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Ultrastructure of STH Cells of the Pars distalis of Hepatectomized Mice*

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Summary. An electron microscopic analysis was performed on the pars distalis of the hypophysis of hepatectomized mice. Intact and sham operated mice served as controls. The STH cells presented striking changes that were most intense and widespread in those animals sacrificed at midnight of the second day after hepatectomy. These changes can be summarized as follows: 1) Hypertrophy of the Golgi complex with increased number of immature granules within the Golgi zone. This change appeared also in otherwise unmodified STH cells. 2) Strong dilatation of the endoplasmic reticulum whose cisternae contained much electron dense material. 3) Granules with partially diminished electron density, some of them in spatial relation with the plasma membrane and others swelling and bursting within the cytoplasm. All transitions between unchanged 350–400 mμ granules and extremely altered ones, were seen. 4) Release sites, characterized by dense zones in the plasmalemma, close to aggregates of electron lucent microvesicles, and almost empty granule membranes. 5) Increase in the density of the mitochondria which appeared grouped near the Golgi zone. 6) Increase in the number of large lysosomes of the autophagic vacuole type. 7) Irregular nuclear outlines. These data suggest increased synthesis and release of growth hormone in STH cells stimulated by hepatectomy.

Key-Words: Pars distalis—STH cells—Hepatectomy.

In 1953 Cordier reported that the pars distalis of the pituitary of *Xenopus* larvae shrinks, and the acidophil cells disappear, at the time of appearance of the forelimbs. He interpreted this fact as the "...excrétion intense et aigü de l'hormone somatotrope en rapport avec la croissance rapide des pattes antérieures...". This observation led us to study the ultrastructure of STH cells of the mouse pars distalis during liver regeneration, another significant growth situation.

Many reports point out the existence of circadian rhythms in pars distalis (Clark and Baker, 1964; Halberg and Reinberg, 1967; Meier, 1969; Gomez Dumm and Echave Llanos, 1970) as well as liver (Echave Llanos, 1967a; Echave Llanos, Bade and Badrán, 1967b) variables. These data were taken into account in the present study in which we report on the ultrastructure of STH cells of hepatectomized mice.

Material and Methods

C3H-S male mice were used. This strain, provided by Professor J. W. Wilson (Brown University Providence, Long Island, U.S.A.), has been maintained in our laboratory by inbreeding, since 1966. The animals were weighed weekly after the time of weaning and those

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with abnormal growth curves were eliminated. At the age of 9 weeks they were standardized for periodicity analysis (Halberg, Barnum, Silber and Bittner, 1958; Echave Llanos and Nash, 1970). At the age of 12 weeks they were used in the following way (Fig. 1):

