Immunoreactive Glucagon Responses to Oral Glucose, Insulin Infusion and Deprivation, and Somatostatin in Pancreatectomized Man

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SUMMARY

In a group of pancreatectomized subjects, immunoreactive glucagon (IRG) concentrations were normal after an overnight fast, increased after oral glucose, were not suppressed by somatostatin (SRIF) or insulin, and in two of four subjects they rose with an arginine infusion. Even though the SRIF infusion failed to lower IRG, there was a fall in plasma glucose concentration in both subjects. In two subjects, endogenous hyperglycemia occurred during insulin withdrawal without a rise in IRG, and, in one subject, mild diabetic ketoacidosis developed with only a minimal rise in IRG.

These results support the presence of an extrapancreatic source of IRG in man. Secretion from these extrapancreatic alpha cells appears to be regulated differently than secretion from pancreatic alpha cells. DIABETES *27*:1005-12, October, 1978.

The concept of an extrapancreatic source of glucagon can be traced back to the discovery of an extrapancreatic, hyperglycemic factor by Sutherland and de-Duve in 1948.[1] Although early work by Unger[2] described no circulating immunoreactive glucagon (IRG) in subtotally pancreatectomized (Px) dogs in whom only the uncinate process was left, subsequent studies have shown that insulin suppresses extrapancreatic glucagon secretion in the dog and, when Px dogs are deprived of insulin, circulating IRG is seen.[3-5] A major source of extrapancreatic IRG in the dog is the gastric fundus,[6] and IRG that is immunometrically, physicochemically, and biologically similar to 3,500-dalton IRG has been extracted from porcine duodenum.[6] In addition, the presence of IRG has been documented in the eviscerated rat,[7] and IRG has also been discovered in the salivary gland of this species.[8]

In Px man, Muller, Brennan, and colleagues[9] described normal fasting plasma concentrations of IRG, but no response to arginine stimulation. Miyata, Yamamoyo, and associates[10] followed the course of IRG before, during, and after pancreatectomy in a single subject. IRG, which was present before surgery, disappeared from plasma immediately after surgery, reappeared on the first postoperative day, remained for nine days, and then disappeared again on day 9. Recently, Botha, Vinik, and colleagues described the IRG responses to several experimental protocols in a Px subject.[11] IRG showed no response to arginine or insulin-induced hypoglycemia, failed to be suppressed with somatostatin but was suppressed with tolbutamide, rose after oral glucose, and appeared to be under beta-adrenergic control. The above studies[9-11] were performed using antiserum 30K. Recent work by Barnes and Bloom[12] claimed an absence of circulating IRG in a group of Px subjects, but this study was done with a different antiserum than 30K. In addition, a standard curve was made for each pa-

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tient using glucagon-free plasma obtained by passing each Px subject's plasma through a glucagon immunoabsorbent.

We studied five subjects who had undergone a total surgical pancreatectomy. In this report, we describe their basal concentrations of IRG and the responses to various stimuli and suppressive agents.

METHODS

The five Px subjects included in this report are described in table 1. In addition to the reports describing a total pancreatectomy, residual beta cell secretion was evaluated in four subjects by arginine stimulation. Patients R.B. and E.D., tested before developing insulin antibodies, had no insulin response to arginine infusion, while patient A.H. had no detectable C-peptide immunoreactivity (CPR). Patient J.S., while having basal levels of CPR in the normal range, had no response to the arginine infusion.

Subjects were studied at the Clinical Research Center of the University of Washington Hospital and in the U.S. Public Health Service Hospital after informed consent was obtained. All studies, except the insulin infusion and withdrawal, were performed after an overnight fast with the subjects having taken their last dose of intermediate-acting insulin 24 hours previously. Nineteen-gauge scalp vein needles were inserted into antecubital veins of one or both arms and were kept patent with an infusion of 0.9 per cent saline. All studies include at least a one-hour control period.

