

Characterization of the Responses of Circulating Glucagon-Like Immunoreactivity to Intraduodenal and Intravenous Administration of Glucose

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ABSTRACT The effects of ingested and infused glucose upon circulating glucagon-like immunoreactivity (GLI) were compared in 14 triply catheterized conscious dogs. Within 60 min after the intraduodenal administration of 2 g/kg of glucose, the mean level of glucagon-like immunoreactivity in the vena caval plasma more than doubled, whereas after intravenous infusion of the same dose over a 90 min period no change in the mean vena caval level was observed; during glucose infusion mean glucagon-like immunoreactivity in the pancreatic venous effluent declined, suggesting that hyperglycemia suppresses rather than stimulates pancreatic glucagon secretion.

To determine if the rise in glucagon-like immunoreactivity that occurs during glucose absorption was of pancreatic origin, the effect of pancreatectomy performed 1 hr after the intraduodenal administration of glucose was determined. Although circulating insulin disappeared after resection of the pancreas, the level of glucagon-like immunoreactivity continued to rise, establishing its extrapancreatic origin. In other experiments, measurements of glucagon-like immunoreactivity in plasma obtained simultaneously from pancreatico-

duodenal and mesenteric veins and from the vena cava revealed the increment after intraduodenal glucose loading to be greatest in the mesenteric vein in 8 of 12 experiments, favoring the gut as the likely source of the rise.

To characterize gut glucagon-like immunoreactivity, acid-alcohol extracts of canine jejunum were compared with similar glucagon-containing extracts of canine pancreas with respect to certain physical and biological properties. On a G-25 Sephadex column the elution volume of the jejunal immunoreactivity was found to be smaller than that of glucagon, which suggested a molecular size at least twice that of pancreatic glucagon. Furthermore, the *in vivo* and *in vitro* biological activities of the eluates containing jejunal glucagon-like immunoreactivity appeared to differ from those of eluates containing pancreatic glucagon. The jejunal material lacked hyperglycemic activity when injected endoportally into dogs, was devoid of glycolytic activity in the isolated perfused rat liver, and did not increase hepatic 3',5' cyclic adenylate in the perfused liver; however, like glucagon it appeared to stimulate insulin release. It seems quite clear the material in intestinal extracts either is a different substance or a different form from that of true pancreatic glucagon, although it cross-reacts in the radioimmunoassay with antibodies to glucagon.

It is concluded, (a) that hyperglycemia does not stimulate and probably suppresses the secretion of

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pancreatic glucagon; (b) that during intestinal absorption of glucose, a rise in glucagon-like immunoreactivity occurs; (c) this immunoreactivity is derived from an extrapancreatic site, probably the gut; (d) that the glucagon-like immunoreactivity extractable from jejunum is not the same as pancreatic glucagon but is a larger molecule devoid of hyperglycemic and glycogenolytic activity, a cross-reactant in radioimmunoassay for glucagon; and (e) that the eluate in which jejunal immunoreactivity is contained can stimulate insulin release in conscious dogs.

INTRODUCTION

Both Samols et al. (1) and Lawrence (2) have reported that the concentration of immunosassayable glucagon *rises* after the ingestion of a large glucose load. These findings appeared to conflict with earlier observations by our lab (3) which had indicated that hyperglycemia induced by the intravenous administration of glucose *suppressed* glucagon secretion. The findings of Lawrence and of Samols, coupled with the demonstration by Samols et al. (4) of glucagon's insulin-stimulating activity, have led these workers to propose that glucagon is a hormone of glucose abundance, the function of which is to enhance the insulin secretory response to ingested glucose. This intriguing proposal is at odds with the more traditional view, supported by the observations of our group (3, 5), that glucagon is a hormone of glucose need (6, 7), the function of which is to enhance hepatic glucose production by means of increased glycogenolysis and gluconeogenesis.

The studies reported here were designed to compare the effects of intraduodenal and intravenous administration of large glucose loads upon the levels of immunoassayable glucagon in plasma, and, if the seemingly conflicting observations cited above are verified, to elucidate the mechanism of the apparent differences in response of glucagon to absorbed and infused glucose.

METHODS

Experimental preparation. 2 days or more before the first experiment, nembutalized mongrel dogs were operated on under sterile conditions. A polyethylene catheter was threaded through a small mesenteric venous radicle and guided into the superior pancreaticoduodenal vein in a retrograde direction to a distance of 3-5 cm from its junction with the portal vein. The cath-

ter was stabilized by means of a suture piercing the vein, which secured it to the side of the vessel. A second catheter was threaded through a mesenteric venous radicle and into a major mesenteric vein draining an area of the upper jejunum. A third catheter, a Teflon tube, was inserted through the left jugular vein; with its opening reposing in the vena cava between the heart and the hepatic veins it was secured with sutures that encircled and occluded the jugular vein. Finally, a plastic gastric tube was passed through a duodenostomy incision and sutured in place with its tip in the third portion of the duodenum. All tubes and catheters were exteriorized and heavily bandaged with tape. The pancreatic vein catheter was infused continuously at a constant rate, in order to deliver 100 U of heparin in a volume of 20 ml/hr. After recovery, the dogs were placed in narrow cages which prevented them from turning or otherwise disturbing the catheters. Each dog received 600,000 U of penicillin G intramuscularly daily after surgery. Recovery was uneventful for most dogs, and survival for 10 days or more in apparently good health was common. Dogs were maintained on a Purina Chow diet containing 53.5% carbohydrate, 8% fat, and 24% protein before and after the surgical procedures.

Only dogs who appeared to be in good health postoperatively were employed in this study. Diarrhea, loss of appetite or vigor, weight loss in excess of 1.5 kg, and unexplained temperature elevation were regarded as evidence of poor health and as grounds for disqualification from the study. Dogs with a hematocrit value below 35% were transfused on the eve of the experiment. Experiments were begun after the 2nd postoperative day.

Each dog was sacrificed upon completion of the study and an autopsy performed. The position of each catheter was determined and the pancreatic and jejunal effluent veins were examined for the presence of clots. Only data derived from dogs with a patent pancreaticoduodenal venous system were included.

Experimental procedure. Dogs were studied after an overnight fast. In a fully conscious state they were suspended in a cradle sling with their extremities touching the floor. Specimens of blood were obtained from all three catheters at frequent intervals with syringes rinsed with EDTA. The blood was immediately centrifuged at 4°C for 20 min and the plasma stored in groups at -15° to -20°C until the time of assay. Assays were performed from 1 to 70 days after freezing in all but two of the experiments.

The effects of intraduodenal glucose loading were studied first; a sterile 20% solution of glucose was administered in a dose of 2 g/kg of body weight via the intraduodenal catheter over a period of 5 min. 2 days later the effects of intravenous glucose administration were studied in the same dog; glucose was infused via the crural vein either in an identical dose of 2 g/kg delivered at a constant rate over a 90 min period ("isometric" experiment) or in a varying dose delivered at a varying rate designed to simulate the vena caval plasma glucose curve of the intraduodenal experiment ("isoglycemic" experi-

ment). Blood specimens were obtained at 5–30 min intervals.

Laboratory Methods. Glucose was measured in plasma by the ferricyanide method of Hoffman (8) using the Technicon Autoanalyzer.

Insulin was measured by the method of Yalow and Berson (9).

Glucagon was measured by radioimmunoassay (10), as recently modified to include the proteinase inhibitor "Trasylol,"¹ added during the assay procedure (11).

In its present form the radioimmunoassay for glucagon must be regarded as no more than semiquantitative. Although the reproducibility of replicate assays of known and unknown specimens within a given run is excellent [$\pm 1.2\%$ (11)], repeated assays of a given unknown sample in different runs using the same antiserum do not give the same degree of reproducibility. When an unknown sample is reassayed within 40 days of the initial assay, the results do not differ by more than 10% if the specimen has been thawed no more than twice. When more than 60 days have elapsed between two assays, a second result may be 30–50% lower than the first value.

In a relative sense, however, inter-run reproducibility appears to be high. For example, four specimens of dog plasma assayed nine times over a 6 month period bore precisely the same relationship to each other on eight occasions despite differences in the absolute values in many of the later runs.

Thus it would appear that within a single immunoassay run, a change in the level of circulating pancreatic glucagon and/or extrapancreatic glucagon-like immunoreactivity can be measured with a high degree of reliability and reproducibility. A quantitative comparison of specimens assayed in different runs is meaningful should the storage times not exceed 40 days, but no claim of quantitative precision, in an absolute sense, can be confidently made, despite the use of millimicrograms/milliliter as the unit.

To minimize these problems in this study, all assays were performed with the same antiserum and specimens were assayed after only one thawing. Each set of specimens in each experiment was assayed in a single run to permit a meaningful measure of change. When specimens obtained from three different vessels were being compared, as in Table III, all three sets were assayed in a single run. This was not done in dogs 1-63, 1-66, and 1-67 and the values for these three dogs, although listed in Table III, were excluded from calculations of the mean values. The duration of storage was less than 40 days in dogs 1-64, 1-73, 1-74, 1-77, 2-02, 2-04, and 2-14, and it was less than 70 days in dogs 1-94, 1-95, 1-96, and 1-99. It is believed that these data permit a valid estimate of relative change in the concentration of glucagon and/or glucagon-like immunoreactivity in a given vessel, as well as comparison of their relative concentrations in different vessels.

Dextran column chromatography of tissue extracts. Acid-alcohol extracts of tissue prepared by the method of

Kenny (16) were reconstituted in a 0.2 M glycine buffer solution (pH 8.8) containing 0.25% human albumin, to achieve a concentration of approximately 2 μg of glucagon-like immunoreactivity per milliliter of the solution. Trasylol, 6000 U/0.3 ml, was added to each milliliter of the extract solution at the time of reconstitution.

