Pancreatic Acinar Cell Regeneration

IV. Regeneration after Surgical Resection

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PANCREAS organ potential for regeneration in the adult animal has generally been regarded as minimal.[1,2] At this laboratory it has been demonstrated that after destruction of most of the adult rat pancreas acinar epithelium by ethionine there was cytological,[3-8] biochemical,[6,9] and organ weight restitution [5,7,8] of the gland from residual acinar tissue within 3–4 weeks after cessation of the antimetabolite. Islet cell regeneration has been demonstrated by others following alloxan administration [10-13] and after 95% partial pancreatectomy.[14]

There are conflicting reports about pancreas regeneration after partial surgical resection, and in most studies very few animals have been used,[15-17] pancreas weight controls were not adequate,[16-18] results were equivocal,[19] or hyperplasia was not substantiated by mitotic counts or autoradiographic studies with tritiated thymidine.[14]

We resected the splenic and gastric segments [20] of the adult rat pancreas and compared the change in weight of the residual duodenal segment with the weight of the duodenal segment of control animals for up to a year after operation. DNA synthesis was studied by means of the autoradiographic technique with thymidine-H^3 during the same period.

Experimental Design

Animals were divided into lots by use of a table of random numbers,[21] one lot to be sacrificed on each of Days 0.5, 1, 1.5, 2, 2.5, 3, 5, 7, 14, 30, 60, 180, 270, and 360 after surgery. Each lot was subdivided into control (c), abdominal sham-operated (sh) and partial pancreatectomized (ppx) groups. The animals of the sh and ppx groups were operated upon over a period of 5 days, the schedule of operations also being decided by randomization.

Splenic and gastric segments of the pancreas were resected, leaving intact the residual parabiliary and duodenal segments [20] (Text-fig. 1). At sacrifice, the total pancreas weight and segment weights were determined.[20] The combined weight of

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the parabiliary and duodenal segments were designated PBDW, total pancreas weight as TPW, and total body weight as TBW. Subscripts c, sh, and ppx were used to denote, respectively, control, sham-operated, and partial pancreatectomy groups. Weights at Day 0 and at the day of sacrifice were indicated by the subscripts o and s, respectively. TPW_{o-csh} represented total pancreas weight at Day 0 in the combined control and sham-operated animals and TBW_{s-ppx} indicated total body weight at sacrifice in the animals with partial pancreatectomy.

When radioactivity measurements and autoradiograms after the administration of thymidine-H^3 indicated peak values at 36 to 48 hr. after operation, additional c, sh, and ppx animals were similarly selected and sacrificed at 28, 30, and 32 hr. for these studies.

Islet cell results are not included in this study.

Materials and Methods

Animals

A total of 277 male Wistar rats (CFN, Carworth Farms, New City, N.Y.) was used. The animals were kept in individual cages in air-conditioned quarters at 74°F. Purina Lab Chow Checkers (Ralston Purina Co., St. Louis, Mo.; content: protein, 25%; carbohydrate, 59%; fat, 6%; plus vitamins, salts and factors necessary for optimum growth) and water were available ad libitum. At the time of surgery the animals weighed between 150 and 200 gm., averaging 181 ± 1.2 gm. (mean ± standard error).

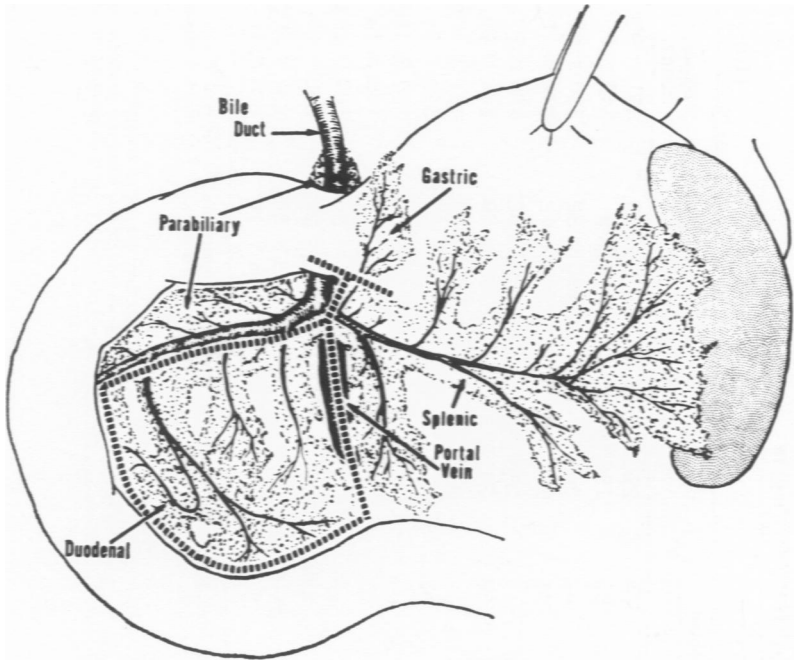
Pancreatic Resection

Under Nembutal anesthesia (0.001 ml. of a 50 mg./ml. solution per gram of body weight) given intraperitoneally (I.P.), the animal's abdomen, under sterile technique, was clipped with an electric shaver, prepared with Betadine and covered with sterile drapes. A left upper paramedian incision was made through the skin, muscle, and peritoneum and ratchet retractors were positioned. The stomach, spleen, and pancreas were mobilized, lifted out of the abdomen, and placed on the draped thorax and abdomen. The splenic segment duct (Text-fig. 1) was ligated with 6-0 silk near its junction with the common duct. The gastric and splenic segments of pancreas were removed by gently picking the tissue away from its vascular and peritoneal attachments with serrated forceps. Hemostasis was accomplished by the application of direct pressure or with a miniature bulldog clamp. Only rarely was it necessary to ligate vessels. During the procedure, the viscera were kept moist with gauze sponges soaked with 0.9% aqueous solution of NaCl. A dissecting microscope (Stereo-Cycloptic Dissecting Microscope, American Optical Co., Rochester, N.Y.) was used at a magnification of seven times to facilitate removal of tissue.

Approximately 4 ml. of warm aqueous solution (0.9%) of NaCl was put in the peritoneal cavity before closure in the ppx and sh animals. The muscle and peritoneum were closed as one with 3-0 silk and the skin with 14-mm. Michel clips. No antibiotics or other drugs were used. Operative mortality was about 5%.

The abdominal sham operation was similar in technique to the partial pancreatectomy except that the splenic duct was not ligated and no tissue was removed. The stomach, duodenum, spleen, and pancreas were exposed and placed on the draped thorax and abdomen. The entire pancreas was kept moist with saline-soaked gauze and handled for 20 min., the average time these organs were exposed in the ppx group during the removal of pancreas tissue.

