CHANGES IN PANCREATIC ACINAR CELLS DURING PROTEIN DEPRIVATION

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ABSTRACT

After 10 days of a protein-free diet the acinar cells of the rat pancreas showed a coarsening of nuclear matrix, depletion of zymogen granules, some loss of ribosomes, and a widening of the spaces between ergastoplasmic membranes. In addition, there could be found, but rarely, a lesion of the ergastoplasm consisting of vacuoles of agranular, disoriented membranes, which was similar to a lesion produced by ethionine. Thereafter, a return toward normal structure occurred which was characterized by beginning increase in the size of the Golgi apparatus at 12 days, appearance of zymogen granules at 18 days, and a relatively normal appearing but smaller cell at 28 days. After 10 to 12 days of protein deprivation a reversal of many of the morphologic effects of protein deprivation was accompanied by a return toward normal of some pancreatic enzyme activities. Possibly this spontaneous return toward normal levels represented a raiding of protein stores, or it may have been an adaptive phenomenon.

INTRODUCTION

As part of a study of the effect of ethionine and a protein-free diet on the morphology of the acinar cell of the pancreas (6, 7, 12), the effect of protein deprivation alone on the acinar cell was studied by electron microscopy.

MATERIAL AND METHODS

Thirty white Wistar male rats (Carworth Farms, New City, New York) weighing approximately 160 grams each were placed on a protein-free diet (Nutritional Biochemicals Corp., Cleveland) (for details of diet see reference 12). Five animals were sacrificed on each of the following days after the start of the experiment: 5, 8, 10, 12, 18, and 28. These represent three separate experiments. The morphology of the pancreatic acinar cells of animals in the protein-free group was compared with that of the pancreatic acinar cells of animals on a stock diet and of animals injected with ethionine while on a protein-free diet (12). Details of tissue processing for light and electron microscopy have been reported (6, 12).

For studies of basophilia, small pieces of pancreas from animals maintained on a stock diet and a protein-free diet were fixed in phosphate-buffered (pH 7.0) 10 per cent U.S.P. formalin, embedded together in paraffin, and sectioned at 4 μ. Slides were incubated for 2 hours in magnesium sulfate–McIlvaine buffered (pH 6.5) 0.01 per cent "protease-free" crystalline deoxyribonuclease (DNase) (Worthington Biochemical Co., Freehold, New Jersey), and stained with azure B (9).

OBSERVATIONS

A. *Stock Diet and Ethionine, Protein-Free Diet Groups*

The findings in animals of the stock diet and ethionine, protein-free diet groups have been

described in detail (6, 12). Fig. 1 shows a pancreas of the stock diet group.

B. *Protein-Free Diet*

Days Five to Eight

No clear-cut consistent changes were recognized. Occasionally, acinar cells showed depletion of zymogen granules and diminution of ergastoplasm. No focal lesion in the cytoplasm similar to that induced by ethionine (12) was recognized.

Days Ten to Twelve

There was a conspicuous reduction in the number of mature zymogen granules and, in some instances, absence of them at the apex of the acinar cell (Fig. 2). The apical cytoplasm contained round, membrane-bounded structures having a diameter equal to that of the normal zymogen granules but containing a substance whose density was much less than that of the zymogen granule of the acinar cell of the stock diet animals (Fig. 1). The space between elements of the endoplasmic reticulum appeared to be increased in size, and numerous obliquely sectioned membranes were seen (compare Figs. 6 and 7).

The Golgi zone was more prominent and the vacuoles appeared to be increased in number. The normal arrangement of Golgi components (vacuoles, vesicles, and distended or compressed cisternae) seen in stock diet animals was not present (compare Figs. 4 and 5). Rather, the cisternal and vacuolar constituents, instead of being flattened, elongated profiles as they are in acinar cells of the stock diet group, were more oval, round, and irregular profiles (Fig. 5). In some cases, Golgi vesicles were seen to be partly encircled by the lamellae of vacuolar elements. The Golgi apparatus was larger and the zone was filled with many small vesicles, some containing a slightly osmiophilic substance (Fig. 5).

Stellate-shaped lipid droplets located basally in the cell were present in significant numbers, sometimes in clusters (Fig. 7). They were similar to those seen occasionally in the stock diet group. It was consistently noted that no demonstrable relationship existed between the lipid droplets and the mitochondria similar to that described in the pancreas of fasted-refed guinea pigs (17, 18).

Focal lesions of ergastoplasm consisting of vacuoles with agranular membranes, myelin-like figures, and enmeshed zymogen granules and mitochondria were seen rarely (Figs. 8 and 9). These were identical with some structures found in the degenerating pancreas of animals on an ethionine, protein-free diet (12) but they occurred with far less frequency; fewer than 1 per cent of the cells surveyed revealed the lesion. Decrease of free cytoplasmic ribosomes was noted.

Day Eighteen

A homogeneous, fine granularity of the nucleus was present. This was similar to that noted in the acinar cells of the stock diet and the ethionine-injected animals of day 12. Although normal acinar patterns predominated, some atrophy of the cells was noted. Reduced ergastoplasm and increased cisternal areas persisted. Most of the cells observed were still free of zymogen granules, except for some cells which contained a few full sized dense zymogen granules scattered throughout the apex. Profiles of Golgi elements were seen but were not so prominent as those in the pancreas of animals of days 10 to 12. Basal lipid droplets (occasionally as many as ten per cell) were prominent at this stage (Fig. 10).

