Studies on Persistent Circulating Immunoreactive Glucagon (IRG) and Immunoreactive Insulin (IRI) Found in Eviscerated Rats with a Functional Liver

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Summary. Eviscerated rats (animals without gastrointestinal tract or pancreas, but with intact liver and kidneys) are diabetic with blood glucose levels of 287 ± 10 mg% ($n = 35$) 24 h after surgery. Immunoreactive insulin (IRI) and immunoreactive glucagon (IRG) persisted in these animals at plasma levels of 36 ± 4 μ U/ml and 0.29 ± 0.02 ng/ml, respectively. Twenty-four h fasted sham-operated controls, on the other hand, had blood glucose levels of 101 ± 3 mg%, plasma IRI levels of 62 ± 8 μ U/ml and plasma IRG levels of 0.38 ± 0.05 ng/ml ($n = 21$). IRG levels were not increased in eviscerated animals by surgical stress, fasting, arginine infusion, or insulin-induced hypoglycaemia, nor did they decrease following somatostatin infusion. IRI levels were similarly unresponsive. An unexplained decrease in IRG followed arginine infusion. Gel filtration studies showed plasma IRI and IRG to consist mainly of the larger molecular weight components with little of the smaller “native hormone” species. The disappearance rates of injected 125 I-insulin and 125 I-glucagon did not differ from sham-operated controls. Removal of the submaxillary glands from eviscerated animals had no effect on the circulating levels of IRG. Bilateral nephrectomy doubled plasma IRG. It is suggested that persistent IRG and IRI in eviscerated rats represents retained immunoreactive materials with slow rates of degradation, although an unresponsive extravisceral source of IRG can not be ruled out.

Key words: Eviscerated rat, immunoreactive glucagon (IRG) immunoreactive insulin (IRI), gel filtration, submaxillary glands.

and man [1–18]. These extrapancreatic sources include the gastrointestinal (G.I.) tract and the submaxillary glands. The stomach [10, 11, 13] in the dog has been documented as a major source for “true glucagon”. In the rat, gastric glucagon has also been reported [16], while the submaxillary glands have been found in the rat, but not in the dog [17], to contain large amounts of glucagon which reacts with antibodies as does pancreatic glucagon [18]. At present, however, the only proven extrapancreatic source of *circulating* IRG is the stomach [1, 14, 15].

Penhos et al. [19] reported the persistence of substantial levels of both immunoreactive insulin (IRI) and IRG in the plasma of rats which had undergone total evisceration (surgical removal of the entire G.I. tract including pancreas and spleen, but leaving functional liver and kidneys). These rats became diabetic and exhibited persisting levels of both IRI and IRG for as long as 96 hours post-evisceration. With regard to this phenomenon, a number of questions can be posed: 1) Are there, in fact, extravisceral sources for both circulating IRG and IRI in the rat? 2) What is the molecular nature of these persistent circulating “hormones” found in the eviscerated rat? 3) Finally, can the plasma levels of IRG and IRI respond to agents which stimulate or inhibit IRI and IRG of pancreatic origin? The results which we present in these studies attempt to provide answers to these questions.

Materials and Methods

A. Evisceration and Other Surgery

Experiments were conducted using adult male, Sprague-Dawley rats weighing 250–300 g (Flow Animal Laboratories, Dublin, Va.). All rats were fed ad libitum prior to surgery and were anaes-

The existence of extrapancreatic sources of immunoreactive glucagon (IRG) has been reported in a variety of species including the rat, dog, monkey

thetized with 125 mg/kg sodium amobarbital (Amytal, Lilly). Total evisceration was achieved by the method of Penhos et al. [19], which maintains functioning liver and kidneys while removing the entire G.I. tract between the oesophagus and rectum, including the pancreas and spleen. Circulation to the liver was maintained by the hepatic artery. In some of these animals, bilateral nephrectomy was performed immediately after evisceration using the same incision. In other eviscerates, bilateral submaxillary glandectomy was performed in the following manner: An incision of the submandibular skin was extended from the anterior angle of the lower jaw to an imaginary line connecting the posterior angles of the mandible. A careful dissection of both submaxillary glands was carried out with an atraumatic curved forceps; the vessels and ducts of each gland were ligated and sectioned and the glands removed.

