# Sterol synthesis in intestinal villi and crypt cells of rats and guinea pigs

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**Abstract** The villi of the small intestine of rats and guinea pigs have been demonstrated to be capable of as rapid synthesis of sterols as the crypt cell fractions. This finding is not in agreement with the generally accepted belief that the crypt cells are responsible for essentially all of the synthesis of sterols in the intestine. Three methods of measuring synthesis were used: incubation with [$^{14}$C]-acetate of villi, crypt cell, and muscle fractions obtained by the method of graduated scraping; incubation of whole intestine pieces followed by fractionation into the same fractions; and in vivo studies in rats injected with [$^{14}$C]-acetate and killed 7–40 min later, after which the intestine was fractionated by scraping or by serial sectioning of frozen tissue. All methods were in agreement in showing sterol synthesis in villi to be as active per mg protein as in crypt cells. Measurements of the isotope content of the total nonsaponifiable lipid fractions led to the same conclusions as did the isotope content of the digitonin-precipitable sterol fractions. For a given period of incubation, the ratio of the two values was the same in villi and crypts, ruling out the possibility of synthesis of sterols in crypts prior to transfer to villi. The fractionation into villi, crypts, and muscle fractions by scraping greatly reduced the total sterol synthetic activity in rat but not in guinea pig small intestine, indicating the hazard of using only this in vitro technique to make quantitative estimates of the sterol synthetic capacity of tissue fractions.

**Supplementary key words** digitonin-precipitable sterols · [1-$^{14}$C]acetate · [2-$^{3}$H]acetate

Since the early studies of Srere et al. (1), the small intestine of the rat has been known to have a rapid rate of sterol synthesis; similar results have also been reported for the rabbit (2) and the guinea pig (3). Dietschy and Siperstein (4) found that the synthetic activity was greatest in the distal ileum and proximal duodenum and considerably less in the proximal ileum and jejunum. They also developed a method for fractionating the intestine into villi, crypt, and muscle fractions by graduated scraping and reported that, in vitro, both the villi and smooth muscle fractions were "essentially devoid" of synthetic activity (4). In early studies in this laboratory, the villi fraction was found to be as active as the crypt cell fraction per mg of tissue (5), and we have therefore reinvestigated this question in rats and also in guinea pigs using three experimental approaches: *1*) incubation with [$^{14}$C]acetate of villi, crypt cell, and muscle fractions obtained by scraping as described by Dietschy and Siperstein (4); *2*) incubation of pieces of whole intestine by the method of Srere et al. (1) followed by fractionation into villi, crypts, and muscle; and *3*) short term in vivo studies in animals injected with labeled acetate. All three methods agreed in showing that villi are as active as crypt cells in sterol synthesis per mg tissue protein in both rats and guinea pigs. Estimates of the total contributions to intestinal sterol synthesis indicate that villi and crypts are essentially equal.

## METHODS

### Animals

Male Sprague-Dawley rats (200–250 g) maintained on commercial rat chow ad libitum were used. The animals were killed during the basal phase of the circadian rhythm by exsanguination under light ether anesthesia. The small intestine was dissected free of mesentery, washed with cold 0.9% NaCl solution, and cut open along the mesenteric border.

### Fractionation of tissue and incubation procedures

*Method 1* (in vitro). Villi, crypts, and muscle were separated by the graduated scraping technique described by Dietschy and Siperstein (4); samples of each were taken for histologic analysis and the fractions were incubated with saturating amounts of

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; MVA, mevalonate; VLDL, very low density lipoproteins; DPS, digitonin-precipitable sterols.

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[1-$^{14}$C]acetate in Krebs-Ringer bicarbonate buffer at pH 7.2 in 95% $O_2$/5% $CO_2$ at 37°C for 30 min. The tissues were analyzed for protein and the isotope contents of the nonsaponifiable fraction and the sterol fraction were determined as described below. The specific conditions for individual experiments are given in the tables.

*Method 2* (in vitro). Pieces of whole intestine about 5 cm long were dissected free of adventitia and incubated as described in Method 1 and subsequently separated into villi, crypts, and muscle fractions by scraping. Samples were taken for histologic analysis and the remainder was worked up as in Method 1.