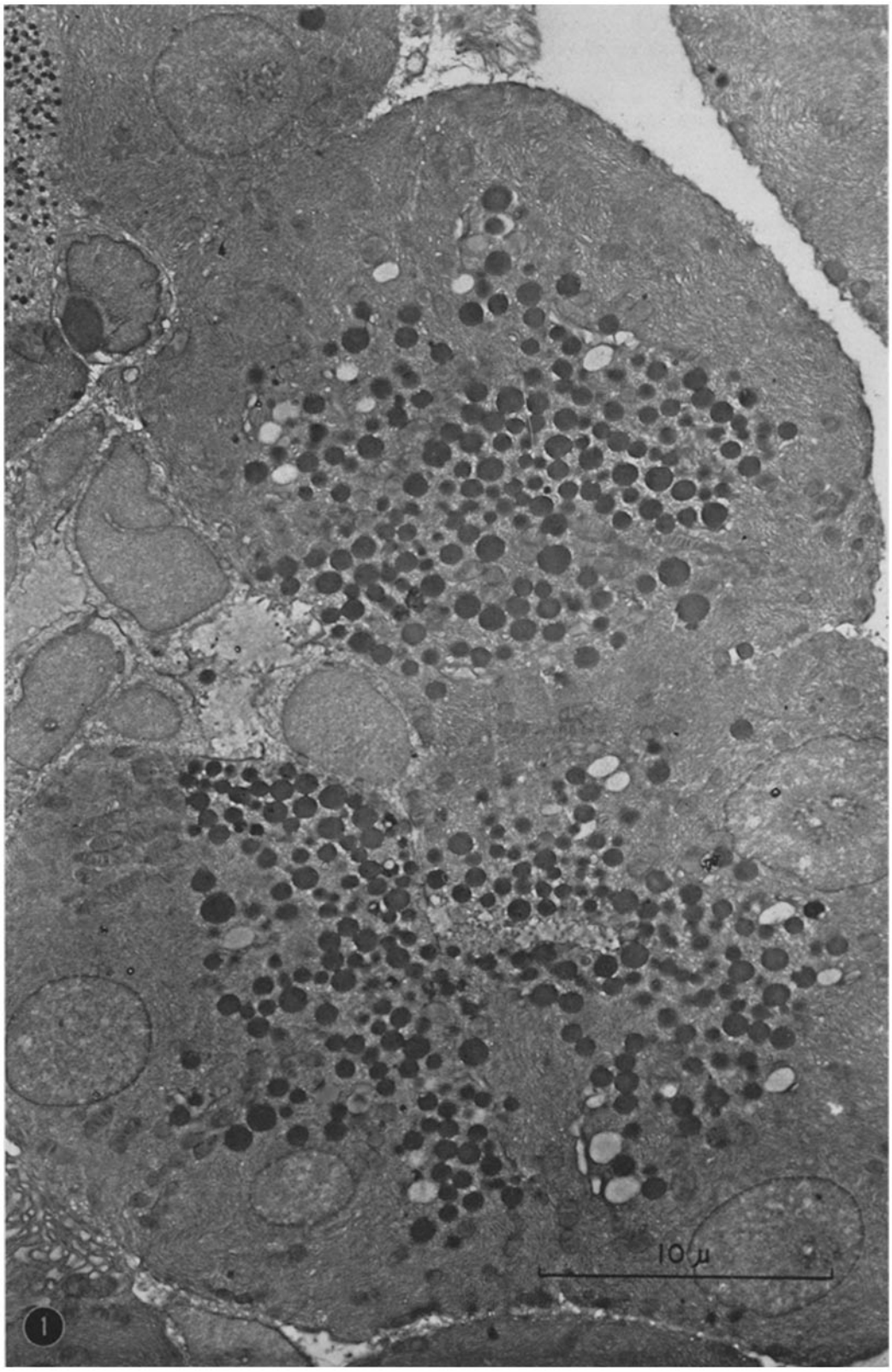
Day Twenty-Eight

Most of the acinar cells were smaller than those of the stock diet group or of the protein-free animals at days 10 to 12. The decrease in cell size represented predominantly a loss of apical cytoplasm (compare Figs. 1, 2, and 3).

Many cells at day 28, in contrast to days 10 and 12, contained more mature, dense zymogen

FIGURE 1

Pancreas of a rat on a stock diet. Normal acinar structure. The nuclei appear round and homogenously granular. The cytoplasm is dense, with the basal regions containing closely packed elements of the endoplasmic reticulum, and the apex is filled with numerous mature zymogen granules. × 4000.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granules and partly resembled the cells of the animals on stock diets in this respect. Golgi elements were seen but they were not so prominent as those in the protein-free animals of days 10 to 12.

Prominent mitochondria were seen interspersed between sparse, widely separated elements of the ergastoplasm (Figs. 3 and 11).

Some cells appeared to be free in the interstitium, apparently unassociated with visible acini. These cells had round, centrally placed nuclei with a rim of cytoplasm containing a minimal amount of ergastoplasm (Fig. 11). Serial "step" sections of plastic-embedded material approximately 2 μ thick showed, at the light microscope level, that these "dissociated" cells were related to an acinar lumen.

Lipid droplets were reduced in size and many appeared to be surrounded by a clear rim. Various patterns of lipid droplets, present basally, included the following: clear rimmed, dense lipid droplets; lipid droplets markedly decreased in density; or clear areas, occurring in clusters, of the same size as the dense lipid droplets. Some clusters were noted to contain a combination of these features.

Nucleolar Diameter

Nucleoli appeared to be enlarged at day 10, particularly in comparison with the nucleoli of the previous days. They also appeared larger than the nucleoli of the acinar cells of the stock diet animals.

DISCUSSION

Cytoplasmic Lesions

Ergastoplasm

The rare cytoplasmic lesions of the ergastoplasm observed at days 10 and 12 were identical with some of those seen at earlier periods in the ethionine study. The presence of similar lesions in the protein-free and ethionine groups is further evidence that the predominant effect of ethionine is on protein metabolism. These findings are consistent with the recent suggestion (4) that the initial biochemical lesion is in RNA metabolism. The infrequent occurrence of the lesion in the protein-free group, as well as its failure to duplicate the many forms seen in the ethionine group, suggests that the interference with protein metabolism is of a lesser degree in the protein-deficient animals than it is in the ethionine series. The experimental design does not permit us to state with certainty how much of the large array of ethionine lesions is caused by ethionine *per se* and how much by ethionine and the protein deficiency it produces.

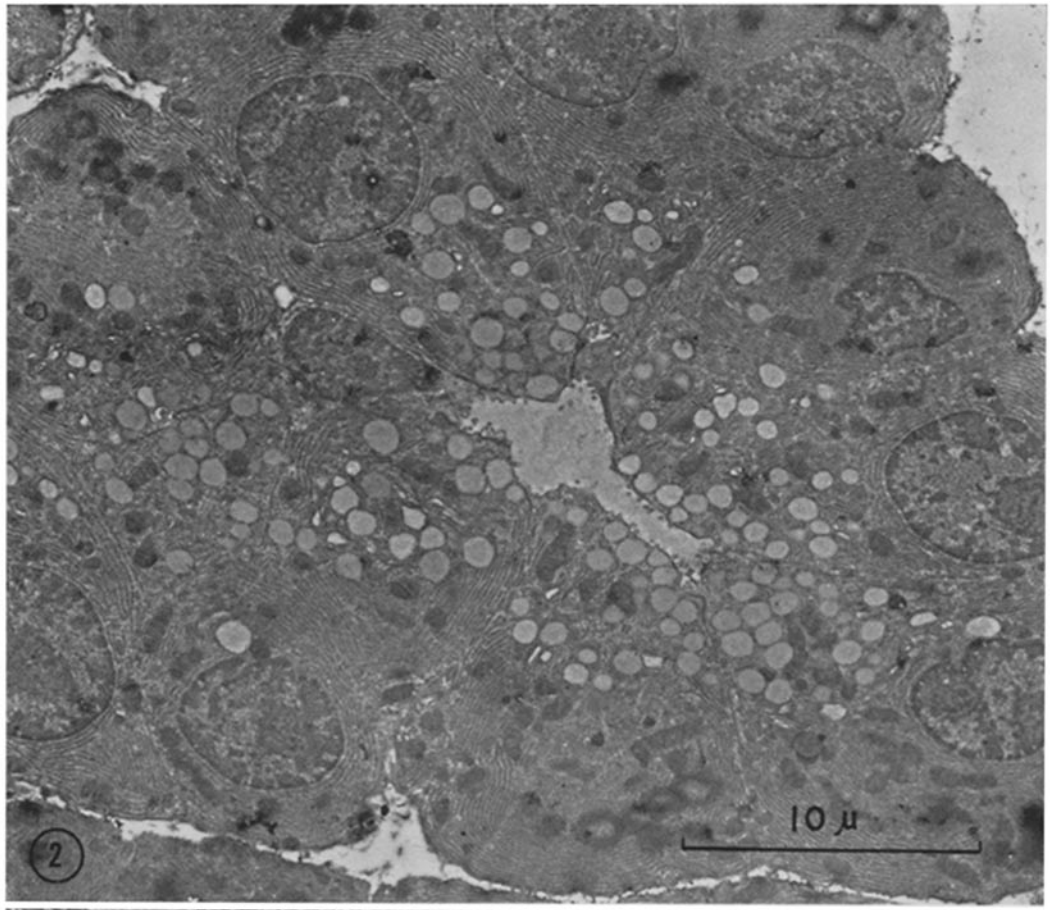
The increased osmiophilia of the ergastoplasmic lesion suggests a possible derangement of phospholipid metabolism with an "unmasking" of membrane phospholipid for osmium reduction. The loss of free ribosomes is possibly a reflection of the same disturbance in RNA metabolism

