Regulation of Cholesterol Synthesis in Cultured Canine Intestinal Mucosa*

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The regulation of intestinal cholesterol synthesis was studied utilizing canine ileal mucosa maintained in organ culture for 6 h. Viability was monitored by light and electron microscopy, measurement of cellular enzymes, and the ability to actively transport a glucose analogue. The activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (EC 1.1.4.3.4), the rate-limiting enzyme of cholesterol synthesis, increased 4-fold during a 6-h culture. A parallel increase occurred in the rate of acetate incorporation into digonitin-precipitable sterols during this period. This increase could be prevented by the addition of cycloheximide to the culture. Pure cholesterol, 7-ketoleolesterol, and 25-hydroxycholesterol, when present during the last 4 h of culture, also caused significant suppression of the rise in HMG-CoA reductase activity (final HMG-CoA reductase activity with the three sterols was 77 ± 4%, 68 ± 5%, and 58 ± 3% of control postculture value). Bile salts at low, nontoxic concentrations also inhibited the increase of enzyme activity (2 mM taurocholate = 63 ± 3% of control, 0.5 mM taurochenodeoxycholate = 76 ± 6% of control). In contrast, dog lipoproteins separated by ultracentrifugation failed to significantly affect intestinal cholesterol synthesis in these short term organ cultures.

Intestine is the second most active site of cholesterol synthesis (1, 2) hence the regulation of cholesterol synthesis by this organ has been investigated in several laboratories. Nevertheless, a clear understanding of the regulatory mechanism has not yet emerged. Could reported that cholesteryl feeding almost completely inhibited cholesterol synthesis in dog liver while the inhibition in intestinal mucosa was only about 30% and was not considered significant (3). Dietschy and Siperstein (4) demonstrated that bile diversion resulted in increased intestinal cholesterol synthesis in the rat and suggested that the rate of this process was regulated by the amount of bile salt traversing the intestine. Following the demonstration of a circadian rhythm of cholesterol synthesis in rat liver (5) and intestine (6), Shofer et al. (7) reinvestigated the problem. They found that during the basal period of the diurnal cycle neither cholesterol nor bile salts, when fed alone, inhibited intestinal 3-hydroxy-3-methylglutaryl coenzyme A reductase (EC 1.1.4.3.4), the rate-limiting enzyme of cholesterol synthesis, but that combined cholesterol and bile salt feeding was required to reduce intestinal cholesterol synthesis. In contrast, they observed that chronic bile salt feeding alone resulted in elimination of the diurnal increase in synthesis usually observed at midnight.

Inhibition of cholesterol synthesis by cholesterol feeding has been demonstrated in the intestine of hamsters (8) and guinea pigs (9). In mice, although no feedback inhibition was demonstrated with cholesterol feeding, the cholesterol analogues 7-ketoleolesterol and 25-hydroxycholesterol did suppress intestinal cholesterol synthesis (10). Recently, Anderson and Dietschy (11) reported that the rate of intestinal sterol synthesis is markedly stimulated in rats made hypolipoproteineminec by the drug 4-aminopyrazol (3,4D) pyrimidine and that this increase in cholesterol synthesis could be partially prevented by infusion of rat or human lipoproteins (12). This suggests that circulating lipoproteins may have a role in regulating the rate of intestinal cholesterol synthesis.

However, simultaneous control of overall nutritional status, luminal cholesterol, content, bile flow, and serum lipoproteins is virtually impossible to attain in vivo. Thus it is difficult to be certain which factors are actually responsible for the observed changes in intestinal cholesterol synthesis. Accordingly, the intestinal organ culture technique of Browning and Trier (13) was adapted to study this problem, since it allows rigid control of the environment to which the intestinal mucosa is subject. The present study establishes the validity of this technique for investigating the regulation of intestinal cholesterol synthesis and reports the effect of free cholesterol, oxygenated cholesterol derivatives, bile salts, and lipoproteins on this process in short term intestinal organ culture.