Oral glucose tolerance tests (OGTT) were performed by ingesting 100 gm. of glucose in three minutes. Arginine infusions consisted of 30 gm. of arginine hydrochloride given by constant rate, intravenous infusion over 30 minutes. An insulin tolerance test (ITT) was performed in one patient, with 26 U. of regular insulin given as a bolus injection. Linear somatostatin (SRIF) was given as a constant infusion of 500 μg. per hour for three hours. In two subjects, their intermediate-acting insulin was withheld for one day, and their blood sugar was controlled by regular insulin administered subcutaneously before each meal. At 10 p.m., an infusion of monocomponent regular insulin of about 1 U. per hour was begun and continued until the plasma glucose was roughly 100 mg. per deciliter. At this time the insulin was discontinued and the patients were allowed to become hyperglycemic. In one subject, an infusion of monocomponent regular insulin (0.4 U. per kilogram per hour) along with glucose (0.6 gm. per kilogram per hour) (to prevent hypoglycemia) was given for two hours. Insulin was then discontinued and the patient was allowed to go into mild ketoacidosis while receiving hourly intravenous replacement of calculated fluid and salt losses, with monitoring of IRG, plasma glucose, beta-hydroxybutyrate, serum bicarbonate, and urinary epinephrine.

Blood samples were collected in heparinized tubes; the tubes for IRG and glucagon-like immunoreactivity (GLI) also contained benzamidine at a final concentration of 0.05 M.[13] All tubes were kept on ice until separation by centrifugation, then at $-20°$ C. until assayed. Urine collections were titrated to pH $<$ 3.0 and then kept at $-20°$ C. until assayed for catecholamines. Plasma and blood glucose were measured by the glucose oxidase method, while IRG, GLI (antiserum 78J), immunoreactive insulin (IRI), CPR,[14] growth hormone, and cortisol were measured by radioimmunoassay. Plasma-free insulin was determined after polyethylene glycol extraction.[15] In our IRG assay (antiserum 30K), plasma was treated with acetone, which extracts 75 per cent of 3,500-dalton IRG, excludes 160,000-dalton IRG, and co-extracts $<$10 per cent of 9,000- to 20,000-dalton IRG and $<$2 per cent of peak-I GLI.[13,16] All the molecular weight (Mol. wt.) glucagon species are measured in untreated plasma, while predominantly 3,500-dalton IRG is assayed in extracted plasma. Unless otherwise stated, IRG refers to values for extracted plasma.

RESULTS

Each Px subject had fasting plasma IRG concentrations within our normal range (table 1). Mean basal IRG values in the Px subjects were 46 $\pm$ 9 pg. per milliliter (mean $\pm$ S.D.) and 113 $\pm$ 20 pg. per milliliter for extracted and unextracted plasma, respectively, as compared with 61 $\pm$ 20 and 130 $\pm$ 46 pg. per milliliter, respectively, in 23 normal controls.

TABLE 1

Data on the pancreatectomized subjects studied

PATIENT	AGE	SEX	REASON FOR PANCREATECTOMY AND DATE	FASTING PLASMA IRG pg/ml	
				UNEXTRACTED	EXTRACTED
A.H.	30	M	INSULINOMA - 1968	184	45
R.B.	47	M	ADENOCARCINOMA - 1976	70	35
+ E.D.	47	F	CHRONIC PANCREATITIS - 1974	72	49
+ J.S.	56	F	ADENOCARCINOMA - 1973	189	60
+ M.C.	59	F	ADENOCARCINOMA - 1972	48	39
NORMALS (N=23)				130 ± 46 (S.D.)	61 ± 20 (S.D.)

+ DECEASED

Oral glucose tolerance test (OGTT). Two subjects (A.H. and R.B.) received an OGTT (figure 1). Both subjects showed a rise in IRG, most prominent at 30 to 60 minutes. Simultaneous GLI levels measured in both subjects also showed a rise at 30 to 60 minutes, but there was no consistent percentage relationship between the GLI and IRG values.