Figure 2

Pancreas of a rat on a protein-free diet, day 12. The cell size is reduced. The nuclei appear smaller, their membranes are slightly irregular, and the matrices show fine and coarse granulation. The ergastoplasm is less compact and numerous lipid droplets appear at the base of the cell. Normal zymogen granules are absent; in their place are vacuoles equal in size to mature granules but containing a substance (prozymogen ?) of reduced density. The acinar lumen is prominent as a result of the reduction in the size of acinar cells. × 4000.

Figure 3

Pancreas of a rat on a protein-free diet, day 28. The cells are markedly reduced in size. The coarse nuclear granulations, reduced amounts of ergastoplasm, and absence of mature zymogen granules are still conspicuous. At this stage, the apical vacuoles are also reduced in number. An occasional lipid droplet surrounded by a clear "halo" is seen. × 4000.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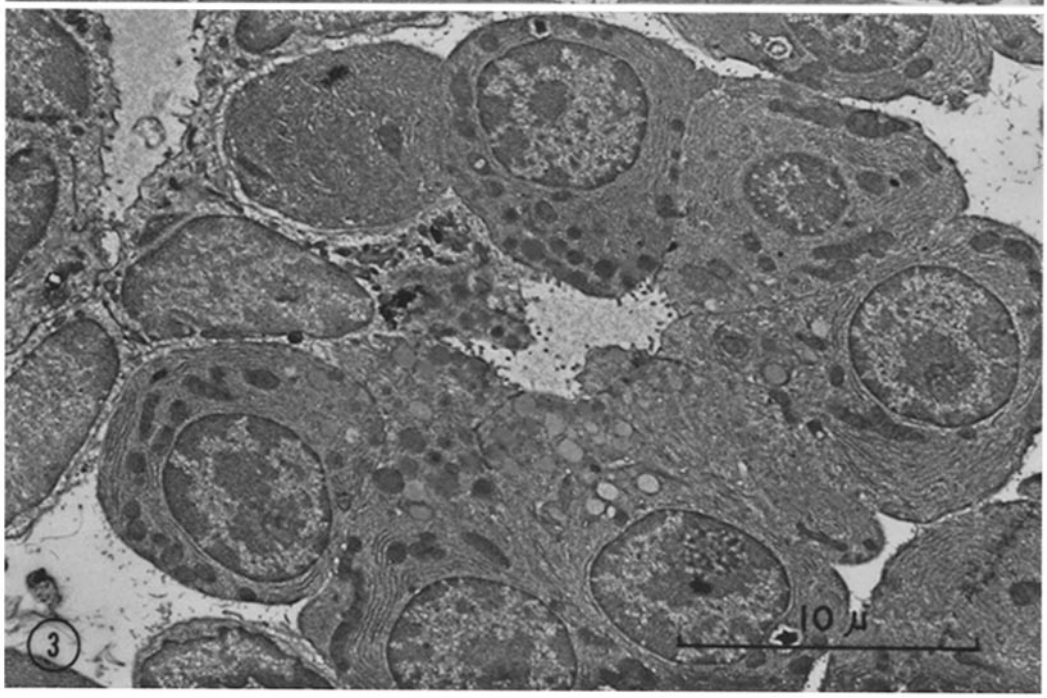
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which affected the membrane-associated ribosomes.

It is of interest that whorls of heavily staining, concentric ergastoplasm with vacuolar lesions (Fig. 8) were present rarely in the protein-free animals. The lesion was seen more frequently in the degenerative changes of the ethionine group. It was suggested that it might represent abortive or abnormal synthesis of ergastoplasm and differed from nebenkern (12).

Lipid Droplets

A common finding in the animals of both the protein-free and the ethionine, protein-free groups was the frequent occurrence, more marked in the former, of basal clusters of lipid droplets.

Increased deposition of cellular lipid has been associated with decreased protein synthesis. King *et al.* (13) reported increased cholesterol and fatty acid content per cell in cultured "L" cells and Ehrlich ascites cells exposed to *p*-fluorophenyl alanine. Lipid droplet deposition produced by ethionine, another amino acid analogue known to inhibit protein synthesis and degradation (20), appears to differ only in degree from that produced by a protein-free diet. Protein deficiency *per se*, whether caused by an analogue ultimately interfering with protein synthesis or by a protein-free diet, would appear to be the common feature of the lesions resulting from the two regimens.

Why the animals in the protein-free group should show more lipid droplets than the animals in the ethionine, protein-free group is not readily apparent, if protein deficiency is the cause of lipid deposition, since it is likely that the over-all protein deficiency is more marked in the latter. One possible reason may be that the animals on an ethionine, protein-free diet consumed only a small fraction of the amount of food eaten by the animals on a protein-free diet. The combination of a higher consumption of food and the absence of dietary protein in the protein-free group may have given rise to a greater depletion of protein in the pancreas because of its role in the production of digestive enzymes. Possibly of greater significance might have been the more severe depletion of the fat depots in the animals of the ethionine, protein-free group, for it has been shown in some procedures producing fatty degeneration that less fat is present in the lesions if the fat depots have been previously reduced.

