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Increased Glucagon Secretion in Protein-fed Rats: Effects of Refeeding a Normal Diet (40250)

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Chronic feeding of a high protein diet to rats (1 week or more) results in elevated glucagon in peripheral blood (1), increased glucagon secretion by isolated pancreatic islets and loss of the suppressive effect of glucose on release of the hormone (2). The increase of glucagon secretion develops gradually during protein feeding and is not fully manifest until the diet has been fed for 7 days (2). Because of the gradual onset of pancreatic alpha cell hyperfunction, the question arose as to the rapidity with which elevated glucagon secretion would revert to the basal level upon resumption of a normal diet. To answer this question, we have evaluated alpha cell function in rats fed a high protein (HP) diet for one week, in rats fed a control diet and in animals fed the HP diet for one week and then returned to the control regimen for 1, 2, 3, 5 or 7 days.

Methods and materials. Male, Holtzman rats weighing 200–225 g were used in this study. Animals were housed in individual cages and fed Purina rat chow *ad lib.* during a several day period of adjustment to their surroundings. At the onset of experiment I, rats were divided into three groups, one of which was fed a HP diet for one week, a second which was fed a control diet for a similar period and a third which consumed the HP diet for one week and was then returned to the control diet for 1, 2, 3, 5 or 7 days. Composition of the HP diet, which contained 70% casein, and control food has been described (3). The days on which animals were started on the experiment were staggered so that blood samples could be obtained from rats of the three groups at the same time. When the experiment was concluded, animals were anesthetized with sodium pentobarbital (4.5 mg/100 g body wt), the abdomen opened, blood taken from the portal vein for glucose and hormone assays. All specimens were obtained between 9:00 and 10:00 AM. Three ml of blood from each rat were placed in a tube containing 1500 units of Trasylol and 2.6 mg EDTA and centrifuged in the cold. The plasma was removed and kept frozen until glucagon assays were done using Unger's antibody 30K (4). Remaining blood from each animal was collected in a heparin-containing tube, centrifuged and the plasma removed. Insulin assays on plasma were done by the method of Morgan and Lazarow (5) and glucose analysis was carried out by the glucose oxidase method (6).

Experiment II was designed and carried out in the same manner as the previous study, however, at the end of the experiment rats were killed, the pancreases distended by injection of Hank's solution into the common bile duct and removed from the carcass *in toto.* Pancreases were minced with a fine scissors and subjected to enzymatic digestion according to the method of Lacy (7). One hundred islets of uniform size were isolated, placed on a Millipore filter which was housed in a small chamber and perifused for 30 min with Krebs-Ringer bicarbonate solution containing 0.2% bovine serum albumin and 1.7 m*M* glucose. The medium was then changed by addition of arginine to attain a 20 m*M* concentration and perifusion continued for an additional 30 min. At this time the arginine-containing medium was replaced by the original solution and perifusion carried out for a final 20-min period. Samples of perifusion media were stored in the frozen state for subsequent radioimmunoassay of insulin and glucagon.

Results. In Experiment I, portal vein glucose values of control and HP rats were virtually identical (Table I), thus, confirming our previous finding that blood glucose is not altered in animals consuming a high protein, carbohydrate free diet (1). Glucose levels in rats initially fed the HP diet and subsequently

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TABLE I. EFFECT OF PROTEIN FEEDING ON PORTAL VEIN GLUCOSE, INSULIN AND GLUCAGON MEAN VALUES ± SEM.

	Plasma glucose (mg/100 ml)	Plasma insulin (uU/ml)	Plasma glucagon (pg/ml)
Control diet (6)[b]	174 ± 17	289 ± 61	133 ± 10
High protein diet (7)	175 ± 5	254 ± 39	716 ± 45[a]
High protein + control diet			
1 day (4)	192 ± 16	218 ± 50	684 ± 42[a]
3 days (4)	176 ± 7	279 ± 61	719 ± 52[a]
5 days (3)	163 ± 9	149 ± 46	665 ± 57[a]
7 days (4)	157 ± 13	152 ± 19	196 ± 68

[a] Significantly different from control, $P = <0.001$.
[b] Number of animals.

returned to the control regimen varied from a low of 157 ± 12.5 mg/100 ml to a high of 192 ± 15.7 mg/100 ml, however, these values were not statistically different from those of the control or HP groups (Table I). Although portal vein insulin showed considerable variation between groups, differences were not significant because of the wide range of values within each group. Protein feeding resulted in a fivefold increase of portal vein glucagon (control = 133 ± 10 pg/ml; HP = 716 ± 45, $P = <0.001$) which was maintained for 5 days after resumption of normal food intake (Table I). In rats fed the control diet for 7 days after cessation of protein feeding, portal vein glucagon had returned to the control level (control = 133 ± 10 pg/ml; HP + control-7 = 196 ± 68, $P = >0.05$).

