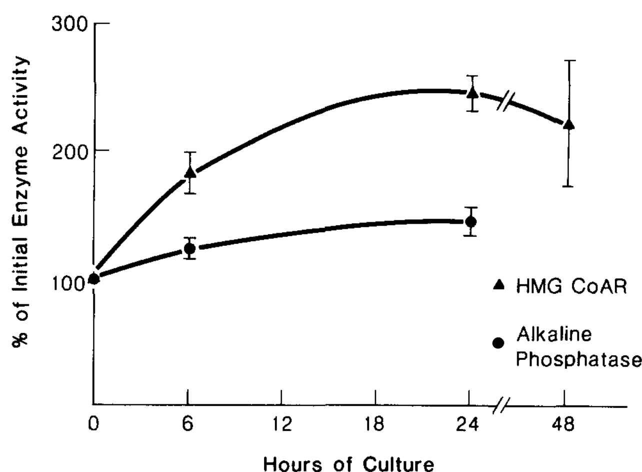

Fig. 3 Effect of organ culture on intestinal enzymes. Jejunal mucosa was cultured as described under Methods. After the designated period, tissue was removed, homogenized, and assayed for total activity of HMG-CoA reductase and alkaline phosphatase. Results are expressed as percent of initial activity and represent mean ± SE. Number of cultures for HMG-CoA reductase: 6 hr, n = 22; 24 hr, n = 28; and 48 hr, n = 4. For alkaline phosphatase, n = 10 and 24.

small but activity in this tissue also increased 2.5- to 3.5-fold (**Table 3**). In contrast to normal mucosa, however, we were unable to demonstrate significant suppression of enzyme activity by the presence of pure cholesterol under these conditions.

DISCUSSION

Activity of HMG-CoA reductase expressed under the conditions of our assay of mucosa from the intestinal tract of healthy human volunteers was found to be rather uniform and low (10 pmol of mevalonate formed per mg of protein per min). Human activity is approximately one-third of the values which we have observed in canine (35 pmol per mg per min) or rodent (28 pmol per mg per min) intestine (7, 19). We have also measured total expressed HMG-CoA reductase activity in hepatic microsomes from two patients and we found activity of 34.5 and 43.1 pmol per mg of microsomal protein per min. These values compare favorably to human activity reported by Salen et al. (25) and are much lower than activity in rodent liver (19). These data may be used to estimate total human intestinal reductase activity to be 850 nmol of mevalonate formed/min and total hepatic activity to be 2200 nmol/min [based on a microsomal recovery of 50% as reported for rat liver (26)]. Low specific activity of human gut and liver HMG-CoA reductase may relate to a diet high in cholesterol or to proportionally high blood cholesterol and LDL levels.

Kinetic parameters of human intestinal HMG-CoA reductase were similar to those reported for the enzyme

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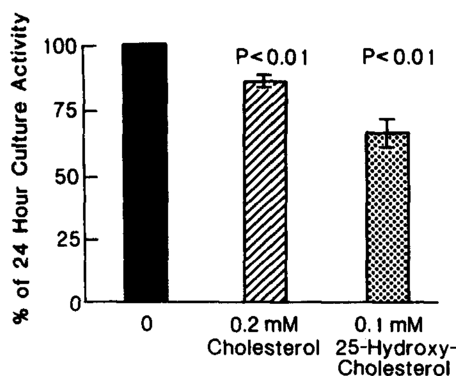

Fig. 4 Effect of cholesterol and 25-hydroxycholesterol on expressed HMG-CoA reductase activity in normal cultured human intestinal mucosa. Mucosal fragments were pair-cultured for 24 hr in the presence and absence of sterol dissolved in 10 μl of ethanol. Following culture, specimens were removed, homogenized, and assayed for HMG-CoA reductase activity. Data for tissue cultured with sterol are expressed as mean ± SE percent of pair control culture:

$$\frac{\text{reductase activity with sterol}}{\text{reductase activity without sterol}} \times 100\%.$$

For cholesterol, n = 65 cultures and for 25-hydroxycholesterol, n = 24 cultures.

in other tissues and species. The enzyme is known to exist in a phosphorylated inactive form and to be dephosphorylated by cytosolic reductase phosphatase to active enzyme (27). Sodium fluoride, a phosphatase inhibitor, prevents activation. Our findings indicate that most (85%) of the intestinal enzyme is present in an active form when NaF-inhibited activity is compared to total expressed activity without fluoride. Other investigators have made similar observations (28). In contrast, many reports have indicated that only 20% of hepatic HMG-CoA reductase is present in the active form under basal conditions (19, 27). If one applies the percent of active enzyme to the values for total enzyme activity present, it can be estimated that human intestine may actually produce a larger quantity of newly synthesized cholesterol than does liver. Reversibility of the dephosphorylation reaction in intestine was shown by the striking reduction in measured enzyme activity when magnesium and ATP were present. Thus, reductase kinase activity is present in intestinal cell homogenates (27–29).

