Secretion and Content of Insulin and Glucagon in Human Fetal Pancreas Slices *in Vitro*[1] (37310)

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Although there is evidence indicating when islets of Langerhans and insulin appear in the fetal pancreas (1–4), relatively little is known about the synthesis and release of insulin and glucagon in human fetal pancreas, and their responsiveness to various biochemical stimuli. In the present investigations, comparisons of the following hormone levels were made: (a) total extractable insulin and glucagon in the pancreas at different fetal ages, including ascertaining the time for the first appearance of these hormones, and (b) the effect of arginine, glucose, and glucagon on their secretion.

Materials and Methods. Fresh pancreas from twenty human fetuses (aged 25–138 days) was obtained immediately after therapeutic abortions, performed either by hysterotomy or curettage. Fetal age was determined by crown-rump and foot-length measurements. The pancreas was weighed, minced, divided into three aliquots, and added to tubes containing synthetic interstitial fluid bicarbonate buffer (pH 7.4) with 2% BSA (5). Additions were made so that the final volume of each was 2.5 ml, and one tube contained 50 mg/100 ml glucose, another 300 mg/100 ml glucose, and the third 5 m*M* arginine. All tubes were gassed continuously with 95% O_2 and 5% CO_2 and incubated for 60 min at 37° in a Dubnoff metabolic shaker. At appropriate time intervals, 0.1-ml aliquots of incubation medium were quickly frozen in a dry ice–ethanol mixture and stored at −20° until assayed for proinsulin, insulin, and glucagon. Immunoreactive insulin (IRI) and proinsulin were determined by the method of Zaharko and Beck (6), and glucagon (IRG) by the method of Hazzard *et al.* (7). The results are expressed as units of hormone per milligram wet-weight pancreas.

Immediately after incubation, the pancreatic preparations were combined and homogenized in 70% acetone. The acetone homogenate was centrifuged and decanted. The supernatant was evaporated via a rotary evaporator and reconstituted in 0.05 *M* veronal buffer, pH 8.6, containing 0.25% BSA. Aliquots were assayed for total IRI and IRG. Also, aliquots of extracts from six pancreases were chromatographed on a 1 × 50 cm G-50 fine Sephadex column, equilibrated and eluted in 1 ml fractions at approximately 15 ml per hour at 4° using the veronal buffer above. ^{125}I-insulin and ^{125}I-glucagon and native hormones were used as markers to determine the respective positions of insulin and glucagon. Porcine proinsulin standards and human insulin and glucagon standards were used in their respective immunoassays.

A chromatographic fraction which combined with glucagon antibody had an estimated molecular weight about three times that of glucagon. Further characterization of it was performed using essentially the same procedures (*e.g.*, 1 *M* acetic acid rechromatography and partial tryptic digestion) as described by Rigopoulou *et al.* (8, 9). Protein was measured by the Lowry method (10).

Results. Release of IRI and IRG from pancreatic slices was higher during incuba-

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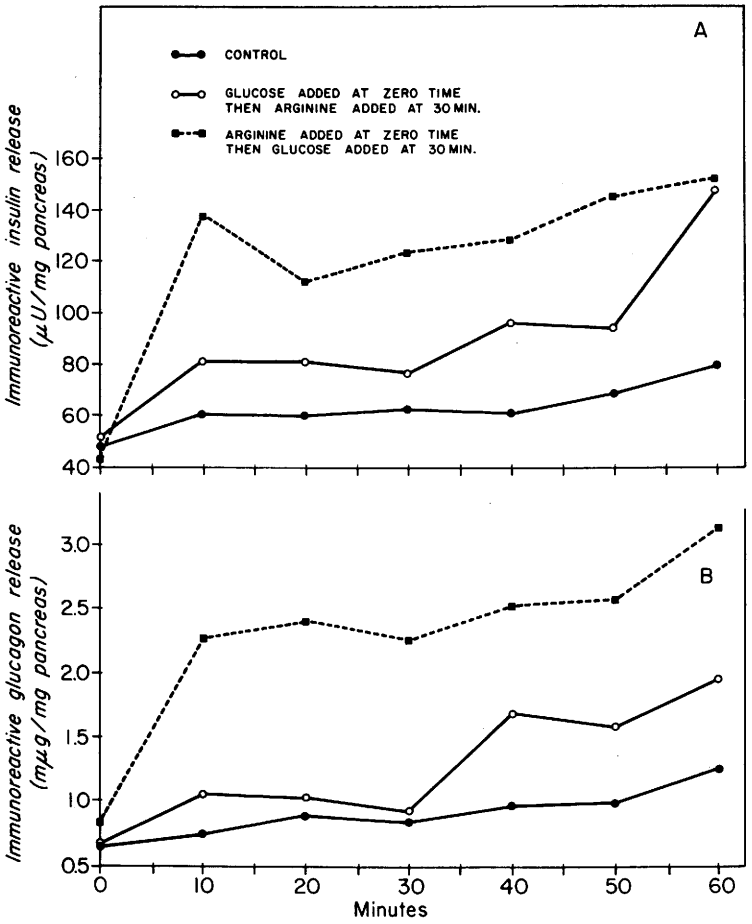

