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Disruption of Endoplasmic Reticulum Is the Primary Ultrastructural Lesion of the Pancreas in the Selenium-Deficient Chick (42697)

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Abstract. Severe uncomplicated selenium (Se) deficiency was produced in chicks by feeding, from 1 day of age, a purified diet that contained 0.010 ppm Se but was adequate with respect to all other known nutrients. The deficiency was characterized by depressions in rate of growth and efficiency of feed utilization and by reductions in the plasma activity of Se-dependent glutathione peroxidase (SeGSHpx) by 85–97% from levels in chicks fed the basal diet supplemented with 0.20 ppm Se as Na_2SeO_3. Histological observations of the target organ of Se deficiency in the chick, i.e., the pancreas, using transmission electron microscopy, showed severe losses of endoplasmic reticulum (ER) and absence of secretory granules in acinar cells of Se-deficient animals. These effects were not uniform within individuals, as Se-deficient pancreases also showed areas of unaffected acini. By 14 days of age, Se-deficient pancreases contained many apparently undifferentiated cells, which were absent from pancreases of Se-fed chicks. It is noteworthy that abnormal mitochondria were not observed in any pancreas sections. It is concluded that the metabolic consequences of severe uncomplicated Se deficiency in the chick result from the disruption of ER and loss of functional acinar cells, rather than to damage to mitochondria as previously suggested.

Chicks fed diets containing exceedingly low amounts of selenium (Se) but adequate amounts of all other known nutrients, including vitamin E, develop dysfunction and atrophy of the exocrine pancreas (1, 2). This condition, termed nutritional pancreatic atrophy (3), is of special interest inasmuch as its prevention shows particular specificity for Se. Unlike other pathologic conditions associated with nutritional Se deficiency in the chick or other species, nutritional pancreatic atrophy is prevented only by Se in chicks fed vitamin E at levels within ca. 30-fold the dietary requirement for that vitamin.[2]

The biochemical basis of the degeneration of the acinar pancreas in severe Se deficiency is not clear, although it is thought to involve the loss of SeGSHpx activity in those cells. Gries and Scott (2) used light microscopy to describe the histopathology of pancreatic degeneration in the Se-deficient chick as involving hyaline body formation and intracellular vacuolization of acinar cells and by infiltration by fibroblasts. Noguchi *et al.* (6) found that atrophied pancreases from Se-deficient chicks contained reduced amounts of zymogen. In subsequent work, the respiratory function of mitochondria isolated from Se-deficient chicks with early-stage pancreatic atrophy (i.e., without fibrosis) was found to be normal (3, 7); however, the yield of mitochondria from Se-deficient pancreases was only 72% of that of Se-adequate controls (7). Whitacre and Combs (7) found that early-stage Se-deficiency involved marked reductions in the synthesis of RNA and protein in the pancreas (but not in the liver). On the basis of these findings, they suggested that the primary metabolic lesion in severe Se deficiency may be the loss of mitochondrial integrity due to diminished antioxidant protection via SeGSHpx in the cytosol and mitochondrial matrix space. Whitacre and Combs (7) supported their hypothesis with limited observations of pancreatic acinar ultrastructure using transmission electron microscopy which they interpreted as showing

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[2] Whitacre and Combs (4) found that dietary levels of vitamin E greater than 300 IU/kg can prevent nutritional pancreatic atrophy in the Se-deficient chick. However, among animals fed levels of vitamin E nearer the dietary requirement (i.e., 10 IU/kg (5)) or in generous excess thereof, as in these experiments, degeneration of the acinar pancreas is prevented only by Se.

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both loss of ER and mitochondrial disruption in affected cells.

Upon publication of the report of Whitacre and Combs (7), one of us (E.J.R.) challenged the authors' interpretation of the published electron micrographs on the basis that the apparent effects on mitochondrial structure appeared to be artifacts of sectioning and that the primary effect was actually one of loss of ER, which lesion was compatible with the authors' biochemical observations. In order to clarify the question of the actual primary ultrastructural lesion due to severe Se deficiency in the chick pancreas, we have collaborated in the following study.

