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The Effect of Partial Starvation and Glucagon Treatment on Intestinal Villus Morphology and Cell Migration (39378)

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The importance of gastrointestinal hormones in the functional physiology of alimentation has long been recognized. The observation that one of these hormones, gastrin, could stimulate incorporation of amino acids in gastric and duodenal mucosa, followed the earlier suggestion that gastrin hormones might influence processes related to cell growth and maturation in the gut (1, 2). Subsequently, other trophic effects of gastrointestinal hormones have been reported (3–8).

Altered blood levels of the pancreatic islet cell hormone glucagon (9) have been associated with augmented intestinal transport of amino acids and sugars in diabetic (10–12), partially starved (13), and glucagon-treated rats (14). Further, changes in small bowel motility, absorptive function, and structure have been reported in a patient with tumor secreting enteroglucagon (6, 7), a gut-derived hormone with immunologic and some functional identities with pancreatic glucagon. These observations raise the possibility that prolonged exposure to augmented glucagon serum concentrations might alter intestinal mucosal structure as well as function. This hypothesis is examined in this report with studies of jejunal morphology and cell migration kinetics in animals with hyperglucagonemia produced by repeated injections of the hormone or by partial starvation (9).

Animal preparation. Male Sprague–Dawley rats weighing between 150 and 200g were used uniformly throughout this study. Animals treated with glucagon were given ip injections of 50 μg of Lilly glucagon in diluent every 6 hr for varying periods of time. Control animals for glucagon studies received 0.05 ml of glucagon diluent alone (containing 0.2% phenol as a preservative) or 0.05 ml of normal saline. The last injection was always given 1 hr before autopsy. Throughout the period of glucagon treatment, control and experimental animals were allowed free access to food and water. In this study, the term "partial starvation" refers to the diet of rats that have been given 6 g of Rockland Complete Mouse-Rat Diet per day per rat instead of the normal food intake of control rats, 22 g per day. Food was given to the starved animals in the evening. Control animals were allowed free access to food and water until autopsy.

Glucagon assay. Blood for glucagon assay was drawn from ether-anesthetized animals by aortic catheterization. All blood was collected into chilled tubes containing 2000 KIU of Trasylol. The blood was allowed to clot, and serum separated under refrigeration was then stored frozen until assayed for glucagon. Serum glucagon concentrations were measured by a standard double-antibody radioimmunoassay (9). ^{125}Iodine-labeled glucagon with a specific activity of greater than 350–400 mCi/mg was purchased from Cambridge Nuclear. The assay sensitivity was better than 20 pg/ml. Antibody to porcine, insulin-free glucagon was produced in guinea pigs. The second antibody used was rabbit anti-guinea pig immunoglobulin. The antiserum was not wholly specific for pancreatic glucagon, as it reacted minimally with purified enteroglucagon (kindly supplied by L. Heding of Novo Industries). Standard curves obtained at the beginning and at the end of the assay were

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identical. All determinations were performed in triplicate. Variance within the assay was less than 10%. Interassay variance was negligible.

Morphology and cell migration by autoradiography. After 7 days of semistarvation or 5 days of 6-hourly glucagon injections, animals were given ip injections of 100 μCi of [*methyl*-^{3}H]thymidine in a 1-ml volume of 0.9% saline. At 1.5, 8, 20, and 32 hr after the injection, animals were sacrificed, and a segment of proximal jejunum 1-cm long was removed 12-cm distal to the pyloric valve. This segment was placed serosal side down onto a piece of cardboard and immersed in 20 ml of buffered formalin for 2 days. The 10% buffered formalin was made by mixing 7 g of monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$), 17 g of anhydrous dibasic sodium phosphate ($NaHPO_4$), 160 ml of formaldehyde, and enough water to make 1600-ml total volume at a pH of 7.0. The tissue was then dehydrated, embedded in paraffin, cut so that each section was at least 50 μm apart, and the sections put onto glass slides. The slides were coded with random numbers and dipped in undiluted Kodak NTB-2 liquid emulsion, allowed to dry, placed in light-tight boxes with desiccant, and stored in uniform orientation in a refrigerator for 8 weeks. At the end of this period, the slides were developed in Kodak Dektol Developer for 5 min at 19° and washed in running water at the same temperature for 30 min. The sections were then stained with Nuclear Fast Red and cover-slipped.

