Pancreatic Acinar Cell Regeneration

I. Cytologic, Cytochemical, and Pancreatic Weight Changes

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REGENERATION, whether it be the restitution of whole planaria from minute fragments of the organism,[1] restoration of limbs of amphibia,[2] or the return of the mammalian liver to its original weight shortly after partial resection,[3,4] has been the subject of many investigations.[5–8] For it has been recognized that although regeneration is an orderly process, it involves some release, even if only partial and temporary, of normal controlling mechanisms. A proper delineation of the essential factors operating to initiate, sustain, and terminate regeneration might lead to a better understanding of the control mechanisms characteristic of growth, differentiation, or possibly, of neoplasia.

A model of regeneration—the pancreas acinar cell—is presented. We reported originally that there was histologic restitution of the rat pancreas after the destruction of pancreatic acinar epithelium by ethionine[9] and this has been confirmed.[10,11] The quantitative studies contained herein indicate that, in addition, within a few weeks after the ethionine destruction of an estimated 90% of rat pancreatic acinar cell mass, there is a return of total pancreas weight to the pre-ethionine level. Ultrastructural and cytochemical patterns are also restored to normal during the regenerative process.

In 2 other reports, concomitant changes in the enzymatic activity of pancreatic enzymes, nucleic acids, and protein[12] and variations in the synthesis of deoxyribonucleic acid (DNA) in the cell nuclei during ethionine-induced acinar cell degeneration and regeneration, as demonstrated by autoradiography,[13] will be described.

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Experimental Design

Animals were given daily intraperitoneal injections of DL-ethionine for 10 days while on a protein-free diet (PFE).[14-16] The day of the first injection was called Day 1 of the experiment and all experiments lasted at least 36 days. Surviving animals received no ethionine after Day 10 and from Day 11 to sacrifice were placed either on a stock diet (PFE-SD)[15,17,18] or were kept on the protein-free diet (PFE-PF).[14-16,19] Control animals were kept on a stock diet (SD) throughout or were placed on a protein-free diet (PF) for 10 days and then fed the SD from Day 11 to sacrifice (PF-SD).

At sacrifice, total and segmental pancreas weights[20] and total body weight were determined. A prior study[14] of rats on an ethionine protein-free regimen (PFE-PF) and a control group on a protein-free diet (PF-PF) was used for a comparison of pancreas weights. Light microscopy (LM), electron microscopy (EM), and histochemical and cytochemical techniques were applied to tissue specimens. Control and experimental animals were sacrified on the same day in an order chosen by random numbers.

The first 10 days of the experiment for the PFE groups represented a period of acinar cell degeneration and necrosis whereas the period Day 11 to Day 36 was one of repair and regeneration.

This and 2 other reports[12,13] represent 13 experiments. While an experiment was done primarily for the determination of enzymatic activity, the detection of morphologic or cytochemical changes, or autoradiographic nuclear labeling, additional tissue was taken at sacrifice for examination by a second or third technique.

All findings were verified by at least one repeated experiment.

Methods and Materials

Animals

Male Wistar rats (CFN strain, Carworth Farms, New City, N. Y.), after acclimatization for at least a week in our animal quarters, were assigned by a table of random numbers[21] to the groups. They were housed in individual cages in air-conditioned rooms, kept at about 74° F., and had access to water ad libitum. The average total body weight at Day 0 of most experiments was between 160 and 180 gm., but the inclusion of an experiment with heavier animals brought the average of all animal weights to 185 gm. Sacrifice was by decapitation between 10:00 A.M. and 12:00 noon. During a 36-day experiment the average mortality of the PFE-SD groups was about 20% and that of the PFE-PF animals about 50% (Table 1). Animals found dead were excluded from all examinations.

Diet

Stock Diet (SD). The SD diet was Purina Laboratory Chow checkers (Ralston Purina Co., St. Louis), said by the supplier to have a composition of approximately 59% carbohydrate, 25% protein, 6% fat, and the vitamins, minerals, and factors known to be necessary for optimum growth.

Protein-free Diet (PF). The PF diet (Nutritional Biochemicals Corp., Cleveland, Ohio) was said by the supplier to contain corn starch, 70%; alphacel (cellulose), 15%; vegetable oil, 10%; and salt mixture, U.S.P. XIV, 4%. Blended into the diet was the producer's Vitamin Diet Fortification Mixture which supplied Vitamins A, D, and the B-complex (1 gm./100 gm. of food) and the other vitamins, factors, and minerals known to be required for optimum growth.

Ethionine

DL-ethionine (Nutritional Biochemicals Corp.) was prepared daily by dissolving the amount to be used in distilled water, warming to 35° C., and bringing it to a pH of 7.4 by the addition of 3 N NaOH. Ethionine, 0.7 gm./kg. of total body weight, was injected intraperitoneally in a total volume of 2–3 ml. of distilled water.

Total Pancreas and Total Body Weights

Our method of identifying and weighing pancreas tissue and pancreas segments has been described, and we have reported a regression equation relating the total wet weight of pancreas and pancreas segments to the total body weight of the rat.[20] Total body weight was determined upon arrival at our laboratory, at Day 0, 3 times a week, and on the day of sacrifice (Table 1).

Histologic and Cytologic Preparation

For light microscopy (LM) studies, thin slices of pancreas tissue were fixed in 10% aqueous solution of formaldehyde (U.S.P.), with calcium carbonate chips added. Dehydration was in increasing concentrations of alcohol, and tissue was cleared in xylol, embedded in paraffin, and cut at 2 μ and 5 μ. Hematoxylin and eosin staining was used. Pancreas tissue from about 1000 animals was examined.

Cytochemical Preparation

Unless otherwise specified below, pieces of pancreas 1–2 mm. in thickness were fixed for 18 hr. in 10% aqueous solution of formaldehyde (U.S.P.) containing 2% sodium acetate, but were dehydrated, cleared, embedded, cut, and stained as for LM studies. The pancreases of about 150 test and control animals were examined.

Nucleic Acids. Deoxyribonucleic acid (DNA) was studied by the Feulgen reaction using a hydrolysis period of 12 min. in 1% HCl at 60° C.[22] For ribonucleic acid (RNA), a methyl green–thionine stain at pH 5.6 was used with pancreas fixed in formaldehyde for only a few hours.[23] Acridine orange fluorochrome staining for DNA and RNA was performed after fixation in glutaraldehyde.[24] Control sections for the nucleic acids were incubated with deoxyribonuclease or ribonuclease prior to the respective nucleic acid stain used.[22]

Zymogen Granules. Zymogen granules were stained with a tetrachrome aqueous stain containing 1% acid fuchsin, 1% methyl green, 1% thionine, and 1% orange G, 0.5%, in acetate buffer of pH. 5.0.[25]

Alkaline Phosphatase. Tissue was fixed in anhydrous acetone at 0° C. for 18 hr., dehydrated in 2 changes of cold acetone, cleared in cold chloroform, and infiltrated in paraffin at 55° C. for 1 hr. and embedded. The method of Gomori[26] with sodium beta-glycerophosphate as substrate was used. Incubation periods were 30–60 min. Unfixed pieces (1 cu. mm. in size) of pancreas were treated by Gomori substrate and squash preparations made to determine if any cytoplasmic alkaline phosphatase activity was lost in fixation.

Period Acid-Schiff (PAS). The MacManus-Hotchkiss procedure with diastase control was used.[27]

Fat. Oil red O was used to demonstrate fat in tissue cut from fresh frozen sections.[28]

Reticulum. The Gomori stain for examination of reticulum[27] was used.