Materials and Methods. *Animals and diets.* Day-old male, Single Comb White Leghorn chicks were produced from a Se- and vitamin E-deficient breeding flock as described previously (7). Chicks were housed in thermostatically controlled, wire-floored, battery brooder cages with a 15-hr day. Feed and water were provided *ad libitum.* The basal diet was the amino acid-based purified diet of Gries and Scott (2) as modified by Bunk and Combs (3). This diet contained sodium taurocholate, monoolein and linoleic acid to promote vitamin E absorption. It was found to contain 0.010 ppm Se by fluorometric analysis (8), and was supplemented with vitamin E (100 IU/kg) as all-*rac*-α-tocopheryl acetate. The same diet supplemented with 0.20 ppm Se as Na_2SeO_3 was used as a positive control.

Biochemical methods. Blood was obtained by anterior cardiac puncture using a heparinized syringe; plasma was prepared from whole blood by centrifugation at 1000*g* for 10 min. The activity of SeGSHpx in plasma was determined by the glutathione reductase-coupled assay of Paglia and Valentine (9) as modified by Lawrence and Burk (10) using 0.25 *M* H_2O_2 as the acceptor substrate. Protein was determined by the method of Lowry (11), and results were expressed as nanomoles NADPH oxidized per minute per milligram plasma protein.

Microscopy. Chicks were killed by cervical dislocation, and pancreases were immediately exposed via a laparotomy. Small slices from each of 10 chicks per treatment group were immediately fixed in a mixture of paraformaldehyde and glutaraldehyde (12) and were postfixed in osmium ferricyanide. The postfixation was carried out for 1 hr at room temperature in a mixture consisting of 10 ml 0.2 *M* sodium phosphate buffer, 2.5 ml 6.4% potassium ferricyanide, 2.5 ml 4% osmium tetroxide, and 5 ml distilled water, all of which reagents were combined just before use. After washing in buffer and immersion for 10 min in 0.15% tannic acid in 0.1 *M* phosphate buffer, the fixed tissues were washed extensively with distilled water and were dehydrated via an ethanol series and acetone, after which they were embedded in Epon-type plastic. Sections were cut using a Huxley ultramicrotome. Semithin sections were stained with toluidine blue and were examined using phase-contrast microscopy. Thin (silver) sections were stained in the grid with uranyl acetate and lead citrate and were examined using a Hitachi HU-11 electron microscope.

Results. Chicks fed the basal diet without supplemental Se showed significantly reduced rates of growth and efficiency of feed utilization. These differences were accompanied by significant differences in the plasma activities of SeGSHpx between the two treatment groups (see Table I).

At six days of age, normal pancreatic exocrine tissue, as viewed by electron microscopy, included both light-colored, immature cells and darker, well-differentiated acinar cells (Fig. 1A). The latter contained closely packed profiles of endoplasmic reticulum (ER) and numerous zymogen granules. In Se-deficient pancreases, a few cells showed dark areas of apparently degenerating material that was associated with ER cisternae, as shown in Fig. 1B.

By 8 days of age, profound changes had taken place in Se-deficient pancreatic tissue. Some acinar cells (Fig. 1C) contained, in addition to ER and zymogen granules, vacuoles and autophagic bodies. Other acinar cells were decreased in size and contained few ER profiles and few or no zymogen granules (Fig. 1D). The nuclei of these cells were elongated in shape but still contained a relatively normal pattern of chromatin which, in the chick, is characterized by moderate-sized areas of heterochromatin adjacent to the nuclear envelope. The periphery of these cells, which normally is smooth, had become ir-

TABLE I. EFFECT OF UNCOMPLICATED Se DEFICIENCY ON CHICK GROWTH AND PLASMA GLUTATHIONE PEROXIDASE (SeGSHPX) ACTIVITY

Dietary Se	Age	Gain (g/chick)	Feed/gain	Plasma SeGSHpx (nmole NADPH/min/mg)
0.01	6	10 ± 2*[a,b]	1.86 ± 0.04[a]	0.18 ± 0.09*[b,c]
	8	12 ± 1*	1.88 ± 0.03	0.73 ± 0.16*
	10	18 ± 3*	1.94 ± 0.02*[b]	0.65 ± 0.15*
	12	20 ± 2*	2.21 ± 0.08*	0.55 ± 0.27*
	14	21 ± 3*	2.40 ± 0.11*	0.66 ± 0.14*
0.21	6	18 ± 2	1.80 ± 0.03	6.34 ± 0.67
	8	23 ± 3	1.76 ± 0.05	9.00 ± 0.44
	10	28 ± 2	1.78 ± 0.04	4.31 ± 0.21
	12	36 ± 4	1.85 ± 0.03	5.48 ± 0.37
	14	45 ± 4	1.90 ± 0.03	10.93 ± 1.02

