Pancreatic Acinar Cell Regeneration

II. Enzymatic, Nucleic Acid, and Protein Changes

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THE PRECEDING REPORT[1] described cytologic, cytochemical, and histologic restitution of the pancreas tissue and restoration of the gland weight after destruction of most of the acinar epithelium by ethionine. Here we record the changes in some enzyme activities during pancreatic acinar cell degeneration and regeneration. The enzyme activities measured were: alpha amylase (E.C.3.2.1.1.), chymotrypsinogen (E.C.-3.4.4.5.), lipase (E.C.3.1.1.3), beta glucuronidase (E.C.3.2.1.31), acid phosphatase (E.C.3.1.3.2), and alkaline phosphatase (EC.3.1.1.31). Fat-free dry weight, nitrogen, and RNA and DNA concentrations were also determined.

Methods and Materials

The general design of experiments and the details concerning diets, animals, and sacrifice times have been presented in the preceding paper.[1]

Pancreas enzyme activities or concentrations of compounds in individual animals of a group sacrificed on the same day, from multiple experiments (at least 2), were averaged and expressed as a percentage of the average value obtained from animals receiving the control stock diet (SD) and sacrificed on the same day. The SD values were set at 100%. Occasionally, on a sacrifice day when the ethionine reduced the amount of pancreas available, it was necessary to pool the tissue from 2 or 3 animals for a determination. Enzymatic activities and nucleic acid concentrations were expressed in terms of milligrams of nitrogen. The latter was expressed as milligrams per milliliter of stock pancreas homogenate.

Mean and standard error of the mean were determined by the usual statistical procedures.[2]

Preparation of Homogenates

At sacrifice, the pancreas was removed, weighed, and chilled with ice water as quickly as possible. The tissue was homogenized at 0° C. in 0.15 M aqueous KCl, and

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diluted to give a final tissue concentration of 300 μg./ml. of stock solution. All enzyme methods were adapted to utilize sample volumes of 10–100 μl. Dilutions of the stock were made to achieve these volumes. All homogenates were maintained at 0° C. and assayed as soon as practical on the day of sacrifice.

Fat-free Dry Weight

Small aliquots of pancreas tissues were weighed rapidly at sacrifice (Mettler analytical balance), placed on preweighed squares of aluminum foil, and dried in an oven at 105° C. to a constant weight over a 3-day period. They were then extracted three times with a 3:1 (v/v) alcohol-ether solution, dried in the oven, and reweighed. The results were expressed as a ratio

$$\frac{\text{fat-free dry weight}}{\text{total wet weight}}$$

and changed to per cent. The latter was expressed as a percentage of the SD value which was taken as 100%.

Nitrogen

Nitrogen was determined by a micro-Kjehldahl method on 10–25 μl. of sample.[3]

Deoxyribonucleic and Ribonucleic Acid

The nucleic acids were separated and assayed by a combination of the Schneider method using perchloric acid and spectrophotometric estimation[4] and the modification of the technique of Scott, Fraccastoro, and Taft.[5] RNA was expressed as milligrams per milligram of nitrogen on the basis of its absorption as compared to a yeast RNA standard (Nutritional Biochemicals Co., Cleveland). DNA (Burton-Dische reaction[6]) was compared to a known sample of deoxyribose (Mann Research Labs., New York) with the DNA expressed in micromole equivalents per milligram of nitrogen.

Enzymes

Amylase. The test was a micromodification (25 μl. of sample) of the method of Fingerhut *et al.*[7] with a 15-min. incubation period. Split starch products were determined by reduction of ferricyanide to ferrocyanide. The latter coupled with phosphomolybdic acid produced the final color. One microgram of amylase could be assayed as determined by the use of crystallized amylase (Worthington Biochemicals, Freehold, N.J.) as the standard.

Chymotrypsinogen. Chymotrypsinogen was converted to chymotrypsin with 50 μg. of chymotrypsin-low trypsin (Mann Research Labs.). Reaction was complete in 10 min. at room temperature. A micromodification of the method of Hummel[8] using trypsin-resistant N-benzoyl-L-tyrosine ethyl ester (BTEE) substrate was used. The method could assay 1 μg. of crystallized chymotrypsin (Worthington).

Lipase. With an expanded-scale pH meter, fatty acid formation was titrated at pH 8.0 with .01 N NaOH under nitrogen by the method of Marchis-Mouren, Sarda, and Desnuelle.[9] The substrate was stabilized olive oil emulsion (Sigma Chemical Co., St. Louis, Mo.) with both calcium chloride and sodium taurocholate added as "activators." Reaction rates were linear for up to 10 min. with suitable dilution. It was possible to assay 500 μg. of the impure lipase standard (Worthington).

"Lysosomal" Hydrolases

Triton X-100 (Rohm & Haas, Philadelphia, Pa.) was added during homogenization by the method of deDuve *et al.*[10]

Acid Phosphatase–Alkaline Phosphatase. Acid and alkaline phosphatases were

determined by a micromodification of the method of Kind and King.[11] Disodium phenylphosphate was employed as substrate. Use of 4-amino antipyrine and ferricyanide permitted color development to proceed in the presence of protein. If turbidity developed, filtration through millipore filters prior to absorption measurement was performed. Acid phosphatase determinations were performed at a pH of 4.6 and alkaline phosphatase at pH 10.0.

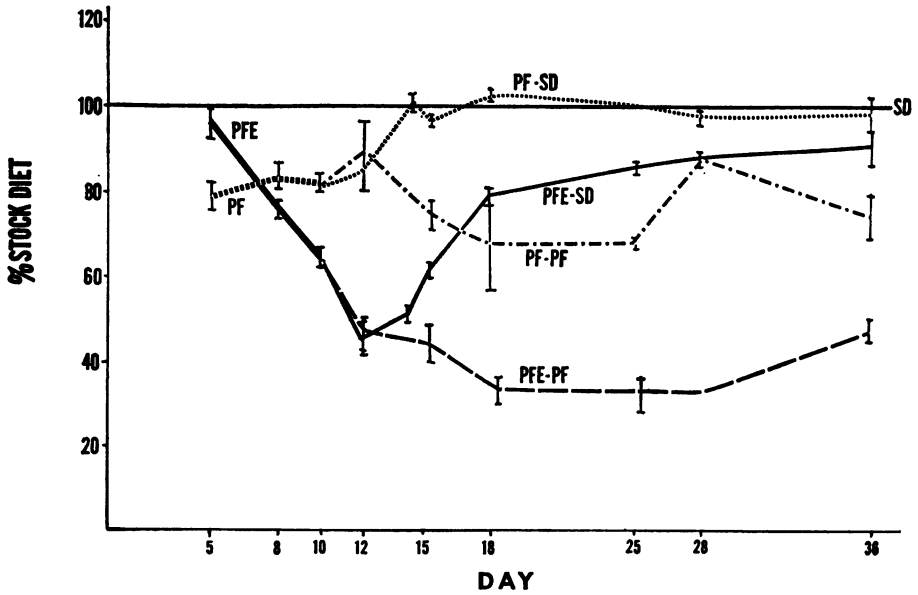
Beta Glucuronidase. A micromodification of the method of Fishman, Springer, and Brunetti,[12] using phenolphthalein glucuronide, was employed. The amount of substrate split in micromoles per minute at pH 4.5 and 37° C. was regarded as a unit.

Results

Fat-free Dry Weight

Under the PFE-SD regimen (Table 1 and Text-fig. 1), the fat-free dry weight decreased to 45% of the SD value at Day 12 and thereafter increased until it reached 90% of the SD value at Day 36. The PF-SD values decreased less and returned relatively quickly to SD values.

The PFE-PF values dropped to lower levels than the PFE-SD values and remained at a low level. PF-PF values decreased less and remained at a higher level than those of the PFE-PF regimen.