Evaluation of Concentrations. The relative amount and intensity of staining substance were estimated visually.

Table 1. Number of Animals Sacrificed per Day for Total Pancreas and Total Body Weights (PFE-SD Experiments 3-7 and PFE-PF Experiments 3, 4, and 6)

Regimen	Day																				Total sacrif.*	Spont. deaths	Total
	0	5	7	8	10	11	12	13	14	15	18	21	22	25	28	35	36	46	48	56			
SD	—	8	10	8	8	8	15	4	4	16	21	4	3	4	19	2	3	1	1	1	140	0	140
PF-SD	—	2	10	9	11	15	14	11	17	13	13	10	6	4	15	1	2	—	1	—	154	1	155
PFE-SD	—	14	16	12	18	22	24	16	27	27	29	20	6	17	27	8	3	3	5	3	297	70	367
TOTAL	—	24	36	29	37	45	53	31	48	56	63	34	15	25	61	11	8	4	7	4	591	71	662
SD	16	8	—	—	18	—	2	—	—	8	2	—	6	—	18	—	18	—	—	—	96	1	97
PF-PF	—	12	—	—	22	—	6	—	—	12	6	—	6	—	22	—	20	—	—	—	106	11	117
PFE-PF	—	23	—	9	23	—	15	—	—	14	18	—	8	—	30	—	27	—	—	—	167	135	302
TOTAL	16	43	—	9	63	—	23	—	—	34	26	—	20	—	70	—	65	—	—	—	369	147	516

* Only the animals sacrificed (960) were used for weight determinations.

Electron Microscopic Preparation

In addition to the EM techniques previously employed with a PFE-PF regimen,[15,16,19] buffered glutaraldehyde [29] followed by post-fixation in osmic acid was used with the PFE-SD specimens. Epon embedding [30] followed by double staining of thin sections with uranyl acetate [31] and lead citrate [32] or with methanol uranyl acetate [33] were also employed. The pancreases of approximately 250 animals were examined.

Statistical Analysis of Pancreas and Total Body Weights

PFE-SD. Ethionine affected total body weight (TBW) and total pancreas weight (PW) differently so that PW could not be easily referred to total body weight. Instead, the pancreas weight at sacrifice, PWs, was related to the estimated pancreas weight at Day 0, PWo, as a ratio, PWs/PWo. The PWo was obtained from our regression analysis equation relating normal PW to normal TBW.[20] The PWs/PWo ratio, converted to a percentage, was determined for each animal and the ratio percentages for all animals in the same group which were sacrificed on the same day from different experiments were averaged. The group daily average percentages for the sacrifice days were used for the regression analysis curve of the group.

The total body weight at sacrifice, TBWs, was similarly expressed as a percentage of the total body weight at Day 0, TBWo—i.e., TBWs/TBWo $\times$ 100, as in the pancreas weight studies.

The variables PWs/PWo and TBWs/TBWo were related to the number of days on the SD, PF-SD, or PFE-SD regimen. The association was performed by fitting a curve (multiple regression or least squares analysis) to these predictors, including squares and cross products of days on PF, PFE, and SD in order to reflect nonlinearity, and 95% confidence bounds were drawn for the curves of each group [34] (Text-fig. 1 and 2 and Table 1).

Fitted curves were also obtained separately for the PWs/PWo and TBWs/TBWo values against the day of experiment, against the individual dietary components (PF, PFE, or SD) and against TBWo. Similar curves with graphs were also fitted for absolute weight changes in milligrams of PWs and in grams for TBWs.

The TBWs/TBWo and PWs/PWo ratios of 591 animals from 5 experiments (PFE-SD 3–7) were used for analysis (Text-fig. 1 and 2 and Table 1).

PFE-PF. The PWs/PWo and TBWs/TBWo ratios of 369 PFE-PF, PF-PF, and SD animals from other studies [14,34] were subjected to regression analysis in a similar manner to that used for the PFE-SD animals (Text-fig. 1 and 2 and Table 1).

The regression coefficients were tested in PF-SD and PFE-PF by using multiple comparisons. Conventional t test statistic was compared to

$$\sqrt{(F_{26,\,\infty,\,10\%})\,(26)} \text{ in PFE-PF or } \sqrt{(F_{24,\,\infty,\,10\%})\,(24)} \text{ in PFE-SD}$$

to determine whether a regression coefficient was significant, allowing for possible correlation between per cent PW and per cent TBW.[34]

Statistical Processing

The regression analyses and confidence intervals [34] were performed with the aid of a 1620, Model II, International Business Machines Corporation computer. Manual techniques were also used in portions of the regression analysis and in other statistical analyses performed by the usual methods.[21]

Results

Cytological and Cytochemical Changes

Our previous LM and EM studies of the normal [35–39] and degenerating pancreas under the PFE-PF regimen have been reported.[13,14,16,25] The regimens for Days 1 to 10 were identical in the PFE-SD and PFE-PF animals and the effects are summarized.

Degenerative Changes—Days 1 Through 10 [9,14–16,36,40]

PFE-SD and PFE-PF. During Days 1–5 there occurred swelling of the acinar cell, marked decrease of staining with thionine of the basal acinar cell cytoplasm, and a reduction in the size, number, and staining with thionine of the nucleoli. The normal sharp lobular and acinar outlines disappeared. Small cytoplasmic vacuoles, usually free of fat or glycogen and often containing PAS-positive, diastase-resistant material, were apparent by LM at Day 2 and became large and prominent later. Mitochondria and zymogen granules remained relatively unaffected until late in degeneration. Feulgen staining was decreased in concentration in some swollen acinar cell nuclei early in degeneration and increased later when acinar nuclei became smaller.

Two hours after the first ethionine injection on Day 1, EM revealed the ethionine lesion of a focal loss of free ribosomes and absence of ribosomes from ergastoplasmic membranes as well as an increased osmiophilia of the ergastoplasmic membrane. Even at this time there were present vacuoles with a few osmiophilic membranes replacing ergastoplasm (Fig. 1). Later, larger cytoplasmic vacuoles contained highly osmiophilic remnants of endoplasmic reticulum coalesced into plaques and myelin figures. Large vacuoles, after Day 5, often contained cellular debris, mitochondria, or zymogen granules, as well as damaged membranes.

The degenerative changes progressed rapidly after Day 10 to marked cytoplasmic vacuolation, necrosis, or dissolution of the acinar cells (Fig. 2). At Day 10 only a small percentage of the cells of the pancreas remnant were acinar cells, and these were usually severely damaged (Fig. 3). Many macrophages, often with PAS-positive cytoplasmic material and plaques of osmiophilic membranes and debris, and a smaller component of polymorphonuclear leukocytes were present. Acinar reticulum framework and basement membrane appeared relatively intact, although wrinkled and distorted. Relative concentration of capillary endothelium was increased (see Fig. 2 [12]).

Repair and Regeneration—Days 11–36 [9,14–16,36,40]

PFE-PF. At Days 10–12, scattered sparsely throughout the pancreas were large, distinctive cells containing a round nucleus with prominent

nucleoli and a cytoplasm which stained intensely with thionine and acridine orange (Fig. 4A).[14,19,40] EM revealed that they were acinar cells with considerable ergastoplasm, often whorled, and containing the characteristic ethionine lesion.[14,19] They had relatively little apical cytoplasm or zymogen granules. At Day 15 the EM studies showed that the acinar cells were larger, had hypertrophied Golgi apparatus and increased apical cytoplasm, and contained homogeneous, lightly staining amorphous substance in the apical cisternae. The ethionine lesion was still present in most acinar cells. At Day 18, more and larger acinar cells were present, mitotic figures were observed, and ethionine lesions were rare. Many small zymogen granules were present. At Days 28 and 36, relatively normal-appearing acinar cells and acini were formed, although lobule and acinar formation were sometimes indistinct.

