Metabolic Activities of Sloughed Cells from the Jejunal Mucosa of Dogs with Thiry-Vella Loops.* (31712)

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Mucosal epithelial cells of the upper small intestine move from the crypts of Lieberkühn to the apex of the villus in about 3 days, cluster around the tip of the villus in groups of slightly rounded cells, and then are sloughed into the lumen(1-3). Although representing only a small proportion of the total epithelial cells of the gut at any given time, the sloughed cells contribute appreciably to the succus entericus, or mucosal secretion, as a source both of enzymes and of cellular material. Although freshly sloughed cells appear to be intact microscopically, their ability to catalyze synthetic as well as degradative metabolic processes has not previously been assessed. Furthermore, factors which control the sloughing rate have not been well defined; for example, regimens of fasting and refeeding have affected sloughing inconsistently(4,5).

In the present investigation, the permeability of sloughed cells and their capacity to catalyze sterol biosynthesis and hydrolytic reactions are examined, and an improved procedure is given for evaluating the sloughing rate of epithelial cells in short term experiments.

Materials and methods. Nine fasted mongrel dogs ranging in weight from 11-16 kg were anaesthetized with pentobarbital and ether. Using aseptic techniques a 20-30 cm segment of the most proximal jejunum was isolated, and the remaining bowel was reconnected with an end-to-end anastomosis. Both ends of the isolated innervated segment were drained through the abdominal wall with the use of nylon cannulas fitted with latex finger cots.

In order to study lipid synthesis, either a 2 ml aliquot of intestinal juice or a 2 × 2 cm segment of excised jejunum was incubated with 10 ml of 0.1 M phosphate buffer pH 7.4, 1.0 ml of 1% glucose and 0.1 ml of C^{14}-acetate (2.3 mg acetate containing 3.3 × 10^7 cpm/ml) for 1 hr at 25°C either in stationary flasks or at 37°C on a rotary shaker. At the end of the incubation period, each sample was saponified for 60 minutes at 90°C with 5 ml of 20% KOH in 95% ethanol. The cooled samples were extracted 3 times with 20 ml of petroleum ether, and the combined extracts were washed 3 times with equal volumes of distilled water and then dried over Na_2SO_4. Two ml aliquots of each sample were evaporated and counted in a gas flow proportional counter. The radioactivity in the non-saponifiable fraction was corrected for background and expressed as counts per minute per tissue sample.

To assess the membrane integrity of cells, tissue sections were differentially stained for 30 minutes in 0.3% Nigrosin in 0.1 M phosphate buffer pH 7.4, and then either examined immediately or fixed in ethanol-acetic acid (3:1).

The collected intestinal secretion was diluted to 15 ml with saline and was centrifuged at 35,000 *g* for 30 minutes in the cold at 8-10°C. The solid fraction was then diluted to 10 ml with distilled water and was dispersed by sonication for 20 seconds in a 20 Kc MSE ultrasonicator.

Alkaline phosphatase activity was determined by the method of Garen and Levinthal(6). In essence, 0.3 ml of a supernatant solution diluted 3-20 times, or of a solid fraction diluted 200-1500 times, was added to 3 ml of 0.005 M p-nitrophenyl phosphate in 0.1 M veronal-glycine buffer, pH 9.2 at 27°C. The optical density at 400 mμ was determined at one-minute intervals in a Bausch and Lomb Spectronic 20 colorimeter. In order to measure sucrase activity, 1.0 ml

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of the undiluted supernatant solution or of the solid portion diluted 2-10 times was added to a calibrated cuvette containing 1 ml of 10% sucrose in 0.01 M phosphate buffer and 4.5 ml of double strength Glucostat reagent (glucose oxidase and o-dianisidine from Worthington Biochemical Corp.) at pH 7.4 and at 27°C. The optical density at 450 mμ was subsequently read at frequent intervals in a Coleman spectrophotometer. The amount of sucrase present was linearly related to the inverse of the time required to produce a given change in optical density. The unit of activity was defined as that amount of enzyme which produced an increase in optical density of 1.0 *unit* in 15 minutes.

Results. a) *Cholesterol synthesis by jejunal sections and sloughed cells.* Radioactive acetate is readily incorporated into cholesterol by the rat intestine *in vivo*(7) and by rat intestinal sections *in vitro*(8). Similarly, jejunal sections of the dog incubated *in vitro* incorporated significant amounts of radioactive acetate into non-saponifiable lipid, of which cholesterol is the major component (Table I). In contrast, samples of intestinal juice which contained a similar number of epithelial cells were completely unable to synthesize sterol from acetate at either 25°C or 37°C (Table I). Even when 2.5 ml of 10% glucose solution was added to the collection bag to ensure a ready energy source for sloughed cells, the incorporation of acetate into non-saponifiable lipid at 37°C was nil. This apparent metabolic defect of sloughed cells was extended by histological study of

TABLE I. Incorporation of C^{14}-Acetate into Non-Saponifiable Lipid.