TEXT-FIG. 1. After aliquots of pancreas had been defatted and dried to constant weight, results were expressed as a ratio of fat-free dry weight to total wet weight. This value was compared to stock diet wet weight values, which were set at 100%. PF indicates protein free diet; PFE, protein free diet with ethionine injections; and SD, stock diet. In this and subsequent text-figures, regimen for experimental animals for the first 10 days was either PF or PFE. Except in experiment illustrated by Text-fig. 10, subsequent diets were either SD or PF. In all curves of text-figures, verticle lines indicate means, and horizontal bars, the standard errors.

Table 1. Mean Values (± S.E.) for Fat-free Dry Weight, Nitrogen, DNA, and RNA

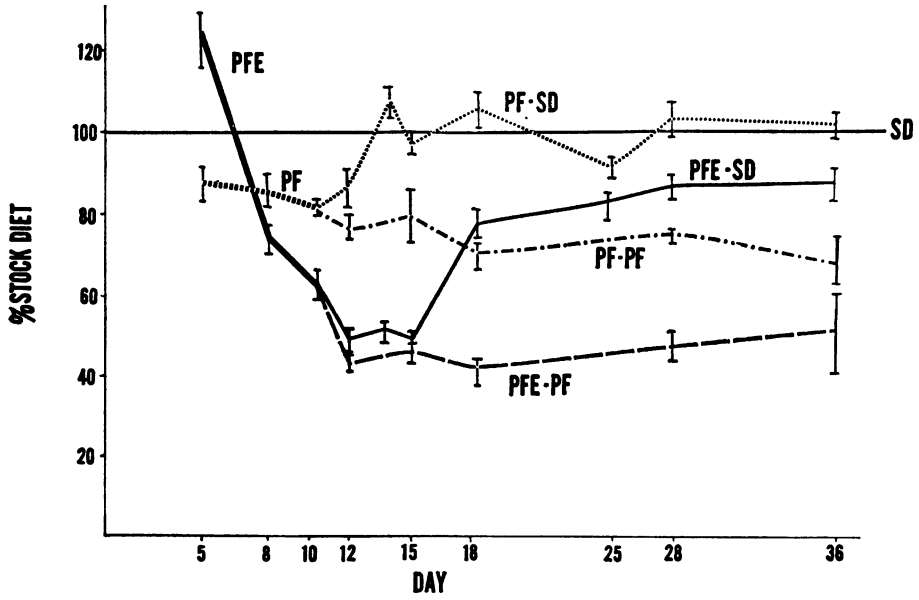
Day	PF regimen *		PFE regimen *	
	Fat-free dry wt. (mg. dry wt./mg. wet wt.†)			
5	79 ± 4 (12)		96 ± 3 (18)	
8	83 ± 2 (12)		77 ± 4 (18)	
10	81 ± 2 (19)		64 ± 2 (26)	
	(PF-SD)	(PF-PF)	(PFE-SD)	(PFE-PF)
12	84 ± 1 (10)	89 ± 7 (9)	45 ± 5 (9)	46 ± 4 (9)
15	96 ± 2 (17)	74 ± 3 (9)	62 ± 2 (18)	44 ± 5 (8)
18	102 ± 2 (16)	67 ± 9 (9)	79 ± 2 (17)	33 ± 3 (9)
25	100 ± 1 (5)	67 ± 2 (6)	85 ± 2 (6)	32 ± 4 (9)
28	97 ± 2 (15)	87 ± 2 (21)	88 ± 3 (16)	32 ± 1 (21)
36	98 ± 4 (10)	74 ± 5 (14)	90 ± 4 (9)	47 ± 3 (9)
	Nitrogen (mg./ml. homogenate†)			
5	87 ± 4 (26)		123 ± 6 (31)	
8	85 ± 4 (25)		73 ± 4 (28)	
10	81 ± 3 (45)		62 ± 4 (32)	
	(PF-SD)	(PF-PF)	(PFE-SD)	(PFE-PF)
12	86 ± 5 (21)	76 ± 4 (24)	49 ± 3 (11)	43 ± 4 (16)
15	97 ± 3 (17)	79 ± 7 (5)	48 ± 5 (13)	46 ± 3 (8)
18	105 ± 4 (20)	71 ± 4 (31)	77 ± 4 (15)	41 ± 4 (18)
25	91 ± 3 (9)	—	82 ± 3 (10)	—
28	103 ± 5 (19)	73 ± 1 (16)	86 ± 3 (21)	47 ± 4 (21)
36	101 ± 4 (16)	67 ± 7 (17)	87 ± 4 (23)	52 ± 10 (20)
	DNA (μmole deoxyribose/mg. N†)			
5	184 ± 12 (16)		138 ± 14 (12)	
8	188 ± 14 (27)		221 ± 23 (20)	
10	213 ± 12 (24)		283 ± 15 (20)	
	(PF-SD)	(PF-PF)	(PFE-SD)	(PFE-PF)
12	144 ± 14 (11)	242 ± 10 (8)	279 ± 24 (6)	315 ± 16 (6)
15	96 ± 10 (7)	—	223 ± 27 (4)	—
18	85 ± 5 (10)	308 ± 15 (14)	160 ± 11 (7)	272 ± 15 (12)
25	107 ± 5 (9)	—	141 ± 10 (10)	—
28	97 ± 9 (9)	301 ± 12 (12)	151 ± 14 (9)	358 ± 23 (18)
36	107 ± 4 (12)	316 ± 11 (14)	141 ± 11 (13)	454 ± 15 (18)
	RNA (mg./mg. N†)			
5	136 ± 8 (18)		61 ± 2 (7)	
8	128 ± 7 (17)		52 ± 7 (7)	
10	123 ± 7 (17)		43 ± 3 (16)	
	(PF-SD)	(PF-PF)	(PFE-SD)	(PFE-PF)
12	108 ± 5 (10)	141 ± 6 (18)	38 ± 2 (10)	37 ± 4 (11)
15	102 ± 4 (10)	132 ± 8 (10)	54 ± 2 (8)	38 ± 2 (10)
18	93 ± 4 (10)	133 ± 5 (17)	73 ± 2 (10)	49 ± 4 (10)
25	—	—	—	—
28	107 ± 8 (9)	139 ± 13 (16)	95 ± 2 (10)	54 ± 7 (14)
36	114 ± 6 (10)	115 ± 2 (10)	96 ± 2 (14)	60 ± 2 (13)

* After 10 days of protein-free diet (PF) or protein-free diet plus ethionine (PFE), animals were continued on stock diet (SD), left column under each regimen, or were continued on the PF diet, right column under each regimen. Numbers in parentheses represent the number of animals used for analysis. For control SD values, there were 209 animals used for fat-free determination, 190 for nitrogen, 126 for deoxyribonucleic acid, and 87 for ribonucleic acid. Control animals were sacrificed and analyzed at the same time as test animals.

† Values are given as percentage of stock diet (SD) control.

Nitrogen

Nitrogen value changes (Table 1 and Text-fig. 2) were similar in direction and degree to the changes in pancreas wet weight[1] or fat-free dry weights, except at Day 5 when values were elevated in the PFE regimen. In the PFE-SD animals there was a decrease to about 50% of the SD value at Days 12 and 15 and a subsequent rise to 87% of the SD level at Day 36. There was less decrease in the PF-SD animals and a more rapid rise to SD values.