MATERIALS AND METHODS

Materials—Cholesterol was purchased from Calbiochem and recrystallized twice from hot glacial acetic acid. No impurities were
detected by thin layer chromatography (Silica Gel H with benzene-ethyl acetate (1:1) or by gas liquid chromatography (3% OV-17 on Gas-Chrom Q at 230°C). 7-Ketocholesterol and 25-hydroxycholesterol were obtained from Steraloid Inc. (Wilton, N. H.) and showed less than 3% impurities by gas liquid chromatography. Isotopes were obtained from New England Nuclear. [3-14C]HMG-CoA was synthesized by the method of Goldfarb and Pitot (14). Myelobald-Silica Gel G chromatography sheets were obtained from Eastman Kodak, and fatty acids, cofactors, and enzyme substrates were obtained from Sigma. Organ culture dishes were obtained from Falcon Plastics. Roswell Park Memorial Institute 1640 tissue culture media was purchased from Difco Labs, Dulbecco's medium and fetal calf serum were purchased from Grand Island Biologic Co., and fatty acid-free bovine serum albumin from Miles Laboratories.

Organ Culture—Ileal mucosa was obtained from healthy adult, mixed breed dogs. The animals were fed a standard diet of Wayne dog diet (Elanco, Indianapolis, Ind.), and sodium pentothal administered between 0800 h and 1000 h. A segment of ileum 10 to 20 cm proximal to the ileocecal valve was removed, rinsed in Dulbecco's medium, and the mucosa was removed at the level of the muscularis mucosa using a sterile scalpel blade. Mucosa was then sectioned into 1- to 2-mm fragments and placed immediately into organ culture or homogenized for enzyme determinations.

Organ culture organ was performed as previously described (15). The wells were filled with sterile tissue culture medium enriched with 10% delipidated fetal calf serum, penicillin (0.2% 100 ml), streptomycin (1.35 mg/100 ml), neomycin (7.5 mg/100 ml), glutamine (20 mg/100 ml), glucose (368 mg/100 ml), and insulin (0.5 mg/100 ml). Incubation was carried out at 37°C in a humidified 85% O2, 5% CO2 environment. At the end of the incubation periods, tissue was removed and rinsed in cold 0.15 M NaCl.

Lipoprotein Preparation—Heparinized blood was obtained from dogs fed two eggs 4 h prior to venipuncture. Lipoproteins were separated from plasma by the method of Havel et al. (16). All lipoprotein isolations were dialyzed for 24 h against 4 liters of 0.15 M NaCl with 0.3 mM EDTA at pH 7.4. Fetal calf serum was delipidated by the method of Kirsten and Watson (17). Cholesterol content of culture medium containing 10% delipidated fetal calf serum was less than 1 ppm.

Histology—Mucosal fragments were fixed in formalin immediately after obtaining or after periods in organ culture with and without lipid in the medium. Fixed tissue was sectioned and stained with hematoxylin and eosin for light microscopy. Electron microscopy was performed by Dr. Eve Reaven at the Palo Alto Veterans Administration Hospital by standard techniques.

3-O-Methylglucose Uptake—Mucosal fragments obtained prior to culture or immediately following culture were quickly rinsed in Krebs-Ringer bicarbonate buffer without glucose at pH 7.4. Fragments were homogenized in 0.15 M NaCl and portions of the homogenate were taken for protein determination and the remainder centrifuged to remove the protein. Portions of the incubation medium and the remaining mucosal supernatant were counted in a scintillation counter using Aquasol as the fluor. The [carboxyl-14C]linulin counts were used to determine the rate of sterol synthesis. This is consistent with the finding of Smithson and Gray (18) that the uptake was linear for 30 min and was saturable. For routine studies, a 3-O-methylglucose concentration of 5 mM was used. At this concentration, 45% of the transport was active and the process was not saturated.