Within 24 hr of its reconstitution, 0.6 ml of the tissue extract solution, to which a trace ($< 0.8 \mu\text{g}$) of glucagon-¹²⁵I had been added, was applied to a 110 \times 0.7–1.0 cm column of Sephadex,² G-25 Fine (20–80 μ particles), which had been previously equilibrated with the glycine-albumin buffer, and tested with molecular weight markers, blue dextran, cytochrome *c*, insulin-¹²⁵I, and glucagon-¹²⁵I. A flow rate of 0.2–0.25 ml/min was maintained in all runs. After collection of the void volume of the column, fractions of 1 or 2 ml were collected in an automatic fraction collector and assayed for glucagon-like immunoreactivity and for radioactivity. Eluates were kept at 4°C until the time of counting and were then stored at –20°C until the time of the radioimmunoassay, approximately 1 wk later.

Liver perfusion. The glycogenolytic activity of eluates of jejunal and pancreatic extracts was examined with the isolated, perfused rat liver preparation described by Mortimore (18). Livers from fed rats were perfused with recirculation for 20 min with medium consisting of 20% red blood cells suspended in Krebs-Henseleit bicarbonate buffer containing 3% bovine serum albumin. The perfusion system was then changed to one in which the medium flowed once through the liver and the glucose concentration in the effluent was measured at 1 min intervals. When a constant glucose level was observed, indicating that a steady rate of glucose production had been attained, the eluates were infused at a known rate into the medium entering the liver and the resulting change in glucose output was compared with that produced by standard amounts of crystalline glucagon. The system was capable of responding to as little as 4 μg of glucagon.

RESULTS

Effect of intraduodenal glucose administration upon the vena caval level of glucagon-like immunoreactivity. In a group of 14 dogs the intraduodenal administration over a period of 5 min of 2 g of glucose/kg of body weight was followed by a rise in the mean vena caval plasma glucose level to a peak of 216 mg/100 ml (151–280 mg/100 ml) at 30 min. In all but one of these experiments this was associated with a rise of 100% or more in the vena caval concentration of glucagon-like immunoreactivity, occurring between 20 and 120 min; the mean level for the group increased from 0.81 $\mu\text{g}/\text{ml}$ at zero time to 2.10 $\mu\text{g}/\text{ml}$ (SD ± 0.81) at its peak 60 min after the glucose. This represented a statistically significant change

¹ Bayer A 128, FBA Pharmaceuticals, Inc., New York.

² Pharmacia Fine Chemicals, Inc., Piscataway, N. J.

TABLE I
Effects of Intraduodenal Administration of Glucose upon Glucagon-Like Immunoreactivity (GLI) and Insulin (Means of 14 Experiments)

| Measurement | Source | Control period, min | | Time after glucose administration, min | | | | | | | | | |
|-------------|--------|---------------------|-------|--|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | | -10 | 0 | 5 | 10 | 20 | 30 | 45 | 60 | 90 | 120 | 150 | 180 |
| Glucose | VC* | 107.9 | 103.8 | 129.1 | 166.0 | 199.4 | 215.5 | 201.3 | 179.9 | 138.8 | 117.2 | 116.5 | 115.1 |
| | ± SD | 8.3 | 11.6 | 19.1 | 24.2 | 33.5 | 41.5 | 47.3 | 56.9 | 48.9 | 33.2 | 30.6 | 27.5 |
| GLI | VC | 0.87 | 0.81 | 0.89 | 1.11 | 1.29 | 1.48 | 2.01 | 2.10 | 2.03 | 1.70 | 1.60 | 1.61 |
| | ± SD | 0.26 | 0.26 | 0.28 | 0.33 | 0.31 | 0.57 | 0.86 | 0.81 | 0.92 | 0.89 | 0.71 | 0.87 |
| Insulin | PV† | 174.6 | 155.4 | 482.5 | 856.2 | 1281 | 1431 | 961.6 | 751.2 | 408.0 | 232.9 | 195.5 | 191.0 |
| | ± SD | 95.9 | 88.4 | 382.3 | 700.8 | 1432 | 2354 | 930.6 | 888.8 | 416.2 | 176.1 | 153.1 | 167.7 |

Note: These mean values for GLI are obtained from experiments summarized in their entirety in Table III.

* Vena cava.

† Pancreaticoduodenal vein.

from the baseline level of glucagon-like immunoreactivity ($P < 0.001$). These results confirm the findings obtained by Lawrence (2) and Samols et al. (1) in human subjects given a large oral glucose load.

The mean values are shown in Table I and Fig. 1a, and the results of individual experiments appear in Table III.

Effect of intravenous glucose administration upon vena caval and pancreaticoduodenal vein glucagon-like immunoreactivity. To determine if the apparent hyperglucagonemia that followed intraduodenal glucose administration was a consequence of the associated hyperglycemia, 11 dogs received an infusion of glucose by crural vein. In six of these experiments 2 g of glucose/kg of body

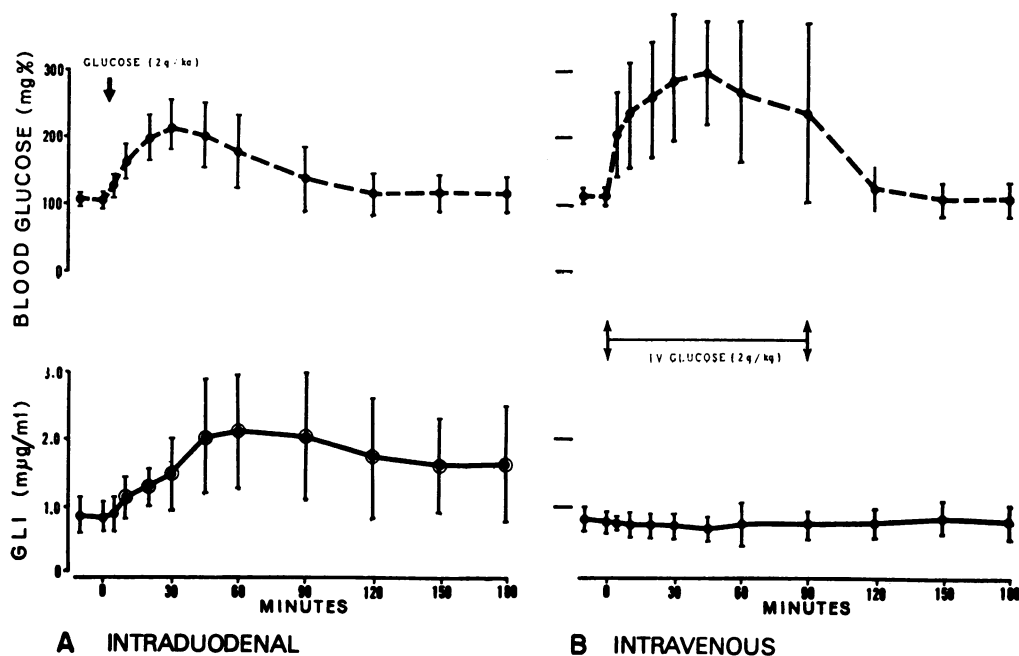


FIGURE 1 The mean levels and SD of plasma glucagon-like immunoreactivity (GLI) in the vena cava after the administration of a large glucose load: A, intraduodenally to a group of 14 dogs; B, intravenously to a group of 11 dogs. A circle about a point on the mean curve of glucagon-like immunoreactivity indicates that it differs significantly ($P < 0.01$) from the mean value at zero time.

TABLE II
Effects of Intravenous Administration of Glucose upon Glucagon-Like Immunoreactivity (GLI) and Insulin (Means of 11 Experiments)

| Measurement | Source | Control period, min | | Time after start of glucose infusion, min | | | | | | | | | |
|-------------|--------|---------------------|-------|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | | -10 | 0 | 5 | 10 | 20 | 30 | 45 | 60 | 90 | 120 | 150 | 180 |
| Glucose | VC* | 110.5 | 111.7 | 203.4 | 231.6 | 258.1 | 285.9 | 291.8 | 263.7 | 234.5 | 113.7 | 101.7 | 102.8 |
| ± SD | | 11.2 | 14.3 | 68.1 | 80.0 | 85.7 | 96.2 | 75.8 | 107.8 | 135.4 | 36.4 | 24.3 | 22.7 |
| GLI | VC | 0.82 | 0.78 | 0.78 | 0.74 | 0.72 | 0.73 | 0.69 | 0.77 | 0.74 | 0.74 | 0.82 | 0.77 |
| ± SD | | 0.20 | 0.17 | 0.13 | 0.20 | 0.18 | 0.21 | 0.18 | 0.31 | 0.20 | 0.19 | 0.26 | 0.24 |
| GLI | PV† | 1.20 | 1.55 | 1.05 | 1.10 | 0.88 | 0.86 | 0.87 | 0.98 | 1.02 | 1.17 | 1.29 | 1.48 |
| ± SD | | 0.42 | 0.93 | 0.35 | 0.35 | 0.26 | 0.19 | 0.20 | 0.45 | 0.25 | 0.39 | 0.53 | 0.73 |
| Insulin | PV | 159.2 | 179.8 | 1059 | 867.9 | 779.4 | 820.7 | 962.3 | 809.1 | 547.6 | 185.7 | 146.5 | 179.4 |
| ± SD | | 117.4 | 156.5 | 1117 | 855.9 | 505.4 | 597.4 | 881.4 | 877.8 | 544.0 | 167.9 | 113.4 | 137.2 |

* Vena cava.

† Pancreaticoduodenal vein.

weight was administered at a constant rate over a 90 min period so as to deliver at least as much glucose as in the previous intraduodenal experiment ("isometric" experiment); in the other five experiments glucose was infused at a varying rate designed to simulate the hyperglycemic curve of the previous intraduodenal administration of glucose ("isoglycemic" experiment). In all but one of the experiments employing the intravenous route the hyperglycemia was at least as great as in the experiments employing the intraduodenal route; the peak vena caval glucose concentration averaged 292 mg/100 ml (from 195 to 496 mg/100 ml), as compared to the peak value of 216 mg/100 ml in the intraduodenal experiments. Despite the greater hyperglycemia in the intravenous experiments, the level of glucagon-like immunoreactivity in vena caval plasma did not increase in any of the 11 experiments during the infusion, nor did the mean value change (Table II and Fig. 1b).