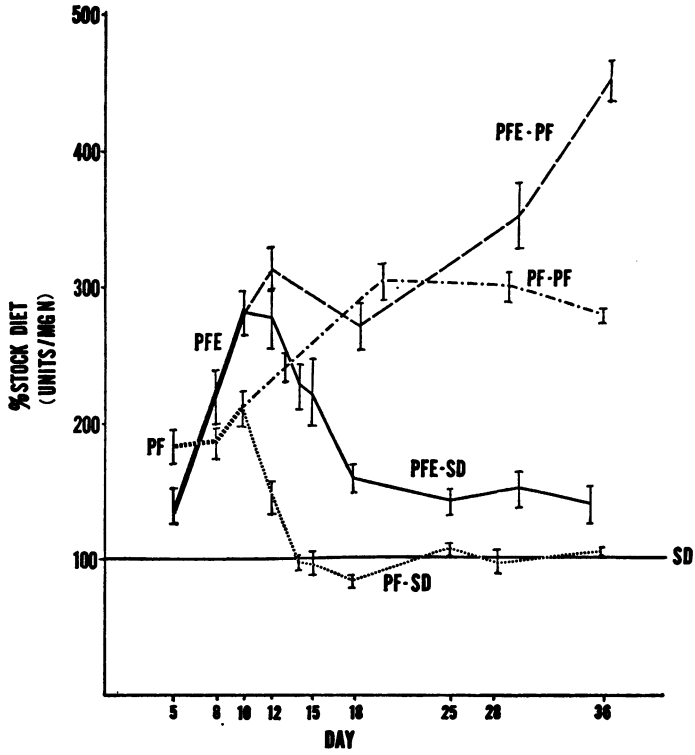
TEXT-FIG. 2. Nitrogen values on aliquots were determined by the micro-Kjehldahl method. The ratio of milligrams of nitrogen to milliliters of homogenate was compared to values (set at 100%) in control animals receiving the stock diet.

The PFE-PF group level decreased to about 45–50% of SD values at Days 12 and 15 and remained at this level. The PF-PF decreased to a low of 67% at Day 36.

Deoxyribonucleic Acid

At most days of sacrifice in all experimental groups there was an increase of DNA per milligram of nitrogen (Table 1 and Text-fig. 3). In both the PFE and PF regimens there was an increase of DNA at Days 5, 8, and 10. PFE-SD concentrations were over double the SD levels at Days 12 and 15 and thereafter dropped closer to SD concentrations. The elevated PF-SD values returned to control levels at Day 15.

The PFE-PF animals through Days 12, 18, 28, and 36 showed the high



TEXT-FIG. 3. Deoxyribonucleic acid was determined on aliquots of pancreas with the Burton[9] diphenylamine reaction. Results were expressed as the ratio of micromoles deoxyribose to milligrams of nitrogen. This ratio was then compared to normal stock diet ratios, set at 100%.

concentrations, up to 4–5 times SD values. The PF-PF values were usually about 3 times the controls at most sacrifice days.

Ribonucleic Acid

The PFE regimen caused a gradual reduction to a low of 38% of SD levels at Day 12 (Table 1 and Text-fig. 4). With the SD diet there was a slow return in the PFE-SD group to control values at Day 36. In the PF-SD animals the values were about at the SD range after Day 10.

In the PFE-PF animals there was some recovery from a low of 37% at Day 12 to 60% of the SD level at Day 36. In the PF-PF groups all values were above the SD levels.

Amylase, Lipase, and Chymotrypsinogen

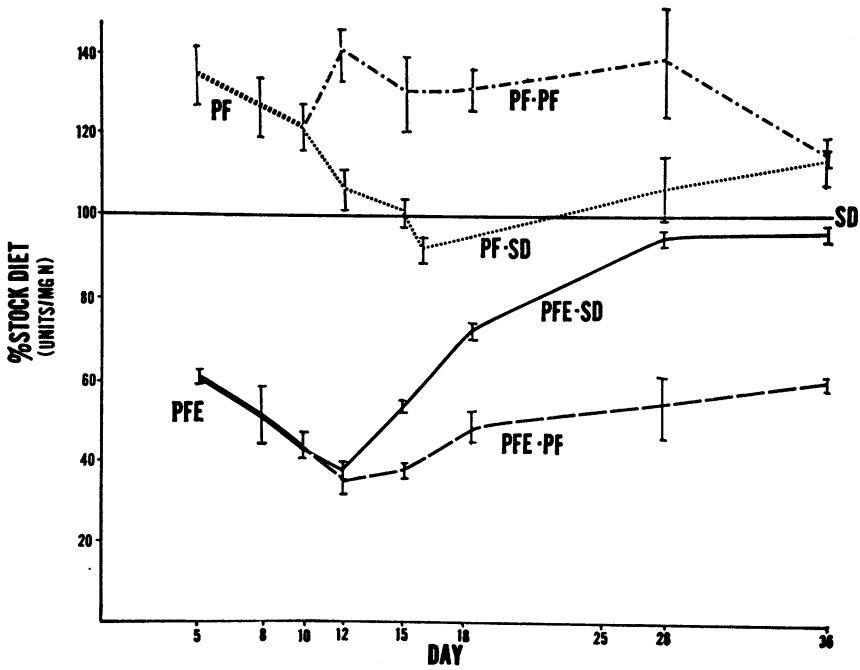
Amylase, lipase, and chymotrypsinogen (Table 2 and Text-fig. 5–7) patterns of response were somewhat similar to each other in trend during the degenerative phase but different in degree (Table 2). At Day 12 in

the PFE-SD group these enzymes had fallen to low levels—amylase to 24%, lipase to 3%, and chymotrypsin to 32% of the SD values. The rates of return of these 3 enzymes to SD activity were different. Chymotrypsin reached the SD level at Day 15, lipase at an estimated Day 22, and amylase not until Day 36.

In the PFE-PF animals the lowest value of amylase activity, 37% at Day 12, gradually increased to a value of 75% at Day 36. There was a slight rise in the PFE-PF chymotrypsinogen values from a value of 19% at Day 15 to 45% at Day 36. The very low lipase values of less than 5% at Days 12 and 15 in the PFE-PF animals were increased to only 15% at Day 36.

"Lysosomal" Hydrolases

Four enzymes of the lysosome class of enzymes were studied originally —acid phosphatase, beta glucuronidase, ribonuclease, and deoxyribonuclease.[13] The latter 2 showed considerable variation not only from animal to animal but from experiment to experiment. However, their changes in activity followed the same general trend as the other 2 enzymes. Since the acid phosphatase and beta glucuronidase assays gave less variable



TEXT-FIG. 4. RNA, separated by the Schneider-Scott technique,[4,5] was expressed as the ratio of milligrams RNA to milligrams of N. This ratio was compared to stock diet ratio, set at 100%.

and more reproducible results, they are reported as representatives of the lysosomal enzymes. Alkaline phosphatase is included for comparison.

Beta Glucuronidase and Acid Phosphatase. These two enzymes showed many features in common (Table 3 and Text-fig. 8 and 9). Both enzymes showed elevated values throughout most days of the experi-

Table 2. Mean Values (± S.E.) for Amylase, Lipase, and Chymotrypsinogen.