*Method 3* (in vivo). [1-$^{14}$C]Acetate or [2-$^{3}$H]acetate was injected into rats either in the jugular vein or intraperitoneally, and the animals were killed 7–40 min later. The small intestine was removed, washed with cold saline, and appropriate portions were fractionated as quickly as possible.

Fractionation was done by two methods. In one series of experiments the villi, crypts, and muscle cell fractions were separated by scraping as described in Method 1. In a second series fractionation was done by cutting serial sections of frozen tissue. The distal ileum was cut into 1-cm long segments, opened along the mesenteric border, and frozen immediately in a cryostat with the serosal surface down according to the method of Das and Gray (6). Serial 10-$\mu$m sections were cut perpendicular to the long axis of the villi from the top of the villi to the bottom of the crypts. The 20th section and every subsequent 10th section were used for histologic examination. The intervening sections were pooled for analysis for protein and assay for [$^{14}$C]-cholesterol.

### Assay of radioactivity

The incubation was terminated by the addition of an equal volume of 1 N NaOH; after removing an aliquot for the determination of protein concentration, 4–5 volumes of ethyl alcohol containing 10% of 10 N KOH were added, and the samples were saponified by heating on the steam bath for 2 hr.

Nonsaponifiable compounds were extracted three times with petroleum ether, and the combined extracts were washed with water. An aliquot was evaporated to dryness under $N_2$ and its $^{14}$C or $^{3}$H content was determined in a liquid scintillation spectrometer with toluene scintillation fluid. Spot checks for quenching by addition of a $^{14}$C- or $^{3}$H-labeled internal standard were done routinely.

A second aliquot was evaporated to dryness; the residue was taken up in 95% alcohol and a 1% digitonin solution was added. The sterol digitonides were isolated, and the $^{14}$C or $^{3}$H content was determined in a Tricarb liquid scintillation spectrometer by previously described methods (7).

The concentration of protein was determined by the method of Lowry et al. (8) using the same concentration of NaOH in standards as in unknowns.

### Histologic examination

Aliquots of tissues, separated either by scraping or with a cryostat, were sectioned and stained with hematoxylin–eosin and examined with a microscope to estimate the relative amounts of villi, crypts, and muscle tissue in each fraction. Every fraction in every experiment was examined, and the results were tabulated. Photomicrographs of random fields of sections showed that villi fractions contained insignificant numbers of crypts or muscle cells. The crypt fractions, however, always contained villi and usually muscle cells.

## RESULTS

### In vitro synthesis (Method 1)

Preliminary experiments on the incubation of the separated villi, crypt, and muscle tissue fractions prepared and incubated in Krebs-Ringer medium with [1-$^{14}$C]acetate as described by Dietschy and Siperstein (4) gave very variable and generally very low rates of incorporation of $^{14}$C into intestinal sterols, but higher rates were observed when unlabeled glucose was added to a medium containing 10 $\mu$moles of acetate or more per g of tissue. Since villi are fragile and lose activity by being scraped off the intestinal wall, the time of incubation was shortened from 2 hr to 30 min. In all other respects the experimental conditions were as described by Dietschy and Siperstein (4).

The results indicated that the villi fraction was at least as active in converting [$^{14}$C]acetate to digitonin-precipitable sterols per mg of tissue protein (**Table 1**). The villi fraction was actually slightly more active, but the difference between villi and crypt fractions was not significant. The muscle fraction was considerably less active, and its activity may be attributable to the incomplete removal of crypts.

In all three fractions the $^{14}$C in the digitonin-precipitable sterols constituted 40 ± 1% of that in the total nonsaponifiable fraction after 30 min of incubation. This difference is due to the cholesterol precursors, particularly squalene, present in the nonsaponifiable fraction in small amounts but with high specific activities. The ratio of dpm in digitonin-precipitable sterols to that in the total nonsaponi-