[a] Means ± SE for triplicate lots of 10 chicks per treatment.
[b] Asterisk (*) indicates significant difference within day from positive (Se-adequate) control.
[c] Means ± SE for 10 chicks per treatment.

regular, with extensions of cytoplasm protruding into the extracellular spaces. The interstitium, which is very small in normal tissue, was by then extensive and filled with cellular debris (Fig. 1D), i.e, material apparently extruded from the atrophied acinar cells. At this time, some portions of the interstitium contained large amounts of collagen.

By 10 days of age, the scant ER remaining in typical acinar cells was either largely degranulated or filled with dense deposits (Fig. 2A), and zymogen granules were generally absent. The interstitium was largely cleared of the cellular debris that had been present at Day 8 and most areas contained either slender dark cellular processes (Fig. 2B) or collagen fibrils against an electron-lucent background.

By Day 12 the majority of acinar cells contained vesiculated ER (Fig. 2C) and no zymogen granules. The cytoplasm of these cells had become lighter in color so that their overall appearance was not unlike that of the light-colored, apparently immature cells present at Day 6. A few dark, still functional, acinar cells were also present. Extrusion of degenerating material from cells into the ducts was observed (Fig. 2D).

At Day 14, i.e., the last time period examined, the remaining acinar cells (Fig. 3B) contained little ER and no zymogen granules. This contrasts markedly with the normal appearances of cells from control (Se-fed) chicks of the same age (Fig. 3A). Chicks of the latter group had maintained the densely packed ER and the zymogen granules that are characteristic of normal cells, as well as the characteristic pattern of nuclear chromatin of the chick. Cells from Se-deficient chicks had lost this pattern; they showed uniform appearing chromatin surrounding an area of dark chromatin, as is characteristic of pyknosis. Light-colored cells like those first prominent at Day 12, evidently acinar cells that had undergone adaptive changes such that they resembled immature cells, were now more plentiful than the darker acinar cells. Interstitial spaces contained portions of degenerating cells and some collagen (Fig. 4), but much less collagen than expected for a fibrotic pancreas, and less than was present at Day 8. The pale areas which appear fibrotic at the light microscope level were, in fact, occupied at this time mainly by the light-colored, altered acinar cells and by cytoplasm from darker acinar cells in advanced stages of degeneration (Fig. 4). Large areas of tissue were affected in this manner by Day 14; however, even at this time, islands of relatively little-damaged, still functional, acini could be found.

Discussion. Mitochondrial degeneration does not appear to be the initial lesion nutritional pancreatic atrophy of the Se-deficient chick, although mitochondria are indeed lost subsequently, as whole acinar cells degener-

