3-Hydroxy-3-methylglutaryl coenzyme A reductase in isolated villous and crypt cells of the rat ileum

Juanita L. Merchant and Renu A. Heller

Department of Biological Sciences and the Department of Medicine, School of Medicine, Stanford University, Stanford, CA 94305

Abstract The localization of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase, E.C. 1.1.1.34) in the villous and crypt cells of the small intestine was accomplished after separating these cells from the mucosal layer by sequential dissociation in a "dual-buffer" system. Consistent separation was demonstrated by using the marker enzymes alkaline phosphatase, specific to the villous cell, and thymidine kinase, specific to the crypt cell. Cells obtained were 95–100% viable, and no relative difference in lability was observed, as evidenced by the equal distribution of acid phosphatase. This method of cell separation was an improvement over the "scraping" technique which damaged cells severely and produced villous preparations that contained little or no reductase activity. The HMG-CoA reductase specific activity in whole cell homogenates of the ileal villi was 0.47 and of the crypts was 0.27 nmol/min per mg of protein, considerably higher values than have been reported earlier. Also in comparison to the crypts, the villi incorporated 1.5-fold more [^{14}C]-acetate into sterols, a ratio similar to that describing the distribution of HMG-CoA reductase in the two cell populations. These results unequivocally establish that the villi have higher HMG-CoA reductase activity than the crypts and confirm an earlier report from this laboratory that the villi are a major site of sterol synthesis. The sterol biosynthetic capacity of the small intestine was highest in the ileum and decreased towards the jejunum. The HMG-CoA reductase specific activity of the ileum averaged 0.30 and that of the jejunum 0.10 nmol/min per mg of protein; however, the cholesterol content of the ileum was slightly lower than the jejunum. These results are discussed to suggest the possibility that the sterol content of the ileum may largely be due to in situ synthesis.

Supplementary key words HMG-CoA reductase · sterol synthesis · jejunum

The enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (E.C. 1.1.1.34) catalyzes the reduction of HMG-CoA to mevalonic acid in the liver and the small intestine. The properties of the intestinal and liver HMG-CoA reductase are similar enough to suggest that they are possibly the same enzyme. Both exhibit a circadian rhythm (1, 2), have similar kinetics (3), require NADPH (3), and cross-react with antibody to the liver reductase (4). It is therefore possible that they have the same primary regulator.

It is known that dietary cholesterol regulates its endogenous synthesis in the liver probably by altering the activity of HMG-CoA reductase (5, 6). However, it is not established whether cholesterol regulates its own synthesis in the small intestine. The decrease or increase in sterol synthesis with bile infusion or diversion from the intestinal lumen led Dietschy and Siperstein to conclude that bile acids may also regulate intestinal sterol synthesis (7). When the effect of feeding bile acids and cholesterol on the circadian rhythm of HMG-CoA reductase was examined by Shefer et al. (8), an inhibition of only the circadian rise was observed with bile acids alone. Combined feeding of bile acids and cholesterol, however, depressed HMG-CoA reductase activity further. Westergaard and Dietschy (9) have recently suggested that bile acid micelles enhance the uptake of long chain fatty acids and cholesterol into the intestinal mucosal cell by decreasing the resistance to diffusion created by the unstirred water layer. This implies that the entry of dietary cholesterol into the cell is facilitated by bile acids and therefore both bile acids and cholesterol play a role in controlling HMG-CoA reductase in the small intestine. A direct correlation between the rate or efficiency of cholesterol or bile acid uptake and the alteration in HMG-CoA reductase activity has not been made in vitro.

The intestinal mucosa has been fractionated by several investigators by using mechanical or biochemical methods. The mechanical methods range from scraping the mucosa with a microscope slide (7) or slicing the mucosa into layers with a "planing" apparatus (10) or a cyostat (11) to mechanical vibration of an everted intestine in different buffers (12, 13). The biochemical methods include enzymatic

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; MVA, mevalonate; DTT, dithiothreitol.

digestion (14, 15) or incubation of the gut in calcium-binding agents such as citrate (16) or EDTA (17). The last two methods employ buffer systems without proteases, and this further ensures minimal damage to the epithelial cells as opposed to cells fractionated by mechanical scraping or slicing. Cells prepared by these two buffer methods have been used in studies on differentiation (13, 18), transport (19), and mitochondrial metabolism of sugars, fatty acids, and amino acids (20). We found the method of Weiser (18) to be most satisfactory in fractionating the mucosa into viable cell populations. This method had been used previously to study the incorporation of monosaccharides into membrane glycoproteins during crypt cell differentiation into villi (18).

The HMG-CoA reductase activities and cholesterol synthesis in the villous and crypt cell fractions obtained by scraping the intestinal epithelium were determined by Shefer et al. (3) and by Dietschy and Siperstein (7) and Muroya, Sodhi and Gould (21), respectively. Dietschy and Siperstein (7) observed little or no incorporation of acetate into cholesterol in the villous cells and had concluded that only the rapidly dividing crypt cell region was the active site. Similarly, Shefer et al. (3) reported little or no HMG-CoA reductase activity in villi. Muroya, Sodhi, and Gould (21) determined rates of sterol synthesis from [^{14}C]acetate in villous and crypt cells by use of three assay methods, incubation of villous and crypt cell fractions obtained by scraping, incubation of pieces of whole intestine prior to fractionation, and short-term in vivo experiments in which fractionation was carried out both by graduated scraping and by a cryostat method. All the results were in agreement in showing that villous cells, free of crypt and muscle cells, were capable of sterol biosynthesis and, based on per milligram of tissue protein, had at least as high a sterol synthetic rate as crypt cells.

Mak and Trier (22) reported radioautographic evidence that showed a higher incorporation of [^{3}H]-mevalonate into sterol by the villi, indicating a selectively higher capability of these cells to synthesize sterol from products beyond the rate-limiting step. Although this approach does not contribute information on the presence of HMG-CoA reductase, the results are consistent with those of Muroya et al. (21), in that both villous and crypt cells were shown to synthesize sterols. To resolve the ambiguity of whether the villous cells synthesize significant amounts of sterol from [^{14}C]acetate and also contain high HMG-CoA reductase activity, we have examined villous and crypt cell populations prepared by an entirely different technique. These cells were obtained by a sequential dissociation from the intestinal epithelial surface using the buffer system described by Weiser (18). Cell separation and viability differences were tested by specific marker enzymes and cell stability was checked over the experimental period. Our results have revealed that the distribution of HMG-CoA reductase activity as well as sterol synthesis from acetate was 1.5–2.0-fold higher in the villi than in the crypts.

METHODS

Animals

Male, Sprague-Dawley rats weighing 200–300 g were maintained under a reversed lighting cycle (lights on 4 PM to 4 AM) and fed Purina rat chow ad libitum. The animals were acclimated to this light cycle for at least 2 weeks before being used. Intestines were obtained from rats killed in the middle of the dark phase, a time of maximum sterol synthesis, denoted as D-6, or from those killed in the middle of the light phase, a time of minimum synthesis, denoted as L-6 (1).

