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Ultrastructural changes in pancreatic acinar cells of rats after administration of 4-hydroxyaminoquinoline-l-oxide

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Summary. Ultrastructural changes in the exocrine pancreas 24, 48, 72 and 168 hours after a single intravenous injection of 4-hydroxyaminoquinoline-1-oxide (4-HAQO), at a dose of 14 mg. per kg., were observed in male rats. At 24 hours after administration, multiple focal degenerative lesion-like large vacuoles in the acini, and a decrease in zymogen granules with dilation of the rough endoplasmic reticulum cisternae in the acinar cells were marked. At 48 hours, acinar cell degeneration and necrosis progressively increased. The nucleus, especially, appeared to be disorganized and lysosome-like bodies with various sizes were frequently observed. At 72 hours, acinar cell degeneration persisted in the acini, and the interstitial space with infiltration of inflammatory cells appeared edematous. In addition, ductular-like cells, which resembled intercalated duct cells, possessing a light cytoplasm with occasional mitoses were observed around the duct lumen. At 168 hours, the exocrine pancreas was occupied with proliferated ductular-like cells. Furthermore, acinar cells and acini regenerated to the normal pattern were sometimes found. Thus, the exocrine pancreas degenerated progressively up to 48 hours after administration of 4-HAQO, gradually came to be repaired by degrees from 72 hours and then partly appeared to be regenerated at 168 hours. It is suggested that the ductular-like cell might be the precursor of the acinar cell in the regenerating process after injection of 4-HAQO.

Key words: Ultrastructure - 4-hydroxyaminoquinoline-l-oxide - Exocrine pancreas - Rat - Regeneration

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Introduction

Hayashi and Hasegawa (1971) reported the high incidence of pancreatic tumors in rats after a single intravenous injection of 4-hydroxyaminoquinoline-1-oxide (4-HAQO).

Thereafter, the administration of 4-HAQO has been performed as an experimental procedure for the occurrence of not only a tumor with a comparatively low dose in long-term observations (Konishi et al., 1976; Shinozuka et al., 1976; Rao et al., 1982) but also the severely acute necrotic changes with a relatively high dose (Konishi et al., 1974; Reddy et al., 1975; Rao and Reddy, 1976) in the pancreas of rodents. However, at the electron microscopic level, the exact details of the regeneration in the exocrine pancreas damaged by 4-HAQO have not been described.

Besides, there are many descriptions of ultrastructural degeneration in the pancreas of rodents following the systemic administration of other chemicals, such as ethionine (Herman and Fitzgerald, 1962; Fitzgerald et al., 1968; Wenk et al., 1974), azaserine (Hruban et al., 1965), excess methionine (Boquist, 1969) and excess puromycin (Longnecker et al., 1975) and so on. The changes in the excocrine pancreas damaged by treatments had marked ultrastructural similarities, although the materials and methods in each experiment were varied. Furthermore, some of the above-mentioned authors reported on the regeneration or recovery of the degenerated pancreas after chemical treatment (Herman and Fitzgerald, 1962; Fitzgerald et al., 1968; Wenk et al., 1974; Longnecker et al., 1975). As to the source of regenerated cells, there are two views in their reports: (1) the duct cells; (2) the acinar cells.

The present investigation used the tumorigenetic agent 4-HAQO which at least seemed to have an ability to make cells differentiate. The study was undertaken to obtain information about the degenerative and regenerative

changes and the origin of the regenerated cells in the exocrine pancreas of rats administered 4-HAQO in electron microscopy.

Materials and methods

Male Wistar rats weighing about 170 grams (aged 7 weeks) were used in this study. 4-HAQO, at a dose of 14 mg/kg of body weight, was dissolved in 0.005 N hydrochloric acid (HCl) solution (0.3 ml) before the experiments and then injected at one time into the saphenous vein under ether anesthesia. Control rats were given a corresponding volume of 0.005 N HCl solution in the same manner. The animals were kept under normal laboratory conditions (room temperature: 20-25°C) and freely given water and a standard diet (CE-2, NIHON CLEA Co.). At least more than four rats in each group were sacrificed at 24, 48, 72 and 168 hours after the injection of 4-HAQO.