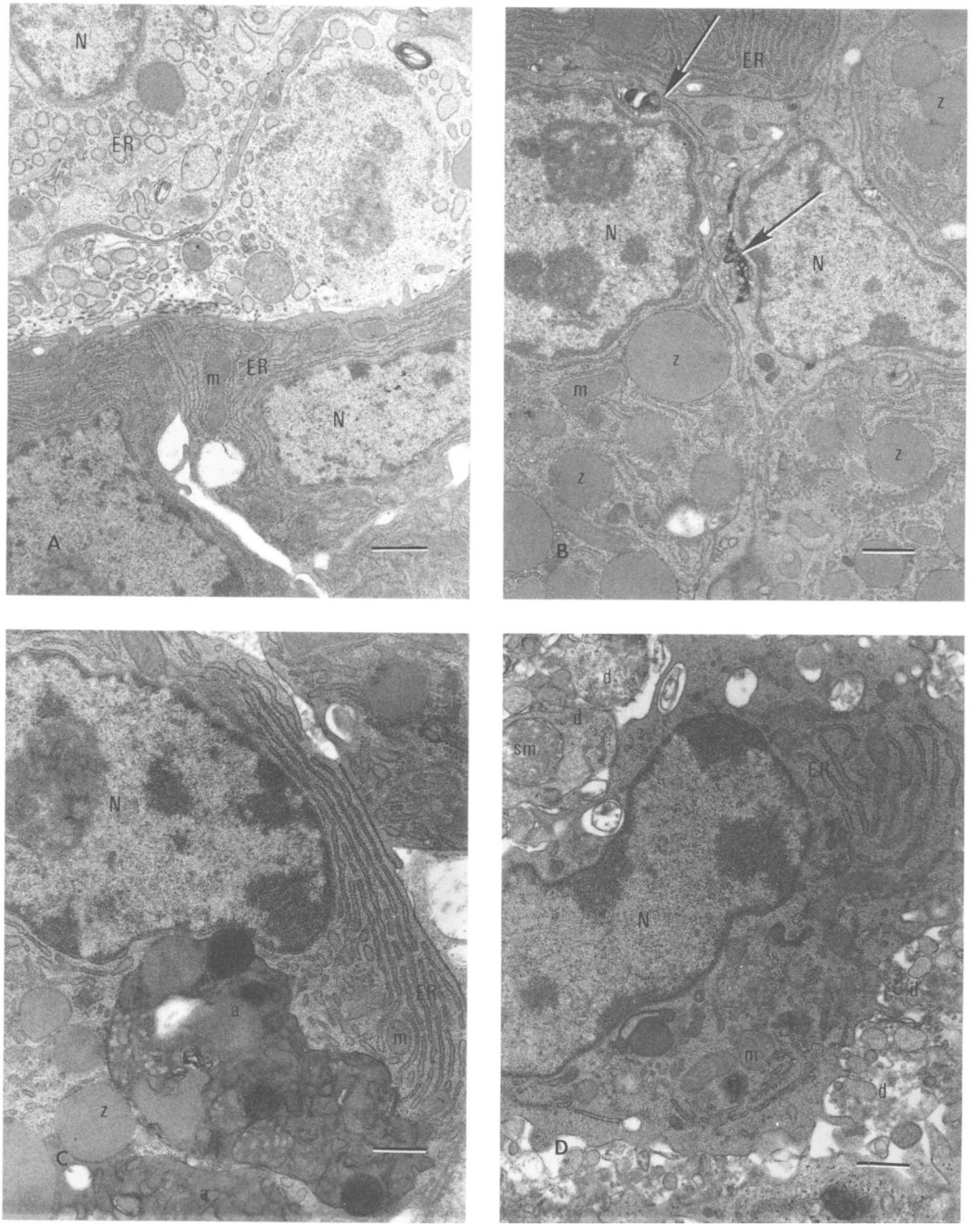

FIG. 1. Electron micrographs showing ultrastructure of normal pancreas and changes with time in Se-deficiency: Nucleus (N), endoplasmic reticulum (ER), zymogen granule (z), autophagic body (a), mitochondria (m), cellular debris (d); all tissue post-fixed in osmium ferricyanide and stained with uranyl acetate and lead citrate; bars indicate 1.0 μm. (A) Six-day-old Se-fed chick. Normal pancreas contains both light-colored, immature, acinar cells (top) and dark-colored, mature, cells (bottom). (B) Six-day-old Se-deficient chick. The first effects of Se-deficiency are seen as dark deposits in or associated with ER (arrows). (C) Eight-day-old Se-deficient chick. Many acinar cells from Se-deficiency show evidence of autophagy (a). (D) Eight-day-old Se-deficient chick. In more severely affected portions of pancreas, large areas of interstitium are filled with cellular debris, while adjacent acinar cells were decreased in both size and content of ER. Mitochondria within these cells still appear normal, although some mitochondria in the cellular debris were swollen (sm).