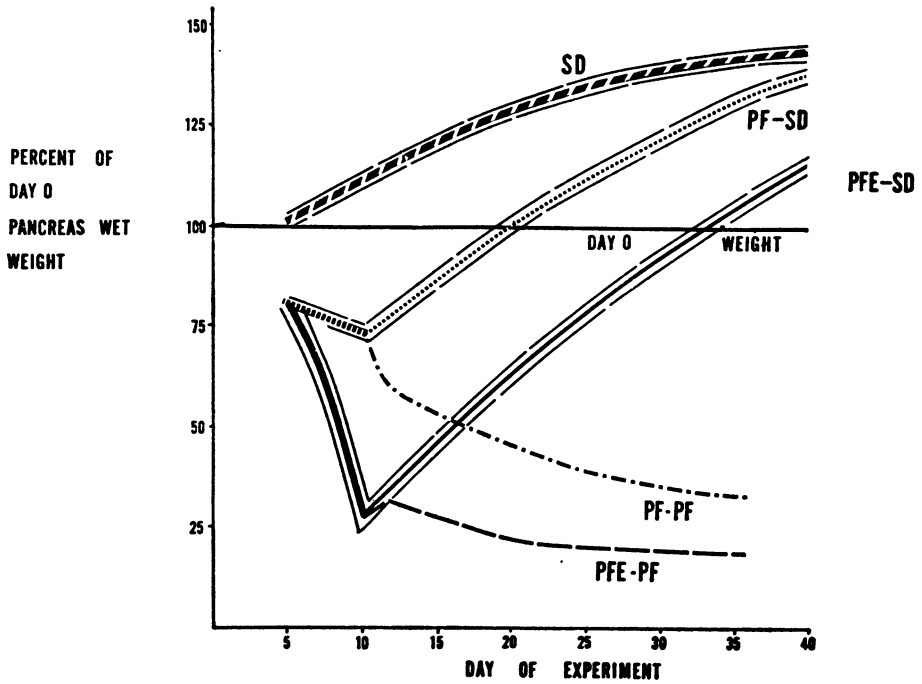
PFE-SD. Repair and regeneration were faster and more extensive in the PFE-SD animals. At Day 12 acinar cells with the intense thionine (Fig. 4A) and acridine orange-staining cytoplasmic material (Fig. 4B–4E) were present.[14,19,40] The whorls of ergastoplasm, the osmiophilic plaques of membranes, and membrane-lined vacuoles of the ethionine lesion were conspicuous in these cells. They had an increased number of cytoplasmic ribosomes and small intracytoplasmic filaments. Cisternal vacuoles with a slightly electron-dense substance were noted. These findings were similar to those of the PFE-PF animals of Day 15.

At Days 14 and 15 in the PFE-SD animals, there were good acinar and lobule formation, an increase of acinar cell cytoplasm, and frequent mitotic figures. In the acinar cells, multiple huge nucleoli stained an intense red to orange with the acridine orange dye, as did the cytoplasm (Fig. 4D and 4E). At Day 15 EM revealed abundant, relatively normal ergastoplasm with very few remnants of the ethionine lesion. Mitochondria, the Golgi apparatus, and nuclei appeared normal. Some cells had zymogen granules of the usual appearance, but in the apical cytoplasm of most acinar cells the cisternae showed a lightly stained material, often with a denser central area (Fig. 5). At Day 18 the PFE-SD acinar cell resembled the PFE-PF cell of Day 28.

The acinar epithelium of Days 25 and 28 was essentially normal except that the acinar cell, its nucleus, and its nucleoli appeared to be larger than normal (Fig. 6). Focal areas in the pancreas showed an absence of acini and the presence of fat.

In the normal rat pancreas acinar cell, only rarely did we find any cytoplasmic structure which might fall into the various categories of lysosomal bodies.[41–46] During degeneration in the PFE regimen, most acinar cells showed the ethionine lesion.[14,16,38] After Day 15 in the PFE-SD animals and after Day 18 in the PFE-PF animals, there were very few acinar

cells with ethionine lesions present. We have been unable, therefore, to correlate an increase or decrease in number of a definitive ultrastructural body, consistently present in the normal rat pancreas acinar cell, with the ethionine-induced degenerative or regenerative changes. There was no cell organelle which might be considered to be the structural unit or sac containing the lysosomal enzymes, as originally postulated.[41-46]



TEXT-FIG. 1. Regression analysis curves of the total pancreas wet weight (PW) changes of 2 different regimens: (1) SD, PF-SD, and PFE-SD, and (2) SD, PF-PF and PFE-PF. Average pancreas weight at sacrifice (PWs) in each group was referred to respective average total pancreas wet weight at Day 0 (PWo) as a ratio PWs/PWo. Analysis was made of 591 animals from 5 PFE-SD experiments (Experiments 3–7) and 369 animals from 3 PFE-PF experiments (Experiments 3, 4, and 6; Table 1). Total body weight of the animal at Day 0 was used for estimation of PWo.[30] Curves are representative of animals of total body weight of 185 gm. Ordinate is ratio PWs/PWo in per cent, with Day 0 set at 100%; 95% confidence bounds were determined for the PFE-SD regimen and its controls. The change in direction at Day 11 for PF-PF and PFE-PF data reflects the fact that Days 5–10 were based on PF and PFE data.

Pancreas Weight (PWs/PWo)

PFE-SD. The proportion PWs/PWo and its variation with regimen, day of experiment, and total body weight at Day 0 can be expressed, on the average as:

$$1.92 - (.011)(TBWo) + .00003\ (TBWo)^2 + .023\ (dPF) + .001\ (dPF)^2 + .035\ (dPFE) - .008\ (dPFE)^2 + .04\ (dSD)^* - .0003$$

$(dSD)^2 - .0003\,(dPF)\,(TBWo) - .0009\,(dPFE)\,(TBWo) - .0001\,(dSD)(TBWo) + .0006\,(dPF)(dSD) + .001\,(dPFE)(dSD)^*$. The factors with asterisks were significantly different from zero by the F test.[34]

PF and PFE regimens decreased PWs. Both regimens when followed by the stock diet (PF-SD and PFE-SD) were additive to the SD effect as measured by their respective coefficients, .0006 and .001, each multiplied by the number of days on the respective diet times the number of days on stock diet.

The PWs in the PFE animals at Day 10 was less than 30% of the PWo value and less than 25% of the SD PWs at this time. After Day 10 the PWs increased in the PFE-SD animals so that the PWo in animals of 185 gm. would be reached at about Day 34. The slope of pancreas weight gain after Day 10 was higher in the PF-SD and PFE-SD animals than in the SD animals whose slope decreased at the end of the experiment (Text-fig. 1).