Zymogen Granule Formation and the Golgi Apparatus

The decrease in the number of zymogen granules during the first 10 days was associated with an increase in the amount of the small vacuolar component of the Golgi apparatus. By day 12, when prozymogen was present in the apex of the acinar cell, there was considerable increase in the amount of Golgi substance present in the cell. At days 18 and 28 the relative decrease in the amount of Golgi apparatus was accompanied by an increase in the number of mature zymogen granules as compared with day 12. Therefore, it could be postulated that a precursor relationship exists between the prior extensive hypertrophy of the vacuolar portion of the Golgi apparatus and the subsequent appearance of mature zymogen granules. These results would appear to be consistent with the hypothesis (3, 5, 17, 22) that the Golgi apparatus is involved in the formation of zymogen granules. Similar changes occurred in

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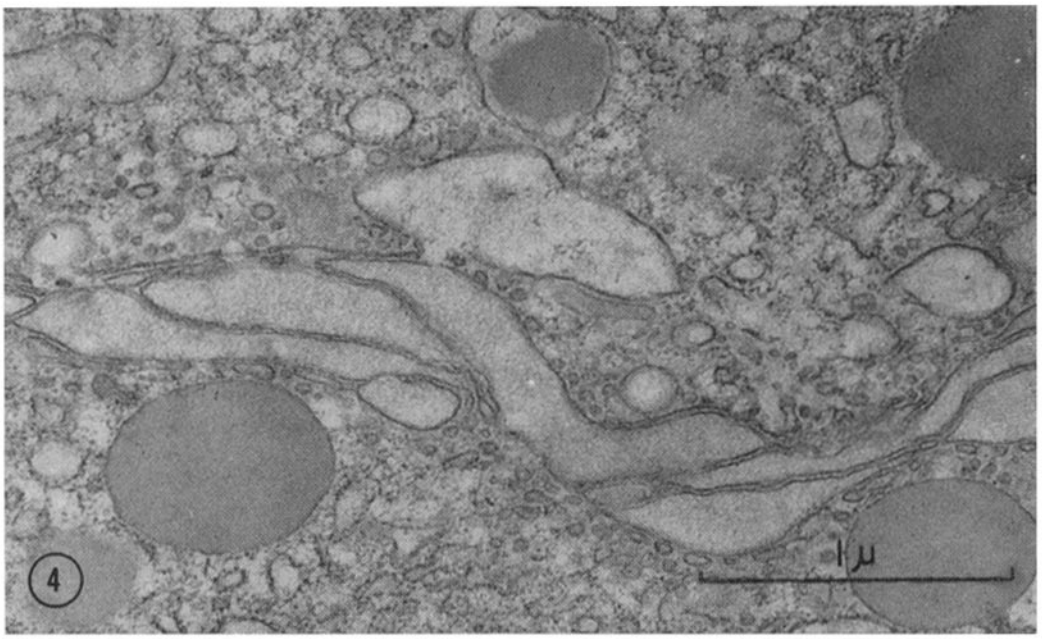
FIGURE 4

Pancreas of a rat on a stock diet. Oriented vesicles and elongated vacuoles of the Golgi apparatus. × 41,000.

FIGURE 5

Pancreas of a rat on a protein-free diet, day 10. Vacuoles are present which may occasionally contain a material slightly stained by the osmium. Rarely is prozymogen present as in the stock diet animals. The Golgi complex is enlarged, is spread through a larger area of the cytoplasm, and contains vacuoles increased in number and size. × 41,000.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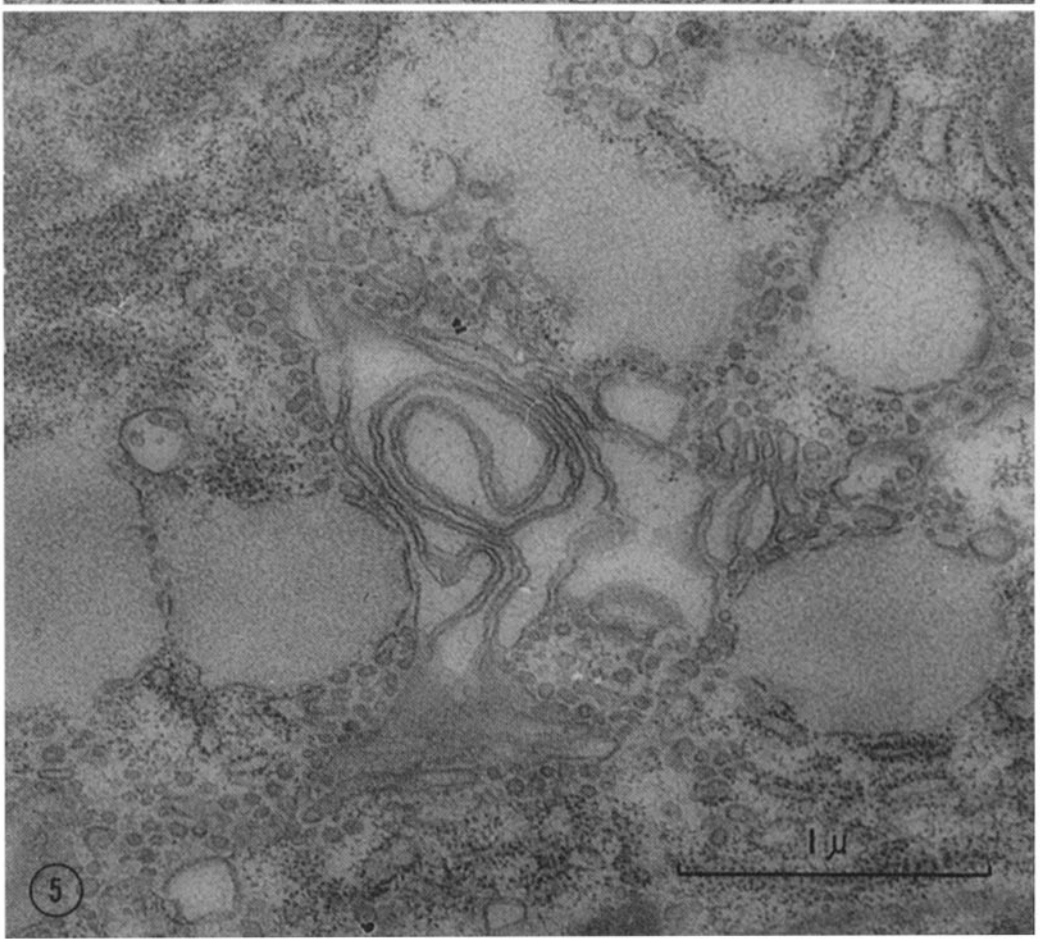
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para basal bodies of flagellates following fasting (11).

Mitochondria

No mitochondrial lesion was noted. A close relation between mitochondria and lipid droplets has been demonstrated in the pancreas of fasted guinea pigs (17). The increased numbers of lipid droplets following protein deprivation seen in this study were not observed to have similar contact with mitochondria. Such failure of lipid-mitochondrial associations in the rat as compared with the guinea pig may be related to species difference. Another striking difference also exists; *i.e.*, the intracisternal granules are prevalent in fasted guinea pigs, as described by Palade (17), but are infrequent in rats on a fasting regimen.

Nucleoli and RNA Metabolism

An apparent increase in the volume of the nucleoli of the acinar cell of the protein-free animal at day 10 was not accompanied by any loss of nucleolar basophilia as estimated visually on azure B-stained material. These results parallel those of Stenram (23–25), who observed that the diameter and RNA content of the nucleoli in rat liver cells increased markedly under conditions of protein deprivation similar to those employed in this experiment. This author stated, however, that no such changes were found in the nucleoli of cells of the exocrine pancreas or the spinal cord, or in cells of a hepatoma (26). In bacteria, increased RNA synthesis has been observed under conditions of inhibited protein synthesis produced by chloramphenicol (19). More recently, Borek and Ryan (2) reported increased RNA synthesis following deprivation of methionine in a methionine-requiring auxotroph of *E. coli*. The experiments reported here on pancreatic cell changes resulting from a protein-free diet as well as those reported by Stenram (23–25) on liver cell changes with a protein-free diet may reflect a similar mechanism.