In the operations for the supplementary experiments of 28, 30, and 32 hr. after operation, the dose of Nembutal was about one-third the amount used previously



TEXT-FIG. 1. Anatomic division of rat pancreas as used in this laboratory.[20] Splenic and gastric segments, comprising about 55% of the gland, were resected. Heavy dotted lines indicate segment resection lines. (From Richards *et al.*,[20] *Lab Invest*; reproduced with permission.)

and a small amount of ethyl ether anesthesia was used by dripping it onto sterile gauze in a face cone from which the animal breathed. The original operations were performed by one of us (M.L.) and the supplemental ones by another person (D.G.).

The number of animals used for a group average is indicated in the tables.

Total Pancreas and Pancreas Segment Weights

At sacrifice, the pancreas was examined in situ and the segments [20] (Text-fig. 1) carefully identified and excised. Particular care was taken to define the medial border of the duodenal segment of pancreas at the portal vein. Fat and lymph nodes were dissected free and discarded. The pancreas was rapidly blotted of blood and weighed to the nearest milligram on a Roller-Smith torsion balance. Small amounts of inflammatory tissue (no more than 10–15 mg.), found at necropsy at the operative margin of the duodenal and splenic segments in a few ppx animals, were not included in the residual pancreas weights although they were fixed for histologic and autoradiographic studies.

From regression analysis curves previously developed in this laboratory,[20] which related total pancreas and segmental pancreas weights to total body weight, we were able to determine TPW_0 and $PBDW_0$, with 95% confidence, as a function of TBW_0. After the first postoperative week there were no significant differences in TBWs between groups (Table 1).

Table 1. Total Body (TBW), Total Pancreas (TPW), and Parabiliary-Duodenal (PBDW) Weights

(1)	(2)	(3)	(4)	(5)	(6)	(7)		(8)	(9)	(10)
Postop. day	Group	No. of rats	TBW_0 (gm.)	TPW_0* (mg.)	$PBDW_{0\text{-}ppx}$* (mg.)	Resected Mg.	Resected %†	TBW_s (gm.)	TPW_s (mg.)	$PBDW_s$ (mg.)
0.5–7	csh	8	195 ± 1	755	350	—	—	210 ± 3	798 ± 10	351 ± 9
	ppx	23	195 ± 1	755	350	397	53	192 ± 2	347 ± 9	347 ± 2
14	csh	4	195 ± 2	755	350	—	—	245 ± 14	890 ± 32	390 ± 45
	ppx	4	189 ± 3	740	343	377	51	231 ± 4	394 ± 12	394 ± 12
30	csh	9	169 ± 1	676	322	—	—	293 ± 9	1078 ± 32	376 ± 22
	ppx	8	157 ± 5	664	308	356	53	281 ± 15	399 ± 29	399 ± 20
60	csh	12	181 ± 6	723	335	—	—	366 ± 12	1126 ± 52	437 ± 17‡
	ppx	11	185 ± 10	732	340	383	52	359 ± 11	519 ± 36	519 ± 36
180	csh	17	182 ± 4	725	336	—	—	490 ± 9	1249 ± 41	522 ± 18‡
	ppx	14	181 ± 4	723	335	380	52	499 ± 12	746 ± 18	746 ± 18
270	csh	16	176 ± 3	709	329	—	—	540 ± 15	1152 ± 38	509 ± 23‡
	ppx	16	176 ± 3	709	329	375	53	505 ± 16	760 ± 25	760 ± 25
360	csh	16	175 ± 2	708	328	—	—	561 ± 16	1208 ± 38	532 ± 20‡
	ppx	15	174 ± 2	705	327	384	54	552 ± 16	794 ± 16	794 ± 16

TBW_0 and TBW_s are total body weight at Day 0 and day of sacrifice, respectively. TPW_0 and TPW_s are total pancreas weights at Day 0 and day of sacrifice, respectively. $PBDW_0$ and $PBDW_s$ are parabiliary and duodenal segment pancreas weights at Day 0 and day of sacrifice, respectively. Figures are mean ± S.E.

Day 0.5–7 values are average of values of sacrifice days 0.5, 1, 2, 3, 5, and 7.

Resected (Col. 7) indicates the weight of the resected splenic and gastric pancreas segments in the partially pancreatectomized (ppx) animals.

* Estimates obtained from regression curves developed in this laboratory,[30] which related them to TBW_0. Note that the weight of the resected gastric and splenic segment tissue (Col. 7) plus estimated parabiliary and duodenal segment weight at Day 0 ($PBDW_{0\text{-}ppx}$, Col. 6) is usually within 5% of the estimated total pancreas weight at Day 0 ($TPW_{0\text{-}ppx}$, Col. 5).

†Percent of estimated $TPW_{0\text{-}ppx}$ (Col. 5).

‡ $p < 0.01$ (Col. 10).

Expressions of Regeneration

TPW_{s-csh}, $PBDW_{s-csh}$, and $PBDW_{s-ppx}$ values in milligrams are shown in Text-fig. 3 and included in Tables 1 and 2. Expressions of regeneration (Table 2) used were as follows.

1. Ratio of Difference in Weight Between $PBDW_{s-ppx}$ and $PBDW_{s-csh}$ to $PBDW_{s-csh}$. Because of the slow rate of regeneration, during which considerable normal growth occurred, $PBDW_{s-csh}$ weight might be considered to be made up of the $PBDW_{o-csh}$ plus the normal growth of these segments from Day 0 to the day of sacrifice. In the ppx group, the $PBDW_{s-ppx}$ would be made up of $PBDW_{o-ppx}$ plus the normal expected growth from Day 0 to the day of sacrifice and, in addition, the weight of the regenerated tissue if regeneration occurred. The difference between the two, related to $PBDW_{s-csh}$ (Table 2, Col. 2), could, in one sense, be considered to be a measure of regeneration in the ppx group:

$$\frac{PBDW_{s-ppx} - PBDW_{s-csh}}{PBDW_{s-csh}} \times 100 \quad (1)$$

Actually, some of the weight difference might be attributed to normal weight gain of regenerated tissue.

