

## TRANSIENT SHORTENING OF MICROVILLI INDUCED BY CYCLOHEXIMIDE IN THE DUODENAL EPITHELIUM OF THE CHICKEN

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### INTRODUCTION

As intestinal epithelial cells migrate from the crypts of Lieberkühn to the tip of a villus, their microvilli become longer and narrower and presumably increase in number per cell (1). These morphological alterations are accompanied by marked changes in the activities of the hydrolytic enzymes associated with the microvilli (2, 3), although the correlation between the morphological and enzymatic changes is not yet clear.

Little is known about the cellular mechanisms

that maintain the microvilli or that control the alterations during migration of the epithelial cells. Recent estimates of protein turnover rates in the rat intestine suggest, however, that microvilli are not static structures, but probably represent a steady state governed by rates of synthesis and degradation of their molecular components (4).

Inhibitors of RNA and protein synthesis have been effective tools for studying regulatory processes in a variety of cell types. Although such drugs elicit paradoxical effects on microvillar enzymes in developing stages of the intestine

(5, 6), they have thus far proved to have little or no effect on the microvilli of adult intestinal epithelial cells.

Previous studies with cycloheximide, which inhibits protein synthesis in many types of cells (7, 8), have indicated that this inhibitor has little effect on the rodent intestine. In rats, for example, doses of 1.5 mg/kg of body weight were reported to have no effect on the ultrastructure of crypt cells, including microvilli (9). This dose was, however, sufficient to inhibit the incorporation of leucine- $^{14}\text{C}$  into protein in epithelial cells, both in the crypts and on the villi (10). Furthermore, mitotic activity of crypt cells was eliminated within 2 hr (10). After the administration of the drug the activities of thymidine phosphorylase and adenosine deaminase, neither of which is a brush border enzyme, were rapidly decreased (11).

In mice, cycloheximide in doses of 40 mg/kg resulted in the atrophy of Golgi cisternae and in the appearance of autophagic vacuoles in the duodenal epithelium. This dose level also eliminated the alkaline phosphatase activity associated with the Golgi apparatus, but no effects on the structure of the brush border or the alkaline phosphatase activity associated with the brush border were noted (12).

In this preliminary paper we report that administration of cycloheximide to young chickens results in a rapid and reversible shortening of the microvilli in the duodenum and in a correlated reduction in the activities of two enzymes localized in these organelles.

#### MATERIALS AND METHODS

White Leghorn chickens, 2 wk posthatching, were injected subcutaneously on the underside of the thigh with cycloheximide (Calbiochem, Los Angeles, Calif.; 0.75 mg/kg of body weight) in 0.25 ml sterile 0.9% NaCl. Control animals received vehicle only. Animals were given free access to food and water and sacrificed at 0, 8, 16, 24, and 36 hr after injection.

For electron microscopy, segments of the proximal and distal limbs of the duodenal loop were placed in cold 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0. Individual villi were dissected from the tissue, kept in the fixative for 2 hr, postfixed in 1%  $\text{OsO}_4$ , and processed for electron microscopy. Thin sections were stained in 2% aqueous uranyl acetate and lead citrate (13).

For enzyme and protein assays, the remaining apex of the duodenal loop was chilled in ice-cold 0.9% NaCl, slit open and washed to remove food and mucus, then frozen and stored at  $-20^\circ\text{C}$ . Alkaline phosphatase, leucyl naphthylamidase, and pro-

tein content were assayed according to the methods previously described (2).

#### RESULTS AND DISCUSSION

Doses of 1.5 mg/kg cycloheximide proved to be lethal to chickens in our study. Sublethal doses of 0.75 mg/kg caused the microvilli to shorten in absorptive cells all along the length of villi in the duodenum, although the effect may be slightly more pronounced in cells near the tips of the villi. In order to compare changes in size and shape of microvilli at varying times after injection of cycloheximide, measurements were always made on the upper one-fourth of the villi; care was taken to avoid necrotic cells in the extrusion zone at the tip of the villus. By 8 hr after injection of the drug, microvilli on the apical portions of the villi measured only  $0.5\ \mu$  in length, as compared to  $2.0\ \mu$  in controls, a decrease of about 75% (Figs. 1, 4, 5). The average diameters of the shortened microvilli are about 12% greater than in controls ( $0.098\ \mu$  compared to  $0.085\ \mu$ ). Preliminary ultrastructural observations on sacrifices between 0 and 8 hr indicate that the decrease in length results from a progressive shortening of the microvilli rather than from a shedding of these structures from the surface of the cells. By 24 hr after administration of cycloheximide, the microvilli have regained the lengths and diameters seen in control specimens.

