RESTITUTION OF PANCREATIC ACINAR CELLS FOLLOWING ETHIONINE

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ABSTRACT

The regeneration of the pancreatic acinar cell was studied at four time periods after ethionine had destroyed most of the acinar cells. Within 2 days of the last ethionine injection, small basophilic cells (pre-acinar cells) with whorls of ergastoplasm or nebenkern were present. These cells also contained a decreased amount of Golgi substance, small zymogen granules, and a fine granularity of the nuclear matrix. They showed persistence of the characteristic ergastoplasm lesion produced by ethionine. Eight days after the last ethionine injection, the nebenkern was replaced by approximately normal appearing ergastoplasm and the nucleoli and Golgi bodies were enlarged. Zymogen granules were less dense but more abundant. Mitochondria were considerably enlarged. Most cells showed no ethionine lesions or only small foci of damage. Eighteen days after the cessation of ethionine, a good approximation of the normal acinar cell was present. The whorls of ergastoplasm appeared at a time (day 12) when basophilia was pronounced. Other studies showed that nucleic acid and protein precursors began to show an increased concentration in acinar cells at this time. The appearance of nebenkern during a phase of cellular recovery and its absence during a phase of replication when mitotic indices were high suggest that its presence is more indicative of ergastoplasmic synthesis than of cell multiplication as such. Possibly the increased density of zymogen granules was a reflection of this increased protein synthesis. The increase in size of Golgi apparatus occurred prior to the replenishment of zymogen granules and thus satisfied a precursor relationship for a possible role in the formation of these secretory structures. Evidence suggests that some injured acinar cells recover from the ethionine and protein-free regimen and give rise to most of the new acinar cells formed. It is possible that, under the severe conditions which prevailed, the centroacinar ductule cells may also have given rise to some acinar cells.

INTRODUCTION

The problem of cellular regeneration is of fundamental importance and has intrigued biologists for many years. Experimentally, limited regeneration of the pancreas has been produced by partial pancreatectomy or ligation of the ducts (10), but attempts to achieve significant restitution comparable to that of the liver after partial hepatectomy have failed. Less is known of pancreatic regeneration following toxic injury. Parker (12) observed mitotic figures in human acinar cells following various infections and in guinea pig acinar cells after chloroform intoxication, and suggested that the acinar cells of the pancreas possessed great capacity to regenerate.

Significant restitution of the pancreas after extensive destruction of acinar cells was noted by one of us after the administration of ethionine, an analogue of methionine (4). The problem of the precursor cell responsible for restitution has been studied in this laboratory (3), and preliminary

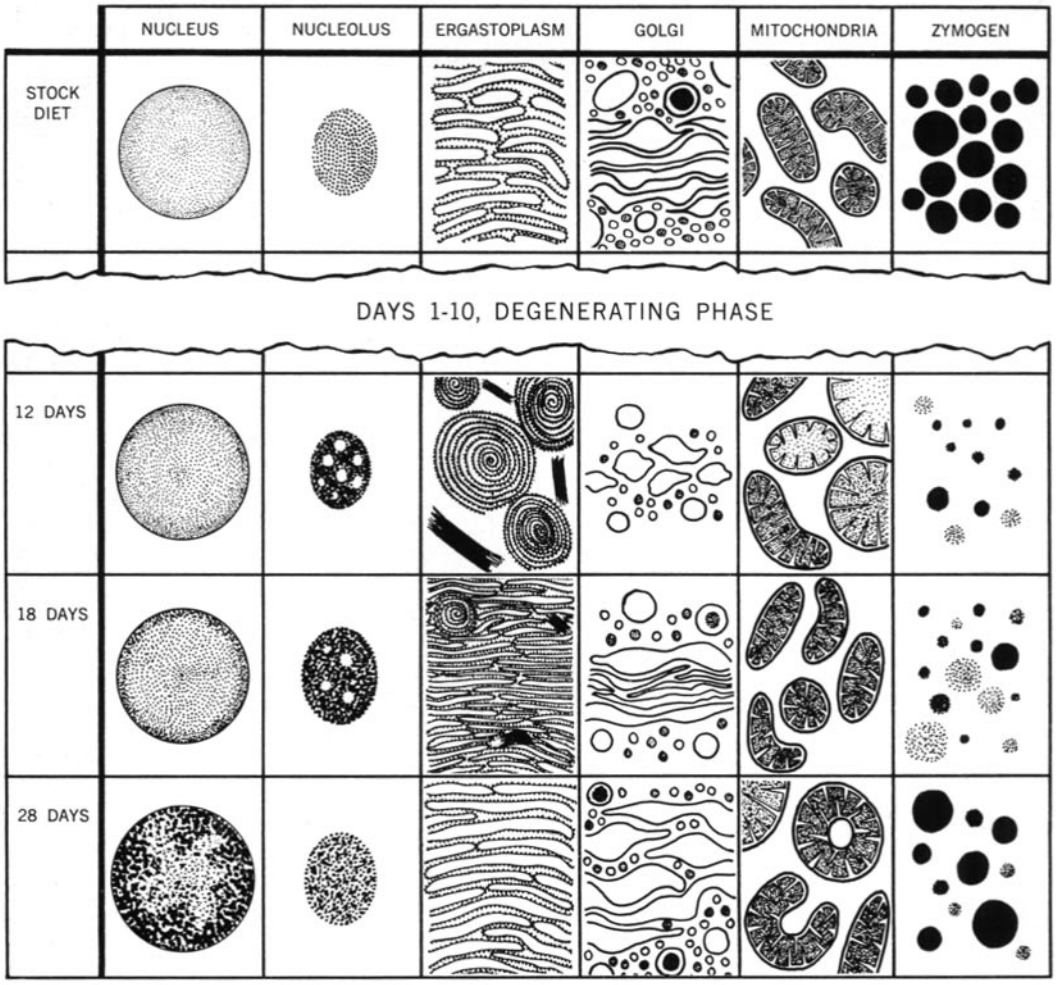
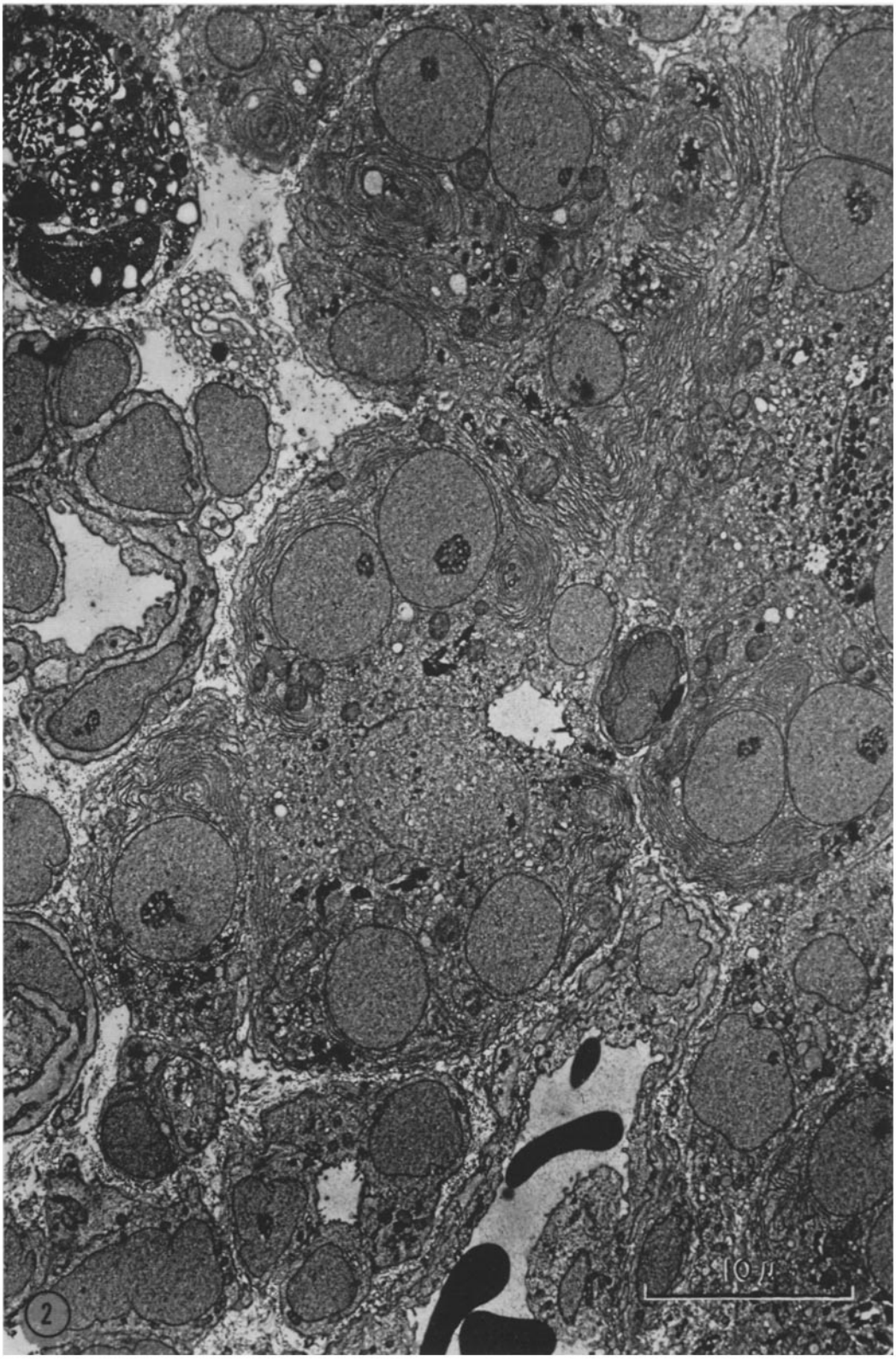


