PROTEIN TURNOVER IN INTESTINAL MUCOSAL VILLUS AND CRYPT BRUSH BORDER MEMBRANES

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SUMMARY. Relative turnover rates of intestinal brush border proteins have been studied by double labelled technique. Brush borders were isolated from cells at all levels along the villus, and from the crypts. Proteins with large molecular weight (>150,000) demonstrated more rapid turnover compared with other brush border proteins at all levels along the villus. This rapid turnover was not seen in crypt brush borders. These findings support the concept of protein turnover in intestinal brush borders, and demonstrate differences between the proteins in rapidly growing crypt cells and non growing villus cells.

INTRODUCTION. The large proteins of the intestinal brush border membrane are degraded and/or replaced at a rate faster than that of the whole membrane (1). At least some of this rapid turnover can be accounted for by the action of pancreatic proteases, since 95% pancreatectomy abolishes the differential turnover among brush border proteins, and elastase feeding restores it (2). However, the intestinal mucosa is not a homogenous tissue. Crypt and villus cells differ in intrinsic biochemical properties, in the properties of their brush border membranes, and, of course, in their anatomical location. The types of protein present in the crypt membranes differ from those in villus cells (3). It is not clear whether large proteins in cells of the lower as well as the upper villus turn over rapidly. For this to happen, presumably pancreatic protease should have access to the cells along the entire villus. Finally, although brush border protein turnover seems more rapid (10-15 hours) than cell migration (24-36 hours), it is possible

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that migration from crypt to villus extrusion at the villus tip accounts for some of the protein turnover measured by the double labelled technique. If the pattern of turnover were the same in the lower villus membranes as in the upper villus, this would favor turnover occurring in situ on the membrane even in the outer half of the villus where cell extrusion is occurring. To clarify these issues we have investigated the relative turnover of membrane proteins in the rat intestine from crypt to villus tip.

MATERIAL AND METHODS. Wistar rats (160 gm) were fasted overnight and injected intrajejunally with 50 μc of u.l. ^{14}C leucine (>270 mCi/mmole) at 1 PM, and with 100 μc of 4,5 ^{3}H leucine (30-50 Ci/mmole) at 11 PM (1). In both instances the entire intestinal lumen was filled with fluid (about 3.0 ml). Both isotopes were obtained from New England Nuclear Corp. (Boston, Mass.). Animals were sacrificed at 9 AM. The 10 hour delay before sacrifice is necessary to allow incorporation of label into brush border membranes. This method has been validated for intestinal mucosa in the rat (1), and confirmed in the mouse (4).

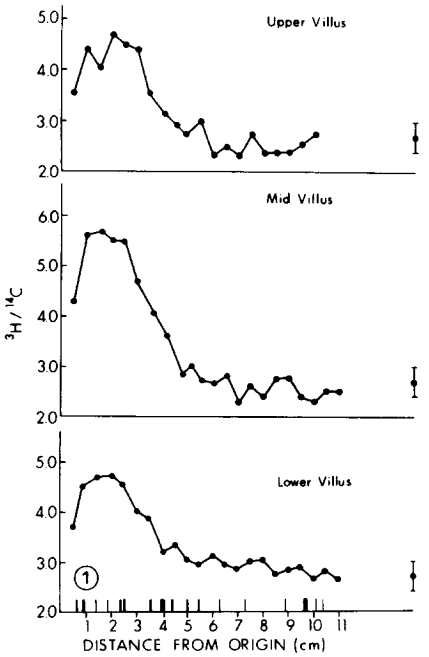
Intestinal cells were prepared sequentially from villus tip to crypt base using EDTA washes by the method of Weiser (5). Cells were collected in 10 fractions using nine washes as previously described (5) and adding a tenth wash of 15 minutes. The collected cells were pooled as follows: fractions 1 and 2 = upper villus, 3 and 4 = mid villus, 5 and 6 = lower villus, 7 and 8 = upper crypt, 9 and 10 = lower crypt. This procedure removed almost all epithelial cells from the underlying lamina propria. Sucrase activity fell markedly and thymidine kinase activity began to rise by fraction 7. Thus, the separation of villus fractions (1-6) from crypt fractions (7-10) was confirmed.

Brush borders were prepared from each group of cells according

to the method of Schmitz et al. (6), using the unfragmented brush borders as the final preparation. Sucrase activity in the brush border fraction was increased 11-13 fold over the homogenate, and there was less than 3% contamination with DNA. The microvillus pellet was resuspended in 0.2 ml of buffer and analyzed for protein (7). The yield of membrane protein from each group of cells derived from three animals was about 250 μg.