PFE-PF. The PWs/PWo ratio of the animals on the PFE-PF regimen could be expressed as:

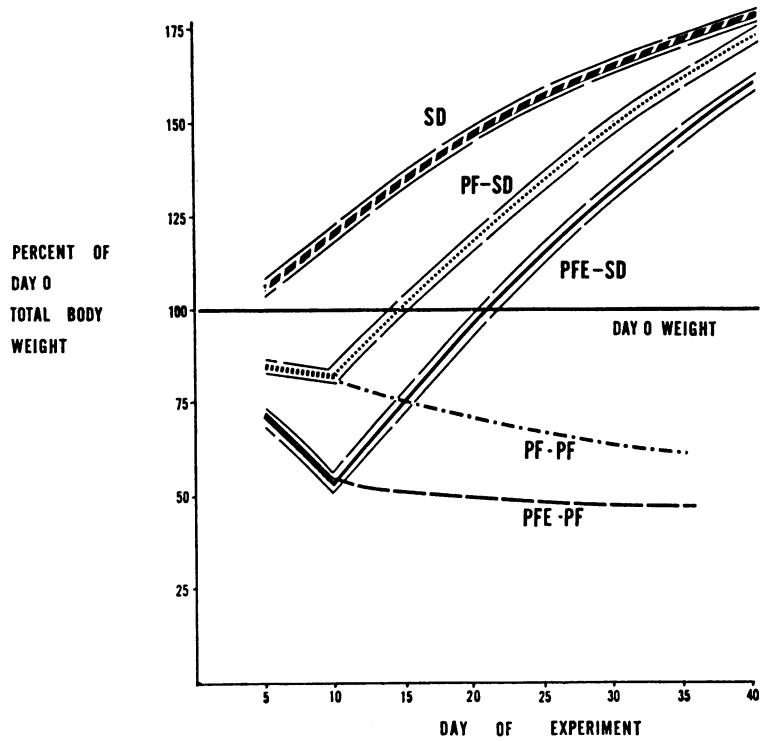
$202.8 + 2.6\,(dSD) - 3.4\,(dPF) + 0.6\,(dPFE) - 1.3\,(TBWo) - 0.06\,(dSD)^{2*} + 0.04\,(dPF)^2 - 0.6\,(dPFE)^2 + 0.004\,(TBWo)^2 + 0.1\,(dPF)(dPFE)^* + 0.003\,(dSD)(TBWo) + 0.003\,(dPF)(TBWo) - 0.0003\,(dPFE)(TBWo)$. The factors with asterisks were significantly different from zero.[34]

In the animals given the PFE-PF and PF-PF regimen there were decreases in pancreas weight after Day 10 (Text-fig. 1).[35]

Residual Pancreas Acinar Cell Tissue

It was difficult to estimate from histologic sections of the pancreas from the PFE animal at Days 10 or 12 the relative mass of acinar tissue or the number of acinar cells present. From a study of the same tissue that indicated the approximate relative number per unit area of each type of cell in the SD and PFE-SD pancreas at Days 10 and 12,[13] the loss of over 70% of the pancreas weight (the loss being almost entirely acinar cell tissue) and the fact that necrotic acinar cells, edema, and inflammatory cells made up part of the weight of the residual pancreas at Days 10–12, we estimate that at these days, morphologically intact acinar cells in the pancreas residual tissue were less than 15–20% of the SD total number of acinar cells. If the enzyme activities present in the residual pancreas at Days 10–12 are indicative of the amount of acinar cell tissue present and if they are corrected for decreases of total pancreas weight and nitrogen concentration,[12] they seem to indicate that less than 10% of the SD amount of functioning acinar tissue remained.

Little morphologic damage to duct, ductular, vascular, or islet cell



TEXT-FIG. 2. Regression analysis curves of average total body weight (TBW) of the SD, PF-SD, and PFE-SD regimens and SD, PF-PF, and PFE-PF regimens. Average TBW at sacrifice referred to the respective average TBW at Day 0 as a ratio TBWs/TBWo. Same number of animals analyzed as in Text-fig. 1. Ordinate is TBWs/TBWo in per cent, Day 0 set at 100%; 95% confidence bounds were determined for the PFE-SD regimen and its controls.

tissues was apparent,[16] and these tissues became relatively more concentrated per unit of residual tissue with the decrease of acinar cell tissue (Text-fig. 2[12]).

Total Body Weight

PFE-SD. The proportion TBWs/TBWo and its variation with regimen, day of experiment, and total body weight at Day 0 was fitted, on the average by:

$2.44 - .016\,(TBWo) + .00004\,(TBWo)^2 + .011\,(dPF) + .0007\,(dPF)^2 - .033\,(dPFE) + .00004\,(dPFE)^2 + .051\,(dSD) - .0003\,(dSD)^{2*} - .0001\,(dPF)(TBWo) - .00002\,(dPFE)(TBWo) - .00008\,(dSD)(TBWo) + .0004\,(dPF)(dSD) + .001\,(dPFE)(dSD)^*$. Factors with an asterisk were significantly different from zero by the F test.[34]

TBWs/TBWo was 53% at Day 10 and by Day 22 TBWo had been regained (Text-fig. 2).

PFE-PF. The proportion TBWs/TBWo can be expressed:

$239.3 + 4.4\,(dSD) + 0.2\,(dPF) - 6.7\,(dPFE) - 1.5\,(TBWo) - 0.05\,(dSD)^{2*} + 0.01\,(dPF)^2 + 0.2\,(dPFE)^2 + 0.004\,(TBWo)^2 + 0.06\,(dPF)(dPFE) - 0.002\,(dSD)(TBWo) - 0.009\,(dPF)(TBWo) + 0.007\,(dPFE)(TBWo)$. The factors with asterisks were significantly different from zero.[34]

There were decreases in total body weight after Day 10 in both the PFE-PF and PF groups (Text-fig. 2). The graphic results of total pancreas and total body weights reflected the fitted curves.

If one were to assume no correlation between PW per cent and TBW per cent, additional significant factors would be: square of days on ethionine and on stock diet in PW of PFE-SD data; initial body weight, its square and days on stock diet in TBW of PFE-SD data; and days on PF and squares of days on PFE in PW of PFE-PF data.

Discussion

Pancreatic Acinar Cell Regeneration

The return of the pancreas in the adult rat to its initial weight and structure within 3–4 weeks after the destruction of most of the acinar tissue by ethionine is surprising. The pancreas has not generally been considered as an organ with much potential for regeneration,[6,47,48] although some investigators have suggested that the gland might possess limited ability to regenerate.[49–53] In 1885 di Mattei reported that a few days after cutting into the pancreas of a dog or guinea pig, mitotic figures were noted in acinar cells.[51] Subsequent attempts to assess the ability of the pancreas to regenerate after surgical partial resection had been largely qualitative, restricted in the number of animals,[49,52,53] or the results were equivocal.[50] Our demonstration of regeneration following ethionine [9] and the present quantitative estimate of the degree of regeneration indicate the regenerative performance after a metabolic analogue. Recent evidence of regeneration after surgical resection has also been convincing.[54–56]

When the PWs of the PFE-SD animals reached the PWo at Day 34, the PWs was approximately 70% of the SD value. At Day 40 it was about 80% of the SD level and its rate was decreasing. Liosner's studies over many years showed that after removal of one of paired organs, the other kidney, ovary, lung, thyroid lobe, or adrenal reached approximately 70–80% of the weight of both organs in control animals.[57] This upper limit implies the existence of a common control mechanism regulating the amount of replacement of these organs.

