

Modulation of Proinsulin Messenger RNA After Partial Pancreatectomy in Rats

Relationships to Glucose Homeostasis

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Abstract

These studies of partial pancreatectomy assess pancreatic proinsulin messenger RNA (mRNA) levels as an index of *in vivo* insulin biosynthesis, and show relationships to glucose homeostasis. Rats were subjected to sham operation, 50% pancreatectomy (Px), or 90% Px, and were examined after 1, 3, or 14 wk. Proinsulin mRNA was measured by dot hybridization to complementary DNA. After 50% Px there was a nearly complete adaptation of proinsulin mRNA. After 90% Px a marked increase of proinsulin mRNA occurred, but it was insufficient and it was not maintained with time. The deficit in insulin production is related to development of hyperglycemia.

Sham-operated controls showed no worsening of fasting or fed blood glucose or of intraperitoneal glucose tolerance within the period of observation. Total proinsulin mRNA and pancreatic insulin content rose in proportion to body weight.

50% Px produced no change from controls in body weight or blood glucose. The concentration of proinsulin mRNA in the 50% pancreatic remnant paralleled that of controls after 1 and 3 wk, but then increased after 14 wk, such that total proinsulin mRNA approached control levels. This adaptive response was reflected by changes in serum insulin, but not by pancreatic insulin content, which was only 30% of control after 14 wk. Intraperitoneal glucose tolerance was impaired mildly, and did not worsen with time after pancreatectomy.

90% Px led to elevated fed blood glucose and reduced serum insulin after 3 wk, and fasting hyperglycemia was seen after 14 wk. Proinsulin mRNA concentration in the 10% pancreatic remnant showed an adaptive increase after 1 and 3 wk, such that total proinsulin mRNA reached 40% of control. After 14 wk, however, remnant proinsulin mRNA concentration was no longer increased; total proinsulin mRNA and pancreatic insulin content were severely reduced. Intraperitoneal glucose tolerance was impaired more dramatically than with the 50% Px animals, and worsened with time after operation.

These observations indicate ability to increase proinsulin mRNA levels as an adaptation to pancreatectomy. Insufficiency of this adaptation is associated with the development of hyperglycemia, and the loss of this adaptation correlates with a worsening of glucose tolerance.

Introduction

Diabetes mellitus represents a heterogeneous group of disorders characterized by hyperglycemia, and by varying degrees of

apparent insulin insufficiency. Despite an appreciation of the importance of insulin production, there has been no adequate quantitative assessment of capacity to produce insulin in humans. In insulin-dependent diabetes mellitus a deficit in insulin production has been established by the loss of endogenous insulin secretion (1, 2), and by a paucity of B cells in pancreas at autopsy (3–6). In noninsulin-dependent diabetes mellitus the deficit of insulin production is less well characterized. Reductions of B cell number (6, 7) and of extractable pancreatic insulin (8, 9) have been observed at autopsy, although these findings have not been consistent (3–5) and their functional significance is unknown. Insulin resistance has been shown to be important in increasing the metabolic demands for insulin, but pathogenetic relationships between insulin sensitivity and capacity to produce insulin remain unclear (10). Estimation of ability to produce insulin has been inferred largely from tests of insulin secretion; ultimately, a lack of ability to secrete insulin in quantities sufficient to meet metabolic demands is evident.

The importance of a quantitative assessment of insulin biosynthesis has led to numerous studies in experimental animals. In isolated pancreatic islets, measurements of isotope incorporation into insulin have revealed ability to modulate insulin biosynthesis with *in vitro* manipulation (11–14), but applicability of these findings to synthesis *in vivo* is unclear. In rats given streptozotocin as neonates, examination of islets has shown increased [³H]leucine incorporation into insulin (15), but demonstration of reductions in pancreatic insulin content and in [³H]leucine incorporation into proinsulin *in vivo* (16, 17) have limited an interpretation of the islet findings. Measurement of proinsulin messenger RNA (mRNA)¹ has conveyed potential for estimating *in vivo* capacity to produce insulin (16, 17). Modulation of proinsulin mRNA concentration has been shown in studies assessing the physiologic regulation of insulin production (17–22). A correlation has been shown in rats between pancreatic proinsulin mRNA concentration and rates of *in vivo* incorporation of [³H]leucine into proinsulin, showing comparable ability to assess rates of insulin biosynthesis (16, 17). A correlation between tissue mRNA levels and protein synthetic rates also has been shown for other protein systems *in vitro* (23, 24) and *in vivo* (25, 26).

These studies were designed to assess the relationship between functional capacity to produce insulin and development of hyperglycemia. Pancreatectomy has been recognized as a model allowing investigation of the correlation between limitation of islet number and expression of diabetes (27–31). Morphologic observations in early studies have shown adaptive increases in B cell number (27, 28), often followed by evidence of islet destruction (28–30). More recent study of 90% pancre-

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1. Abbreviations used in this paper: cDNA, complementary DNA; mRNA, messenger RNA; Px, pancreatectomized; SSC, 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0.

atectomy in rats has indicated that abnormalities of glucose tolerance are associated with a loss of glucose-stimulated insulin secretion characteristic of diabetes in man (31). In the experiments described below, proinsulin mRNA measurement has been used to assess the functional response of insulin production to varying degrees of pancreatectomy, with attention to age and body weight. This assessment has been made in conjunction with glucose tolerance testing, to evaluate further the association of functional capacity to produce insulin and ability to maintain glucose tolerance.

Methods

Pancreatectomy. Male Sprague-Dawley rats, fed ad lib. on Purina Rodent Chow (Ralston Purina Co., St. Louis, MO), were selected for pancreatectomy at 4 wk of age, at ~100 g body wt. Litter mates were sorted randomly for sham, 50%, or 90% pancreatectomy. Anesthesia was accomplished using sodium pentobarbital, 3–10 mg/100 g, injected intraperitoneally, followed in most cases by inhalation of diethyl ether. Skin of the ventral abdominal wall was washed with 70% ethanol, and a midline longitudinal incision was made from the sternum to the pubic symphysis. The skin and the muscular anterior abdominal wall were opened separately.