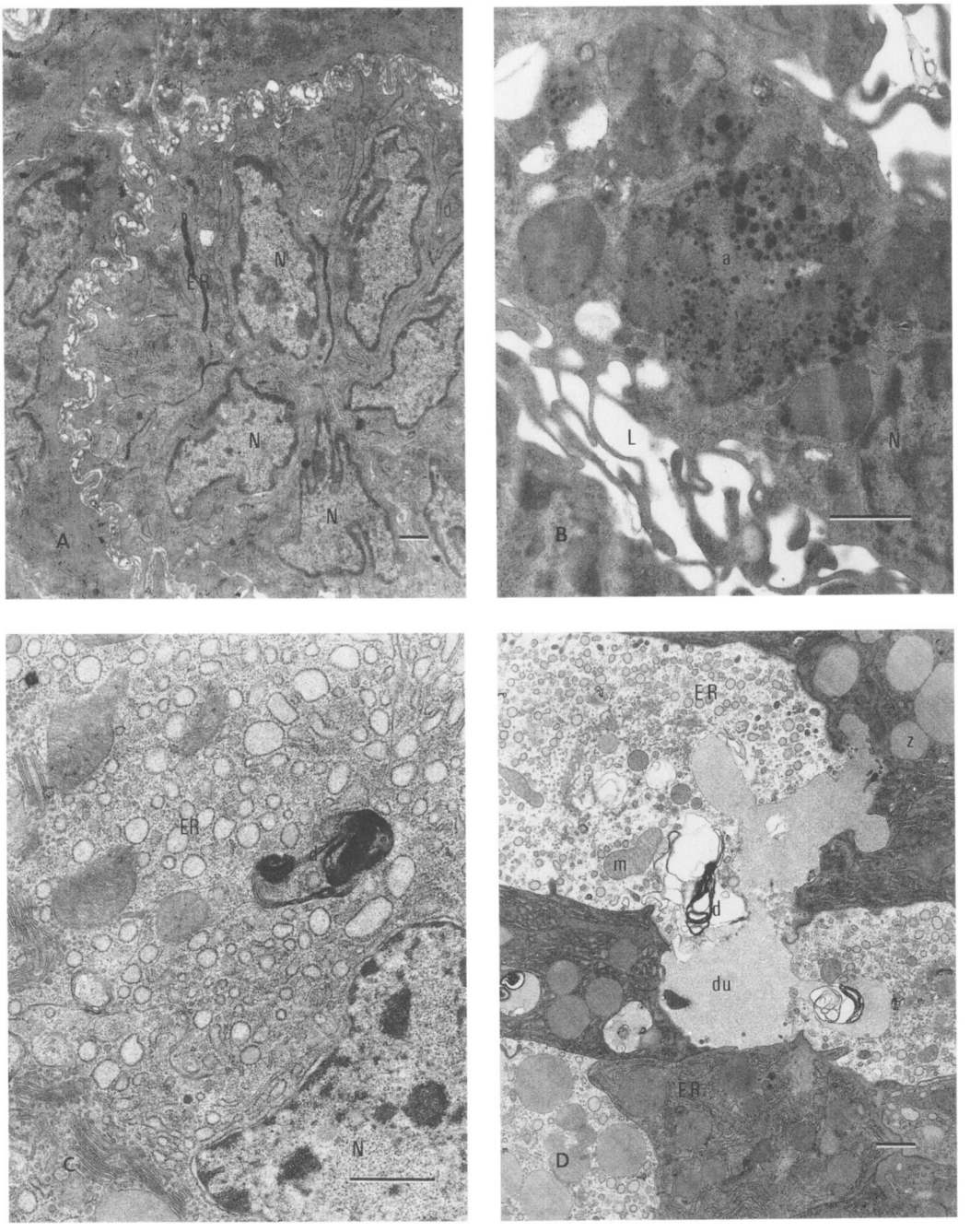

FIG. 2. Electron micrographs showing ultrastructure of Se-deficient pancreases of 10- and 12-day-old chicks. Nucleus (N), endoplasmic reticulum (ER), autophagic body (a), zymogen (z), pancreatic duct (du). All tissue postfixed in osmium ferricyanide and stained with uranyl acetate and lead citrate. Bars indicate 1.0 μm. (A) A still-intact acinus is shown at low magnification. The acinar cells are atrophied with irregularly shaped nuclei, and contain little ER, some of which are filled with dark deposits. (B) In more severely affected areas, interstitium at 10 days is cleared of most of the cellular debris, leaving electron-lucent areas (L) interlaced by slender cytoplasmic projections from the atrophied acinar cells. Debris may have been cleared from the pancreas by finding its way into the ducts (see Fig. 2D). (C) At 12 days, ER of a majority of acinar cells had become vesiculated, and cytoplasm of many of these appeared lighter in color than previously. (D) At 12 days, some relatively undamaged acinar cells could still be found. Here dark, still functional cells and light, altered cells adjoin each other in a single acinus. Zymogen granules from the functional cells and degenerating material (d) from the altered cells are released into the duct at the center of the acinus.