2. Ratios of $PBDW_{s-ppx}$ and $PBDW_{s-csh}$ to TBW_s. The $PBDW_s$ in both groups were expressed as ratios in terms of TBW_s (Table 2, Col 2 and 3).

$$\frac{PBDW_{s-ppx}}{TBW_{s-ppx}} \times 100 \quad (2)$$

$$\frac{PBDW_{s-csh}}{TBW_{s-csh}} \times 100 \quad (3)$$

3. Ratio of $PBDW_s$ to $PBDW_o$ as Percent The $PBDW_s$ was referred to its respective $PBDW_o$ and changed to percent (Table 2, Col. 5 and 6):

$$\frac{PBDW_{s-ppx}}{PBDW_{o-ppx}} \times 100 \quad (4)$$

$$\frac{PBDW_{s-csh}}{PBDW_{o-csh}} \times 100 \quad (5)$$

4. Ratio of $PBDW_s$ to TPW_o as Percent. The $PBDW_s$ was expressed as a percent of the corresponding TPW_o (Table 2, Col. 7 and 8):

$$\frac{PBDW_{s-ppx}}{TPW_{o-ppx}} \times 100 \quad (6)$$

$$\frac{PBDW_{s-csh}}{TPW_{o-csh}} \times 100 \quad (7)$$

5. Ratio of $PBDW_s$ to TPW_s as Percent. The relationship of $PBDW_s$ to the TPW_s in the two groups was expressed as a percent (Table 2, Col. 3 and 4):

$$\frac{PBDW_{s-ppx}}{TPW_{s-csh}} \times 100 \quad (8)$$

$$\frac{PBDW_{s-csh}}{TPW_{s-csh}} \times 100 \quad (9)$$

In Expression 8, resection of pancreas splenic and gastric segments in the ppx animals meant that there was not a comparable TPW_{s-ppx} for the denominator; so the control TPW_{s-csh} value was used.

Autoradiography

DNA synthesis was studied by tritium autoradiography [22] using tritiated thymidine [23] and the stripping film [24] or liquid emulsion techniques.[25,26] Animals were sacrificed by decapitation between 10 A.M. and 12 noon, 1 hr. after they were given (I.P.) thymidine-H^3, 0.25 μc./gm. body weight, 0.36 c./mM, in aqueous solution (Schwarz Bioresearch, Orangeburg, N.Y.).

At sacrifice, a portion of the duodenal pancreas, as close to the duodenum and as far away from the operative site as possible, was fixed in an aqueous 10% solution of formaldehyde (U.S.P.) containing $CaCO_3$ chips. After dehydration in alcohol and embedding in paraffin, histologic sections were cut at 2 μ. Autoradiograms were prepared with Kodak NTB-2 liquid emulsion, exposed for 30 days at 5° C., processed under standard conditions, and stained with hematoxylin and eosin.[27]

Two or more autoradiograms from the duodenal pancreas of each animal, with a few exceptions, were counted by at least two counters who used a randomized field selection technique.[28,29] The labeling of acinar cell nuclei was expressed as the labeling index—i.e., the percent of acinar cell nuclei counted which showed nuclear radioactivity.[24] The mean labeling index of a group on a day of sacrifice was determined by averaging the labeling indexes of all animals in the group. Our technique has been subjected to an analysis of variance,[30,31] and tables of maximum possible relative error (MPRE) and sensitivity (S) have been developed.[31,32]

Pancreas Radioactivity

Animals were given (I.P.) 0.25 μc./gm. TBW of thymidine-H^3, 0.36 c./mM (Schwarz Bioresearch, Orangeburg, N.Y.) 1 hr. prior to sacrifice. Duplicate samples of pancreas taken from areas as close as possible to the duodenal loop were homogenized separately in 1 ml. of Hyamine (Packard) in a glass homogenizing tube with a glass motor-driven pestle. The homogenate was added to 19 ml. of counting fluid (PPO-POPOP-toluene) in a counting vial.[33] Beta particle radioactivity was measured in a calibrated Tri-Carb liquid spectrometer (Model No. 527, Packard Instrument Co., LaGrange, Ill.) in duplicate samples, each counted twice for 10 min. Counts were corrected for background by counting the vials containing counting fluid and the nonradioactive pancreas and Hyamine, and subtracting the counts obtained.

Pancreas Fat-free Dry Weight

For the determination of dry weight, parabiliary pancreas tissue of known wet weight was de-fatted in diethyl ether-alcohol (1:3 v/v) for 3 days and in diethyl ether for 1 day. The specimen was dried in an oven at 100° C. to a constant weight. The defatted dried weight was expressed as a percent of the wet weight and was determined at all sacrifice days after Day 7.

Biochemical Determinations

Standard techniques, or modification of them for small amounts of rat pancreas tissue, were used for determination of amylase,[9,34] chymotrypsinogen,[9,35] nitrogen,[9,36] and DNA.[9,37-39]

In those animals in which serum determinations were to be made, 1 ml. of Nembutal was given (I.P.), the thorax was opened, and approximately 10 ml. of blood was drawn in a syringe from the beating left ventricle. Serum levels of electrolytes, total protein, albumin, calcium, glucose, urea nitrogen, total bilirubin, alkaline phosphatase, and glutamic oxalacetic transaminase were measured in a Technicon SMA-12 AutoAnalyzer.[40] Serum amylase was determined by a manual method.[34]

Statistical Methods

Standard statistical methods were used.[21] Since total body weights in the groups were not statistically different except during the days of the first postoperative week, when the sh and ppx animals had lower TBW_s, the c and sh group values were combined into a csh group. All values were expressed as the mean ± one standard error of the mean. A harmonic mean analysis was done on Table A (in reprints only), and multiple comparisons were carried out.[21,30]

Results

Total Body Weight

With the exception of the first postoperative week, there were no significant differences between the average TBW_o or TBW_s of the control, sham-operated, and partial pancreatectomy animals (Table 1, Col. 4 and 8, respectively).