50% pancreatectomy entailed resection of the splenic portion of the pancreas. The spleen was mobilized by breaking mesenteric connections to the stomach, small bowel, and retroperitoneum. Pancreatic tissue was teased from splenic and pancreatic vessels using a cotton-tipped applicator, resulting in devascularization of ~50% of the total pancreatic substance, as determined by weight (data not shown). This portion of the pancreas was removed with sharp dissection ~5 mm from the pylorus, leaving a 50% pancreatic remnant.

90% pancreatectomy was performed according to the method of Foglia (30), as recently adopted by Bonner-Weir et al. (31). The splenic portion was removed as described above, but dissection at the pylorus was extended to leave only a small remnant at the gastroduodenal artery. After mesenteric connections to the colon were broken, the duodenal portion of the pancreas was dissected dorsal and caudal to the common bile duct. Care was taken to preserve duodenal vasculature. The result of this dissection was a 10% remnant, appearing as a crescent of pancreatic tissue lying between the bile duct and the proximal portion of the duodenum.

Sham pancreatectomy was accomplished by gently teasing the entire pancreas with a cotton applicator, after breaking splenic and duodenal mesenteric connections.

After pancreatic manipulation, the muscular abdominal wall was closed with 4-0 silk suture, and the skin was closed with wound clips. Animals were given 1–2 ml of isotonic sodium chloride subcutaneously for volume replacement; no antibiotics were administered. Animals chosen for subsequent studies were drinking and eating ad lib. within 12 h of operation.

Sacrificed animals. Animals were sacrificed in the fed state at 1, 3, and 14 wk after pancreatectomy or sham operation. Before sacrifice, fed blood glucose was measured from tail samples using Dextrostix glucose oxidase reagent strips and an Ames Glucometer (Miles Laboratories Inc., Elkhart, IN). Animals were more heavily anesthetized with sodium pentobarbital, 10–20 mg/100 g body wt. Before respiratory arrest, the abdomen was opened along previous suture lines, and dissection was extended into the thorax. Blood was obtained from the right ventricle for subsequent measurement of serum glucose and insulin.

Intra-abdominal adhesions were broken, and the spleen, pancreas, and duodenum were mobilized. The pancreas or pancreatic remnant was removed by sharp dissection with the spleen, pyloric portion of the stomach, and the duodenal loop en bloc; the common bile duct and accompanying vasculature were cut proximally. Pancreatic tissue was then dissected from the spleen and gut by a combination of blunt and sharp dissection. Accompanying fat and lymphatic tissue were

discarded. In the 90% pancreatectomized (Px) animals at 1 and 3 wk after pancreatectomy, the entire 10% pancreatic remnant was homogenized in a guanidine thiocyanate solution for subsequent purification of RNA, as described below. In the 50% Px animals, and in the 90% Px animals after 14 wk, the pancreas was divided randomly into two equal portions after dissection. One portion was homogenized in guanidine thiocyanate; the other was frozen in liquid nitrogen and stored at –80°C for subsequent extraction of insulin. In the sham-operated animals pancreatic tissue was divided into head and tail portions, such that the head approximated equivalent 50 or 10% remnants in the Px animals by weight and anatomic location. The remnant equivalent thus defined was divided randomly for assessment of insulin and of proinsulin mRNA. The tail portion was homogenized separately in guanidine thiocyanate, thus allowing subsequent comparison of head and tail proinsulin mRNA concentrations for both 50% Px and 90% Px remnants.

Serum insulin. Blood obtained from ventricular puncture was allowed to clot at room temperature, was centrifuged, and serum was decanted. Serum was stored at –80°C until all samples were available, then insulin was measured using a double antibody radioimmunoassay (RIA) with purified rat insulin standards (Novo Research Institute, Copenhagen, Denmark).

Pancreatic insulin. The frozen aliquots of pancreas were crushed and homogenized in 4 ml of acid ethanol (75% ethanol, 0.15 M HCl) using a Polytron homogenizer (Brinkmann Instruments Co., Westbury, NY). After 48 h of incubation at 4°C, the suspension was centrifuged for 10 min at 10,000 rpm using a Sorval SS 34 rotor in a Sorval RC-5 refrigerated centrifuge (DuPont Instruments, Des Plaines, IL). The supernatant was decanted. The pellet was resuspended in 2 ml of acid ethanol, and incubated at 4°C for 24 h. After centrifugation, the second supernatant was pooled with the first. The pellet was discarded. A 1:6,000 dilution of this acid alcohol extract was prepared in a phosphate-buffered saline solution containing 0.25% bovine serum albumin, and insulin was measured by double antibody RIA. All RIAs were performed concurrently.

Pancreatic RNA. RNA was purified from pancreatic tissue after homogenization at highest speed with the Polytron homogenizer (Brinkmann Instruments Co.) in 10 ml of 4 M guanidine thiocyanate, containing 0.5% *N*-lauroyl sarcosine, 0.025 M sodium citrate, 0.1 M 2-mercaptoethanol, and 0.1% Antifoam A (Sigma Chemical Co., St. Louis, MO), according to the method of Chirgwin et al. (32). Immediate precipitation of nucleic acids from the initial homogenate was necessary due to the high concentrations of RNAase present in rat pancreas, and repeated ethanol precipitation from guanidine hydrochloride was found to be superior to cesium chloride density centrifugation of RNA in limiting RNA degradation. Total RNA yield was assessed by absorbance at 260 nm in 0.01 M triethanolamine hydrochloride, pH 7.4, using a spectrophotometer (DU; Beckman Instruments, Inc., Fullerton, CA). Purity of RNA was estimated from ratios of optical density at 260 vs. 280 nm; in all cases this ratio was >2.05.

Degradation of RNA was assessed by electrophoresing 20 µg of total pancreatic RNA on 2% agarose gels, after glyoxalation using the technique of McMaster and Carmichael (33). Densitometric analysis of gels stained with Stainsall (Sigma) revealed ratios of 28S/18S ribosomal RNA of ~1.5. Subsequent analysis of proinsulin messenger RNA by Northern blot (34) and dot blot (35) analysis (see below) failed to reveal significant differences in proinsulin mRNA concentration when 28S/18S ratios remained in the range of 1–2 (data not shown). Purified pancreatic RNA was frozen at –80°C in water before blotting and hybridization analysis.