Materials

The substrate, *R,S*-[3-^{14}C]HMG-CoA was prepared using the methods of Goldfarb and Pitot (23) and Hilz et al. (24). Chemical materials were purchased from the following commercial sources: *R,S*-[3-^{14}C]HMG acid (2.25 mCi/μmol), *R,S*-[5-^{3}H]mevalonic acid (dibenzylethylene diamine salt) (3.25 mCi/mmol), sodium [2-^{14}C]acetate (20 mCi/μmol), sodium [1-^{14}C]-octanoate (25 μCi/μmol) from New England Nuclear, Boston, MA; unlabeled HMG acid and [6-^{3}H]thymidine (7 Ci/mmol) were from Schwarz-Mann, Orangeburg, NY; *p*-nitrophenolphosphate, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, NADP, bovine serum albumin (Fraction V), L-glutamine, and *N*-tris(hydroxymethyl)methyl glycine (Tricine) were from Sigma, St. Louis, MO; D-glucose from Mallinckrodt, St. Louis, MO; dithiothreitol from Calbiochem, San Diego, CA; Swim's S-77 medium from Grand Island Biological Company, Grand Island, NY; mylar-backed silica gel chromatography sheets from Eastman Kodak, Rochester, NY; DEAE paper discs (DE-81, 1.6 cm) from Whatman, Clifton, NJ; and coenzyme A from P–L Biochemicals, Milwaukee, WI.

Method of cell isolation

Epithelial cells were isolated from the mucosal layer of 16–20 cm of rat intestinal segments according to the method described by Weiser (18) with some modifications. Intestinal segments were excised and collected in ice-cold saline. Each segment was flushed

with 0.9% NaCl and further manipulations were carried out at room temperature. Each gut loop was distended by holding one end closed with the index finger and thumb and filling with Buffer A equilibrated to 37°C. Buffer A contained 1.5 mM KCl, 96 mM NaCl, 27 mM sodium citrate, 8 mM KH_2PO_4, 5.6 mM Na_2HPO_4, at pH 7.3. Each distended gut loop was placed in 50 ml of Buffer A in a 250-ml Nalgene plastic flask and incubated at 37°C in a gyratory water bath at a rotation speed of 160–200 rpm for 15 min. Buffer A was decanted and the segments rinsed on the outside and then distended with Buffer B kept at 37°C which contained 0.137 M NaCl, 2.69 mM KCl, 8.10 mM Na_2HPO_4, 1.47 mM KH_2PO_4, 1.5 mM EDTA, 0.5 mM dithiothreitol at pH 7.4. Weiser (18) described a method whereby short incubations in Buffer B consecutively released cells starting from the most apical villous cells to the lower crypt cells. We found that a first incubation in Buffer B for a maximum of 40 min released most of the villous cells and another incubation of 20 min gave a predominantly crypt cell population. The cells were removed by syringing Buffer B through the gut loop and applying gentle pressure along the length of the intestine with the fingertips. This cell isolation method is hereafter referred to as the "dual-buffer" system. Villi and crypts were pelleted in plastic centrifuge tubes at 500 *g* for 5 min, the supernatant was aspirated off, and the cells were weighed. They were suspended in a hypotonic medium that contained 1.5 mM Tris, 10 mM KCl, and 10 mM $MgCl_2$ at pH 7.4 and lysed by homogenizing with 10 passes in tight-fitting Kontes Dounce tissue grinders (size K-885300).

Incubation studies

The cells, 0.1–0.2 g wet weight per ml of Swim's S-77 medium containing 50 mM Tricine, 22.2 mM D-glucose, 2.05 mM L-glutamine, 1.5% bovine serum albumin, and 10 mM bicarbonate at pH 7.4 were incubated in 50-ml Nalgene flasks in a gyratory water bath at 37°C in an atmosphere of 95% O_2 and 5% CO_2. At designated times, cells were removed and an aliquot was stained with 0.05% trypan blue. Cells excluding the dye were considered viable. The remaining suspension was pelleted and washed twice with Buffer B at 500 *g* to remove excess BSA that would interfere with protein determinations. The cells were then lysed and diluted to the appropriate protein concentration with the Dounce medium described above and assayed for enzyme activities.

In vitro sterol synthesis

Approximately 0.2–0.4 g of isolated villi or crypts suspended in 2 ml of Swim's medium or 2 ml of whole cell homogenate in Swim's medium was incubated in a final concentration of 0.052 mM NADP, 1.0 mM glucose-6-phosphate, and 2.4 mM glutathione for 5 min at 37°C before initiating the reaction with the addition of 10 μmol of sodium [2-^{14}C]acetate (0.2 μCi/μmol) or 0.15 μmol of sodium [1-^{14}C]octanoate (27 μCi/μmol). The reaction was terminated after 30 min by precipitating the tissue with an equal volume of 10% tricholoroacetic acid, and the pellet was collected by centrifugation at 2000 *g* for 10 min, leaving a clear TCA supernatant that contained no labeled sterol. The amount of sterol synthesized was determined as described below.

In vivo sterol synthesis

Intraperitoneal injections of 17 nmol of [1-^{14}C]-acetate (7 μCi/nmol) were given 30 min before the D-6 hour. Villi and crypts were isolated from 20-cm ileal segments as described above, and the incorporation of [^{14}C]acetate into sterols was measured in whole cell homogenates.

Determination of sterol synthesis from [^{14}C]acetate

Cell pellets were saponified over a steam bath for 2–3 hr after the addition of 0.3 ml of 33% KOH, 1 ml of 1 mg/ml carrier cholesterol, 18,000 dpm of [^{3}H]cholesterol to determine extraction efficiencies, and 2 ml of 95% ethanol until all tissue was dissolved. Nonsaponifiable lipids were extracted three times in a total volume of 20 ml with petroleum ether and washed with water plus phenolphthalein. The petroleum ether layer was removed, taken to dryness under nitrogen, redissolved in 1 ml of acetone–absolute ethanol 1:1 and acidified with one drop of 10% acetic acid. Sterols were precipitated with 1 ml of 1% digitonin in 50% ethanol and kept at room temperature for at least 2 hr. The sterol–digitonides were washed with 1 ml of 50% ethanol, 5 ml of absolute ethanol–anhydrous ether 1:1, and then with 5 ml of anhydrous ether. The remaining digitonide pellet was dried under nitrogen, redissolved in 1–2 ml of methanol, and counted in toluene scintillation fluid.

Kinetic studies on villous and crypt homogenates

A 7% (wt/vol) villous homogenate and a 6% (wt/vol) crypt homogenate in Dounce medium were assayed for HMG-CoA reductase under the standard assay conditions described below using 100–700 μl aliquots. The activity per min of both villous and crypt homogenates plotted as a function of protein concentration is shown in **Fig. 1A**. Villous homogenate activity remained linear for up to 1.9 mg of protein while that of crypt homogenates was linear up to

1.3 mg of protein. Fig. 1B represents a time course over 60 min for villous and crypt cell homogenates and shows that the reaction rate for both cell types remained linear for at least 40 min under these assay conditions.