Pathologic Changes

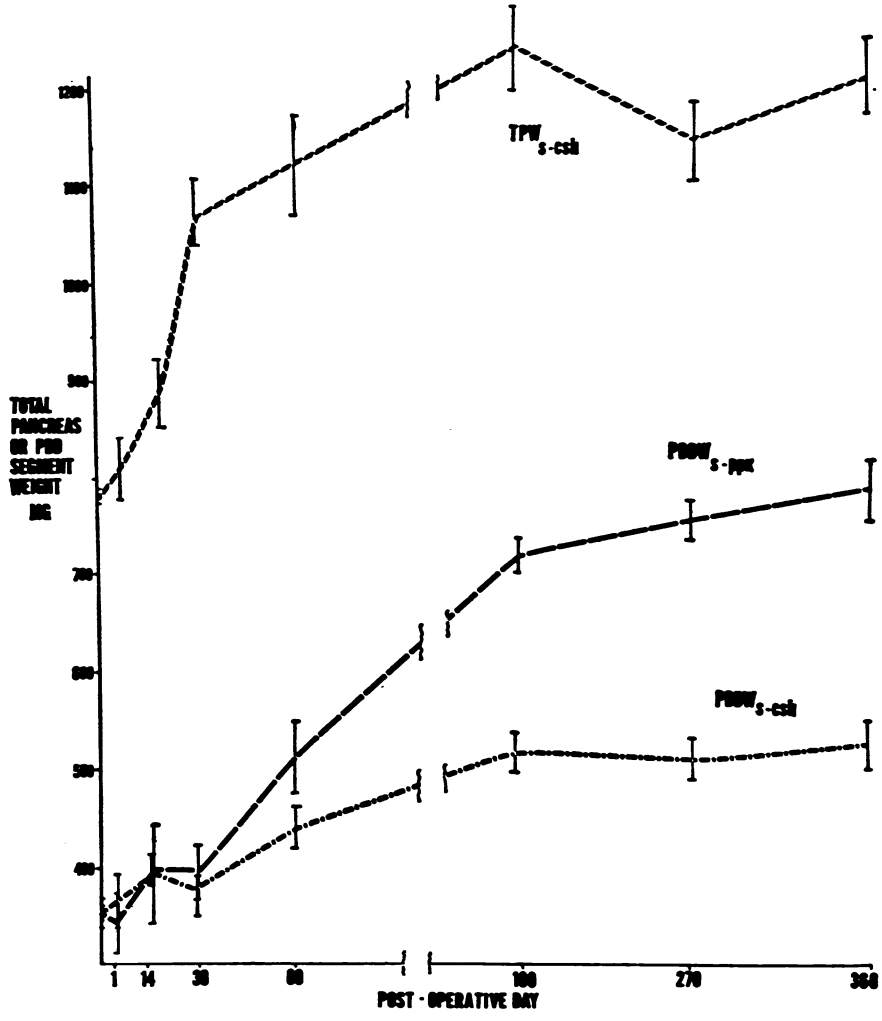
During the first few postoperative days there was a gross inflammatory response at the operative site in the ppx group. Slight edema and hyperemia occurred in the duodenal and parabiliary areas in the sh and ppx groups. Some peritoneal surfaces were lightly adherent to each other. At 2 weeks after operation, the peritoneal surfaces were free of adhesions and there was no gross evidence of inflammation. No pancreas tissue was present in the splenic or gastric segment areas at any time. The residual duodenal and parabiliary pancreas segments in the ppx animals appeared after 6 months to be thicker than the corresponding segments in the c and sh animals.

Microscopically, during the first few postoperative days there was, in many sh and ppx animals but not in all, a moderate peripancreatic and a slight interstitial inflammatory response in the duodenal and parabiliary segments of the pancreas. Most inflammatory cells were spindle-shaped—presumably fibroblasts or endothelial cells—with some macrophages and varying numbers of polymorphonuclear leukocytes, including eosinophils. Interstitial edema was prominent for the first week. Mitotic figures, uncommon in the controls, were increased in the ppx animals at Days 1–3 after operation. In the tissue at the operative site within the first week there was some necrosis with the expected acute inflammatory reaction. No degenerative changes were noted in the acinar cells of the PBD segments in any animals.

In the ppx animals of 2, 6, 9, and 12 months, there was no increase of collagen or inflammatory cells or other change to explain the increase in weight of the residual duodenal and parabiliary segments.

Pancreas Wet Weights

Our resection of splenic and gastric segments removed 52.8% ± 2% of the total pancreas. Through postoperative Day 30 there were no significant differences between $PBDW_{s-ppx}$ and the $PBDW_{s-csh}$ (Table 1, Col. 10; Table 2, Col. 2; and Text-fig. 2). At 60, 180, 270, and 360 days after operation, significant differences were observed ($p<0.01$; Tables 1 and 2 and Text-fig. 2).



TEXT-FIG. 2. Total pancreas weight (in milligrams) in control-sham operated group (TPW_{s-csh}), parabiliary-duodenal weight in partial pancreatectomy group ($PBDW_{s-ppx}$), and parabiliary-duodenal weight in the control-sham operated group ($PBDW_{s-csh}$) on postoperative sacrifice days.

Expression 1, the difference between the PBD_s segment weights expressed in terms of $PBDW_{s\text{-}csh}$, gave 49% difference at Days 270 and 360. Correction for individual differences in average TBW_s (Expressions 2 and 3) gave higher values, 60% and 53%, respectively (Table 2, Col. 2 and 3, figures in parentheses).

Expressions 8 and 9 (Table 2, Col. 3 and 4) indicated that the $PBDW_{s\text{-}ppx}/TPW_{s\text{-}csh}$ ratio was about 50% higher than the $PBDW_{s\text{-}csh}/TPW_{s\text{-}csh}$ ratio at Days 180, 270, and 360. The results show that the $PBDW_{s\text{-}ppx}$ segment attained a weight of about 66% of the $TPW_{s\text{-}csh}$ at Days 270 and 360, whereas the $PBDW_{s\text{-}csh}$ was about 44% of the $TPW_{s\text{-}csh}$.

When the $PBDW_s$ in the two groups were referred to their corresponding $PBDW_o$ (Expressions 4 and 5), it was found that the ppx group ratio was about 50% higher at Days 270 and 360 (Table 2, Col. 5 and 6). The results suggest that weight difference between the ppx and csh groups began between 2 weeks and 1 month after operation, increased roughly linearly until about 6 months, and then increased at a much lower rate (Table 2, Col. 5 and 6). Similar results were obtained when the $PBDW_s$ of both groups were expressed in terms of their respective TPW_o (Table 2, Col. 7 and 8, Expressions 6 and 7).

At all sacrifice days the ratio of $TPW_{s\text{-}csh}$ to $TBW_{s\text{-}csh}$ was greater than that of $TPW_{s\text{-}ppx}$ to $TBW_{s\text{-}ppx}$ because of the loss of resected pancreas in the latter (Table 1, Col. 8 and 9). The difference became less with time because of the regeneration of tissue in the ppx animals. In this comparison, $PBDW_{s\text{-}ppx}$ became $TPW_{s\text{-}ppx}$ because of the resection of gastric and splenic segments (Table 1, Col. 9 and 10).

When the $PBDW_{s\text{-}ppx}$ weight was expressed as a ratio of $TPW_{o\text{-}ppx}$, the regenerating PBD_{ppx} segments reached the preoperative total pancreas weight at postoperative Day 180 (Table 2, Col. 7).

Fat-free Dry Weight

At no point was there a significant difference between the control, sham, and pancreatectomy values.