TABLE 1. Sterol synthetic activity in rat small intestinal fractions, determined in vitro (Method 1)

| Tissue Fraction | Radioactivity Incorporated into Nonsaponifiable Compounds | Radioactivity Incorporated into Digitonin-precipitable Sterols | Percent of Nonsaponifiable Activity in Digitonin-precipitable Sterols | Sterol Synthesis | Protein Content of Incubated Tissue |
|---|---|---|---|---|---|
| | *dpm/mg protein* | *dpm/mg protein* | % | *nmol/g tissue/hr* | *mg* |
| Villi | 117 ± 11 | 47 ± 8 | 40 | 1.07 ± 0.18 | 12.0 |
| Crypts | 70 ± 19 | 29 ± 8 | 41 | 0.64 ± 0.17 | 43.8 |
| Muscle | 33 ± 5 | 13 ± 2 | 39 | 0.28 ± 0.10 | 44.1 |

Intestinal villi, crypts, and muscle were separated by the scraping method from three 5-cm long portions of the distal part of the ileum as described in Methods. The fractions were incubated separately in 3.5 ml Krebs-Ringer bicarbonate buffer, pH 7.2, containing 20 μmol glucose and 5 μmol (2.5 μCi) of [1-$^{14}$C]acetate for 30 min at 37°C with shaking. The mean values ± SE for groups of six rats are given. The amount of protein in each incubation flask was determined as described in Methods. The amount of acetate used per g of tissue was 62.5 μmol for villi and 17 μmol for crypts and for muscle. The absolute synthetic rates were calculated using protein content of intestinal tissue fractions as 15%; 12 cholesterol carbons are derived from the [1-$^{14}$C] atom of acetate.

fiable fraction was remarkably constant for a given period of incubation.

The total amount of tissue in the villi fraction was considerably smaller than that in the crypt cell fraction as indicated by the protein content (Table 1). However, the villi fraction consisted of only villi, whereas the crypt cell fraction always contained both villi and crypt cells and usually muscle cells as well. The contribution of villi to total intestinal sterol synthesis cannot therefore be obtained accurately by considering only the villi fraction. The freedom of this fraction from crypt and other types of cells confirms the conclusion that villi cells are as capable of active sterol synthesis as crypt cells.

### In vitro synthesis (Method 2)

Incubation of whole pieces of rat small intestine resulted in considerably more incorporation of acetate into sterols then incubation of the isolated villi, crypt cells, and muscle or the mixture of all three (**Table 2**). Merely scraping off the villi and then adding this fraction back also resulted in a reduction of synthetic activity of over 80%. Because the scraping procedure decreased the activity so much, a second in vitro method was used in which whole pieces of rat intestine were incubated for 30 min before fractionation into villi, crypts, and muscle fractions by scraping.

The incorporation of $^{14}$C in the digitonin-precipitable sterol fraction per mg of protein by this method was as rapid in villi as in crypts in the distal ileum (**Table 3**, Expt. 1). There was no significant difference between villi and crypts, but muscle was considerably lower. The rates of sterol synthesis in both villi and crypts by this in vitro method were at least 10 times as high as by the first in vitro method given in Table 1.

Results for different parts of the small intestine

TABLE 2. Effect on synthetic activity of fractionation of villi, crypts, and muscle by scraping

| Tissue Incubated | No. of Rats | Nonsaponifiable Fraction | | Percent of Whole Intestine Value |
|---|---|---|---|---|
| | | Mean | Range | |
| | | *dpm/mg protein* | | % |
| Whole intestine | 6 | 1408 | 500–2545 | 100 |
| Villi | 5 | 97 | 34– 146 | 6.9 |
| Crypts | 5 | 121 | 18– 268 | 8.6 |
| Muscle | 5 | 29 | 6– 42 | 2.0 |
| Villi + crypts + muscle | 4 | 217 | 85– 310 | 15.4 |
| Villi + residual intestine | 2 | 242 | 174– 309 | 17 |

The distal 25 cm of rat ileum was cut into five pieces 5 cm in length. In each experiment one segment was incubated without fractionation and the others were fractionated by scraping as indicated. The segment incubated directly was varied from the most distal to the most proximal. Fractionation and incubation were done as described in Methods and Table 1, except that [2-$^{14}$C]acetate was used at a level of 15 μmol per g of tissue or higher.