Enzyme assays

HMG-CoA reductase assays were conducted at pH 7.4 in a cofactor mixture consisting of 10 μmol of dithiothreitol, 30 μmol of EDTA, 70 μmol of NaCl, 2 enzyme units of glucose-6-phosphate dehydrogenase, 30 μmol of glucose-6-phosphate, and 3 μmol of NADP to which 100–700 μl of whole cell homogenates was added and the volume made up to 970 μl with Buffer C containing 0.25 M sucrose, 0.05 M KCl, 0.04 M KH_2PO_4, 0.03 M EDTA and 10 mM dithiothreitol. This mixture was preincubated at 37°C for 5 min, and the reaction was initiated with 30 μl of *R,S*-[3-^{14}C]HMG-CoA containing 337 nmol (460 dpm/nmol). This substrate concentration was saturating for both villous and crypt homogenates. The reaction was terminated after 30 min with 100 μl of 10 N NaOH. Labeled [^{14}C]mevalonate was extracted with anhydrous diethyl ether using 20 nmol of mevalonic acid as carrier and containing [5-^{3}H]-mevalonic acid (200,000 dpm) as an internal standard (25).

Alkaline phosphatase was assayed at pH 9.4 (18), and acid phosphatase at pH 5.2 (26) utilizing *p*-nitrophenolphosphate as substrate for both. Thymidine kinase was assayed using 1.7 μmol of [6-^{3}H]thymidine (0.73 μCi/μmol) instead of [^{14}C]thymidine as described by Klemperer and Haynes (27). Supernatant aliquots of 25 μl, containing the phosphorylated product, were plated in duplicate on 1.6 cm Whatman DEAE paper discs (28). These discs were swirled in 25 ml of 1 mM ammonium formate at room temperature in a gyratory water bath at a rotation speed of 200–240 rpm for 15 min, the solution decanted, and the discs rinsed once with 25 ml of ammonium formate and twice with distilled water. These were oven dried and counted in toluene scintillation fluid.

Cholesterol determination

An average of 47 mg of cell homogenate in 4 ml was saponified for 3 hr at 75–80°C over a steam bath in a mixture containing 1 ml of 33% KOH, 10 ml of 70% ethanol to which 100 μl of [^{14}C]cholesterol (40,000 dpm) was added to obtain the extraction efficiencies. After cooling, nonsaponifiable lipids were extracted three times with 20 ml volumes of petroleum ether and taken to dryness. The dried sample was redissolved in 1–2 ml of isopropanol and aliquots were taken to determine the extraction efficiency by counting in toluene scintillation fluid. The cholesterol content was determined using the colorimetric assay described by Leffler (29).

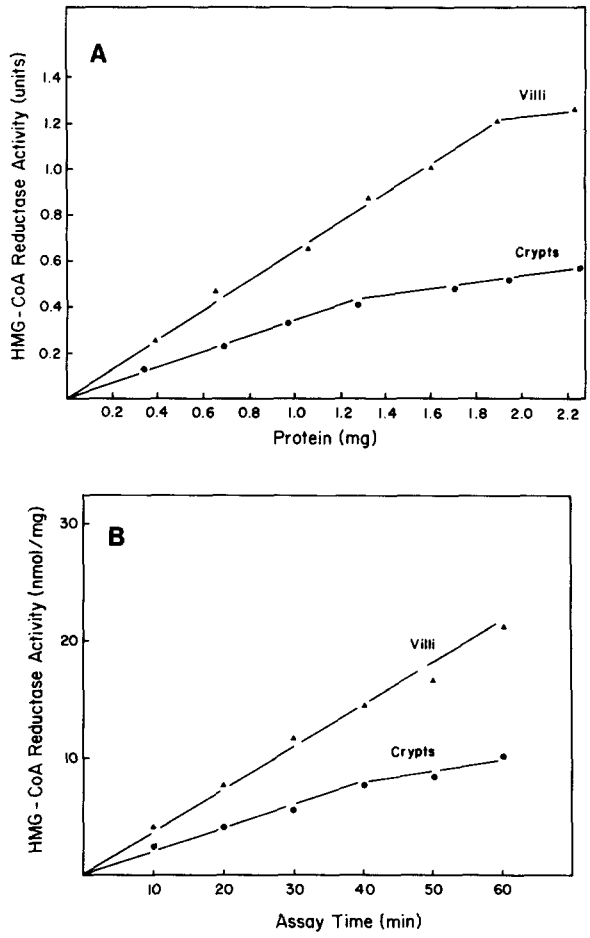

Fig. 1. Kinetic studies on villous and crypt homogenates. Villous and crypt cells were isolated from the ileum at the D-6 hour and assayed for HMG-CoA reductase as described in Methods. One unit of enzyme activity is defined as the amount that produces 1 nmol of MVA per min. Each time point represents the mean of duplicate assays. *A*. Increasing aliquots of the total cell homogenates were assayed for 30 min. *B*. Villous homogenate containing 1.4 mg of protein and crypt homogenate containing 1.3 mg of protein were assayed for varying lengths of time.

Protein determination

Each aliquot including the standards was brought up to 1.0 ml in 0.2 N NaOH, and the protein content was determined by the biuret method (30) using bovine serum albumin as a standard.

RESULTS

Distribution of HMG-CoA reductase activity in epithelial cells of the jejunum and ileum

To confirm that cells isolated by the dual-buffer system reflected some of the properties observed pre-

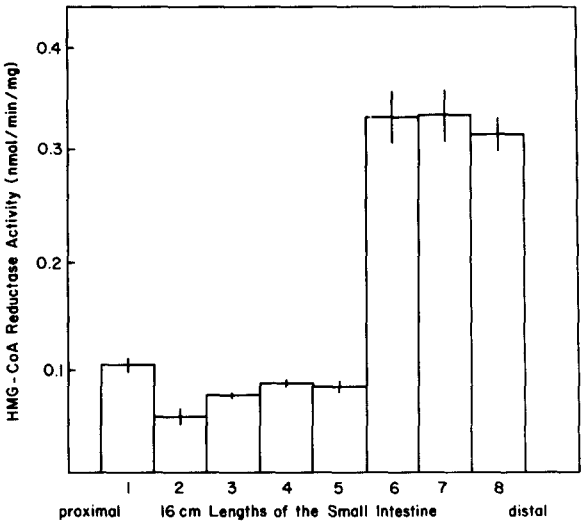

Fig. 2. Distribution of HMG-CoA reductase activity in epithelial cells of the jejunum and ileum. The small intestine from the ligament of Trietz to the ileocecal junction was removed at the D-6 hour. Intestinal segments approximately 16 cm in length were excised and numbered 1 through 8 starting from the ligament. Villous and crypt cells from each segment were isolated, lysed in 4 ml of Dounce medium, and the homogenates were assayed in duplicate for HMG-CoA reductase as described in the text. The error bars represent SD.

viously (3, 7), the small intestine from the ileocecal junction to the ligament of Treitz was divided into eight segments of approximately 16 cm in length. Villous and crypt cells were isolated together from each segment and whole cell homogenates were assayed to determine HMG-CoA reductase specific activities at various sites along the intestinal tract. Activity in the distal segments was 2.5–3.0 times greater than in the proximal ones (**Fig. 2**) and, assuming a close correspondence between the activity of the enzyme and the sterol synthetic capacity of the tissue, this observation indicated greater sterol synthesis in the ileum. These results confirm the earlier studies on HMG-CoA reductase by Shefer et al. (3) and on sterol synthesis by Dietschy and Siperstein (7) and Muroya et al. (21). Since the major site of active sterol biosynthesis in the small intestine appears to be in the ileum, our subsequent experiments were performed with cells from this tissue.