Pancreas Radioactivity

In the ppx and sh animals there generally was an increase in tissue radioactivity above control values at 1–2.5 days after operation (Table 3 and Text-fig. 3). The radioactivity was usually higher in the ppx group than in the controls at these times and often higher in the sham-operated animals than in the controls. The ppx value was significantly increased above the control level at 30 hr. and was higher than that of the sham-operated animals in most cases from 1 to 2.5 days.

Table 2. Pancreas Weights as Given by Expressions 1–9

(1)	(2) EXPR. 1 (2 & 3)	(3) EXPR. 8 (2 & 3)	(4) EXPR. 9	(5) EXPR. 4	(6) EXPR. 5	(7) EXPR. 6	(8) EXPR. 7
Postop. day	$\frac{PBDW_{a\text{-}ppx} - PBDW_{a\text{-}csh}}{PBDW_{a\text{-}csh}} \times 100$ (%)	$\frac{PBDW_{a\text{-}ppx}}{TPW_{a\text{-}csh}} \times 100$ (%)	$\frac{PBDW_{a\text{-}csh}}{TPW_{a\text{-}csh}} \times 100$ (%)	$\frac{PBDW_{a\text{-}ppx}}{PBDW_{o\text{-}ppx}} \times 100$ (%)	$\frac{PBDW_{a\text{-}csh}}{PBDW_{o\text{-}csh}} \times 100$ (%)	$\frac{PBDW_{a\text{-}ppx}}{TPW_{o\text{-}ppx}} \times 100$ (%)	$\frac{PBDW_{a\text{-}csh}}{TPW_{o\text{-}csh}} \times 100$ (%)
1	−2 (1)	44 (45)	45	99	101	46	47
14	−1 (8)	44 (46)	44	115	111	53	52
30	−6 (11)	37 (39)	35	130	117	60	54
60	19 (18)	46 (47)	39	153	130	71	61
180	43 (42)	60 (59)	42	223	156	103	72
270	49 (60)	66 (70)	44	231	155	107	72
360	49 (53)	66 (67)	44	243	163	113	75

Numbers in parentheses (Col. 2 and 3) indicate values when correction was made for different TBW in ppx and csh groups (Expressions 2 and 3).

Autoradiographic Labeling

After an initial postoperative drop, there was a significant increase ($p < 0.02$) at 36 hr. after operation in the labeling index of the acinar cells in the ppx animals above the average indexes of the combined control and sham-operated animals (Fig. 1 and Table A,* and Text-fig. 4). The peak in the ppx animals was three times that of the control and sham animals and about six times the values found for comparable controls used in this laboratory over many years.[33] At all other subsequent times there were no significant differences between groups.

The number and labeling indexes of peripancreatic and interstitial cells in the duodenal segment of the ppx and sh animals, although not counted, appeared to be increased at 1 and 2 days after operation (Table 3). The labeling was primarily in the spindle-shaped cells or macrophages. Counting data suggested that these labeling indexes in the ppx and sh animals reached a peak at about 30–32 hr. after operation (Table 3).

* Table A, not presented in this report is available with authors' reprints.

Table 3. Radioactivity in Duodenal Segment of Pancreas after Thymidine-H^3

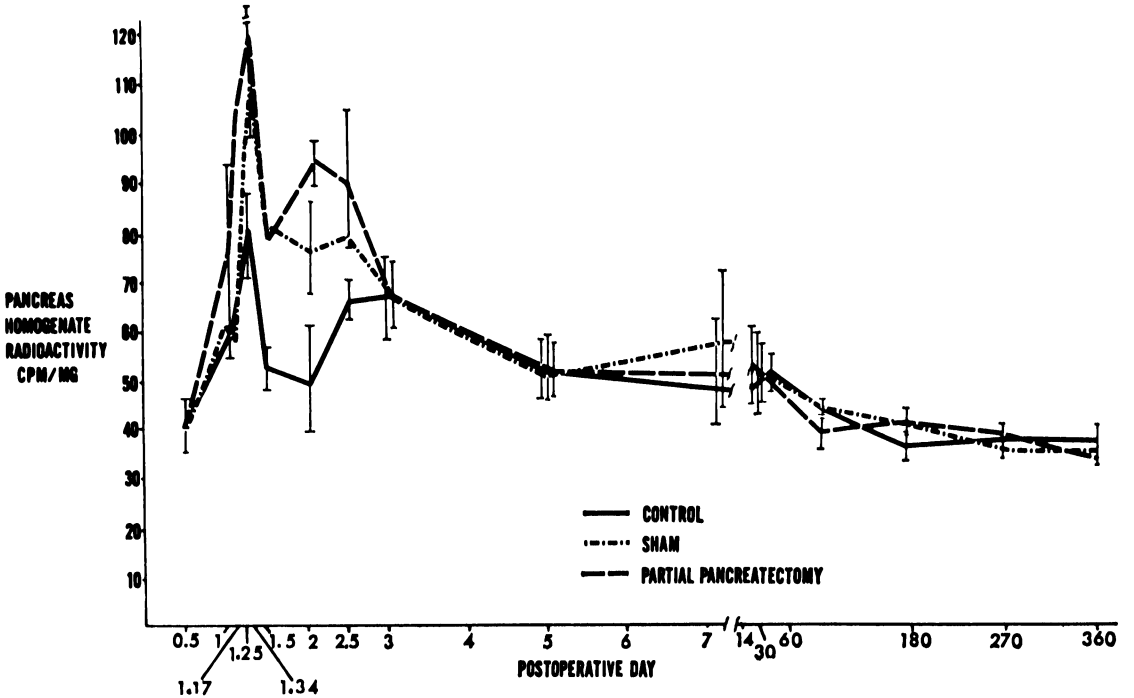
Postop. day	Radioactivity (cpm/mg. wet wt.) Control (c)	Sham-operated (sh)	Partial pancreatectomy (ppx)
0.5	41 ± 5 (2)	40 ± 3 (4)	41 ± 2 (5)
1.0	—	61 ± 13 (4)	74 ± 18 (7)
1.17	62 ± 3 (6)	58 ± 3 (6)	102 ± 7 (6)
1.25	81 ± 9 (6)*	96 ± 13 (6)*	110 ± 10 (6)*
1.34	76 ± 13 (5)	110 ± 10 (6)	119 ± 19 (5)
1.5	54 ± 3 (2)†	82 ± 10 (4)†	80 ± 7 (7)†
2.0	48 ± 12 (2)†	77 ± 11 (4)†	94 ± 4 (6)†
2.5	65 ± 4 (2)	79 ± 4 (3)	91 ± 17 (5)
3.0	67 ± 5 (2)	66 ± 9 (4)	67 ± 8 (7)
5.0	52 ± 7 (2)	48 ± 9 (2)	52 ± 4 (4)
7.0	—	60 ± 12 (2)	55 ± 9 (3)
14.0	46 ± 3 (2)	49 ± 10 (2)	51 ± 6 (4)
30.0	53 ± 7 (4)	50 ± 9 (5)	49 ± 5 (8)
60.0	45 ± 4 (5)	45 ± 6 (7)	40 ± 3 (11)
180.0	37 ± 4 (12)	40 ± 3 (5)	41 ± 2 (14)
270.0	39 ± 4 (8)	35 ± 3 (8)	42 ± 3 (16)
360.0	38 ± 3 (8)	36 ± 3 (8)	34 ± 4 (15)
Total	(68)	(80)	(129)

Thymidine-H^3, 0.25 μc./gm. total body weight, given intraperitoneally. Measurements made 1 hr. later.