FIG. 1. Secretion profiles of (A) immunoreactive insulin and (B) immunoreactive glucagon obtained from human fetal pancreatic slices *in vitro*. Each point represents the mean of triplicate immunoassays for each time interval shown. Synthetic interstitial fluid buffer (pH 7.4, 50 mg/100 ml glucose, and 2% BSA) was used. As shown in panel A, arginine (5 m*M*) caused significantly increased insulin release whether added before or after glucose. Glucose (300 mg/100 ml) may have increased insulin when added before arginine, but not when added after it. In panel B, arginine markedly increased glucagon release when added before glucose but caused less increase when given after glucose. Glucose did not significantly affect glucagon release.

tion in 5 m*M* arginine than in 300 mg/100 ml glucose (final concentration), as illustrated in Fig. 1. Arginine, whether present initially or added at 30 min, caused a significant release of insulin into the incubation medium; insulin secretion was slightly increased over controls when 300 mg/100 ml glucose was present initially (Fig. 1A).

Arginine produced comparable increments of glucagon and insulin release (Fig. 1); it produced a twofold increase in glucagon release during the first 10 min of incubation. When arginine was added after glucose, a onefold increase in IRG resulted. Glucose alone resulted in slight increases of IRG when compared to the control profile. Increases in IRI release occurred after glucose or glucagon, but a much greater release was produced by arginine (Fig. 2).

Increasing amounts of insulin and glucagon in the pancreas were associated with increasing fetal age (Table I). These hormones increased in parallel manner ($r = 0.96$, $p < 0.001$). Whereas neither hormone was present

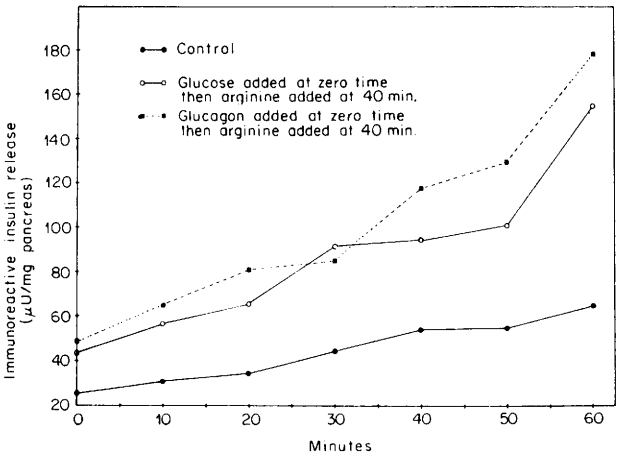

FIG. 2. Secretion profiles of immunoreactive insulin obtained from human fetal pancreatic slices *in vitro* when challenged by glucose (300 mg/100 ml), arginine (5 m*M*), and glucagon (0.05 mg/100 ml) are shown. Only a small increase in insulin release followed glucagon or glucose, but a greater increment occurred when arginine was subsequently given. Each point is the mean of triplicate assays.

TABLE I. Total Insulin and Glucagon in Human Fetal Pancreases.

Gestational age (days)	Insulin (μU/mg)	Glucagon (ng/mg)
25	0	0
47	543[a]	4.0
50	512	2.3
53	321	3.7
65	150	10.4
69	432	2.7
72	320	3.7
74	105	0.5
78	490	10.7
84	2180	13.8
85	790	3.1
87	1760	19.9
88	2780	14.2
90	1085	11.9
108	1680	30.9
112	2890	51.9
115	4640	76.3
130	8390	121.6
138	3260	49.4
	Mean	
47– 69 (7)	333 ± 64[b]	4.0 ± 1.2[b]
72– 90 (6)	1189 ± 359	9.7 ± 2.2
108–138 (5)	4172 ± 1159	66.0 ± 15.6

[a] Values are the mean of duplicate assays of 70% acetone extract.