FIGURE 1

Schema of acinar cell regeneration. For purposes of comparison the cell organelles of the stock diet animal appear in the uppermost row of diagrams and the regenerating cell organelles at days 12, 18, and 28 in the lower three rows. The features of degeneration during days 1 to 10 (indicated here by the notation Days 1–10, Degenerating Phase) have been described and illustrated in the preceding article (7).

FIGURE 2

Pancreas of protein-free ethionine rat, day 12. Acinar cells contain large, round or oval nuclei with finely granular nucleoplasm and prominent, dense, vesiculated nucleoli. The osmiophilic plaques of ergastoplasm are present. Several cells contain ergastoplasmic whorls or nebenkern. The parallel ergastoplasmic lamellae at the base of the cell often have a wavy pattern or are "spread." Dense zymogen granules, smaller than normal, appear at the apex of a cell at the middle right. A duct outlined by five cells is discernible at lower left. At upper left an area of ethionine degeneration is conspicuous. × 4000.


10 μ
2

observations on structural changes during regeneration have been reported (8). We report here some morphologic changes, discernible by electron microscopy, in the regenerating pancreatic acinar cell of the rat after daily injections of ethionine had caused destruction of most of the acinar cells. A schematic summary of the morphologic changes is given in Fig. 1.

METHODS AND MATERIALS

Details of our experimental design have been described in the previous communication (7). In summary, rats received ten daily injections of ethionine while on a protein-free diet. This was the period of acinar cell degeneration. Ethionine injections were stopped after day 10 and the rats kept on the protein-free diet. The following 18 days of the experiment were designated as the period of regeneration. Specimens of the pancreas were obtained from three similar experiments, in each of which animals were sacrificed at days 12, 15, 18, and 28, corresponding respectively to 2, 5, 8, and 18 days after the last injection of ethionine. At least six animals were examined at each time period. Equal numbers of control animals from the stock diet and protein-free groups were sacrificed at the same time periods and all tissues processed under the same conditions.

OBSERVATIONS

For purposes of comparison, it should be mentioned that a low magnification micrograph of the pancreas of a rat on stock diet was presented in the previous communication (7), in which there is also a listing of literature relevant to the normal structure.

Days Twelve to Fifteen

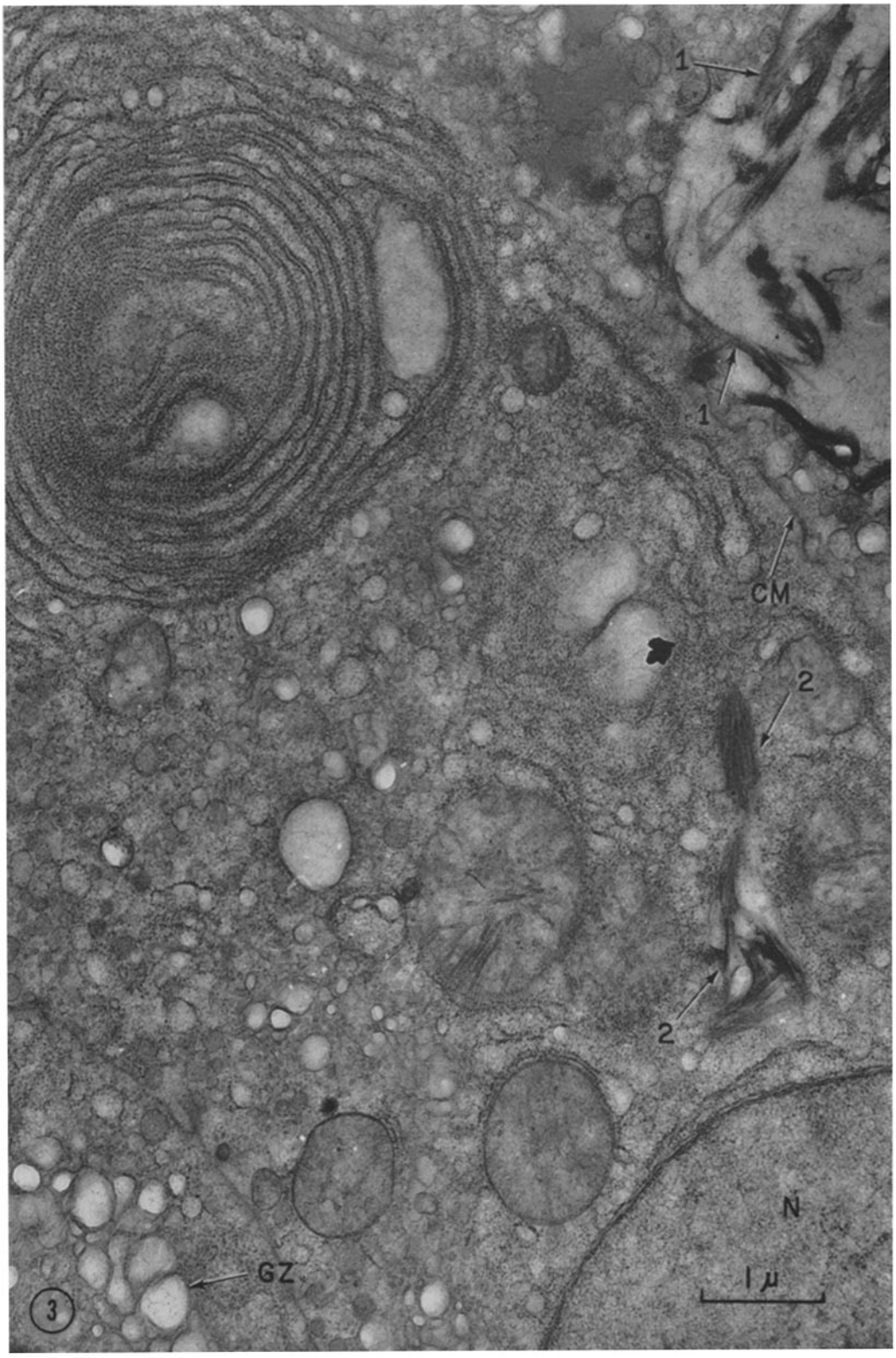
A major portion of acinar cell tissue had been destroyed at day 10 and most cells showed some morphologic damage at that time. At day 12 some of the cellular debris noted at day 10 had been removed. Isolated foci of single cells or clusters of a few cells were present, often in acinar pattern. These cells were obviously not inflammatory or connective tissue cells. Macrophages, some showing phagocytosis, fibroblasts, collagen fibrils, and dilated blood vessels were present. Dilated acinar lumens with small microvilli persisted (Fig. 2).