Other morphological changes in the microvilli were also noted in the 8-hr specimens. The angle formed between the microvilli and the cell surface was often less than the  $90^\circ$  that is typical of contro

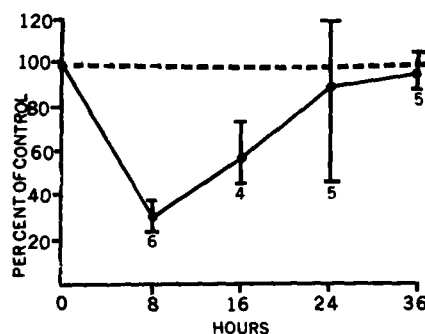


FIGURE 1 Ordinate: microvillus length as per cent of control; abscissa: hours. Changes in the lengths of duodenal microvilli after injection of cycloheximide at time 0. Actual dimensions are given in the text. Dots represent mean values obtained from at least two villi per animal; vertical bars indicate the range. Numbers beneath vertical bars indicate the number of animals.

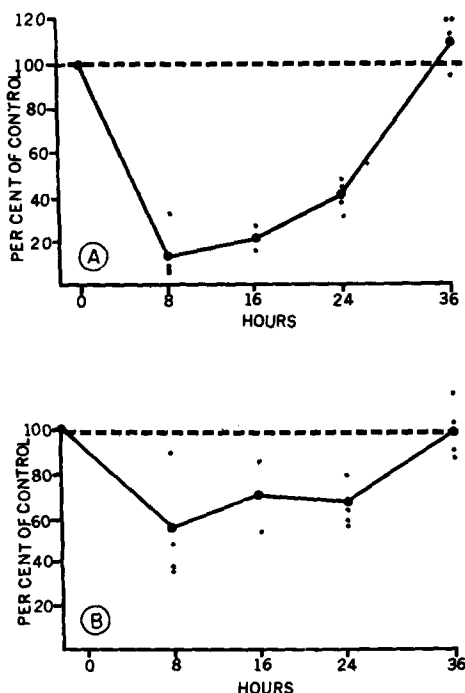


FIGURE 2 Activities of microvillar enzymes in the duodenum after cycloheximide injection at time 0. A total of 14 animals served as controls, with a minimum of two controls for each time point. Ordinate: values for cycloheximide-injected animals expressed as per cent of the control values for that time point. Each dot represents one animal; solid lines connect the mean values. Abscissa: time (hours) after injection. A: alkaline phosphatase. Mean activity of control samples was 1.71  $\mu$ moles/min per mg protein (range: 1.22–1.99). B: leucyl naphthylamidase. Mean activity of controls was 131 nanomoles/min per mg protein (range: 89–185).

specimens (Figs. 3, 4). There is also a decrease in the number of microvilli per unit distance of cell surface. In general, the microvilli of the 8-hr experimental animals tend to resemble those of the normal embryonic duodenum of the chick at about 13 days of incubation (14). Of particular interest is the apparent disruption of the terminal web (Fig. 4). The core filaments of the microvilli do not penetrate as far into the apical cytoplasm as in the normal epithelial cell; mitochondria and other organelles were seen to closely approach the apical cell surface.

Small regions of necrosis were observed near the tips of villi in some, but not all, of the specimens from cycloheximide-treated animals. We emphasize, however, that the shortened microvilli are not confined to these few necrotic cells. None of the shortened microvilli showed the

vesiculation that characterized microvilli of dying cells in the extrusion zone of the villus. The glycocalyx, missing in necrotic cells,<sup>1</sup> was present on the microvilli shortened by cycloheximide treatment (Fig. 6).

As another means of determining the effects of cycloheximide on the microvilli, we assayed the activities of two enzymes that are known to be localized primarily in the brush border: alkaline phosphatase (AlPase) and leucyl naphthylamidase (LNAase) (15, 16).<sup>2</sup> The specific activities of both AlPase and LNAase decreased markedly as a result of cycloheximide treatment (Fig. 2). The decrease in AlPase activity at 8 hr after injection (12% of control) was more dramatic than that of LNAase (55% of control). The activities of both enzymes returned to control levels by 36 hr after cycloheximide injection.

The over-all shape of the villi is also altered in cycloheximide-treated animals. As viewed in a dissecting microscope, each villus seems to assume a blunt paddle shape, more characteristic of the jejunum or ileum of chickens, in contrast to the long, finger-like shape typically seen in the duodenum. This change, too, is transient; normal shapes are resumed by 16 hr after injection of cycloheximide.

At this point, we have no evidence that the effects described here are due to interference with protein synthesis by cycloheximide. We cannot yet rule out the possibility that the alterations in duodenal microvilli are due to a side effect of the drug. Experiments are in progress to determine the degree of inhibition of protein synthesis caused by the dose levels we employed and to compare the effects of other protein synthesis inhibitors.