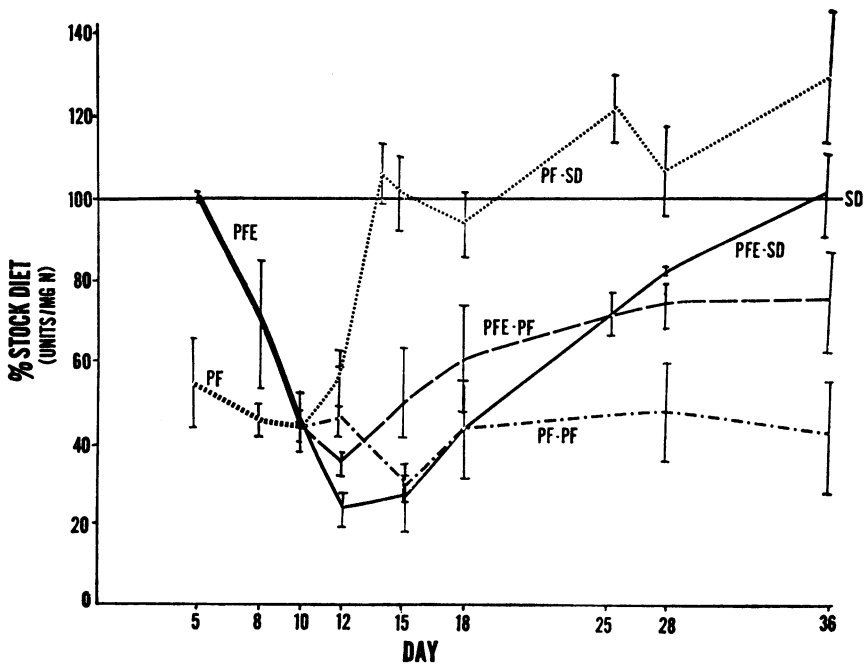
Day	PF regimen *		PFE regimen *	
	Amylase (U./mg. N†)			
5	55 ± 11 (12)		101 ± 1 (16)	
8	46 ± 4 (15)		64 ± 14 (19)	
10	24 ± 4 (22)		46 ± 7 (16)	
	(PF-SD)	(PF-PF)	(PFE-SD)	(PFE-PF)
12	56 ± 7 (11)	47 ± 8 (11)	24 ± 4 (10)	37 ± 5 (10)
15	101 ± 8 (10)	30 ± 12 (5)	26 ± 2 (8)	50 ± 15 (12)
18	94 ± 8 (8)	44 ± 12 (9)	44 ± 11 (9)	61 ± 12 (9)
25	122 ± 8 (8)	—	72 ± 5 (10)	—
28	107 ± 11 (9)	48 ± 12 (9)	83 ± 1 (9)	73 ± 10 (9)
36	135 ± 16 (12)	42 ± 14 (10)	101 ± 10 (12)	75 ± 15 (10)
	Lipase (U./mg. N†)			
5	58 ± 8 (11)		110 ± 9 (10)	
8	40 ± 3 (17)		99 ± 7 (13)	
10	39 ± 7 (25)		31 ± 8 (11)	
	(PF-SD)	(PF-PF)	(PFE-SD)	(PFE-PF)
12	62 ± 4 (12)	16 ± 2 (12)	3 ± 1 (7)	3 ± 1 (12)
15	129 ± 14 (10)	8 ± 2 (6)	22 ± 2 (8)	4 ± 1 (12)
18	116 ± 7 (10)	26 ± (13)	40 ± 11 (6)	12 ± 4 (14)
25	113 ± 6 (9)	—	125 ± 6 (9)	—
28	108 ± 8 (8)	17 ± 1 (11)	146 ± 16 (9)	12 ± 2 (21)
36	135 ± 17 (12)	27 ± 9 (10)	159 ± 13 (13)	15 ± 6 (20)
	Chymotrypsinogen (U./mg. N†)			
5	78 ± 8 (9)		101 ± 7 (9)	
8	69 ± 9 (11)		74 ± 13 (13)	
10	46 ± 3 (21)		52 ± 6 (13)	
	(PF-SD)	(PF-PF)	(PFE-SD)	(PFE-PF)
12	114 ± 11 (11)	57 ± 9 (9)	32 ± 3 (9)	39 ± 11 (9)
15	154 ± 9 (11)	25 ± 8 (5)	106 ± 13 (8)	19 ± 1 (9)
18	120 ± 6 (11)	39 ± 6 (9)	135 ± 15 (9)	48 ± 7 (10)
25	111 ± 10 (9)	—	140 ± 7 (10)	—
28	115 ± 8 (11)	39 ± 4 (9)	153 ± 14 (9)	42 ± 2 (8)
36	159 ± 14 (11)	49 ± 8 (10)	169 ± 14 (13)	45 ± 8 (10)

* After 10 days of PF diet or PFE diet, animals were continued on stock diet (SD), left column under each regimen, or on the PF diet, right column under each regimen. Numbers in parentheses represent the number of animals used for each time point. For control SD activity values there were 105 animals sacrificed for the amylase, 110 for the lipase, and 105 for chymotrypsinogen.
† Values given as percentage of stock diet (SD) control.

ments. In the PFE regimen the peak activities were at Days 10 or 12, and in the PF regimen they were earlier, at Days 8 or 10. The PFE peaks were higher than the PF peaks. The institution of SD feeding at Day 11 in the PFE-SD animals was associated with a quicker return to control values than were the enzyme activities of the PFE-PF group, in which the PF diet gave slower return to SD levels. A similar difference occurred between the PF-SD and PF-PF groups. The beta glucuronidase peak levels were reached earlier than were the acid phosphatase levels. The peak activity of beta glucuronidase was very much greater than that of the acid phosphatase in the PFE regimen.

Deoxyribonuclease (DNase) and Ribonuclease (RNase). In general, DNase and RNase activities in the PFE-SD animals varied much as did acid phosphatase and beta glucuronidase with peak values at Day 12 and a decrease toward normal values subsequently.[13] They did not attain the very high levels of beta glucuronidase; they had values approximating those of the acid phosphatase, and they showed increased activities at the earliest time point examined (Day 5).

Alkaline Phosphatase. The curves of alkaline phosphatase activity in the PFE-PF and PF-PF groups were similar to those of acid phosphatase



TEXT-FIG. 5. Amylase. The change in reducing activity of enzymatically hydrolyzed starch was determined on aliquots by the method of Fingerhut *et al.*[7] Results were converted to the ratio of reducing units to milligrams of N. These were compared to corresponding stock diet ratio, set at 100%.

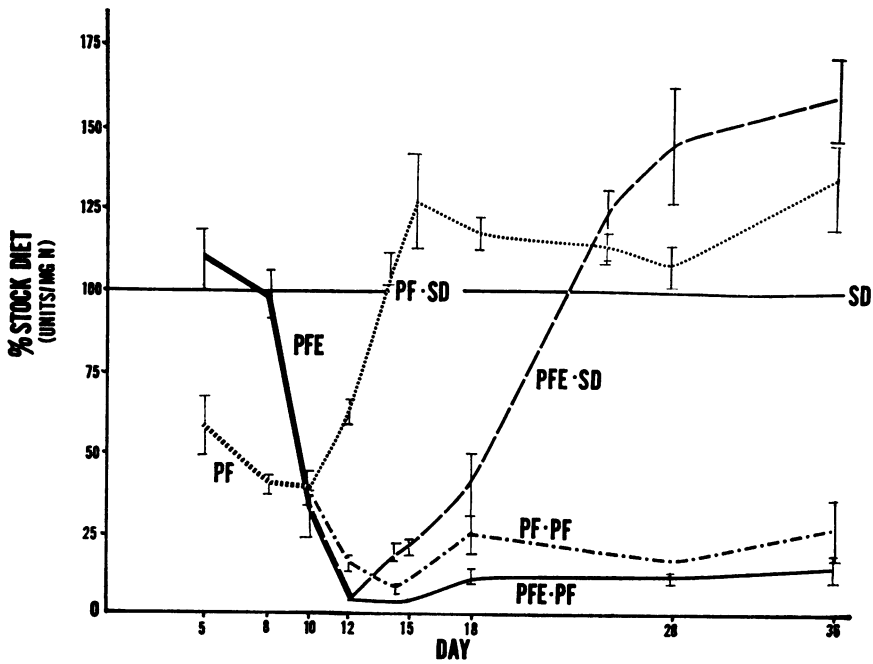
and beta glucuronidase, generally with intermediate values. There were peaks at Day 12 in both the PFE-PF and PF-PF groups and the PFE regimen was associated with higher peak activity than was the PF regimen (Table 3 and Text-fig. 10).

Discussion

Amylase, Lipase, and Chymotrypsinogen

The increase of amylase, lipase, and chymotrypsinogen activities per unit of wet pancreas tissue at Day 5 in the PFE animals was higher than implied by the values given (Table 2 and Text-fig. 5–7) because of the elevated N values. This may have been related to enzyme retention in the gland.[14–16] The very low value of these enzymes may also have resulted from decreased synthesis[17] as well as from the obvious loss of acinar tissue.