Tissue	Temp (°C)	No. of experiments	Net radioactivity in non-saponifiable fraction (cpm)
Jejunal* segments	25	2	844, 1010
	37	2	246, 486
Sloughed* cells	25	6	0
	37	6	0

* In experiments with both jejunal and sloughed cells, about 10^8 epithelial cells were present. The number of sloughed cells was directly counted, whereas the number of epithelial cells in a 2 × 2 cm jejunal segment was calculated by use of the following data: surface area of an epithelial cell, 36 μ^2; villus size, 1 mm × 100 μ diameter; villi on the intestinal surface, 25/mm².

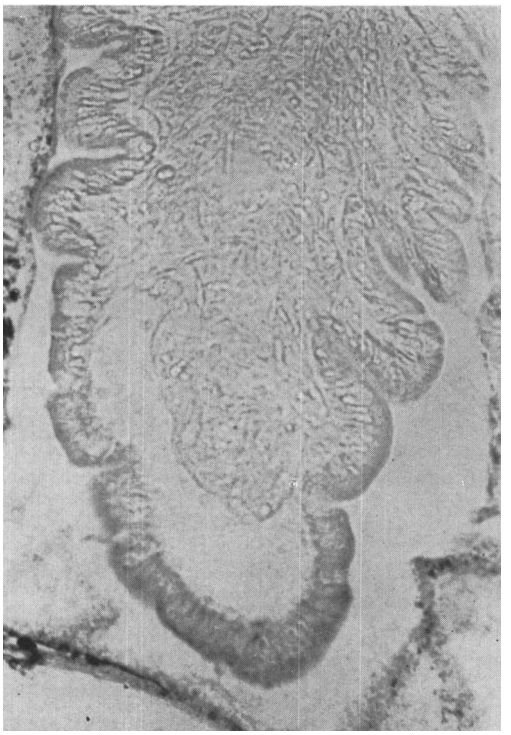

FIG. 1. Differential staining of epithelial cells of a jejunal villus with Nigrosin. The dye is localized in cells at tips of villus.

Nigrosin treated sections of the jejunum. Nigrosin, a member of the quinone-imine group of dyes, is continuously secreted from viable cells with intact membranes but remains within cells with damaged cell membranes(9). As shown in Fig. 1, the epithelial cells at the tip of the villus retain Nigrosin, whereas cells further down the villus do not. Apparently sloughed cells, and even cells still attached to the tip of the villus, are damaged biochemically although their histological appearance is relatively normal.

b) *Localization and stability of sucrase and alkaline phosphatase activity in sloughed epithelial cells.* Although sucrase and alkaline phosphatase are mainly associated with the brush border of intact epithelial cells(10), these enzymes might be liberated from sloughed cells or be destroyed by bacterial or proteolytic action during their passage through the gut. As shown in Tables II and III, however, both sucrase and alkaline phosphatase are still largely associated with the sloughed cells after passage through the jejunal loop.

TABLE II. Sucrase Activity in Cells and Supernatant Solution from Intestinal Juice Centrifuged at 35,000 *g* for 30 Minutes.

Dog No.	Particulate fraction	Supernatant solution	% Activity in cells
5A67	20.0	1.5	93.0
"	30.0	2.1	93.5
"	58.0	2.3	96.2
"	64.0	2.6	96.1
5B47	31.0	.8	97.5
"	40.0	2.4	94.3
5N11	28.5	.6	98.5
"	14.5	1.0	93.5
"	8.0	.6	93.0

TABLE III. Alkaline Phosphatase Activity in Cells and Supernatant Solution from Intestinal Juice Centrifuged at 35,000 *g* for 30 Minutes.

Dog No.	Cells	Supernatant	% Activity in cells
5A67	12.0	.44	96.5
"	90.0	2.95	96.8
5B47	56.0	.66	98.8
"	27.2	.62	97.8
5N11	6.0	.60	90.0
"	40.5	2.70	93.7

Furthermore, sucrase retains its activity well upon storage at body temperature for almost 7 hours in the presence of bacteria and of other cells (Fig. 2).

c) *Utilization of the sucrase activity as a measure of rate of cell sloughing.* The rate of cell sloughing is somewhat difficult to quantitate due to cellular clumping and the irregular passage of particulate material of the intestinal juice into the collection bag. Because the sucrase of sloughed cells was stable during the collection and storage of intestinal juice, it was evaluated as a measure of cell number. While the rate of sloughing varied greatly when intestinal juice was merely collected from the distal cannula, a much greater constancy in the sloughing rate was observed when the intestinal segment was flushed periodically with saline (Fig. 3).