However, because of dilution and binding to the liver of secreted glucagon a physiologically significant increase in the pancreatic contribution of glucagon may not be reflected by a measurable increment in its concentration in peripheral plasma (3, 12). To exclude the possibility of undetected secretion of pancreatic glucagon during the foregoing infusion experiments, the glucagon concentration was also measured in the pancreaticoduodenal vein.

In 10 of the 11 experiments pancreaticoduodenal vein glucagon concentration failed to rise during

the infusion; furthermore, in all four dogs in which glucagon-like immunoreactivity at zero time exceeded 1.8 m μ g/ml, the level declined by at least 50% during the period of hyperglycemia. The mean level of glucagon-like immunoreactivity in the pancreaticoduodenal vein for the entire group declined from 1.6 m μ g/ml at zero time to a nadir of 0.9 m μ g/ml at 20 min; this change was statistically significant ($P < 0.01$). After the termination of the glucose infusion, as the plasma glucose level was rapidly declining, a rise in glucagon concentration was noted in six of the experiments. The results of these experiments are recorded in Table II.

These results confirm previous reports from this laboratory which indicated that hyperglycemia does not stimulate pancreatic glucagon secretion and may, in fact, suppress it (3).

The source of the apparent rise in plasma glucagon induced by intraduodenal administration of glucose. If, as shown above, the apparent rise in immunoreactive glucagon observed after enteric administration of glucose is not explained by hyperglycemia, it may well be a consequence of the presence of glucose in the gut, which causes either an increase in pancreatic glucagon secretion through humoral or neural stimulation of alpha cells, or the release from the gut of the "glucagon-like immunoreactivity" known to be present in extracts of the gastrointestinal tract (13-15). If the increase in plasma glucagon-like immunoreactivity is pancreatogenous, the former alphacyto-

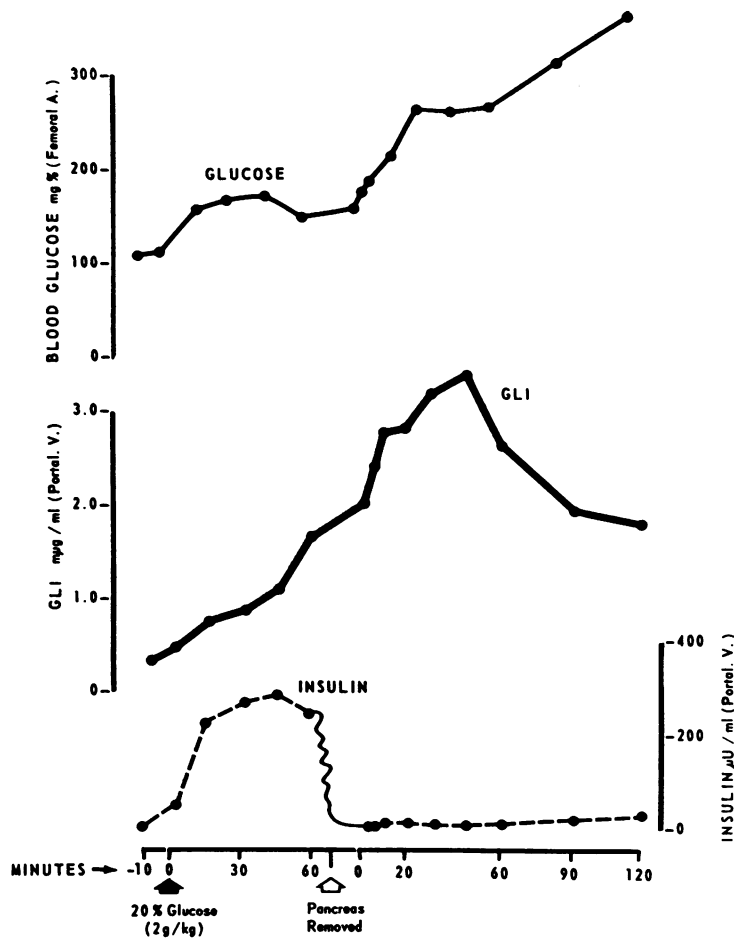


FIGURE 2 The results of one experiment show the effect of acute removal of the pancreas upon the rising level of glucagon-like immunoreactivity (*GLI*) in the portal vein after the intraduodenal administration of a large glucose load.

tropic mechanism would be supported; if it is of enteric origin, the latter concept would be favored.

To determine if the increase in glucagon values was enterogenous or pancreatogenous, two Nembutalized, acutely operated dogs were given 2 g of glucose/kg of body weight intraduodenally. 1 hr later, when the portal vein levels of both insulin and glucagon-like immunoreactivity were rising substantially, preplaced sutures were suddenly tightened about all vascular connections of the pancreas, and the organ quickly resected. The insulin level abruptly fell almost to zero and the plasma glucose level rose, but the course of portal vein glucagon-like immunoreactivity continued upward (Fig. 2). These findings leave little doubt that the rise in glucagon-like immunoreactivity that follows the intraduodenal administration of glucose is extrapancreatic in origin.

The gastrointestinal tract is highly suspect as a source of nonpancreatic glucagon-like immuno-

reactivity. Immunoassay for glucagon of acid-alcohol extracts of the upper gastrointestinal tract of man, rat, beef, and dog, prepared by the method of Kenny (16), has demonstrated the presence of a large and widely distributed quantity of glucagon or glucagon-like immunoreactivity (15). Fig. 3 shows the distribution of glucagon-like immunoreactivity in the gut of a dog; the total immunoreactivity approaches in immunologic equivalence the glucagon content of the pancreas. To determine if the small bowel might, therefore, be the extrapancreatic source of the rise in glucagon-like immunoreactivity, which occurs during glucose absorption, an attempt was made in 14 unanesthetized dogs to measure glucagon-like immunoreactivity of plasma obtained simultaneously from the vena cava, the pancreaticoduodenal, and the mesenteric veins before and during 180 min after the intraduodenal administration of 2 g/kg of glucose. The results of the 11 complete experi-

TABLE III
Effects of Intraduodenal Administration of Glucose upon Glucagon-Like Immunoreactivity (GLI) in Vena Cava, Mesenteric, and Pancreaticoduodenal Veins