The nucleus of the cells forming acini was round and homogeneously granular. The coarse granularity described in the degeneration phase and occasionally present in the stock diet animals was absent (Fig. 2). The round nuclei frequently contained conspicuous nucleoli many of which had small clear vesicles (Fig. 2). The basal region of these cells contained various forms of ergastoplasm. In some cells parallel profiles of membranes resembling the normal state were present. Concentrically arranged, distinct ergastoplasmic membranes in the form of a whorl were common. Since these membranes were sometimes less closely packed than the normal, oblique views were observed. Such "grazed" membranes were particularly common at the center of a whorl (Fig. 3). We believe that this cell is the precursor of the reconstituted acinar cell of day 28, and have called it the pre-acinar cell (3). Our previous study suggested that it was either a damaged acinar or ductule cell recovering from the ethionine effect.

Interspersed between the ergastoplasmic membranes were two abnormal structures. One was the densely packed osmiophilic structure, sometimes fibrillar, other times lamellar, seen in the earlier degenerative phase (osmiophilic plaques). These fused masses represent what we believe to be the late stage of the ergastoplasmic lesion (Figs. 3 to 5). The second organelle involved was the mitochondrion. Some mitochondria were swollen and round, contained clear areas, and were obviously disintegrating, with cristae which were either disrupted or short. Other mitochondria were of approximately the same size and shape as the swollen ones, but in addition they were packed with numerous cristae and contained a homogeneously dense interior (Fig. 4).

FIGURE 3

Pancreas of ethionine protein-free rat, day 12. Areas of degeneration are present (arrows 1 and 2) as well as a prominent whorl of regenerating ergastoplasm which appears at the upper left. Some strands of the whorl appear to be continuous with normal appearing ergastoplasm. Vacuoles and vesicles are conspicuous. A Golgi zone (*GZ*) appears in the lower left corner. *N*, nucleus; *CM*, cell membrane. × 19,000.


1
1
CM
2
2
N
GZ
1 μ
3

No increase in bodies called lysosomes were apparent during this stage of regeneration.

Cells having round nuclei, large amounts of ergastoplasm, and normal appearing mitochondria contained within the centrosphere region Golgi structures which appeared to be smaller than normal (Fig. 4).

The apex of the acinar cell was conspicuous by its lack of the usual large, mature zymogen granules, measuring 1 to 2 μ in diameter. Instead, two forms of smaller granules were present, both approximately 0.25 to 0.5 μ in diameter. One type was similar to the normal except for size. The other was less dense and appeared to be associated with small Golgi vesicles (Fig. 4).

Small ducts lined with cuboidal cells were still conspicuous (7). Infrequently, a cell resembling the duct cell, *i.e.*, cuboidal with a large, indented nucleus and interdigitating cell membranes, exhibited, in addition, a feature more characteristic of the acinar cell: ergastoplasmic formation and, rarely, zymogen granules. Such a cell bordered on a dilated lumen with its base often bulging into the collagen framework.

Days Fifteen to Eighteen

Acinar and lobular patterns approximated the normal. Collagen was extensive and the vascular endothelium was prominent. Large areas of extracellular lipid and some interstitial cellular debris were present.

Since many of the features of regeneration were prominent at this time, an extensive description of what we regard as regenerating cells will be given. Progressive stages of regeneration were characterized by changes in the granularity of the nuclear matrix, the amount of ergastoplasm present, the amount and pattern of Golgi apparatus, the size, shape, and number of mitochondrial cristae, and the presence of either zymogen granules or partially filled vacuoles at the apex of the cells.

The regenerating acinar cell was generally round or oval. It contained a large nucleus with one or two relatively large, non-vacuolated osmiophilic nucleoli. The nucleus at day 18, unlike that at day 12, contained coarse and fine granules and was similar to that observed in the earlier degenerative stages and in some animals on a stock diet. The nuclear membrane was double, and the nuclear "pores" communicated with an irregularly shaped, clear area within the nucleus (Fig. 7).

