

## Chronic Glucagon Administration Enhances Intestinal Transport in the Rat<sup>1</sup> (37059)

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(Introduced by R. W. Wissler)

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The pancreatic islet cell hormones glucagon and insulin modulate many of the metabolic responses of liver, muscle, and fat cells. Insulin is the primary factor in the adjustment of the function of these tissues in the postprandial state while glucagon acts to mediate the response to fasting. Since the goal of these hormonal controls is to maintain an appropriate distribution and utilization of substrate by body tissues, it is possible that these hormones might act toward this end by adjusting the rate of intestinal uptake of amino acids, sugars, and other essential nutrients. Unlike liver, muscle and adipose tissue, intestinal epithelium appears to be largely unresponsive to changes in plasma insulin levels (1). However, the active intestinal transport of amino acids and sugars has been shown to be markedly increased in at least two instances which are accompanied by prominent hormonal alterations, diabetes (2, 3) and partial starvation (4). In both instances the absolute or relative level of plasma glucagon is increased (5, 6). In order to see if hyperglucagonemia itself could cause increased transport, we have studied intestinal uptake of amino acids and sugars in rats treated with repeated doses of glucagon. We have found that experimental exogenous hyperglucagonemia does produce changes in

intestinal transport similar to those previously observed in diabetes and semistarvation.

*Methods.* Sprague-Dawley male rats weighing between 150 and 200 g received 6-hourly intraperitoneal injections of 50  $\mu$ g of glucagon<sup>4</sup> in a diluent containing 0.2% phenol as a preservative, 0.05 ml normal saline, or 0.05 ml of diluent alone. The last injection was always given 1 hr before sacrifice. Feeding and behavior patterns were monitored. Throughout the period of treatment, control and experimental animals were allowed free access to food and water until the time of sacrifice. Assay of intestinal absorption was carried out by minor modifications of the method of Crane and Mandelstam (7) and Olsen and Rosenberg (2). The rats were sacrificed by stunning and decapitation. Segments of proximal jejunum were removed, washed in chilled, oxygenated Krebs-Ringer bicarbonate buffer, and incubated in 2.4 ml of the same buffer containing the actively transported but nonutilizable <sup>14</sup>C labeled substrates, 3-O-methylglucose (3-OMG) and 1-aminocyclopentane-1-carboxylic acid (ACPC), at a concentration of about 2.0  $\mu$ g/ml of incubation medium. After incubation for 40 min at 37° in stoppered flasks containing an atmosphere of 95% O<sub>2</sub> and 5% CO<sub>2</sub>, the rings were removed, rinsed, blotted, weighed and then equilibrated in distilled water by boiling for 6 min. Aliquots of tissue fluid extract and media were counted in a liquid scintillation spectrometer with an efficiency for <sup>14</sup>C of 60%. After making adjustments for the extracellular (inulin) space and for tissue water, the sugar and amino acid concentration in the intracellular

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TABLE I. Effect of Glucagon Administration on Inulin Space and Tissue Water.

	% Inulin space (mean $\pm$ SD)	% Tissue water (mean $\pm$ SD)	No. of animals	Significance
Control	8.63 $\pm$ 3.38	81.8 $\pm$ 2.2	9	NS
Glucagon	9.97 $\pm$ 2.91	82.0 $\pm$ 3.1	9	NS

fluid and that in the extracellular medium were calculated. This intracellular to extracellular fluid concentration (ICF/ECF) ratio was utilized as a measure of intestinal transport.

*Results.* Table I demonstrates that there was no difference in tissue water or extracellular inulin space after treatment with glucagon for 6 days.

The sugar, 3-O-MG, and the amino acid, ACPC, are actively transported by the intestinal epithelium, but neither is metabolized or incorporated into macromolecules by the epithelial cells. Table II demonstrates the intracellular to extracellular fluid ratios of 3-O-MG and ACPC in intestinal rings from control animals and from animals treated with glucagon for a period of 6 days. The differences were highly significant.

The effect of duration of treatment with glucagon upon the observed changes in intestinal function is demonstrated in Fig. 1. After as little as 2 days of treatment an increase in uptake of ACPC was invariably observed, but it was not until 4 days of treatment that this difference became significant. A different pattern, shown in Table III, was seen after treatment with injections of 50  $\mu$ g of glucagon every 6 hr for 26 hr or less. In all experiments the uptake of ACPC into tissue from animals treated with glucagon for 14 hr prior to sacrifice was decreased compared to the uptake observed in control tissues. However, the differences were not significant.