Arginine infusion. As previously reported,[17] arginine infusions in two subjects (A.H. and J.S.) showed a rise in IRG, while in one subject (E.D.) there was no response (figure 2). A repeat arginine infusion a year later in patient A.H. again showed the rise in IRG. Another Px subject (R.B.) was studied twice with arginine infusion, once when his fasting plasma glucose was 118 mg. per deciliter, and on another occasion when his previous day's insulin dose had been decreased and his fasting plasma glucose was 348 mg. per deciliter. This was done to evaluate the possibility that residual exogenous insulin activity could be suppressing the extrapancreatic alpha cells' responsiveness to arginine. Arginine infusion failed to elicit a rise in IRG on either day.

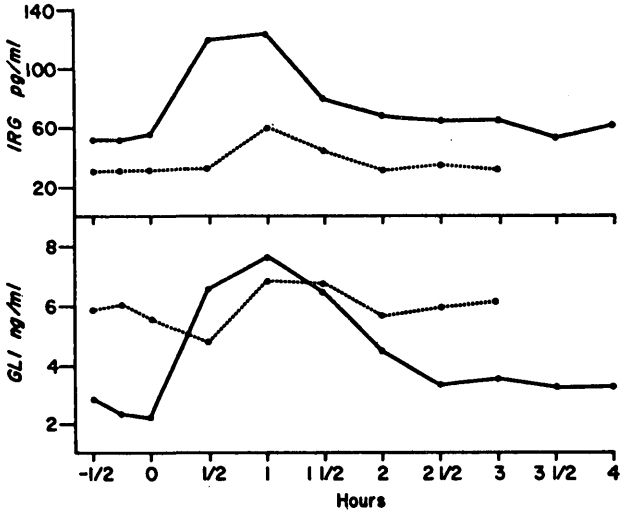

FIG. 1. IRG and GLI responses during oral glucose tolerance test (100 gm. at time 0) in subjects A.H. (——) and R.B. (----).

Insulin tolerance test (ITT). One subject (A.H.) received a bolus injection of 26 U. of regular insulin, with a fall in blood glucose from 260 to 25 mg. per deciliter and resultant symptomatic hypoglycemia (table 2). This was accompanied by a rise in growth hormone, cortisol, and urinary epinephrine and norepinephrine, further indicating that hypothalamic glucopenia had been achieved. IRG concentrations made no significant change during the ITT.

SRIF infusion. During the SRIF infusion (figure 3), both subjects showed a definite fall in plasma glucose: in patient A.H. from 216 to 138 mg. per deciliter and in patient R.B. from 169 to 128 mg. per deciliter. IRG and GLI values showed no change in either subject.

Insulin infusion and withdrawal. During the insulin infusion (figure 4), plasma glucose fell from 282 to 107 mg. per deciliter in patient A.H. and from 232 to 75 mg. per deciliter in R.B. During the subsequent period of insulin withdrawal, plasma glucose rose to 344 mg. per deciliter in patient A.H. and to 174 mg. per deciliter in R.B. No significant changes in IRG concentrations occurred in either subject dur-