Enzyme Determinations—Mucosal homogenates were assayed for sucrase activity by the method of Dalqvist (19). Alkaline phosphatase was assayed by the method of Roseay et al. (20) as previously described. Glucose-6-phosphate dehydrogenase was measured using 10 mM glucose-6-phosphate as the substrate in 50 mM Tris buffer (pH 6.5) containing 50 mM Tris buffer (pH 6.5) containing 10 mM mersaptoethanol. The reaction was terminated by adding an equal volume of 10% trichloroacetic acid. The liberated phosphorus was measured by a standard method (21). All enzyme activities were expressed as units of protein.

Uptake and Esterification of Cholesterol and Fatty Acids—Mucosal tissue was cultured for 5 h in standard culture media (12). [1-14C]Cholesterol or [9,10-3H]oleic acid were dissolved in 10 μl of absolute ethanol and added to the media with [carboxyl-14C]linulin. After standing overnight, the samples were centrifuged and an additional 60 min. The tissue was homogenized in 1 ml of 0.15 M NaCl and portions of the homogenate were taken for protein determination and for scintillation counting to determine the extracellular fluid space. The lipids in the remaining tissue homogenate were extracted as described by Folch et al. (22) and were separated by thin layer chromatography on silica gel with the solvents, petroleum ether:diethyl ether:glacial acetic acid (85:15:1). Areas corresponding to authentic cholesterol ester, triglyceride, free fatty acid, free cholesterol, and phospholipids were scraped from the plate with a razor blade and added to 10 ml of Aquasol for scintillation counting.

Assay for HMG-CoA Reductase Activity—HMG-CoA reductase was measured in whole mucosal homogenates by a modification of the method of Shapiro et al. (23). Tissue fragments were homogenized in 0.7 ml of 0.2 M potassium phosphate buffer (pH 7.2) containing 0.2 M sucrose using 30 strokes of a Dounce homogenizer. Each assay contained 0.1 to 0.2 mg of mucosal protein and 0.05 ml of an aqueous solution of cofactors. Final cofactor concentrations were 25.5 mM glucose 6-phosphate, 1 unit/ml of glucose-6-phosphate dehydrogenase, 5 mM NADP, 15 mM dithiothreitol, 70 mM NaCl. The assay was initiated by the addition of [1-14C]HMG-CoA (27 μM) (specific activity 12 ncil/mmol). Incubations were terminated after 15 min by addition of 0.025 ml of concentrated HCl followed by 0.5 ml of 4% trichloroacetic acid, 2 ml of 30-500000 pmm/ml). The precipitate was washed in acetone, then ether, then dissolved in methanol, and the radioactivity was determined by scintillation counting. The rate of sterol synthesis was expressed as picomoles of mevalonate formed/mg of protein/min.

The reaction rate was directly proportional to the protein concentration in the range 0.05 to 0.5 mg/incubation and was linear for incubations up to 20 min. HMG-CoA and NADP concentrations were chosen so that the reaction was zero order with respect to them. Measurement of the HMG-CoA reductase activity of pure dog lymphocytes (the other major cellular element of intestinal mucosa) revealed that their specific activity (1.1 pmol/mg/min) was too low to contribute significantly to the activity of the whole homogenate (15.9 pmol/mg/min).

Sterol Synthesis from Acetate—Mucosal tissue was incubated for 2 h in the basic culture medium containing 2 mM sodium [2,4,6]acetate (specific activity 3.6 μc/mmol). The tissue was then rinsed and homogenized in 0.15 M NaCl. Lipids were extracted with CHCl3:MeOH (2:1), washed with excess cold acetate, saponified in ethanolic KOH, and the cholesterol precipitated as the digluconate. The precipitate was washed in acetone, then ether, then dissolved in methanol, and the radioactivity was determined by scintillation counting. The rate of sterol synthesis was expressed as picomoles of acetate incorporation/mg of tissue protein/hr.

Chemical Methods—Cholesterol was determined by the method of Leffler (24), and protein by the method of Lowry et al. (25).