Proinsulin mRNA. 5 µg of total pancreatic RNA (determined by absorbance at 260 nm) in 5–10 µl water were added to 100 µl of 20× SSC (SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), then applied directly to nitrocellulose filters using a 96-well Hybri-Dot apparatus (Bethesda Research Laboratories, Gaithersburg, MD). Samples from sham, 50% Px, and 90% Px groups were applied in duplicate on a single filter for subsequent hybridization. Samples from 1-, 3-, and 14-wk groups were placed on separate filters, but hybridized concur-

rently. Pooled RNA from the pancreata of 10 male Sprague-Dawley rats weighing 100–150 g, killed in the fed state, was used as internal standard. This RNA was divided into aliquots of 20, 15, 10, 7.5, 5, 3, 2.5, 2, 1.5, 1, and 0.625 μg ; duplicates of each concentration were placed on all filters for comparison with experimental samples (Fig. 1). On separate filters the intensity of hybridization of the RNA standards was assessed relative to that of dilutions of the complementary DNA (cDNA) probe (see below) applied in duplicate, and a proinsulin mRNA concentration in the standards of 1.0 ± 0.2 pg/ μg total RNA was deduced (data not shown). 20- μg aliquots of rat kidney and spleen RNA prepared as described above were also applied to the filters to assess nonspecific binding. Each dot was washed with a second aliquot of $20\times$ SSC, then filters were dried and baked at 80°C for 2 h.

Proinsulin mRNA was assessed by hybridization to a 440-base pair cDNA probe, pCRI354, containing 354 bases complementary to rat preproinsulin I mRNA (36). The probe was obtained from *Hind* III digestion of a parent pBR322 plasmid and subsequent isolation employing agarose gel electrophoresis. This cDNA was radiolabeled with α [^{32}P]deoxycytidine by nick translation (37) to a specific activity of $\sim 3 \times 10^8$ cpm/ μg , and 5×10^6 cpm were used per blot. After prehybridization at 42°C for 24 h in a solution of 50% deionized formamide, $5\times$ SSC, 0.05 M sodium phosphate pH 6.5, $1\times$ Denhardt's solution, and 250 $\mu\text{g}/\text{ml}$ salmon sperm DNA, blots were added to a hybridization solution, prepared from prehybridization solution with 5% dextran sulphate, and hybridization to the radiolabeled cDNA was accomplished at 42°C for 18 h. To minimize uneven hybridization, blots were sandwiched between glass plates.

After hybridization filters were washed in a solution of $2\times$ SSC and 0.1% sodium dodecyl sulfate (SDS) at room temperature, then in

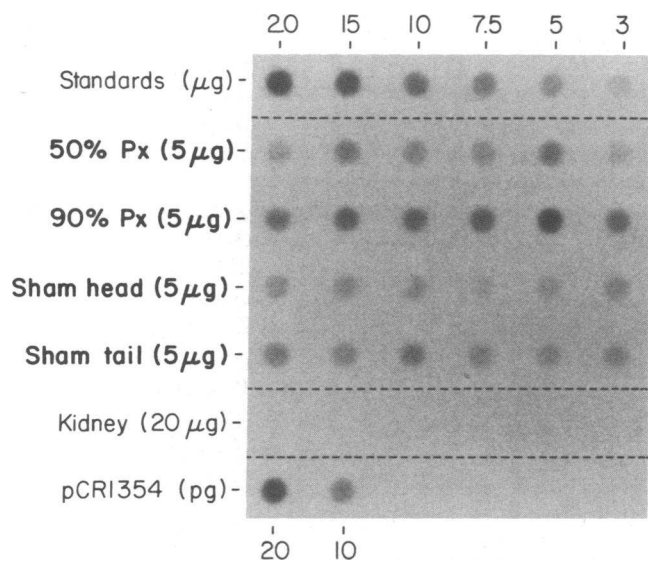


Figure 1. This portion of an autoradiograph shows relative concentrations of proinsulin mRNA in aliquots of total RNA, determined by dot hybridization to a cloned [^{32}P]radiolabeled cDNA probe, pCRI354, as described in Methods. On the top row pooled rat pancreatic RNA standards were applied, containing increments of total RNA as listed in the scale above. In the next four rows (bold face), 5 μg of total pancreatic RNA was applied to each dot, from 50% Px, 90% Px, and sham-operated control rats 3 wk after operation. The sham samples were prepared separately from a 10% remnant equivalent head (see text) and the corresponding body and tail. No signal is observed from 20- μg samples of kidney RNA, applied as a negative control. On the bottom row, two dilutions of unlabeled pCRI354 cDNA insert were applied as listed in the scale below. Differences among the experimental groups are described in Results, in Table II, and in Fig. 4.

$0.1\times$ SSC and 0.1% SDS twice for 15 min at 50°C . Blots were rinsed in $0.1\times$ SSC at room temperature and were air dried. Autoradiographs were prepared using Kodak XAR-5 film (Eastman Kodak Co., Rochester, NY) with Cronex intensifying screens (DuPont, Newtown, CT). Autoradiography was performed for 24–48 h at -80°C . Densitometric analysis was performed using a scanning densitometer (Hoefer Scientific Instruments, San Francisco, CA); peak height was used to assess relative proinsulin mRNA concentration. Suitability of autoradiographs for subsequent analysis was assessed by a linear range of intensity among the pooled rat pancreatic RNA internal standards, and lack of signal from spleen or kidney RNA (Fig. 1).

Glucose tolerance testing. In those rats ultimately sacrificed after 14 wk, intraperitoneal glucose tolerance tests were performed at 3, 6, and 12 wk after operation, using a protocol previously described (31). 2 wk separated the final test from the time of sacrifice, allowing potential effects of fasting and glucose injection to be minimized (20, 21). Food was withheld for 12 h before testing. Fasting blood glucose was measured from tail samples using the Glucometer as described above. Animals then were injected intraperitoneally with dextrose, 200 mg/100 g fasted body wt, administered as a 20% solution in water, then blood glucose was measured by tail bleeding at 10, 30, and 50 min after injection. Blood glucose concentrations of >400 mg/dl were estimated from 30-s Dextrostix determinations.

Data analysis. Parameters measured in experimental groups were compared with those in controls using a nonpaired *t* test, and exact *P* values were calculated.