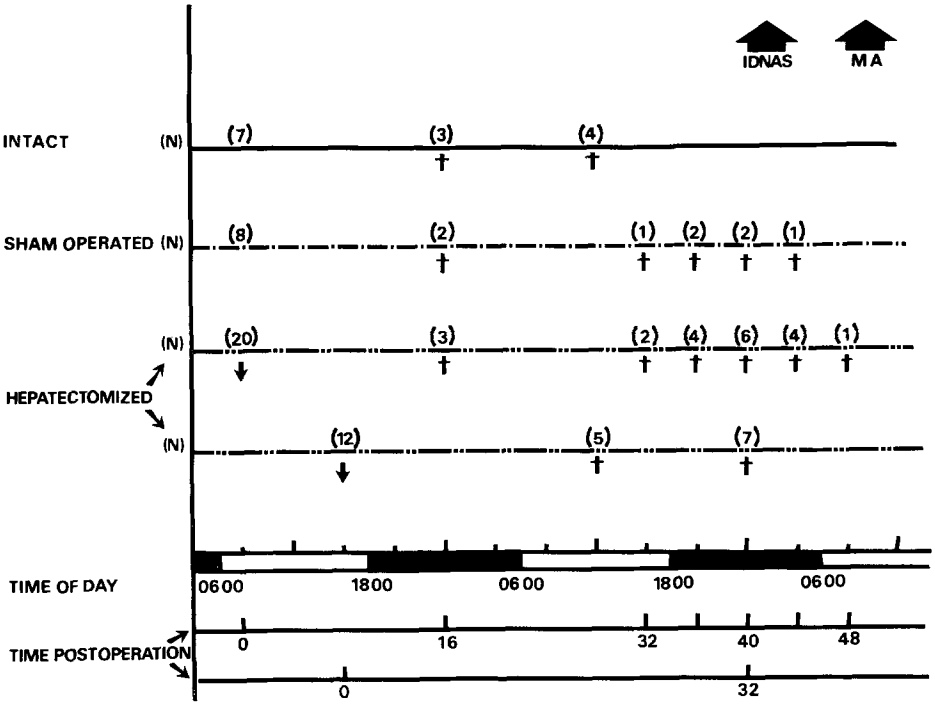


Fig. 1. Experimental design. Time of Day: Indicates the regimen of illumination with light from 6 a.m. to 6 p.m. alternating with 12 h darkness. *N* sample size. ↓ Time of day for operation. † Time of day for sacrifice of animals. The two large arrows (upper right) indicate the DNA synthesis and mitotic activity peaks according to unpublished results from our laboratory

Seven control intact mice were sacrificed either at noon (4 animals) or at midnight (3 animals). Eight animals were sham operated, with liver handling, at 8 a.m. and sacrificed according to the following schedule: 00/16 (time of day/hours postoperation): 2 animals; 16/32:1 animal; 20/36:2 animals; 00/40:2 animals and 04/44:1 animal.

Twenty animals were hepatectomized (Brues, Drury and Brues, 1936) at 8 a.m. and sacrificed as follows: 00/16:3 animals; 16/32:2 animals; 20/36:4 animals; 00/40:6 animals; 04/44:4 animals; 08/48:1 animal. Twelve mice were hepatectomized at 4 p.m. and sacrificed at 12/20:5 animals and 00/32:7 animals.

Once the animals were decapitated and exsanguinated, the skull was opened, the brain removed and the hypophysis exposed by eliminating the meningeal covers. A drop of cold Millonig (1962) fixative was then placed onto the sella turcica and, after a few seconds, the pituitary gland was removed. It was then immersed into another drop of fixative and the lateral wings of the pars distalis were separated. They were divided into 6 pieces, anterior, middle and posterior, each of them also divided into medial and lateral (Purves and Giesbach, 1951). Some hypophyses were fixed in glutaraldehyde of formaldehyde (Pease, 1964) and post-fixed in OsO_4. The time of fixation was up to 8 h for aldehydes and 1–2 h for osmium tetroxide.

The material was dehydrated and embedded in araldite (Luft, 1961). Sections were obtained with glass knives and an LKB ultratome III Ultramicrotome, mounted on copper grids and stained with permanganate (Lawn, 1960) and lead citrate (Reynolds, 1963). They were examined in a Siemens Elmiskop 1 at 60 kV.