RESULTS

Tissue Viability—Both light and electron microscopy were used to assess the structural integrity of cultured canine...
Lipids - An important function of bile salts is the facilitation of lipid absorption. The presence of 5% albumin caused no significant effect. With culture continued for longer than 8 h a progressive deterioration of the histology was noted. The inclusion of 10 µl of alcohol with or without sterols did not alter the histology. Taurocholate at a concentration of 5 mM, however, caused serious disturbances of the histologic integrity of the epithelial cells, but concentrations below 2 mM with 5% albumin present had no significant effect.

The active transport of a nonmetabolizable sugar (3-O-methylglucose) was also measured to assess the viability of the tissue during culture and to monitor possible damage to metabolic function induced by various alterations in the culture medium. The initial uptake rate of 3-O-methylglucose is virtually identical before and after 6 h of culture (Table I). Moreover, the addition of cholesterol, 7-ketocholesterol, and 25-hydroxycholesterol to the medium did not disturb the rate of this sensitive transport function. Very low concentrations of bile salts in the presence of delipidated bovine serum albumin also caused no measurable alteration of the transport rate. However, addition of taurochenodeoxycholate to the medium, at a concentration shown previously (26) to injure intestinal epithelial function, profoundly depressed the cultured intestine's ability to actively transport the sugar.

Thus, canine intestine remains fully viable by both morphologic and metabolic criteria during a 6-h culture. Moreover, although the presence of bile salts in the medium at a concentration usually found in vivo impairs viability, cholesterol and its 25-hydroxy and 7-keto derivatives do not, nor do bile salts in concentrations below the critical micellar concentration in the presence of 5% albumin.

**Ability of Cultured Mucosa to Absorb and Metabolize Lipids** - An important function of bile salts is the facilitation of lipid absorption. The dependence of a particular lipid upon bile salt for absorption is generally inversely proportional to its water solubility. Because cholesterol is hydrophobic, its absorption is very dependent upon the presence of bile salts (27). To assess whether any cholesterol was absorbed in the absence of bile salts, radiolabeled free cholesterol dissolved in alcohol was added to the medium and the amount of isotope in the cell was determined after correction for extracellular fluid space. It can be seen from Table II that radiolabeled cholesterol was found in the cell. Although the calculated value for absorption may represent an overestimate because some exchange of labeled and unlabeled sterol, without net transfer, may have occurred, it is clear that cholesterol dissolved in the medium, that is, cholesterol as such, was absorbed and not a cholesterol derivative. That cholesterol from the medium enters the cells' metabolic pathways was demonstrated by the fact that more than 3% of the radiolabel was recovered as cholesterol ester.

Finally, the results obtained when a radiolabeled fatty acid was added to the medium are shown in Table II. With oleic acid, a higher concentration of lipid dissolved in the medium could be achieved, resulting in greater absorption. Moreover, the bulk of this fatty acid entered the metabolic pathways involved in fat assimilation (28).

On the basis of these experiments we concluded that lipid added to the medium can be absorbed by the epithelial cells and enter, at least in part, the metabolic pathway characteristic of dietary lipid. It is also apparent that cholesterol absorption in the absence of detergent is quite limited.

**Effect of Organ Culture on Intestinal HMG-CoA Reductase** - To assess the effect of organ culture on cholesterol synthesis, the activity of HMG-CoA reductase was measured in intestinal fragments before and at various times after culture in a lipid-free medium. As shown in Fig. 1 the activity of this enzyme increased rapidly, doubling after 2 h, and rising to more than three times the control value after 6 h of culture. The activity remained at this level for the next 4 h then appeared to decline somewhat by the 12th h of culture. The activity of alkaline phosphatase, a plasma membrane enzyme, did not change during culture, suggesting that this effect was specific for HMG-CoA reductase and not the result of loss of cell protein. The constancy of sucrase, an enzyme active only in villous cells, demonstrates that it was not due to an alteration in proportion of crypt and villous cells present (Fig. 1). That it was not the result of generalized microsomal hypertrophy was shown by the fact that the enzyme glucose-6-phosphatase did not increase in activity (97% ± 8% of preculture value after 6 h).