Results

Body weight, fed blood glucose, and serum insulin after pancreatectomy. The effects of pancreatectomy upon body weight, blood glucose, and serum insulin in the fed state are summarized in Table I and in Fig. 2. All animals showed comparable development and an increase in body weight after pancreatectomy. No differences in weight were seen between the sham-operated control animals and the 50% Px animals. The mean

Table I. Response of Body Weight, Blood Glucose, and Serum Insulin to Partial Pancreatectomy

wk	Body weight	Fed blood glucose	Fed serum insulin
	g	mg/dl	ng/ml
Sham Px			
1	135 ± 4 (18)	136 ± 6 (6)	0.4 ± 0.1 (12)
3	228 ± 7 (17)	153 ± 9 (6)	3.1 ± 0.6 (12)
14	466 ± 8 (13)	102 ± 4 (6)	3.1 ± 0.7 (6)
50% Px			
1	144 ± 4 (12)	121 ± 5 (6)	0.8 ± 0.2 (6)
3	215 ± 8 (12)	132 ± 2 (6)	2.0 ± 0.3 (6)
14	482 ± 16 (7)	132 ± 5 (5)*	3.0 ± 0.8 (6)
90% Px			
1	118 ± 4 (11)	139 ± 20 (6)	0.4 ± 0.1 (6)
3	195 ± 8 (14)*	213 ± 21 (6)‡	1.4 ± 0.3 (6)‡
14	357 ± 44 (4)*	316 ± 85 (4)*	0.8 ± 0.2 (4)‡

Body weight, blood glucose, and serum insulin concentrations obtained in the fed state from sham, 50% Px, and 90% Px rats at 1, 3, and 14 wk after operation, as described in Methods. Values are mean \pm SEM and the number of observations (*n*) for each group. Footnotes denote difference of 50% or 90% Px groups from sham control at corresponding time: * *P* < 0.01; ‡ *P* < 0.05.

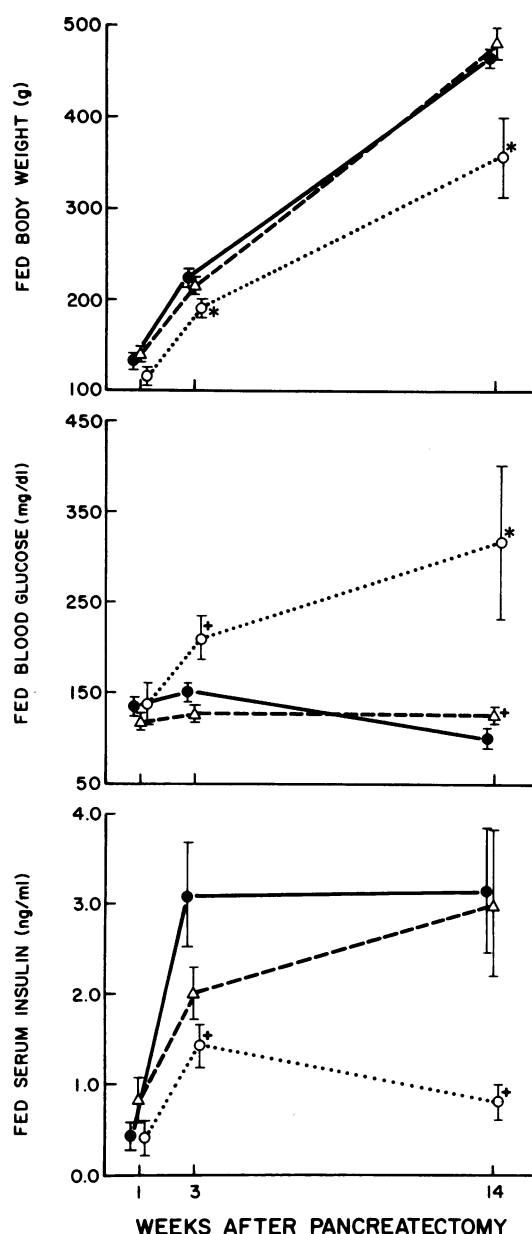


Figure 2. Body weight (top), blood glucose (middle), and serum insulin concentration (bottom), obtained in the fed state from sham, 50% Px, and 90% Px rats, as described in Methods, are plotted against time after operation. Illustrated are mean \pm SEM for the number of observations shown in Table I. Footnotes denote difference of 50% Px or 90% Px from control at corresponding time. † $P < 0.05$; * $P < 0.01$. —●—, sham pancreatectomy; ---△---, 50% pancreatectomy; ...○..., 90% pancreatectomy.

body weight of the 90% Px animals was lower than that of controls after 3 wk ($P < 0.005$) and after 14 wk ($P < 0.001$).

Fed blood glucose was not elevated in the sham-operated animals. There was a minimal difference between the 50% Px animals and the sham-operated controls after 14 wk (50% 132 ± 2 vs. sham 102 ± 4 mg/dl, $P < 0.05$). The 90% Px animals were not different from controls at 1 wk after pancreatectomy, but showed a higher fed blood glucose after 3 wk ($P < 0.05$) and a more marked elevation after 14 wk ($P < 0.0005$). After

14 wk glycosuria was evident in two of the four 90% Px animals.

Fed serum insulin levels were not different among the groups at 1 wk after pancreatectomy. After 3 wk, lower serum insulin levels were noted in the 90% Px animals than in the sham-operated controls ($P < 0.05$). After 14 wk there was no difference between control and 50% Px animals, but serum insulin levels of 90% Px animals remained low ($P < 0.05$).

Pancreatic weight and insulin content after pancreatectomy. Changes in pancreatic weight and in total pancreatic insulin content are shown in Table II and in Fig. 3. An increase in pancreatic weight was shown in all three groups. After 14 wk, the weight of the 50% pancreatic remnant was 50% of control, and the weight of the 10% remnant was 14% of control.

Total pancreatic insulin content increased in the sham-operated animals from 1 wk to 14 wk after operation ($P < 0.001$). Insulin content also increased in the 50% Px animals after 3 wk ($P < 0.05$), but not after 14 wk, at which time pancreatic insulin concentration was reduced ($P < 0.05$), and total pancreatic insulin was 30% of control. In the 90% Px animals pancreatic insulin was measured only after 14 wk, at which time the total pancreatic insulin content was 15% of control.