Totally eviscerated rats and their sham-operated controls were maintained for up to 72 h post-evisceration by the administration of twice daily subcutaneous injections of 0.154 mol/l saline (1 ml/100 g body weight) to minimize dehydration.

Sub-total evisceration was achieved by removing the pancreas as well as the G.I. tract distal to the duodenum and proximal to the rectum. In this procedure, the coeliac trunk and superior mesenteric arteries were left patent to supply the remaining stomach, duodenum, liver and spleen. A polyethylene tube (Intramedic P.E. 280, Clay-Adams) 2 cm in length was used to connect the distal end of the duodenum with the proximal end of the rectum, thus permitting the excretion of remaining G.I. secretions. Pancreatectomy was achieved by a gentle scraping of the organ with the edges of a curved forceps, taking care not to disrupt the bile duct or the vessels supplying the stomach, duodenum, spleen or liver. These animals and their sham-operated controls were used 24 h after surgery, having been maintained on twice daily subcutaneous 0.154 mol/l saline injections (1 ml/100 g body weight).

B. Infusion of Test Substances

The administration of L-arginine (Sigma Chemical Co.), cyclical somatostatin (kindly provided by Dr. A.V. Schally), crystalline porcine insulin (Eli Lilly Co.) and 0.154 mol/l saline to anaesthetized eviscerated rats and their controls was accomplished by intravenous infusion through the femoral vein with a Harvard Infusion Pump. The vein was cannulated with a 30 gauge needle attached to PE-10 plastic cannula (Intramedic, Clay-Adams). Infusions

were carried out over a 20 to 30 min period. In all cases, the test substances were dissolved in heparinized 0.154 mol/l saline (200 U.S.P. U/ml) and were infused at a rate of 0.03 ml/min. Arginine (772 mg/kg) was infused over a 20 min period; insulin (0.4 U/kg) was infused over 30 min (except as otherwise stated); somatostatin (120 µg/kg) was infused over 30 min following a loading dose of 120 µg/kg administered as a 0.2 ml bolus; heparinized 0.154 mol/l saline alone was infused over 30 min to provide control values.

Immediately following the infusion period, the animals were bled via the abdominal aorta. A sample of whole blood was taken for glucose determination by the glucose oxidase method, while the remainder of the whole blood (4 to 6 ml) was centrifuged in tubes containing disodium EDTA (10.5 mg) and Trasylol (1,000 K.I.U., FBA Pharmaceuticals). The plasma was removed for insulin and glucagon radioimmunoassay. IRI was assayed by the double antibody method of Morgan and Lazarow [20] while IRG was assayed according to the procedure of Unger, using 30 K antibody [21] and compared with a non-specific glucagon antibody measuring intestinal glucagon-like material. In eviscerated rats, IRG reacted no better with the gut antibody than with 30 K, indicating its immunologic similarity to pancreatic glucagon. IRG measurements were sensitive to 0.05 ng/ml and IRI to 3 µU/ml. Interassay variation was minimized by inclusion of the same plasma standards with each assay.

C. Plasma Disappearance of Injected Glucagon and Insulin

Biologically active moniodo ^{125}I -insulin and ^{125}I -glucagon were prepared by a modification [22] of the method of Hunter and Greenwood [23]. Labeled hormones (2×10^6 CPM in 0.5 ml 0.154 mol/l saline) were injected intravenously in anaesthetized eviscerated and control rats fasted for 24 h. Tail vein blood samples (0.1 ml) were collected in heparinized micropipettes at intervals of -1, 2, 4, 6, 8, 10, 20, 30, 45 and 60 min post-injection and placed on ice in 1.1 ml 0.154 mol/l saline containing 5 g/l salt-poor human albumin (Cutter Laboratories) and 1,000 K.I.U. Trasylol. Blood cells were removed by centrifugation and 1 ml of the supernatant was recovered for analysis. In order to separate labelled intact hormone from non-specific counts, glucagon-containing samples were incubated at 4° C with charcoal, while insulin-containing samples were treated with 1 ml 10% trichloroacetic acid (w/v). All samples were then centrifuged at 4° C for