We were unable to measure both total (without NaF) and active (with NaF) fraction of intestinal HMG-CoA reductase in all experiments because of the small quantities of tissue available. When measured in 20 separate samples, the active fraction was equivalent for normal jejunum (85%), normal ileum (86%), and cultured normal mucosa (57%). The active fraction of total HMG-CoA reductase was not measured in sprue or hyperlipidemic mucosa, but it is most likely that total expressed activity adequately reflects cholesterol synthesis rate in these tissues because it has previously been shown that both total and dephosphorylated enzyme activity correlate with cholesterol synthesis in rat liver (30). Furthermore, Stange and Dietschy (31) have presented data to indicate that enzyme phosphorylation/dephosphorylation is only involved in very short term modulation of cholesterol synthesis, long term (greater than 60 min) regulation being related to total enzyme.

The observation that HMG-CoA reductase activity is no higher in human ileum than in jejunum contrasts with data from animal species (4, 5, 9). Low human ileal activity may relate to high dietary cholesterol or serum cholesterol levels of man. Alternatively, since bile salts also suppress intestinal cholesterogenesis, greater meal frequency in man may maintain greater bile salt exposure (4, 8, 9). The findings of HMG-CoA reductase activity also differ from results showing greater incorporation of [^{14}C]acetate into cholesterol by human ileal mucosa compared to jejunal mucosa (17). However, [^{14}C]acetate incorporation may not accurately reflect cholesterol synthesis since the size of unlabeled acetate pools may vary (18). In particular, the acetate pool of jejunal mucosa may be high since it is exposed to more nutrients.

It is likely that cholesterol synthesis occurs in the gastrointestinal mucosa for two primary purposes: for lipid transport to the circulation and for use in cell membranes during enterocyte replication. The high level of HMG-CoA reductase in sprue patients, a condition of rapid cell turnover, is evidence for the latter. These studies also provide several pieces of evidence for the existence of feedback regulation of HMG-CoA reductase by cholesterol in human intestine. First, HMG-CoA reductase was found to be significantly

TABLE 3. Culture of mucosa from hyperlipoproteinemic subjects

	Increase in Activity During Culture	Effect of Cholesterol: Paired Culture[a]
Hypercholesterolemia	267 ± 35% (n = 13 cultures, from 4 subjects)	118 ± 14% (n = 8 cultures)
Hypertriglyceridemia	351 ± 85% (n = 10 cultures, from 4 subjects)	102 ± 9% (n = 6 cultures)
Normal	250 ± 12% (n = 48 cultures, from 24 subjects)	86 ± 3% (n = 65 cultures)

Jejunal mucosa from hyperlipidemic patients was maintained in organ culture for 24 hr. Values for normal mucosa are shown for comparison. Shown in the first column, HMG-CoA reductase activity increased during culture in all tissues. In the second column, however, no significant feedback inhibition of enzyme activity was observed in hyperlipidemic mucosa when cholesterol was present in culture media (in contrast to normals).

[a]Cholesterol effect is calculated as:

$$\left[\left(\frac{\text{activity in the presence of cholesterol}}{\text{activity in the absence of cholesterol}}\right) \times 100\%\right].$$

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higher in gastric mucosa, a site where very little cholesterol absorption occurs. Second, elevated expressed activity was found in sprue patients and these patients display diminished cholesterol absorption. Third, bypassed jejunal mucosa, which is out of continuity with luminal sterol, showed higher expressed activity than did ileal mucosa exposed to the fecal stream. This activity was increased in spite of decreased cell turnover in the bypassed mucosa. Finally, the mucosal culture studies revealed an increase in expressed HMG-CoA reductase activity when mucosa was cultured in cholesterol-free medium; the increase was inhibited by 12–30% when cholesterol or 25-hydroxycholesterol was present in the medium.

The hyperlipidemic patients studied showed basal activity of jejunal HMG-CoA reductase activity that was not significantly different from normal. Nevertheless, it is possible that this level of cholesterol synthesis may be inappropriately high for these patients' blood cholesterol and LDL concentrations. During these patients' mucosal culture, enzyme activity was stimulated in a manner similar to normal subjects. However, we were unable to demonstrate feedback inhibition of activity by cholesterol in this tissue under the conditions tested. The significance of this intriguing finding is unclear, but it may reflect abnormal feedback regulation by free cholesterol in intestine as has been demonstrated for LDL cholesterol in other tissues (32).

The authors acknowledge the assistance of Sheila Haroldson and Valarie Wesley in the preparation of this manuscript. Work was supported by the Research Service of the Veterans Administration.

Manuscript received 6 September 1983.

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