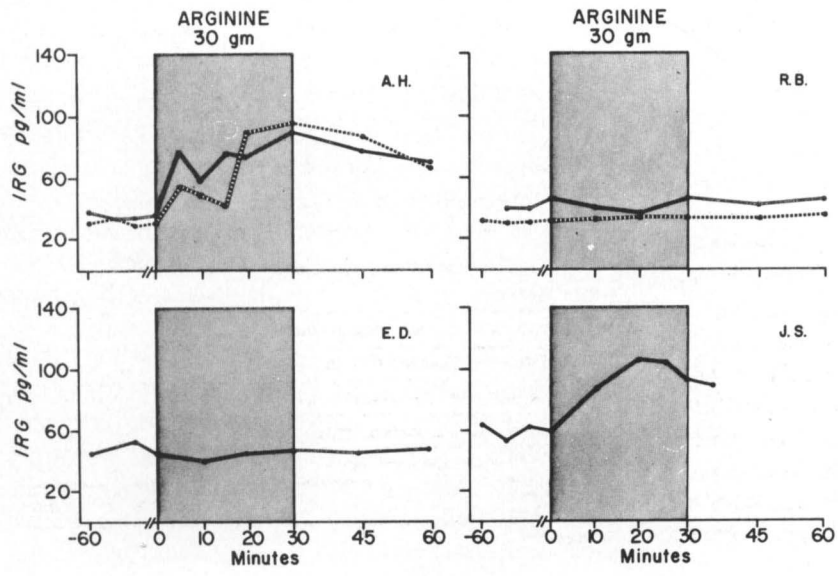

FIGURE 2

IRG response to arginine infusions in four patients, designated by initials. Subjects A.H. and R.B. were studied on two separate occasions (—— and ----) (see RESULTS for details).

TABLE 2

Responses of blood glucose, growth hormone, cortisol, IRG, and urinary norepinephrine and epinephrine to the insulin tolerance test (26 U. at time 0) in patient A.H.

Minutes	Glucose (mg./dl.)	Growth hormone (ng./ml.)	Cortisol (μg./dl.)	Norepinephrine (ng./min.)	Epinephrine (ng./min.)	IRG (pg./ml.)
0	260	11	12.5	96	21	53
10	188					48
20	135					43
30	112					46
40	86					48
50	60			208	266	51
60	35					51
75	25					48
90	38					48
105	43		26			51
120	39	>50				43

ing either the insulin infusion or withdrawal period. GLI values fluctuated throughout the study without any consistent relationship to the concentration of plasma glucose or IRG.

One subject (A.H.) received an infusion of regular insulin at a rate of 0.4 U. per kilogram per hour for two hours and a simultaneous infusion of glucose at 0.6 gm. per kilogram per hour without a fall in IRG (figure 5). Insulin and glucose were then discontinued, and fluid and salt losses were replaced hourly by vein. After about 15 hours the patient was in mild ketoacidosis with a serum bicarbonate of 13 mEq. per liter, a beta-hydroxybutyrate concentration of >3 μM per milliliter and a plasma glucose of 354 mg. per deciliter. Urinary epinephrine values did not rise during withdrawal of insulin. IRG showed a transient rise after the patient was inadvertently served lunch and then returned to essentially basal values, except for a slight increase of 20 pg. per milliliter at the end of the period of insulin withdrawal.

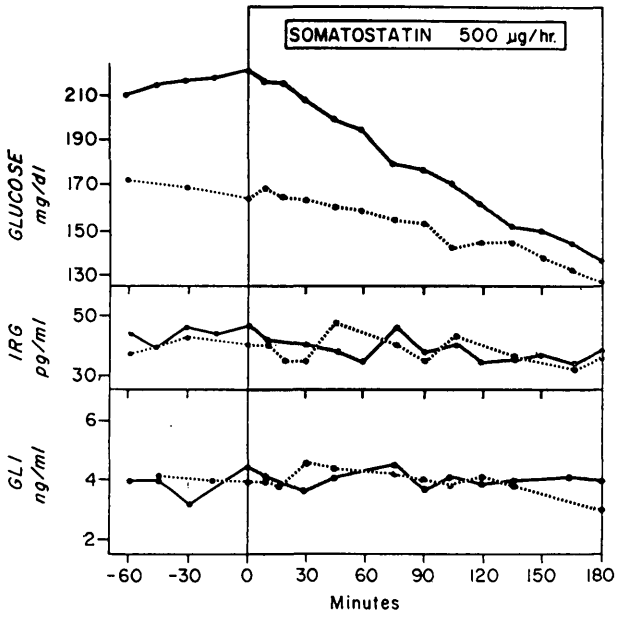

FIG. 3. Glucose, IRG, and GLI responses to SRIF infusion in subjects A.H. (——) and R.B. (----).