To prove that the increase in HMG-CoA reductase reflected
Fig. 1. Effect of organ culture on intestinal HMG-CoA reductase. Canine intestine was cultured as described under "Materials and Methods." After the designated period, the tissue was removed and the activity of HMG-CoA reductase, sucrase, or alkaline phosphatase was measured. Results are mean ± S.E. of six or more determinations.

Table III

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<th>Culture time</th>
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<tr>
<td>h</td>
<td>pmol/min/mg</td>
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<tr>
<td>0-2</td>
<td>5.5 ± 1.8</td>
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<td>4-6</td>
<td>11.1 ± 2.3</td>
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Table IV

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<thead>
<tr>
<th>Duration of culture</th>
<th>Material added</th>
<th>HMG CoA reductase</th>
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<td>0</td>
<td>16.8</td>
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<td>6</td>
<td>71.5</td>
<td>426</td>
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<tr>
<td>b</td>
<td>0.2 mM cycloheximide at 0 h</td>
<td>10.7</td>
<td>94</td>
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<tr>
<td>0</td>
<td>5.8</td>
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<td>2</td>
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<td>6</td>
<td>21.9</td>
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<td>14.1</td>
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An increase in sterol synthesis, the rate of incorporation of acetate into digitonin-precipitable sterols was measured. From the data in Table III it can be seen that the rate of acetate incorporation is twice as great between 0 and 6 h as between 0 and 2 h of culture. This is very close to what would be predicted from the reductase data of Fig. 1.

Effect of Cycloheximide in Culture Medium—A similar increase in HMG-CoA reductase occurs when human fibroblasts (29), rat hepatoma cells (30), or isolated rat livers (31) are maintained in lipid-free medium. In each of those instances the increase could be prevented by the addition of cycloheximide to the medium. When cycloheximide was added to medium at the start of intestinal culture, the increase in HMG-CoA reductase at 6 h was completely prevented (Table IV, Experiment A). If cycloheximide was added after 2 h of culture, and the activity measured 4 h later, the HMG-CoA reductase activity was 50 ± 2% of the 6-h cultured control (Fig. 2). Cycloheximide did not affect the histology or the activity of alkaline phosphatase (2.0 ± 0.03 pmol of p-nitrophenol/g of protein/min if cycloheximide is added either initially or after 2 h of culture, mean of two experiments), but did cause a decrease in the active transport of 3-O-methylglucoside (50% of control). Although quantitative interpretation is difficult since cycloheximide affects other processes and may have diminished the viability of the intestine somewhat, these results suggest that protein synthesis is required for the induction and maintenance of the increase in HMG-CoA reductase.

Effect of Cholesterol and Other Sterols on Intestinal HMG-CoA Reductase Activity—To ascertain whether pure cholesterol could affect the activity of intestinal HMG-CoA reductase, the sterol was added to the organ culture medium in 10 μl of alcohol after 2 h of culture in delipidated medium. After 4 additional h of culture the activity of HMG-CoA reductase was measured. The results are shown in Fig. 2. Although neither alcohol alone nor 40 μM cholesterol significantly affected the activity of the enzyme, higher concentrations of cholesterol caused a consistent and significant (p < 0.01) decrease in enzyme activity (Fig. 2). Since the sterols were added after 2 h of culture when the reductase was about 65% of the 6-h value, cholesterol appeared to prevent over half the usual rise that occurred between 2 and 6 h of culture. This is shown by Experiment B in Table IV, where reductase activity was measured at the start of culture, and after 2 and 6 h, with and without addition of cholesterol.

In tissues other than intestine it has been shown that 7-ketocholesterol and 25-hydroxycholesterol, which are more soluble than cholesterol, are also more potent inhibitors of HMG-CoA reductase than pure cholesterol (32). Both of these sterols significantly inhibited intestinal HMG-CoA reductase and, at comparable concentrations, 25 hydroxycholesterol was significantly (p < 0.001) more potent than cholesterol while 7-ketocholesterol appeared to be of intermediate potency (Fig. 2).

These data demonstrate that cholesterol in the absence of bile salts can exert an inhibitory effect on intestinal cholesterol synthesis.