| Dog | Measurement | Source | Control period, min | | Time after glucose administration, min | | | | | | | | | | |
|------|---------------------|--------|------------------------|-----|--|-----|-----|-----|-----|-------|-----|-------|-----|-------|--|
| | | | -19 | 0 | 5 | 10 | 20 | 30 | 45 | 60 | 90 | 120 | 150 | 180 | |
| 1-63 | Glucose (mg/100 ml) | MV* | 88 | 94 | 194 | 247 | | | | | | | | | |
| | | VC† | 94 | 100 | 120 | 146 | 170 | 208 | 202 | 186 | 188 | 166 | 184 | 180 | |
| | GLI (μg/ml) | MV | 1.2 | 1.1 | 1.3 | 1.1 | | | | | | | | | |
| | | VC | 0.7 | 0.4 | 0.4 | 0.4 | 0.5 | 0.2 | 0.9 | 0.9 | 0.8 | 0.7 | 0.7 | 1.4 | |
| | | PV‡ | 1.6 | 1.4 | 1.4 | 1.4 | 1.5 | 1.6 | 1.5 | 1.4 | 1.7 | 1.6 | 1.4 | 1.4 | |
| 1-64 | Glucose | MV | 94 | 102 | | 272 | 480 | 486 | 460 | 388 | 217 | 214 | 146 | 125 | |
| | | VC | 106 | 106 | 130 | 189 | 228 | 251 | 247 | 245 | 222 | 188 | 147 | 125 | |
| 1-64 | GLI | MV | 0.7 | 0.8 | | 1.2 | 1.6 | 1.7 | 2.1 | 2.6 | 2.4 | 3.0 | 2.6 | 2.8 | |
| | | VC | 1.0 | 0.8 | 1.0 | 1.2 | 1.4 | 1.8 | 2.2 | 2.6 | 3.0 | 2.8 | 2.8 | 2.8 | |
| | | PV | 0.7 | 0.8 | | 1.2 | 1.9 | 1.8 | 2.2 | 2.4 | 2.6 | 2.6 | 2.4 | 2.2 | |
| 1-66 | Glucose | MV | 110 | 120 | 211 | 286 | 348 | 340 | 344 | 264 | 161 | 125 | 141 | 196 | |
| | | VC | 106 | 113 | 141 | 191 | 254 | 280 | 272 | 236 | 164 | 126 | 134 | 148 | |
| | GLI | MV | 0.4 | 0.4 | 0.5 | 0.7 | 0.6 | 0.9 | 0.8 | 0.6 | 0.4 | 0.5 | 0.5 | 0.5 | |
| | | VC | 0.4 | 0.4 | 0.5 | 1.0 | 0.7 | 0.6 | 0.6 | 0.5 | 0.4 | 0.5 | 1.0 | 0.4 | |
| | | PV | 2.2 | 2.2 | 1.7 | 1.9 | 1.7 | 1.9 | 1.9 | 1.9 | 1.7 | 1.7 | 1.7 | 1.8 | |
| 1-67 | Glucose | MV | 116 | 118 | 187 | 212 | 296 | 310 | 300 | 280 | 227 | 142 | 110 | 106 | |
| | | VC | 121 | 124 | 138 | 166 | 229 | 250 | 254 | 242 | 196 | 122 | 104 | 108 | |
| | GLI | VC | 0.7 | 0.7 | 0.7 | 0.8 | 1.1 | 1.2 | 1.2 | 1.1 | 0.9 | 0.9 | 1.0 | 0.7 | |
| | | PV | 2.4 | 2.0 | 1.9 | 2.4 | 2.4 | 2.6 | 2.4 | 2.2 | 2.0 | 2.0 | 2.2 | 2.4 | |
| 1-95 | Glucose | MV | 142 | 127 | 200 | 390 | 392 | 358 | 234 | 136 | 146 | 196 | 173 | 175 | |
| | | VC | 124 | 118 | 158 | 197 | 256 | 272 | 206 | 118 | 110 | 144 | 111 | 124 | |
| | GLI | MV | 1.0 | 0.9 | 1.6 | 2.0 | 1.8 | 1.8 | 1.8 | 1.9 | 1.5 | 1.0 | 1.2 | 1.2 | |
| | | VC | 1.1 | 1.0 | 1.2 | 1.5 | 1.8 | 1.8 | 2.2 | 4.0 ? | 1.6 | | 1.4 | | |
| | | PV | 0.9 | | 1.3 | 2.4 | 1.9 | 1.9 | 2.0 | 2.0 | 2.0 | 1.8 | 2.8 | 3.6 | |
| 1-99 | Glucose | MV | 108 | 117 | 203 | 203 | 210 | 208 | 183 | 142 | 98 | 136 | 106 | 125 | |
| | | VC | 118 | 107 | 141 | 202 | 193 | 172 | 143 | 112 | 86 | 106 | 92 | 103 | |
| | GLI | MV | 1.5 | 0.9 | 1.4 | 1.3 | 1.7 | 1.9 | 2.6 | 3.2 | 2.8 | 2.6 | 1.4 | 1.5 | |
| | | VC | 0.9 | 0.9 | 0.7 | 1.0 | 1.1 | 1.2 | 1.9 | 2.2 | 2.2 | 1.6 | 1.0 | 1.4 | |
| | | PV | 1.4 | 1.3 | 1.1 | 0.9 | 1.0 | 1.4 | 1.6 | 1.9 | 2.2 | 2.2 | 1.3 | 1.1 | |
| 2-02 | Glucose | MV | 90 | 88 | 210 | 222 | 216 | 204 | 187 | 149 | 125 | 136 | 130 | 130 | |
| | | VC | 11 | 108 | 170 | 196 | 202 | 194 | 166 | 162 | 138 | 140 | 130 | 142 | |
| | GLI | MV | 0.9 | 0.8 | 1.0 | 1.1 | 1.3 | 1.5 | | 3.2 | 3.2 | 3.2 | 3.2 | 2.6 | |
| | | VC | 1.1 | 1.2 | 1.1 | 1.3 | 1.3 | 1.8 | 1.9 | 3.0 | 3.0 | 3.4 | 2.8 | 2.8 | |
| | | PV | 5.4 | 3.4 | 2.0 | 2.4 | 2.4 | 3.0 | 5.0 | 4.8 | 4.0 | 4.6 | 4.0 | 3.6 | |
| 2-04 | Glucose | MV | 104 | 100 | 234 | 294 | 324 | 348 | 369 | 306 | 111 | 76 | 92 | 98 | |
| | | VC | 100 | 93 | 112 | 144 | 190 | 204 | 192 | 180 | 95 | 72 | 92 | 90 | |
| | GLI | MV | 1.2 | 0.7 | 0.8 | 1.0 | 1.1 | 3.6 | 1.5 | 1.8 | 1.8 | 2.0 | 1.4 | | |
| | | VC | 0.7 | 0.8 | 0.7 | 0.8 | 1.1 | 1.3 | 1.6 | 1.6 | 1.8 | 1.5 | 1.2 | 3.6 ? | |
| | | PV | 7.2 | 2.6 | 1.2 | 1.4 | 1.4 | 1.4 | 1.8 | 2.0 | 2.0 | 5.8 ? | 1.9 | 2.4 | |
| 2-14 | Glucose | MV | 112 | 115 | 242 | 280 | 314 | 314 | 296 | 256 | 184 | 154 | 105 | 129 | |
| | | VC | 112 | 106 | 130 | 146 | 175 | 174 | 140 | 131 | 114 | 108 | 107 | 114 | |
| | GLI | MV | 2.0 | 1.2 | 1.2 | 1.4 | 1.9 | 2.4 | 3.0 | 2.4 | 3.0 | 2.4 | 1.8 | 1.5 | |
| | | VC | 1.2 | 1.1 | 1.2 | 1.4 | 1.7 | 2.4 | 3.0 | 3.0 | 3.0 | 2.4 | 1.8 | 1.8 | |
| | | PV | 5.8 | 5.0 | 2.6 | 3.4 | 3.6 | 3.8 | 4.6 | 4.6 | 6.0 | 6.6 | 7.2 | 6.4 | |
| 1-73 | Glucose | MV | 117 | 114 | 185 | 272 | 292 | 344 | 302 | 266 | 199 | 127 | 111 | 103 | |
| | | VC | 114 | 113 | 117 | 136 | 168 | 185 | 187 | 156 | 116 | 110 | 104 | 102 | |
| | GLI | MV | 0.8 | 0.8 | 1.0 | 1.2 | 1.6 | 1.8 | 2.0 | 2.6 | 3.0 | 3.2 | 3.2 | 2.8 | |
| | | VC | 1.0 | 1.0 | 1.0 | 1.4 | 1.8 | 1.8 | 2.4 | 2.6 | 2.4 | 2.4 | 2.4 | 2.2 | |
| | | PV | 1.2 | 1.0 | | | 1.8 | 2.0 | 2.4 | 3.0 | 3.6 | 4.2 | 4.2 | 3.4 | |
| 1-74 | Glucose | MV | 96 | 96 | 268 | | 338 | 348 | 250 | 386 | 87 | 87 | 106 | 158 | |
| | | VC | 104 | 97 | 110 | 135 | 149 | 151 | 143 | 129 | 86 | 84 | 104 | 115 | |
| | GLI | MV | 1.0 | 1.2 | 1.4 | 1.6 | 1.8 | 1.8 | 2.0 | 2.0 | 1.8 | 1.4 | 1.4 | 1.2 | |
| | | VC | 1.2 | 1.0 | 1.2 | 1.4 | 1.2 | 1.8 | 2.0 | 2.0 | 1.8 | 1.4 | 1.2 | 1.2 | |
| | | PV | 2.2 | 2.2 | 1.6 | 1.6 | 1.8 | 2.0 | 2.4 | 2.2 | 2.6 | 4.2 | 2.2 | 1.8 | |
| 1-77 | Glucose | MV | 104 | 98 | | 266 | 284 | 292 | 288 | 250 | 182 | 102 | 100 | 101 | |
| | | VC | 102 | 100 | 128 | 157 | 168 | 182 | 165 | 136 | 94 | 80 | 95 | 96 | |
| | GLI | MV | 0.8 | 0.8 | | 1.4 | | 1.8 | 1.8 | 2.2 | 1.8 | 1.8 | 1.6 | 1.4 | |
| | | VC | 1.0 | 0.8 | 1.2 | 1.4 | 1.6 | 1.8 | 2.6 | 2.4 | 2.4 | 1.0 | 1.4 | 1.0 | |
| | | PV | 1.0 | 1.0 | | 1.4 | 1.6 | 1.8 | 2.2 | 2.4 | 2.2 | 2.0 | 1.6 | 1.4 | |

TABLE III (Continued)

| Dog | Measurement | Source | Control period, min | | Time after glucose administration, min | | | | | | | | | | |
|---------------|-----------------|--------|------------------------|-------|--|-------|-------|-------|-------|-------|-------|-------|-------|-------|-----|
| | | | -10 | 0 | 5 | 10 | 20 | 30 | 45 | 60 | 90 | 120 | 150 | 180 | |
| 1-94 | Glucose | MV | 98 | 88 | 308 | 306 | 392 | 304 | 244 | 177 | 127 | | | 90 | 94 |
| | | VC | 95 | 85 | 107 | 158 | 218 | 240 | 234 | 186 | 118 | 97 | 158 | 86 | |
| | GLI | MV | 0.7 | 0.7 | 1.0 | 1.2 | 2.8 | 2.4 | 3.8 | 3.6 | | | 2.6 | 3.4 | 2.0 |
| | | VC | 0.7 | 0.7 | 0.9 | 1.2 | 1.8 | 1.9 | 4.0 | 3.6 | 3.4 | 6.4 ? | 2.4 | 2.8 | |
| | | PV | 0.7 | 0.8 | 1.1 | 1.5 | 1.7 | 2.4 | 3.0 | 3.4 | 4.2 | 3.6 | 3.4 | 2.4 | |
| 1-96 | Glucose | MV | 87 | 95 | 186 | 240 | 274 | 262 | 266 | 330 | 248 | 274 | 68 | 86 | |
| | | VC | 103 | 83 | 105 | 161 | 192 | 254 | 268 | 300 | 216 | 98 | 69 | 79 | |
| | GLI | MV | 0.7 | 0.6 | 0.8 | 1.0 | 2.4 | 2.0 | 2.4 | 2.4 | 2.2 | 2.0 | 1.2 | 0.8 | |
| | | VC | 0.5 | 0.6 | 0.6 | 0.7 | 0.9 | 1.1 | 1.6 | 1.8 | 1.7 | 1.8 | 1.3 | 0.8 | |
| | | PV | 0.7 | 0.8 | 0.9 | 0.9 | 1.0 | 1.2 | 1.4 | 1.4 | 1.4 | 1.3 | 0.9 | 0.7 | |
| Mean | Glucose ± SD | MV | 104.7 | 105.1 | 219.0 | 272.6 | 320.0 | 316.8 | 286.4 | 256.2 | 162.5 | 147.4 | 113.7 | 125.1 | |
| | | VC | 14.8 | 13.3 | 37.7 | 54.1 | 73.5 | 71.7 | 75.0 | 85.7 | 51.6 | 56.4 | 27.5 | 33.2 | |
| | Glucose ± SD | MV | 107.9 | 103.8 | 129.1 | 166.0 | 199.4 | 215.5 | 201.3 | 179.9 | 138.8 | 117.2 | 116.5 | 115.1 | |
| | | VC | 8.3 | 11.6 | 19.1 | 24.2 | 33.5 | 41.5 | 47.3 | 56.9 | 48.9 | 33.2 | 30.6 | 27.5 | |
| | GLI ± SD | MV | 0.98 | 0.82 | 1.07 | 1.26 | 1.69 | 1.97 | 2.16 | 2.37 | 2.17 | 2.14 | 1.91 | 1.66 | |
| | | VC | 0.43 | 0.23 | 0.33 | 0.33 | 0.60 | 0.65 | 0.80 | 0.80 | 0.84 | 0.87 | 0.95 | 0.80 | |
| GLI¶ ± SD | MV | 0.90 | 0.86 | 0.94 | 1.42 | 1.37 | 1.61 | 2.17 | 2.30 | 2.23 | 1.88 | 1.73 | 1.72 | | |
| | VC | 0.27 | 0.26 | 0.26 | 0.27 | 0.38 | 0.48 | 0.83 | 0.83 | 0.83 | 0.87 | 0.69 | 0.90 | | |
| GLI** ± SD | MV | 2.47 | 1.89 | 1.48 | 1.71 | 1.83 | 2.05 | 2.56 | 2.68 | 2.93 | 3.25 | 2.90 | 2.64 | | |
| | VC | 2.42 | 1.41 | 0.57 | 0.79 | 0.75 | 0.77 | 1.19 | 1.11 | 1.33 | 1.61 | 1.78 | 1.59 | | |

* Mesenteric vein.