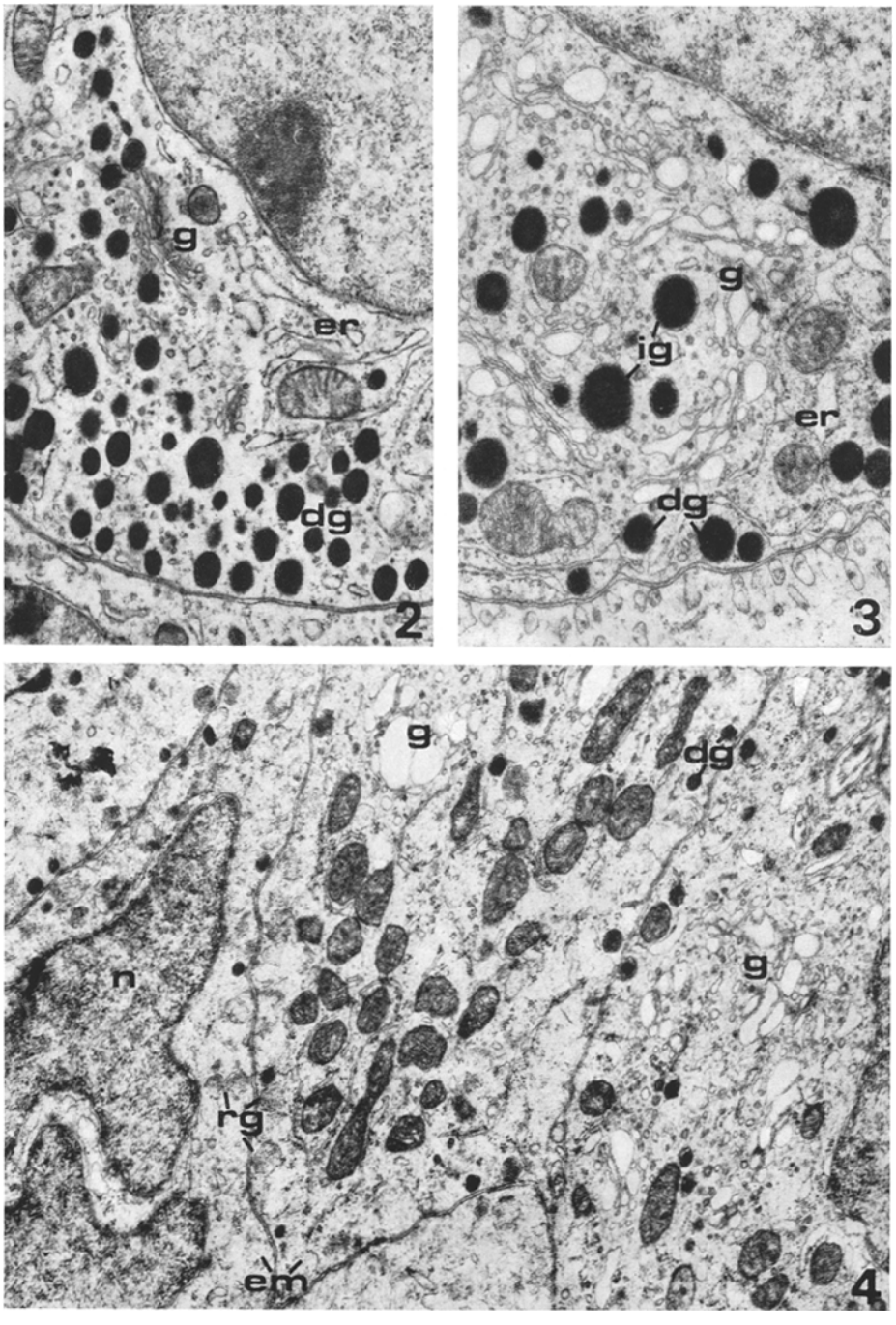
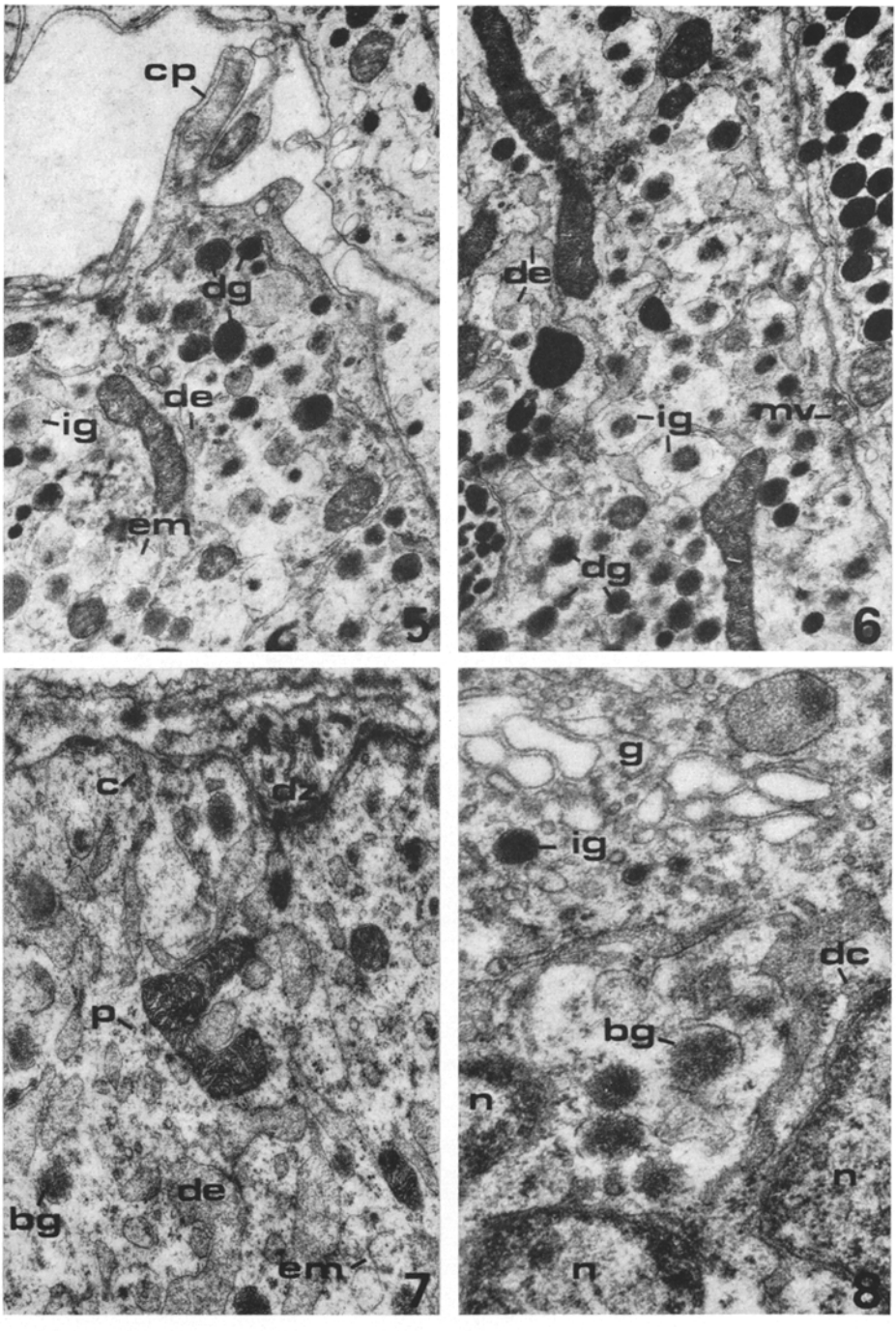