An estimate of the total amount of amylase, lipase, and chymotrypsinogen enzyme activities present in the residual pancreas of the PFE-SD animal at Days 10–12, made by correcting for tissue loss and the decrease

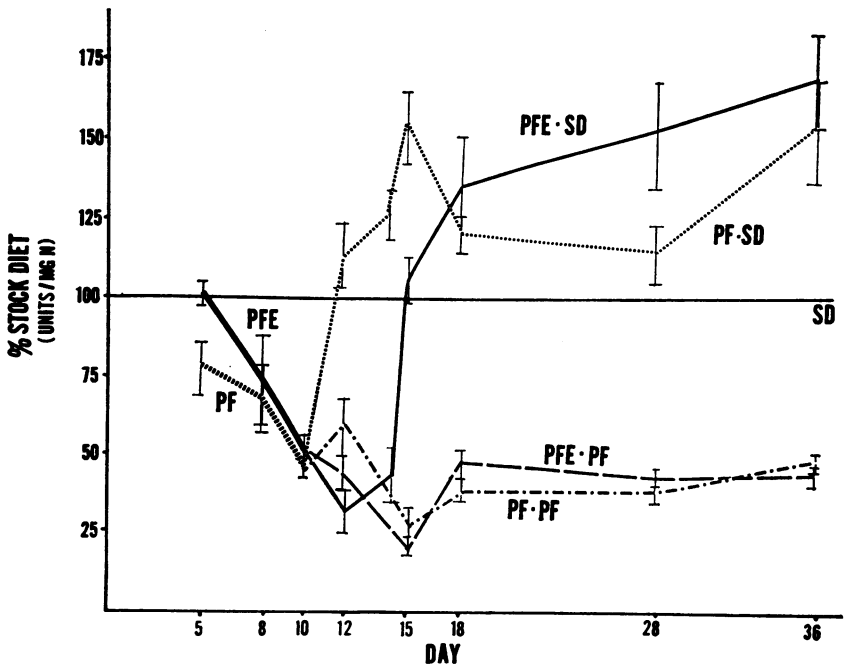


TEXT-FIG. 6. Lipase. Fatty acid split from olive oil emulsion containing $CaCl_2$ and taurocholate was titrated at pH 8.0 with 0.01 N NaOH. The ratio of milliliters per minute to milligrams N was compared to the corresponding stock diet value, set at 100%.

of N, indicates that the residual pancreas tissue total enzyme activity for any one of these enzymes would be less than 5% of the SD gland total enzyme activity. Others have obtained similar results.[18,19]

The return of enzyme activities to an SD level at such different days was surprising. Chymotrypsinogen reached this point at Day 15 when cytologic restitution, as seen by EM, although good in most respects, showed a lack of dense zymogen granule formation (Fig. 5[1]). Possibly, chymotryptic activity might have come from nuclei.[20] Amylase activity attained SD levels a week after EM cytologic restitution was essentially complete (Fig. 6[1]). Recovery of pancreatic amylase was found to be slow after fasting and nonprotein diet.[15] Ethionine is incorporated in the amylase of *B. subtilis*.[22,23] Its continued administration in mammals results in a decreased plasma amylase.[19,21]

We found the order of appearance of enzymes in the pancreas of the embryonic rat to be amylase, lipase, and chymotrypsinogen.[24] The reversal of the embryonic order of appearance of these 3 enzymes during regeneration is unexplained.



TEXT-FIG. 7. Chymotrypsinogen was converted to chymotrypsin with chymotrypsin-free trypsin. Chymotryptic activity was then determined by the rate of splitting of the trypsin-insensitive synthetic substrate BTEE.[8] The ratio of enzyme units to milligrams N was compared to stock diet control, set at 100%.

Desnuelle's group has demonstrated that prior diet affects tissue enzyme synthesis and secretion. A high carbohydrate diet (75%) for a month increased subsequent pancreas amylase activity and secretion. A high protein diet for the same time gave rise to increased synthesis and secretion of chymotrypsin.[25,26] Our SD diet was relatively high in protein (25%), and possibly this was a factor in the rapid return of the chymotrypsinogen in our PFE-SD animals. Our SD diet contained 60% carbohydrate, an amount which appears to be adequate for normal growth. Higher con-

Table 3. Mean Values (± S.E.) for Beta Glucuronidase, Acid Phosphatase, and Alkaline Phosphatase

Day	PF regimen *		PFE regimen *	
	Beta glucuronidase (U./mg. N†)			
5	270 ± 7 (12)		226 ± 29 (17)	
8	480 ± 31 (9)		1973 ± 194 (9)	
10	338 ± 38 (17)		2741 ± 69 (13)	
	(PF-SD)	(PF-PF)	(PFE-SD)	(PFE-PF)
12	105 ± 12 (10)	466 ± 35 (11)	1363 ± 116 (10)	1740 ± 125 (10)
15	105 ± 15 (6)	—	799 ± 154 (10)	—
18	86 ± 12 (10)	420 ± 33 (10)	545 ± 108 (9)	1236 ± 102 (11)
28	117 ± 18 (10)	385 ± 34 (11)	197 ± 21 (21)	1136 ± 115 (9)
36	102 ± 6 (10)	109 ± 10 (20)	125 ± 9 (10)	330 ± 11 (10)
	Acid phosphatase (U./mg. N†)			
5	111 ± 3 (12)		89 ± 6 (17)	
8	107 ± 10 (7)		215 ± 25 (7)	
10	145 ± 18 (17)		260 ± 21 (12)	
	(PF-SD)	(PF-PF)	(PFE-SD)	(PFE-PF)
12	141 ± 16 (10)	124 ± 5 (10)	362 ± 20 (9)	361 ± 24 (10)
15	87 ± 4 (6)	—	135 ± 9 (8)	—
18	98 ± 6 (10)	153 ± 16 (10)	136 ± 12 (10)	343 ± 19 (12)
28	103 ± 9 (10)	152 ± 16 (10)	108 ± 3 (10)	258 ± 18 (10)
36	95 ± 3 (9)	133 ± 14 (10)	94 ± 4 (10)	111 ± 14 (10)
	Alkaline phosphatase (U./mg. N†)			
5	187 ± 18 (9)		290 ± 6 (10)	
8	208 ± 19 (9)		422 ± 8 (8)	
10	372 ± 19 (9)		740 ± 9 (10)	
12	539 ± 18 (9)		1133 ± 20 (9)	
15	—		—	
18	446 ± 15 (9)		954 ± 19 (9)	
28	120 ± 18 (9)		634 ± 16 (9)	
36	145 ± 13 (10)		214 ± 11 (10)	

* After 10 days of PF diet or PFE diet, animals were continued on stock diet (SD), left column under each regimen, or on the PF diet, right column under each regimen. Numbers in parentheses represent the number of animals used for analysis. For control SD values there were 55 animals used for the beta glucuronidase, 55 for the acid phosphatase, and 25 for the alkaline phosphatase values.
† Values given as percentage of stock diet (SD) control. See text for description of units.

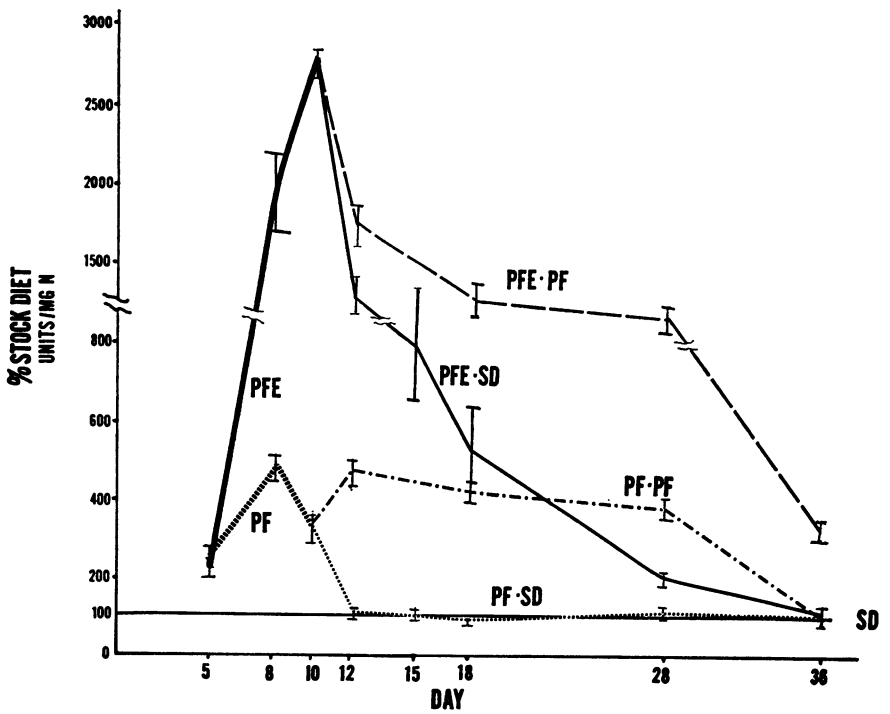
centrations of carbohydrate have not been tested in our regenerating pancreas model.