DISCUSSION

The normal basal levels of IRG we found in Px man are in agreement with four earlier studies.[9-11,18] However, in the study by Miyata, Yamamoyo, and associates,[10] IRG disappeared from plasma on day 9 after pancreatectomy. The subjects in our group were studied from one week to six years after their surgery without significantly different basal concentrations of glucagon. Four IRG fractions—2,000 dalton; 3,500 dalton; 9,000 dalton; and 160,000 dalton—have been described in the plasma of Px dogs.[19] In the study by Villanueva, Hedo, and Marco,[18] gel filtration of their subject's plasma revealed IRG in the 160,000- and 2,000-dalton fractions but not in the 3,500-dalton area. Chromatography of the plasma from the Px subject studied by Botha, Vinik, and colleagues[11] revealed IRG of two fractions, one of large molecular weight and one in the range of 3,500-dalton IRG. Our attempt to distinguish which molecular weight glucagon species accounts for the IRG in our Px subjects' plasma did not give reliable results. We have shown that our acetone extraction procedure removes species of IRG with molecular weight greater than 9,000 daltons,[16,17] thus eliminating the possibility that we are measuring large molecular weight or big plasma glucagon. Conceivably, our subjects' extracted IRG could have been partly 2,000-dalton glucagon.

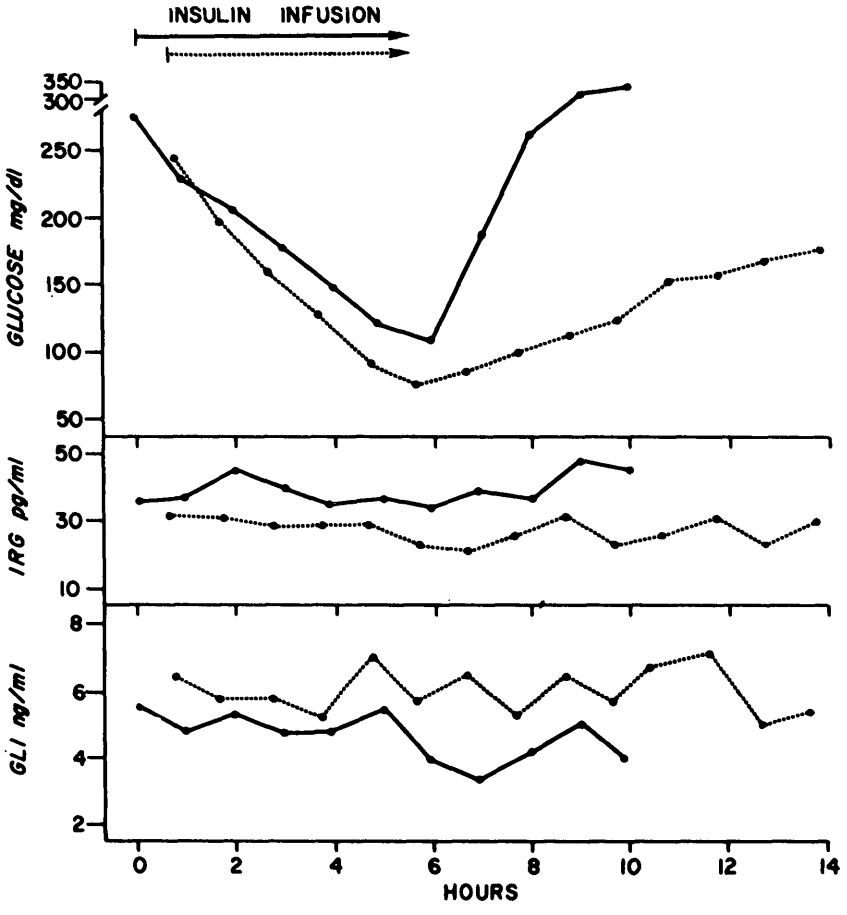

FIGURE 4

Glucose, IRG, and GLI responses to insulin infusion (roughly 1 U. per hour) and withdrawal in subjects A.H. (——) and R.B. (----).