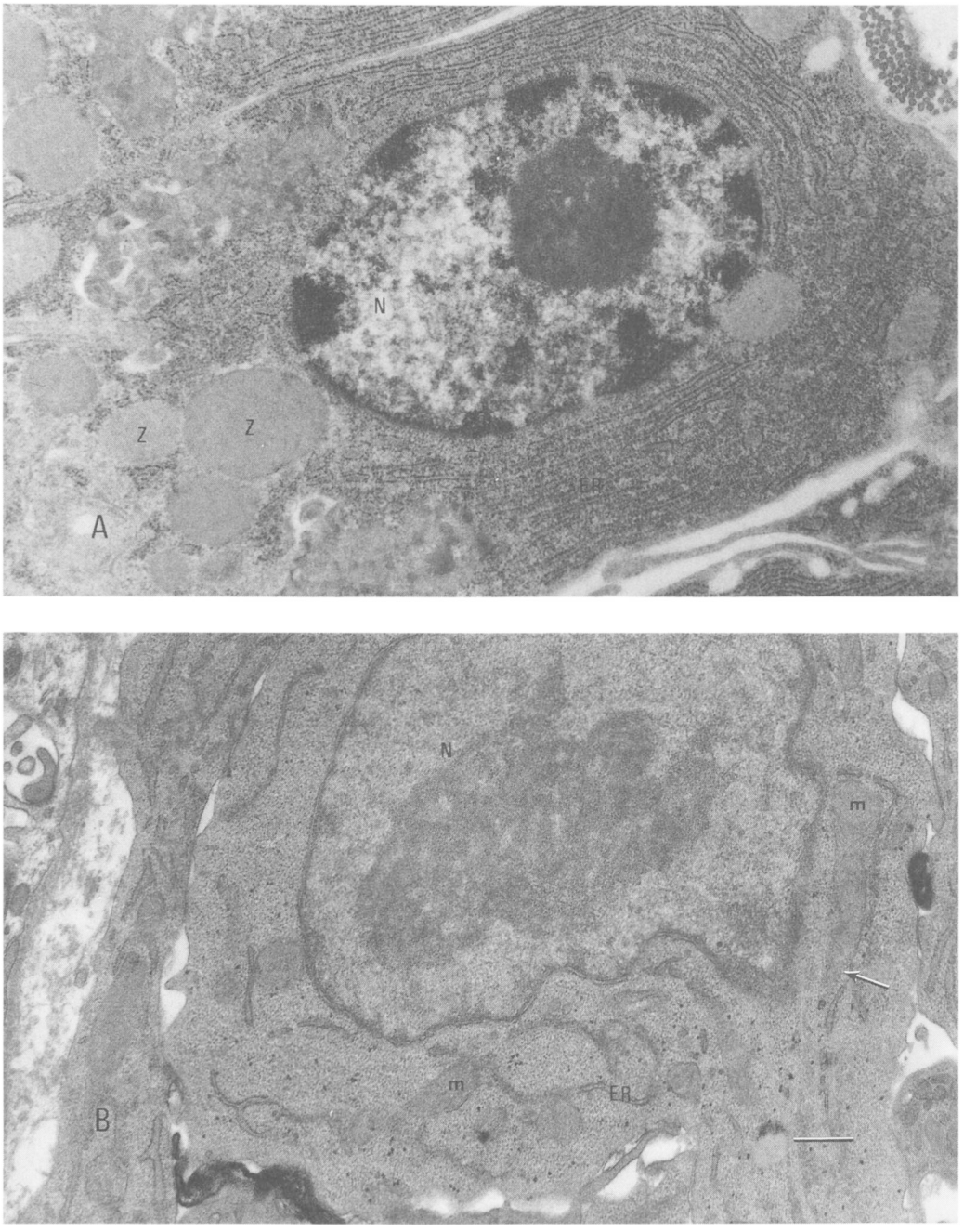

FIG. 3. Electron micrographs showing ultrastructure of typical Se-adequate (A) and Se-deficient (B) acinar cells at 14 days. Post-fixed in osmium ferricyanide and stained with uranyl acetate and lead citrate. Bar indicates 0.5 μm. The Se-deficient cell is one that is unaltered in that it has not reverted to an immature type, but that shows considerable alteration from normal structure: loss of ER, absence of zymogen granules, and loss of nuclear heterochromatin. Mitochondria (m) are normal. The arrow indicates an area of tangential section through the membrane.