Fig. 2. STH cell from an intact mouse sacrificed at midnight. *g* small Golgi complex. *dg* dense granules. *er* slightly dilated endoplasmic reticulum. ×12,000

Fig. 3. STH cell from a hepatectomized mouse sacrificed at midnight of the second day of regeneration. *g* hypertrophic Golgi complex. *ig* immature granules. *dg* dense mature granules. *er* endoplasmic reticulum. ×18,000

Fig. 4. Diffuse changes in several STH cells. *n* irregularly outlined nucleus. *rg* granules with greatly reduced electron density. *g* Golgi complex. *dg* dense granules. *em* empty vesicle membranes. Hepatectomy: 4 p.m. Sacrifice 00/32. ×10,000



Figs. 5—8

Results

The changes to be described were observed in hepatectomized mice all along the sampling, but they were most pronounced in those animals sacrificed at midnight of the second day of regeneration, when they were seen in more than half of the STH cells. Occasional STH cells with mild changes of the same type occurred in some of the sham operated mice. Modified STH cells were easily differentiated from other pars distalis cells (Barnes, 1963; Sano, 1962).

In animals that showed the most conspicuous cellular changes the hypophysis presented congested capillaries with many red blood cells and an increased number of blood platelets. In some zones of the capillary bed the endothelium was lacking and parenchymal cells seemed to be in immediate contact with blood. The following changes were observed in STH cells of hepatectomized mice:

1. Hypertrophy of the Golgi complex with conspicuous vacuoles and cisternae and many microvesicles, some of them with dense content. Many immature granules were seen within the Golgi zone (Figs. 3, 4, 8). This pattern, observed also in some unmodified STH cells was in sharp contrast to the extreme reduction of this organelle in intact mice sacrificed at midnight (Fig. 2) (Gomez Dumm and Echave Llanos, 1970).

2. Dilatation of the endoplasmic reticulum, including the perinuclear cisternae (Figs. 6–8). Characteristics were intracisternal electron dense material, few membrane-bound ribosomes and many free ribosomes of the polysome type (Fig. 7).

3. Diminution in number of secretory granules, most marked in those cells with maximal dilatation of the endoplasmic reticulum (Fig. 7).

4. Regular (Fig. 9) or irregular (Figs. 5, 6) diminution in granule electron density. Some of these granules showed their membrane broken and the content spilled into the cytoplasm (Figs. 7, 8). All transitions between intact granules and accumulations of granular material were seen in these cells.