Distribution of three marker enzymes in villous and crypt cells of the ileum

Cells were separated by the method of Weiser (18). In order to assess the adequacy of the separation, the activities of alkaline phosphatase, a villous enzyme, thymidine kinase, a crypt cell marker, and acid phosphatase, an enzyme distributed equally in the two cell types, were assayed. **Table 1** reports these results. Alkaline phosphatase was found to have at least 3-fold higher activity per mg of protein in the villi than in the crypts; the villi fraction contained 80–90% of the total alkaline phosphatase activity. The crypt fraction contained at least 4-fold higher thymidine kinase specific activity than the villi and accounted for approximately 80% of the total activity. Acid phosphatase specific activities were approximately the same in villi and crypts while the percent distribution of activities remained about 50:50. These results demonstrate that a high degree of separation of villous and crypt cells was obtained by this method, and that comparatively equal activities of acid phosphatase assured us that differences in alkaline phosphatase and thymidine kinase were not due to differences in cell stability.

Distribution of HMG-CoA reductase in villous and crypt cells of the ileum

Using these isolated cell preparations that had been characterized for their degree of cross-contamination, the distribution of HMG-CoA reductase in them was analyzed. In whole cell homogenates prepared at the D-6 or L-6 hours, HMG-CoA reductase activities were 1.6–1.8-fold higher in the villous population than in the crypt population. From D-6 rats the activities were 0.47 nmol/min per mg for the villi and 0.27 nmol/min per mg for crypts (Table 1); whereas from L-6 rats they were 0.27 nmol/min per mg for villi and 0.17 nmol/min per mg for crypts. The villi represented 75% of the total HMG-CoA reductase activity. Since the same percent distribution was found in repeated experiments, the specific activity ratio of villi to crypts or the percent distribution could now be used as an indicator of epithelial cell separation.

The HMG-CoA reductase activities at the D-6 hour of cells isolated by the dual-buffer system were compared with those of the mucosal scrapings obtained by the method of Dietschy and Siperstein (7). As presented in **Table 2**, more protein was recovered from both the villous and crypt fractions when using the scraping technique than with the dual-buffer method. This could possibly be the reason why the specific activities of HMG-CoA reductase in the "scraped" preparations were always lower. It is conceivable that the scraping technique removes tissue that contained little or no HMG-CoA reductase activity in addition to the active cell populations. It is important to note, however, that, again in comparison to the crypt cell fraction, the total as well as the specific activities of HMG-CoA reductase were higher in the villous population.

TABLE 1. Enzyme distributions in villi and crypts from the ileum

Enzyme Specific Activity	Villi		Crypts	
	Units/mg[d]	Mean % Total Activity[e]	Units/mg[d]	Mean % Total Activity[e]
Alkaline phosphatase[a] (5)[c]	4.70 ± 1.55 [7.0][f]	86 $P < 0.01$	1.26 ± 1.11 [3.2]	14
Thymidine kinase[b] (5)	1.59 ± 0.59 [7.0]	21 $P < 0.01$	11.22 ± 5.09 [3.2]	79
Acid phosphatase[a] (5)	8.98 ± 1.24 [6.85]	53 N.S.	8.69 ± 1.30 [6.85]	47
HMG-CoA reductase (4)	0.470 ± 0.10 [7.0]	75 $P < 0.02$	0.270 ± 0.07 [3.2]	25

[a] One unit of alkaline phosphatase or 1 unit of acid phosphatase is the amount that releases 1 μmol of *P*-nitrophenolphosphate per min.
[b] One unit of thymidine kinase is the amount that produces 1 pmol of thymidine phosphate per min.
[c] The values in parentheses represent the number of experiments performed in duplicate.
[d] Mean activity ± SD.
[e] Calculated from the number of units in the original homogenate volume.
[f] Total protein.
Villous and crypt cells isolated in the dual-buffer system were homogenized in Dounce medium and assayed for alkaline phosphatase, thymidine kinase, acid phosphatase, and HMG-CoA reductase as described in the text. Protein values for alkaline phosphatase, thymidine kinase, and HMG-CoA reductase were determined on the same samples. Protein values for acid phosphatase were determined separately on pooled samples of villi or crypts. The *P* values are the results of the Student's *t* test. All values are derived from cells obtained from D-6 rats.

Villous and crypt cell viabilities

Fig. 3 shows the percent viability, as measured by the ability of the cells to exclude trypan blue, and the HMG-CoA reductase activities of isolated villous and crypt cells that were incubated in Swim's medium for 2 hr. Over the first 30 min of incubation at 37°C, 98% of the villi and crypt cells excluded trypan blue, but beyond this point the villi showed a gradual increase in the uptake of the dye. HMG-CoA reductase activity in both cell types remained constant during this entire period. These results suggest that for these preparations the optimal time period for an in vitro study of sterol synthesis was 30 min, where the viabilities of the villous and crypt cells were about equal.

Sterol synthesis from [^{14}C]acetate by villous and crypt cells

To confirm that the HMG-CoA reductase activities accurately reflected the overall rate of sterol biosynthesis, the incorporation of acetate into digitonin-precipitable sterols was measured. The villi were observed to synthesize as much or more sterol from [^{14}C]acetate than the crypts in three different systems: (*a*) in cell homogenates, (*b*) in intact isolated cells, and (*c*) in vivo (**Fig. 4**). In all three cases, the separation of the cell types was demonstrated by marker enzymes or HMG-CoA reductase specific activity and the amount of sterol synthesized determined as described under Methods.

The villi incorporated at least 1.5–2.0-fold more acetate into digitonin-precipitable sterols than the crypts in cell homogenates and in vivo; however, incorporation by the isolated cells was about equal. This inconsistency is difficult to explain, but may be due to an excessive dilution of exogenous [^{14}C]acetate by acetyl-CoA derived endogenously from oxidation of fatty acids, which has been shown to occur at a

TABLE 2. Comparison of HMG-CoA reductase activity in villi and crypts obtained by the scraping and dual-buffer methods

	Total Protein	Total Activity	Specific Activity
	mg	*units*	*units/mg*
Scraping			
Villi (4)[a]	62.9	11.3	0.18 ± 0.03[b]
Crypts (4)	15.8	1.1	0.07 ± 0.01
Dual-buffer			
Villi (4)	7.0	3.3	0.47 ± 0.10
Crypts (4)	3.2	0.9	0.27 ± 0.07

[a] Number of experiments performed in duplicate.
[b] Mean ± SD.
Ileal segments 20 cm long were excised from D-6 rats and the villous and crypt cells were isolated by either scraping the mucosa with a microscope slide (7) or by incubating the gut loop in Buffer A and then Buffer B as described in Methods.