Nuclear Matrix

In the protein-free animals at day 18, a diffuse fine granularity of nuclear matrix was noticeable which was generally absent in the animals at other periods. This finding was noted also, but less frequently, at day 12 in the ethionine, protein-free group and in the stock diet group. Whether these findings were due to artifact or related to morphologic and biochemical changes (16) occurring spontaneously in the protein-free animals at this period is difficult to determine. That they represent changes in the nuclear matrix is suggested by the findings from associated studies in enzyme activities and autoradiographic studies, which showed return toward the stock diet values at this time (8, 15).

Cell Dissociation

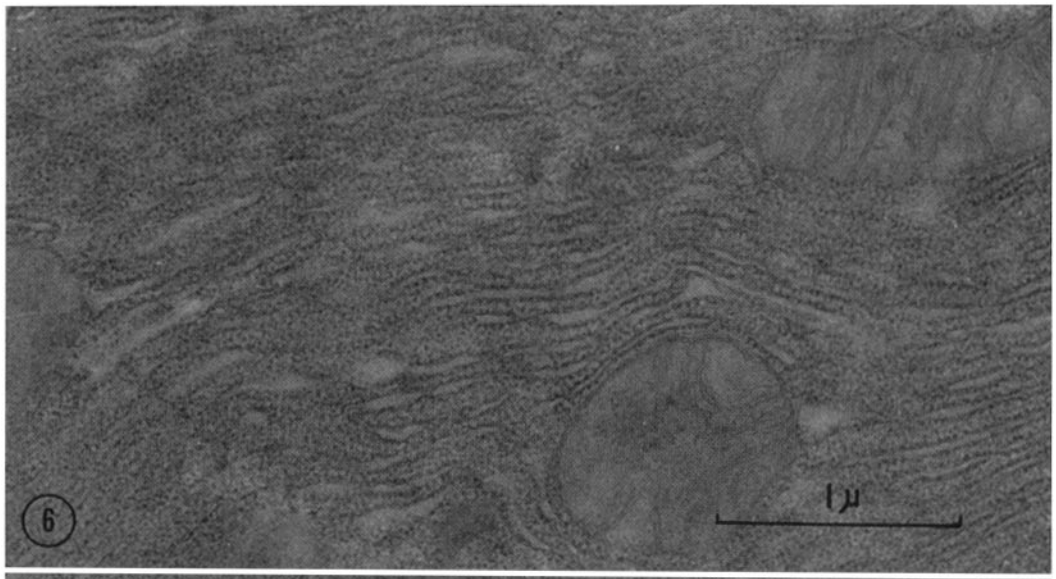
Adams *et al.* (1), in attempting to produce in rats a disease state similar to that of human kwashiorkor, noted that after a low protein and high carbohydrate diet for 5 and 7 weeks the rats showed atrophy of the pancreatic acinar cells, decrease in the number of zymogen granules, proliferation of ducts, and periacinar round cell infiltration. Friedman and Friedman (10) confirmed these findings and suggested that the isolated acinar cells seen in some histologic sections might represent a dissociation of pancreatic acini into individual cell units. Our findings using histologic "step" serial sections revealed that "isolated" cells which appeared to be unattached to ducts were actually part of a normal duct–acinar system.

Figure 6

Pancreas of a rat on a stock diet. The compact arrangement of the ergastoplasm and its numerous attached ribosomes is conspicuous. × 32,000.

Figure 7

Pancreas of a rat on a protein-free diet, day 10. The ergastoplasmic membranes are less compact and are "tilted" so that they have been sectioned obliquely, revealing ribosome patterns. A cluster of lipid droplets appears in the lower region of the cell. A small portion of interacinar space appears in the lower left corner, and the faint outline of a thin basement membrane may be seen adjacent to the acinar cell. × 32,000.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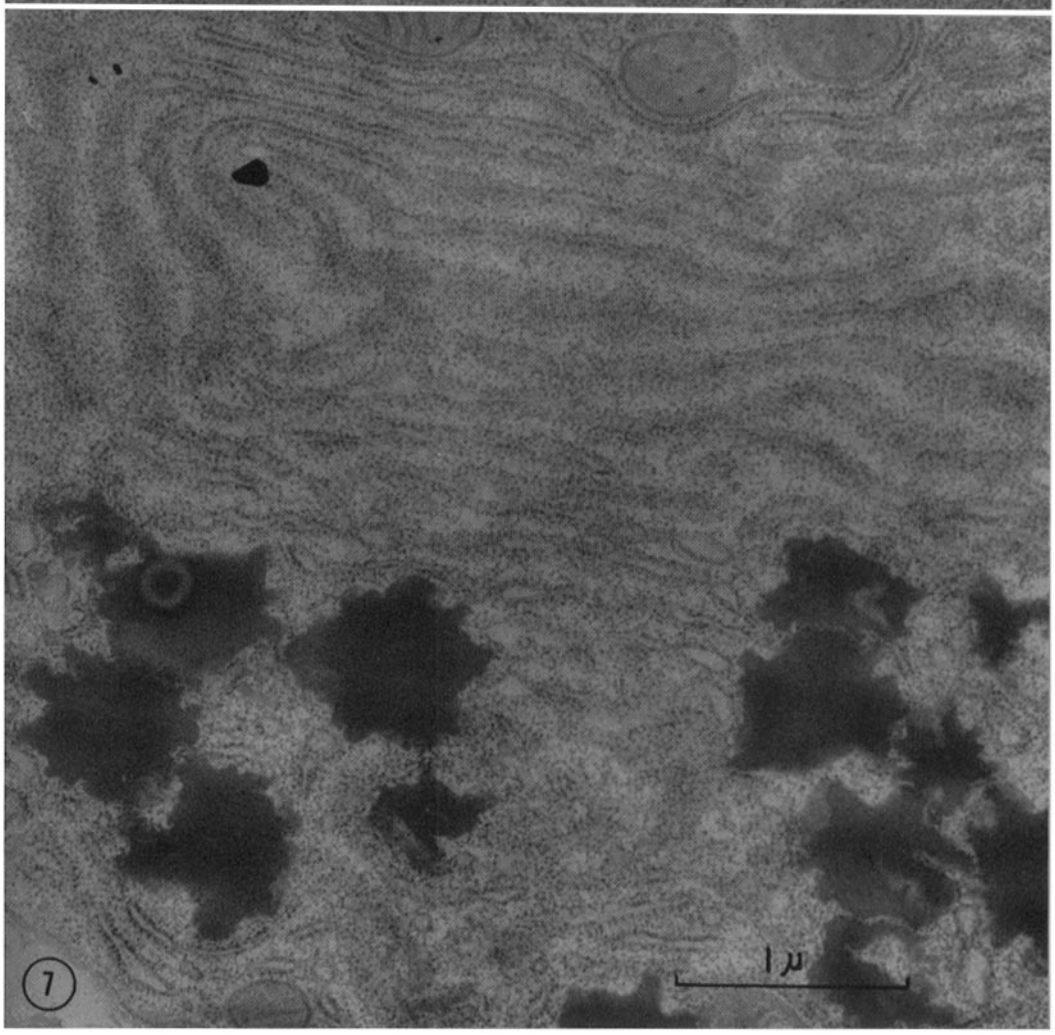
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Return of Biochemical Function and Morphologic Changes

