dieldrin, endrin, and heptachlor residues by vaporization from soil surfaces as well as from root uptake and translocation. The major source of DDT contamination is vaporization from the soil. Although earlier researchers have shown that dieldrin concentration in leaves was 50 times higher from field-grown corn than from greenhouse-grown corn protected from aerial contamination (*5*), they did not demonstrate that this large difference was the result of dieldrin vaporizing from the soil.

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Insulin Release from Isolated Human Fetal Pancreatic Islets

Abstract. *Pancreases were obtained from five human fetuses 12 to 16 weeks old. The islets of Langerhans were isolated with collagenase, and then incubated with buffer, glucose, tolbutamide, or glucagon added to the medium. The insulin released into the medium was measured by immunoassay. Glucagon produced the only significant increase above base line; glucose and tolbutamide failed to enhance secretion of insulin. The data suggest that isolated human fetal islets of this gestational age develop responsiveness to glucagon earlier than to glucose or tolbutamide.*

In the human fetus the islets of Langerhans can be detected by the 12th week of pregnancy; pancreatic insulin can be demonstrated either by acid-ethanol extraction or with fluorescent antibody (*1*, *2*). Circulating insulin has been measured as early as 12 weeks (*2*, *3*). To our knowledge, the responsiveness of the isolated human fetal pancreatic islets to various stimuli has not been studied, although the newborn has been investigated intensively. We now report studies on isolated human fetal islets challenged with glucose, tolbutamide, or glucagon.

Therapeutic abortion was performed on five nondiabetic women; gestational age was estimated by fetal measurement and ranged from 12 to 16 weeks. The fetal pancreas was placed in cold Hanks solution (*p*H 7.4) and transported to the laboratory. The interval between completion of the abortion and initiation of incubation with collagenase was less than 20 minutes. Islets were isolated as described by Lacy and Kostianovsky (*4*) with the following modifications: 15 mg of collagenase (5) per pancreas was used, and the incubation time necessary to obtain adequate digestion was extended to 40 minutes. The islets could easily be recognized under the dissecting microscope. They were cleaned and removed from exocrine tissue with two needles and transferred with a 100 μl pipette into small tubes containing Krebs-Ringer bicarbonate (KRB) buffer with 2 percent bovine serum albumin. The *p*H was adjusted to 7.4 with 1*N* NaOH. Additions to the KRB buffer were as follows: glucose (3 mg/ml), sodium tolbutamide (0.1 mg/ml), and glucagon (0.005 mg/ml).

Each pancreas supplied a minimum of 16 islets. Because of the very limited number, we placed only two islets in each incubation flask; however, all incubations were performed in duplicate and are reported as the mean of these duplicate determinations. Each experiment contained four groups: buffer alone; buffer and glucose; buffer and tolbutamide; and buffer and glucagon. The final volume was 1 ml per flask. All flasks were incubated at 37°C in a Dubnoff metabolic shaker for 90 minutes under continuous gassing with 95 percent O_2 and 5 percent CO_2. Afterward the islets were allowed to settle for 20 minutes at room temperature. Two 0.1-ml aliquots were then removed for insulin determination in duplicate by a double antibody immunoassay (*6*) with human insulin used as a standard. To assess any possible contamination of glucagon with insulin, we also incubated control tubes, without islets, from each experimental group. In addition, a recovery study was performed by adding human insulin to glucagon which was of bovine-porcine origin.

The recovery of human insulin added to glucagon was adequate (data not shown). Immunological analysis of the control glucagon samples revealed insulin contamination; 5 μg of glucagon contained between 5 to 15 μU of immunoreactive insulin (IRI) per milliliter; therefore, 15 μU of IRI were subtracted when glucagon was present in the incubation medium. The mean basal insulin secretion was 41 ± 9 μU/ml (Table 1). The addition of glucose (3 mg/ml) induced no significant change from basal insulin secretion, although three out of five exhibited slightly higher values. The mean insulin secretion was 45 ± 8 μU/ml. Tolbutamide also failed

Table 1. Insulin release by isolated human fetal pancreatic islets. Each value represents the mean of two experiments. Within parentheses are individual values. Results are expressed as microunits of immunoreactive insulin (IRI) per milliliter released into the incubation medium. The values for glucagon have been corrected for possible insulin contamination. The mean and standard error of the mean for each group are: buffer, 41 ± 9 μU/ml; glucose, 45 ± 8 μU/ml; tolbutamide 35 ± 9 μU/ml; glucagon, 70 ± 12 μU/ml.