TABLE 3. Sterol synthetic activity in rat small intestine (in vitro method 2)

| Intestinal Segment | Radioactivity Incorporated into Digitonin-precipitable Sterols | | | | Protein Content of Incubated Tissue | | | Sterol Synthesis | | | Percent of Non-saponifiable Activity in Digitonin-precipitable Sterols | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Villi | Crypts | Muscle | Villi / Crypts | Villi | Crypts | Muscle | Villi | Crypts | Muscle | Villi | Crypts |
| | *dpm/mg protein* | | | *ratio* | *mg* | *mg* | *mg* | *nmol/g tissue/hr* | | | | |
| Expt 1[a] | | | | | | | | | | | | |
| Ileum, distal | 559 | 370 | 19 | 1.51 | | | | 12.58 | 8.32 | 0.42 | 59 | 60 |
| ± SEM | 91 | 123 | 3 | | | | | 2.06 | 2.78 | 0.07 | | |
| *P* value | N.S. | | <.02 | | | | | N.S. | | <.02 | | |
| Expt 2[b], Rat A | | | | | | | | | | | | |
| Ileum, distal | 775 | 992 | 35 | 0.84 | 10.4 | 52.7 | 35.4 | 17.46 | 20.77 | 0.79 | 66 | 68 |
| Ileum, proximal | 152 | 81 | 7 | 1.88 | 18.7 | 49.5 | 30.6 | 3.42 | 1.83 | 0.16 | | |
| Jejunum | 71 | 45 | 5 | 1.58 | 18.4 | 46.4 | 31.8 | 1.60 | 1.01 | 0.14 | | |
| Duodenum | 566 | 210 | 20 | 2.70 | 15.4 | 43.2 | 42.4 | 12.75 | 4.73 | 0.45 | 62 | 56 |
| Expt 2[b], Rat B | | | | | | | | | | | | |
| Ileum, distal | 636 | 476 | 20 | 1.34 | 15.1 | 46.4 | 47.2 | 14.33 | 10.72 | 0.45 | 59 | 56 |
| Ileum, proximal | 38 | 19 | 3 | 2.00 | 20.6 | 42.4 | 44.4 | 0.86 | 0.43 | 0.07 | | |
| Jejunum | 21 | 11 | 2 | 1.91 | 22.7 | 42.4 | 37.6 | 0.47 | 0.25 | 0.05 | | |
| Duodenum | 263 | 63 | 6 | 4.17 | 19.2 | 47.2 | 38.4 | 5.92 | 1.42 | 0.14 | 56 | 47 |

[a] Intestinal segments of the distal part of the ileum (approximately 1 g) from six rats were each incubated in 5 ml of Krebs-Ringer bicarbonate buffer, pH 7.2, containing 28 μmol glucose and 10 μmol (5 μCi) of [1-$^{14}$C]acetate for 30 min at 37°C under 95% $O_2$, 5% $CO_2$ with shaking. At the end of the incubation, villi, crypts, and muscle were immediately separated by scraping according to the methods described in Table 1. Actual rates of sterol synthesis were calculated as in Table 1. Activities in villi and muscle fractions were compared with those in crypts statistically by Student's *t* test.

[b] Intestinal segments (approximately 1 g) from different portions of the small intestine were incubated and fractionated as described above.

show that the proximal ileum and the jejunum were much less active in sterol synthesis than the distal ileum (Table 3, Expt. 2). Duodenum samples taken from between the common duct and the pylorus were more active than the proximal ileum or jejunum but less active than the distal ileum. In every section of the intestine studied the villi gave as much or more activity than the crypts per mg of tissue protein.

The percent of $^{14}$C in the total nonsaponifiable fraction found in $^{14}$C-labeled digitonin-precipitable sterols was similar for villi and the crypts, both in distal ileum and in duodenum. These data indicate that both villi and crypts contained approximately the same relative amounts of $^{14}$C-labeled sterol precursors as $^{14}$C-labeled sterols, supporting the conclusion that the sterols were synthesized in the tissue cells in which they were found.

TABLE 4. Relationship between time of incubation of whole intestinal pieces and sterol synthesis in villi, crypts, and muscle

| Time | Digitonin-precipitable Sterols | | |
|---|---|---|---|
| | Villi | Crypts | Muscle |
| *min* | *nmol/g* | | |
| 10 | 2.19 | 2.40 | 0.053 |
| 20 | 5.06 | 4.13 | 0.10 |
| 40 | 15.00 | 11.50 | 0.24 |

The terminal 20 cm of the ileums from three rats were cut into 5-cm long sections and randomized. They were incubated as described in Table 3, except that the incubation times were as given above. They were then fractionated by scraping and the digitonin-precipitable sterols were isolated.