After laparotomy, some portions removed from various parts of the pancreas tissue for electron microscopy were cut into small blocks immediately and immersed in a modified Karnovsky's fixative, containing 4.0% paraformaldehyde and 1.25% glutaraldehyde in 0.1 mol/l cacodylate buffer (pH 7.4), for 2 hours. The blocks were post-fixed for 1 hour in 1% osmium tetroxide in the same buffer and then dehydrated in a graded ethanol series and embedded in Epon 812.

Ultrathin (0.05 μm) sections were stained with uranyl acetate and lead citrate and viewed under an electron microscope (JEM 100S) at 80 kV.

Results

Control

The ultrastructure of the pancreatic acinar cells in control rats showed no marked difference in the various hours after administration of 0.005 N HCl solution and almost conformed to previous description (Ekholm et al., 1962a, Wistar rats weighing between 150 and 200 grams). Thus, each acinus was composed of a number of acinar cells and a few lumina bordered by few or no centroacinar cells. The acinar cells were filled with numerous zymogen granules in the apical portion and abundant rough endoplasmic reticulum (rER) which was prominent in the basal and paranuclear portions (Fig. 1).

24 hours after injection

Severely degenerative focal lesions of pancreatic acinar cells (one to five) were frequently recognized throughout the pancreas tissue, although the whole acinus was not completely degenerated. These altered cells were atrophied, looking like large autophagic vacuoles of the other acinar cells. The nucleolus appeared somewhat larger than the control and the interchromatin of the nucleus aggregated on one side of the nucleoplasm. The cisternae of the rER were markedly dilated and were seen to the vesicular or saccular structure in the tangential view. Few zymogen granules were seen and the mitochondria, in general, were swollen. Most other pancreatic acinar cells relatively maintained their structure; however, the zymogen granules decreased in number and varied in size and, compared with the control lysosome-like bodies and vacuoles were more often seen. (Fig. 2). No clear-cut changes were recognized in the centroacinar or duct cells.