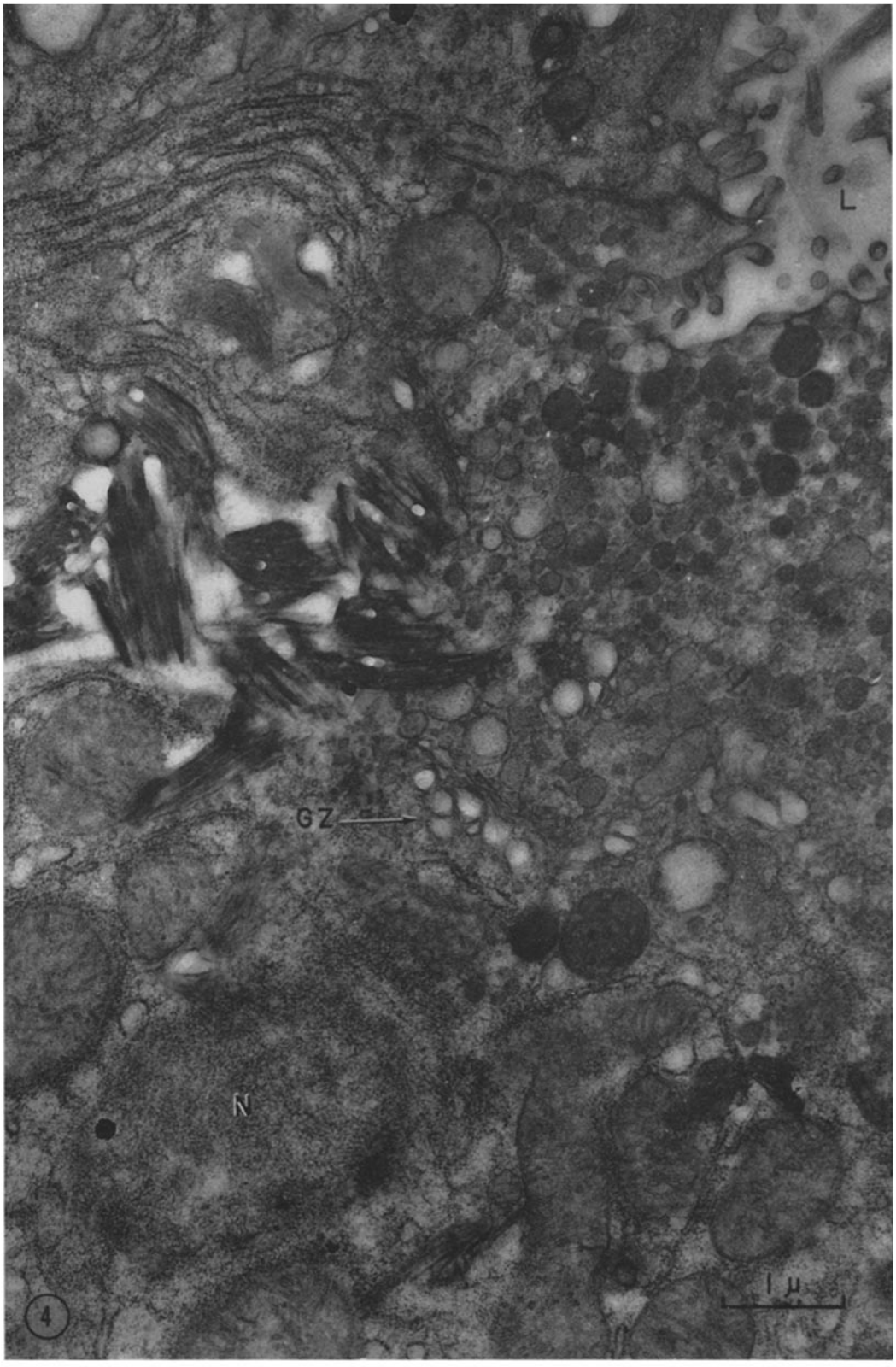
The ergastoplasm assumed different forms during regeneration. Early in regeneration, it was sparse and frequently restricted to regions immediately adjacent to the nucleus or cell membrane. The nebenkern, or whorled ergastoplasm, similar to that seen more prominently at days 12 and 15, was present only rarely at day 18. Occasionally, normal ergastoplasmic membranes appeared below the nucleus and were randomly oriented within a lesion area, frequently being interspersed with the granule-free osmiophilic membranes of the ethionine lesion (Fig. 6).

Clusters of lipid droplets were present between ergastoplasmic membranes at the base of the cell between the nucleus and basement membrane. At day 18 the droplets were characterized by a clear empty space at their periphery, giving a "halo" appearance (Fig. 7).

The Golgi complex appeared in the centrosphere region and was increased in amount in the cells of the day 18 animals as compared with the normal or the day 12 animals. The Golgi region extended across the width of the cell from one

FIGURE 4

Pancreas of protein-free ethionine rat, day 12. A lumen (*L*) with microvilli appears at the upper right. The lower three-fourths of the micrograph is occupied by an acinar cell which contains both degenerative and regenerative structures. The degenerative osmiophilic plaques appear in the middle left. A Golgi zone reduced in size (*GZ*) is seen just below the center of the micrograph and above a nucleus (*N*). At the upper left are the profiles of five ergastoplasmic cisternae parallel to the lateral plasma membrane. The apex of the cell just beneath the lumen contains granules which vary in size and density. The density of the largest of these approaches that of a mature zymogen granule; the smaller granules are less dense and some are surrounded by a distinct single membrane. × 19,000.


L
GZ
N
4
1 μ

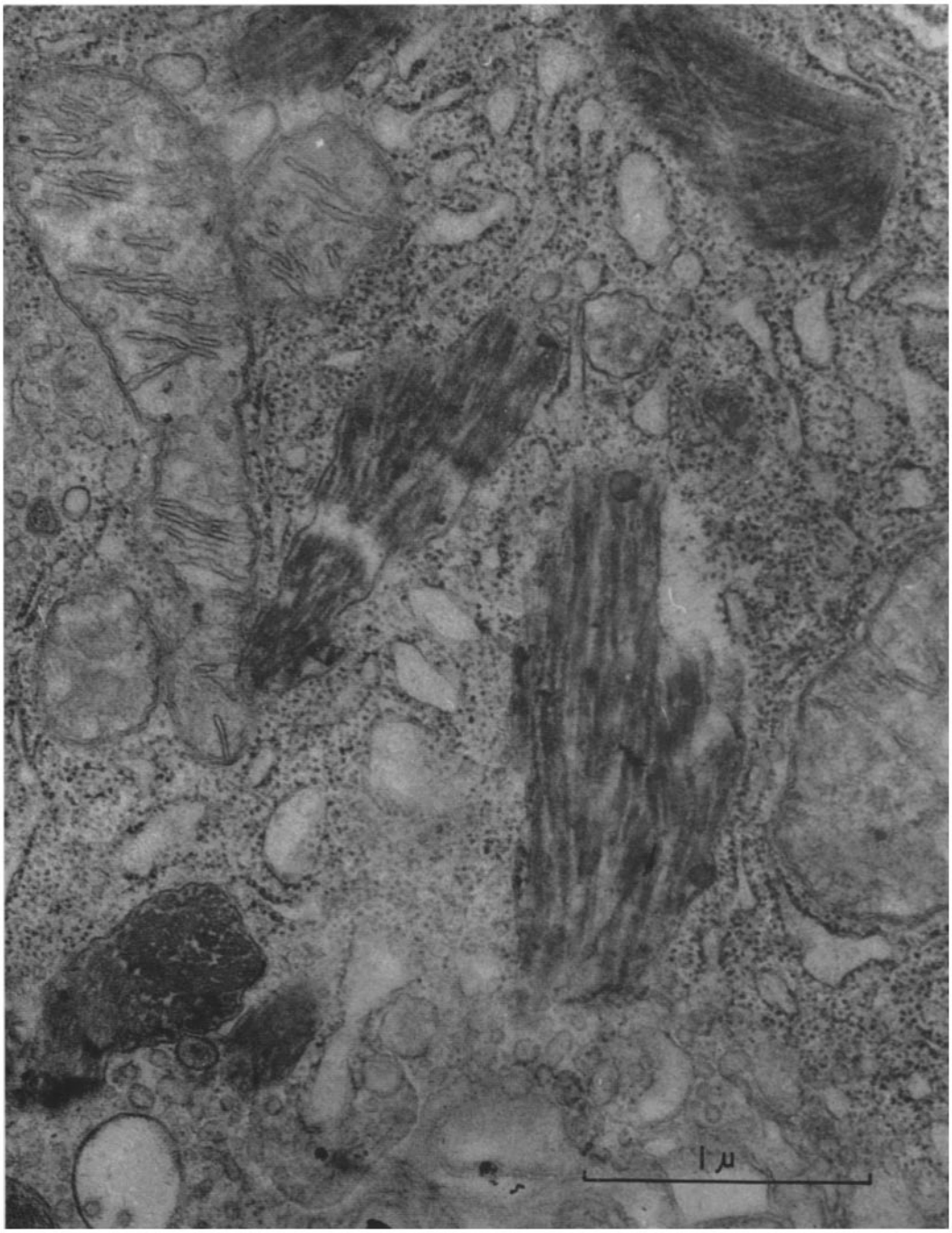


FIGURE 5

Pancreas of protein-free ethionine rat, day 15. Osmiophilic plaques, remnants of degenerating ergastoplasm, are scattered throughout the cytoplasm. Newly formed or undamaged ergastoplasm may be seen as membrane-bounded profiles with attached ribosomes. × 42,000.