† Vena cava.

‡ Pancreaticoduodenal vein.

|| Dog 1-63 is excluded from calculations of the mean value as noted in Methods.

¶ Dogs 1-63 and 1-67 are excluded from calculations of the mean value as noted in Methods.

** Dogs 1-63, 1-66, and 1-67 are excluded from calculations of the mean value as noted in Methods.

ments, and of 3 incomplete experiments (dogs 1-64, 1-66, and 1-67) not included in the calculation of means, are shown in Table III. However, change in concentration is more meaningful than absolute values, and the mean changes are summarized in Table IV and Fig. 4.

In 8 of the 11 experiments, the rise in the concentration of glucagon-like immunoreactivity after the intraduodenal administration of glucose was higher in the mesenteric vein than in either the vena cava or the pancreaticoduodenal vein. As shown in Table IV and Fig. 4, the first statistically significant mean increment ($P < 0.01$) in glucagon-like immunoreactivity above the value at zero time occurred in the mesenteric vein within 5 min, which suggests an enteric contribution. The initial change in the pancreaticoduodenal vein at this time is a downward one, providing further evidence that at least initially, the increment must be of extrapancreatic origin; the subsequent rise does not become statistically significant until the 90 min specimen, after which it exceeds the vena

caval level, and suggests a late pancreatic contribution. At 5 min, the mean mesenteric vein increment is three times greater than the mean vena caval increment, as would be expected from the anticipated dilution of hepatic venous blood by non-splanchnic venous return; thereafter this mesenteric vein-cava gradient, though present throughout the experiment, narrows considerably. Possible explanations for this apparent failure to observe the predicted gradient will be discussed.

The rise in the mesenteric vein is statistically significant ($P < 0.01$) throughout the entire experiment beginning with the 5 min specimen; in the vena cava it becomes significant at 10 min, however, the rise in the pancreaticoduodenal vein does not become significant until 90 min after glucose administration. This order of appearance of a statistically significant rise may reflect the site of origin of the rise.

The characterization of glucagon-like immunoreactivity of jejunal extracts. Because of the likelihood that the rise in glucagon-like immuno-

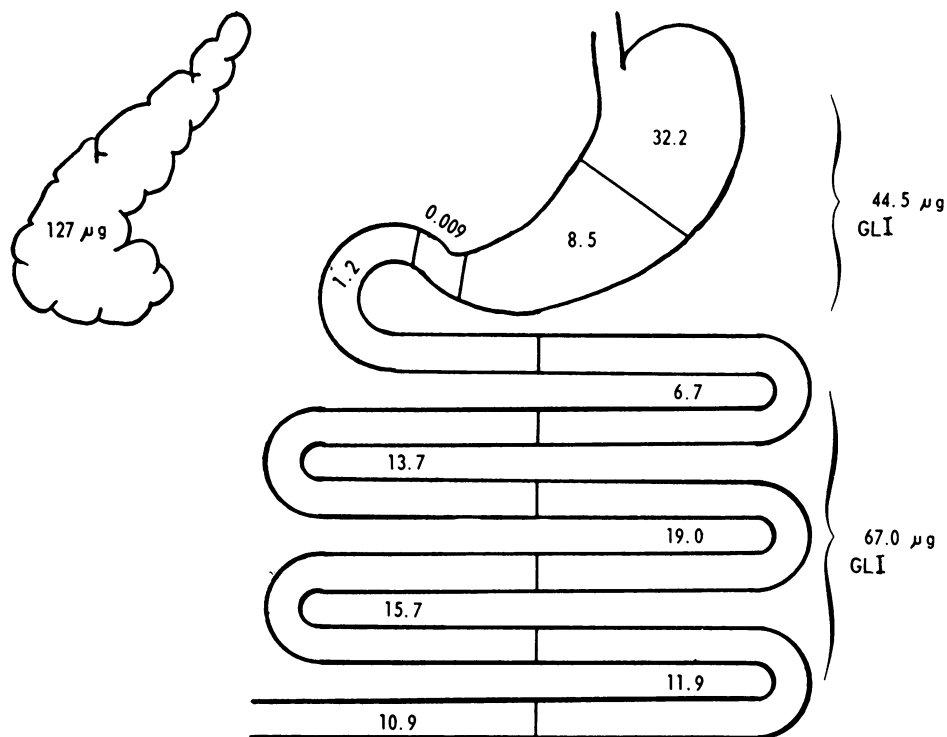


FIGURE 3 The distribution of glucagon-like immunoreactivity acid-alcohol extracts of the tissues of the upper gastrointestinal tract of a dog.

reactivity after intraduodenal administration of glucose was of enteric origin, it seemed of interest to determine whether the immunoreactivity present in acid-alcohol extracts of small bowel was true glucagon, indistinguishable from that of extracts of pancreas, or a different substance which cross-reacts in the immunoassay for glucagon.

The elution volume of a substance after its chromatography on a dextran column is primarily related to its molecular size. To determine whether jejunal glucagon-like immunoreactivity is similar to pancreatic glucagon in molecular size, the elution volumes of the glucagon-like immunoreactivity of jejunal and pancreatic extracts were compared.

TABLE IV
Mean Increment of Plasma Glucagon-Like Immunoreactivity in the Mesenteric Vein (MV), Vena Cava (VC), and Pancreaticoduodenal Vein (PV) after Intraduodenal Glucose Administration (2 g/kg)

| Source | Time after glucose administration, min | | | | | | | | | |
|-----------|--|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| | 5 | 10 | 20 | 30 | 45 | 60 | 90 | 120 | 150 | 180 |
| MV | 0.25 | 0.44 | 0.87 | 1.15 | 1.35 | 1.56 | 1.35 | 1.33 | 1.09 | 0.83 |
| ± SD | 0.21 | 0.23 | 0.57 | 0.64 | 0.74 | 0.76 | 0.75 | 0.85 | 0.95 | 0.78 |
| P = Value | <0.005 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.005 | <0.01 |
| VC | 0.08 | 0.34 | 0.48 | 0.76 | 1.31 | 1.45 | 1.37 | 1.02 | 0.81 | 1.12 |
| ± SD | 0.17 | 0.20 | 0.30 | 0.47 | 0.78 | 0.72 | 0.74 | 0.72 | 0.68 | 0.93 |
| P = Value | ns | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.005 | <0.005 | <0.005 |
| PV | -0.65 | -0.17 | -0.05 | 0.24 | 0.76 | 0.88 | 1.13 | 1.53 | 1.10 | 0.84 |
| ± SD | 0.99 | 0.96 | 0.95 | 0.91 | 0.89 | 0.95 | 1.02 | 0.77 | 1.26 | 1.10 |
| P = Value | ns | ns | ns | ns | <0.025 | <0.025 | <0.005 | <0.001 | <0.025 | <0.05 |

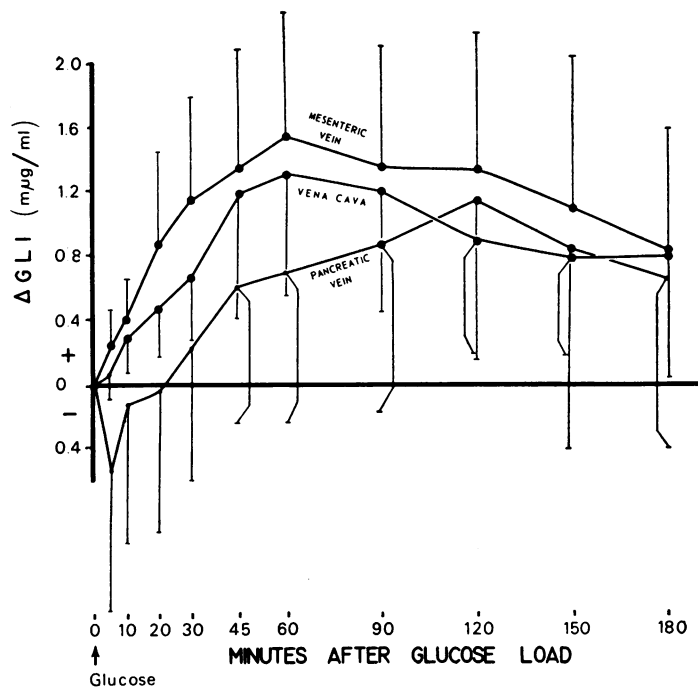


FIGURE 4 Mean increments and SD of glucagon-like immunoreactivity in the mesenteric vein, vena cava, and pancreaticoduodenal vein after intraduodenal administration of 2 g/kg in a group of 13 dogs. A heavy dot on the mean curve of glucagon-like immunoreactivity indicates that it differs significantly ($P < 0.01$) from the mean value at zero time.