48 hours after injection

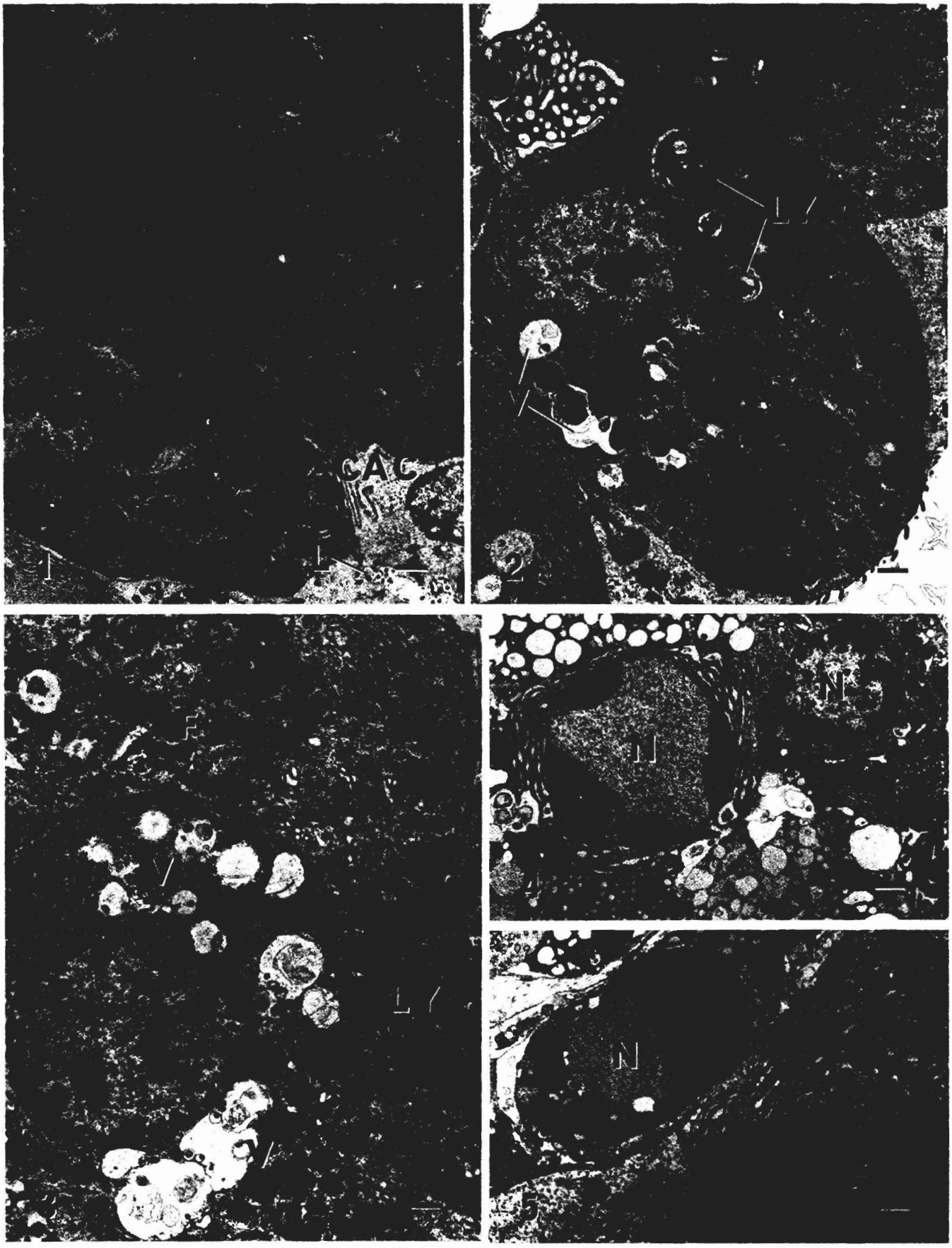
Compared with the previous stage, the damage due to the agent abruptly extended throughout and normal architecture in the pancreatic acini could no longer be recognized (Fig. 3). The various degrees of degeneration in each pancreatic acinar cell were more pronounced, being severe or moderate. Numerous degenerated cells, considered apparently necrotic, were seen. In general, the nucleus was deformed, with reduction of chromatin. Moreover, the disorganization was often striking at this stage, as illustrated in Figures 4 and 5. The rER showed vesicular, saccular or vacuolar cisternal dilation with material of lucent electron density. The decrease of ribosomes attached to the rER membrane was marked. The zymogen granules disappeared, or conspicuously decreased in number, with varied sizes and irregular, especially angular, shapes. The lysosome-like bodies increased, scattering in the cytoplasm of the acinar cells. In the apical portion of the cell, multiple small and rod-shaped (0.2-1.5 μm) structures, suspected to be primary lysosomes, and autophagic vacuoles containing rER, ribosomes, mitochondria or other degenerated cytoplasm were often observed. Furthermore, in some acinar cells, the cytoplasm contained groups of complicated filaments (Fig. 3). The centroacinar cells also showed degeneration to various extent and most had vacuoles and lysosome-like bodies in the cytoplasm.

72 hours after injection

Most of the acini continued to show degeneration, including necrosis of cells. In the edematous interstitial space, there was a lot of debris, consisting of collagen fibers and degenerated acinar cells; inflammatory cells, particularly macrophages, frequently appeared. Additionally, a new duct system, composed of many lumina and ductular-like cells was observed in the acini, intermingled with degenerated acinar cells. The ductular-like cells around the dilated ducts appeared to be rectangular or trapezoid; the nuclei were elliptical with marginal indentations and often had a few nucleoli and scant chromatin (Figs. 6,7). Occasionally, somewhat-rounded cells with mitoses were observed protruding into the duct (Fig. 8). As it contained very little rER and relatively few small oval-shaped mitochondria, and the cytoplasm of the ductular-like cells was light and sometimes included vacuoles, autolysosome-like bodies and lipid droplets. In addition, acinar-like cells, the nuclei of which were round, with poor zymogen granules and an rER, were found close to the ductular-like cells (Fig. 7).