Although the data obtained in Experiment I and previous reports from our laboratory (2) demonstrate increased pancreatic glucagon secretion in protein fed rats, the effect of arginine stimulation of the hyperfunctioning alpha cell has not been determined. Perifusion of isolated islets from control rats with an arginine-containing medium for 30 minutes elicits a biphasic pattern of glucagon secretion (Fig. 1). Exposure of islets from HP fed rats to arginine also results in biphasic glucagon release, however, the amount of hormone secreted in both phases is greater than that released by control islets (Fig. 1). Islets of rats that initially consumed the HP diet and more subsequently fed the control diet for 1 or 2 days responded to arginine in the same manner as did HP islets (Fig. 1). Estimates of glucagon secretion made by planimetric measurement of the area under the glucagon curves during arginine stimulation are presented in Table II. Glucagon secretion by HP islets and HP + control (1 and 2 days) islets did not differ and both exceeded that by control islets (control = 56.4 ± 4.2 pg/islet/30 min; HP = 84.6 ± 5.3; HP + control, 1 and 2 days = 72.4 ± 3.7). Glucagon release by islets from HP + control (3 day) rats was the same as that of HP islets and greatly exceeded hormone secretion by control islets (control = 57.8 ± 3.5 pg/islet/30 min; HP = 94.0 ± 5.5; HP + control (3 day) = 89.6 ± 8.5). After 5 days of refeeding the

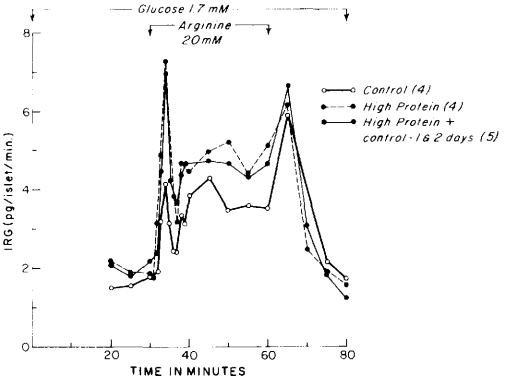

FIG. 1. Glucagon secretion in response to arginine occurs in two phases—an initial short burst followed by a second phase of sustained hormone release. Removal of arginine from the medium is followed by a short period of elevated glucagon secretion which spontaneously reverts to the basal level. Consumption of the control diet for 1 or 2 days after one week of protein feeding does not lower the enhanced hormone release.

TABLE II. EFFECTS OF PROTEIN FEEDING ON ARGININE-INDUCED GLUCAGON SECRETION.

	Glucagon secretion (pg/islet/30 min)*
Control (4)	56.4 ± 4.2
High protein (4)	84.6 ± 5.3[a]
High protein + control (5) 1 and 2 days	72.4 ± 3.7[a]
Control (7)	57.8 ± 3.5
High protein (8)	94.0 ± 5.5[b]
High protein + control	
3 days (6)	89.6 ± 8.5[c]
5 days (3)	60.8 ± 8.1
7 days (3)	51.6 ± 2.3

* Mean values ± SEM.
[a] Significantly different from control, $P = <0.01$.
[b] Significantly different from control, $P = <0.001$.
[c] Significantly different from control, $P = <0.005$.

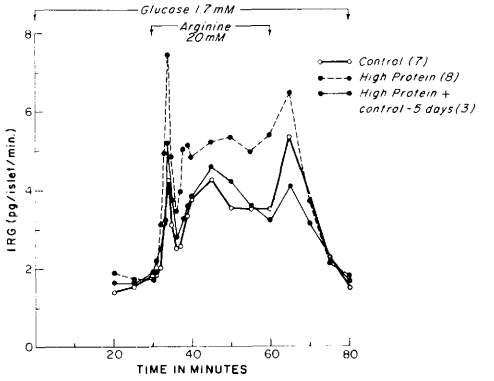

FIG. 2. After 5 days on the control regimen, subsequent to one week of protein feeding, glucagon secretion has returned to the basal level.

control diet, the elevated secretion of glucagon which had been induced by the HP regimen returned to the control level (Fig. 2, Table II). Glucagon secretion by HP + control (7 days) islets remained at the control level (Table II).