Membrane proteins were then separated by SDS gel electrophoresis. Fifty μg of membrane protein containing 940-1400 ^{3}H counts and 320-580 ^{14}C counts were applied to four successive wells for each pooled fraction of the villus. Samples were boiled in 1% SDS and 1% 2-mercaptoethanol for 3 minutes prior to application. The gel slab was 1 mm thick and used a tris buffer system (8). After electrophoresis, this gel was rinsed and cut into 5 mm pieces using a multiple razor blade holder. The pieces corresponding to four wells were transferred to counting vials, covered with 0.4% Omnifluor (New England Nuclear Corp.) in toluene containing 3% Protosol (New England Nuclear Corp., Boston, Mass.) and incubated overnight at 37°. The vials were then chilled and counted in a Packard Model 2000 liquid spectrometer. All samples less than twice background were rejected. Recovery of counts was 70 - 80%. The data was reported as $^{3}H/^{14}C$ ratios for each gel slice, and represent the mean of two separate experiments.

RESULTS. Figure 1 shows the results found in the brush border proteins from villus cells. The pattern of turnover was similar for all levels along the villus, with the most rapid turnover occurring in the larger proteins (left side of each gel). The control experiments were performed by injecting both isotopes simultaneously into the jejunum and sacrificing the animals 10 hours later. On the other hand, the proteins of the crypt cell brush



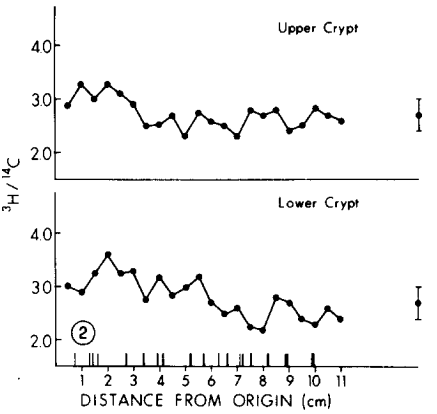


Figure 1. Relative turnover of membrane proteins along the villus. The dot and lines on the right of the graph refer to the mean $\pm$ 1 standard error of the mean for an experiment in which both isotopes, ^3H and ^{14}C, were administered to the animal simultaneously. The vertical lines at the bottom of the graph depict the Coomassie blue stain of a fixed and unsliced gel of membrane proteins run simultaneously. These patterns were rather similar at all three levels of the villus.

Figure 2. Relative turnover of membrane proteins in the intestinal crypt. Symbols are identical to those in Figure 1.

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border (Figure 2) showed no differences in turnover whatever, since the variation in $^3H/^{14}C$ ratio did not differ from the mean $\pm$ one standard error (shown on right) of a control experiment in which both labelled amino acids were administered simultaneously. Although the large molecular weight proteins were not so abundant in crypt as they were in the villus brush borders, large proteins were present. These large proteins, (MW >150,000), migrating 1-3 cm from the origin, did not display the relatively rapid turnover found in proteins of similar size in villus cells. It is unlikely,

however, that the large proteins of crypt and villus cells are identical, since many enzyme activities corresponding to proteins of large size are a unique property of the mature villus cell membrane.

DISCUSSION. During the 10 hours of the double labelled protein of the experiment, cells on the villus might migrate and be extruded into the lumen whereas those in the crypt do not. Thus, this difference might be thought to account for the difference found. This explanation seems unlikely for two reasons. First, the large proteins from the lower villus brush border show the same rapid turnover as those from the upper villus. Since the double labelled part of the experiment only takes 10 hours, and transit time from villus base to tip is 24-36 hours, it seems unlikely that the lower villus would be much affected by this cell loss. Second, since fasting decreases cell migration, one might assume that the rate of migration during the experiment is not so rapid as measured in fed animals.

These data support the concept that protein turnover occurs in the luminal membranes of villus cells. If some of this turnover is related to the actions of pancreatic proteases, the data suggest that these proteases are available to cells of the lower villus as well as the upper villus. The present findings demonstrate that turnover of large proteins is a phenomenon occurring at all levels along the villus. This mechanism thus maintains a constant level of brush border enzymes along the villus (9) despite the continued synthesis of protein by villus cells (3).

The lack of turnover (or low rate of turnover) found in the dividing crypt cells may relate to data obtained from bacteria. E. Coli in logarithmic phase shows a low level of protein catabolism, and the level depends upon the rate of growth (10), with degradation

increasing as growth slows. When cells enter the stationary phase, comparable to villus cells, the rate of protein breakdown increases further. It is not certain whether the slow turnover of crypt cell brush borders is due to the intrinsic properties of the membrane itself or to the lack of availability of pancreatic proteases to the surface of the crypt cell. However, it is clear that the heterogeneity in turnover rates of brush border proteins seen in mature villus cells is not seen in the crypt brush border. It would seem reasonable, when using the double labelled technique for intestinal mucosa, to use only the mucosal cells on the villus, leaving the crypt cells behind during the process of scraping the cells from the muscle wall.

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