Effect of Bile Salts on Intestinal HMG-CoA Reductase—Because of prior studies indicating that bile salts play an important role in the regulation of intestinal cholesterol synthesis (4), we studied their effect on HMG-CoA reductase in cultured intestine. Following the initial 2 h of culture, taurocholate (1 or 2 mM) or taurochenodeoxycholate (0.25 or 0.5 mM) was added to the medium at the start of culture, and after 2 h of culture, mean of two experiments) but did cause a decrease in the active transport of 3-O-methylgluctoside (50% of control). Although quantitative interpretation is difficult since cycloheximide affects other processes and may have diminished the viability of the intestine somewhat, these results suggest that protein synthesis is required for the induction and maintenance of the increase in HMG-CoA reductase.

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0.5 mM) was added to media with 5% albumin. Control cultures received only 5% albumin. All cultures were continued for 4 more h. HMG-CoA reductase activity of mucosa was significantly suppressed by both of these bile salts (Table V). This effect is particularly striking since even below the critical micellar concentration, bile salts had an effect comparable to the oxygenated sterols. Addition of 0.2 mM cholesterol and 1 mM taurocholate (Table V) suppressed reductase activity more than either cholesterol or taurocholate alone at these concentrations. However, 0.2 mM cholesterol plus 2 mM taurocholate did not result in more suppression than 2 mM taurocholate alone. Thus, bile salts appear to be capable of independently regulating the rate of intestinal cholesterol synthesis.

**Effect of Lipoproteins on Intestinal HMG-CoA Reductase** — It has been shown in several cultured tissues (17, 29) and intact liver (33) that the addition of cholesterol to the media as lipoprotein results in potent inhibition of HMG-CoA reductase activity. In *in vivo* these tissues are exposed to cholesterol only as components of lipoproteins. In contrast, the intestine is in the unique position of being exposed to cholesterol as a dissolved molecule at its brush border surface and as a component of the serum lipoproteins at its serosal surface. Thus, it was important to determine whether the serum lipoproteins could regulate intestinal HMG-CoA reductase. The data in Table VI demonstrate that the addition of cholesterol containing lipoproteins to intestinal organ culture medium has little effect on HMG-CoA reductase. The concentrations used were severalfold greater than physiologic and greater than that which suppresses reductase in other systems.

Failure of lipoproteins to suppress cholesterol synthesis could have been due to their failure to penetrate the lamina propria, and thus failure to reach the cell surface where binding and internalization would presumably occur. This possibility was assessed by determining the volume of extracellular fluid associated with different concentrations of taurocholate and 0.2 mM cholesterol plus 2 mM taurocholate. The extracellular fluid volume as determined with [carboxyl-^14^C]insulin under the same circumstances was found to be 8.1 ± 0.7 μl/specimen. Thus the space in which the lipoproteins were distributed was at least as great as the space in which a standard marker of extracellular fluid volume was distributed. These results do not provide any insight about whether the lipoproteins bound to or were internalized by the intestinal epithelial cells. However, the data do suggest that the lipoproteins could diffuse through the extracellular space of the cultured intestine as well as insulin implying that failure of the lipoproteins to penetrate the lamina propria was not the explanation for their failure to suppress HMG-CoA reductase.

**DISCUSSION**

The present studies utilizing cultured canine intestine were undertaken to resolve the conflicting reports in the literature regarding the regulation of intestinal cholesterol synthesis. Dog intestine was chosen for two reasons. First, the intestine of dog and rabbit could be successfully cultured, whereas that of the rat could not. Second, sterol and lipoprotein metabolism (34) in dog appears to be more comparable to man’s than does that of the rabbit (35).
The viability of tissue after 6 h of culture was demonstrated by light and electron microscopy as well as by its ability to actively transport a glucose analogue. The latter criterion is especially useful since transport processes seem to be sensitive indicators of metabolic dysfunction. In addition, the ability of the cultured mucosa to remove and metabolize lipid from the medium was demonstrated. On the basis of these results, it was concluded that cultured canine intestine was an appropriate system for studying the regulation of intestinal cholesterol synthesis, since the epithelial cells were fully viable and retained characteristic functions of intestinal epithelium.