To obtain additional evidence on this point, pieces of distal ileum were incubated for 10, 20, or 40 min and then fractionated into villi, crypts, and muscle fractions. The amounts of synthesis of digitonin-precipitable sterols per mg protein were approximately the same in villi as in crypts at 10 min, as well as at the later times (**Table 4**). An initial lag of about 5 min in the conversion of acetate through the many intermediates into sterols has been observed in other experiments, not included in the table; this lag makes the rate of synthesis appear to be increasing with time in all three fractions.

### In vivo syntheis (Method 3a)

Rats injected with radioactive acetate and killed a short time later had newly synthesized sterols in villi as well as in crypts when these fractions were separated by the scraping method (**Table 5**). When

TABLE 5. Sterol synthesis in rat tissue fractions as determined in vivo (Method 3a)

SERIES A

| Time | Liver | Serum | Villi | Crypts | Muscle | Villi/Crypts |
|---|---|---|---|---|---|---|
| *min* | *dpm in digitonin-preceipitable sterols per mg protein* | | | | | *ratio* |
| 40 | 161 | 66 | 550 | 597 | 68 | 0.92 |
| 20 | 265 | 51 | 324 | 427 | 30 | 0.76 |
| 10 | 287 | 20 | 286 | 476 | 35 | 0.60 |

[1-$^{14}$C]Acetate with a specific activity of 2.0 $\mu$Ci/$\mu$mol was injected into the jugular vein of rats under ether anesthesia in a dose of 20 $\mu$mol (40 $\mu$Ci)/100 g body weight. Intestinal villi, crypt, and muscle cells were isolated by scraping from the distal 1-g portion of ileum as described in Methods. Results for one rat are given for each time period.

SERIES B

| | Specific Activity | | | Percent of Non-saponifiable Fraction Activity Present as Digitonin-precipitable Sterols | |
|---|---|---|---|---|---|
| Time | Villi | Crypts | Villi/Crypts | Villi | Crypts |
| *min* | *dpm per mg sterol* | | *ratio* | % | % |
| 20 | 4,320 | 1,520 | 2.78 | 58 | 50 |
| 11.5 | 9,240 | 8,390 | 1.10 | 20 | 42 |
| 10 | 2,610 | 1,710 | 1.53 | 43 | 39 |
| 10 | 3,930 | 3,750 | 1.05 | 15 | 11 |
| 8.5 | 12,090 | 15,920 | 0.76 | 31 | 30 |
| 7 | 11,380 | 12,360 | 0.92 | 14 | 16 |

[2-$^{14}$C]Acetate with a specific activity of 100 $\mu$Ci/$\mu$mol was injected intraperitoneally into rats in a dose of 200 $\mu$Ci per rat. Villi, crypt, and muscle fractions were obtained by scraping 1-g portions of the duodenum above the entrance of the common bile duct as described in Methods.

expressed either as dpm in sterols per mg of tissue protein or as specific activity (dpm per mg sterols), synthesis was about as rapid in villi as in the crypt cell fraction in both distal ileum and proximal duodenum.

As expected, the digitonin-precipitable sterols represented a relatively small fraction of the total nonsaponifiable activity during the first 7–10 min after injection of the precursor but, by 20 min, over 50% was present in this fraction.

### In vivo synthesis (Method 3b)

The method of Das and Gray (6) for separating villi and crypts was used for purposes of comparison with results obtained by the graduated scraping method; 44 samples of the distal ileum from rats killed 20 min after the intravenous injection of [$^{14}$C]acetate were frozen and sliced on a cryostat into 10 $\mu$m slices cut perpendicularly to the long axis of the villi. The sections were pooled into four fractions, except for the sections taken for histologic analysis as indicated in **Table 6.** Fraction A was entirely villi; while none of the other fractions consisted entirely of crypts, Fraction D contained the highest proportion.

