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 digalactin-precipitable sterols during this period. This increase could be prevented by the addition of cycloheximide to the culture. Pure cholesterol, 7-ketocholesterol, 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 with the three sterols was 77 ± 4%, 68 ± 5%, and 58 ± 3% of control postculture value). Bile salts at 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 cholesterol 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), Shefer 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-ketocholesterol 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 hypolipoproteinemic 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 re-crystallized 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% OV17 on Gas-Chrom Q at 230°C). 7-Ketocholesterol and 25-hydroxycholesterol were obtained from Steraloids Inc. (Wilton, N. H.) and showed less than 3% impurities by gas liquid chromatography. Isotopes were obtained from New England Nuclear. [3-4C]HMG-CoA was synthesized by the method of Goldberg and Pintar (14). Mylar-backed 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 Biological 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 chow (Allied Mills, Inc., Chicago, Ill.) for at least 2 weeks and the mucosa was removed, rinsed in Dulbecco's medium, and the mucosa was removed from the ileocecal valve was segment of ileum 10 to 20 cm proximal to from Eastman Kodak, and fatty acids, cofactors, and enzyme sub-

Aqueous solution of cofactors. Final cofactor concentrations were 0.7 ml of 0.1 M potassium phosphate buffer (pH 7.2) followed by DL-[5-3H]mevalonic acid, 2 pmol (20,000 dpm/pmoll. Incubations were terminated after 15 min by addition of 0.025 ml of concentrated HCl followed by oxalacetic acid, 2 pmol (30,000 dpm/pmoll). After standing over-night, the samples were centrifuged and a portion of the supernatant was applied to activated (100°C for 30 min) Silica Gel G thin layer chromatography sheets and developed in benzene:acetone (1:1). The Rf region 0.5 to 0.9 was removed by scraping with a razor blade and added to 100 ml of Aquasol and the radioactivity was measured in a Packard Tri-Carb liquid scintillation counter. Control assays with HCl added prior to addition of substrate showed essentially no [3H]mevalonate formation. Activity 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 constituent focus) 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 [2,4-14C]acetate (specific activity 3.6 µCi/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 digitonide. 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 intestines. Since cold and excess unlabeled glucose gave similar results, cold was used for the routine studies. Thus, a 3-O-methylglucose concentration of 5 mm was used. At this concentration, 85% of the transport was active and the process was not saturated.
Lipids—An important function of bile salts is the facilitation of lipid absorption in the presence of 5% albumin.

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 the same 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 (28) 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, the portion of which is absorbed, was taken up. 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 4 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 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 absorption in the absence of detergent is quite limited.
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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.

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 4 and 6 h as between 0 and 2 h of culture. This is very close to what would be predicted from the data in Table I.

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 × 10² 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-methylglucose (96% 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 taurocholate (0.25 or

![Graph](https://via.placeholder.com/150)

**TABLE III**

Acetate incorporation into digitonin precipitable sterols

Intestinal mucosa was cultured as described under "Materials and Methods." [2-^14C]Acetate was added to the medium for the period indicated, after which the tissue was removed and lipids were extracted and saponified. The digitonide precipitate was prepared, isolated, dissolved in methanol, and the radioactivity measured. Results are mean ± S.E. of three experiments.

<table>
<thead>
<tr>
<th>Culture time</th>
<th>Incorporation rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>h</td>
<td>pmol/min/mg</td>
</tr>
<tr>
<td>0–2</td>
<td>5.5 ± 1.8</td>
</tr>
<tr>
<td>4–6</td>
<td>11.1 ± 2.5</td>
</tr>
</tbody>
</table>

**TABLE IV**

Effect of cycloheximide and cholesterol on intestinal HMG-CoA reductase

Dog intestine was cultured as described under "Materials and Methods." In Experiment a, samples from the same intestine were assayed for HMG-CoA reductase before culture and after 6 h of culture with or without cycloheximide. In Experiment b, samples from another intestine were assayed before and after culture for 2 or 6 h. In some samples, cholesterol was added to the medium at the start of culture and in others it was added after 2 h of culture. Results are mean of two determinations.

<table>
<thead>
<tr>
<th>Duration of culture</th>
<th>Material added</th>
<th>HMG CoA reductase Final % increase</th>
<th>Initial % increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>h</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Experiment a**

<table>
<thead>
<tr>
<th>h</th>
<th>Material added</th>
<th>Initial % increase</th>
<th>Final % increase</th>
<th>% increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>16.8</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>71.5</td>
<td>100</td>
<td>426</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.2 mm cycloheximide at 0 h</td>
<td>10.7</td>
<td>64</td>
<td></td>
</tr>
</tbody>
</table>

**Experiment b**

<table>
<thead>
<tr>
<th>h</th>
<th>Material added</th>
<th>% increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.8</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>12.5</td>
<td>216</td>
</tr>
<tr>
<td>6</td>
<td>21.9</td>
<td>378</td>
</tr>
<tr>
<td>6</td>
<td>14.9</td>
<td>257</td>
</tr>
<tr>
<td>6</td>
<td>14.1</td>
<td>243</td>
</tr>
</tbody>
</table>
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activity was determined. Results are mean ± S.E. of six or more

blotted to remove any medium adherent to the edges of the
determinations.