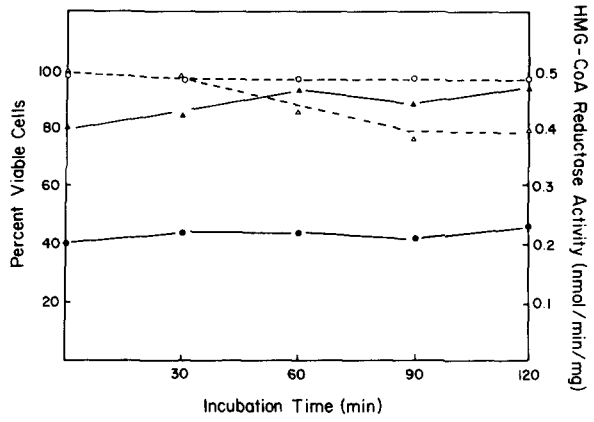

Fig. 3. Villous and crypt cell viabilities. After fractionation, the villous or crypt cells were placed immediately in modified Swim's S-77 medium and incubated for 2 hr at 37°C in an atmosphere of 95% O_2 and 5% CO_2. Every 30 min and up to 2 hr, the cells were removed and an aliquot taken to determine the uptake of 0.05% trypan blue. Cells excluding the dye were considered viable. The percent viable cells and the HMG-CoA reductase specific activities were both plotted as a function of the incubation time in min. Each time point represents the average of duplicate assays. △---△, Villous viability; ○---○, crypt viability; ▲——▲, villous HMG-CoA reductase activity; ●——●, crypt HMG-CoA reductase activity.

more rapid rate within the villi than within the crypts (20). In cell homogenates, rapid equilibration of acetate may circumvent this problem. When [^{14}C]octanoate was used as the substrate instead of [^{14}C]acetate, the incorporation into sterols was more than 2-fold higher in the villi than in the crypts. These results indicate that sterol synthesis in the villous cells might be underestimated in comparison to the crypt cells if dilution is not taken into account when [^{14}C]acetate is used as the substrate, but perhaps overestimated when [^{14}C]octanoate is used, due to their higher fatty acid oxidation capacity.

Cholesterol content of jejunal and ileal segments

The total cholesterol content was determined from 16-cm segments of the small intestine starting from the ligament of Trietz to the ileocecal junction. The dual-buffer system yielded relatively equivalent amounts of protein from both the jejunum and the ileum. There were no large differences in total cell cholesterol content (**Fig. 5**); however, the lowest cholesterol to protein ratios occurred in those segments that exhibited the highest HMG-CoA reductase activities (3, Fig. 2) and sterol synthesis (7, 21).

Subcellular fractionation of villous and crypt cells

The subcellular localization of HMG-CoA reductase in villous and crypt cells was reported by Shefer et al. (3) to be predominantly distributed in the mitochondrial and microsomal fractions, unlike the liver where HMG-CoA reductase is located only in the microsomes. To corroborate this finding, we fractionated the villous and crypt populations ultracentrifugally in Buffer D containing 0.25 M sucrose, 0.05 M KCl, 0.04 M KH_2PO_4, and 0.03 M EDTA. The original 7% villous and 6% crypt homogenates in Dounce medium were assayed for HMG-CoA reductase and the three marker enzymes. These homogenates were diluted 2-fold with Buffer D, and the "nuclear" fraction was collected after centrifugation at 1500 *g* for 10 min and washed once with Buffer D. The villi contained a "fat" layer that floated to the top of the post-nuclear supernatant; this was also removed and assayed for HMG-CoA reductase. The "mitochondrial" fraction was collected after centrifugation at 22,300 *g* for 15 min and washed once with Buffer D. The post-mitochondrial supernatant was fractionated into "microsomal" and "cytosol" fractions at

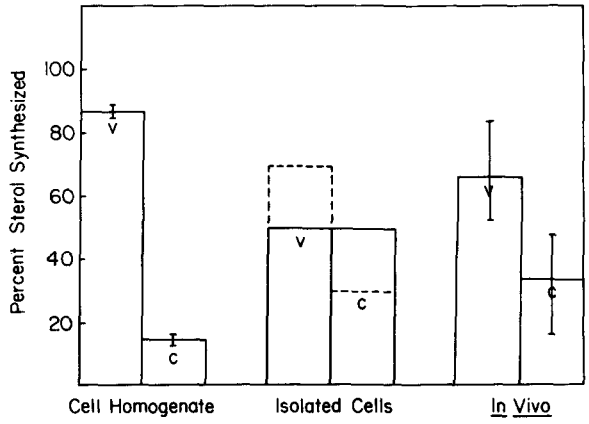

Fig. 4. Sterol synthesis from [^{14}C]acetate by villous and crypt cells of the ileum. Approximately 0.2–0.4 g of isolated villous or crypt cells were suspended or homogenized in 2 ml of Swim's medium and incubated with 10 μmol of sodium [2-^{14}C]acetate (0.2 μCi/μmol) or 0.15 μmol of [1-^{14}C]octanoate (25 μCi/μmol). In vivo determinations involved injecting 17 nmol of sodium [1-^{14}C]acetate (7 μCi/nmol) intraperitoneally 30 min before the D-6 hour. Villi and crypts were isolated separately as described under Methods, and their protein contents as well as total radioisotope incorporated were as follows: Cell homogenates: total villous protein, 15.1 mg; total dpm incorporated, 6045, total crypt protein, 11.8 mg; total dpm incorporated, 653. Isolated cells from [^{14}C]acetate: total villous protein, 15.1 mg; total dpm incorporated, 18,790; total crypt protein, 11.8 mg; total dpm incorporated, 18,830. From [^{14}C]octanoate: total villous protein, 6.4 mg; total dpm incorporated, 400; total crypt protein, 8.0 mg; total dpm incorporated, 250. In vivo: Total villous protein, 45.6 mg; total dpm incorporated, 14.5×10^5; total crypt protein, 23.9 mg; total dpm incorporated, 1.9×10^5. The recovery in these determinations was consistently around 50–60% and was corrected for in the samples. The solid lines represent sterol synthesis from [^{14}C]acetate, and the dotted lines represent synthesis from [^{14}C]octanoate. The error bars indicate SD.

144,000 *g* for 60 min. All fractions were maintained at 0°C until assayed.

Table 3 shows the specific activities of each fraction, and the percent total activity recovered. HMG-CoA reductase activities were present in all cell fractions due, we believe, to cross-contamination and aggregation of organelles in the presence of mucus. Of particular concern was the loss of a considerable amount of enzyme activity during fractionation. The microsomal villous and crypt HMG-CoA reductase activities were 0.83 and 0.52 nmol/min per mg, respectively, representing a 1.6-fold difference. These results correlated well with the total cell homogenate HMG-CoA reductase activities (a difference of 1.7-fold) and sterol synthesis from acetate (1.5–2.0-fold difference).

TABLE 3. Distribution of HMG-CoA reductase in ileal villous and crypt subcellular fractions

Fraction	Villi		Crypts	
	Sp Act	%	Sp Act	%
Homogenate	0.478	100	0.275	100
Nuclei	1.44	20	0.780	26
Fat layer	2.09	7		
Mitochondria	1.44	6	1.44	10
Microsomes	0.829	5	0.512	5
Cytosol	0.014	1	0.027	3
Total recovery		39		44

"Enriched" villous and crypt populations were lysed in Dounce medium and assayed for the three marker enzymes and HMG-CoA reductase. The whole cell homogenates were then fractionated ultracentrifugally as described in Results. Each fraction was assayed for HMG-CoA reductase activity and the volume was measured to determine the percent recovery. The villous fraction contained a substantial "fat" layer that was removed and assayed; very little fat was observed in the crypt fraction and the layer was not removed.