Pancreatic RNA and proinsulin mRNA after pancreatectomy. Changes in total pancreatic RNA, proinsulin messenger RNA concentration, and total proinsulin messenger RNA are shown in Table II and Fig. 4. Changes in total pancreatic RNA resembled those in pancreatic weight. In the control animals an increase in pancreatic RNA was shown from 1 to 14 wk after sham operation ($P < 0.005$). At 1 wk after pancreatectomy, total RNA was 64% of control in the 50% pancreatic remnant, and 16% of control in the 10% remnant. After 14 wk total RNA was 54% of control in the 50% remnant, and 13% of control in the 10% remnant, reflecting the extent of initial resection.

The concentration of proinsulin messenger RNA, as determined by dot hybridization of total RNA aliquots (Fig. 1), increased in the sham-operated animals from 1–14 weeks after operation ($P < 0.001$). The concentration of proinsulin messenger RNA in the 50% Px animals was not different from control after 1 or 3 wk, but was increased to 165% of control after 14 wk. Contrastingly, in the 90% Px animals, proinsulin mRNA concentration (Fig. 1) was markedly increased at 1 wk after pancreatectomy, to 220% of control, and after 3 wk, to 250% of control. After 14 wk, however, the concentration of proinsulin messenger RNA was reduced from the 3 wk value ($P < 0.01$), and was no longer different from control.

The impact of changes in proinsulin mRNA concentration was apparent after calculation of total pancreatic proinsulin mRNA, which is the product of proinsulin mRNA concentration and total pancreatic RNA. Over the 13-wk observation period the sham-operated controls exhibited a more than threefold increase in total proinsulin mRNA ($P < 0.001$). As proinsulin mRNA concentration in the 50% Px animals was not different from control at 1 or 3 wk after pancreatectomy, total levels of proinsulin mRNA in the 50% remnant were not demonstrably augmented. After 14 wk, however, the increase in proinsulin mRNA concentration observed in the 50% Px animals established a rise in total proinsulin mRNA ($P < 0.001$), such that levels were no longer different from those in sham-operated controls. In the 90% Px animals the marked increases in proinsulin mRNA concentration seen at 1 and 3 wk after

Table II. Response of the Pancreatic Remnant to Partial Pancreatectomy

	Pancreatic weight	Total pancreatic RNA	Pancreatic insulin concentration	Total pancreatic insulin	Pancreatic PImRNA concentration	Total pancreatic PImRNA
wk	g	mg	mg/g	mg	ng/mg RNA	ng
Sham Px						
1	0.70±0.003 (18)	11.4±1.1 (6)	21.1±2.0 (6)	16.9±1.7 (6)	0.83±0.11 (6)	12.2±2.1 (6)
3	1.61±0.48 (17)	14.3±1.0 (6)	21.4±3.0 (6)	27.3±4.0 (6)	1.10±0.16 (6)	21.5±3.2 (6)
14	2.03±0.063 (13)	22.3±2.4 (6)	30.7±4.7 (12)	61.8±10.2 (12)	1.76±0.16 (6)	39.7±4.5 (6)
50% Px						
1	0.49±0.013 (13)*	7.3±0.02 (6)*	19.9±1.9 (6)	10.1±0.8 (6)*	0.74±0.06 (6)	5.48±0.06 (6)‡
3	0.63±0.016 (12)*	11.1±0.08 (6)‡	26.4±4.4 (6)	17.0±3.5 (6)	1.22±0.20 (6)	13.8±2.8 (6)
14	1.02±0.118 (6)*	12.0±0.08 (6)*	17.6±0.37 (6)*	18.4±4.3 (6)*	2.91±0.27 (6)*	34.8±4.2 (6)
90% Px						
1	0.18±0.080 (11)*	1.8±0.03 (6)*			1.88±0.29 (6)*	3.17±0.5 (6)*
3	0.17±0.009 (14)*	3.0±0.02 (6)*			2.76±0.28 (6)*	8.38±1.1 (6)*
14	0.28±0.059 (4)*	2.9±0.04 (4)*	28.3±13.5 (4)	9.3±4.9 (4)*	1.37±0.30 (4)	3.84±1.0 (4)*

Pancreatic weight, total RNA, insulin concentration, total insulin, proinsulin mRNA (PImRNA) concentration, and total proinsulin mRNA content, obtained at the time of sacrifice of sham, 50% Px, and 90% Px rats at 1, 3, and 14 wk after operation, as described in Methods. Values are mean±SEM (n) for each group. Footnotes denote difference of 50% or 90% Px groups from sham control at corresponding time: * $P < 0.01$; ‡ $P < 0.05$.

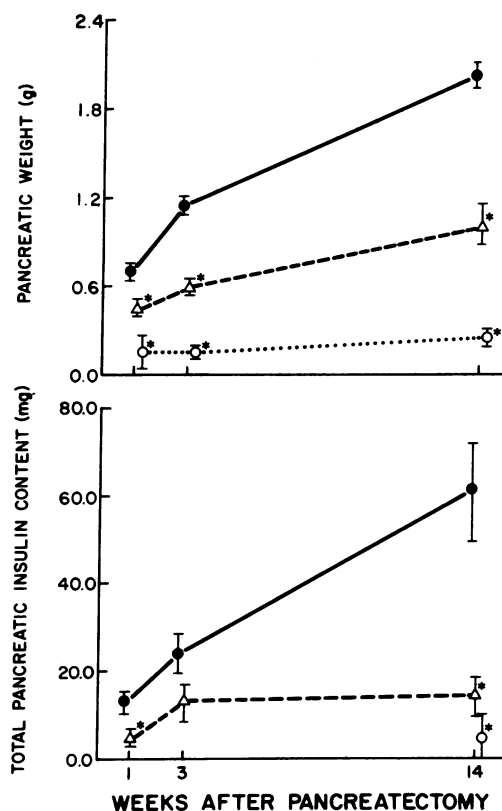


Figure 3. Pancreatic weight (top) and pancreatic insulin content (bottom) obtained after sacrifice of sham, 50% Px, and 90% Px rats in the fed state as described in Methods, are plotted against time after operation. Illustrated are mean±SEM for the number of observations shown in Table II. Footnotes denote difference of 50% Px or 90% Px from control at corresponding time. † $P < 0.05$; * $P < 0.01$. —●—, sham pancreatectomy; ---△---, 50% pancreatectomy; ...○..., 90% pancreatectomy.

pancreatectomy were associated with a significant rise in total proinsulin mRNA such that the 10% remnant regained 26% of control levels after 1 wk, and 40% of control after 3 wk. The drop in proinsulin mRNA concentration seen in the 90% Px animals after 14 wk also was associated with a marked change in relative total proinsulin mRNA content, yielding levels reduced to 10% of control.