Fig. 1. Effect of fasting and post-surgical stress on plasma IRI, IRG and blood glucose levels in totally eviscerated and sham-operated rats. The number of animals in each group is shown in parentheses

Fig. 2. Effect of arginine (ARG), somatostatin (SRIF) and insulin (INS; 0.4 U/kg) infusions on plasma IRI, IRG and blood glucose levels in totally eviscerated, subtotally eviscerated and sham-operated rats. Animals were fasted 24 h post-operatively before infusion. Blood samples were taken immediately following infusion. The number of animals in each group is shown in parentheses

Fig. 3. Effect of total evisceration versus sham-operation on the plasma disappearance rates of injected 125 I-glucagon and 125 I-insulin. Curves are plotted as the percent of charcoal precipitable (intact) 125 I-glucagon or TCA precipitable (intact) 125 I-insulin remaining in the plasma with time, using the 2 min values as 100%. Each curve represents the mean of 4 experiments. The SEM is shown

20 min at 2,000 RPM. The supernatants, containing hormone fragments and other non-specific counts, were decanted, while the precipitates were counted to determine the rates of disappearance of the labelled intact hormones from the plasma. It should be noted that this method must be critically examined in view of the observations of Berger et al. [24]. These authors showed that mere counting of precipitates does not necessarily measure disappearance rates of hormones, but measures also the appearance of label in larger fractions which would all be precipitated and counted. Appearance of these larger fractions will increase with time and could greatly affect the second component of the disappearance curves.

D. Column Chromatography

Two to 4 ml of pooled plasma (3 rats) were placed on columns for IRG or IRI separation. Plasma IRG components were separated at 4° C on Biogel P-30 columns (45 × 1.5 cm), prepared and eluted with 0.2 mol/l glycine buffer containing 2.5 g/l human albumin and 1% (v/v) normal sheep serum (pH 8.8) [25, 26]. Fractions of 1 ml were collected in 500 U Trasylol and assayed directly for IRG. ¹²⁵I-glucagon was included with each sample as a marker. Addition of unlabelled glucagon to serum resulted in a peak in the area of the ¹²⁵I-glucagon. Plasma IRI components were separated as described by Gutman et al. [27] at 4° C on Sephadex G-50 superfine columns (Pharmacia Fine Chemicals) eluted with 0.05 mol/l veronal buffer (pH 8.6) with 50 g/l bovine albumin, Fraction V (Sigma Chemical). Fractions of 1.5 ml were collected and assayed directly for IRI. ¹²⁵I-insulin was included with each sample as a marker.

In all experiments, the mean and standard error of the mean were calculated. Statistical significance was determined using the t test for group data.

Results

Response of Eviscerated Rats to Fasting

In Figure 1, it can be seen that eviscerated rats rapidly developed hyperglycaemia, with a significant elevation of blood glucose as early as 4 h post-operatively ($p < 0.05$). The blood glucose level continued to rise for 48 h, plateauing at levels greater than 400 mg/100 ml. Persistence of plasma IRI and IRG in the eviscerated rat was noted throughout the period of fasting. Plasma IRI levels fell rapidly during the first 4 h after surgery from 104 μ U/ml to

26 μ U/ml ($p < 0.01$), but no further significant change was noted up to 72 h. Plasma IRG levels fell significantly 4 h after surgery from 0.27 ng/ml to 0.20 ng/ml ($p < 0.05$), but then rebounded after 24 h to 0.29 ng/ml ($p < 0.05$). By 72 h, however, the plasma IRG levels had again fallen to 0.14 ng/ml ($p < 0.01$).

In contrast with the eviscerated rats, sham-operated animals maintained normal blood glucose levels in the face of steadily falling plasma IRI and steadily rising plasma IRG levels (Fig. 1).