Our regression equation would predict that with the PFE-SD regimen, an animal of 200 gm. total body weight will sustain a higher percentage of pancreas weight loss after ethionine than would an animal of 100 gm.,

but that the latter would regain his PWo faster. An animal of 300 gm. will be the slowest to regain the PWo level. Total body weight would appear to be an important factor in determining the rate of pancreas regeneration, as it is in liver regeneration.[3]

Pancreas acinar cell regeneration after ethionine is different from many types of regeneration. There is morphologic injury to the cell which regenerates. No dedifferentiation, blastema formation, or migration of cells occurs, as in the epimorphosis of amphibian or crustacean limbs.[2,5-7] There is no remodeling of the entire organism—morphallaxis—as illustrated by one type of planarian regeneration.[1,6] Pancreas regeneration does not resemble the constant regeneration of skin, mucous membranes, bone marrow, lymphopoietic cells, or other cell types that have a sustained, relatively high rate of DNA synthesis.[47,58] It would appear to have many features in common with renal tubule regeneration.[59,60] Pancreatic acinar cell regeneration after ethionine is a diffuse, multicentric restoration, in situ, of relatively normal acini by repair, hyperplasia, and hypertrophy of injured differentiated residual acinar cells lying within the original acinar reticular framework.

Cytologic Findings and Regeneration

The decreased number and size of nucleoli with the PFE regimen[14,16] have also been reported by others[11,40] and are consistent with the role of the nucleolus in RNA synthesis.[61-64] A reported decrease in acinar cell nucleolar size during a PF regimen[65] did not occur in our prior[66] or present experiments. The very large acinar cell nucleoli noted at Day 12 (Fig. 4B) and thereafter in the PFE-SD animals are analogous to the enlarged nucleoli of liver regeneration[61,62] and are consistent with increased RNA and protein synthesis.[63,64]

We have suggested that the whorls of ergastoplasm in the acinar cell of Day 12[14-19] may be indicative of increased protein synthesis. Autoradiographic studies at Day 12 with leucine-H^3 of the acinar cells with intense basophilia[14,19,40] demonstrated that whereas at Day 10 the number of grains per unit area of cytoplasm in acinar cells in the PFE-PF animals decreased to values below SD levels, at Day 12 there was a return to the SD values.[35] Johnson and Roman also found an increase of protein synthesis prior to an increase of DNA synthesis in kidney regeneration.[59]

Variations in the number and the degree of maturation of zymogen granules and the return of acinar cell enzyme activities will be discussed in the following paper.[12]

The restriction of the ethionine lesion primarily to acinar cells, the persistence of some acinar cells at all times, and the dual occurrence of

the ethionine lesion and whorls of ergastoplasm in the same acinar cell that showed features of regeneration indicate that the reversibly damaged acinar cell (Fig. 4A) is the source of the acinar cell regeneration.

Acinar Cell Injury, Tissue Loss, and Regeneration

The greater regenerative response of acinar cells in the PFE-SD animals than in the PF-SD animals was associated with injury, necrosis, or disappearance of acinar epithelium and considerable regeneration in the former and relatively little damage or regeneration in the latter. Our regression equation suggests that the *rate of regeneration* was related to the number of days on ethionine and thereby, presumably, to the amount of acinar cell injury or destruction. Age, total body weight,[67] and other factors[67] may modify the regenerative response to ethionine.[13] Our regression equation shows that the number of days on a PFE regimen times TBW was not significant, implying—since dosage was determined on a TBW basis—that ethionine inflicts damage per unit of total body weight, irrespective of TBW.

Regeneration may not be related to the intracellular damage per se but to the decrease in amount of pancreas acinar tissue.[54–56] However, the more rapid restitution after ethionine than after surgical resection suggests that an additional factor(s) may be involved in post-ethionine regeneration.[13,17]

Use of the Model

The distribution of the rodent pancreas throughout the mesentery is a disadvantage. The organ has many digestive and autolytic enzymes. As in most organs, it has a population of cell types. With the PFE regimen there is considerable toxicity and mortality. It is difficult to estimate accurately the number of acinar cells or the amount of pancreas tissue destroyed by ethionine.

The organ is representative of a mammalian glandular tissue with a high rate of protein synthesis;[68] its acinar cell has characteristic enzymes, and hundreds of milligrams of tissue may be obtained from one rat. With experience, consistent ratios of total pancreas wet weight to total body weight may be obtained.[20] The organ has an ultrastructural compartmentalization[37] that, as shown by the biochemical and EM studies of Palade and co-workers, exhibits a distinctive pathway of protein synthesis and secretion.[69–72] Post-ethionine regeneration is primarily of one cell type—the acinar cell—even though there is some increase in the DNA synthesis of other cell types.[13] The change from a relatively low to a relatively high rate of DNA synthesis is sustained for 2–3 weeks, and the organ weight return to the pre-ethionine level occurs in about 3–4 weeks.

The effects of various dietary regimens and compounds on hyperplasia and hypertrophy of process can be studied over a period of a few weeks, and it may be possible that the relatively longer period of increased DNA synthesis is advantageous for a study of factors involved in hyperplasia. Comparative studies of post-ethionine pancreas regeneration with other organs after partial resection, especially pancreas and liver, should give information concerning the possibility of their being common mechanisms involved in these otherwise disparate types of regeneration.

Summary

1. After destruction of most of the acinar cell epithelium of the adult rat pancreas by an ethionine, protein-free diet regimen and a subsequent stock diet refeeding without ethionine (PFE-SD), there was regeneration of acinar tissue and restitution of the cytologic, cytochemical, and ultrastructural features of the pancreas to normal configurations within 2–3 weeks.

2. After an ethionine-induced loss of about 70% of the pancreas weight, there was an increase of pancreas weight over a period of 3–4 weeks in PFE-SD animals to the pre-experimental weight of the gland.

3. It is believed that the reversibly injured acinar cell is the cell which gives rise to the regenerating acinar tissue.

4. The pancreatic acinar cell injury induced by ethionine may be a useful model in the studies of degeneration and regeneration involving a well-differentiated mammalian exocrine cell which normally has a high rate of protein synthesis and secretion and a low rate of DNA synthesis.

5. Comparison of pancreatic acinar cell regeneration after ethionine with pancreas and liver regeneration after partial resection of these organs may indicate whether in these different organs and different types of regeneration there are called into play common mechanisms which initiate, sustain, and terminate regeneration.

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[*Illustrations follow*]

Legends for Figures

Figures 1, 2, 5, and 6 are electron micrographs of sections fixed in buffered OsO_4 and stained with uranyl acetate and lead citrate.

Fig. 1. Pancreas of PFE animal 2 hr. after an I.P. injection of ethionine. Mitochondria, zymogen granules, and Golgi complex are normal. Endoplasmic reticulum (ER) is mostly intact except for ethionine lesion (Le) where ER is disrupted by vacuoles containing osmiophilic plaques of membranes, myelin figures, and debris. × 28,800.