[b] The values of IRI and IRG are the mean (± SEM) for each group of fetuses shown.

in the pancreas of a fetus aged 25 days, both were present in the next age group tested. Table I shows the amount of pancreatic insulin and glucagon in fetuses of three age groups. Although each hormone was present in significant levels in the 47–69 day group, there were appreciable increases in the 72–90 day group and marked increases in those aged 108–138 days.

Sephadex G-50f elution profiles of acetone extractable insulin, proinsulin and glucagon of a fetus aged 130 days are shown in Fig. 3; these results are representative for fetal ages 70–130 days. The elution profile of IRG displayed two distinctive peaks. Approximately 80% of the material reacting with glucagon antibody appeared in the larger peak (peak II) that cochromatographed with ^{125}I-glucagon and native glucagon markers (3500 MW); the remaining 20% of IRG appeared in a smaller peak (peak I). IRG-peak I eluted well ahead of the ^{125}I-insulin (6000 MW), and therefore represents a molecular weight greater than insulin but less than proinsulin. To eliminate the possibility that IRG-peak I represents aggregates of glucagon molecules, IRG-peak I fractions were concentrated and rechromatographed using 1 *M* acetic acid for reconstitution, equilibration, and elution. The peak was still

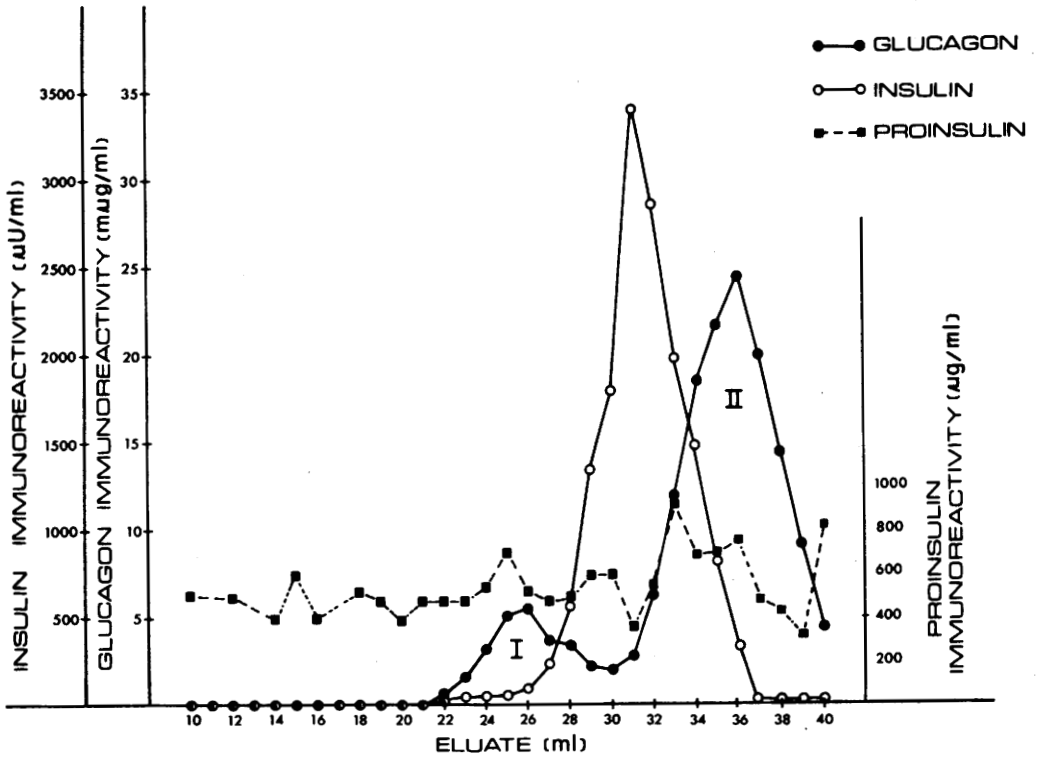

FIG. 3. Typical Sephadex G-50f chromatographic elution profiles of immunoreactive insulin, glucagon, and proinsulin from a normal human fetal pancreatic extract. The glucagon immunoreactivity elution pattern shows a peak for a component of molecular weight (IRG-peak I) larger than glucagon. Veronal buffer (0.05 *M*, pH 8.6, 0.25% BSA and merthiolate) was used for equilibration and elution. Each point represents the mean of duplicate values. Results shown are representative of eight fetal pancreases tested.

present after this treatment. Figure 4 shows that following mild tryptic digestion the peak on rechromatography of the original IRG-peak I fraction peaked about 3 ml after the original nontrypsinized material, indicating a slight reduction in molecular size.