There are at least two possible mechanisms for the differences between the basal IRG values seen in our Px subjects as well as in those of other workers employing antiserum 30K[9-11,18] and those reported by Barnes and Bloom.[12] First, the latter authors used a different antiserum, which may selectively recognize different molecular weight species of glucagon than antiserum 30K. Second, in the same study, each patient's standard curve was run with glucagon-free plasma, which is plasma that was treated with their glucagon immunoabsorbent. This may have created an arbitrarily zero standard, since their immunoabsorbent was standardized with only 3,500-dalton IRG. Their glucagon-free plasma may still contain IRG, which would be measured by antiserum 30K.

The IRG rise in response to oral glucose seen in two of our subjects may be similar to the paradoxic IRG response to oral glucose seen in some diabetic subjects.[20] Interestingly, a similar response was seen in the subject studied by Botha, Vinik, et al.[11] Both subjects in our study also had a GLI rise after oral glucose. But, since we were unable to find any constant percentage relationship between GLI and IRG and since less than 2 per cent of peak-I GLI is measured as IRG in our assay, it is unlikely that the IRG rise was merely cross reactivity between GLI and antiserum 30K.

Failure of IRG to rise after arginine in two of the four Px subjects may have been related to suppression of IRG by exogenous insulin or, possibly, to more extensive surgery, including duodenectomy and partial gastrectomy, which had removed the majority of the source of extrapancreatic IRG. The failure of IRG to rise with arginine in patient R.B., who was studied both in the presence and relative absence of exogenous insulin, is evidence against the possibility that residual insulin activity prevented a potential IRG rise in the two subjects who showed no response to arginine. The Px subject of Botha, Vinik, et al.[11] apparently had only a cuff of duodenum removed, yet had no IRG response to arginine. It is possible that the cells producing IRG were already secreting at maximum capacity and could not respond further to arginine in these subjects.

The studies with SRIF are unique in that they demonstrated not only the failure of IRG to be suppressed

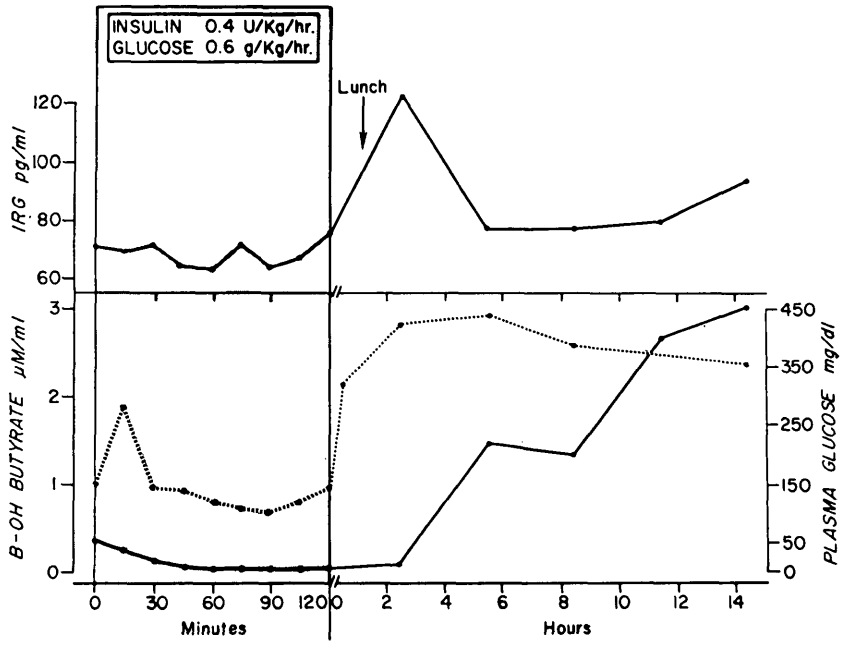

FIGURE 5

IRG, beta-hydroxybutyrate (——), and glucose (----) responses to insulin-glucose infusion and insulin deprivation in subject A.H. Note change in time scale after insulin-glucose infusion.