The slides were examined in a Bausch and Lomb microscope that contained a wide-field eyepiece with a grid calibrated for measuring. The grid contained 400 square units, each unit having 0.05175-mm sides at low power. Measurements were made of villus height, crypt depth, and the distance on the villus covered by radioactive grains visualized over nuclei. All measurements were made at 10× magnification, mucosal side of the muscularis mucosae as a base line. Only villi were measured which were oriented so as to demonstrate a vertical section through the crypt; thus an unbroken line of cells could be followed from the base of the crypt to the tip of the villus. After all the slides had been examined and recorded, the numbering code was broken.

Data are presented as the sample mean plus or minus the standard deviation. Straight lines in graphs were drawn by the method of least squares (16). The letter "b" is used to denote the regression coefficient of each line. Regression coefficients were compared with Student's *t* test to establish significant differences between plotted lines.

Results. Production of exogenous hyperglucagonemia. To examine the effect of ip injections of 50 μg of glucagon on serum levels of the hormone, sera were assayed at various intervals after an injection. The results are shown in Fig. 1. In a previous study, control animals had mean serum glucagon levels of 77 pg/ml, under the conditions of this assay (9). After injection, serum glucagon levels were above the normal range for the first 2 hr, but thereafter values returned to the range of physiologic function.

Effects of partial starvation and glucagon treatment on intestinal villus morphology and cell migration. To examine whether prolonged exposure to elevated plasma concentrations of glucagon might exert a trophic effect on jejunal mucosa, measurements of villus height and rate of villus cell migration were determined in animals made hyperglucagonemic with repeated doses of glucagon or by partial starvation. Partial starvation for 7 days has previously been shown to be associated with marked hyperglucagonemia in the rat (9). After 7 days of partial starvation or 5 days of 6-hourly glucagon injections, the mean villus length in both experimental groups was found to be significantly reduced. The crypt-to-villus ratio was unal-

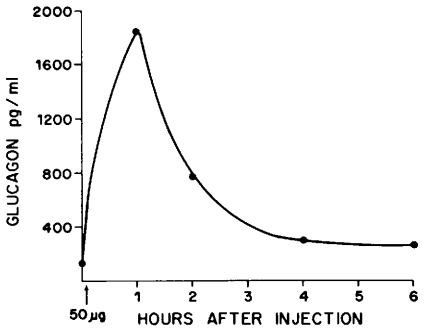

FIG. 1. The effect of exogenously administered glucagon on serum levels of the hormone. Fifty micrograms of glucagon were injected intraperitoneally.

tered by either treatment modality. These data are presented in Table I. Further, as shown in Fig. 2, the rate of cell migration was diminished with both partial starvation and glucagon treatment, but only glucagon therapy was found to reduce the rate of cell migration significantly ($p < 0.01$): (b = 7.8 $\times$ 10^{-3} mm/hr), compared to controls (b = 13.6 $\times$ 10^{-3} mm/hr).

Discussion. Active transport of amino acids and sugars has been shown to be augmented in two conditions associated with hyperglucagonemia in the rat, namely, partial starvation (9, 13) and diabetes (10–12). Recent studies reported from this laboratory have shown that chronic administration of glucagon to rats will also increase intestinal transport of amino acids and sugars (14). These functional alterations seen in association with hyperglucagonemia might be explained by a morphologic effect of the hormone on the intestinal mucosa.

It was the purpose of this study to determine whether the hyperglucagonemia previously shown to be associated with augmented intestinal transport might also be accompanied by altered intestinal morphology or cell migration kinetics. In both partially starved and glucagon-treated animals the mean villus length was found to be significantly reduced when compared to that of controls. The rate of cell migration was diminished by both partial starvation and glucagon treatment, but only the latter was found to reduce the rate of villus cell migration significantly compared to controls.