5. Transitions between electron lucent granules and empty membranes, both in contact with the plasmalemma, were very common (Fig. 4). Extrusion images (Farquhar, 1961) were very scarce.

6. Electron dense zones in the plasmalemma and intercellular space, in spatial relation with aggregates of electron lucent microvesicles or granules within the

Fig. 5. Altered STH cell. *dg* dense granules. *ig* granules with partially decreased electron density. *de* incipient dilatation of endoplasmic reticulum containing dense material. *em* empty vesicle membranes. *cp* cytoplasmic process into the interstitial space. Hepatectomy: 4 p.m. Sacrifice: 00/32. ×12,000

Fig. 6. Unmodified (upper right) and highly altered STH cells. *de* dilated endoplasmic reticulum containing dense material. *ig* granules with uneven diminution of electron density. *dg* dense granules. *mv* aggregate of microvesicles. Hepatectomy: 4 p.m. Sacrifice: 00/32. ×12,000

Fig. 7. Extremely altered STH cell. *de* much dilated endoplasmic reticulum containing dense material. *dz* dense zone in the plasmalemma. *c* cell surface concavity containing microvesicles. *p* polysomes. *bg* bursting granule. *em* empty granule membranes. Hepatectomy: 4 p.m. Sacrifice: 00/32. ×12,000

Fig. 8. Detail of an altered STH cell. *n* part of irregularly outlined nucleus. *dc* dilated perinuclear cisternae containing dense material. *bg* bursting granule. *g* hypertrophic Golgi complex. *ig* immature granule. Hepatectomy: 4 p.m. Sacrifice: 00/32. ×35,000