Discussion. Experiments for this study were designed to test the *in vitro* effects of some known stimulators on insulin and glucagon secretion from human fetal pancreas slices (11–13), and to determine levels of extractable IRI and IRG in fetuses of different ages. Arginine (5 m*M*) produced much more release of each hormone than either glucose (300 mg/100 ml) or glucagon (0.05 mg/100 ml). Others have observed that arginine is a potent stimulator of insulin and glucagon secretion (14). In our studies and in those by Monteros *et al.* (15) using isolated human fetal islets, 300 mg/100 ml glucose only slightly increased insulin secretion. This lack of significant increase in insulin secretion by glucose in fetal pancreases has been observed by others (2, 12, 16). However, in contrast to Monteros *et al.* (15) we did not observe a significant increase in IRI when the pancreas was challenged with glucagon. Figures 1 and 2 show that arginine added at 30 min resulted in significantly less increase in IRI and IRG release than when it was initially present. Possibly this is related to increased rates of proteolysis in the pancreatic preparations.

We found a poor correlation in the rate of insulin and glucagon release with fetal age. This was doubtless due to sample variability such as differences in size of pancreatic pieces, interval post-abortion, and abortion

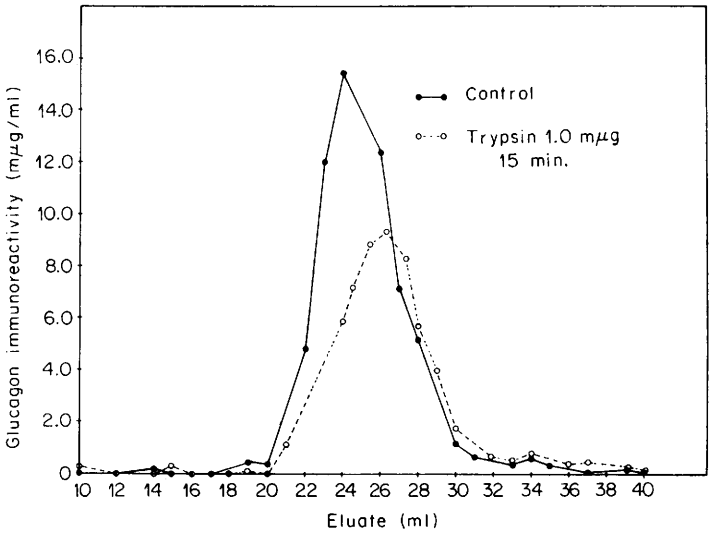

FIG. 4. Elution profiles of glucagon immunoreactivity before and after partial tryptic digestion of the higher molecular weight fraction obtained from Sephadex G-50f (50 × 1 cm) column. Buffer is 0.05 *M* veronal, pH 8.6, with 0.25% BSA. Each point represents the mean of duplicate values.

method. However, the specimens were satisfactory for measuring the effect of potent stimuli. The highly significant correlation between total amounts of extractable IRI and IRG (Table I) lends support to the "bifunctional unit" hypothesis for the control of insulin and glucagon secretion proposed by Unger (17, 18).

The chromatographic studies demonstrate a glucagon antibody reacting fraction with a molecular weight greater than glucagon (but less than proinsulin marker), which continues to react with glucagon antibody after its molecular weight is reduced by partial tryptic digestion. These results are in keeping with those reported by Rigopoulou *et al.* (8, 9) and Noe and Bauer (19, 20). We are determining whether peak I and its tryptic product have biologic activity like glucagon.

Summary. Total quantities of immunoreactive insulin and glucagon in human fetal pancreases (aged 25–135 days) were determined. Small amounts of these hormones were demonstrable by the 50th day, larger amounts by the 90th day and relatively large quantities by the 110th day. Arginine (5 m*M*) produced a very significant increase in the secretion of each hormone from slices of pancreas, while glucose (300 mg/100 ml) did not affect glucagon release and had relatively little effect upon insulin release. Using Sephadex gel filtration, we have found a fraction with a molecular weight three times that of glucagon which combines with glucagon antibody. Studies are being performed to determine if this larger molecular compound contains glucagon.

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