Response of Eviscerated Rats to Nephrectomy and Submaxillary Glandectomy

Twenty-four hours after surgery, no significant change in blood glucose or plasma IRI levels was noted in 8 nephrectomized or 5 submaxillary glandectomized eviscerated animals when compared to 35 eviscerated controls with these organs intact. Plasma IRG levels were not significantly altered in submaxillary glandectomized eviscerates, 0.26 ± 0.05 ng/ml, but showed significant elevation, 0.53 ± 0.07 ng/ml ($p < 0.01$) in the nephrectomized group.

Response of Eviscerated Rats to Stimulators and Inhibitors of Pancreatic IRG

The control animals responded to arginine infusions with a doubling of plasma IRI ($p < 0.01$) and a tripling of plasma IRG ($p < 0.01$) (Fig. 2). No change in blood glucose occurred. SRIF infusion significantly lowered both plasma IRI ($p < 0.01$) and plasma IRG ($p < 0.05$) in the controls, again without affecting the blood glucose levels. Infusion of insulin (0.4 U/kg) reduced blood glucose to hypoglycaemic levels (50 mg/100 ml, $p < 0.01$) and caused a significant increase in plasma IRG ($p < 0.05$).

In contrast with sham-operated animals, eviscerated rats, when infused with arginine, showed a rise in blood glucose ($p < 0.01$), no change in plasma IRI and a significant decrease ($p < 0.01$) in plasma IRG. Administration of SRIF resulted in no change in blood glucose, plasma IRI or plasma IRG. The eviscerated rat appeared to be insulin resistant in that 0.4 U/kg of insulin infused over 30 min produced only a 19% decrease in blood glucose concentration. No change in plasma IRG was observed. Because hypoglycaemia was not achieved, additional experiments (Table 1) were undertaken in which 32 U of insulin per kg of body weight were infused into 7 eviscerated rats over a period of 2 h. These animals developed hypoglycaemic blood glucose levels (62 ± 6 mg/100 ml) only after 2 h of infusion.

Still no significant change in plasma IRG was detected ($0.24 < 0.07$ ng/ml).

Subtotally eviscerated rats, when compared with totally eviscerated rats in the basal state (Fig. 2), showed higher blood glucose levels ($p < 0.01$), as well as significantly higher plasma IRG levels ($p < 0.01$). Plasma IRI levels did not differ. In response to arginine, neither blood glucose nor plasma IRI levels changed significantly. However, in the 10 rats studied, plasma IRG levels *decreased* significantly ($p < 0.01$), as in the case of totally eviscerated animals. Administration of SRIF affected only plasma IRG levels with a fall of 64% ($p < 0.01$). Following insulin infusion (0.4 U/kg body weight), blood glucose decreased 45% ($p < 0.01$), but failed to achieve hypoglycaemic levels. No change in plasma IRG was observed. Thus, in an effort to achieve hypoglycaemia in these animals as well, 4 additional subtotal eviscerates (Table 1) were infused with 32 U of insulin per kg body weight over a 2 h period. Although hypoglycaemia was achieved after 2 h of infusion (48 ± 14 mg/100 ml), no significant change in plasma IRG was noted (0.38 ± 0.14 ng/ml).

Insulin and Glucagon Disappearance Studies

Both glucagon and insulin disappearance curves exhibit an early equilibration phase of non-first order kinetics followed by a later phase which appeared linear when plotted on semi-log paper. This latter phase represents the rate of removal of the labelled hormones from the plasma [28]. As can be seen in Figure 3, the insulin and glucagon disappearance curves in both sham-operated animals and eviscerated rats examined after 24 h exhibit a relatively rapid equilibration phase followed by a discrete straight-line phase. From the slopes of the extrapolated lines, it can be seen that the irreversible disappearance time for 50% of the ^{125}I -insulin circulating in the plasma is 64 min for shams and 58 min for eviscerates (N.S.). The irreversible disappearance times for 50% of the circulating ^{125}I -glucagon in the plasma of shams and eviscerates was 39 and 38 min, respectively (N.S.).