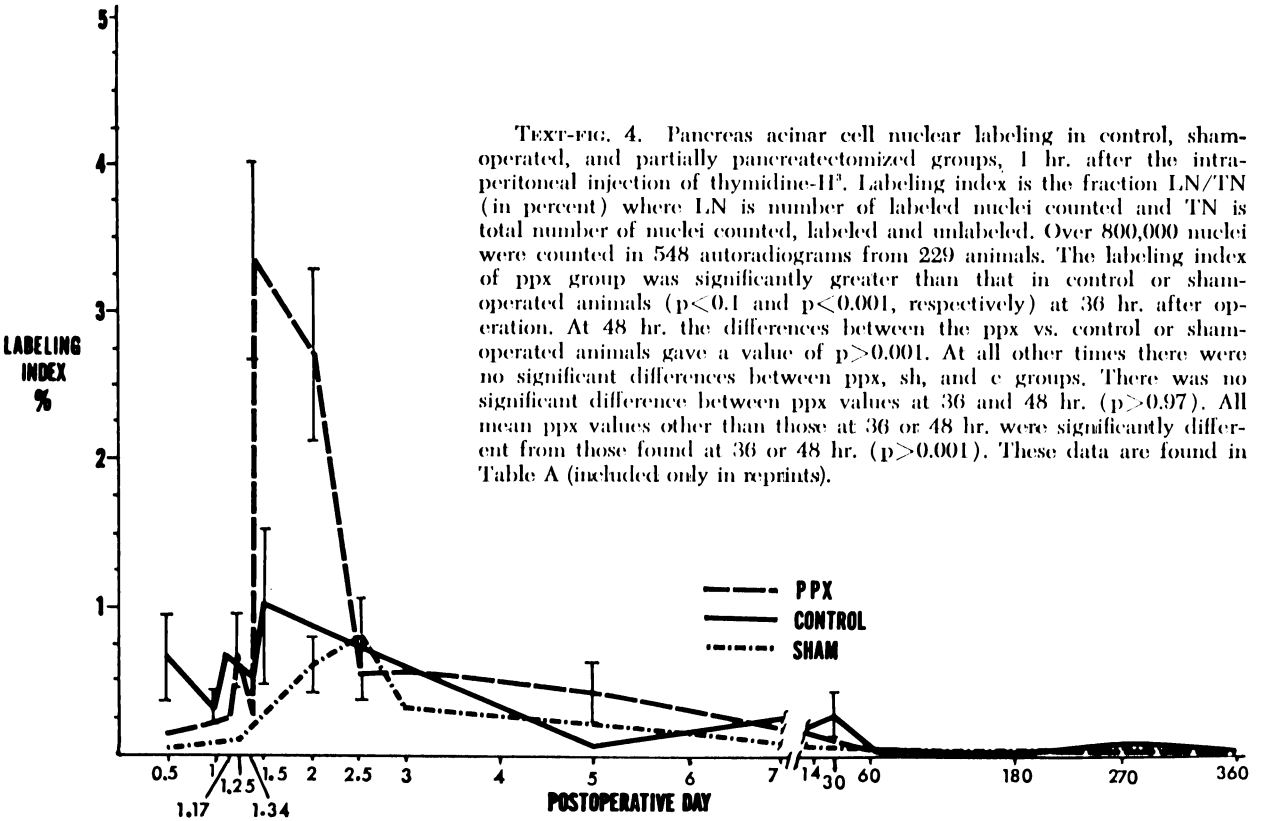
Numbers in parentheses indicate number of animals sacrificed.

* At Day 1.25 (30 hr.) the radioactivity of the ppx group was significantly greater than that of the control groups, using multiple comparisons—3 comparisons for each group ($p < 0.075$).

† At 1.5 and 2 days after operation there was no significant difference between ppx and sh groups ($p > 0.50$), but both were significantly different from c groups ($p < 0.01$).



TEXT-FIG. 3. Pancreas homogenate radioactivity 1 hr. after injection of thymidine-H^3 on postoperative days in control, sham-operated, and partial pancreatectomy groups. Radioactivity expressed in cpm/mg. of homogenate (Table 3).



TEXT-FIG. 4. Pancreas acinar cell nuclear labeling in control, sham-operated, and partially pancreatectomized groups, 1 hr. after the intraperitoneal injection of thymidine-H^3. Labeling index is the fraction LN/TN (in percent) where LN is number of labeled nuclei counted and TN is total number of nuclei counted, labeled and unlabeled. Over 800,000 nuclei were counted in 548 autoradiograms from 229 animals. The labeling index of ppx group was significantly greater than that in control or sham-operated animals ($p<0.1$ and $p<0.001$, respectively) at 36 hr. after operation. At 48 hr. the differences between the ppx vs. control or sham-operated animals gave a value of $p>0.001$. At all other times there were no significant differences between ppx, sh, and c groups. There was no significant difference between ppx values at 36 and 48 hr. ($p>0.97$). All mean ppx values other than those at 36 or 48 hr. were significantly different from those found at 36 or 48 hr. ($p>0.001$). These data are found in Table A (included only in reprints).