Marchis-Mouren and Cozzone showed that chymotrypsin has a much shorter half-life than amylase[27] and Dickman, Holtzer, and Gazzinelli that chymotripsinogen was synthesized in beef pancreas slices more rapidly than total protein.[28] These differences may be related to the more rapid return of the chymotrypsin to SD levels.

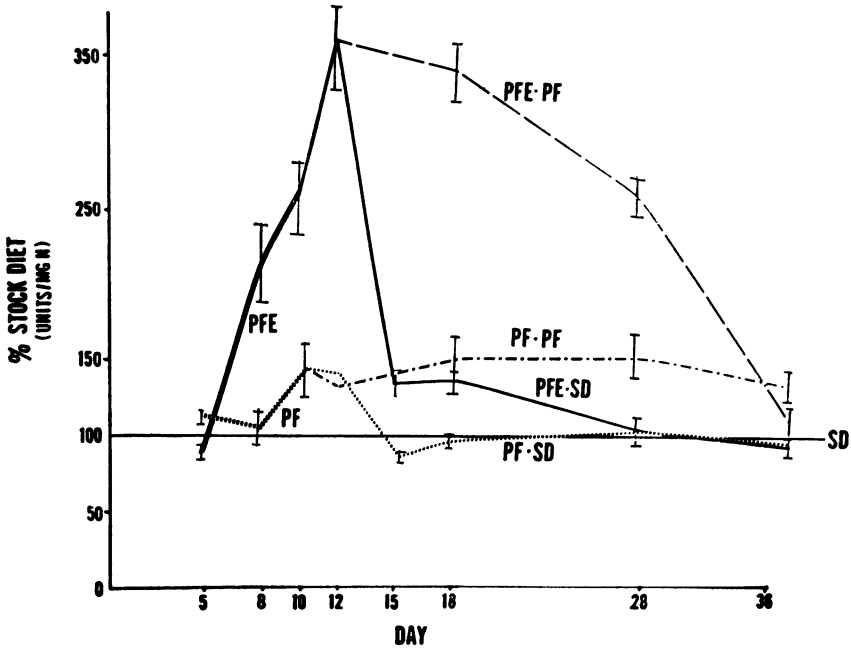
It is not known whether in regeneration there are clones of regenerating acinar cells which produce different amounts of these enyzmes or have different rates of regeneration or whether the regenerating individual acinar cell has the same relative amount of these 3 enzymes as indicated by the total homogenate activities.

Lysosomal Enzymes

The original concept of deDuve *et al.*[10] that certain latent enzymes which sedimented as a unit during ultracentrifugation were related in hydrolytic function has been accepted by many observers. Novikoff,



TEXT.-FIG. 8. Splitting of phenolphthalein glucuronide[12] was used to measure beta glucuronidase activity. Units per milligram N in experimental animals were compared to corresponding value for stock diet, set at 100%.



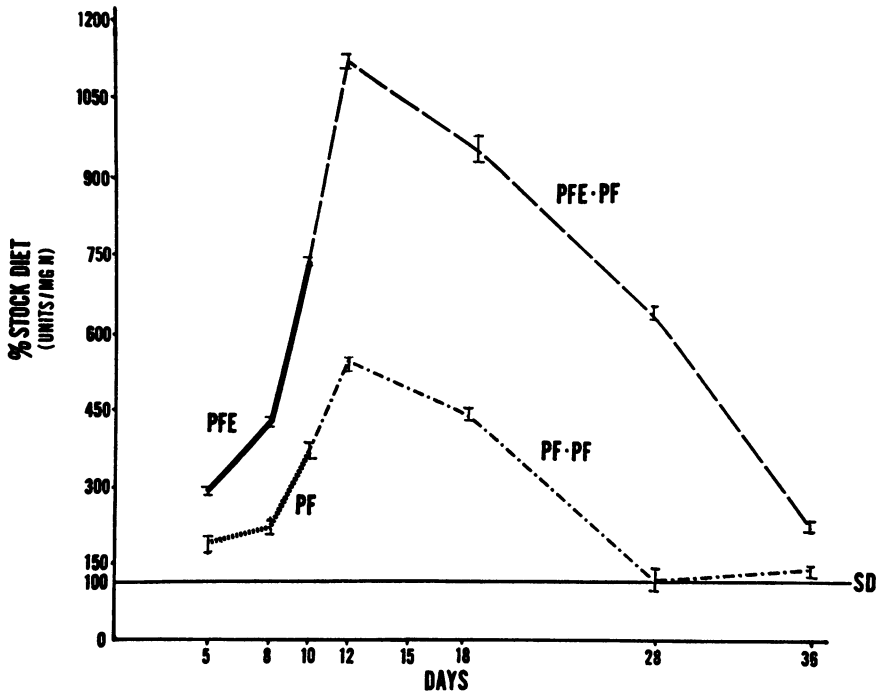
TEXT-FIG. 9. Acid phosphatase. Using the action of enzymatic splitting of phenylphosphate at pH 4.6,[11] the amount of phenol formed in K.-A. units was determined with 4-aminoantipyrine and alkaline ferricyanide.

Beaufay, and deDuve[29] suggested that these lysosomal enzymes were enclosed in a single ultrastructural unit in the cytoplasm of normal cells and were released by trauma or changes in pH, or during degenerative or inflammatory processes. Holtzer and van Lancker, on the basis of biochemical and electron microscopy studies, concluded that there are differences in the hydrolytic enzyme activation system in the mouse pancreas during autolysis from the lysosomal mechanism activating liver autolysis.[30] Slater and Greenbaum have reported findings which indicate that lysosomes are not involved in the initiation of hydrolytic changes in the liver,[31] and Levvy and Conchie have questioned the lysosomal concept.[32]

During ethionine degeneration of the pancreas, there was a rise of acid phosphatase, beta glucuronidase, RNase, and DNase enzymatic activities consistent with the lysosomal hypothesis. There were differences between the degrees of change in enzyme activity, beta glucuronidase activity being far greater than acid phosphatase (Text-fig. 8 and 9), and some enzymes changed earlier or later than others. Some enzyme activities were still higher than control values at Days 28 and 36 when morphologic restitution by EM appeared complete (Fig. 5[1] and 6[1]). Enzyme activities

were highest at Days 10 and 12, when acinar cells, the type showing the most morphologic evidence of ethionine damage of any cell type, were most scarce. Although acid phosphatase reaction product was demonstrable in the ethionine lesions of the acinar cell (Fig. 1), it also was present in inflammatory cells, primarily macrophages, which must be considered important contributors to homogenate lysosomal enzyme activities. The very high level of alkaline phosphatase activity which paralleled the course of lysosomal enzyme activities (Table 3 and Text-fig. 10) seemed related, in part, to volumetric concentration (secondary to loss of acinar tissue) although there was increased enzyme activity of endothelium per unit of capillary length (Fig. 2).