DISCUSSION

Our purpose in conducting the studies reported in this paper was to resolve the controversy over which intestinal cell type (villous or crypt) is responsible for sterol synthesis. To do this, cells were dissociated sequentially from the intestinal mucosa in a dual-buffer system described by Weiser (18) that reproducibly separated them into "enriched populations" of villi and crypts. The cells obtained were 95–100% viable and could be maintained in vitro for the duration of the experimental period without loss of functional integrity. Cross-contamination between cell populations was demonstrated by the marker enzymes alkaline phosphatase, thymidine kinase, and acid phosphatase. Weiser (18) had demonstrated that 90% of the alkaline phosphatase activity was contained in the villi and only 10% was found in the crypts, and this enzyme had been used as a marker for villi (13, 18, 31–33). We found alkaline phosphatase activity to be 3-fold higher per mg of protein in the villi than in the crypts, and the villi accounted for 80–90% of the total activity. Thymidine kinase occurs exclusively in the crypts due to rapid mitosis of the undifferentiated cells and is therefore employed as a marker (10, 18, 31–33). We obtained thymidine kinase activities in the crypt cells that were at least 4-fold higher than the villi and accounted for approximately 80% of the total activity. Acid phosphatase is known to be equally distributed (32) between the two cell types and was used to ensure that the observed differences in alkaline phosphatase and thymidine kinase were not due to differences in cell stability. In our preparations, acid phosphatase specific activities were approximately the same in villi and in crypts, while the percent distribution of activities remained about 50:50. Fractionated cells characterized by these marker enzymes were assayed for the presence and distribution of HMG-CoA reductase. The specific activities obtained were 0.47 nmol/min per mg from the villi and 0.27 nmol/min per mg from the crypts, representing a difference

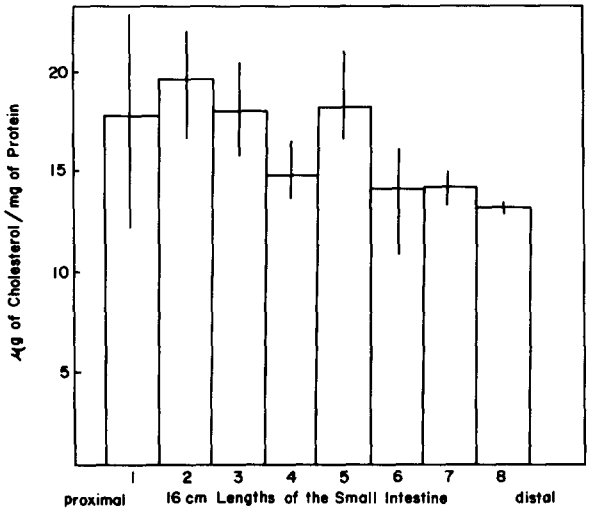

Fig. 5. Sterol content of epithelial cells from the jejunum and ileum. The small intestine from the ligament of Trietz to the ileocecal junction was removed at the D-6 hour. Intestinal segments approximately 16 cm in length were excised and numbered 1 through 8 starting from the ligament. The villous and crypt cells were removed simultaneously as described under Methods. Cells obtained from each 16-cm segment were homogenized, then saponified and the nonsaponifiable lipids were extracted with petroleum ether. The cholesterol content was determined by the method of Leffler (29). Results represent the mean and standard deviations of three experiments.

of 1.6–2.0-fold and distribution of 75% of the total activity in the villi and 25% in the crypts. Sterol synthesis from [^{14}C]acetate and [^{14}C]octanoate correlated well with the HMG-CoA reductase activities. These results demonstrate that the villi synthesize as much, if not more, sterol as the crypts.

Our results however, contradict the earlier conclusions of Dietschy and Siperstein (7) that sterol synthesis in mucosal scrapings of the small intestine was almost exclusively located in the crypt cell region and was essentially absent from villous tissue. Shefer et al. (3) had studied the localization of HMG-CoA reductase activity in mucosal cells that were also obtained by scraping the intestine and arrived at the same conclusions regarding the sterol biosynthetic ability of the two cell types. HMG-CoA reductase activity was reported to be located exclusively in the crypts and practically absent from the villi. Their reported activities in nuclei-free supernatant of villi were 0.004 and of crypts were 0.062 nmol/min per mg.

Subsequent to these reports Mak and Trier (22) demonstrated by radioautographic methods that the ileal villous cells convert more mevalonic acid to cholesterol than do the crypts. The localization of sterol biosynthesis in these cells was determined after incubating the everted gut loop in [^{3}H]mevalonate for 60 min. The sterols were precipitated in a buffered digitonin–aldehyde solution and fixed. Heavy labeling was reported in the upper two-thirds of the villi, "moderate" labeling in the lower third, and little labeling in the crypt zone. Since mevalonate occurs after the rate-limiting step in the biosynthetic pathway, these results may suggest, though not conclusively prove, a greater capacity of the villi to synthesize sterols.

Recent evidence from the work of Muroya et al. (21) demonstrates that both the villi and the crypts synthesize equal amounts of sterols from acetate, both in vivo and in vitro. They used three separate methods to determine the relative biosynthetic ability. First, the scraping technique (7) separated villous and crypt fractions which were then incubated with [^{14}C]-acetate. This method was reported to yield low and variable results. Second, pieces of the ileum were first incubated in [^{14}C]acetate for 30 min, and then the villi and crypts were scraped off. The rate of incorporation in each fraction was significantly higher with this method and, per milligram of protein, the villi were as active or more active than the crypts. Similar results were also obtained with the third method in which rats were injected intravenously or intraperitoneally and killed up to 40 min later, and the mucosal layer was then separated into villous and crypt fractions by using a cryostat or the scraping technique. These methods used for the separation of villous and crypt fractions are very different from ours, yet gave results that were in close agreement.

Weiser (18) had reported in a study on the incorporation of D-glucosamine into glycoproteins that cells obtained by the scraping method showed only ⅓ the capacity for incorporation compared to cells prepared in the dual-buffer system. Muroya et al. (21) also presented evidence to show that the scraping technique damaged cells severely. In our hands, the HMG-CoA reductase activities of fractions obtained by scraping were only ⅓ of the dual-buffer-prepared villous activity and ⅕ of the crypt activity. While the lower specific activities of the scrapings may be attributed to the larger protein recovery (Table 2), it does not account for the discrepancy between the observations of Dietschy and Siperstein (7) and Shefer et al. (3) and those of Muroya et al. (21) and ours. While an absolute comparison of data is difficult because neither cell viability nor separation of cell types was verified with marker enzymes by Shefer et al. (3), their crypt cell microsomal preparation made at the L-6 hour showed activity comparable to our D-6 total homogenate preparation; but our villous homogenate activity was about 60-fold higher. Likewise, the [^{14}C]acetate to CO_2 oxidation capacity of the crypt muscle preparations of Dietschy and Siperstein (7) was 10-fold more than the villous preparation but, according to Iemhoff and Hülsmann (20), the villous mitochondria produce 34–38-fold more CO_2 from [^{14}C]octanoate than the crypt mitochondria. Their villi and crypts were isolated by vibrating an everted intestine in Tris buffer as described by Harrison and Webster (13) and then fractionating to assay 11 mitochondrial enzymes and to study oxidation of octanoate and palmitate as well as the metabolism of glutamate, 3-hydroxybutyrate, and pyruvate. They reported an overall higher rate of metabolism in the villi so that oxidation rates that are lower in the villi would imply cell damage.