plasma membrane to the other and also into the apex of the cell. The complex consisted of closely arranged agranular membranes and large vacuoles. Scattered small clusters of vesicles were frequent in this region (Fig. 8). On occasion, one or two small osmiophilic granules, possibly representing prozymogen, were present within the Golgi vesicles.

Interspersed between ergastoplasmic membranes were round or oval mitochondria much larger than those of the stock diet animal. Internally, the mitochondria contained a slightly dense or osmiophilic amorphous matrix which was interrupted by an array of closely packed parallel cristae. Swollen, empty mitochondria such as were seen in the pancreas of the day 12 animal were absent at this time.

The apex of the regenerating cell was conspicuous by the lack of zymogen granules (Fig. 8). In place of zymogen granules were a few vacuoles approximately 1 to 2 μ in diameter. These vacuoles contained either a moderate amount of homogeneous, low electron-scattering material or a small (0.5 to 1.0 μ), centrally placed, homogeneous particle the density of which closely resembled that of a mature zymogen granule. In addition, numerous small vesicles or droplets filled with varying amounts of a light, homogeneous material were present at the apex. These vesicles had a considerable variation in size and distribution although all were less than 0.25 μ in diameter. The smaller were more frequent on the apical side of the parallel membranes of the Golgi region, and the larger were more numerous in the uppermost apical region. The density of the vacuoles appeared to decrease from Golgi zone to apex (Fig. 8).

Ducts were still conspicuous at this stage. One or two cells, larger than duct cells, contained varying amounts of ergastoplasm, Golgi apparatus, large mitochondria, and partially filled apical vacuoles. These cells were observed bordering a lumen in association with two, three, or four typical duct cells. They bulged out into the surrounding collagen framework.

Day Twenty-Eight

The outline of the pancreas at this stage of regeneration resembled the normal pattern. Suggestive lobular structure was present. Fair acinar configuration was widespread. Ducts were still conspicuous. Collagen fibrils were numerous. In some of the acinar cells small patches of degeneration containing heavily osmiophilic plaques and large empty vacuoles persisted. Often these cells were components of well formed acini (Fig. 9).

Acinar cells were in various stages of regeneration. Some acinar cells were smaller than normal, and had well rounded nuclei containing coarse granular patches and round, prominent nucleoli. These cells bordered on an extensive lumen and their free edge contained some microvilli. The basal cytoplasmic region contained ergastoplasm, loosely packed and frequently presenting profiles on oblique sectioning. The mitochondria were unusually large, contained numerous cristae, and occurred singly, packed one upon another, or bordered cell membranes. They appeared as straight, tortuous, or curved rods, or sometimes they were "doughnut-shaped." In the centrosphere region of the cytoplasm the cells contained an extensive, large, essentially normal Golgi apparatus. Scattered about the Golgi zone were numerous small vesicles approximately 0.25 μ in diameter and containing varying amounts of lightly staining homogeneous material. Between these small vesicles were larger round or oval vacuoles ranging in size from 0.5 to 1.0 μ.

Other cells at day 28 were larger and had a more abundant basal ergastoplasm arranged concentrically around the nucleus. Mature zymogen granules were more frequent at the apex of the cell. The cytoplasm of the more normal appearing acinar cells frequently contained a small (1.0 μ), oval light region, bounded by a single membrane, which contained a fine fibrillar network. Such structures were found in the ergastoplasmic region and to some degree resembled the ethionine lesion described in the degenerative period (arrows, Fig. 9).

DISCUSSION

Morphologic Features of Regeneration

Ergastoplasm

A problem of considerable interest to us has been the recognition of the first morphologic signs of regeneration. At days 8 to 10 the majority of acinar cells had been destroyed and in those remaining there was extensive damage to the ergastoplasm (7). In the attempt to determine which cell type gave rise to regeneration of acinar epithelium, autoradiography with tritiated com-

pounds (5) was used. Our autoradiographic studies of the same animals have shown that day 12 was the point of inflection between the progressively decreasing concentration of H^3-thymidine, H^3-cytidine, and H^3-leucine in acinar cells during degeneration and the increasing concentration of these compounds during regeneration (3, 18). Such findings would suggest that nucleic acid and protein metabolism were recovering at day 12 from the ethionine-induced repression and returning toward normal levels. The presence of whorls of ergastoplasm in a large cell with intense basophilia (our "pre-acinar" cell (3)) at this time suggests that they were related to this phase of cellular recovery. We believe that the pre-acinar cell of day 12 further matures into an acinar cell easily recognizable as such at day 18. At the latter time the newly formed acinar cell or the recuperating, previously damaged cell was larger and closely resembled the relatively normal acinar cell seen at day 28.

Since the acinar cell concentration of nucleic acid and protein-precursors, and the mitotic index both increased to levels above those of the stock diet pancreas at days 18 and 28 (3, 18) and since very few whorls of ergastoplasm were present at these stages, it would appear that the whorls are not an accompaniment *per se* of cell division. Their prior presence at day 12 suggests a reparative role, possibly representing new cytoplasmic foci of ergastoplasmic synthesis responding to some injury to, or depletion of, the ergastoplasm. Their rare presence in the pancreas of the protein-free and stock diet animals may be consistent with some excessive metabolic demand of growth, secretion, infection, or stress.

Although the relationship of nebenkern to the formation of ergastoplasm is debatable, (Haguenau) (6), under the conditions of our experiment, the nebenkern could be considered as a stage of formation of new ergastoplasm as suggested by Weiss (20).

Mitochondria

Notable at days 18 and 28, when the autoradiographs showed the highest percentage of labeled nuclei after H^3-thymidine (18), was the presence of mitochondria larger than normal which contained greater numbers of cristae. The importance of mitochondria as sources of energy in growth and regeneration is now axiomatic, and our findings would fit into what must be an over-all increased demand for energy during a tremendous surge of cell replication. Such large mitochondria would be of considerable interest in terms of their enzyme composition and content.

Golgi Apparatus

The Golgi apparatus became smaller during the period of degeneration. At day 12 there was some increase in size, and at day 18 a further increase to greater than normal size. There was a return to approximately the normal size of the Golgi apparatus at day 28. The Golgi enlargement at day 18 preceded the appearance of mature zymogen granules at day 28, thus fulfilling a precursor relationship, and might be interpreted, therefore, as a compensatory hypertrophy of the system forming, or being involved in the formation of, zymogen granules. The light osmium-stained material and small granules seen in the Golgi vesicles at day 18 might be considered to be prozymogen, the postulated forerunner of the mature zymogen granule substance (2, 14). It would thus appear that the increase in size of Golgi structures is a feature of regeneration possibly associated with the formation of prozymogen and zymogen granules.