Table 4. Analyses of Pancreas Homogenate

Postop. day	Group	No. of rats	N (mg./ml. homogenate)	DNA* (per ml. homogenate)	DNA* (per mg. N)	Amylase† (U./mg. N)	Chymotrypsinogen† (U./mg. N)
180	csh	17	7.0 ± 0.3	—	—	34.8 ± 2.9‡	—
	ppx	14	7.0 ± 0.2	—	—	25.9 ± 2.5	—
270	csh	16	7.3 ± 0.3	2.5 ± 0.1	0.36 ± 0.02	23.0 ± 1.4	20.1 ± 1.4§
	ppx	16	6.5 ± 0.3	2.4 ± 0.1	0.37 ± 0.02	20.5 ± 1.2	24.4 ± 1.5
360	csh	16	7.1 ± 0.4	2.4 ± 0.1	0.33 ± 0.02	26.8 ± 1.7	22.3 ± 1.2
	ppx	15	7.1 ± 0.4	2.4 ± 0.0	0.34 ± 0.02	27.1 ± 1.8	22.8 ± 1.5

Methods as described in Marsh et al.[9] Values are mean ± standard error.
* DNA expressed as μM deoxyribose.
† Expressed as units of enzyme activity in relation to activity of purified control standards.[9]
‡ Differences between ppx and csh groups, $p < 0.01$.
§ Differences between ppx and csh, $0.05 > p > 0.02$.

It is emphasized that there was no direct correlation between interstitial cell number or labeling and the labeling of contiguous acinar cells. Many autoradiograms showing the highest labeling indexes were free of inflammation (Fig. 1) and the opposite was also true: Inflammation was present in some autoradiograms with no increase in the labeling index of contiguous or nearby acinar cells.

Pancreas Biochemical Analyses

At Day 180 there was a significantly lower amylase activity per milligram nitrogen in pancreas homogenate in the ppx rats than in the c rats ($p < 0.01$; Table 4). Chymotrypsinogen activity per milligram N was elevated in the ppx animals at Day 270 ($0.05 > p > 0.02$). Values of other tests showed no significant differences between the two groups.

Serum Analyses

In the data from 13 biochemical tests on serum at postoperative Days 270 and 360 (Table 5), there were no significant differences between the groups.

Table 5. Serum Analyses

Post-op. day	Group	No. of rats	Na^+ (mEq./L.)	K^+ (mEq./L.)	Cl^- (mEq./L.)	CO_2 (mEq./L.)	Ca^{++} (mg./100 ml.)
270	csh	15	145 ± .51	5.8 ± .13	100 ± .38	28 ± .76	10.4 ± .09
	ppx	15	144 ± .61	6.1 ± .09	101 ± .58	27 ± .54	10.5 ± .10
360	csh	12	145 ± .60	5.6 ± .12	100 ± .64	28 ± .51	10.4 ± .07
	ppx	11	145 ± .82	5.8 ± .08	99 ± .10	27 ± .83	10.4 ± .11

Serum analyses were performed in the SMA-12 AutoAnalyzer[40] except for amylase determinations, which were done manually.[34] All values are mean ± standard error.

Discussion

Hypertrophy

By the criteria that we have used to describe weight differences (Expressions 1–9) and the labeling index changes, the $PBDW_{s\text{-}ppx}$ pancreas weights of 50–60% above the $PBDW_{s\text{-}csh}$ control values at 9 months to 1 year after operation indicate significant regeneration.[41–45]

The increase of $PBDW_{o\text{-}ppx}$ from a value of about 44% of the $TPW_{o\text{-}ppx}$ to a 9-months postoperative figure of about 66% of the $TPW_{s\text{-}csh}$ is similar to Liosner's findings in other organs that when one of a pair of organs is removed, restoration to about 70–80% of the weight of the two organs occurs.[46] The pancreas after ethionine shows a similar amount of regeneration.[5,7,8,33]

Pancreatic regeneration after partial pancreatectomy proceeded at a slower rate than did rat liver regeneration after partial hepatectomy wherein total organ weight was restored to the preoperative level within 1–3 weeks.[47–50] There is also a more rapid weight gain in the contralateral kidney after unilateral nephrectomy,[51,52] in the remaining adrenal following unilateral adrenalectomy and adrenal enucleation,[53] and in the pancreas after ethionine destruction of acinar tissue.[5,7,8] In the present study it was 6 months after operation before the $TPW_{s\text{-}ppx}$ attained the Day 0 pancreas weight, and during such a long postoperative period some of the weight gain could be attributed to growth of the regenerated pancreas. The tapering off in weight gain differential between the ppx and control groups 6 months after operation may have been related, in part, to the same mechanism which occasioned a similar leveling off of weight in the $TPW_{s\text{-}csh}$ group (Text-fig. 2).

Cameron's study suggested regeneration of the guinea pig pancreas after partial pancreatic resection.[19] Recently, Segida, using a small number of guinea pigs, stated that there was restoration of pancreas weight 4 months after resection of one-third of the gland. Surprisingly, she also

Table 5. Serum Analyses (continued)

Total proteins (gm./ 100 ml.)	Albumin (gm./ 100 ml.)	Urea nitrogen (mg./ 100 ml.)	Glucose (mg./ 100 ml.)	Total bilirubin (mg./ 100 ml.)	Amylase (Somogyi U.)	AlkPase (K.-A. U.)	GOT (K.-A. U.)
7.2 ± .07	2.7 ± .10	18 ± .64	198 ± 4.7	0.6 ± .06	3042 ± 264	40 ± 2.9	199 ± 15
7.3 ± .08	2.8 ± .07	17 ± .48	229 ± 18.5	0.7 ± .07	3287 ± 369	41 ± 2.7	213 ± 16
7.3 ± .10	3.1 ± .14	20 ± .87	178 ± 13.6	0.4 ± .05	2154 ± 330	39 ± 2.5	201 ± 14
7.2 ± .12	3.0 ± .13	19 ± .67	179 ± 8.3	0.5 ± .05	3106 ± 516	38 ± 2.2	206 ± 12

reported that at 6 months the hypertrophied glandular tissue had disappeared.[16] Martin and Lacy indicated that there was considerable regeneration of pancreas in young rats after resection of most of the pancreas.[14]

Hyperplasia

Acinar Cell. The peak of the labeling index curve at 36 hr. in the ppx animals resembled, in time, the peak of regenerating liver (24–30 hr. after hepatectomy).[49,50] The kidney after the administration of toxic compounds,[51] the remaining kidney after unilateral nephrectomy,[52] the adrenal after unilateral adrenalectomy and enucleation,[53] the salivary gland after isoproterenol,[54] and the pancreas after ethionine [5,7,33] appear to reach peaks of mitosis or of labeling indexes a few days after operation, or after the administration, or cessation, of the initiating compound.