We have not been able to demonstrate consistently by EM in the normal pancreas acinar cell any organelle which is a likely candidate for the postulated lysosomal body. Throughout degeneration and in the early regenerative period there were ergastoplasmic vacuolar lesions containing plaques of osmiophilic membranes and debris.[33-36] Wachstein, Fernandez, and Ortiz had demonstrated that these ergastoplasmic lesions, as well as infiltrating histiocytes, had associated with them acid phos-



TEXT-FIG. 10. Alkaline phosphatase. The method of Kind and King[11] was used as in acid phosphatase. Determinations were performed at a pH of 10. Only PF-PF and PFE-PF dietary regimes were tested.

phatase reaction product,[37] and Takino, in our laboratory, has verified their findings (Fig. 1).[38] The ethionine pathologic lesion, in one sense, might be regarded as a lysosomal body since its appearance was most extensive at Days 10–12 when the enzymatic activities were highest. However, the enzyme activities were still well above SD levels at Days 28 and 36 (Text-fig. 8 and 9) when ethionine lesions were absent (Fig. 5[1] and 6[1]). The high levels of lysosomal enzyme activities in the PF-PF regimen (Text-fig. 8 and 9), in which ethionine-like lesions were very rare,[36] further demonstrate the lack of correlation of the ethionine lesion with the high lysosomal enzyme activities.

Ethionine Action

Stekol and colleagues,[39–41] Schmidt *et al.*,[42] Farber and co-workers,[43,44] and Parks [45] have suggested that the slow turnover of S-adenosylethionine, formed after ethionine injection, acts effectively as a trap of adenosine triphosphate (ATP). This results in a lower level of ATP in the liver which, in turn, it is suggested, prevents ribosomal aggregation and m-RNA formation.[46] Although the injection of ATP precursors prevents the drop in liver ATP levels and also prevents the morphologic damage to the liver,[47] ATP or ATP precursors have no effect on pancreas ATP levels or on the morphologic changes produced in the pancreas by ethionine.[48] Alkylations of nucleic acids,[40,43] the formation of an abnormal protein by the incorporation of ethionine, instead of methionine, into protein,[49,50] and the reduction of the availability of a one carbon compound which interferes with peptide chain initiation by formyl methionine [51] have been suggested as key points whereby ethionine causes its effects.

The net result of ethionine action on the pancreas, regardless of its initial action, is eventually a decrease of protein synthesis. This is illustrated by our PF regimen which, on rare occasion, produced effects qualitatively similar to, but much less drastic, than those of ethionine.

Protein (Nitrogen) and Fat-free Dry Weight

The loss of total pancreas wet weight during Days 5–10 in the PFE animals [1] was, with one notable exception, accompanied by losses in nitrogen and fat-free dry weight. These changes were consistent with the cytologic damage and loss of acinar cells.[1] The increase of N at Day 5 in the PFE animals may be related to the reported increase of protein synthesis occurring early after ethionine injection,[52] or, possibly, to the resultant lack of alimentary stimulation and the organ retention of protein.[14–16]

The return in the PFE-SD animals of nitrogen and dry weight to about

90% of SD values at Day 36 corresponded roughly to the increase of organ weight and restitution of the gland.[1]

The persistently low values of nitrogen, dry weight, and pancreas weight in the PFE-PF animals after Day 10 is in accord with the known requirements for protein in the normal diet and during regeneration.[53,54] The relatively good histologic and cytologic restitution of the pancreas in these animals [55] is surprising in view of the poor restitution of pancreas weight,[1] fat-free dry weight (Text-fig. 1), or nitrogen (Text-fig. 2). Protein stores elsewhere may have been depleted to provide precursors for cytologic restitution.[56,57]

Deoxyribonucleic Acid

Only relatively slight histochemical and morphologic changes were present in the acinar cell nuclei at Day 5, and a decrease of acinar, islet, and duct cell nuclear labeling with thymidine-H^3 occurred [58] so that the increase of DNA in the PFE and PF animals may have been related to an increase of inflammatory cells. The further increase of DNA in the PFE regimen from Day 5 to Day 10, despite necrosis of acinar cells, would appear to have been the resultant of at least 2 factors, a decrease of nitrogen (a decrease of the denominator of the DNA/N ratio) and cellular infiltrate.

The persistent elevation of DNA from Days 10 through 15 in the PFE-SD group occurred when there was an increased DNA synthesis of acinar, duct, and interstitial cells.[58,59] A drop in DNA, an increase in N, and a decrease in inflammatory cells were present after Day 15. The decrease of acid phosphatase and beta glucuronidase activities is consistent with the decrease of inflammatory cells.

The increase of DNA through Day 12 in the PFE-PF groups may have been related to the decrease of nitrogen and to inflammatory cell infiltrate because autoradiography demonstrated a decrease in the labeling of acinar or duct cell nuclei with thymidine-H^3.[35,39] Accompanying the marked increase of DNA at Day 28 were increased acinar cell nuclear labeling with thymidine-H^3,[35,59] persistently low N, inflammatory infiltrate, and, possibly, polyploidy.[60]

The increased values of DNA in the PF-PF group occurred even though there was a decrease of acinar cell nuclear labeling,[35,59] and it would appear that the inflammatory cell infiltrate and low N were responsible for these findings.

Ribonucleic Acid

The decrease of tissue RNA in the PFE animals to about 40% of SD values at Days 10–12 (Table 1 and Text-fig. 4) suggests less loss of RNA

from acinar cells than was implied by the cytochemical and cytologic findings.[1] Correction for the low N values at Days 10 and 12 would give RNA values per unit wet weight of pancreas of about 20% of the SD values.[1] Castrini showed a loss of RNA in the pancreas by histochemical methods after Day 3.[61] The observed ductular cell hypertrophy and the presence of inflammatory cells at Day 12 would have increased tissue homogenate RNA concentration so that the acinar cell RNA was probably even lower than the calculated 20%.

The increase of RNA after Day 10 in the PFE-SD groups was roughly parallel to the increase of pancreas weight,[1] fat-free dry weight percentage composition, nitrogen, and amylase. Both an increased number of acinar cells and an increase of RNA per acinar cell (Fig. 5[1] and 6[1]) contributed to the over-all RNA increase.

The increase in the RNA of the PF regimen is of interest because of the EM findings from this laboratory of a loss of zymogen granules and apical cytoplasm (loss of N) with relative preservation of ribosomes and nuclei and the enlargement of nucleoli (major sources of RNA).[35] Decreased N and the presence of inflammatory cells may have been responsible for the change. The results of Mandel, Jacob, and Wentzerith[62] appear similar.

Summary

1. After 10–12 days of ethionine-induced degeneration of rat pancreas acinar cell epithelium, there was a decrease of pancreatic amylase, lipase, and chymotrypsinogen enzyme activities in the residual pancreas tissue to about 25%, 5%, and 30%, respectively, of control SD levels. Correction of these values for loss of pancreas tissue and decreased nitrogen would imply that the total pancreas activities of these enzymes was less than 5% of control values.

2. After cessation of ethionine and the institution of SD feeding, the chymotrypsin values per unit of nitrogen returned to SD levels at Day 15, lipase was at control values at about Day 22, and amylase activity was not restored to SD levels until Day 36.

3. Lysosomal enzymes in the PFE-SD and PFE-PF animals showed increased enzymatic activity during degeneration and reached a peak at Days 8–12. Beta glucuronidase was markedly elevated. A protein-free diet (PF) increased lysosomal enzyme activity but less so than did the PFE regimen. SD refeeding from Day 11 caused the elevated values to return more rapidly towards control values than did the PF diet.

4. There was no correlation between any cytoplasmic organelle present in the normal, degenerating, or regenerating pancreatic acinar cell and the increase or decrease in lysosomal enzyme activities during acinar cell degeneration or regeneration.

5. DNA concentration was increased in the PFE-SD animals above SD levels throughout the experiment, particularly during the early regeneration period (Days 12–15) when there was an influx of inflammatory cells and an increase in DNA synthesis of acinar and nonacinar cells.

6. RNA concentration decreased during degeneration to low values in the PFE-SD animals and thereafter slowly increased to approximately control values at Day 36—a return which paralleled the increase of amylase, nitrogen, and fat-free dry weight percentage.

7. Since the PF diet produced changes similar to those given by the PFE regimen, although not to the same degree, and the inclusion of protein in the SD diet during regeneration was associated with the return of structure and function to normal, it appears that most of the ethionine effects on the pancreas occurring in the model were related to the decrease of protein synthesis.