Discussion. As epithelial cells of the upper gut move from the area of mitotic activity in the crypts to the extrusion zone at the villus tip, a marked change occurs in their structure and metabolic capabilities. Undifferentiated columnar cells of the crypt are capable of both merocrine and apocrine secretion, have large mitochondria, and relatively short microvilli (11). At the base of the villus, however, absorption cells appear with conspicuous microvilli and smaller mitochondria (11). As the columnar epithelial cells proceed up the villus, the microvilli become longer and narrower and increase in number, the RNA concentration presumably increases, and the capacity of cells to absorb sugars and other nutrients becomes greater (12). In relation to sugar absorption, the activity of sucrase and other disaccharidases is very low in the crypt cells, increases progressively during migration, and reaches a peak in the apical half of the villus (13). In contrast the synthesis of cholesterol from radioactive acetate decreases as cells move from the crypts towards the villus tip (14).

As an extension of this work, we have shown that sloughed cells do not synthesize sterol from labeled acetate under conditions where cells attached to the villus do. The incorporation of C^{14}-acetate into non-saponifiable lipids is a particularly valuable tool in studying the synthetic capacities of mammalian tissues in the presence of bacteria, since the latter do not synthesize sterol. In conjunction with decreasing synthetic abilities, the integrity of the cell membrane is progressively lost, as demonstrated by Nigrosin staining. While the crypt cells remain free of Nigrosin, those cells at the villus tip are no longer capable of excreting the stain, a further indication of the loss of an energy requiring process.

During sloughing and passage down the gastrointestinal tract, epithelial cells retain their sucrase and alkaline phosphatase ac-

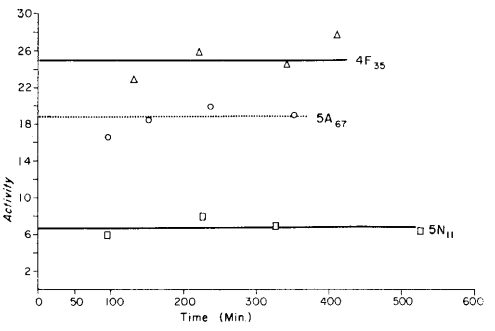

FIG. 2. Effects on sucrase activity of incubating jejunal secretion *in vitro* at 37°C.