All four fractions showed sterol synthetic activity; the fraction richest in crypts (D) gave about the same value as the pure villi fraction (A). Although the intermediate fractions gave somewhat higher values, the differences were not significant. All four fractions also showed almost exactly the same percent of total nonsaponifiable $^{14}$C that was recoverable in digitonin-precipitable sterols.

### Synthesis of sterols in guinea pig intestinal fractions (Method 1 and 2)

In contrast to the low values for sterol synthesis shown by isolated villi and crypt cell fractions from the rat, guinea pig fractions were found to have rapid rates; villi from the distal ileum had a rate of 40 nmol/g tissue per hr and the crypt cell fractions a rate of 18 (**Table 7**). The difference was significant ($P < 0.002$). In both tissue fractions 62–66% of the $^{14}$C in the nonsaponifiable fraction was recoverable as sterol digitonides.

When incubated whole, the guinea pig intestine pieces had a rate of 17 nmoles per g tissue per hr. The estimated values for the contributions of the villi, crypts, and muscle fractions to the total sterol synthesis per g of intestine (Table 7, last column) indicate approximately equal contributions from villi and crypts. However, the crypt fractions contained appreciable amounts of villi so the value given underestimates the contribution of the villi and overestimates that of the crypts. The fractionation of guinea pig intestine into villi, crypt cells, and muscle fractions did not appear to damage the synthetic activity for sterols appreciably whereas in rats there was a loss of over 80% of the activity.

In the guinea pig, unlike the rat, it cannot be assumed that the labeled digitonin-precipitable sterols are largely cholesterol. Ockner and Laster (3) have reported that in vitro studies on [$^{14}$C]acetate incorporation into $C_{27}$ sterols in guinea pig intestinal mucosa resulted in high $^{14}$C incorporation in lathosterol and dehydrocholesterol and very little in cholesterol, even after 4 hr of incubation. However, cholesterol is the principal sterol present, consituting 82% of the $C_{27}$ sterols, and it seems probable that it is formed eventually from the other sterols, but it is possible that the enzymes involved are damaged by the scraping technique. Until appropriate in vivo studies are done, it is essential to refer

TABLE 6. Sterol synthetic activity in rat small intestine as determined in vivo (Method 3b)

| Section | Histologic Analysis | | | Fraction | Sections | Estimated Composition | | | Radioactivity Incorporated into Digitonin Precipitable Sterols | Percent of Non-saponifiable Activity in Digitonin-precipitable Sterols |
|---|---|---|---|---|---|---|---|---|---|---|
| | Villi | Crypts | Muscle | | | Villi | Crypts | Muscle | | |
| | % | % | % | | | % | % | % | *dpm/mg protein* | % |
| | | | | A | 1–19 | 100 | 0 | 0 | 336 ± 88 | 62 |
| 20 | 100 | 0 | 0 | | | | | | | |
| | | | | B | 21–29 | 89 | 11 | 0 | 505 ± 58 | 68 |
| 30 | 78 | 22 | 0 | | | | | | | |
| | | | | C | 31–39 | 54 | 44 | 2 | 561 ± 41 | 65 |
| 40 | 30 | 65 | 5 | | | | | | | |
| | | | | D | 41–49 | 17 | 61 | 12 | 343 ± 54 | 58 |
| 50 | 4 | 56 | 40 | | | | | | | |

[1-$^{14}$C]Acetate was injected into the jugular vein of rats under light ether anesthesia in a dose of 50 $\mu$Ci per 100 g of body weight. At 20 min after the injection, the animals were killed. The distal part of the ileum was excised, washed and frozen immediately as described in Method 3b. Serial 10-$\mu$m sections were made perpendicular to the long axis of villi from the top of the villi to the bottom of the crypts. Sections 20, 30, 40, and 50 in each of 44 tissue blocks were analyzed histologically and the other sections pooled into four fractions, A, B, C, and D. The cell composition of each fraction was taken to be the average of that of the two boundary sections. The four fractions were analyzed for protein and the [$^{14}$C]sterol digitonides were isolated and counted. The mean values ± SE are given.

only to "sterol" synthesis in the guinea pig intestine.