168 hours after injection

Scarcely any lesions in the necrotic acinar cells and edematous interstitial tissue were found, although there was a persistence of inflammatory cells around the acini. On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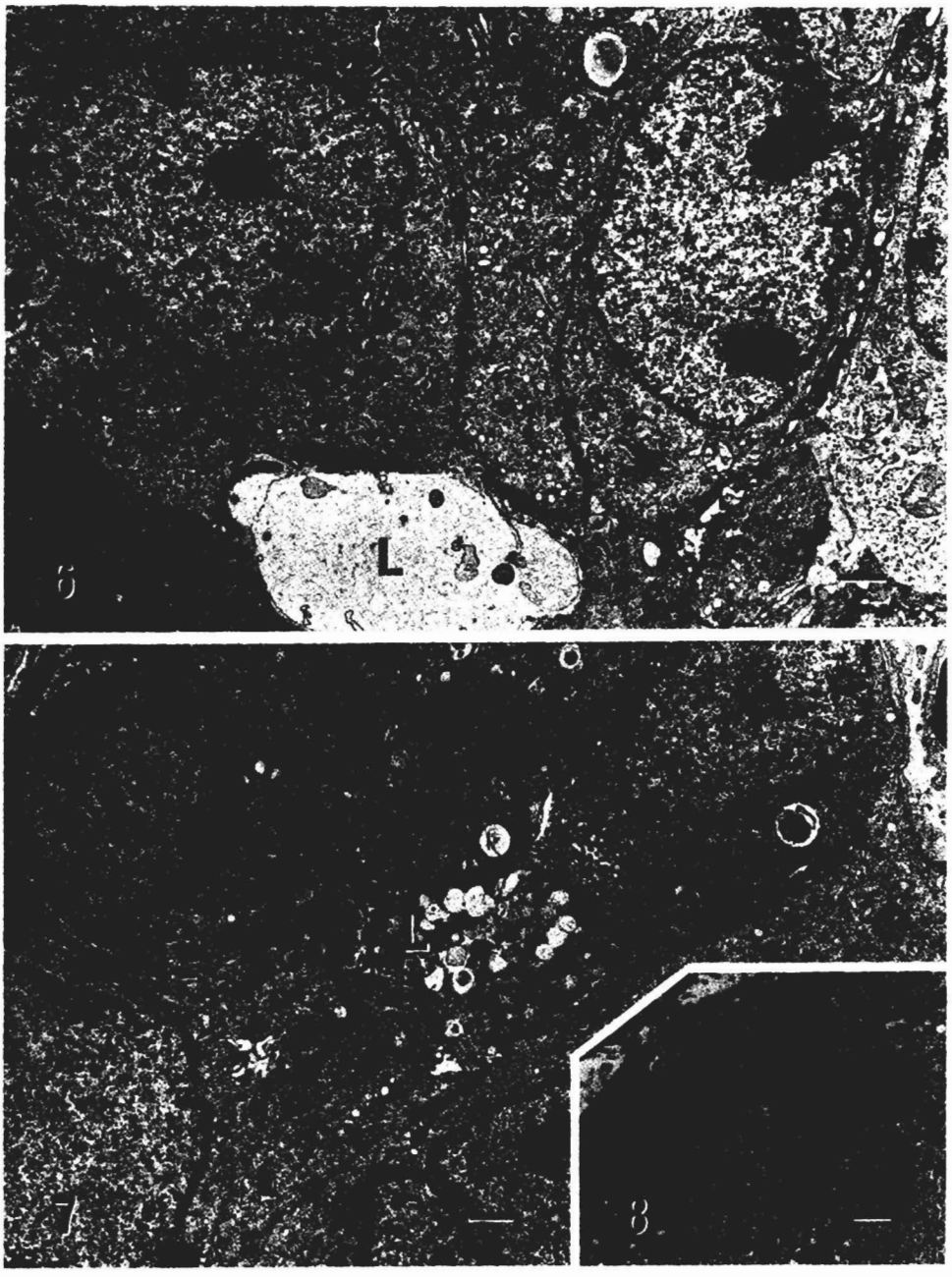
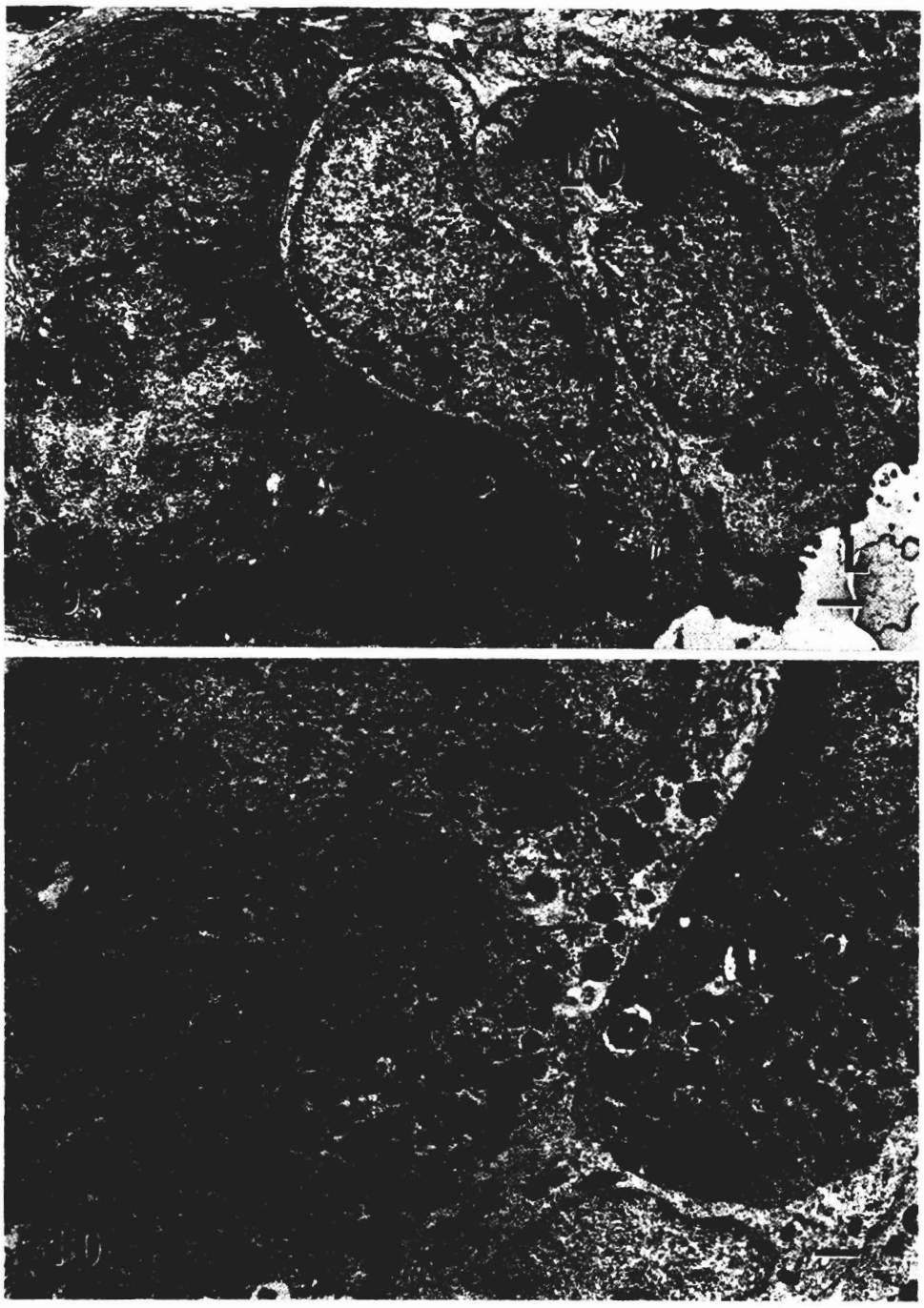