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Legends for Figures

Fig. 1. Electron micrograph showing cytochemical acid phosphatase determination[88] in pancreas of PFE rat at Day 10. Reaction product is localized to ergastoplasmic lesions. Incubation with cytidine monophosphate substrate. Glutaraldehyde fixation, uranyl acetate stain. × 32,000.

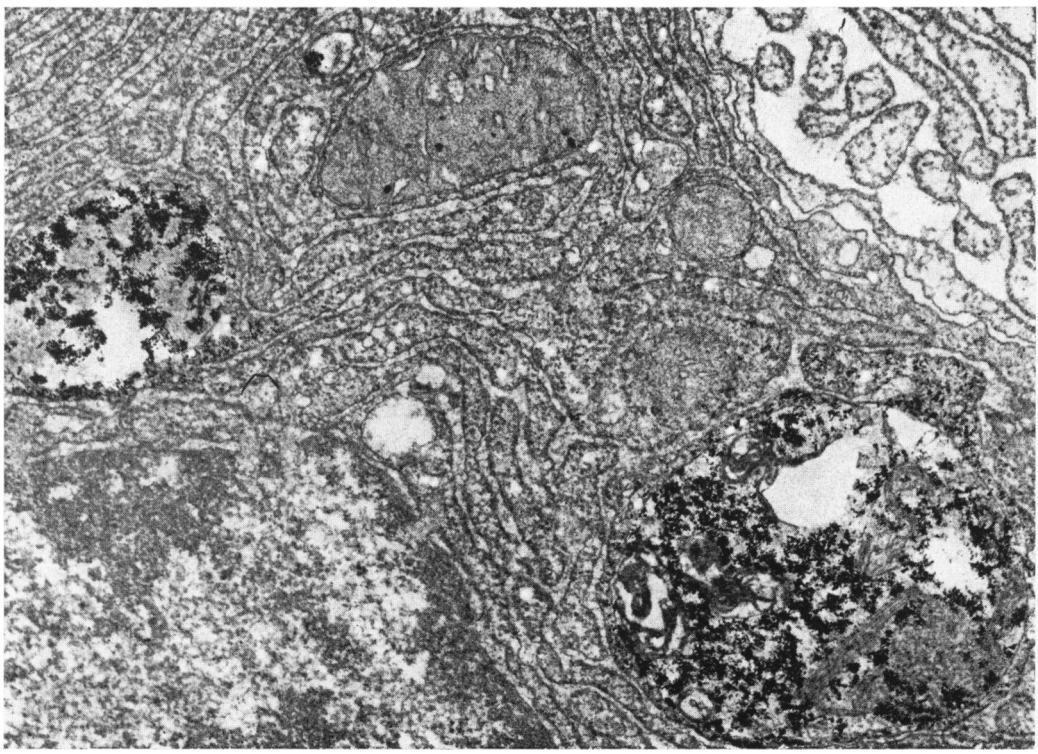
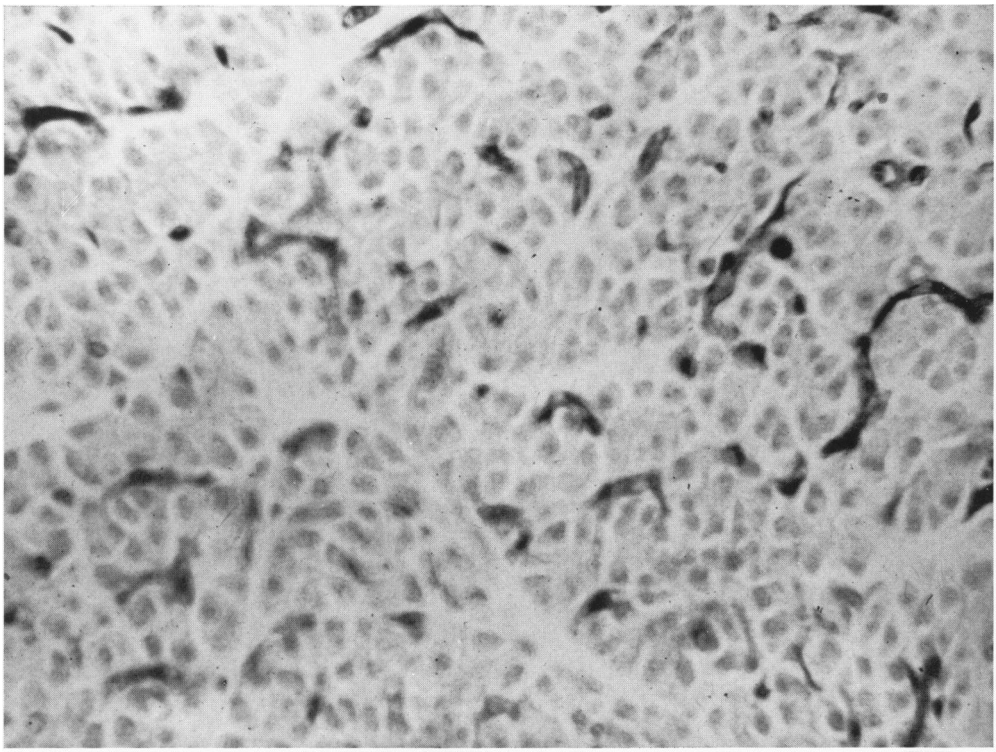


Fig. 2. Cytochemical test for alkaline phosphatase in rat pancreas.[1] **A.** Stock diet control pancreas. Reaction product is limited to foci of capillary endothelium. × 120. **B.** Pancreas of Day 10 PFE animal. There is relatively greater concentration of capillaries associated with destruction of acinar tissue. In addition, capillary endothelium appears to contain more reaction product per unit length of capillary. × 120.



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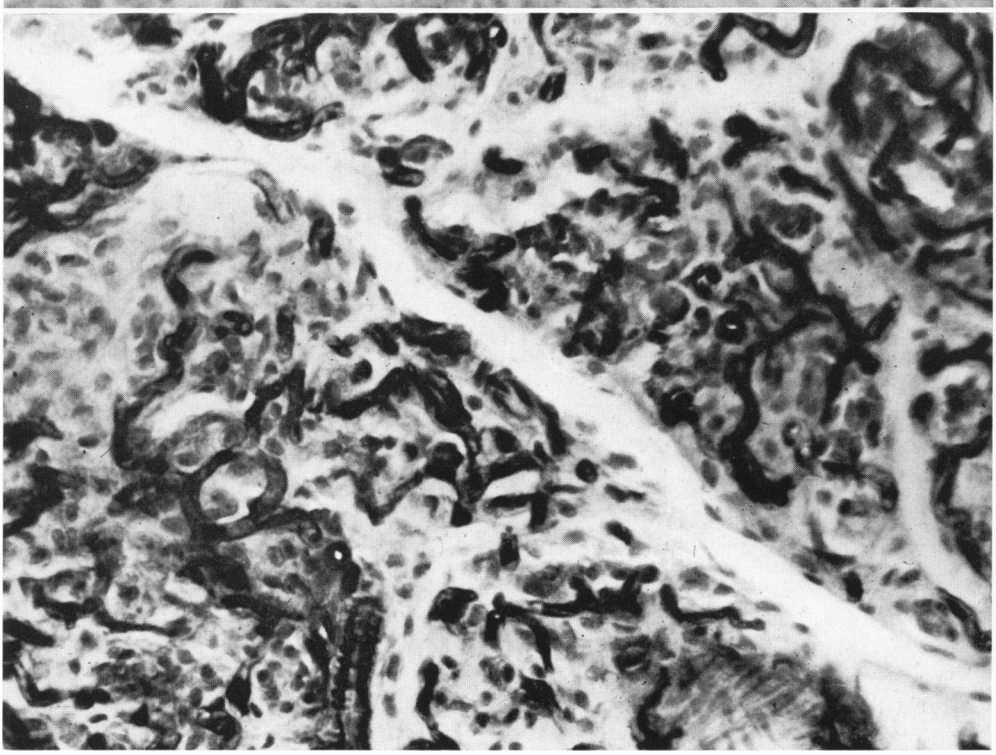

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