## DISCUSSION

The primary objective of these studies was to determine if cholesterol synthesis takes place in intestinal villi as well as in crypt cells. Dietschy and Siperstein (4) reported in 1965 that crypt cells were responsible for virtually all the sterol synthesis in the rat small intestine, based on results from the in vitro method of the incubation with [$^{14}$C]acetate of intestinal fractions obtained by scraping. They did not determine the amounts of tissue incubated, but reported counts per min in the digitonin-precipitable sterols in the villi, crypts, and muscle fractions obtained from 6-cm long pieces of intestine. In the present investigation, the villi fractions had as much sterol synthetic activity as the crypt fractions per mg tissue, measured by several methods, in vitro and in vivo, but the villi fraction obtained by the scraping method was much smaller in amount than the crypt fraction. In our hands it constituted about 12% of the total ileum and was only about one-fourth as large as the crypt frac-

TABLE 7. Sterol synthetic activity in guinea pig small intestine (Method 1, in vitro)

| Tissue Fraction | Radioactivity Incorporated into Nonsaponifiable Compounds | Radioactivity Incorporated into Digitonin-precipitable Sterols | Percent of Nonsaponifiable Fraction Activity Present as Digitonin-precipitable Sterols | Sterols Synthesized | |
|---|---|---|---|---|---|
| | *dpm/mg protein mean ± SE* | *dpm/mg protein mean ± SE* | % | *nmol/g tissue/hr mean ± SE* | *nmol/g whole intestine/hr* |
| Villi | 1421[a] ± 124 | 884[b] ± 90 | 62 | 39.84[b] ± 4.08 | 8.64 |
| Crypts | 612 ± 54 | 406 ± 90 | 66 | 18.30 ± 1.82 | 9.05 |
| Muscle | 111[a] ± 20 | 85[a] ± 15 | 76 | 3.85[a] ± 0.67 | 1.10 |
| Intestinal wall (incubated without scraping) | 576 ± 40 | 371 ± 20 | 64 | 16.71 ± 0.90 | 16.67 |

Intestinal villi, crypts, and muscle fractions were scraped from approximately 1-g portions of the distal part of the guinea pig ileum and incubated separately in 3.5 ml Krebs-Ringer bicarbonate buffer, pH 7.2, containing 100 mg% glucose and 10 $\mu$moles (1 $\mu$Ci) of [2-$^{14}$C]acetate. Incubation was for 60 min at 37°C under 95% $O_2$, 5% $CO_2$ with shaking. The value of nmol of sterol synthesized/g tissue per hr was calculated as in Table 1, except that 15 cholesterol carbons are derived from the [2-$^{14}$C]acetate. The villi fraction contained only 20% of the protein, the crypts contained 48% and the muscle contained 28.9%. From these data and the synthetic rates, the contributions of the fractions were estimated and are given in the last column. Each value represents at least five animals. *P* values for difference between crypts and tissue are shown.

[a] $P < 0.001$.

[b] $P < 0.002$.

tion. This appears to have been the case also in the data reported by Dietschy and Siperstein (4) and may account in part for the low results obtained by them for the villi fraction.

The presence of the enzymes that synthesize sterols from mevalonate in the villi has been demonstrated by Mak and Trier (9) by means of a histochemical approach using rat ileal everted sacs incubated with [5-$^3$H]MVA. They found heavy labeling in the upper two-thirds of the villi, moderate labeling in the lower third, and little labeling in the crypts. While these results do not bear on the distribution of HMG-CoA reductase, they do suggest that crypts may not be capable of much cholesterol synthesis. The results in the present report do not permit a conclusion as to how active crypts are in sterol synthesis, because it was not possible to obtain preparations of crypt cells free of villi by either the scraping method or the cryostat method. However, the villi fraction usually was more active than the mixture of villi and crypts so it is possible that the crypts have considerably less synthetic activity than the villi.

In evaluating the synthetic capability of a tissue and particularly of a tissue fraction in vitro, it is essential to consider the possibility that the experimental procedures used may have partially or completely inactivated the enzyme systems being measured. The inactivation may be more marked in one type of cell than in another, making comparisons of the two invalid. For this reason, we used three methods in the present investigation; the finding that all three give the same result strongly supports our conclusion that villi are approximately as active as crypt cell fractions in sterol synthesis per unit weight of tissue.