Comparison of proinsulin mRNA concentration by anatomic location was accomplished by dividing the pancreas of sham-operated rats into a remnant equivalent head and tail. Data in this analysis were pooled from 3 and 14 wk after operation. The 50% remnant equivalent head was not different from the tail (head, 1.76 ± 0.11 vs. tail, 1.77 ± 0.11 ng/mg RNA, $n = 12$, NS). The 10% remnant equivalent head showed a lower proinsulin mRNA concentration relative to the corresponding body and tail (head 1.10 ± 0.11 vs. body and tail 1.87 ± 0.11 ng/mg RNA, $n = 10$, $P < 0.001$). These differences complement results of previous morphologic observations (38).

Fasting blood glucose and glucose tolerance after pancreatectomy. The results of intraperitoneal glucose tolerance testing at 3, 6, and 12 wk after pancreatectomy are illustrated in Fig. 5. These tests were performed in those rats ultimately sacrificed at 14 wk after operation.

Sham-operated control animals ($n = 13$) showed no change in their glucose profile with time after pancreatectomy. The 50% Px animals ($n = 7$) demonstrated an impairment of glucose tolerance relative to the sham-operated animals by showing elevated blood glucose at 30 min and at 50 min after 3 and 6 wk ($P < 0.01$), and at 50 min after 12 wk ($P < 0.005$). Comparison of the glucose profiles at 3, 6, and 12 wk revealed no evidence to suggest deterioration of glucose tolerance with time in the 50% Px group. Fasting hyperglycemia was not evident.

The 90% Px animals showed a more marked impairment of glucose tolerance than did the control or the 50% Px

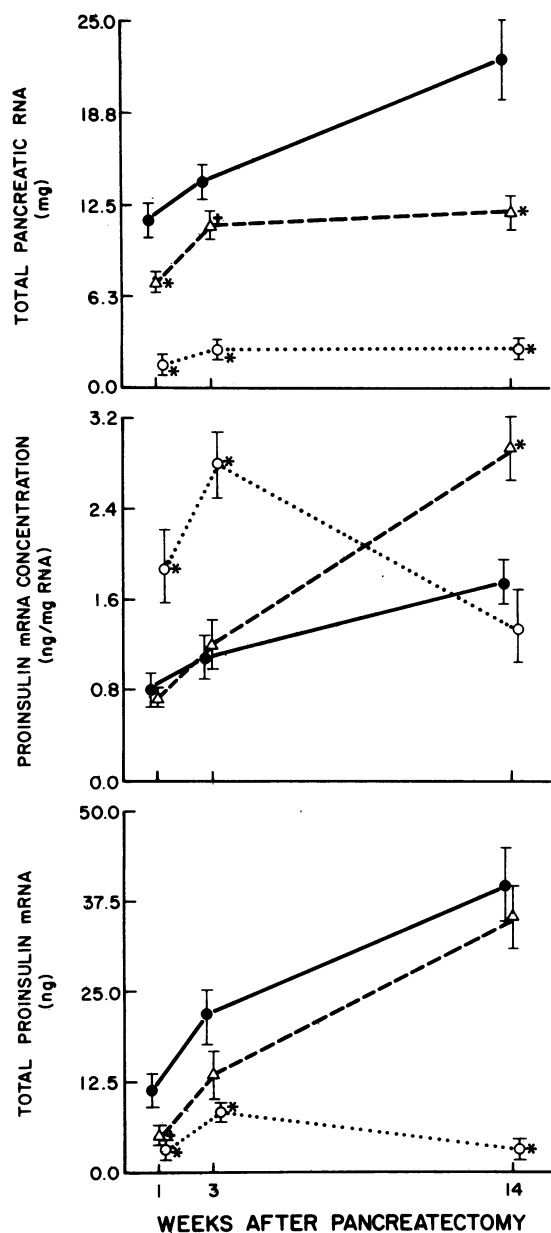


Figure 4. Total pancreatic RNA (top), proinsulin mRNA concentration (middle), and total pancreatic proinsulin mRNA content (bottom) obtained after sacrifice of sham, 50% Px, and 90% Px rats in the fed state as described in Methods, are plotted against time after operation. Illustrated are mean \pm SEM for the number of observations shown in Table II. Footnotes denote difference of 50% Px or 90% Px from control at corresponding time. † $P < 0.05$; * $P < 0.01$. —●—, sham pancreatectomy; ---△---, 50% pancreatectomy; ...○..., 90% pancreatectomy.

animals at all times tested. At 3 wk ($n = 7$) and at 6 wk ($n = 4$) after pancreatectomy this difference was apparent at 30 min and at 50 min after glucose injection ($P < 0.01$). Worsening of glucose tolerance was apparent in the 90% group after 6 wk, as depicted by the 50-min determination (6 wk 436 ± 59 vs. 3 wk 342 ± 16 mg/dl, $P < 0.01$). This abnormality of glucose tolerance has been associated with impaired insulin secretion in previous studies (31). After 12 wk ($n = 4$), a further deterioration of glucose tolerance was observed, including fasting