After 4 additional h tissue was removed and the HMG-CoA reductase

activity was determined. Results are mean ± S.E. of six or more
determinations.

Thus, it was important to determine whether the serum

cholesterol has little effect on HMG-CoA reductase. The concentra-
tion of cholesterol was 0.25 mM (n = 13) 86.5 ± 6.1 0.05

0.5 mM (n = 13) 75.5 ± 6.1 0.005

tissue was removed from the medium and gently

blotted to remove any medium adherent to the edges of the
tissue. The tissue was solubilized and the radioactivity deter-
mained. The radioactivity in 10 μl of medium was determined
and from this the volume of medium associated with the tissue
was calculated to be 13.3 ± 1.4 μl of medium/specimen. The
extracellular fluid volume as determined with [carboxyl-
14C]ulin 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 inulin implying that failure of the lipoproteins to penetrate the lamina propria was not the
explanation for their failure to suppress HMG-CoA reductase activity.

TABLE V

Effect of bile salts on intestinal HMG-CoA reductase

Intestinal mucosa was cultured as described under "Materials and
Methods." After 2 h the medium was changed to one containing bie
salts and 5% bovine serum albumin. After 4 additional h of culture
the tissue was removed and the specific activity of HMG-CoA
reductase determined. Results are mean ± S.E.

<table>
<thead>
<tr>
<th>HMG-CoA reductase</th>
<th>p &lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>% 6-h control</td>
<td></td>
</tr>
<tr>
<td>Taurocholate</td>
<td></td>
</tr>
<tr>
<td>1 mM (n = 22)</td>
<td>89 ± 5.1</td>
</tr>
<tr>
<td>2 mM (n = 24)</td>
<td>62.8 ± 3.1</td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
</tr>
<tr>
<td>0.2 mM + taurocholate, 1 mM (n = 8)</td>
<td>65.8 ± 6.0</td>
</tr>
<tr>
<td>0.2 mM + taurocholate, 2 mM (n = 8)</td>
<td>67.1 ± 4.5</td>
</tr>
<tr>
<td>Taurochenodeoxycholate</td>
<td></td>
</tr>
<tr>
<td>0.25 mM (n = 13)</td>
<td>86.5 ± 6.1</td>
</tr>
<tr>
<td>0.5 mM (n = 13)</td>
<td>75.5 ± 6.1</td>
</tr>
</tbody>
</table>

TABLE VI

Effect of lipoprotein on intestinal HMG-CoA reductase

Intestinal mucosa was cultured as described under "Materials and
Methods." After 2 h the medium was changed to one containing canine lipoproteins. After 4 additional h of culture the tissue was removed and the specific activity of HMG-CoA reductase was deter-
mained. Results are mean ± S.E.

<table>
<thead>
<tr>
<th>HMG-CoA reductase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>0.07 mg/ml of lipoprotein cholesterol added</td>
</tr>
<tr>
<td>% 6-h control</td>
</tr>
<tr>
<td>Chylomicrons</td>
</tr>
<tr>
<td>(&lt;n = 4)</td>
</tr>
<tr>
<td>&lt;1.006 (n = 9)</td>
</tr>
<tr>
<td>1.006–1.063 (n = 13)</td>
</tr>
<tr>
<td>1.063–1.21 (n = 4)</td>
</tr>
</tbody>
</table>

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 extra-
cellular fluid in which lipoproteins added to the culture
medium were distributed. Chylomicrons containing H tri-
glyceride were added to the culture medium. After 4 h of
culture the tissue was removed from the medium and gently
blotted to remove any medium adherent to the edges of the
tissue. The tissue was solubilized and the radioactivity deter-
mained. The radioactivity in 10 μl of medium was determined
and from this the volume of medium associated with the tissue
was calculated to be 13.3 ± 1.4 μl of medium/specimen. The
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cultured intestine as well as inulin implying that failure of the lipoproteins to penetrate the lamina propria was not the
explanation for their failure to suppress HMG-CoA reductase activity.

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 (11), 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 (3,4a)-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 (35) 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 occur. 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 (36).

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 was also noteworthy that at submaximal doses the bile salt and cholesterol effects were additive, but with maximal amounts of each, their effect was not additive. Hence intestine 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 (3,4a)-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 hypolipidemia longer than the 6 h which could be utilized in this study. With prolonged hypolipidemia, 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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