Although it has been well established by experiments on intact rats (9, 10) that the intestine is very active in sterol biosynthesis when measured during any part of the circadian cycle, it is a common experience to have considerably more difficulty in demonstrating significant synthesis in rat intestinal mucosa scraped off the intestinal wall and incubated in vitro than in rat liver slices. The extensive damage produced by scraping (as illustrated in Table 2) is probably responsible for this and explains the success of Srere et al. (1) in demonstrating in vitro cholesterol synthesis in intestinal tissue for the first time by using whole sections of intestine. Although the use of isolated villi and crypts provides the most rigorous proof of the presence of the necessary enzyme systems in each, it is not reliable from the quantitative standpoint for the reasons given above. Incubation of whole pieces of intestine provides somewhat more physiological conditions and in vivo studies of very short duration add confirmatory evidence, even though only relative rates of synthesis can be determined using [$^{14}$C]acetate.

The validity of this pulse labeling method was investigated in previous studies in rats given intraperitoneal injections of a mixture of $^3H_2O$ and [$^{14}$C]-acetate (11). Incorporation of both isotopes into liver and intestinal cholesterol was measured 4 hr later in rats treated in a variety of ways affecting the rates of synthesis of cholesterol. The mean values of the ratio of [$^{14}$C]/[$^3$H] incorporation into intestinal cholesterol for four groups of three animals each fell within a range of 0.80–0.88. Liver cholesterol ratios were also within a narrow range, although the rate of synthesis varied considerably from group to group. It can therefore be assumed that the data obtained by injection of [$^{14}$C]acetate will be similar to that which would be obtained from the use of tritium water.

Although none of the three methods used in the present investigation is ideal for determining absolute rates of sterol synthesis, any one of them shows clearly that intestinal villi are capable of active sterol synthesis and the agreement among the three methods greatly strengthens the conclusions that the villi are at least as active as crypts.

Dietschy and Siperstein (4) suggested the hypothesis that sterol synthesis in the gastrointestinal tract serves the function of providing structural cholesterol for the newly forming cells, and that such synthesis ceases when the crypt cells mature into villi cells. The present observations of active sterol synthesis in villi indicate the need for a modification of this hypothesis, but the function of such rapid sterol synthesis in villi is not obvious. As rats and guinea pigs normally subsist on cholesterol-free diets, the intestine needs a constant supply of cholesterol for incorporation into chylomicrons and lipoproteins of intestinal origin. Absorption of nutrients is well known to take place in the villi, and the synthesis and assembly of chylomicrons and VLDL have been shown to take place in the microsomes of the villi (13). Since cholesterol is a constituent of both types of lipoproteins, an increase in cholesterol synthesis in villi would be expected when the diet is deficient in cholesterol but contains triglycerides.

Intestinal cholesterol synthesis shows a circadian rhythm like that of liver, although the amplitude is much smaller (11, 12). When food is available ad libitum, the rate increases when food ingestion increases, normally at the beginning of the dark phase, and reaches a peak about 6 hr later. In a previous study in which food availability was restricted to the period from 9 AM until 1 PM, the rate of

synthesis in the ileum, measured in vivo, increased rapidly starting at 9 AM and reached a peak about 3 hr later, in contrast to the liver which did not reach a peak until about 6–9 hr after 9 AM (9). Thus, in agreement with the above hypothesis, increases in intestinal cholesterol synthesis appeared to be closely associated with the absorption of food.

The relative contributions of villi and crypts to total sterol synthesis in the distal ileum may be approximated from estimates of the total amounts of tissue in each of the two fractions. Although the crypt cell fraction could not be cleanly separated from villi and muscle, the muscle fraction was approximately 44% of the total (Tables 1 and 3). Dietschy and Siperstein (4) reported that, by histologic analysis of whole intestinal sections, the crypt cell fraction constitutes 25% of the total intestine. The villi fraction then will constitute about 31% of the total. This estimate agrees with that calculated from the data in Table 6 which indicates that the crypt cell fraction contains about 35% villi. Since the villi and crypt cell fractions had approximately the same sterol synthesizing activity per mg of tissue, their contributions to total sterol synthesis will be approximately the same.

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