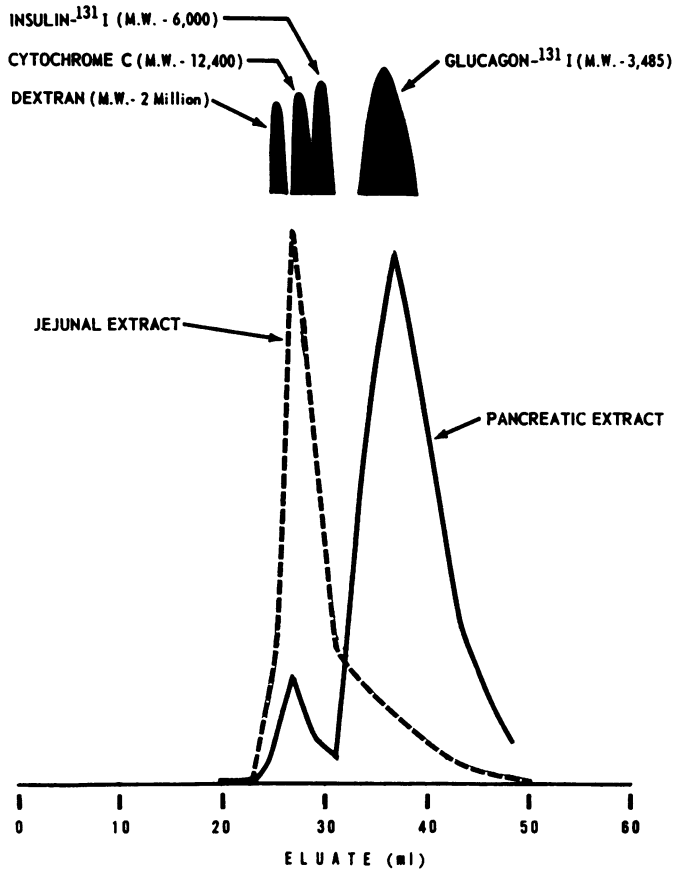


FIGURE 5 The elution curves of glucagon-like immunoreactivity after chromatography of canine pancreatic and jejunal extracts on a Sephadex G-25 column.

Acid-alcohol extracts of an entire, homogenized dog jejunum (all layers) and of a dog pancreas were dissolved in 0.2 M glycine buffer containing 0.25% human albumin and were subjected to chromatography on a 0.9 × 110 cm G-25 Sephadex

column; the elution volume of glucagon-like immunoreactivity was determined by assaying each 2 ml of eluate. The elution volume of glucagon-¹³¹I was also determined by measuring the radioactivity in each 2 ml of eluate, and that of cytochrome c

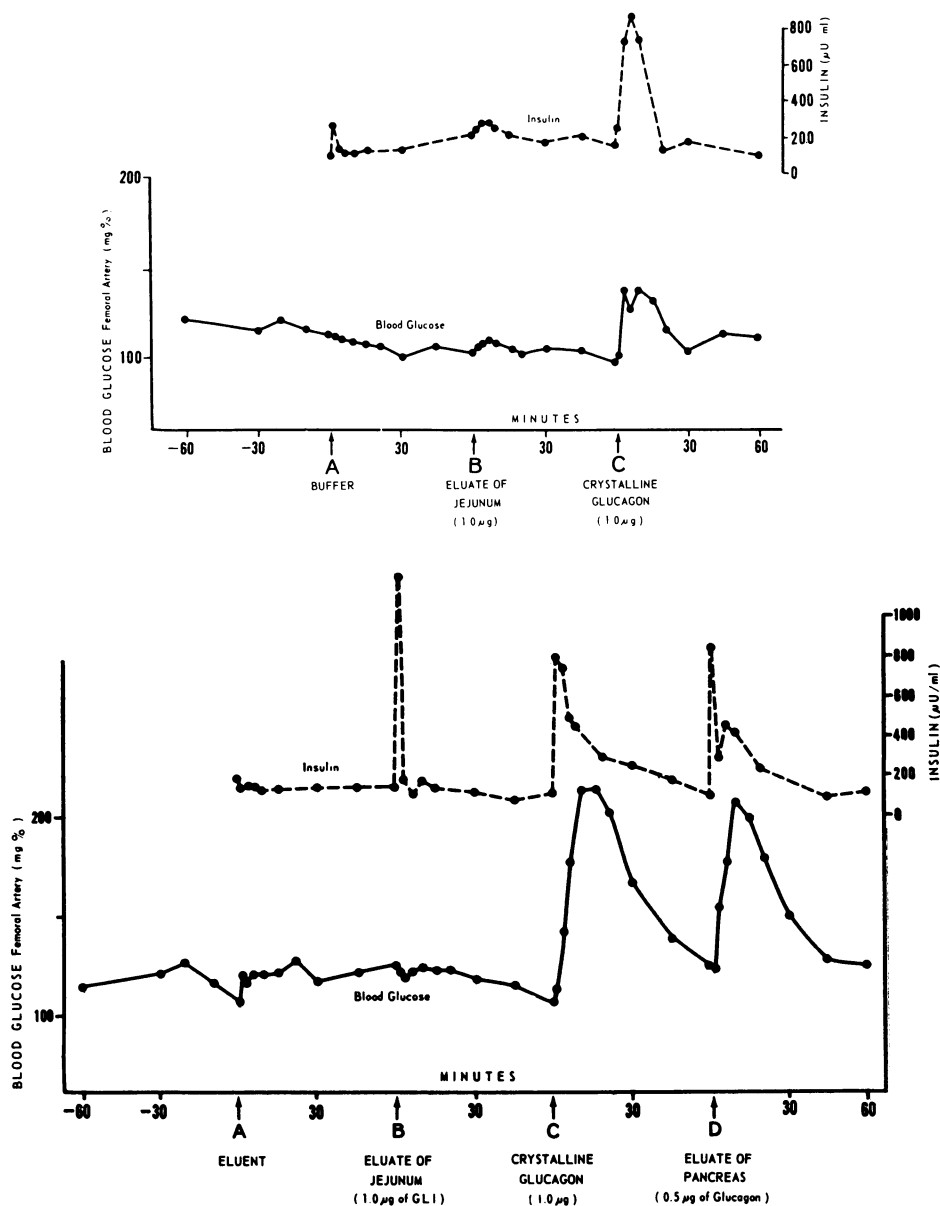


FIGURE 6 Comparison of the hyperglycemic and insulin-stimulating activities of the eluate of jejunal extract containing the glucagon-like immunoreactivity with those of pancreatic extract and crystalline glucagon in two dogs. 7 ml of eluate 24–27 ml of the jejunal extract containing immunoreactivity equivalent to 1 μg of pancreatic glucagon failed to cause appreciable arterial hyperglycemia after its rapid endoportral injection (upper panel), in contrast to 1 μg of crystalline glucagon and eluate 31–40 ml of pancreatic extract containing 0.5 μg of glucagon (lower panel).

and blue dextran by spectrophotometric measurement, thus providing markers of known molecular weight.

The results of an experiment are shown in Fig. 5 and are typical of the findings in 10 such experiments. The elution volume of the glucagon immunoreactivity of pancreatic extract was virtually identical with that of glucagon-¹³¹I which has a molecular weight of less than 4000, whereas the elution volume of the immunoreactivity of jejunal extracts was much less, suggesting a substance of considerably larger molecular size.

A comparison of the biological actions of the eluates of jejunal and pancreatic extracts that contain the glucagon-like immunoreactivity was also made, both in vivo and in vitro. The in vivo hyperglycemic activity of the eluates was studied by means of technic described previously (12), in which the test substance is injected rapidly via the portal vein of a Nembutalized, acutely operated dog and the arterial blood glucose level is measured at intervals of 1–15 min. Fig. 6 indicates that the endoportal injection of 7 ml of jejunal eluate, 24–27 ml (Fig. 5), containing the immunologic equivalent of 1 μ g of glucagon did *not* cause arterial hyperglycemia when injected rapidly into the portal vein of dogs. By contrast, injections of 1 μ g of crystalline beef-pork glucagon dissolved in 7 ml of the same buffer, and in one of the experiments 7 ml of the pancreatic eluate 31–40 ml (Fig. 5) containing only 0.5 μ g of glucagon, caused marked arterial hyperglycemia. The lack of hyperglycemic activity in the fraction of the jejunal extract containing the glucagon-like immunoreactivity has been a consistent finding in four separate experiments (17). However, in Fig. 7 the whole extract of jejunum does appear to have some hyperglycemic activity. The striking but extremely transient rise in pancreaticoduodenal vein insulin concentration, shown in the lower panel (after injecting jejunal eluate) of Fig. 6, has not been a consistent finding with a dose of 1 μ g or less.

However, two experiments have been performed using a higher dose of glucagon-like immunoreactivity. In one of these experiments (Fig. 8, upper panel), beef jejunal eluate containing 4.2 μ g of glucagon-like immunoreactivity was injected rapidly into a peripheral vein of a conscious dog during a continuous glucose infusion at a rate of 100 mg/min. A striking threefold rise in pan-

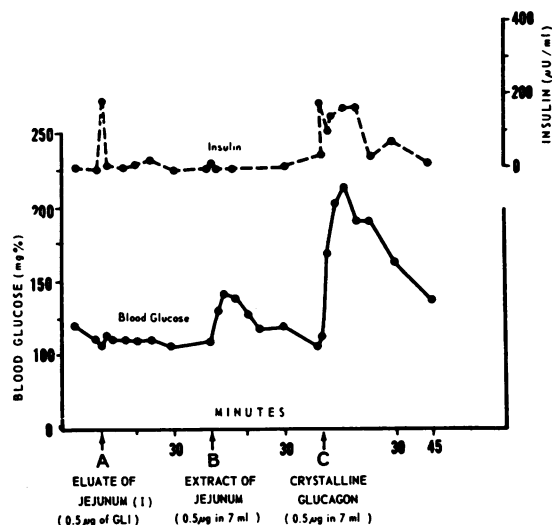


FIGURE 7 Comparison of biological activities of jejunal glucagon-like immunoreactivity and glucagon in vivo. 7 ml of eluate containing 0.5 μ g of glucagon-like immunoreactivity failed to cause arterial hyperglycemia when injected rapidly into the portal vein of Nembutal-anesthetized dog. Injection of whole extract of jejunum containing an identical quantity of immunoreactivity caused a 35 mg/100 ml rise in arterial glucose concentration, and 0.5 μ g of glucagon caused a rise of more than 100 mg/100 ml.

creaticoduodenal insulin level was noted 1 min after the injection; this exceeded in magnitude, if not in duration, the rise noted after an immunologically equivalent dose of beef-pork crystalline glucagon, even though hyperglycemia was not induced. The other experiment (Fig. 8, lower panel) was identical except that dog, rather than beef, jejunal eluate containing 3.0 μ g of glucagon-like immunoreactivity was administered. A 10-fold rise in insulin concentration in the pancreaticoduodenal vein occurred at 1 min after injection, exceeding in height the insulin response to an immunologically equivalent dose of beef-pork crystalline glucagon. In this experiment a 25 mg/100 ml of blood sugar rise occurred after the injection of the eluate, the only experiment in which a hyperglycemic response with this fraction of jejunal extract was observed. The hyperglycemic response to glucagon was also about 25 mg/100 ml, i.e., an unusually weak response.