Zymogen Granule

It has been suggested that the final assembly or packaging of zymogen granules occurs in the Golgi apparatus and that the granules move up

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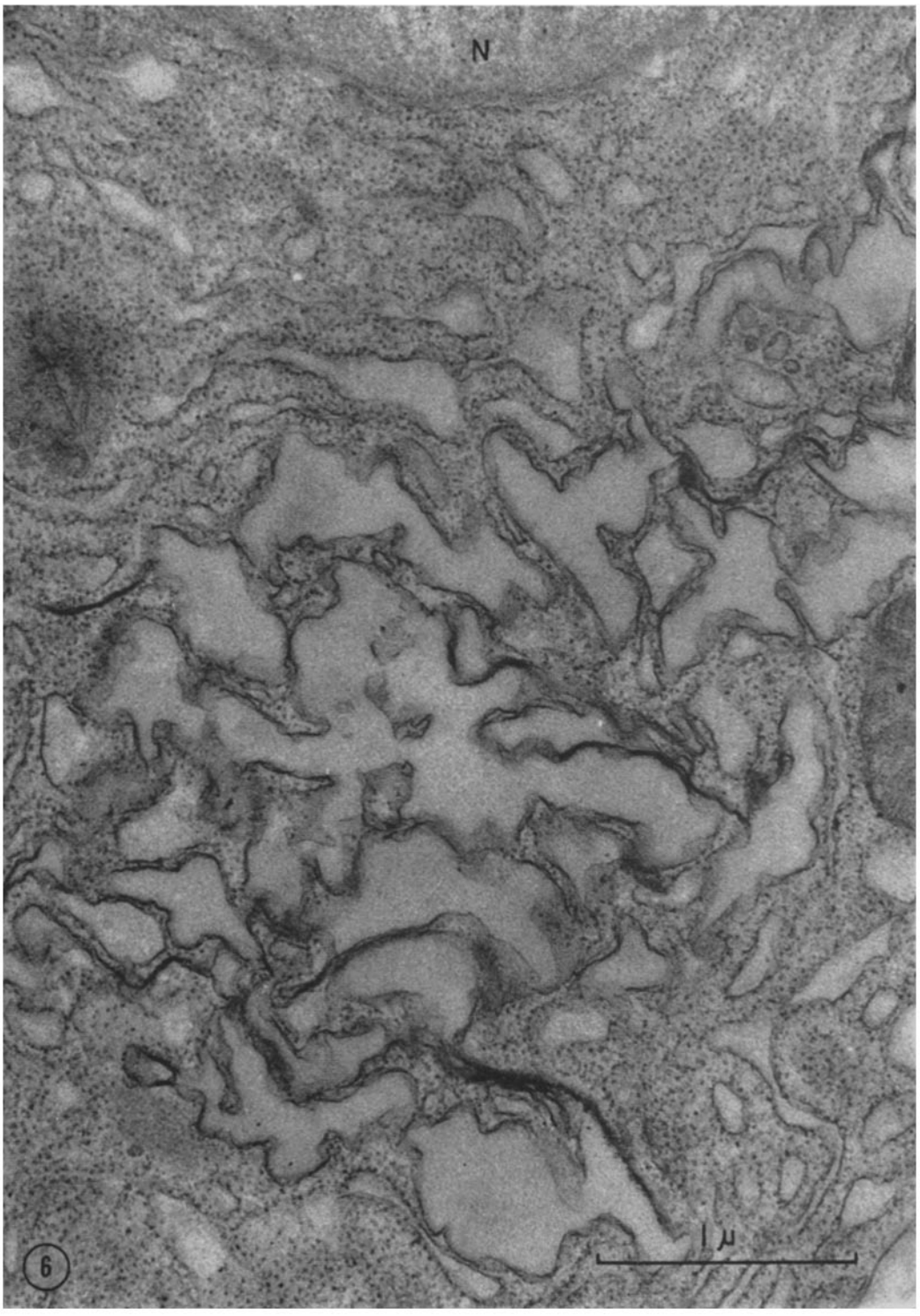
Figure 6

Pancreas of protein-free ethionine rat, day 15. The basal region of an acinar cell appears below the nucleus (*N*). Large, irregular anastomosing channels outlined by osmiophilic agranular membranes are seen. This may be a process of repair of the ergastoplasm extending from normal ergastoplasm peripheral to the lesion. × 39,000.