Column Chromatography

Figure 4 is representative of plasma IRG patterns and Figure 5 of plasma IRI patterns obtained in control and eviscerated rats. Columns utilizing plasma from normal rats, in most cases, showed elevated baseline immunoreactivity. This was not an artifact of elution buffers and could not be explained (not seen with human plasma). This problem was only

Table 1. Blood glucose levels during high and low-dose insulin infusion of totally and subtotally eviscerated rats. Insulin resistance is apparent in both groups relative to sham-operated animals

Time after onset of infusion (min)	Blood glucose levels (mg/100 ml $\pm$ SEM) following insulin infusion				
	0.013 U/kg/min insulin infused over 30 min			0.27 U/kg/min insulin infused over 2 h ^a	
	Totally eviscerated	Subtotally eviscerated	Shams	Totally eviscerated	Subtotally eviscerated
0	287 $\pm$ 10 (n=35)	338 $\pm$ 17 (n=17)	101 $\pm$ 3 (n=22)	287 $\pm$ 10 (n=35)	338 $\pm$ 17 (n=17)
30	233 $\pm$ 14 (n= 8)	185 $\pm$ 69 (n= 4)	50 $\pm$ 2 (n=10)	203 $\pm$ 26 (n= 7)	—
60				144 $\pm$ 22 (n= 7)	140 $\pm$ 44 (n= 4)
90				102 $\pm$ 16 (n= 7)	—
120				62 $\pm$ 6 (n= 7)	48 $\pm$ 14 (n= 4)

^a Sham-operated rats did not survive high insulin dose

rarely encountered with eviscerated rat plasma. Plasma from normal animals showed 3 to 4 peaks of IRG representing molecules previously described [29, 30] as having molecular weights of $> 20,000$ (eluting in the void volume), possibly one of 9,000, 3,500 (eluting with the tracer) and 2,000. It can be clearly observed, however, that plasma from eviscerated animals did not display any prominent peaks associated with the 9,000 or 3,500 molecular weight species. Thus, IRG persisting in the plasma of eviscerates is mainly of the large molecular species which appears in the void volume.

The IRI patterns of both normal and eviscerated rats appeared similar with the exception that the per cent of total IRI due to the 6,000 molecular weight species (eluting with tracer ^{125}I -insulin) is considerably less in eviscerated rats.

Discussion

The presence of circulating immunoreactive glucagon (IRG) following pancreatectomy has been documented in dogs [1–5], rats [6], monkeys [7] and humans [8, 9]. In the dog, levels of IRG appear to be decreased by insulin therapy and stimulated by arginine only in the near absence of circulating insulin [1, 2]. Studies in depancreatized humans suggest a similar phenomenon [8, 9, 31]. The inference to be drawn from those studies is that extrapancreatic sources of IRG exist in dog and man, and can only

Fig. 4. Biogel P-30 column chromatography of plasma IRI pooled from 24 h fasted totally eviscerated and 24 h fasted normal rats. Tracer ^{125}I -glucagon was added to each column. Three ml plasma applied to each column

Fig. 5. Sephadex G-50 Superfine column chromatography of plasma IRI pooled from 24 h fasted totally eviscerated and 24 h fasted normal rats. Tracer ^{125}I -insulin was added to each column. Four ml plasma applied to each column