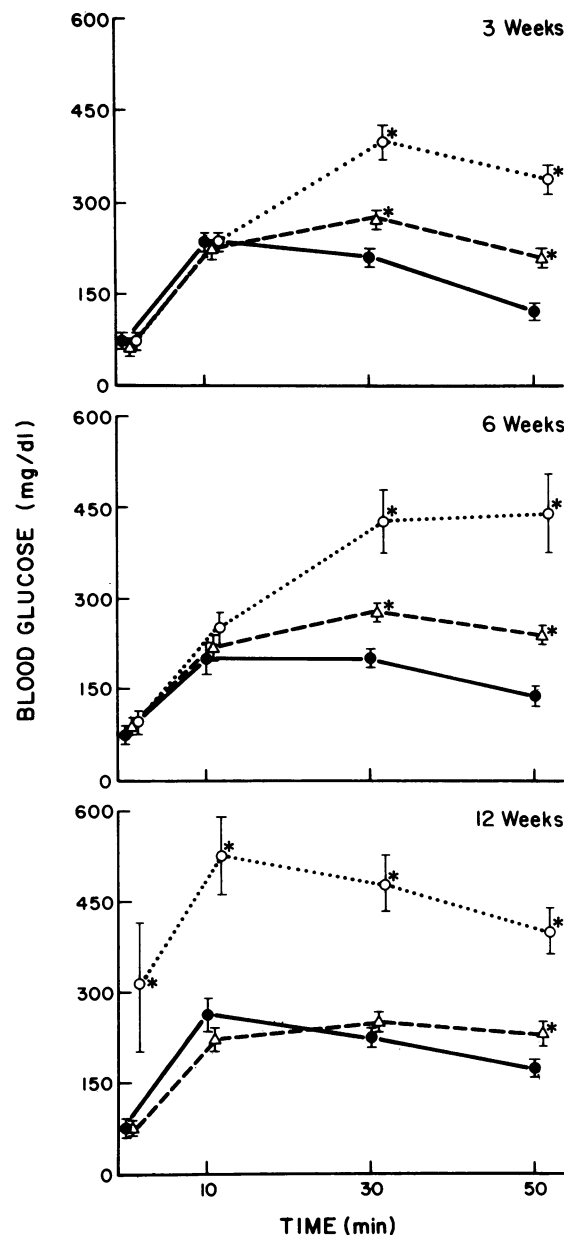


Figure 5. Results of intraperitoneal glucose tolerance testing, performed after 12-h fasting in sham, 50% Px, and 90% Px rats as described in Methods, are plotted against time after glucose injection. Results of tests at 3 wk (top), 6 wk (middle), and 12 wk (bottom) after pancreatectomy are shown. Illustrated are mean \pm SEM for the number of observations indicated in Results. Footnotes denote difference of 50% Px or 90% Px from control at corresponding time. † $P < 0.05$; * $P < 0.01$. —●—, sham pancreatectomy; ---△---, 50% pancreatectomy; ...○..., 90% pancreatectomy.

hyperglycemia (90% 307 ± 101 vs. sham 85 ± 5 mg/dl, $P < 0.001$), and marked hyperglycemia 10 min after glucose injection ($P < 0.001$), accompanying the previously observed abnormalities at 30 and 50 min.

Discussion

The proinsulin mRNA measurements presented in these studies represent an important complement to previous observations

of pancreatectomy, by assessing a functional parameter of *in vivo* insulin production. This parameter may reflect changes in the status of individual B cells and/or changes in B cell number with time, toward the generation of an apparent adaptive response, and eventually toward the loss of that response as observed following 90% pancreatectomy.

A relationship between levels of proinsulin mRNA and the physiologic regulation of insulin production has been demonstrated by previous *in vivo* observations. During fetal pancreatic development there is a specific increase in insulin content relative to total pancreatic protein (39), which is preceded by a comparable increase in proinsulin mRNA (18, 19). Reduction of proinsulin mRNA has been established as a result of fasting (20), and ability to increase proinsulin mRNA concentration has been shown in fasted rats after glucose injection (21). Observations of pancreatic adaptation in rats after removal of insulinoma tissue has correlated increases in proinsulin mRNA with regrowth of atrophic B cells in the presence of hyperglycemia (22). An abundant proinsulin mRNA content has been observed in genetically obese and hyperglycemic db/db mice (Orland, M. J., R. Chyn, and M. A. Permutt, manuscript in preparation), associated with hyperinsulinemia and B cell hyperplasia observed in these animals (40).

The utility of proinsulin mRNA as a quantitative parameter of insulin production has been supported by studies favorably relating proinsulin mRNA to [³H]leucine incorporation into proinsulin *in vivo*. Parallel measurement of these two parameters in 100 g male Sprague-Dawley rats has shown tight correlation in estimating a twofold reduction of insulin biosynthesis imposed by neonatal streptozotocin treatment (16, 17), and in assessing a twofold augmentation of insulin biosynthesis apparent in normal and streptozotocin-treated rats given pharmacologic doses of dexamethasone for 4 d (17). Contrary to the results of short-term labeling studies in isolated pancreatic islets (11), the *in vivo* data did not show an important independent contribution of translational activity to insulin biosynthesis under the conditions specified.

The sham-operated rats in this study demonstrated (a) maintenance of fed blood glucose and intraperitoneal glucose tolerance after surgery, and (b) an increase both in proinsulin mRNA concentration and in total proinsulin mRNA content with time. The measurements of total proinsulin mRNA corroborate observations of B cell number and of insulin content with age in normal animals previously reported (41, 42). Increases in proinsulin mRNA concentration indicate that the regulation of total proinsulin mRNA is not merely a phenomenon reflecting pancreatic growth. The fed blood glucose and intraperitoneal glucose tolerance data suggest that this increase is appropriate to maintain glucose tolerance with time. Increases in total proinsulin mRNA were proportional to changes in body weight; within the study period a ratio of total proinsulin mRNA to body weight, as depicted in Fig. 6, did not show significant variation.

Observation of the 50% Px animals disclosed (a) euglycemia, initially despite a 50% reduction in proinsulin mRNA relative to controls, (b) mild impairment of glucose tolerance, which did not deteriorate, and (c) ability to regain normal total proinsulin mRNA content with time. It was anticipated that 50% pancreatectomy would not result in diabetes. Functional adaptation was indeed suggested by maintenance of fed and fasting blood glucose comparable to that of the sham-operated controls. Ability to maintain euglycemia despite a 50% reduc-