N
1 μ
6

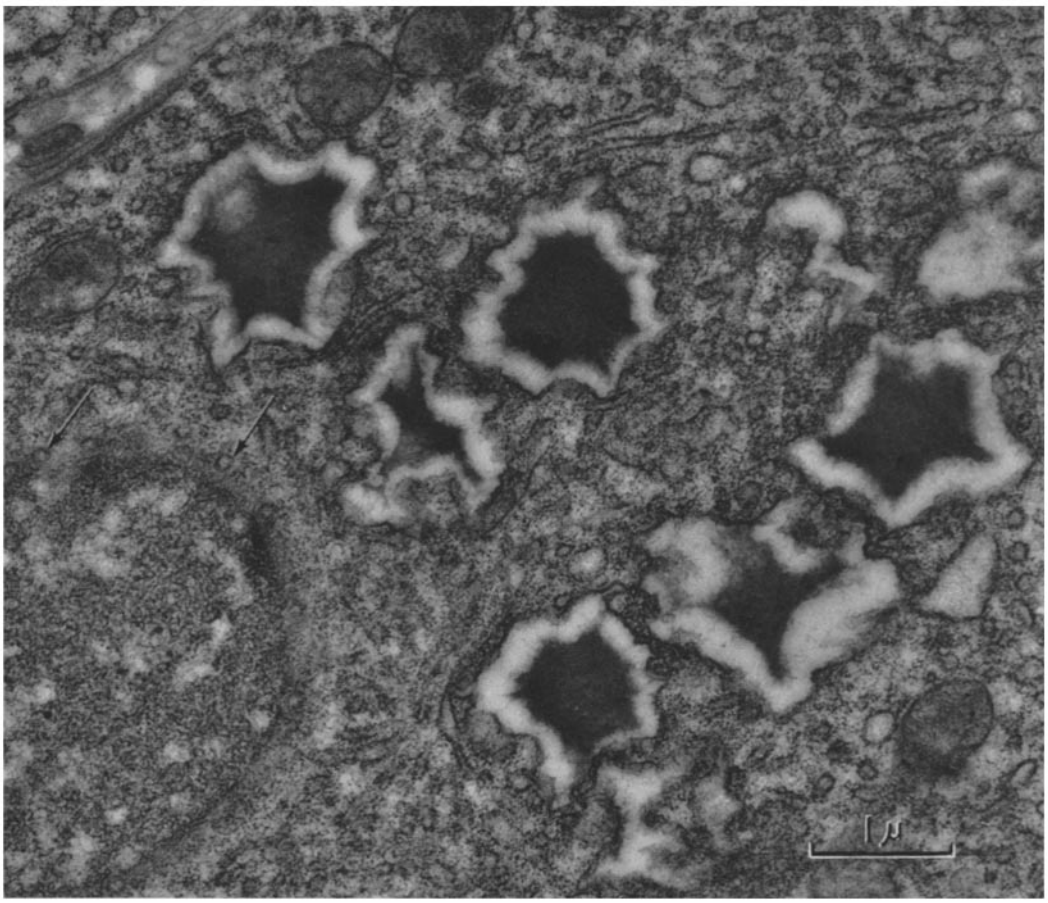
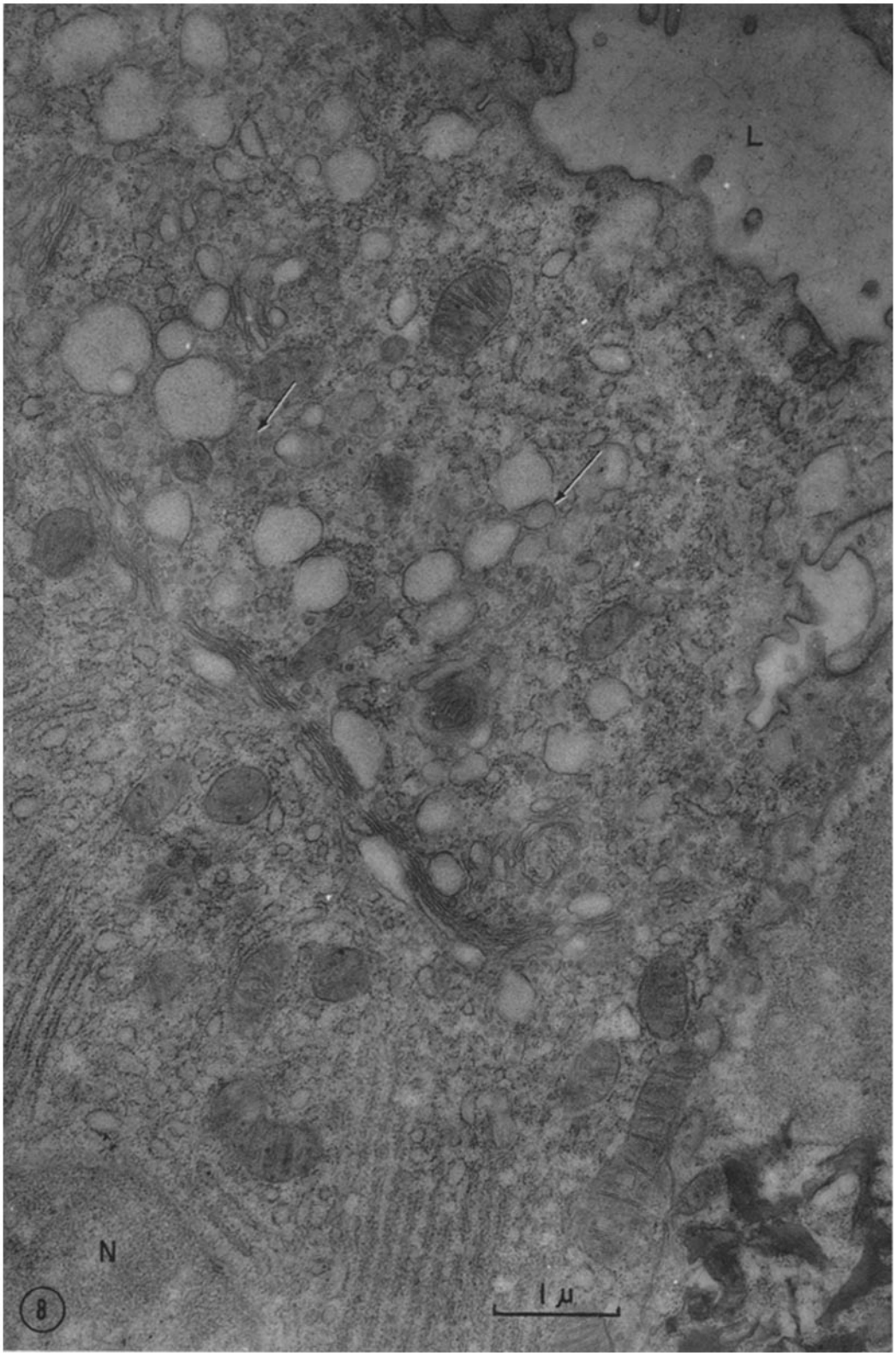


FIGURE 7

Pancreas of protein-free ethionine rat, day 18. An obliquely sectioned nucleus (lower left) demonstrates the presence of nuclear pores (arrows) and characteristic dark and light areas. The ergastoplasm appears as short parallel arrays of disconnected granule-studded membranes within which lie six lipid droplets with an empty periphery presenting a "halo" effect. × 19,000.

FIGURE 8

Pancreas of protein-free ethionine rat, day 18. A small regenerating acinar cell is present. Its lumen (*L*) appears in the upper right corner, and a part of its nucleus (*N*) is in the lower left. Newly formed parallel arranged ergastoplasm appears on either side of the nucleus. An enlarged Golgi apparatus consisting of five to eight parallel arranged membranes and associated vacuoles and vesicles appears in the centrosphere region of the cell. Numerous vacuoles and vesicles of varying sizes appear above this Golgi region in the apical zone. Some contain variable amounts of homogeneously stained material resembling prozymogen (arrows). × 19,000.


L
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from the Golgi zone to the apex of the cell (2, 11, 14).

The presence, observed in our studies, of many small, dense zymogen granules in vacuoles of the apical and Golgi regions of the cell at day 12, and of larger vesicles containing less dense material at day 18, seems somewhat at variance with the proposed model of zymogen secretion. Possibly at the earlier time relatively more precursor zymogen substance was available for the smaller volume of Golgi apparatus than at day 18, when the great increase in volume of Golgi apparatus may have been unmatched by a proportionate increase in the amount of zymogen and thereby would have led to a lesser concentration of zymogen per unit volume of Golgi substance.

Lipid Droplets

The presence of lipid droplets between the nucleus and the base of the cell during the degeneration period is interpreted as evidence of a disturbance of fat metabolism, possibly secondary to decreased nucleic acid or protein metabolism resulting from the effect of ethionine or the protein-free diet. The reduction in number of droplets at days 18 and 28 is regarded as an indication of the return of these functions toward normal levels. The "halo" around the lipid droplets at days 18 and 28 may indicate the beginning of their dissolution and absorption. Similar droplets have been noted in tissue culture cells by Bensch and coworkers (1) as well as by King and coworkers (9), and in our protein-free pancreas controls at day 18 (19).

Nuclear Matrix

Of unusual occurrence during the periods of degeneration and regeneration was the presence in most cells at day 12 of a finely granular nuclear matrix similar to that seen in most animals on a stock diet. Coarse clumping of chromatin was common in the nuclei of degenerated and regenerated cells. The possibility that the fine granularity of the nuclei of the acinar cells at day 12 was associated with a change in the composition of the nuclear matrix and was not an artifact of processing was suggested by the increased concentration of H^3-thymidine in the nuclei and the increased concentration of H^3-cytidine in the nucleoli at this time, in contrast to the very low levels or absence of isotope in the prior period of degeneration (18). There was also an increase of nuclear histone, as indicated by the fast green stain, at day 12 (13). For these reasons it seems possible that the change in nuclear granularity reflects some reparative change in the nucleoplasm. The coarse clumping seen during the periods of degeneration and regeneration was also associated with abnormal uptake of nucleic acid and protein precursors and may indicate disturbances of nuclear matrix.