Table IV demonstrates that glucagon added to the incubation medium (0.5 or 5.0  $\mu$ g/ml) had no observable effect on intestinal uptake of ACPC by tissue rings from control animals during the usual 40 min incubation period.

To determine if rates of uptake, as well as steady state concentrations of ACPC, were increased by glucagon treatment, timed experiments were performed. Figure 2 shows

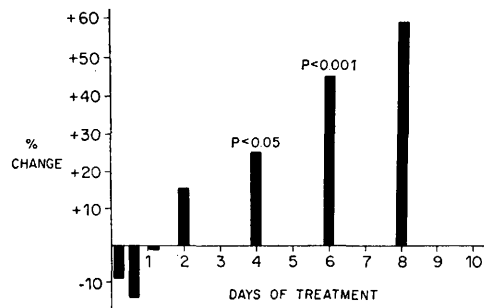


FIG. 1. The effect of glucagon treatment for varying time periods on intestinal transport of ACPC. The bars represent the percentage change in ICF/ECF ratios in treated animals compared with controls.

the ICF/ECF ratios achieved with increasing incubation times by intestinal rings taken from control animals and from animals treated with glucagon for 6 days. After 5 min of incubation, rings from glucagon-treated animals had accumulated more ACPC. This accumulation continued to increase linearly throughout the 40 min of incubation. Thus the rate of uptake or influx was enhanced by

TABLE II. Effect of Chronic Glucagon Administration on Intestinal Transport.

Substrate	Glucagon			Control			Significance
	ICF/ECF ratio (mean $\pm$ SD)	No. of observ. animals	No. of animals	ICF/ECF ratio (mean $\pm$ SD)	No. of observ. animals	No. of animals	
3-O-Methyl Glucose	2.89 $\pm$ 0.49	38	10	2.42 $\pm$ 0.52	38	10	$p < .005$
ACPC	7.03 $\pm$ 1.48	28	12	4.85 $\pm$ 1.48	28	12	$p < .001$

TABLE III. Effect of Acute Glucagon Administration on Uptake of ACPC.

Period of treatment (hr)	ICF/ECF ratio (mean $\pm$ SD)	No. of observations	No. of animals	Significance
Control	5.40 $\pm$ 1.75	21	12	
2	4.95 $\pm$ 2.51	21	12	NS
14	4.66 $\pm$ 1.73	21	12	NS
26	5.31 $\pm$ 1.04	21	12	NS

glucagon treatment.

To exclude the possibility that the observed increase in intestinal uptake of ACPC in glucagon-treated animals might have been the result of changes in eating habits, paired feeding experiments were carried out. Table V shows that when ACPC uptake was com-

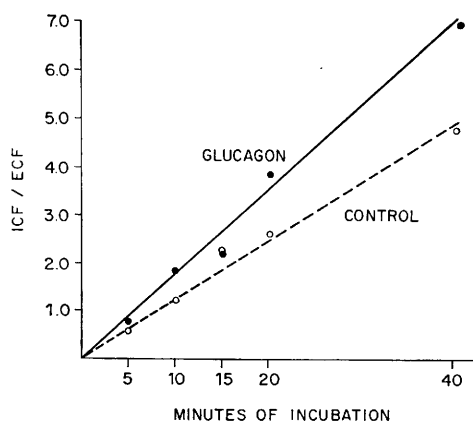


FIG. 2. The effect of time of incubation on uptake of ACPC by intestinal rings from control animals and from animals treated with glucagon for 6 days.

pared in pair fed control and glucagon-treated animals, the uptake by rings from glucagon-treated animals was significantly larger than that of pair fed animals. Furthermore, the values attained by rings from pair fed animals showed no significant difference from those

attained by control animals allowed free access to food. The amount of food (Rockland Complete Mouse-Rat Diet) consumed by control animals (21.9  $\pm$  1.82 g/rat/day) was not significantly different from that consumed by the glucagon-treated animals (22.4  $\pm$  2.18 g/rat/day). Food consumption remained stable from the first day of the experiments to the sixth.