These observations in starved animals are in accord with those of other authors (18–21) who have also observed decreased villus height and a diminished rate of cell migration in starved animals.

Since villus height is decreased with both semistarvation and glucagon therapy, it might be argued that in both instances the animals probably had fewer transporting cells than control animals. Despite this, tissues from glucagon-treated (14) and semistarved animals (8, 12) have been shown to be able to absorb amino acids and sugars more effectively. It appears from Kinter's autoradiographs (17) that, as intestinal epithelial cells migrate upward on the villus and become more mature, they become progressively better able to transport. If this is true, then lengthening the residence time from crypt to villus tip might result in a population of more mature cells on the villus, and therefore increase the capacity for active transport of amino acids. A decreased rate of cell migration was observed in tissues from semistarved and glucagon-treated animals. Glucagon-treated animals appeared to have a slower rate of cellular migration than do semistarved animals, but the semistarved animals showed a greater stimulation of

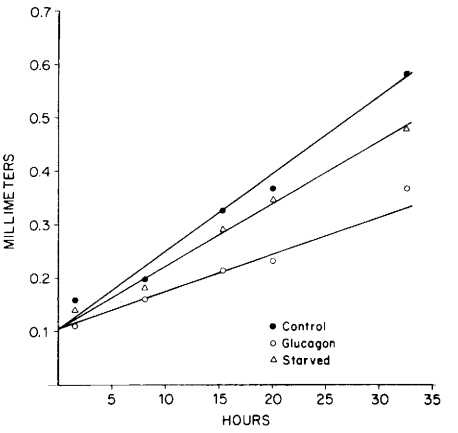

FIG. 2. The rate of cell migration in control, semistarved, and glucagon-treated rats. The rate was significantly decreased in glucagon-treated animals ($p < 0.01$). The difference in rate was not significant for semistarved animals.

TABLE I. THE EFFECT OF SEMISTARVATION AND GLUCAGON TREATMENT ON VILLUS LENGTH AND CRYPT DEPTH.

	Villus length in mm Mean ± SD	Crypt depth in mm Mean ± SD	Number of animals	Significance[a]
Control	0.75 ± 0.03	0.19 ± 0.02	15	–
Semistarved	0.66 ± 0.07	0.16 ± 0.02	14	$p < 0.001$
Glucagon-treated	0.61 ± 0.07	0.16 ± 0.02	14	$p < 0.001$

Note: Villus length and crypt depth were decreased by semistarvation and glucagon treatment.
[a] Significance figures apply to both villus and crypt measurements.

transport function. Therefore, it is not likely that an increased villus dwelling time alone could account for the increased transport seen in these two conditions.

The morphologic and cell migration data presented here and elsewhere (18, 19) raise some important questions about the relationship between intestinal cell migration and function. The technology is presently at hand to examine this problem further. An altered rate of intestinal cell migration and turnover can now be studied, and transport can be quantified, in any of a variety of situations (radiation, starvation, glucagon therapy, sprue, etc.). In this way it may become possible to examine the relationship between cell turnover, migration time, and functional parameters of the intestinal epithelium.

Summary. Male Sprague–Dawley rats were either treated with repeated ip injections of glucagon every 6 hr or partially starved. After 7 days of partial starvation or 5 days of glucagon injections, a time period shown previously to induce increased transport, all animals were sacrificed and a segment of jejunum was removed, fixed in formalin, sectioned, and dipped in Kodak NTB-2 liquid emulsion. After 8 weeks of exposure the autoradiographs were developed; assessments of villus height and crypt depth and measurements of length of the column of exposed grains were made in a calibrated microscope. The mean villus length in both semistarved and glucagon-treated groups was found to be significantly reduced ($p < 0.001$) when compared to control animals. The crypt-to-villus ratio was found to be unaltered by either treatment modality. The rate of cell migration was diminished by both partial starvation and glucagon treatment, but only glucagon therapy was found to cause a significant ($p < 0.01$) reduction in the rate of cell movement when compared to controls.

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