by SRIF but also a dissociation between IRG suppression and plasma glucose levels after SRIF. The extrapancreatic IRG in the Px dog model was suppressed by SRIF.[21] IRG not being suppressed by SRIF was demonstrated by Hetenyi, Kovacevic, and colleagues[22] in the puppy. The Px subject studied by Botha, Vinik, and co-workers[11] also failed to show suppression of IRG with SRIF, but there was no fall in the plasma glucose. The fall in plasma glucose caused by SRIF in studies in vivo has been interpreted as secondary to suppression of IRG.[23,24] Two studies in vitro[25,26] have demonstrated a direct inhibitory effect of SRIF on hepatic glucose production, although the SRIF concentrations in those studies were probably 10 to 100 times greater than the concentrations achieved in patients A.H. and R.B. Also, other studies in vitro have failed to demonstrate such an effect.[27-29] Thus, the fall in plasma glucose without a change in IRG seen in our two subjects could possibly be secondary to a direct hepatic effect of SRIF. Our results would not implicate an SRIF-induced fall in GLI as causing the fall in plasma glucose. Another possible mechanism for the decline in plasma glucose could be the decreased splanchnic blood flow observed with SRIF infusion.[30] Both subjects were studied 24 hours after their last dose of intermediate-acting insulin. It is unlikely that their progressive fall in plasma glucose over a three-hour period was secondary to the residual effect of exogenous insulin, since both subjects became hyperglycemic with insulin deprivation on another day. Thus, SRIF appears to have lowered plasma glucose, at least in these two subjects, by a mechanism other than suppression of IRG. Three other Px subjects[11,31,32] reported to have received SRIF infusions failed to show suppression of plasma glucose. It is clear that further studies are needed in Px patients.

The failure of IRG to be suppressed by insulin and the insulin-glucose infusion is also atypical for both pancreatic and extrapancreatic glucagon.[33-35] Of equal interest was the observation that endogenous hyperglycemia occurred during insulin withdrawal in both subjects without a discernible rise in IRG. The insulin-glucagon molar ratio plus many other factors regulate hepatic glucose metabolism, with hepatic glucose production unlikely at ratios greater than 10.[36,37] Using free IRI and extracted IRG values, we calculated the IRI-IRG molar ratios during the period of insulin withdrawal. With the fall in IRI during insulin withdrawal, there was a fall in the IRI-IRG molar ratio from a peak of 22.5 to 7.3 in subject R.B. and from a peak of 10.3 to 3.1 in A.H., both changes in favor of increased hepatic glucose production. In fact the lower IRI-IRG molar ratio in A.H. than in R.B. was associated with a more rapid rise in plasma glucose concentration (figure 4). In addition, portal vein IRG concentrations may have risen with consequent hepatic effects but not to a degree sufficient to result in detectable changes in peripheral IRG values. Also the glycogenolytic action of GLI may have contributed to the increased hepatic glucose output.[38] Endogenous hyperglycemia is a function of both he-

patic glucose production and glucose utilization; the latter was decreasing after the insulin was discontinued and thus contributed to the resulting hyperglycemia.

During an extended period of insulin withdrawal, patient A.H. developed ketoacidosis. Although IRG showed a transient rise early in the course of insulin withdrawal after he ate a meal, the IRG concentration returned to essentially basal values, except for a minimal rise of 20 pg. per milliliter at the end of the period of insulin withdrawal. These results are consistent with recent work describing the development of ketoacidosis in Px man without a rise in glucagon.[39] But, the basal IRG values during insulin deficiency may contribute to the ketogenic capacity of the liver.

The nature and origin of the IRG we measured in this group of Px subjects is uncertain at the present time. As mentioned previously, we feel confident that extracted IRG is not large molecular weight IRG. Several studies[40-42] have shown failure of IRG to be suppressed to zero after SRIF infusion. This nonsuppressible IRG may be similar to the IRG we measured in Px man. As regards the origin of this IRG, two recent studies in man provide support for an extrapancreatic source of IRG. Lawrence, Kirsteins, and coworkers described IRG in the salivary gland of man,[43] although chromatography has shown this IRG to be of Mol. wt. 29,000 daltons,[44] and immunocytochemical evidence of glucagon-containing cells in the human stomach has been reported.[45]

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