In both of these experiments the level of glucagon-like immunoreactivity in the vena caval plasma was measured throughout. Although the rate and route of the injections were the same and

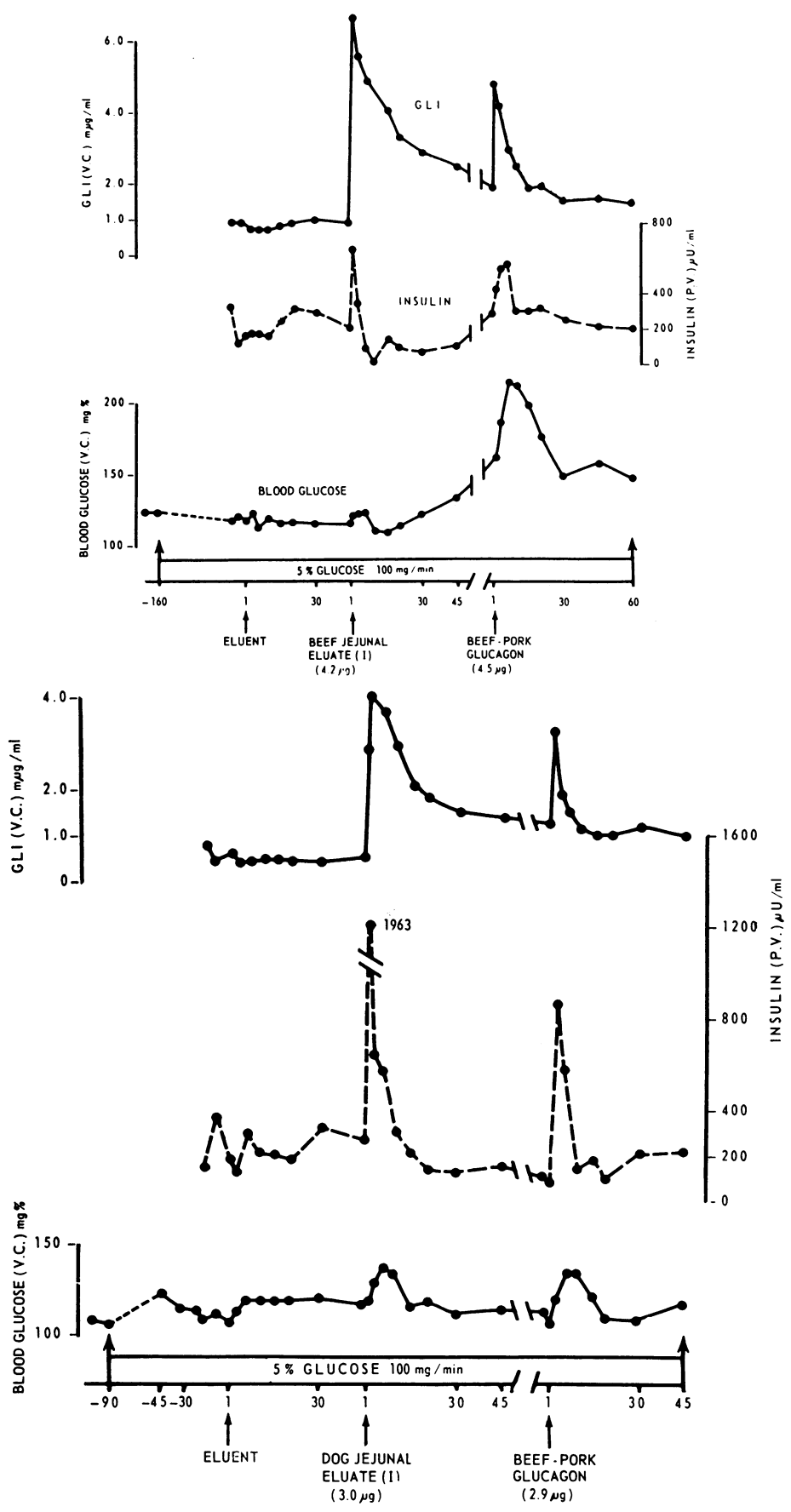


FIGURE 8

the quantities of jejunal glucagon-like immunoreactivity and pancreatic glucagon injected were identical in terms of immunologic equivalence, the 1 min level of glucagon-like immunoreactivity in vena caval plasma was higher after injection of the jejunal material than after injection of crystalline glucagon; the half-time of disappearance of the jejunal material was considerably longer, approximately 20 min, as compared to 7 min for glucagon. The early differences may reflect a difference in the initial distribution of the two substances and, indeed, the calculated volume of distribution at 1 min was 748 and 857 ml for both the beef and dog jejunal material, respectively, whereas it was 1500 and 1450 ml for glucagon in those experiments. The rapid return to the base line concentration after crystalline glucagon injection suggests that glucagon is removed much more rapidly than the gut material.

The *in vitro* biological activity in the eluate of jejunal extract containing the glucagon-like immunoreactivity was examined in the isolated, perfused liver of fed rats and compared with similar eluates of pancreatic extract. Table V reveals that the jejunal eluate that contained most of the glucagon-like immunoreactivity, 24–27 ml (Fig. 5), is devoid of measurable glycogenolytic activity. Of the three jejunal eluates, only eluate 32–41 ml in which pancreatic glucagon was found exhibited measurable glycogenolytic activity equivalent, however, to only 10 $\mu\text{g}/\text{ml}$ of glucagon. By contrast, in the pancreatic eluates, glycogenolytic activity was highest in eluate 31–40 ml, in which the concentration of immunoassayable glucagon was maximal.

The contents of 3',5' cyclic adenylate was measured by the method of Butcher et al. (19)⁸ in livers in which glycogenolytic activity had been noted (Table V). Only one liver, which had been perfused with the pancreatic eluate 31–40 ml and that had a high glucagon content, showed a definite

⁸ These assays were kindly performed by Dr. G. Alan Robison, Vanderbilt University, Nashville, Tenn.

increase in adenylate above the level of the control liver. Neither the jejunal eluate 24–27 ml nor pancreatic eluate 41–50 ml were considered to have induced a definite increase in 3',5' cyclic adenylate and, in this respect, they were devoid of glucagon-like activity.

These preliminary studies of biological activity strongly suggest that glucagon-like immunoreactivity present in eluates of jejunal extracts does not possess either hyperglycemic, glycogenolytic, or adenylyl cyclase-activating activity present in glucagon-containing eluates of pancreatic extracts.

Comparison of insulin response to intravenous and intraduodenal glucose administration. Measurements of insulin concentration in the pancreaticoduodenal vein were made in all experiments and the response to intravenous and intraduodenal administration of glucose was, therefore, compared to determine if the clear-cut and consistent enhancement of peripheral venous plasma

TABLE V
Effect of Perfusion of Jejunal and Pancreatic Eluates upon Glycogenolytic Activity and 3',5' Cyclic Adenylate Content of Isolated Rat Liver

| Sample | Concentration of glucagon-like immunoreactivity | Increase in glucose output | Glycogenolytic activity | Hepatic cyclic AMP concentration |
|---------------------------------|---|---|-------------------------------------|----------------------------------|
| | $\mu\text{g}/\text{ml}$ | $\mu\text{moles}/\text{g liver per hr}$ | $\mu\text{g}/\text{ml of glucagon}$ | $\mu\text{moles}/\text{g}$ |
| Control, glycine-albumin buffer | 0 | 0 | 0 | 0.49 |
| Jejunal eluate, 24–27 ml | 57.6 | 0 | 0 | |
| Jejunal eluate, 32–41 ml | 12.5 | 30 | 10 | 0.51 |
| Jejunal eluate, 42–51 ml | 1.1 | 0 | 0 | |
| Pancreatic eluate, 24–27 ml | 11.4 | 12 | 4 | |
| Pancreatic eluate, 31–40 ml | 45.3 | 240 | 80 | 4.03 |
| Pancreatic eluate, 41–50 ml | 2.9 | 60 | 20 | 0.68 |

FIGURE 8 The effect of the rapid intravenous injection of *GLI*-containing eluate of beef jejunal extract upon the concentrations of pancreaticoduodenal vein insulin, vena caval *GLI*, and glucose during a glucose infusion in unanesthetized dogs (upper panel). The lower panel shows the same effect with eluate of dog jejunal extract.

insulin levels reported in man after oral glucose administration (20–22) also occurs in dogs. In 9 of 13 dogs the pancreaticoduodenal vein insulin was greater after intraduodenal glucose than after intravenous glucose loading; the difference in the means of the peak levels was not statistically significant, thus confirming to a degree the findings of Grossman and Gold ⁴ and of Seltzer (23). However, in all but 3 of the 13 dogs the “insulinogenic index” (24), determined here by dividing the area of the insulin increment by the area of the increment of vena cava glucose during the first 20 min ⁵ after glucose administration, was greater in the intraduodenal experiment (Table VI). This was true in both “isometric” and “isoglycemic” experiments, but the difference between the groups was only slightly significant ($P < 0.05$). However, if one excludes dog 2-14, in which the change in insulin was enormous, the difference between the response to intraduodenal and intravenous glucose becomes statistically significant ($P < 0.01$). This exclusion can be defended on the grounds that this dog is unique, a “hyper-responder,” to both intravenously and intraduodenally administered glucose, but particularly to the latter; consequently, this exclusion reduced the significance of the intergroup difference only because of the magnitude of both responses and the resulting increase of standard deviations, rather than through a lack of a greater response to intraduodenal glucose administration.