The peak of the labeling index or mitotic index is relatively very high in the liver after partial hepatectomy,[49,50] high in the salivary gland after isoproterenol [54] and in the renal tubules after heavy metals,[51] lower in the pancreas after ethionine,[5,7,33] and lower in the kidney after unilateral nephrectomy.[52] The peak of labeling in the acinar cells of three to six times control values after partial pancreatectomy appears to approximate the last model. Presumably, the peak of pancreas regeneration would be higher in younger animals or if more pancreas were removed.[14]

In these models of regeneration, hyperplasia occurred relatively soon after resection, cell destruction, or stimulation. Increases in organ weight took place later. The rate of increase of organ weight appeared to be related, roughly, to the magnitude and duration of increase of the labeling index. In kidney regeneration following unilateral nephrectomy an assessment of the relative contributions of hyperplasia and hypertrophy to regeneration has been made.[52] It should be noted that although the rates of pancreas regeneration differed in post-ethionine and post-partial-pancreatectomy studies, in both models the restoration of total organ weight was approximately to the same percent of control total organ weight.[8,46]

Nonacinar Cell. Although the labeling indexes were not determined for nonacinar, nonislet cells,[33] hyperplasia of these cells appeared to be considerable during the first few days after operation in both the sh and ppx groups.

Labeling Index Increase with Operative Stress and Tissue Injury

The increased pancreas homogenate radioactivity values at 1–2.5 days after operation in the ppx and sh groups were probably related,

in part, to increases in the number and/or the labeling indexes of interstitial cells. The increased labeling of interstitial cells at 36 hr. obscured, in the organ homogenate, the significant difference in nuclear labeling indexes found between the acinar cells of the ppx and sh animals by autoradiography (Text-fig. 4).

Segida reported that in the residual pancreas of the guinea pig subjected to splenic segment resection, there was an increase in acinar cell mitotic activity at 72 hr. The tissue examined was taken from the operative site.[17] Pancreas tissue used for labeling index determinations in our study was carefully selected from the duodenal segment of pancreas near the duodenum, as far from the operative site as possible. This selection was made because previous observations from this laboratory showed that inflammatory and degenerative changes secondary to operative ligation of the splenic artery or duct in the pancreas splenic segment of the rat were associated, at 3–7 days after operation, with significant increases in nuclear labeling in the degenerating acinar cells of the involved pancreas segment.[55,56] It is possible that degenerative changes in tissue released substances which acted as de-repressors, permitting an increase of DNA synthesis.[7,8,33,55,56] However, no degenerative changes were noted in any PBD acinar cells of the present study.

We believe that the significantly increased labeling of the acinar cell in the ppx animals at 36 hr. after operation is not the result of nonspecific operative factors but is a specific integral part of the regenerative process.

Biochemical Changes

The absence of significant differences in most values for amylase and chymotrypsinogen in serum or pancreatic tissue among control, sham, and ppx animals implies that pancreas function was essentially normal at 360 days after operation. Their abnormal values at 180 and 270 days are difficult to interpret. Relatively little pancreas tissue can sustain pancreatic exocrine function,[57] and as little as 5% can prevent the development of diabetes.[58] However, rates of enzyme synthesis and secretion would also have to be examined to determine whether the regenerated tissue functioned at control rates.

Mechanism of Regeneration

After partial pancreatectomy no significant amount of pancreas tissue was found in the area where the splenic and gastric segments had been resected, and the residual duodenal segment appeared thicker than that

of the control animals. More rapid and extensive regeneration occurred after ethionine administration, where there was relative preservation of the reticulum scaffolding of the gland.[8] Possibly the lack of such a connective tissue framework may have limited regeneration in the animals with partial pancreatectomy. After partial hepatectomy, however, the resection of two-thirds of the liver does not prevent rapid extensive liver regeneration. Since the animals used in the ethionine experiments and those used in this study were similar in total body weight, strain, sex, and dietary regimen, and had been exposed to the same environment, these factors could not be invoked to explain the difference in the rates of regeneration.

When an organ is partially resected or destroyed or one of a pair of organs is removed, the sudden decrease of organ mass or the drop in level of one of its products may set up a negative feedback mechanism[59] which leads eventually to regeneration. One of us has suggested that ethionine causes a greater decrease of a repressor substance in the pancreas acinar cell than the decrease of protein(s) required for DNA synthesis. This could lead to an increase of DNA synthesis.[7,8,33] It would appear possible that ethionine and partial pancreatectomy increase DNA synthesis in the pancreas by different mechanisms although, in both cases, a loss of pancreas tissue, or one of its secretory products, could be the factor initiating DNA synthesis. After partial pancreatectomy there might not be present the additional factor of the de-repression which may be present in regeneration after ethionine. Thus, regeneration after partial pancreatectomy would not be boosted beyond the limited impetus given by the loss of pancreas tissue. In post-ethionine regeneration the combination of a loss of pancreas tissue and the relative deficiency of a repressor protein might be responsible for the difference in the rates of regeneration.

Conclusions

1. Following resection of the splenic and gastric segments of the rat pancreas, there was increase in the weight of the residual parabiliary and duodenal (PBD) segments of pancreas so that at 9 and 12 months after operation the residual segments ($PBDW_{s-ppx}$) weighed about 50% more than the corresponding PBD segments in control (c) and sham-operated (sh) animals ($PBDW_{s-csh}$).

2. The average weight of the residual pancreas of the ppx animals increased from about 44% of the total pancreas weight at operation to about 66% of the total pancreas weight of the control animals at 9 and 12 months after operation.

3. DNA synthesis in pancreas acinar cells, as studied by autoradiography with thymidine-H^3, showed a significant—at least three fold—increase above control values 36 hr. after operation.

4. In the regenerated pancreas tissue, histologic and cytologic features were generally similar to those of control animals except for an increase in mitotic figures at 1 and 2 days after operation.

5. Amylase and chymotripsinogen enzyme activities of pancreas tissue examined at 12 months were essentially similar in c, sh, and ppx groups.

6. Compared to the rates of regeneration of such organs as the liver after partial hepatectomy, the kidney after unilateral nephrectomy, the pancreas after ethionine, or the adrenal after unilateral adrenalectomy and enucleation, the rate of regeneration of the adult rat pancreas after partial pancreatectomy in this study was relatively very slow.

7. It would appear from the different rates of pancreas regeneration noted in post-ethionine and post-partial-pancreatectomy studies that different mechanisms may operate in these two models to increase DNA synthesis even though both may be initiated by the loss of pancreas tissue.

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Legend for Figure

Fig. 1. Autoradiogram of pancreas of partially pancreatectomized rat 36 hr. after operation. Animal given 0.25 μc. of thymidine-H^3 I.P. 1 hr. prior to sacrifice. Eleven or twelve acinar cell nuclei are labeled with thymidine-H^3. In controls, on the average, about 1 of 200 nuclei is labeled. Formalin fixation, hematoxylin and eosin staining, Eastman Kodak NTB2 emulsion, 1-mo. exposure. × 1020.