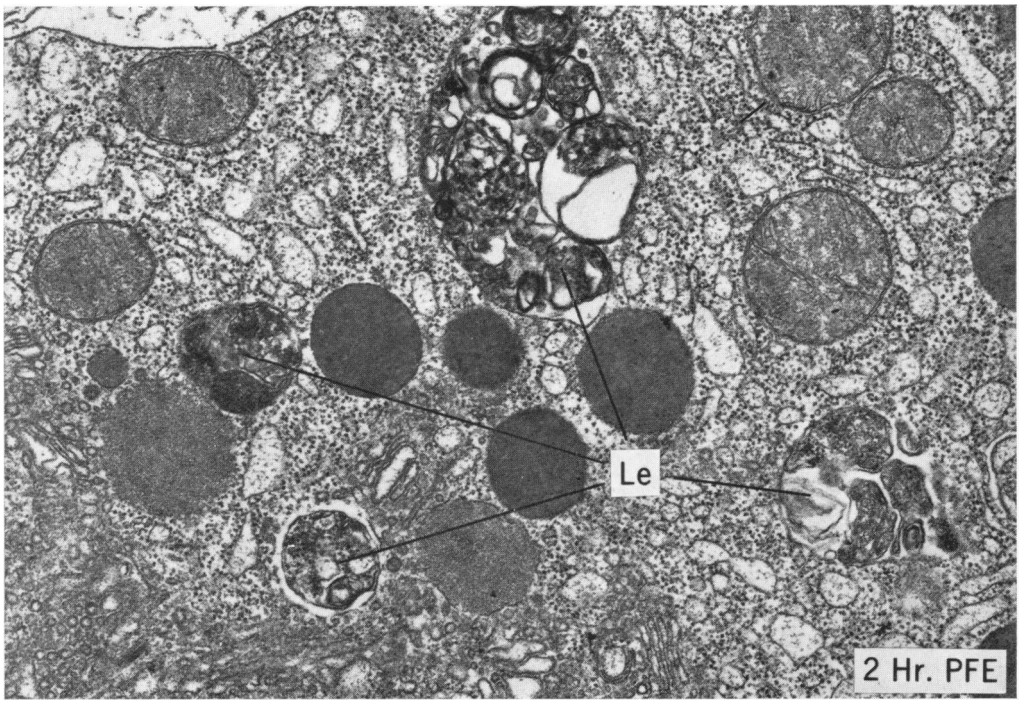

Le
2 Hr. PFE

Fig. 2. Pancreas of PFE animal at Day 10. Normal acini and acinar cell patterns (see Fig. 6 for comparison) are disrupted by lesions (Le) of large vacuoles replacing acinus in some areas and occupying much of the cytoplasm of most acinar cells. Acinar cell vacuoles contain varying amounts of intracellular debris: highly osmiophilic plaques of membranes, myelin figures, distorted swollen mitochondria, and zymogen granules. Macrophages contain similar debris. Acinar cell nuclei are smaller than normal, with irregular nuclear membranes and a decreased number of smaller nucleoli. Intercellular edema and collagen are evident. A few cells are relatively intact with fairly well preserved zymogen granules (6 and 9 o'clock). × 4050.

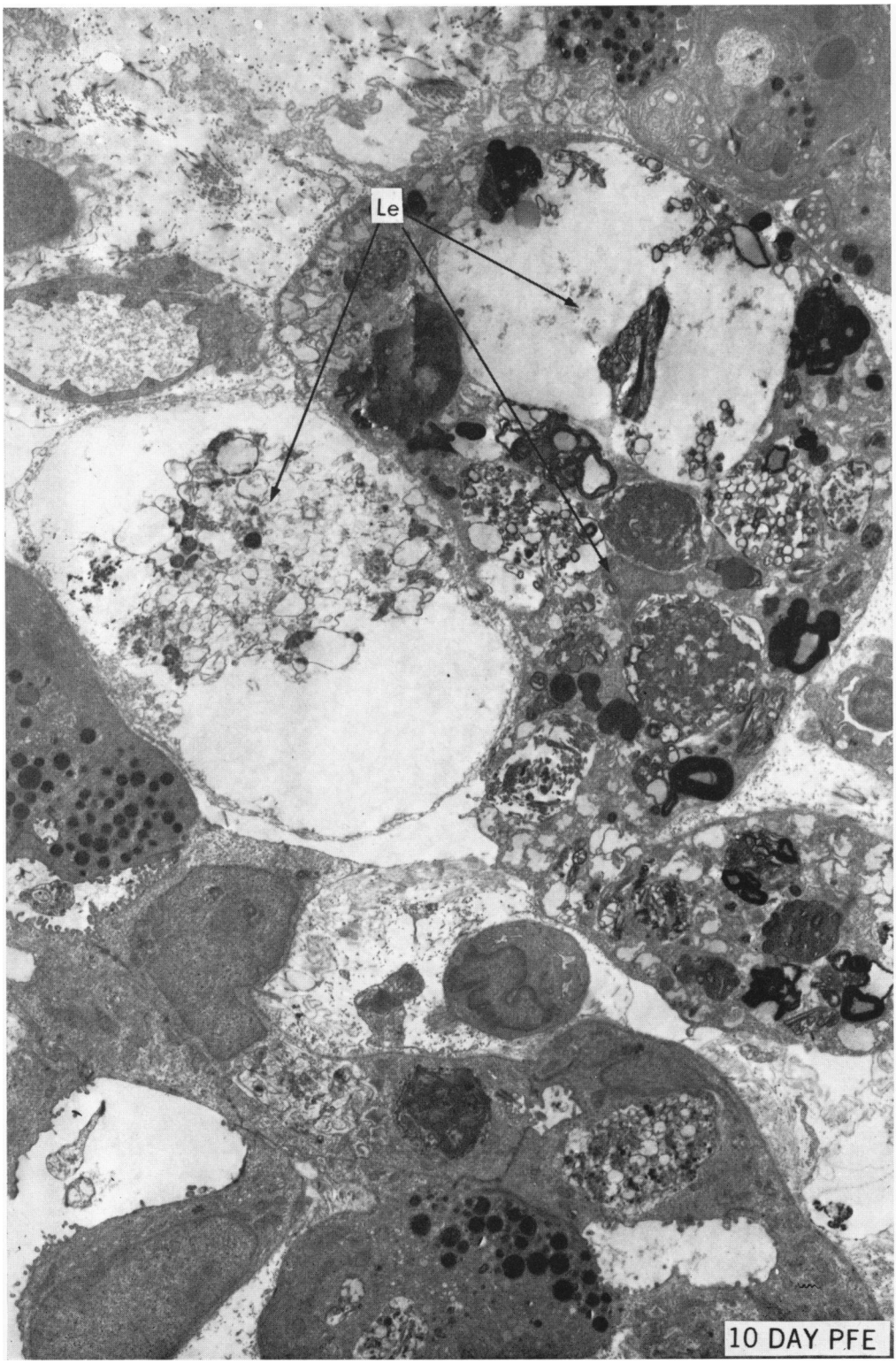
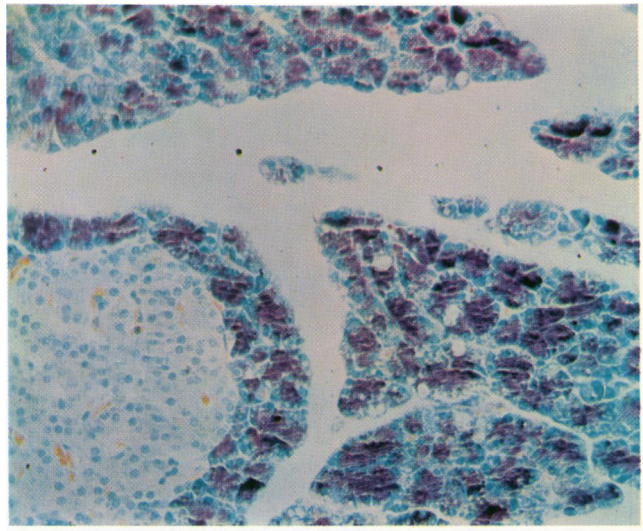
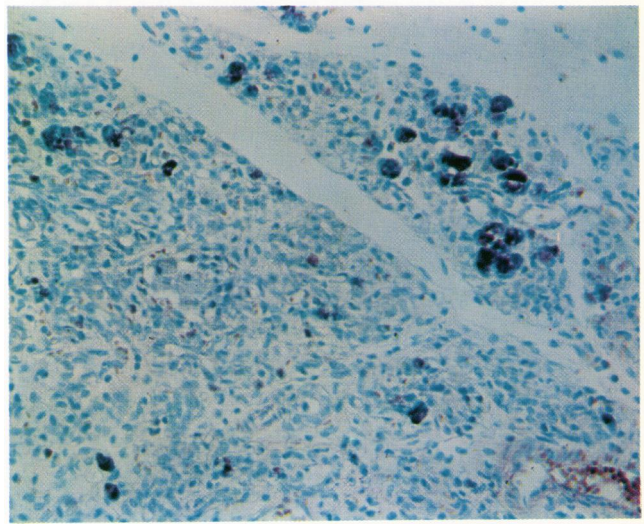