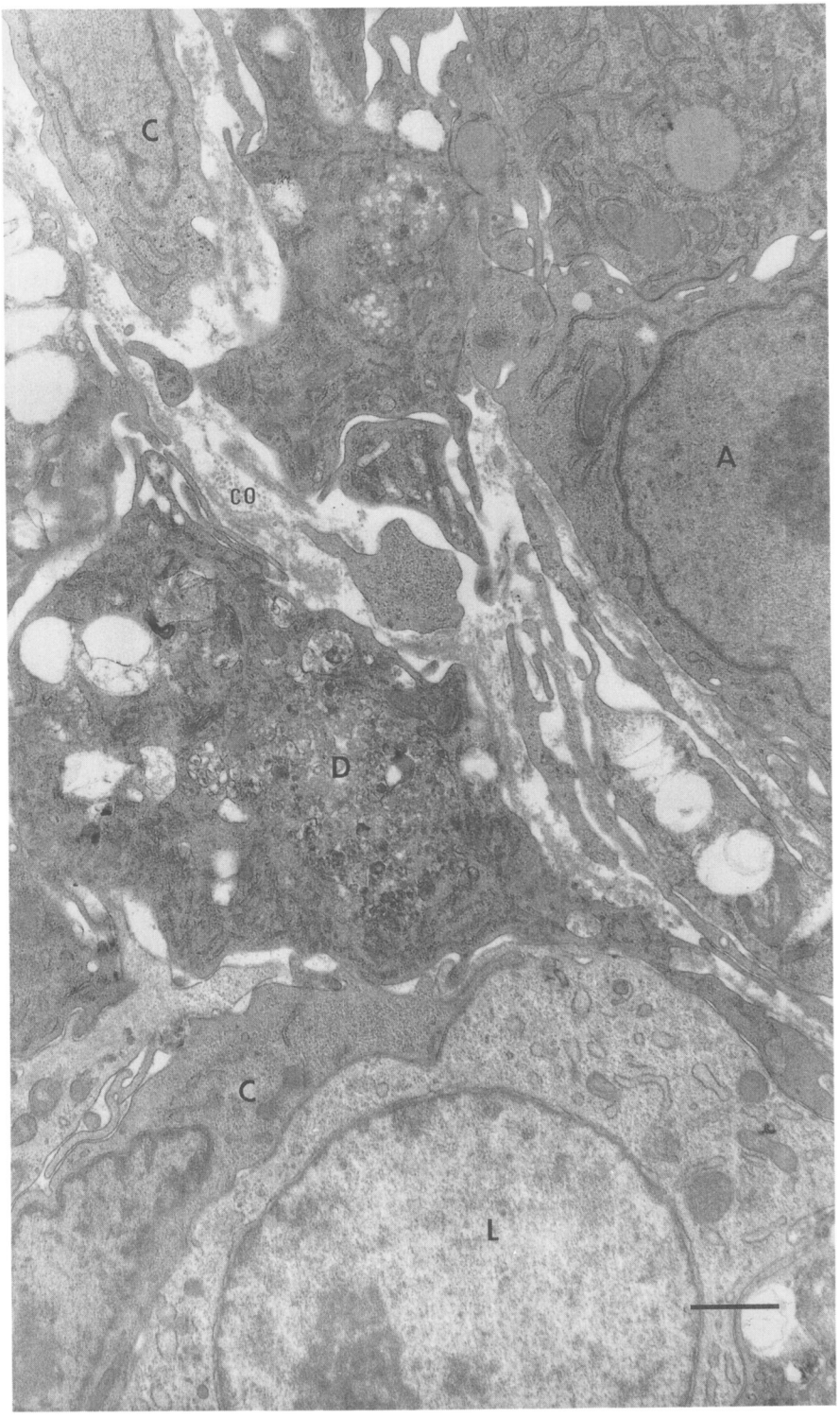

FIG. 4. Electron micrograph of an area showing moderately severe degeneration in the pancreas of a 14-day old Se-deficient chick. Postfixed in osmium ferricyanide and stained with uranyl acetate and lead citrate. Bar indicates 1.0 μm. The disorganized tissue contains degenerating cells (D), centroacinar cells (c), abnormal acinar cells (A), and light colored, altered acinar cells (L), which may be de-differentiated. The interstitium contains degenerating material and some collagen (co).

ate. These observations indicate that, in severe uncomplicated selenium deficiency, pancreatic acinar degeneration is initiated in the ER, ultimately destroying much of that organelle; this event either brings about adaptive changes in the acinar cell or causes its death and degeneration. Some cells appear to adapt by decreasing and altering their ER and by decreasing or ceasing production of zymogen granules. Other cells die and disappear. As more and more cells are affected in this manner, the production of zymogen granules decrease so that the chick eventually lacks the enzymes to digest protein, lipids, and starch, and soon dies of inanition. That these changes result from nutritional Se-deficiency per se, rather than to the effects of reduced feed intake, are indicated by the work of Bunk and Combs (14) who found severe feed restriction not to affect the manifestation of NPA in the chick.

The question arises as to whether loss of the known biochemical function of Se as component of SeGSHpx is adequate to account for these profound changes in cellular structure and function arising from severe nutritional Se-deficiency. This Se-dependent enzyme, the activity of which is reduced in Se-deficient chicks (Table I), catalyzes reduction of hydrogen peroxide to water, the last step in the removal of superoxide. Because the ER in many cell types contains enzymes that produce superoxide as part of their normal function (e.g., the NADPH-dependent cytochrome *P*-450 system (15)), it can be expected that the loss of SeGSHpx due to Se-deprivation may render the ER susceptible to oxidative damage from superoxide or other highly reactive oxygen species (e.g., singlet oxygen, hydroxyl radical). That different balances between the production and removal of such oxygen free radicals, exist in different organs, is suggested by different organ susceptibilities to damage in Se-deficiency among various species (16). For example, in the rat, the target organ is the liver; whereas, in the chick, it is the pancreas. Both of these organs contain extensive ER.