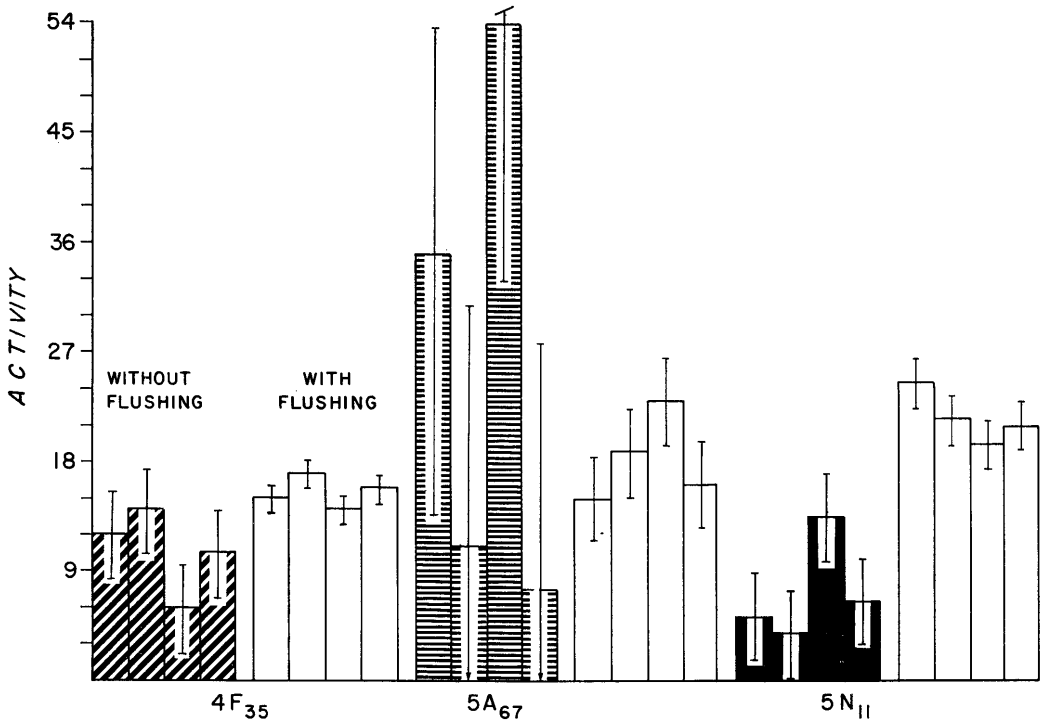

FIG. 3. Effect on sucrase activity of flushing the loops with saline before and after each collection period. I represents the standard deviation in 5 different determinations.

tivity(13), however, and about 95% of both enzymes remain attached to the particulate fraction of the intestinal cell. Nonetheless, this hydrolytic activity probably plays a minor role in intraluminal digestion. Normally the hydrolysis of disaccharides and phosphorylated monosaccharides is an intracellular process occurring in the brush border, and only a relatively small amount of the total enzyme is associated with sloughed cells(10).

To examine factors which control the duplication of intestinal epithelial cells, a rapid and accurate method of determining the rate of cellular sloughing is necessary. Direct enumeration of cells in a microscopic counting chamber is unsatisfactory because of cellular clumping caused by mucous strands, and radioautography is too cumbersome a technique for rapid *in vitro* experiments. Furthermore, the volume of secretion normally collected in a 30-minute period is often too small and sporadic to allow the calculation of dependable average sloughing rates. However, when the isolated segment is perfused both before and after each collection period to insure the total collection of sloughed cells, and when sucrase content, used as a measure of sloughed cells, was determined in consecutive collection periods from the same animal, reproducible results were obtained. This indirect method for the determination of the number of sloughed cells should be useful in the future in evaluating factors which control the sloughing rate.

Summary. Three metabolic activities of sloughed epithelial cells of the dog jejunum were investigated. Sloughed epithelial cells as well as cells in the extrusion zone of the villus took up Nigrosin stain, while cells further down on the villus did not. Furthermore, sloughed cells did not incorporate acetate into non-saponifiable lipid, whereas an equal number of cells in an intact intestinal slice did. Nonetheless, the particulate fraction of the succus entericus contained 95% of the total sucrase and alkaline phosphatase activity, and the sucrase activity was stable during incubation with the intestinal secretion. Based on this stability of sucrase and on the use of a simple flushing procedure, a depend-

able *in vivo* method of determining the sloughing rate is described.

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Effects of Androgens on Concentrations of LH and FSH in the Male Rat Pituitary.* (31713)

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The relative biologic potency among androgenic steroids is dependent, in part at least, on the end-point used in the assay. The present study was undertaken to compare the ability of several androgenic steroids and their biosynthetic precursors to prevent castration changes in pituitary LH and FSH concentration. An estrogenic steroid was included in the study with the hope that these data would further elucidate the control of gonadotropin secretion in the male rat.

Methods. The experiments were done in two series, the first in the fall of 1963, the second in the summer of 1965. Experimental procedures were made as similar as possible. Two hundred to 250 g male Sprague-Dawley rats were castrated or left intact and segregated into treatment groups. They were fed *ad libitum* with Purina Mouse-Breeder chow and tap water and were housed in a controlled environment of 76°F with 10 hours of light and 14 of darkness.

Subcutaneous injections of the materials to be tested were begun on the day of operation and continued 6 days a week for 30 injections. The volume of material injected was 0.25 ml. The steroids used and the doses employed are given in Tables I and II. Peanut oil (Planters) was used as the vehicle for the steroids and was used unadulterated for the control animals.

The rats were killed with chloroform 24 hours following the last injection. The pituitaries were quickly removed, weighed and soaked in acetone. Body weights were measured. The ventral prostates and seminal vesicles (plus coagulation glands) were dissected, plotted and weighed.

The acetone soaked pituitaries were dried *in vacuo,* homogenized in 0.9% saline and diluted appropriately for assay. Stock solutions were kept frozen at −10°C for reassay, usually within one to two weeks.

The pituitaries were assayed for LH content using Parlow's ovarian ascorbic-acid depletion procedure(1). The first series of assays was done using conditions as previously defined(2).† In the second series, the interval between injection and removal of the ovary for ascorbic acid measurement was in-

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† LH assays in both series employed 4 or 5 assay rats at each of the 2 dose levels tested.