Le
10 DAY PFE

Fig. 3. Zymogen granule stain (tetrachrome[25]) of pancreas. **A.** Control animal on stock diet (SD). Purple staining represents zymogen granules in apical cytoplasm of acinar cells. Relatively light area is islet of Langerhans, in which granules do not concentrate tetrachrome stain. × 120. **B.** PFE animal, at Day 10, showing zone of marked destruction. Absence of tetrachrome stain indicates absence of zymogen granules. Most of acinar cells have disappeared. Pancreas is composed primarily of vessels, ductules, ducts, islets, and interstitial and inflammatory cells (primarily macrophages). Even in this field, however, a few acinar cells with zymogen granules remain. × 120.

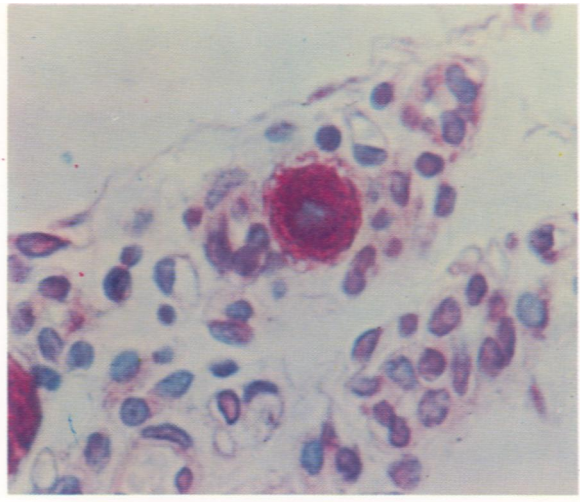


3A



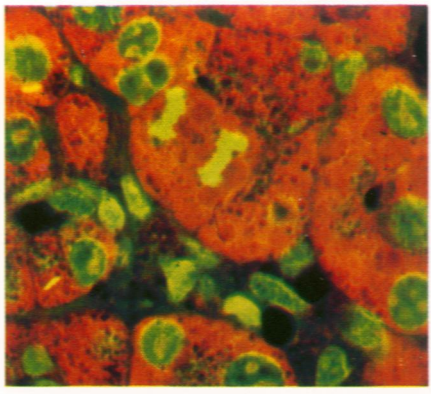
3B

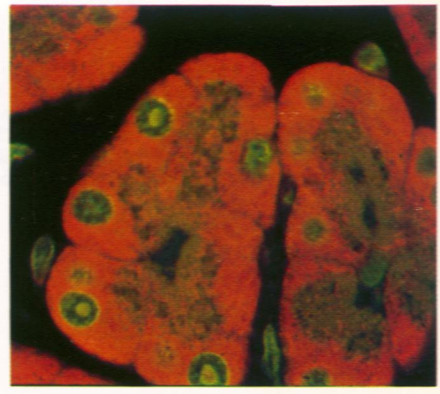
Fig. 4. A. Rat pancreas, Day 12 PFE-PF. Pink-purple stain indicates RNA.[23] Intense cytoplasmic staining of large spherical acinar cell, upper center, is associated with whorls of ergastoplasm often seen by EM at this day.[11,19] Such a surviving acinar cell appears by EM to have repaired much of the ethionine lesion and seems to be the cell which subsequently divides and is responsible for hyperplasia of acinar cell regeneration. Methyl green-thionine stain × 840. **B–E.** Rat pancreas, Day 15 PFE-SD. Green indicates DNA or protein, and orange-pink represents RNA.[24] Figures **B, D,** and **E** are from PFE-SD animals; **C,** from SD control. Mitotic figures in acinar cells of **B** and **D** are indicative of increased replication of DNA.[13] Intense orange-pink staining of cytoplasm of acinar cells is consistent with normal or increased RNA concentration (Fig. 5 and 6). The relative paucity of RNA in cytoplasm of duct and interstitial cells (small, elliptical, green-stained nuclei) is striking and consistent with their ultrastructure.[37] Enlarged orange-red nucleoli of acinar cell (particularly in **D** and **E**) are consistent with an increased concentration and content of RNA. Small round cytoplasmic vacuoles varying from black background (**B**) to yellow (**E**) may represent immature zymogen granule substance, prozymogen (Fig. 5), not taking the green of mature zymogen granules (**C** and Fig. 6). Glutaraldehyde fixation, acridine-orange stain, photographed with fluorescent UV light.[24] × 1020.