the other hand, the exocrine pancreas was occupied by a progressively regenerated duct system and acini. The acinus was seen to have many dilated and irregular lumina and to be almost intermingled with ductular-like cells, normal-appearing acinar cells containing numerous zymogen granules and a large amount of rER and transitional forms between the two above-mentioned cell types (Figs. 9, 10); occasionally, the formation of acini with almost normal architecture was observed. The normal-appearing acinar cells and transitional-formed cells occupied about one-fifth of the whole pancreatic tissue. The ductular-like cells often contained a few lipid droplets in the apical or basal portion (Fig. 9). Only very few cells with mitotic figures were encountered.



Figs. 1-10. Stained with uranyl acetate and lead citrate. Bar: 1 μm.

Fig. 1. Pancreatic acinar cells from control rat. There are abundant round zymogen granules in the apical part and the rER in the paranuclear part of the cell. L: acinar lumen. CAC: centroacinar cell. x 5,400

Fig. 2. Pancreatic acinar cells from rat at 24 hours after injection of 4-HAQO. The nucleoplasm contains little faintly-stained chromatin material. The zymogen granules are of variable size and many vacuoles (V) and lysosome-like bodies (LY) are seen. x 5,400

Fig. 3. Pancreatic acinar cells from rat at 48 hours after injection of 4-HAQO. Many vacuoles (V), containing cytoplasmic debris and groups of complicated filaments (F), are seen. The rER is irregularly arranged. In the apical part of the cell, small round and rod-shaped structures (lysosome-like bodies) (LY) are observed in large numbers. x 5,600

Fig. 4. Pancreatic acinar cells from rat at 48 hours after injection of 4-HAQO. The chromatin of the left nucleus aggregates at the nuclear membrane. The cisternae of the rER are remarkably dilated. N: nucleus. x 5,600

Fig. 5. Pancreatic acinar cells from rat at 48 hours after injection of 4-HAQO. The chromatin of the nucleus is concentrated on one side of the nucleoplasm. N: nucleus. F: complicated filaments. x 5,600

Fig. 6. Pancreas from rat at 72 hours after injection of 4-HAQO. The ductular-like cells have clear cytoplasm and a nucleus with an indented outline; a small number of microvilli protruding into the dilated duct lumen (L) are seen. In the apical part of one cell, a few zymogen granules are found. x 6,000

Fig. 7. Pancreas from rat at 72 hours after injection of 4-HAQO. Generally, the ductular-like cells show developed cytoplasmic organs. The cell at the upper left, containing a oval nucleus, zymogen granules and a well-developed rER, appears to be an acinar-like cell. The dilated lumen (L) is filled with cytoplasmic debris. x6,000

Fig. 8. The cell in mitotic figure, at 72 hours after injection of 4-HAQO. Microvilli are seen facing the lumen. x5,000

Fig. 9. Pancreas from rat at 168 hours after injection of 4-HAQO. A normal-appearing acinar cell is near a ductular-like cell (suspected intercalated duct cell or centroacinar cell). L: acinar lumen. LD: lipid droplets. x 6,000

Fig. 10. Pancreas from rat at 168 hours after injection of 4-HAQO. The acinus is built up of some normal-appearing acinar cells, containing abundant zymogen granules and rER. x 6,000

Discussion

In the ultrastructural study reported here, it was found that the damage to the rat exocrine pancreas progressed appreciably after 48 hours of a single intravenous injection of 4-HAQO, at a dose of 14 mg/kg. The initial morphological changes observed in the pancreatic acinar cells were marked dilation of the rER cisternae, a reduction in the number of zymogen granules, a moderate increase of lysosome-like bodies and an aggregation of interchromatin in the nucleus. Later, the degenerative changes further advanced; the acini and acinar cells showed disorganization of the rER, numerous vacuoles and lysosome-like bodies appeared and the normal acinar pattern was no longer seen. Moreover, cells were frequently observed, the nuclei of which showed aggregation or reduction of chromatin and destruction (so-called pyknosis, karyolysis and karyorrhexis). We considered these remarkably-degenerated acinar cells to be almost analogous to the necrosis observed in the pancreatic acinar cells of rodents within 48 hours after systemic treatment of 4-HAQO, at a dose of 20 mg/kg (Konishi et al., 1974) and at a dose of 22.5 mg/kg (Reddy et al., 1975) at light microscopic level. Furthermore, the results from the present study are similar to those of a previous description which dealt with the acute ultrastructural changes in the exocrine pancreas of the guinea-pig, following the period of 60 hours after intravenous injection of the same agent, at a dose of 22.5 mg/kg (Rao and Reddy, 1976).