That the ER should be particularly liable to free-radical damage is suggested, not only because it is a site of oxygen radical production, but also because its membranes are rich in polyunsaturated fatty acids which are susceptible to free-radical attack. Although the damage from highly reactive free radicals is generally localized at the site of their production (15), secondary products of oxidative damage may affect other sites inside the cell. Alterations in other parts of the cell, such as the nucleus, may arise as adaptations to damage or to altered capacity for protein synthesis. This pattern of damage, which corresponds to that observed in the present study, is consistent with the loss of SeGSHpx as the primary cause. These observations, therefore, do not suggest a metabolic role of Se other than that already recognized.

It has been observed that, if Se is provided to Se-deficient chicks, they recover rapidly from pancreatic atrophy (17). This rapid response to Se-therapy may involve the light-colored altered acinar cells that are observed to emerge as pancreatic damage progresses. The exocrine pancreas has long been known to be capable of remarkable recovery from injury (18), but the source of this ability was identified only recently. Whereas, it was formerly thought that small numbers of undifferentiated pancreatic stem cells may exist, and that these may differentiate and replace the damaged cells, it is now accepted that pancreatic acinar cells can de-differentiate and simplify their structures in response to injury, later to re-differentiate and re-populate the pancreas (19–21). The observations reported here (i.e., the finding, in late-stage Se-deficient pancreases, of ER-poor, apparently de-differentiated cells resembling those of the 6-day-old chick) would appear to exemplify this phenomenon. It is our hypothesis that, if Se-deficiency is corrected before the chick dies of starvation, these cells apparently are able to divide and re-differentiate to form anew normal acinar tissue.

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