be stimulated by arginine when insulin levels are low. Further studies done in dogs, using direct cannulation of the gastric vein, have shown the gastrointestinal tract, particularly the stomach and duodenum, to be a major source of extrapancreatic plasma IRG [1, 14, 15]. In the rat, Pek [16] demonstrated secretion of gastric glucagon. In unpublished observations, our laboratory found that extracts of rat gastric mucosa contain IRG indistinguishable from pancreatic IRG and different from the intestinal glucagon-like materials. Thus, the report of substantial levels of IRG and IRI in the plasma of rats following removal of both the pancreas and G.I. tract stimulated us to explore this model in an attempt to determine the origin of the persistent IRG, its molecular nature and its ability to respond to conditions known to modulate IRG of pancreatic origin. With regard to the latter issue, our observations have shown the following: 1) With prolonged fasting and post-operative stress, eviscerated rats show no increase in plasma IRG over fed levels; 2) There is no lowering of plasma IRG in eviscerated rats following somatostatin infusion; 3) Insulin induced hypoglycaemia does not elicit a rise in IRG levels in eviscerated rats; and 4) Arginine infusion in eviscerated rats causes a *decrease* in IRG levels. However, because of possible species variation, we were not certain how the G.I. sources of IRG in the *rat*, as compared with the dog, would respond to experimental agents given to eviscerated animals. Consequently, we prepared rats in which *subtotal* eviscerations were performed, leaving functional *stomach* and *duodenum*, while removing the pancreas and bowel distal to the duodenum. The response of these animals differed from totally eviscerated rats only in that they exhibited higher basal levels of plasma IRG, and these levels decreased with somatostatin. From these findings, it is apparent that if, in fact, there exists an extravisceral source for circulating IRG in the rat, it differs from sources within the gut and pancreas by its inability to respond to somatostatin. In repeated experiments, IRG levels were observed to *decrease* following arginine infusion in both types of evisceration. We have no explanation for this phenomenon other than to speculate that arginine infusion and its resultant osmotic diuresis may have increased the kidney's metabolic clearance of IRG. Another possibility is that the presence of IRI in the plasma may have prevented arginine stimulation of extrapancreatic IRG sources. Extrapancreatic insulin has been reported in extracts of porcine stomach [32] and in rat parotid gland [33]. The latter may be contributory to the substantial plasma IRI levels present in the totally eviscerated rat.

With regard to the origin of the persistent IRG found in the totally eviscerated rat, the observations of Lawrence et al. [17], confirmed in our laboratory [18], that rat submaxillary glands contain microgram quantities of extractable IRG seemed worthy of consideration. However, removal of the submaxillary glands at the time of evisceration had no effect on circulating IRG levels. The only suggestion in our studies that an extravisceral source is contributing IRG in eviscerated rats is found in the observation that nephrectomy doubled the plasma IRG in eviscerated rats. Such an elevation cannot be explained on the basis of impaired IRG excretion alone, but suggests continued provision of IRG.

On the other hand, if there were no extravisceral contributions to circulating IRG in the eviscerate, could these persistent levels be explained by retention of slowly metabolized hormone components? No substantial difference was found in the disposal rates of injected ^{125}I -glucagon (3500 mol. wt.) or insulin (6000 mol. wt.) from either eviscerated or control rats, suggesting that the removal of *small* molecular weight species was similar in both. This point assumes significance in the light of our chromatographic studies, which indicate that eviscerated rat plasma contains primarily the *larger* molecular weight species of both glucagon and insulin. With respect to glucagon, these findings are in agreement with those of Villanueva et al. [34] who demonstrated the presence of mainly the void volume fraction of IRG in the plasma of a patient who had undergone duodenopancreatectomy with resection of the gastric antrum. Prohormones, such as proinsulin [35, 36], and other large molecular species may have considerably longer disappearance times than the small molecular species. The failure of plasma IRG or IRI to respond to stimulators and inhibitors in the eviscerated rat would be compatible with retention of the larger molecular weight hormone components. However, the existence of an unresponsive extravisceral source for the large IRG fraction cannot be ruled out. In fact, Villanueva et al. [34] were able to demonstrate the persistence of such an IRG fraction in a patient who had been without a pancreas, duodenum and gastric antrum for 3 years.

The diabetic state in the eviscerated animal can be sufficiently explained by the failure of IRI moieties to respond to any stimuli tested together with the demonstrated insulin resistance manifested in both totally and partially eviscerated rats. The added diabetogenic potential of a biologically active IRG moiety, however, must also be considered. The large (> 20000) molecular weight species of IRG has been found in one preliminary study [37] to pos-

sess biological activity at least equivalent to the 3500 molecular weight species. This large molecular species is the one which we have found to prevail in the plasma of eviscerated rats.