Nucleolar Size

The nucleoli of the acinar cells were enlarged at day 18 and conspicuous at day 28. Many studies on the role of the nucleolus in regeneration have been reported (17, 15, 16). Although a consensus of opinion about the enlargement of the nucleolus is available (17), there is some question as to whether the enlargement is primary or secondary. Our studies show only that the nucleolus was less than normal in size at day 12, but was of normal size at day 18 and enlarged at day 28. Since nucleolar concentration of H^3-cytidine (18) had increased above the 8 to 10 day levels to a subnormal level at day 12 and had attained higher

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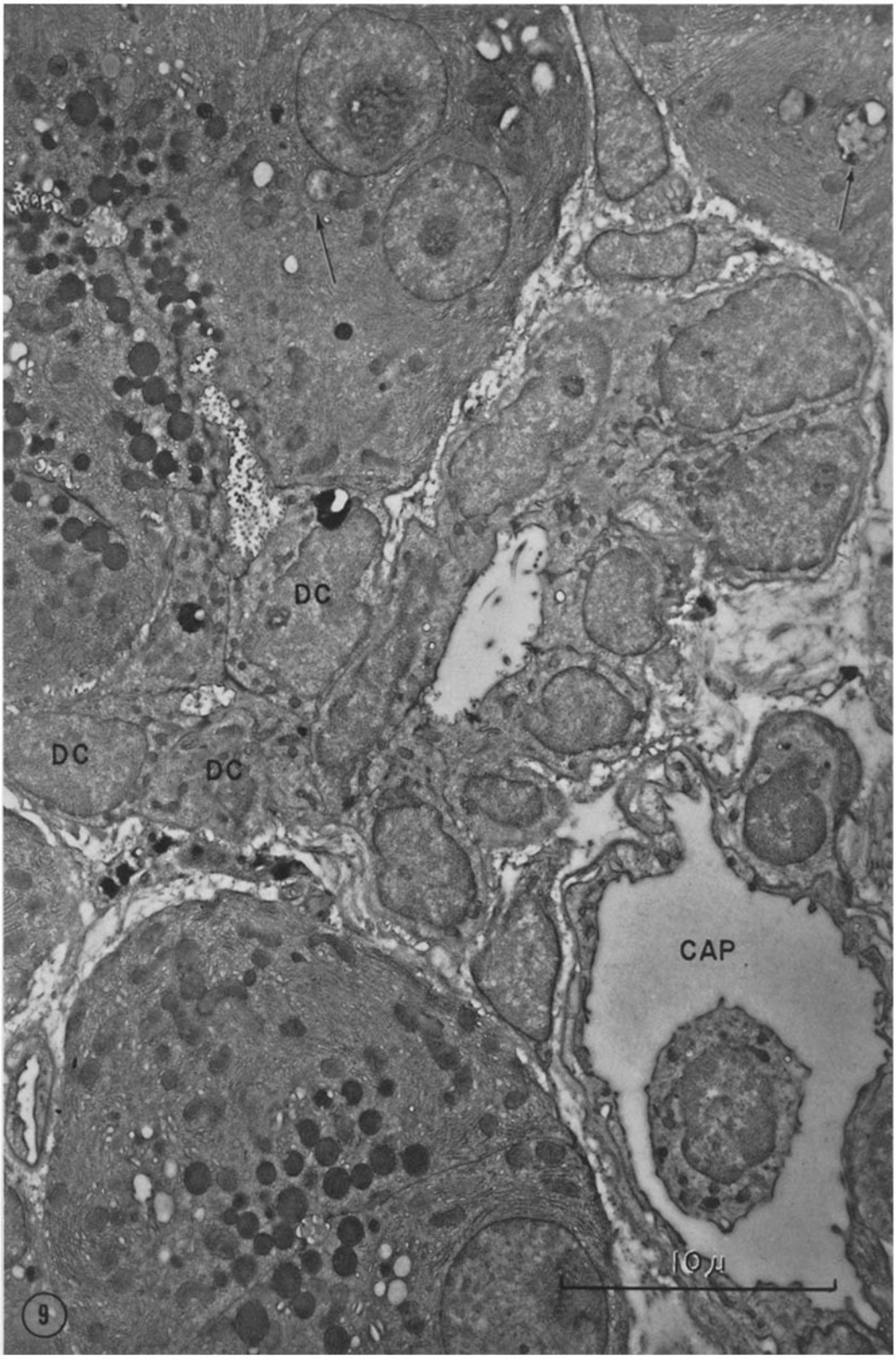
FIGURE 9

Pancreas of protein-free ethionine rat, day 28. Regenerated acinar cells are present. The upper left contains a well developed acinus surrounding a longitudinally sectioned lumen with numerous microvilli. Several duct cells (*DC*) complete the lumen. In the regenerated acinus are present large round nuclei, large nucleoli, vacuoles, lipid droplets, extensive ergastoplasm, well formed mitochondria, and mature zymogen granules. The center of the micrograph contains a longitudinally sectioned duct the cells of which appear cuboidal, but at the right upper corner of the duct there are two large cells which may represent proliferating duct cells. Arrows indicate a small vacuolar structure which is membrane-enclosed and resembles the ergastoplasmic lesion described in the degeneration period. *CAP*, capillary. × 4000.


DC
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10 μ
9

than normal levels at days 18 and 28, the morphologic and autoradiographic results would suggest the possibility of a primary role for the nucleolus in the replication process (days 18 and 28) but a lesser role in the early stage (day 12) of recovery (Compare Figs. 2 and 9).

Acinar Cell Precursor

The problem of the precursor cell in regeneration has been studied in this laboratory (3). The ethionine insult to the acinar cell left a distinctive intracellular marker of ergastoplasmic degeneration. Its presence as osmiophilic plaques in many regenerating cells at days 12 and 18 indicated that most of the pre-acinar and regenerating acinar cells were injured cells which had survived the analogue effect. However, since some duct cells as well as most acinar cells showed evidence of ethionine damage, both were considered possible sources of pre-acinar cells. A type of transition cell with features of both duct cells and acinar cells was seen rarely. The possibility was entertained that this was a ductule cell of the centroacinar type giving rise to a pre-acinar or acinar cell. The electron microscopic findings suggest that most regenerated cells were damaged acinar cells which recovered from the ethionine effect. It is possible, however, that under the stringent conditions imposed by our regimen the ductule cells also gave rise, in small numbers, to acinar cells.

Protein-Free Diet

In a few animals on the protein-free diet there was noted at day 10 an ergastoplasmic lesion similar to that induced by ethionine. Rarely would a cell in the protein-free animals show most of the degenerative changes associated with ethionine (19). Very rarely, a few whorls of ergastoplasm were present in the protein-free animals. No cell regeneration was noted. For these reasons the changes described seem to be characteristic of recovery from the ethionine effect rather than being the result of the diet, although the latter accentuated the action of the analogue.

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