The return toward normal of some pancreatic enzyme activities in the ethionine, protein-free group began shortly after the cessation of ethionine injections (15), as might be expected. It was surprising, however, to note a similar change in some of the enzymatic activities of the protein-free group. These changes were associated with beginning morphologic restitution at either day 12 or day 18. There was no change in experimental conditions. It is possible that a raiding of protein precursor stores elsewhere in the body might have occurred at this time (14) and repair might have been initiated thereby. Conceivably, these changes could have represented adaptive phenomena.

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BIBLIOGRAPHY

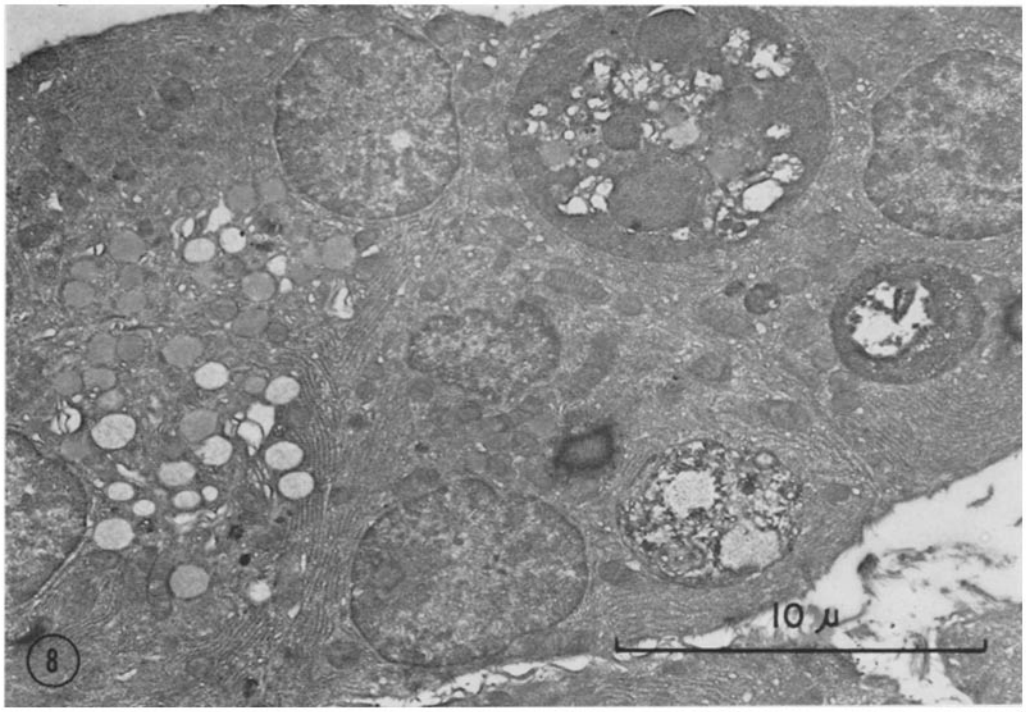
1. ADAMS, C. W. M., FERNAND, V. S. V., and SCHNEIDEN, H., *Brit. J. Exp. Path.*, 1958, **39**, 393.
2. BOREK, E., and RYAN, A. J., *Bacteriology*, 1958, **75**, 72.
3. DALTON, A. J., and FELIX, M. D., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4 suppl. 79.
4. FARBER, E., and MAGEE, P. N., *Biochem. J.*, 1960, **76**, 58.
5. FARQUHAR, M. G., and WELLINGS, S. R., *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 319.
6. FITZGERALD, P. J., *Lab. Invest.*, 1960, **9**, 67.
7. FITZGERALD, P. J., and ALVIZOURI, M., *Nature*, 1952, **170**, 929.
8. FITZGERALD, P. J., and VINIJCHAIKUL, K., unpublished observations.
9. FLAX, M. H., and HIMES, M. H., *Physiol. Zool.*, 1952, **25**, 297.
10. FRIEDMAN, S. M., and FRIEDMAN, C. L., *Canad. Med. Assn. J.*, 1946, **55**, 15.
11. GRIMSTONE, A. V., *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 369.
12. HERMAN, L., and FITZGERALD, P. J., *J. Cell Biol.*, 1962, **12**, 277.
13. KING, D. W., SOCOLOW, E. L., and BENSCH, K. G., *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 421.
14. MADDEN, S. C., and WHIPPLE, G. H. *Physiol. Rev.*, 1940, **20**, 194.
15. MARSH, W., and FITZGERALD, P. J., in preparation.
16. MIRSKY, A. E., and ALLFREY, V. G., *Proc. Nat. Acad. Sc.*, 1958, **44**, 981.
17. PALADE, G. E., *in* Symposium on Subcellular Particles, (T. Hayashi, editor), New York, Ronald Press Co., 1958, 64.
18. PALADE, G. E., and SCHIDLOWSKY, G., *Anat. Rec.*, 1958, **130**, 352.
19. PARDEE, A. B., PAIGEN, K., and PRESTIGE, L. S., *Biochim. et Biophysica Acta*, 1957, **23**, 162.

FIGURE 8

Pancreas of a rat on a protein-free diet, day 10. Survey electron micrograph showing the variety of ergastoplasmic lesions which are seen, but very rarely, in the protein-free group. Two whorls of deeply osmiophilic ergastoplasm with central vacuolar lesions are present. These were more common in the ethionine group (12). × 5000.