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References

1. Blazquez, E., Munoz-Barragan, L., Patton, G. S., Orci, L., Dobbs, R. E., Unger, R. H.: Gastric A-cell function in insulin-deprived depancreatized dogs. *Endocrinology* **99**, 1182–1188 (1976)
2. Vranic, M., Engerman, R., Doi, K., Morita, S., Yip, C. C.: Extrapancratic glucagon in the dog. *Metabolism* **25**, 1469–1473 (1976)
3. Vranic, M., Pek, S., Kawamori, R.: Increased "glucagon immunoreactivity" in plasma of totally depancreatized dogs. *Diabetes* **23**, 905–912 (1974)
4. Diamond, D., Field, J. B.: Persistent pancreatic glucagon but not insulin response to arginine in pancreatectomized dogs. *Endocrinology* **96**, 678–693 (1975)
5. Matsuyama, T., Foa, P. P.: Plasma glucose, insulin, pancreatic and enteroglucagon levels in normal and depancreatized dogs. *Proc. Soc. Exp. Biol. Med.* **147**, 97–102 (1974)
6. Dunbar, J. C., Walsh, M. F., Foa, P. P.: Effects of somatostatin (SRIF), insulin and antiglucagon serum (AGS) in depancreatized rats, p. 88. The Endocrine Society Annual Meeting, San Francisco (1976)
7. Hoganson, G., Huss, G., Hoversten, G., Hastings, R., Jonasson, O.: Blood glucagon alterations in response to arginine and somatostatin (SRIF) in the pancreatectomized and streptozotocin (STZ) diabetic monkey. *Diabetes* **25**, 339 (1976)
8. Palmer, J. P., Werner, P. L., Benson, J. W., Ensink, J. W.: Immunoreactive glucagon responses to arginine in three pancreatectomized humans. *Metabolism* **25**, 1483–1485 (1976)
9. Muller, W. A., Brennan, M. F., Tan, M. H., Aoki, T. T.: Studies of glucagon secretion in pancreatectomized patients. *Diabetes* **23**, 512–516 (1974)
10. Morita, S., Doi, D., Yip, C., Vranic, M.: Measurement and partial characterization of immunoreactive glucagon in gastrointestinal tissues of the dog. *Diabetes* **25**, 1018–1025 (1976)
11. Sasaki, H., Rubalcava, B., Baetens, D., Blazquez, E., Srikant, C. B., Orci, L., Unger, R. H.: Identification of glucagon in the gastrointestinal tract. *J. Clin. Invest.* **56**, 135–145 (1975)
12. Munoz-Barragan, L., Rufener, C., Srikant, C. B., Dobbs, R. E., Shannon, Jr., W. A., Baetens, D., Unger, R. H.: Immunocytochemical evidence for glucagon-containing cells in the human stomach. *Horm. Metab. Res.* **9**, 37–39 (1977)
13. Baetens, D., Rufener, C., Srikant, C. B., Dobbs, R. E., Unger, R. H., Orci, L.: Identification of glucagon-producing cells (A-cells) in dog gastric mucosa. *J. Cell Biol.* **69**, 455–464 (1976)
14. Lefebvre, P., Luyckx, A. S., Brassinne, A. H., Nizet, A. H.: Glucagon and gastrin release by the isolated perfused dog stomach in response to arginine. *Metabolism* **25**, 1477–1479 (1976)
15. Lefebvre, P. J., Luyckx, A. S.: Factors controlling gastric-glucagon release. *J. Clin. Invest.* **59**, 716–722 (1977)
16. Unger, R. H.: Glucagon Symposium. Distribution and prop-