To determine if the presumably enterogenous glucagon-like immunoreactivity released during glucose absorption might be a potentiator of insulin secretion in these experiments, the maximum increment in vena caval insulin concentration was compared with the associated peak insulinogenic index in each of the intraduodenal experiments. No significant quantitative correlation was observed. However, owing to the large individual variation in the magnitude of insulin response to a given glucose concentration, no significant quantitative correlation between glucose and insulin levels was observed in these experiments either; therefore the physiologic implications that can be

⁴ Grossman, M. I., and E. Gold. Personal communication.

⁵ A 20 min “cut-off point” was selected because it tends to maximize differences between insulinogenic indices of intraduodenal and intravenous experiments.

TABLE VI
Comparison of “Insulinogenic Indexes”^{*} after Intraduodenal (i.d.) and Intravenous (i.v.) Glucose Administration

| Dog | Type of experiment | Route of administration | Amount of glucose | Insulinogenic index |
|------|--------------------|-------------------------|-------------------|---------------------|
| | | | g/kg | |
| 1-63 | Isometric | i.d. | 2.0 | 10.8 |
| | | i.v. | 2.0 | 4.0 |
| 1-64 | Isometric | i.d. | 2.0 | 2.8 |
| | | i.v. | 2.0 | 1.1 |
| 1-66 | Isometric | i.d. | 2.0 | 9.6 |
| | | i.v. | 2.0 | 1.5 |
| 1-67 | Isometric | i.d. | 2.0 | 8.2 |
| | | i.v. | 2.0 | 3.6 |
| 1-95 | Isoglycemic | i.d. | 2.0 | 10.0 |
| | | i.v. | 1.3 | 3.8 |
| 1-99 | Isoglycemic | i.d. | 2.0 | 5.9 |
| | | i.v. | 0.9 | 3.5 |
| 2-02 | Isoglycemic | i.d. | 2.0 | 11.6 |
| | | i.v. | 1.3 | 8.0 |
| 2-04 | Isoglycemic | i.d. | 2.0 | 11.3 |
| | | i.v. | 1.4 | 10.3 |
| 2-14 | Isoglycemic | i.d. | 2.0 | 67.6 |
| | | i.v. | 1.4 | 25.5 |
| 2-38 | Isoglycemic | i.d. | 2.0 | 14.4 |
| | | i.v. | 1.4 | 0.8 |
| 2-39 | Isoglycemic | i.d. | 2.0 | 8.9 |
| | | i.v. | 2.8 | 10.5 |
| 2-40 | Isoglycemic | i.d. | 2.0 | 2.0 |
| | | i.v. | 1.4 | 2.8 |
| 2-44 | Isoglycemic | i.d. | 2.0 | 8.4 |
| | | i.v. | 0.9 | 6.5 |

* Area of Δ pancreaticoduodenal insulin concentration/area of Δ vena caval glucose concentration.

derived from quantitative relationships of these data are probably extremely limited.

DISCUSSION

The foregoing results may help to clarify the controversy concerning the effects of hyperglycemia upon glucagon secretion. These results confirm earlier reports from this laboratory (3, 5) that hyperglycemia induced by intravenous infusion of glucose suppresses the secretion of pancreatic glucagon,⁶ and are consonant with the traditional

⁶ It should be noted that in this study suppression never occurred when the base line level of glucagon-like immunoreactivity in the pancreaticoduodenal vein was below 0.9 $\mu\text{g}/\text{ml}$, nor was suppression to a level of less than 0.6 $\mu\text{g}/\text{ml}$ ever observed. This may reflect the presence of a cross-reacting or inhibiting substance in plasma that is not pancreatic glucagon and is not influenced by glucose concentration, but which is measured in the assay and gives a small but falsely high value for the total

concept of glucagon as a hormone of glucose need.

The apparent rise in immunoassayable glucagon after glucose ingestion first observed almost simultaneously by Samols et al. (1) and by Lawrence (2) has been verified in the present studies, although their intriguing proposals concerning the physiologic implications of the rise are disputed by these data. Having previously demonstrated that glucagon was a potent stimulator of insulin secretion (4), a fact that has been amply confirmed (27, 12), Samols had suggested that the apparent hyperglucagonemia was derived either from the pancreas or gut (1), and most probably from the pancreas (14). If, as proposed, glucagon is released during glucose absorption, it might, indeed, be the factor responsible for the augmented insulin response to orally administered glucose (1) observed most dramatically in man.

The results of these studies suggest otherwise, however. First, it appears that hyperglycemia per se, whether induced by intravenous or enteric administration of glucose, does not stimulate the secretion of pancreatic glucagon, but probably suppresses it. Second, it is now virtually certain that the rise in glucagon-like immunoreactivity that follows the enteric administration of glucose is not pancreatic glucagon, since it occurs in the absence of the pancreas; Samols, too, has recently observed the characteristic rise after the intrajejunal administration of glucose to a totally depancreatized human (28). Furthermore, the present studies in triply catheterized dogs indicate that the pancreatic contribution cannot account for the rise, although evidence of enhancement of the pancreatic contribution does appear after 90 min in the form of a reversal of the vena cava-pancreaticoduodenal vein gradient, which coincides with a waning of hyperglycemia.

The experiments in the triply catheterized dogs (Table III) were designed to determine if the gut was the source of the extrapancreatic contribution of glucagon-like immunoreactivity during glucose absorption. It has been reported by Schenk

level of "glucagon." This same possibility has been proposed previously, both on the basis of a lack of parallelism between dilution slopes of fasting plasma and of glucagon (14), and on the basis of assays of plasma fractions after ultracentrifugation (25). It would, furthermore, agree extremely well with the predictions of Sokal regarding the levels of circulating glucagon derived from bioassays of glycogenolytic activity (26).

et al. (29) that the portal vein contribution to the total inferior vena caval venous return is approximately 30%; if this figure is correct, a substance released from the gut would be diluted 2.3 times when mixed in the inferior vena caval blood. In these studies (Table IV) the mean increment in the mesenteric vein level of glucagon-like immunoreactivity at 5 min after the glucose load is 0.25 m μ g/ml and 3.1 times that in the increment in the inferior vena cava (0.08 m μ g/ml); at all subsequent times, however, the increment is less than twice. This failure to observe predicted dilution ratio can be interpreted in one of three ways: (1) extraintestinal sites also contribute to the rise in glucagon-like immunoreactivity; (2) Schenk's estimate of dilution by extraportal venous return is incorrect under the conditions of this experiment; (3) the measurements of glucagon-like immunoreactivity are quantitatively inaccurate. All of these possibilities may pertain. As for the first possibility, a survey of various tissue extracts has failed to reveal the presence of significant quantities of glucagon or glucagon-like immunoreactivity, except in the gastrointestinal tract and pancreas;⁷ however, the apparent early suppression of the pancreatic glucagon secretion is short-lived and, thereafter, a rising pancreatic contribution coupled with a declining intestinal contribution may diminish the ratio of mesenteric vein to vena caval glucagon-like immunoreactivity. For the second possibility, the estimated dilution of portal blood (29) may well be higher than actually occurs during alimentation when an increase in the ratio of portal to nonportal venous return would be expected; by adding one sd from Schenk's estimate of total inferior vena caval return, one derives a portal-to-nonportal ratio that approaches unity. With the third possibility, the reliability of the measurements themselves, the limitations of the assay have already been pointed out and its semi-quantitative nature emphasized. It is unlikely that the precision of the assay is sufficient to permit meaningful comparison of differences of less than 0.3 m μ g/ml. Furthermore, since the jejunal glucagon-like immunoreactivity present in tissue extracts does not "dilute out" proportionately to glucagon (25), it is therefore possible that values for enterogenous glucagon-like immunoreactivity, di-

⁷ Unger, R. H., and A. M. Eisentraut. Unpublished observations.

luted by vena caval blood, fail to reflect the full effect of dilution.

Comparative studies of the glucagon-like immunoreactivity present in extracts of canine jejunum reveal major physical and biological differences from pancreatic glucagon. Its elution volume on Sephadex G-25 columns suggests that its molecular size is at least twice as large as that of pancreatic glucagon. In vivo studies indicate that it lacks the hyperglycemic activity of pancreatic glucagon and of whole jejunal extract as well (Fig. 6). In addition, in vitro studies reveal it to be devoid of glycogenolytic activity and incapable of increasing the level of hepatic 3',5' cyclic adenylate in the isolated perfused rat liver. Sokal has also found that such eluates lack glycogenolytic activity.⁸ In short, this material either is a substance quite different from glucagon, but which cross-reacts with glucagon antibodies, or it is glucagon in a different form, i.e., an aggregate of glucagon or glucagon complexed to another molecule. It does not appear to be the source of the glucagon-like activity noted by Makman and Sutherland (30) to be present in extracts of human gastrointestinal tissue, and which, in these studies, may well be in the 32-41 ml eluate (Table V). It is therefore suggested that the jejunal immunoreactivity be tentatively referred to as a "cross-reacting material" or CRM, as proposed by Samols (28). It is, of course, possible that this material is a storage form of a smaller molecule which may be similar or identical with glucagon or may be glucagon-like. If this material is released from the gut during glucose absorption it may, of course, represent no more than a nonspecific consequence of a large hypertonic load. Nevertheless, the possibility that it is a hormone of blood glucose homeostasis must be considered. The most obvious of the possible hormonal roles under consideration would be one of enhancer of the insulin response to ingested glucose. (In both experiments in which 3.0 μg or more of the gut immunoreactivity was employed, an impressive response of insulin release, comparable to that elicited by immunologically equivalent quantities of glucagon, was noted.)

It now seems certain that increased secretion of pancreatic glucagon is not involved in the response to glucose ingestion, except, possibly, as a permissive stimulator of insulin response. However, it

⁸ Sokal, J. E. Personal communication.

has recently been reported that pancreatic glucagon secretion is enhanced during the ingestion of amino acids (31). It is quite possible that the insulin-stimulating action of glucagon, as proposed by Samols and his colleagues, will prove to be of greatest physiological importance in the insulin response to nutrients other than glucose.

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