Fetus No.	Fetal age (weeks)	Buffer	IRI released (μU/ml)		
			Glucose (3 mg/ml)	Tolbutamide (0.1 mg/ml)	Glucagon (0.005 mg/ml)
1	12	30 (45, 15)	30 (30, 30)	40 (45, 35)	55 (50, 60)
2	14	50 (40, 60)	60 (50, 70)	20 (15, 25)	75 (75, 75)
3	15	35 (25, 45)	45 (40, 50)	20 (15, 25)	60 (55, 65)
4	16	70 (65, 75)	60 (60, 60)	70 (65, 75)	115 (100, 130)
5	16	20 (20, 20)	30 (20, 40)	25 (20, 30)	45 (40, 50)

to enhance insulin release; mean secretion was 35 ± 9 μU/ml. However, glucagon promoted a significant increase in insulin secretion (70 ± 12 μU/ml), even when we allowed for maximum possible contamination of the glucagon with insulin. The results of analysis of variance and Duncan's new multiple range test indicated that insulin output from the group with glucagon was significantly greater than that of the other groups ($P < .01$).

The supply of viable fetal pancreatic tissue is extremely limited, and thus the number of experiments performed is by necessity small. Our results, although limited in number, indicate that isolated pancreatic islets, obtained from human fetuses of an early gestational age, do not respond to either glucose or tolbutamide. This observation could lead one to doubt the viability of the preparations, were it not for the significant insulin release promoted by glucagon.

Milner and Wright (*7*) were among the first to report that in human newborns intravenous administration of glucagon directly stimulated insulin release. Similar observations that glucagon in the absence of glucose can stimulate insulin secretion had been made previously with rat pancreatic slices (*8*) and with isolated perfused rat pancreas (*9*), as recently reviewed by Mayhew *et al.* (*10*). Therefore, our observation that glucagon directly stimulates pancreatic insulin release is not an isolated finding. It is interesting, however, that glucagon did promote insulin secretion when islets obtained from the same pancreas failed to respond to either glucose or tolbutamide. Failure to observe glucose-induced insulin release in vivo has been reported for the fetus of the sheep (*11*) and the subhuman primate (*12*), and for pancreatic slices from the fetal rat (*13*) and 24-day-old rabbit fetus (*14*). On the other hand, Correa *et al.* reported that pancreatic slices taken from fetal calves less than 5 months old responded to glucose (*15*).

The lack of response to glucose by the fetal pancreas apparently disappears during or shortly after delivery (*16*), although it may persist in the premature infant (*17*). In the newborn a definite, although occasional, sluggish insulin response to glucose has been well documented (*16–18*). The age spectrum of pancreatic response to glucagon contrasts interestingly with that to glucose and tolbutamide; and, as shown here in human fetal islets preparations, glucagon alone possesses the capacity to promote insulin release during critical stages of fetal development.

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Inhibition of Cytotoxicity of Lymphocytes by Concanavalin A in vitro

Abstract. *Human lymphocytes treated with the plant protein concanavalin A are stimulated to transform into blasts, without developing cytotoxicity for chicken erythrocytes. Prior treatment of lymphocytes with concanavalin A potentiated phytohemagglutinin-induced blast transformation and DNA synthesis but completely inhibited phytohemagglutinin-induced cytotoxicity. Inhibiton was not due to suppression of the mixed lymphocyte–erythrocyte aggregation normally caused by phytohemagglutinin. Inhibition of cytotoxicity was reversible when concanavalin A was removed from the lymphocytes by treatment with methyl-α-D-mannopyranoside after 1 hour but not after 20 hours. The results indicate that blast transformation and cytotoxicity are separate expressions of lymphocyte stimulation.*

Lymphocytes of human or animal origin are cytotoxic to tissue culture cells or chicken red blood cells (that is, target cells) after stimulation with phytohemagglutinin, antigen, allogenic lymphocytes, or certain antibodies to target cells. The reactions are related to the immunospecific cytotoxicity produced by lymphocytes from donors sensitized to target cell antigens and are believed to constitute models in vitro for cell-mediated tissue damage in vivo (*1*). When cytotoxicity is induced by stimulation of lymphocytes, the strength of the cytotoxic reaction is correlated to the incidence of blast transformation induced by the stimulating agent (*2*). However, cytotoxicity is assumed to be an expression of an energy requiring activation of the lymphocytes rather than of blast transformation per se (*2*, *3*). We report here that treatment of lymphocytes with the plant protein concanavalin A (conA) (*4*) suppresses cytotoxicity but not transformation, providing new evidence for the independence of these phenomena.

Concanavalin A was extracted from commercial jack bean meal (*Canavalia ensiformis*) and purified as described (*5*, *6*). It was dissolved in 0.1*M* phosphate buffer, *p*H 7.9, to give a stock solution of 800 μg/ml. Lymphocytes