In a variety of cultured cell lines (17, 29, 36) and in isolated perfused livers (31), it has been observed that when the cholesterol content of the medium bathing the cells is reduced there is a marked increase in the rate of cholesterol synthesis. Thus, the increase in HMG-CoA reductase and cholesterol synthesis in intestine cultured in the absence of lipids was anticipated. Moreover, this increase seems to require synthesis of new HMG-CoA reductase since, as in other tissues, it could be prevented by the addition of cycloheximide to the medium. This increase does not appear to be an artifact induced by tissue culture techniques since Anderson and Dietschy (11) have demonstrated a similar phenomenon in vivo, in a variety of tissues including intestine, by rendering rats hypolipoproteinemic with the drug 4-aminopyrazol (3,4)n pyrimidine.

One of the primary objectives of this study was to resolve the questions regarding which sterols could affect intestinal cholesterol synthesis. The greatest uncertainty involved the ability of cholesterol to act as a regulator of its own synthesis in intestine as it does in other tissues. The suppression of cholesterol synthesis produced by the addition of cholesterol to the culture medium convincingly demonstrates that autoregulation can occur. Furthermore, as in other tissues (32) the oxygenated sterols 7-ketocholesterol and 25-hydroxycholesterol are even more potent inhibitors of cholesterol synthesis. However, the response to all sterols appears to be less marked than in other cultured tissues. This may be similar to what has been described in liver where the response to 7-ketocholesterol (37) and 25-hydroxycholesterol is also less profound and is short-lived probably because rapid metabolism and excretion of these compounds occurs. Indeed, it is likely that intestine can excrete and possibly metabolize these sterols, thus limiting their entry into the sterol pool responsible for regulating the rate of de novo cholesterol synthesis. Further support for this concept derives from the fact that despite increased cholesterol ingestion, the cholesterol content of intestinal epithelium fails to increase (38).

In previous investigations (4), bile salts reduced the rate of intestinal cholesterol synthesis. This was true in cultured canine intestine. This result was especially impressive because concentrations of bile salts far below the physiologic range had to be used to avoid tissue damage in this system. Despite this, bile salts were more effective inhibitors than cholesterol and were as effective as 25-hydroxycholesterol. It appears to be the only tissue thus far identified where bile salts exert an effect on cholesterol synthesis independently. Whether they directly regulate the activity of HMG-CoA reductase or act by first changing some other aspect of intestinal cholesterol metabolism such as the rate of cholesterol esterification (39) remains to be explored.

In several tissues, including fibroblasts (29) and liver (33, 40), specific lipoproteins are efficient vehicles for the delivery of cholesterol to the cells. In the present study with acutely isolated intestine, addition of lipoproteins failed to suppress cholesterol synthesis. However, in the studies of Anderson and Dietschy (12) when rats were made hypolipoproteinemic with 4-aminopyrazol (3,4)n pyrimidine, cholesterol synthesis in intestine increased after 24 h. This increased rate of synthesis could be decreased by infusion of low density lipoprotein. Their results suggest that the intestine is a lipoprotein-sensitive organ. However both fibroblasts (41) and leukocytes (42) must be cultured for more than 12 h in the absence of cholesterol in order to induce an increase in the number of lipoprotein receptors. In an analogous fashion, development of lipoprotein receptors in intestinal mucosa may require a period of hyperlipidemia longer than the 6 h which could be utilized in this study. With prolonged hyperlipidemia, the number of receptors may increase, as in other tissues, allowing an increase in the capacity of the enterocyte to bind and utilize cholesterol from circulating lipoproteins (12). If this is the case one might hypothesize that the intestine’s cholesterol requirement is partially met by dietary cholesterol. When this is inadequate, increased cholesterol synthesis ensues followed by increased utilization of cholesterol from serum lipoproteins.

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