Numerous observations suggest that the villi are well equipped to transport de novo synthesized cholesterol. The endoplasmic reticulum, where lipoprotein (34–36) and cholesterol biosynthesis is believed to occur, is more developed in the villi than in the crypts, and it is presumed that the cytosolic cholesterol esterfying enzyme also occurs there (37). Also, cholesterol in its esterified form was reported to be transported from mucosal cells to the lymph in association with lipoproteins (38).

The villous and crypt cells differ greatly in their

general morphology (39) and metabolism as demonstrated by higher oxidation rates of fatty acids, amino acids, and sugars. Therefore it is probable that the concentration of biosynthetic precursors may be different in the two cell types, which makes it difficult to compare their cholesterol biosynthetic rates by using substrates that encounter different pool sizes. We tested this hypothesis by measuring the relative amount of sterol synthesized from [^{14}C]octanoate, as suggested by Dietschy and Brown (40). The villi synthesized 2-fold more sterols from octanoate than did the crypts; however, sterol synthesis from [^{14}C]-acetate by villous cells was equal to synthesis by the crypt cells. This large difference in measure of sterol synthesis may in part be due to the fact that the villi oxidize octanoate 38 times faster than the crypts (20). As a result acetyl-CoA derived from octanoate would be more readily available in the villi than in the crypts. It is also conceivable that the villi cells with their higher rates of metabolism may have the largest acetate pool and therefore the greatest dilution of exogenous radiolabeled acetate. We would therefore expect sterol synthesis from [^{14}C]acetate to be greater in the villi than was apparent. In vivo, measurements made by pulse labeling with high specific activity [^{14}C]acetate (7 μCi/nmol) exhibited sterol synthesis in the villi that was 60% of the total (Fig. 4).

Subcellular fractionation of the intestinal mucosa involves problems that need to be commented upon briefly. Studies on fractionation by Shefer et al. (3) showed HMG-CoA reductase to be distributed mainly in the crypt cell mitochondria and microsomes. Their fractionation, however, started with the post-nuclear supernatant and the nuclear pellet, which we find contains considerable amounts of activity, was disregarded. Furthermore, data on the activity in the total homogenate and the percent distribution were not given. We have found that, because of the presence of mucus (41), there is considerable cross contamination of subcellular fractions and that only 20% of the total activity remains in the post-nuclear supernatant, an equivalent amount sediments in the low speed "nuclear" pellet, some is found in the floating "fat" layer, and a large amount is lost or inactivated. At present we are unable to explain the loss of activity. We have also used the fractionation procedure described by Hübscher (42) and found the same distribution of HMG-CoA reductase, i.e., significant sedimentation in the "nuclear" pellet. Therefore, methods will have to be devised to prevent aggregation of subcellular fractions and losses in enzyme activity before the organelle distribution of HMG-CoA reductase can be clearly established.

Biosynthesis of cholesterol from [^{14}C]acetate is low in the jejunum and high in the ileum (7) which correlates well with the HMG-CoA reductase activities of these tissues (3, Fig. 2). However, their cholesterol content is unexpectedly close (Fig. 5). Assuming that the jejunum and the ileum obtain a similar contribution of sterols from the circulating plasma, the fact that absorption of luminal sterols occurs mainly in the jejunum (43) leads us to postulate that a large part of the sterol content of the ileum may be synthesized in situ. This is in agreement with the concept that the sterol concentration in the cells determines the sterol biosynthetic rate (38) and the activity of HMG-CoA reductase. Therefore, efficient absorption of dietary sterols in the jejunum would rapidly produce a high sterol content resulting in low HMG-CoA reductase activity. A low absorption rate in the ileum would result in low sterol concentration in these cells and HMG-CoA reductase activity would not be depressed until endogenous synthesis raised the sterol concentration to a sufficiently high level.

Both the crypt and villi cells actively synthesize sterols but, whereas the crypts may utilize all or part of their supply for growth and development as has been suggested earlier (7), the sterol produced by the villi would be available for transport to the body pool. The villi are mature cells and evidence seems to indicate that they are well-equipped for lipoprotein synthesis. This feature provides one possible mechanism of transport of villous sterols to the lymph and adds support to the proposal of Wilson and Reinke (44) that part of the cholesterol synthesized in the intestinal wall directly empties into the lymph.

This work was done in the laboratory of Dr. R. Gordon Gould. We wish to thank him for stimulating discussions and for the critical review of the manuscript. We also wish to thank Dr. Alan Cooper for helpful criticism of the manuscript. This research was supported in part by NIH Grants HL08476 and HLI18219. Dr. Heller is an Established Investigator of the American Heart Association. Reprint requests should be addressed to RAH, Department of Biological Sciences, Herrin Hall, Stanford University, Stanford, Ca. 94305.

Manuscript received 23 December 1976 and accepted 16 May 1977.

REFERENCES

1. Edwards, P. A., H. Muroya, and R. G. Gould. 1972. In vivo demonstration of the circadian rhythm of cholesterol biosynthesis in the liver and intestine of the rat. *J. Lipid Res.* **7:** 396–401.
2. Shefer, S., S. Hauser, V. Lapar, and E. H. Mosbach. 1972. Diurnal variation of HMG-CoA reductase activity in rat intestine. *J. Lipid Res.* **13:** 571–573.