FIGURE 9

Pancreas of a rat on a protein-free diet, day 12. A lesion at higher magnification. The lesion seems to be encompassed by a single membrane. At the center appears an oval structure containing small vesicles and osmiophilic granules. This may be a "trapped" mitochondrion, the vesicles being remnants of swollen, disrupted cristae, and the dense granules being equivalent to the intramitochondrial granules frequently seen in mitochondria. Encircling this central structure are tangled masses of agranular membranes. These "smooth" membranes are equivalent to the rought surfaced elements of the endoplasmic reticulum (ergastoplasm) prior to the loss of the ribosomes. The irregular clusters of dense bodies along the periphery of the lesion may represent remnants of ribosomes. × 31,000.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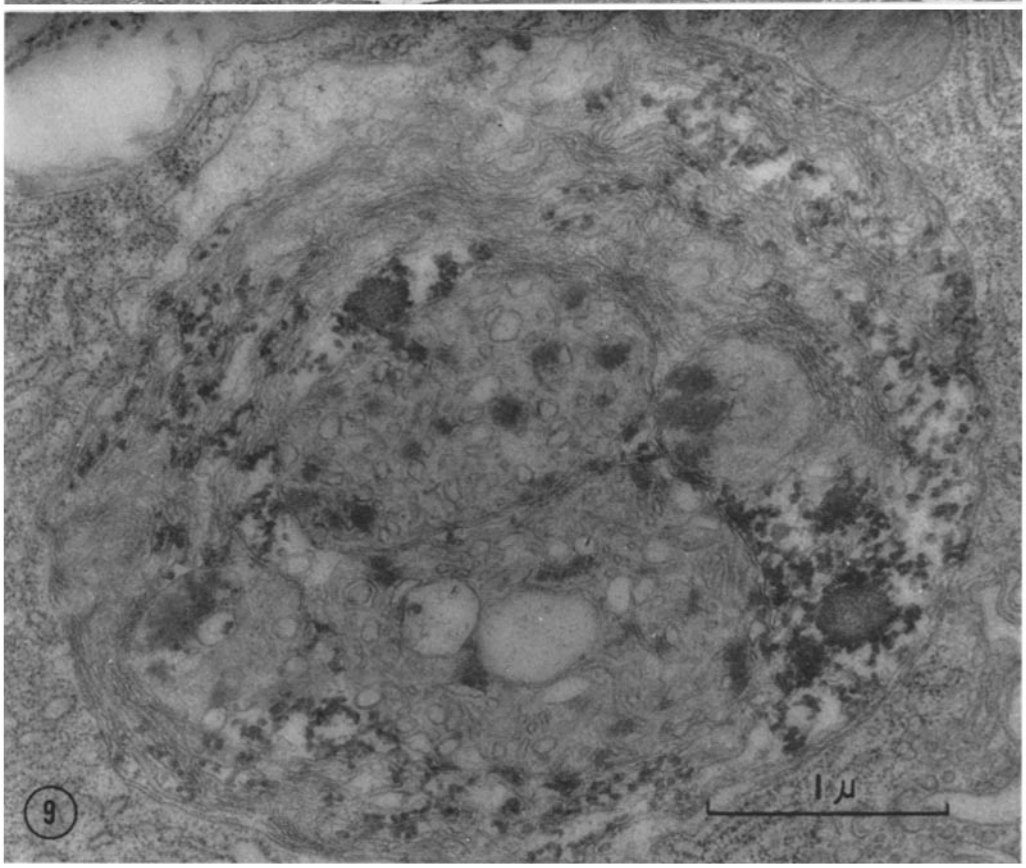
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20. RENDI, R., *Nature*, 1958, **181**, 351.
21. ROQUE, A. L., in preparation.
22. SJÖSTRAND, F. S., and HANZON, V., *Exp. Cell. Research*, 1954, **7**, 415.
23. STENRAM, U., *Exp. Cell. Research*, 1953, **5**, 539.
24. STENRAM, U., *Acta Path. et Microbiol. Scand.*, 1956, **38**, 364.
25. STENRAM, U., *Acta Path. et Microbiol. Scand.*, 1958, **44**, 239.
26. STENRAM, U., *Exp. Cell Research*, 1958, **15**, 174.

FIGURE 10

Pancreas of a rat on a protein-free diet, day 18. Survey electron micrograph illustrating the reduction in the size of cells and in the content of zymogen granules. Unlike the nuclei at day 12 and day 28, these nuclei are homogeneous as in the stock diet animals. The nucleoli are osmiophilic and slightly vesiculated. Numerous prominent lipid droplets are present in the basal regions of the acinar cells. Zymogen content is limited to a few mature granules clustered around the lumen. Compare with Fig. 1 (stock diet animal), Fig. 2 (day 12, protein-free animal), and Fig. 3 (day 28, protein-free animal). × 3000.