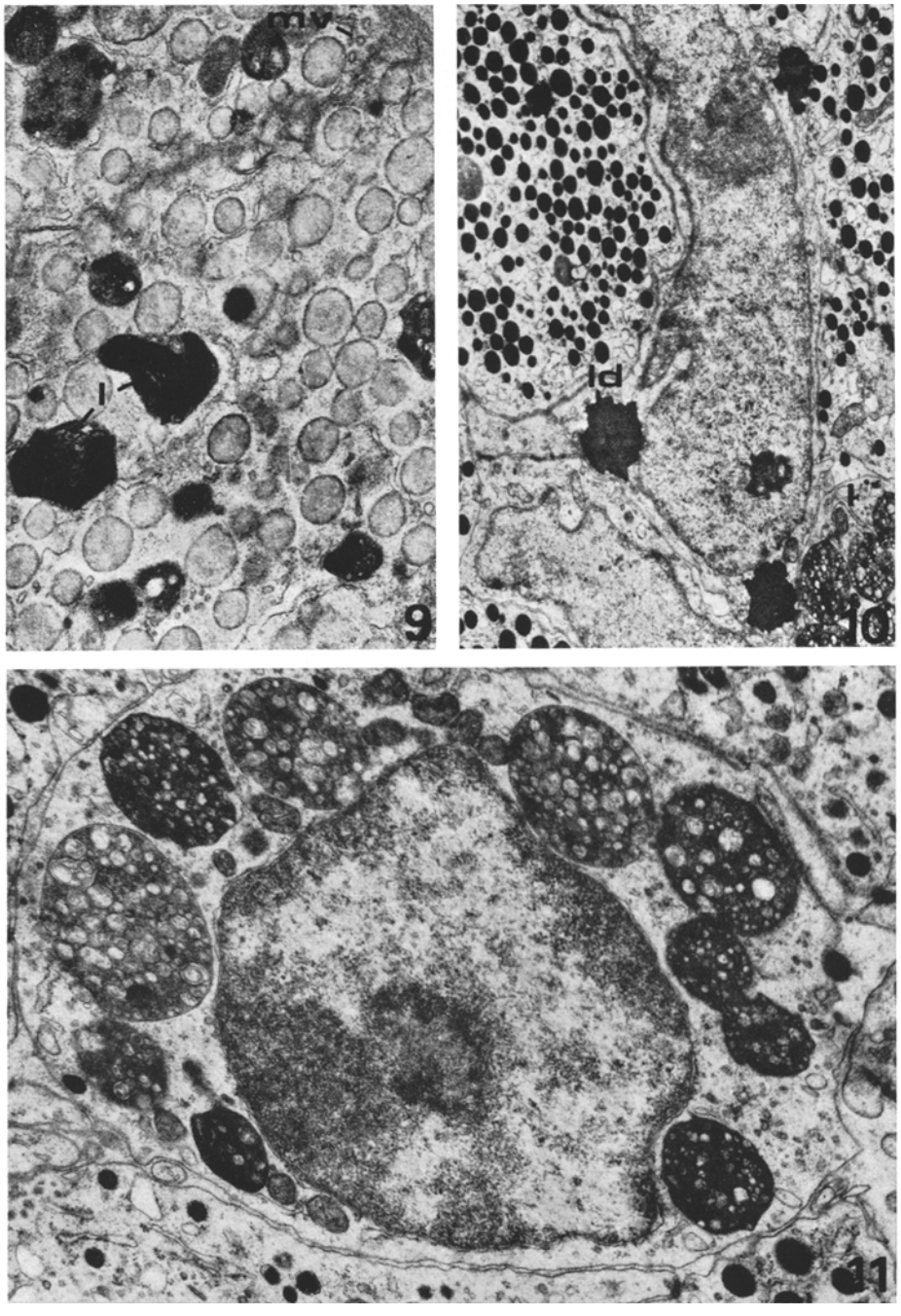


Fig. 9. Two adjacent altered STH cells. The granules show a marked diminution of electron density. Abundant lysosomes in the cytoplasm (*l*). *mv* microvesicles. Hepatectomy: 8 a.m. Sacrifice: 00/40. ×17,000

Fig. 10. Cell without specific granules and three lipid droplets (*ld*) in its cytoplasm. *l* autophagic vacuoles in another cell. Hepatectomy: 4 p.m. Sacrifice: 00/32. ×6,000

Fig. 11. Cell with numerous large lysosomes of the autophagic vacuole type. Hepatectomy: 4p.m. Sacrifice: 00/32. ×12,000

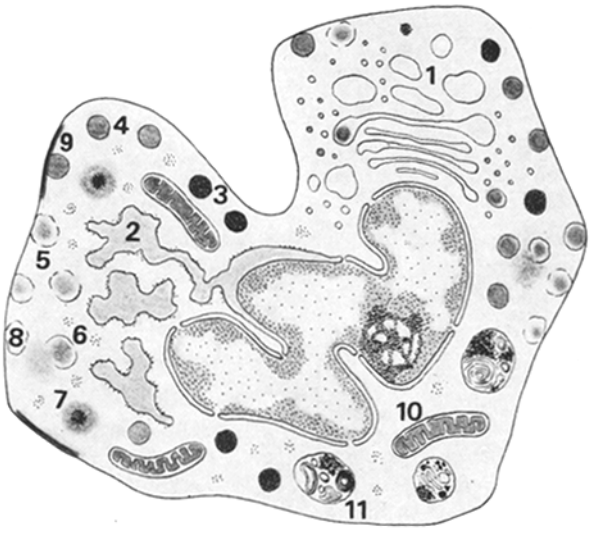


Fig. 12. Diagram to illustrate the changes in hepatectomy STH cells. *1* Hypertrophic Golgi complex. *2* Dilated endoplasmic reticulum, including perinuclear cisternae, and containing electron dense material. *3* Dense granules, still unchauged. *4* Granules with reduced electron density. *5* Granules with uneven diminution of electron density, some of them attached to the plasma membrane. *6* Bursting granule with content spilling into the cytoplasm. *7* Granule content free in the cytoplasm. *8* Empty granule membrane against the plasmalemma. *9* Dense zone in the plasmalemma in relation with a granule whose density is diminishing. *10* Dense mitochondria. *11* Large lysosomes of the autophagic vacuole type. The cellular and nuclear outlines are irregular

cytoplasm (Figs. 6, 7). In other fields, concavities of the cellular surface were occupied by small microvesicles (Fig. 7).

7. Increased density of mitochondria in extremely altered cells (Figs. 6, 7). They were frequently grouped within and around the Golgi zone (Fig. 4).

8. Increased number of lysosomes (Fig. 9) of the autophagic vacuole type (Smith and Farquhar, 1966). Cells without specific granules and a large number of large autophagic vacuoles (Fig. 11) or several lipid droplets (Fig. 10) were a common finding, but they were also sometimes seen in control animals.

9. Very irregular nuclear (Figs. 4, 8) and cellular (Fig. 5) outlines.

The changes described were widespread, or appeared in several (Fig. 4) or isolated STH cells (Fig. 6).