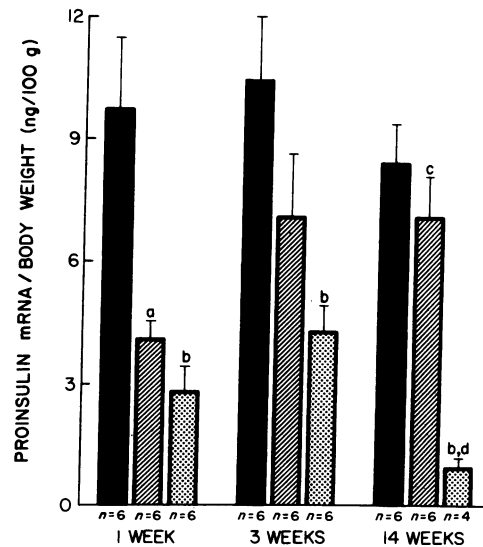


Figure 6. The ratio of proinsulin mRNA/body weight is depicted for sham (■), 50% Px (▨), and 90% Px (□) rats 1 wk, 3 wk, and 14 wk after operation. Ratios were calculated from total pancreatic proinsulin mRNA and fed body weight (Table II). Bars represent mean values of experimental groups; numbers in the groups are listed below each bar. Standard errors are illustrated. Footnotes denote significant differences as follows: *50% Px vs. sham, $P < 0.01$; ^b90% Px vs. sham, $P < 0.01$; ^c50% Px after 14 wk vs. 1 wk, $P < 0.02$; ^d90% Px after 14 wk vs. 1 wk, $P < 0.05$; vs. 3 wk, $P < 0.01$.

tion in insulin biosynthesis has also been observed in 4-wk-old rats after neonatal streptozotocin injection (16, 17). It is possible that changes occur in peripheral insulin sensitivity; thus far, no such changes have been measured. Glucagon production is apt to be limited by pancreatectomy, and limitation of glucagon secretion after arginine stimulation has been documented in the neonatal streptozotocin model (43), suggesting a mechanism for insulin-independent regulation of hepatic glucose production. Eventually, however, in the 50% Px rats a definite response of proinsulin mRNA concentration was demonstrable, such that total proinsulin mRNA and the ratio of proinsulin mRNA to body weight (Fig. 6) were not different from control after 14 wk. Note that this adaptation was not apparent from assessment of pancreatic insulin content, which remained reduced. No conclusions concerning a relationship of proinsulin mRNA/body weight to glucose tolerance test results could be made in the 50% Px group, as significant changes were not observed from 3–14 wk after pancreatectomy.

Observation of the 90% Px animals revealed (a) appearance of fed and fasting hyperglycemia, (b) more marked impairment of glucose tolerance, worsening with time, (c) limitation in the magnitude of proinsulin mRNA adaptation, and (d) loss of proinsulin mRNA adaptation with time. The changes in total proinsulin mRNA adjusted for body weight are illustrated in Fig. 6. Accounting for the reduced proinsulin mRNA concentration evident in the 10% remnant equivalent head, 90% pancreatectomy imposed demand for a greater than 10-fold increase in insulin production to achieve complete adaptation. An adaptation of this magnitude might have been anticipated on the basis of data from isolated rat islets, where significant short-term increases in insulin biosynthesis have been demonstrated (11–14). A maximum 3.5-fold increase in proinsulin

mRNA concentration was observed in this study at 3 wk after pancreatectomy, associated with a rise of proinsulin mRNA/body weight to 40% of control. Although the absolute levels of insulin production remain unknown, serum insulin was reduced, fed hyperglycemia became evident, and intraperitoneal glucose tolerance was impaired more dramatically than after 50% pancreatectomy, attesting to an insufficiency of the adaptive response. Inability to maintain adaptive increases in insulin production was suggested after 6 wk by the worsening of intraperitoneal glucose tolerance, and after 12 wk by the appearance of fasting hyperglycemia and glycosuria. After 14 wk the reduction of proinsulin mRNA was associated with marked hypoinsulinemia.

The factors determining appropriate levels of proinsulin mRNA and rates of insulin biosynthesis are unknown, and mechanisms by which a loss of adaptive proinsulin mRNA response occurred with time in the 90% Px animals are not apparent. Hyperglycemia was not evident as a promoter of the increases in proinsulin mRNA observed among sham-operated or 50% Px animals, although changes in glucose levels within a physiologic range may be significant for proinsulin mRNA regulation. The association of elevated proinsulin mRNA with reduced pancreatic insulin content after 14 wk is interesting in this regard, and suggests a relationship between the depletion of B cell insulin stores and the stimulation of insulin biosynthesis. Prolonged hyperglycemia may be responsible for the deterioration of proinsulin mRNA and of insulin biosynthetic capacity in the 90% Px group, supporting previous observations of B cell degeneration (29, 44). On the other hand, it is possible that further observation may reveal a loss of proinsulin mRNA response in 90% Px animals, or even in 50% Px animals, not related to antecedent hyperglycemic stresses (45).

The results of these experiments are directly applicable to pancreatic diabetes in man, including that related to exocrine pancreatic diseases or to surgery. Pancreatectomy has certainly been related to the development of diabetes in humans, but the degree of pancreatectomy necessary for subsequent development of diabetes has been unclear, both because of an imprecise documentation of the extent of pancreatic resections, and because of the coexistence of pancreatic disease in patients undergoing surgery (46, 47). Hemipancreatectomy is now being performed in normal individuals who are donating tissue to diabetic relatives (48). Observation of these donors has disclosed mild impairment of oral and intravenous glucose tolerance, which seems to improve within 1 yr after operation, but which might be worsened with obesity (49). In an analogous fashion, limitation of the adaptive responses seen in these studies may be important toward anticipating the behavior of islet allografts, and in a consideration of the critical mass of islet tissue necessary to maintain adequate insulin production in transplant recipients.

These observations of functional adaptation also may be applicable to an understanding of primary diabetes in man. Although immune destruction of B cells seems to account for the development of insulin insufficiency in insulin-dependent diabetes, many patients show a remission from hyperglycemia early in their clinical course. This remission may represent an adaptive response of remaining B cells, which in most afflicted individuals is not maintained. The relative contributions of deficient insulin production and of insulin resistance are uncertain in the pathogenesis of noninsulin-dependent diabetes (10), but an ultimate finding is a lack of insulin necessary to

meet metabolic demands. A primary abnormality of insulin resistance, such as that imposed by obesity or by pregnancy, may augment insulin production in a manner similar to pancreatectomy. Tendency to diabetes may be related to an inability to adapt fully to augmented demands, or to an inability to maintain a functional adaptation with time, heralded by a worsening of glucose tolerance similar to that seen in 90% Px rats. Testing of these hypotheses requires further understanding of the spectrum of insulin biosynthetic capacity in man, and may be accomplished when tests that describe insulin production in man are elucidated.

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