The recovery time for the effect of cycloheximide on the morphology (less than 24 hr for the microvillus length) and enzyme activities of the microvilli is much less than the turnover time for this population of epithelial cells (more than 48 hr, [17]). This consideration, and the observation that all microvilli on the villus are shortened, argue that the microvilli of the affected cells in the epithelial population regain their normal properties and that the recovery phenomenon is not due to replacement of the affected cells by new cells emerging from the crypts.

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<sup>1</sup> LeCount, T. S., and R. D. Grey. Unpublished observations.

<sup>2</sup> Grey, R. D. Unpublished observations.

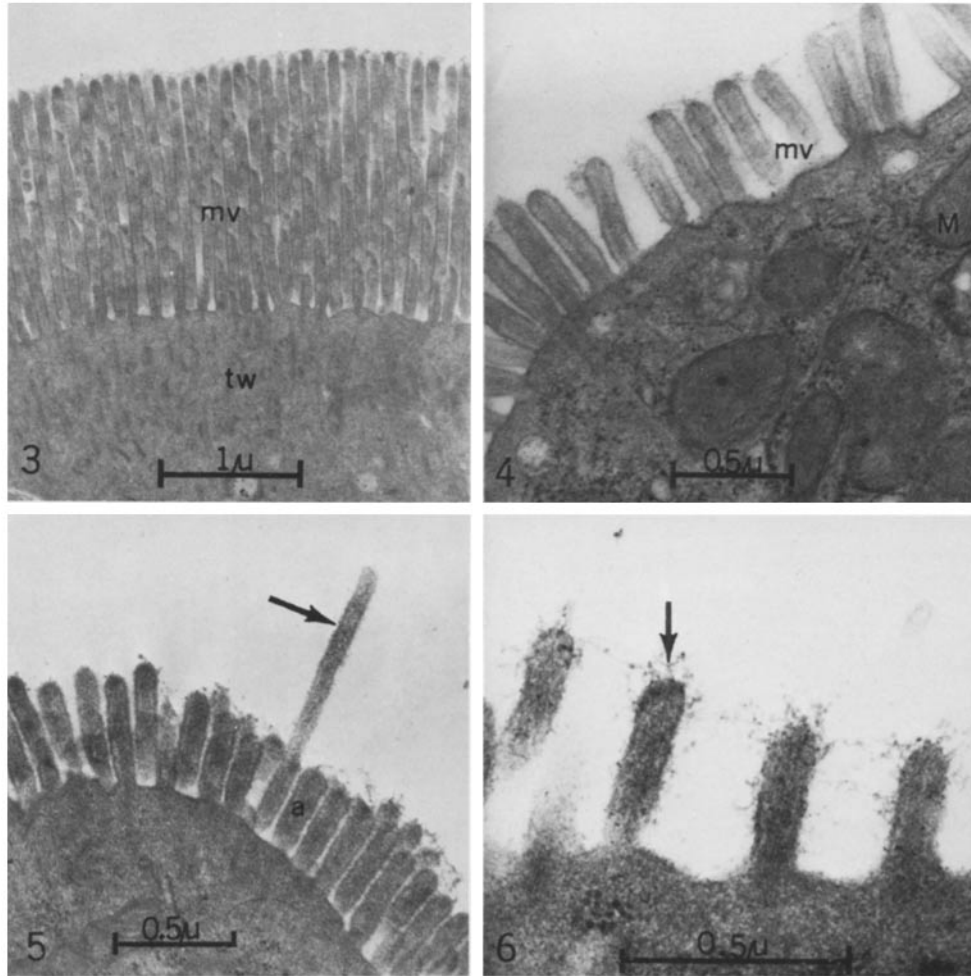


FIGURE 3 Microvilli (*mv*) of duodenal epithelial cell of control chick measure about  $1.9 \mu$  in length. Notice that the apical cytoplasm occupied by the terminal web is devoid of mitochondria and other cell organelles. *tw* = terminal web.  $\times 18,000$ .

FIGURE 4 Microvilli (*mv*) from chick that received  $0.75 \text{ mg}$  cycloheximide per kg body weight 8 hr before sacrifice measure about  $0.48 \mu$  in length. Mitochondria (*M*) closely approach apical surface of cell, suggesting the reduction or loss of the terminal web.  $\times 30,300$ .

FIGURE 5 Microvilli 8 hr after cycloheximide treatment. One microvillus (arrow) has retained most of its length. Adjacent microvillus (*a*) is  $0.39 \mu$  long.  $\times 30,800$ .

FIGURE 6 Microvilli have retained the glycocalyx (arrow) 8 hr after cycloheximide treatment.  $\times 57,900$ .

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