Discussion

Some pictures described for TSH (Farquhar and Rinehart, 1954b), ACTH (Kurosumi and Kobayashi, 1966; Herlant, 1963), FSH and LH (Farquhar and Rinehart 1954a; Herlant, 1963b; Yoshimura and Harumiya, 1965; De Virgilis, 1968) cells, could be a source of error in the identification of hepatectomy STH cells. The morphological differences between these pictures and the ones described here, and the existence of many transitions between unmodified and altered STH cells, allowed us to recognize them. We did not find transitions in the other cell types, including LTH cells (Sano, 1962; Pasteels, 1963; Herlant, 1963a; Smith

and Farquhar, 1968). Furthermore, the relative liver insufficiency in hepatectomized animals, probably parallels an increased blood concentration of sex steroids normally inactivated by the liver (Cameron, 1964). This is not compatible with castration changes in gonadotrops, that could be confused with the pictures here described.

Stress FSH cells (Herlant and Klasterski, 1963a) present some granule changes similar to those described here, but their Golgi complex is inactive, a pattern just the opposite of that after hepatectomy in STH cells. Since growth hormone is released in stress situations (Schalch and Reichlin, 1968), the possibility is not discarded that some of the stress FSH cells could be in reality altered STH cells.

The scarcity of signs of exocytosis (Farquhar, 1961) in hepatectomy STH cells, might be due to the fact that this process appears early (De Virgilis, Meldolesi and Clementi, 1968; Couch, Arimura, Schally, Saito and Sawano, 1969) and is probably very fast. Alternatively many sites of release, similar to those described by Herlant (1963b) in adenohypophysial cells and by Scharrer (1968) in neurosecretory neurons have been found.

The pattern of increased synthesis and release, is suggested by numerous dense mitochondria, spatially related with hypertrophic Golgi elements (Fig. 4), by a dilated endoplasmic reticulum, containing electron dense material (Figs. 4–6), by irregular cellular and nuclear outlines (Figs. 4, 5, 8) and by an increased number of large autophagic vacuoles (Fig. 9) indicating the elimination of membranes in excess.

Furthermore, mouse plasma obtained at midnight of the second day after hepatectomy, is known to increase tenfold the DNA synthesis index of adult intact mouse liver. This effect is also observed with pure growth hormone, but not with plasma of intact mice (Echave Llanos, Gómez Dumm and Surur, unpublished results, 1970). Also, the incorporation of tritiated thymidine into rat hypophysis, is significantly lowered after hepatectomy, in probable relation with the increase of its participation in the regeneration process (Stöcker, Heine and Löffler, 1969).

The fact that cells with mild ultrastructural changes of the type described here are sometimes observed in control sham operated mice, is coincident with the fact that handling of the liver causes a rise in tritiated thymidine incorporation into peripheral hepatocytes (Weinbren, Arden and Stirling, 1969).

These data strongly suggest that hepatectomy causes release of STH from mouse pars distalis. This release seems to increase as regeneration advances, attaining its maximum at about midnight of the second day. Some 2–4 h later the first peak in liver DNA synthesis appears (Echave Llanos, Surur and Balduzzi, unpublished results, 1970) and it is not unreasonable to postulate some causal relationship between the release of STH and the increase of DNA synthesis in regenerating liver. The approach reported here adds new information which may help to settle the problem of the participation of the pars distalis in liver regeneration (Harkness, 1957; Weinbren, 1959; Bucher, 1963).

With respect to the mechanism of release of STH from pars distalis cells of hepatectomized mice, the hypoglycemia appearing after hepatectomy (Epele and Echave Llanos, unpublished results, 1970) which attains its maximum at 4 p.m. of the second day, could play some important role in the liberation of STH releasing factor (Schalch and Reichlin, 1968). The hypoalbuminemia, which takes place

after hepatectomy, could also be a link in the chain of events (Pimstone, Wittman, Hansen and Murray, 1966; Glinos, 1958). Lack of food intake during the first night after hepatectomy (Nash and Echave Llanos, 1969) might play some additional role, since fasting has been reported to produce increase in STH plasma levels (Pimstone, Wittman, Hansen and Murray, 1966) and also of the granule release from STH cells of the pars distalis (Girod et Dubois, 1969).

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