*Discussion.* Glucagon causes an increase in hepatic uptake and deamination of some amino acids (8, 9), and stimulates the release of other amino acids from hepatocytes and fatty acids from adipose tissue (10, 11). Intestinal responses to glucagon have been observed only after acute exposure to the hormone. Ponz, Lluch and Planas (12) using an intestinal loop preparation found that an injected dose of glucagon decreased intestinal absorption of glucose. Varro and Csernay (13) reported that the intra-arterial administration of glucagon during perfusion had no effect on the transport of glucose from the lumen into intestinal epithelial cells, but increased the rate of loss of glucose from the epithelial cells toward the serosa. Neither Nagler, Forrest and Shapiro (14) nor Aulsebrook (15) observed any effect of glucagon added to the incubation medium on intestinal absorption of glucose using everted loop preparations. Recently, Hubel (16) has shown that glucagon can cause an increase in net sodium and water absorption by the perfused

TABLE IV. Effect of Glucagon in the Incubation Medium on Uptake of ACPC.

	ICF/ECF ratio (mean $\pm$ SD)	No. of observations	No. of animals	Significance
Control	4.71 $\pm$ 1.08	24	10	
0.5 $\mu$ g glucagon/ml incubation medium	5.14 $\pm$ 1.36	24		NS
5.0 $\mu$ g glucagon/ml incubation medium	4.35 $\pm$ 0.99	24		NS

TABLE V. Intestinal Uptake of ACPC in Animals Pair Fed with Glucagon-Treated Animals.

Group	ICF/ECF ratio (mean $\pm$ SD)	No. of observations	No. of animals	Significance
Pair fed controls	5.57 $\pm$ 0.82	24	6	
Glucagon treated	7.04 $\pm$ 1.15	24	6	$p < .001$

small bowel of the rat.

We have found, as others have previously, that treatment with glucagon has little effect on intestinal uptake of amino acids or sugars during the first 24 to 48 hr. When glucagon was administered for periods longer than 2 days, however, the uptake of amino acids and sugars by the small intestine was increased. The observed change in transport brought on by glucagon administration has been shown not to be due to differences in food consumption. Probably, it was not due to systemic hyperglycemia secondary to glucagon administration, as Olsen and Rosenberg (2) have shown that infusion of glucose in sufficient amounts to cause chronic hyperglycemia for a period of 5 days, had no observable effect on intestinal uptake in the rat. The increase in cellular concentrations of ACPC and 3-O-MG with glucagon treatment cannot be attributed to changes in the utilization of these substrates by the epithelial cells as neither compound is metabolized. Even after only 5 min of incubation with labeled amino acid the tissues from animals treated with glucagon achieved a higher ICF/ECF concentration ratio. Therefore, the increase in intracellular concentration is best explained by an increased rate of transport into the epithelial cells.

The doses of glucagon used in this study were large, and, for a period of time after the intraperitoneal injections, the plasma concentrations of glucagon were no doubt higher than physiologic. Therefore, it is possible that the observed effects were pharmacologic rather than physiologic. However, there are reasons to postulate that endogenous glucagon elevations result in functional changes in the intestine, since similar changes in transport have also been observed in two known instances of endogenous hyperglucagonemia: diabetes (5) and food deprivation (6). Induction of alloxan diabetes or partial starva-

tion is followed first by a slight decrease and, after 2 to 4 days, by a marked increase in intestinal transport (unpublished data). This pattern is very similar to that observed in exogenously induced hyperglucagonemia.

Data are not available to establish whether this effect of glucagon on the intestine is direct or mediated by other hormonal or metabolic responses. Experimental hyperglucagonemia provides a method of studying the mechanism by which the intestine adapts to prolonged changes in its hormonal environment.

*Summary.* Hyperglucagonemia, induced experimentally in rats by 6-hourly injections of glucagon, resulted in increased intestinal transport of amino acids and sugar as measured by the everted ring technique. Glucagon administration enhanced both accumulation and rate of uptake into the intestinal rings. The effect was observed after a lag of 2 days and increased thereafter with the length of treatment. Glucagon added *in vitro* was without effect.

This experimental system, like diabetes and partial starvation, provides a technique for studying the effect of relative or absolute hyperglucagonemia on the intestine.

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