3. Shefer, S., S. Hauser, V. Lapar, and E. H. Mosbach. 1972. HMG-CoA reductase of intestinal mucosa and liver of the rat. *J. Lipid Res.* **13:** 402–413.
4. Heller, R. A., and M. A. Shrewsbury. 1976. 3-Hydroxy-3-methylglutaryl coenzyme A reductase from rat liver: Its purification, properties, and immunochemical studies. *J. Biol. Chem.* **251:** 3815–3822.
5. Rodwell, V. W., D. J. McNamara, and D. J. Shapiro. 1973. Regulation of hepatic HMG-CoA reductase. *Adv. Enzymol.* **38:** 373–411.
6. Rodwell, V. W., J. L. Nordstrom, and J. J. Mitschelen. 1976. Regulation of HMG-CoA reductase. *Adv. Lipid Res.* **14:** 1–71.
7. Dietschy, J. M., and M. D. Siperstein. 1965. Cholesterol synthesis by the gastrointestinal tract: localization and mechanisms of control. *J. Clin. Invest.* **8:** 1311–1327.
8. Shefer, S., S. Hauser, V. Lapar, and E. H. Mosbach. 1973. Regulatory effects of dietary sterols and bile acids on rat intestinal HMG-CoA reductase. *J. Lipd Res.* **14:** 400–405.
9. Westergaard, H., and J. M. Dietschy. 1976. The mechanism whereby bile acid micelles increase the rate of fatty acid and cholesterol uptake into the intestinal mucosal cell. *J. Clin. Invest.* **58:** 97–108.
10. Imondi, A. R., M. E. Balis, and M. Lipkin. 1969. Changes in enzyme levels accompanying differentiation of intestinal cells. *Exp. Cell Res.* **58:** 323–330.
11. Das, B. C., and G. M. Gray. 1969. Protein in small intestine: localization and correlation with DNA synthesis and sucrase activity. *Biochim. Biophys. Acta.* **195:** 255–257.
12. Sjöstrand, F. S. 1968. A simple and rapid method to prepare dispersions of columnar epithelial cells from the rat intestine. *J. Ultrastruct. Res.* **22:** 424–442.
13. Webster, H. L., and D. D. Harrison. 1969. Enzymic activities during the transformation of crypt to columnar intestinal cells. *Exp. Cell Res.* **56:** 245–253.
14. Harrer, D. S., B. K. Stern, and R. W. Reilly. 1964. Removal and dissociation of epithelial cells from the rodent gastrointestinal tract. *Nature.* **203:** 319–320.
15. Huang, K. C. 1965. Uptake of L-tyrosine and 3-*O*-methylglucose by isolated intestinal epithelial cells. *Life Sci.* **4:** 1201–1206.
16. Stern, B. K., 1966. Some biochemical properties of suspensions of intestinal epithelial cells. *Gastroenterology.* **51:** 855–864.
17. Evans, E. M., J. M. Wrigglesworth, K. Burdett, and W. F. R. Pover. 1971. Studies on epithelial cells isolated from guinea pig small intestine. *J. Cell Biol.* **51:** 452–464.
18. Weiser, M. M. 1973. Intestinal epithelial cell surface membrane glycoprotein synthesis I. An indicator of cellular differentiation. *J. Biol. Chem.* **248:** 2536–2541.
19. Gall, D. G., and D. Chapman. 1976. Sodium ion transport in isolated intestinal epithelial cells II. Comparison of the effect of actively transported sugars on sodium ion efflux in cells isolated from jejunum and ileum. *Biochim. Biophys. Acta.* **419:** 314–319.
20. Iemhoff, W. G., J., and W. C. Hülsmann. 1971. Development of mitochondrial enzyme activities in rat-small-intestinal epithelium. *Eur. J. Biochem.* **23:** 429–434.
21. Muroya, H., H. S. Sodhi, and R. G. Gould. 1977. Sterol synthesis in intestinal villi and crypt cells of rats and guinea pigs. *J. Lipid Res.* **18:** 301–308.
22. Mak, K., and J. S. Trier. 1972. Radioautographic and chemical evidence for [5-^{3}H]mevalonate incorporation into cholesterol by rat villous absorptive cells. *Biochim. Biophys. Acta.* **280:** 316–328.
23. Goldfarb, S., and H. C. Pitot. 1971. Improved assay of 3-hydroxy-3-methylglutaryl coenzyme A reductase. *J. Lipid Res.* **12:** 512–515.
24. Hilz, H., J. Knappe, E. Ringelmann, and F. Lynen. 1958. Methylglutaconase, ein neue Hydratase die am Stoffwechsel verzweigter Carbonsäuren beteiligt ist. *Biochem. Z.* **329:** 476–489.
25. Edwards, P. A. 1975. Effect of plasma lipoproteins and lecithin–cholesterol dispersions on the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase of isolated rat hepatocytes. *Biochim. Biophys. Acta.* **409:** 39–50.
26. Bingham, E. W., and C. A. Zittle. 1963. Purification and properties of acid phosphatase in bovine milk. *Arch. Biochem. Biophys.* **101:** 471–477.
27. Klemperer, H. G., and G. R. Haynes. 1968. Thymidine kinase in rat liver during development. *Biochem. J.* **108:** 541–546.
28. Breitman, T. R. 1963. The feedback inhibition of thymidine kinase. *Biochim. Biophys. Acta.* **67:** 153–155.
29. Leffler, H. H., 1959. Estimation of cholesterol in serum. *Amer. J. Clin. Pathol.* **31:** 310–313.
30. Gornall, A. G., C. J. Bordawill, and M. M. David. 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **177:** 751–766.
31. Charney, A. N., R. E. Gots, and R. A. Giannella. 1974. (Na^+–K^+)-stimulated adenosinetriphosphatase in isolated intestinal villus tip and crypt cells. *Biochim. Biophys. Acta.* **367:** 265–270.
32. Fortin-Magana, R., R. Hurwitz, J. J. Herbst, and N. Kretchmer. 1970. Intestinal enzymes: indicators of proliferation and differentiation in the jejunum. *Science.* **167:** 1627–1628.
33. Schwartz, C. G., D. V. Kimberg, and F. Ware. 1975. Adenylate cyclase in intestinal crypt and villus cells: stimulation by cholera enterotoxin and prostaglandin E_1. *Gastroenterology.* **68:** 94–104.
34. Kessler, J. I., J. Stein, D. Dannacker, and P. Narcessian. 1970. Biosynthesis of low density lipoprotein by cell-free preparations of rat intestinal mucosa. *J. Biol. Chem.* **245:** 5281–5288.
35. Jones, A. L., and R. K. Ockner. 1971. An electron microscope study of endogenous very low density lipoprotein production in the intestine of rat and man. *J. Lipid Res.* **12:** 580–589.
36. Tytgat, G. N., C. E. Rubin, and D. R. Saunders. 1971. Synthesis and transport of lipoprotein particles by intestine absorptive cells in man. *J. Clin. Invest.* **50:** 2065–2078.
37. Gallo, L. L., and C. R. Treadwell. 1963. Localization of cholesterol esterase and cholesterol in mucosal fractions of rat small intestine. *Proc. Soc. Exp. Biol. Med.* **114:** 69–72.
38. McIntyre, N., and K. J. Isselbacher. 1973. Role of the

small intestine in cholesterol metabolism. *Amer. J. Clin. Nutr.* **26:** 647–656.

39. Trier, J. S. 1968. Morphology of the epithelium of the small intestine. *In* Handbook of Physiology: Alimentary Canal. Sect. 6, *Amer. Physiol. Soc.*, Washington, D.C. 1125–1175.
40. Dietschy, J. M., and M. S. Brown. 1974. Effect of alterations of the specific activity of the intracellular acetyl CoA pool on apparent rates of hepatic cholesterogenesis. *J. Lipid. Res.* **15:** 508–516.
41. Hülsmann, W. C., J. W. O. van den Berg, and H. R. de Jonge. 1974. Isolation of intestinal mucosa cells. *In* Methods in Enzymology Vol. 32, Biomembranes Part B. S. P. Colowick and N. O. Kaplan, editors. 665–673.
42. Hübscher, G., G. R. West, and D. N. Brindley. 1965. Studies on the fractionation of mucosal homogenates from the small intestine. *Biochem. J.* **97:** 629–642.
43. Slyvén, C., and C. Nordstrom. 1970. The site of absorption of cholesterol and sitosterol in the rat small intestine. *Scand. J. Gastroenterol.* **5:** 57–63.
44. Wilson, J. D., and R. T. Reinke. 1968. Transfer of locally synthesized cholesterol from intestinal wall to intestinal lymph. *J. Lipid Res.* **9:** 85–92.