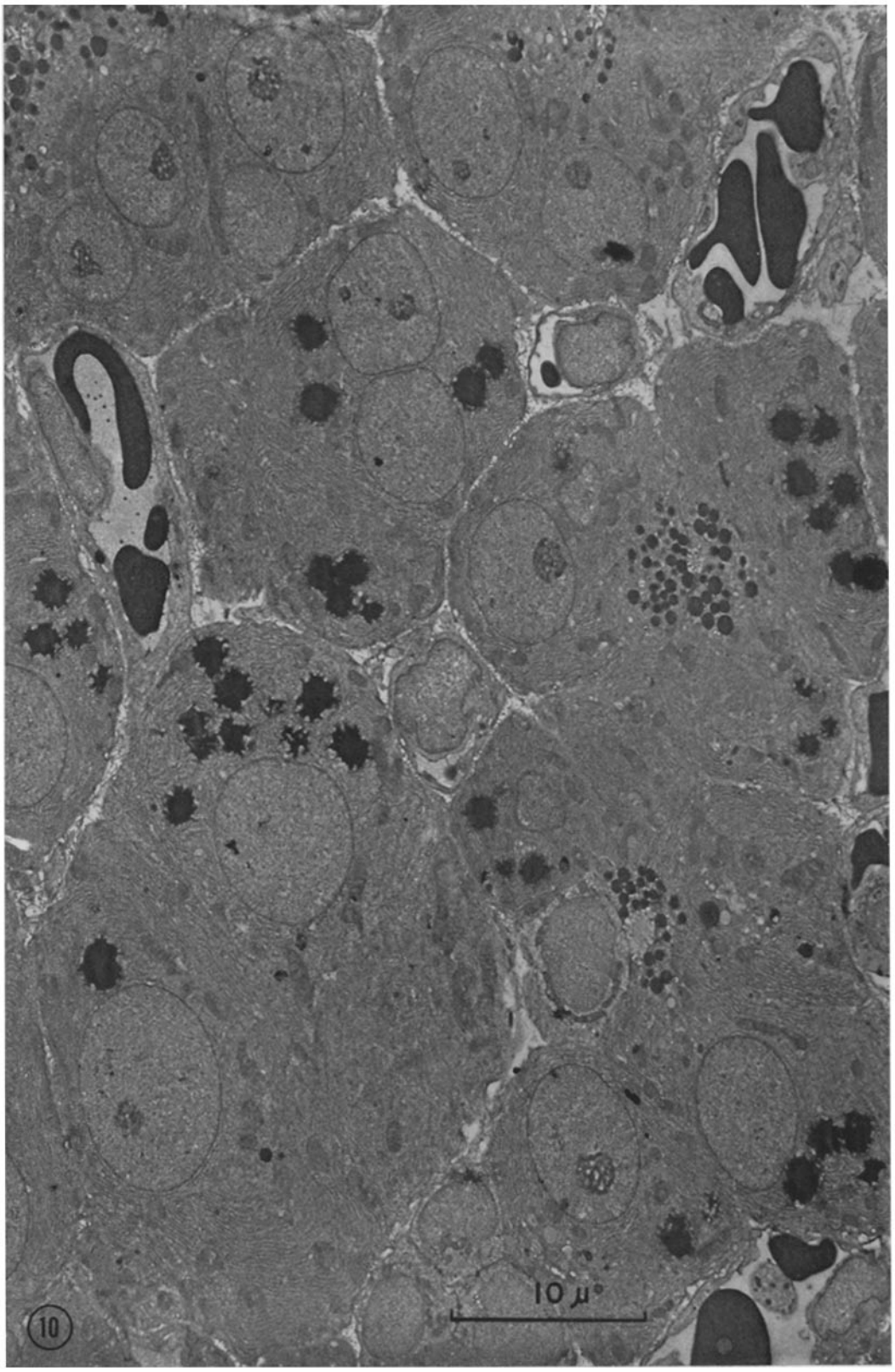
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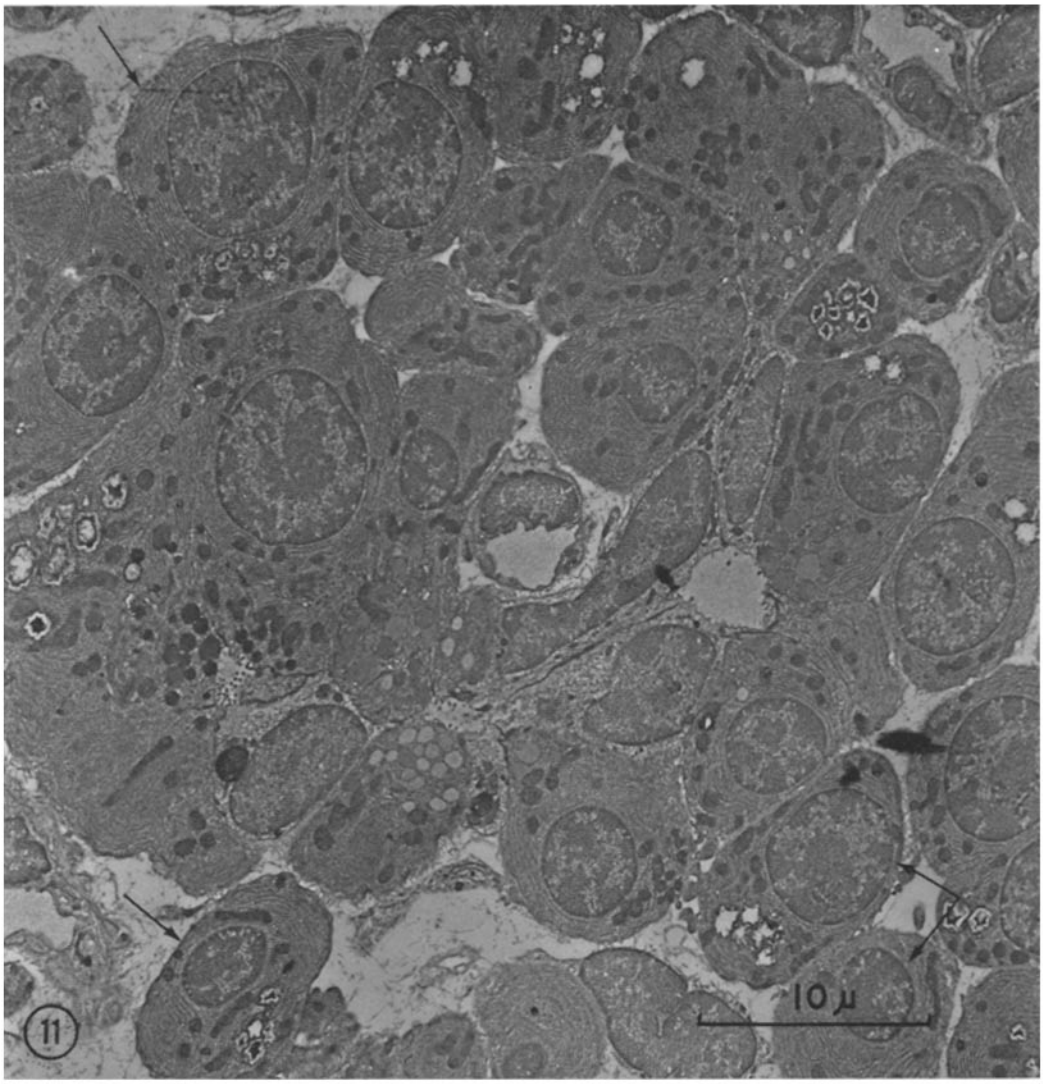
FIGURE 11

Pancreas of a rat on a protein-free diet, day 28. Maximum reduction in cell size is noted at day 28. Frequently, acinar cells appear isolated and unattached to a lumen (arrows). Correlative light microscopy of "step" serial sections of methacrylate blocks showed that such seemingly isolated cells were, in reality, connected with a lumen at some level in the tissue. Note the number and variety of lipid droplets. Some appear outlined by a clear ring or "halo." In other areas similar vacuoles appear to be without fat. × 3000.

FIGURE 12

Pancreas of a rat on a protein-free diet, day 28. A part of an acinus and its lumen appears at the lower right. Note the bulbous acinar cell at the center of the micrograph. Cells such as these seen at different levels of sectioning appear to be isolated. The apex of this particular cell is wedged between a duct cell on the right and an acinar cell on the left. × 4000.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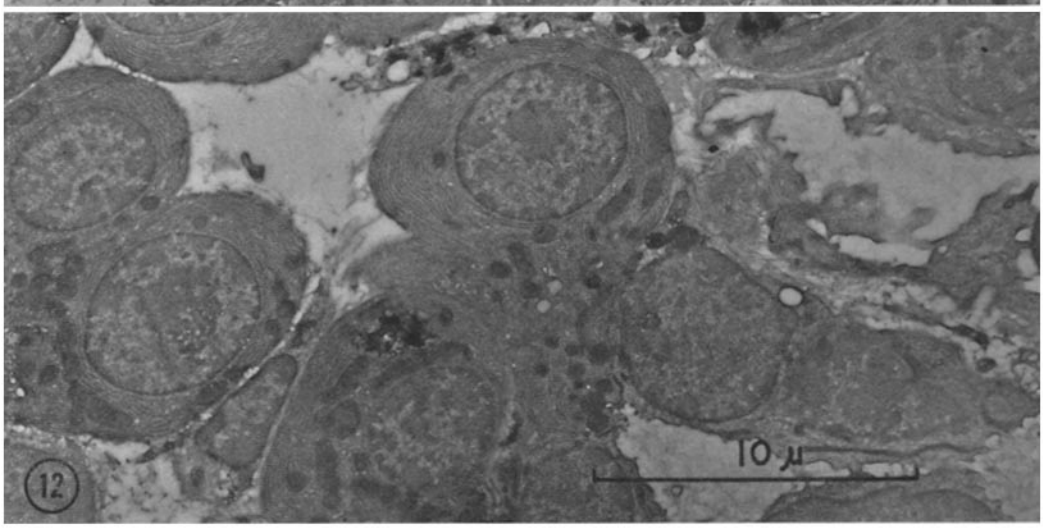
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