4A

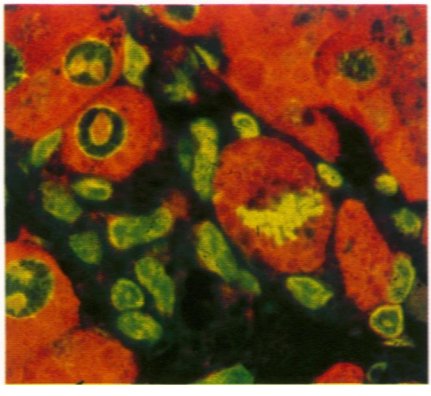
4B

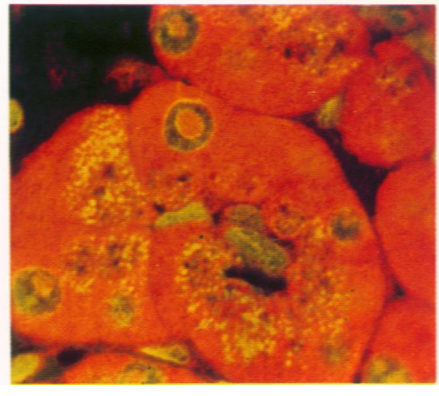




4C

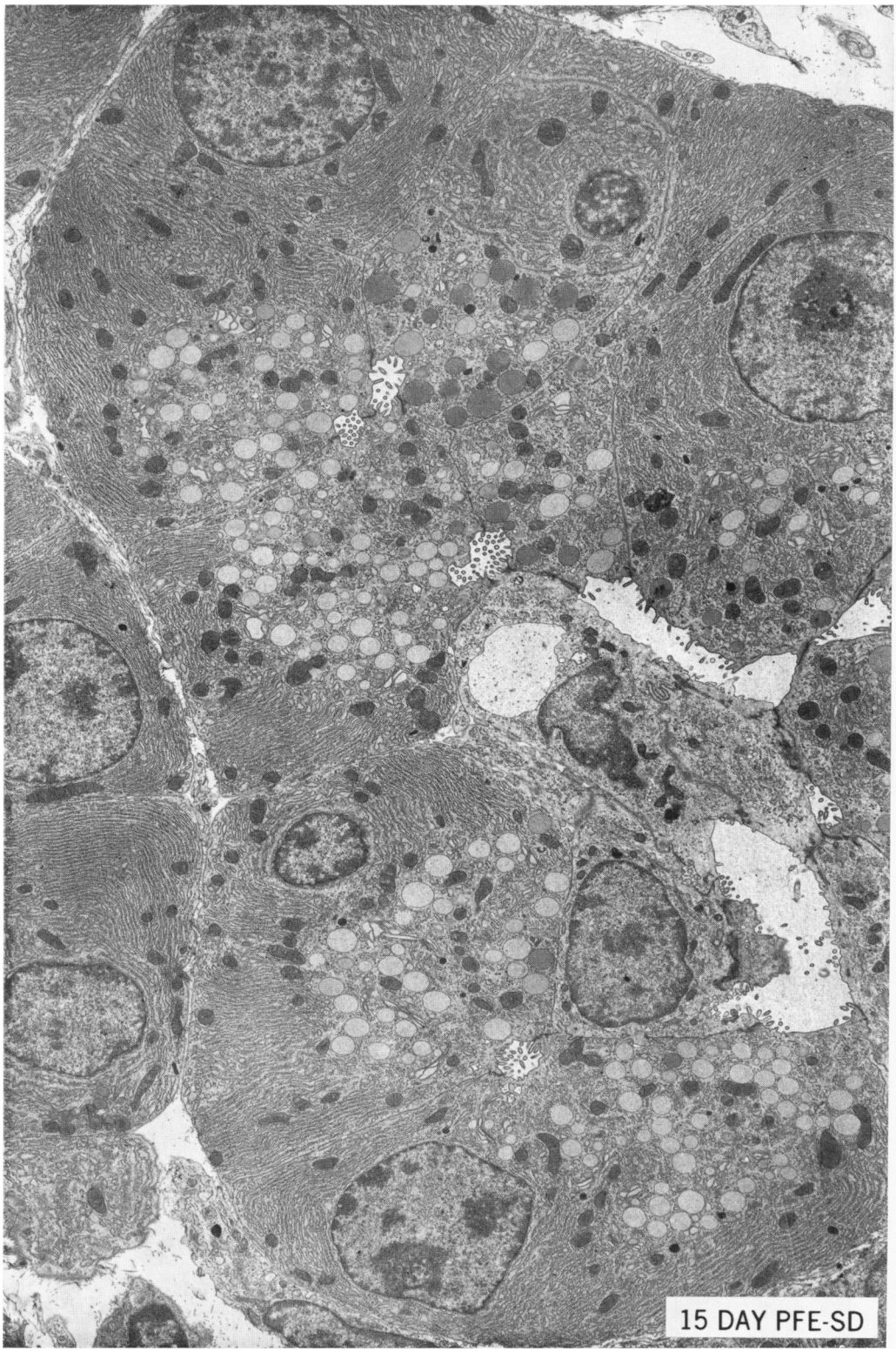
4D





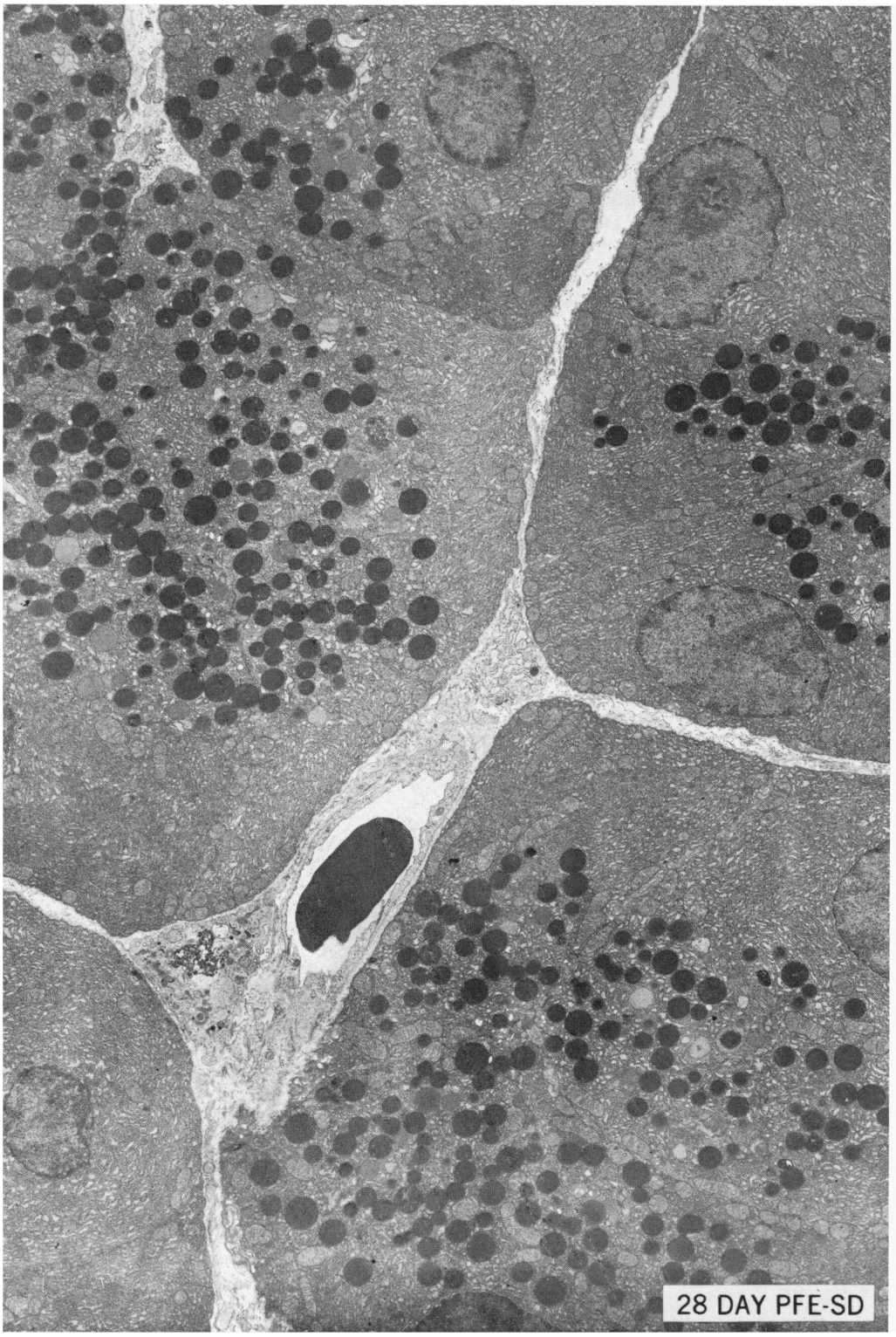
4E

Fig. 5. PFE-SD animal, at Day 15. Acini and acinar cells are relatively normal (compare with Fig. 6). Basal spherical nuclei are large, with prominent surrounding ergastoplasm, large nucleoli, and apical cytoplasm with many cisternae containing a slightly dense material (?prozymogen). Only a rare ethionine lesion is present. There is a relative lack of dense zymogen granules. Acinar cell appears smaller and has less apical cytoplasm than does the normal acinar cell. × 4050.



5

Fig. 6. PFE-SD animal, at Day 28. Structure of acinus and acinar cell is essentially normal. Note abundant, dense zymogen granules, in contrast to lightly staining material present in cisternae of PFE-SD animal at Day 15 (Fig. 5). × 4050.



6