- erties of IRGs in animals and man. *Metabolism* **25** (Suppl. 1), 1414 (1976)
17. Lawrence, A. M., Tan, S., Hojvat, S., Kirsteins, L., Mitton, J.: Salivary gland glucagon in man and animals. *Metabolism* **25** (Suppl. 1), 1405–1408 (1976)
 18. Bhathena, S. J., Smith, S. S., Voyles, N. R., Penhos, J. C., Recant, L.: Studies on submaxillary gland immunoreactive glucagon. *Biochem. Biophys. Res. Commun.* **74**, 1574–1581 (1977)
 19. Penhos, J. C., Ezequiel, M., Lepp, A., Ramey, E. R.: Plasma immunoreactive insulin (IRI) and immunoreactive glucagon (IRG) after evisceration with and without functional liver. *Diabetes* **24**, 637–640 (1975)
 20. Morgan, C. R., Lazarow, A.: Immunoassay of insulin: Two antibody system. *Diabetes* **12**, 115–126 (1963)
 21. Unger, R. H., Eisentraut, A. M., Madison, L. L.: The effects of total starvation upon the levels of circulating glucagon and insulin in man. *J. Clin. Invest.* **42**, 1031–1039 (1963)
 22. Giorgio, N. A., Johnson, C. B., Blecher, M.: Hormone receptors. III. Properties of glucagon-binding proteins isolated from liver plasma membranes. *J. Biol. Chem.* **249**, 428–437 (1974)
 23. Hunter, W. M., Greenwood, F. C.: Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature* **194**, 495–496 (1962)
 24. Berger, M., Halban, P., Muller, W. A., Renold, A. E., Vranic, M.: Mobilization and distribution of subcutaneously injected ³H-insulin: effects of exercise. *Diabetes* **26** (Suppl. 1), 357 (1977)
 25. Valverde, I., Villaneuva, M. L., Lozano, I., Marco, J.: Presence of glucagon immunoreactivity in the globulin fraction of human plasma ("Big plasma glucagon"). *J. Clin. Endocrinol.* **39**, 1090–1098 (1974)
 26. Valverde, I., Dobbs, R., Unger, R. H.: Heterogeneity of plasma-glucagon immunoreactivity in normal, depancreatized and alloxan diabetic dogs. *Metabolism* **24**, 1021–1028 (1975)
 27. Gutman, R. A., Fink, G., Shapiro, J. R., Selawry, H., Recant, L.: Proinsulin and insulin release with a human insulinoma and adjacent nonadenomatous pancreas. *J. Clin. Endocrinol.* **36**, 978–987 (1973)
 28. Stern, M. P., Farquhar, J. W., Silvers, A., Reaven, G. M.: Insulin delivery rate into plasma in normal and diabetic subjects. *J. Clin. Invest.* **47**, 1947–1957 (1968)
 29. Valverde, I., Rigopoulou, D., Marco, J., Faloona, G. R., Unger, R. H.: Molecular size of extractable glucagon and glucagon-like immunoreactivity (GLI) in plasma. *Diabetes* **19**, 624–629 (1970)
 30. Valverde, I., Villaneuva, M. L.: Heterogeneity of plasma immunoreactive glucagon. *Metabolism* **25**, 1393–1395 (1976)
 31. Barnes, A. J., Bloom, S. R.: Pancreatectomized man: A model for diabetes without glucagon. *Lancet* **1976 I**, 219–221
 32. Kuhl, C., Jensen, S. L., Nielsen, O. V.: Porcine gastric insulin. *Endocrinology* **99**, 1667–1670 (1976)
 33. Lawrence, A. M., Kirsteins, L., Mitton, J.: Parotid gland insulin: An extrapancreatic source of insulin in rats. *Diabetes* **25** (Suppl. 1), 328 (1976)
 34. Villaneuva, M. L., Hedo, V. A., Marco, J.: Plasma glucagon immunoreactivity in a totally pancreatectomized patient. *Diabetologia* **12**, 613–616 (1976)
 35. Katz, A. I., Rubenstein, A. H.: Metabolism of proinsulin, insulin and C-peptide in the rat. *J. Clin. Invest.* **52**, 1113–1121 (1973)
 36. Stoll, R. W., Touber, J. L., Winterscheid, L. C., Ensink, J. W., Williams, R. H.: Hypoglycemic activity and immunological half-life of porcine insulin and proinsulin in baboons and swine. *Endocrinology* **88**, 714–717 (1971)
 37. Recant, L., Perrino, P. V., Bhathena, S. J., Danforth, D. N., Lavine, R. L.: Plasma immunoreactive glucagon fractions in four cases of glucagonoma: Increased "large glucagon-immunoreactivity". *Diabetologia* **12**, 219–326 (1976)

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