Discussion. Consumption of a high protein diet by rats results in a marked increase of pancreatic alpha cell function. Glucagon secretion by isolated pancreatic islets is not altered during the first 3 to 4 days of HP feeding, however, by the fifth day glucagon release does not suppress normally in response to high glucose concentrations (2). After 1 week on the HP diet, islet glucagon secretion is greatly elevated and hormone release is not inhibited by raising medium glucose to very high concentrations (2). In the present study, we have demonstrated that the augmented glucagon secretion induced by protein feeding does not return to the basal level for 5–7 days after resumption of a normal diet. Portal vein glucagon which was increased by more than 500% in HP fed rats did not return to normal until 7 days after returning to the control dietary regimen. Glucagon secretion by isolated islets in response to arginine was enhanced by protein feeding and did not revert to a normal pattern until 5 days after resumption of the control diet.

Our observations concerning the effects of protein feeding on pancreatic alpha cell function raise significant questions. First, what is the explanation for the delayed onset of glucagon hypersecretion in response to protein ingestion when previous investigations have demonstrated an acute rise of glucagon release following consumption of a protein meal (8). Secondly, why does the increased glucagon release continue for several days after animals resume a normal dietary intake. Studies of glucagon biosynthesis have been conducted using incorporation of labelled tryptophan into glucagon-like immunoreactive material as a measure of the synthetic process (9–12). In anglerfish islets, ^{3}H-tryptophan is incorporated into several peptides of varying molecular size, the largest of which has a molecular weight (MW) of ~12,000 daltons (9). This large glucagon precursor is rapidly cleaved to a peptide having a molecular weight of ~9000. The second intermediate is apparently then cleaved to a peptide of 4900 MW which in turn is converted to 3500 MW glucagon (9). It was suggested that the last two steps occur much more slowly than conversion of the ~12,000 MW component to the ~9000 MW intermediate. Further evidence that glucagon biosynthesis proceeds slowly was provided by Hellerstrom *et al.* who investigated ^{3}H-tryptophan incorporation into glucagon by isolated guinea pig islets (10). These investigators found that incubation of ^{3}H tryptophan with isolated islets for 17 hr resulted in incorporation of label into a protein with glucagon-like immunoreactivity of ~18,000 MW but there was minimal labelling of protein that eluted from the column with glucagon. Prolonged incubation of ^{3}H-tryptophan with islets in tissue culture (6 days) resulted in labelling of glucagon as well as the ~18,000 MW precursor. Thus, it appears that the delayed increase of glucagon secretion induced by HP feeding in our studies is due to the slow rate of hormone biosynthesis. Since the onset of glucagon synthesis is not rapid, the acute rise of plasma glucagon which occurs in response to a protein meal probably represents release of preformed glucagon and does not involve hormone synthesis.

The belated incorporation of tryptophan into 3500 MW glucagon observed by Hellerstrom (10) suggests slow turnover of enzymes involved in synthesis of the hormone. If enzyme turnover occurs at the rate indicated by Hellerstrom's studies, a 5- to 7-day delay in return to normal of elevated glucagon secretion induced by protein feeding might be

expected. Studies of the turnover of enzymes involved in glucagon synthesis will ultimately determine whether this explanation is correct. Another possible explanation for the delayed decline of glucagon secretion is that protein feeding causes hyperplasia as well as hyperfunction of pancreatic alpha cells in rats (13). A continued high rate of glucagon secretion after cessation of protein feeding may be due to the gradual regression of cellular hyperplasia.

Although glucagon release is stimulated by several mechanisms (e.g., hypoglycemia, strenuous exercise, hypercorticism), these stimuli are not involved in control of alpha cell function under normal physiological circumstances. Starvation which was previously through to be a significant stimulus of glucagon secretion is now known to alter metabolism of the hormone rather than release (14). It therefore appears that the major stimulus to glucagon secretion is protein ingestion, a situation which emphasizes the important role of glucagon in promoting hepatic gluconeogenesis.

Summary. This study was conducted to determine the length of time required for elevated glucagon secretion induced by protein feeding to be reversed upon resumption of a normal diet. Portal vein (PV) glucagon and glucagon secretion by isolated islets stimulated with arginine were determined in rats fed a high protein (HP) diet for 1 week, in control rats and in animals fed the HP diet for one week followed by the control regimen for 1, 2, 3, 5 or 7 days. Protein feeding caused a 500% rise of PV glucagon which was not reversed until day 7 on the control food. Arginine stimulated glucagon secretion by isolated islets which was almost doubled by protein feeding, returned to basal level on the fifth day of refeeding the control diet. These observations are in agreement with previous work which has demonstrated a slow change in the rate of glucagon synthesis.

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