In the present study, the response of regeneration (involving repair due to inflammatory cells) with mitotic figures was recognized mingling with the degenerative lesions in the rat pancreas from 72 hours after the administration of 4-HAQO. The duct system, composed of many ductular-like cells around the dilated lumen, was developed. The ductular-like cells were rectangular or trapezoid, had an indented nucleus with scant chromatin and cytoplasm with poorly-developed rER and few, small mitochondria; thus, they seem to correspond to the intercalated duct cell in the normal pancreas (Ekholm et al., 1962b). Sometimes the intermediate forms between ductular-like cells and acinar cells, containing a small number of zymogen granules, were found near the ductular-like cells. It might be considered that these cells were differentiating en route. At 168 hours after administration of 4-HAQO, the intermediate-type cells, the normal-appearing acinar cells containing numerous zymogen granules and well-developed rER apparently increased. The acini also began to form, although their architecture was incomplete.

It is known that many investigators have studied both the degeneration and regeneration of the pancreas of rats and guinea-pigs in ethionine models, with varying doses and methods in administration, by light or electron microscopies (Fitzgerald and Alvizouri, 1952; Wachstein and Meisel, 1953; Kinney et al., 1955; Herman and Fitzgerald, 1962; Wenk et al., 1974). As regards regeneration, light microscopic studies showed mitotic figures and differentiation of cells (Fitzgerald and Alvizouri, 1952; Wachstein and Meisel, 1953; Kinney et al., 1955). Herman and Fitzgerald (1962) described, at electron microscopic level, the proliferation of ducts and duct cells, as well as the regeneration of cells with relatively abundant rER and a few normal-appearing zymogen granules in the rat pancreas, at 10 days after injection of ethionine. The relation between duct cells and regenerated cells (similar to acinar cells) was not described in detail; however, the morphologic changes of their report were similar to those in the pancreas from rats administered 4-HAQO in the present study.

A single intravenous injection of 4-HAQO in the rat pancreas at a relatively low dose (6, 9 and 13 mg/kg), caused a high incidence of hyperplastic nodules or adenomas between 390 and 400 days (Hayashi and Hasegawa, 1971). Thereafter, some investigators also reported the occurrence of acinar cell foci and nodules between 6 months and about one year in similar

experiments by electron microscopy (Shinozuka et al., 1976; Rao et al., 1982). Injection of 4-HAQO during ethionine-induced regeneration resulted in the development of hyperplastic nodules, adenomas and adenocarcinoma (Konishi et al., 1976). The origin of cells in these pancreatic tumors was not sufficiently detailed, however. The nodules induced by 4-HAQO were composed of a new population of phenotypically-altered acinar cells (Shinozuka et al., 1976). It is unfortunate that the problem concerning the relation between the regeneration of the acinar cells and the occurrence of tumor cells in the pancreas could not be discussed because the period of observation after the injection of 4-HAQO was only up to 168 hours in the present experiment.

In this study it may be considered that, in the rat pancreas, the severely-degenerated acinar cells with large vacuolar structures or necrotic cells, induced by 4-HAQO, extruded to the interstitial space and the lumen, sometimes appearing to be within a macrophage; these cells were then displaced by regeneration of ductular-like cells. Thus, the ductular-like (intercalated duct) cells seem to develop into the acinar-like cells and acinar cells and finally restore the acini. It is suggested that the differentiating ductular-like cell may be the precursor of the acinar cell; in addition, the possibility that abnormal development of these cells may produce the tumor is naturally considered. It appears significant to solve the problem of the occurrence of neoplasms and the differentiation in the exocrine pancreas after recovery from the effects of 4-HAQO. Further studies can clear up this point.

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