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Ultrastructure of STH Cells in the Pars Distalis of the Hypophysis of Mice Bearing Transplanted Hepatomas*

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Summary. An electron microscopic analysis was performed on the pars distalis of the hypophysis of mice bearing transplanted hepatomas. Normal mice served as controls. In animals bearing a fast growing as well as in those bearing a slow growing hepatoma, the STH cells presented changes indicating increased release and synthesis of its secretory product. These changes can be summarized as follows: 1) Increased number of granule-depleted STH cells which were very irregular in size and shape. 2) Very irregular and sometimes bizarre nuclei. 3) Plasma membranes irregular and convoluted. 4) Increased quantity of endoplasmic reticulum which appeared sometimes dilated and contained material of varying electron density. 5) Abundant, irregular and frequently ramified mitochondria. 6) Hypertrophic Golgi system with increased number of microvesicles. 7) Increased number of large lysosomes of different type. 8) Increased number of fat droplets.

Key-Words: Hypophysis — Mouse — Pars distalis — STH cells — Hepatomas.

The histophysiological approach yields important information on the functional significance of pars distalis cell types (Herlant, 1962). The morphological variations of STH cells have received increased attention during the last decade, and their changes have been described during fasting (Girod and Dubois, 1969), after the injection of hypothalamic extracts (de Virgilis, Meldolesi and Clementi, 1968; Coates, Ashby, Krulich, Dhariwal and McCann, 1970) or purified growth hormone releasing factor (Couch, Arimura, Schally, Saito, and Sawano, 1969) and, in our laboratory, during liver regeneration after hepatectomy (Echave Llanos, Gomez Dumm, and Nessi, 1971).

The present report is concerned with observations on STH cells in the pars distalis of mice bearing a mass of transplanted neoplastic tissue with a high growth rate.

Material and Methods

C3H-S Wilson, male mice were used. They were kept under standard conditions for periodicity analysis (Halberg, Barnum, Silber, and Bittner, 1958; Nash and Echave Llanos, 1969; Vilchez and Echave Llanos, 1971) at a temperature of $22 \pm 1°$ C, water and food ad libitum, illuminated (fluorescent light, 40 w) from 6 a.m. to 6 p.m., alternating with 12 hours darkness.

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Two hepatomas, one of them fast growing (SS1K, Wilson) and the other one slow growing (SS1H, Wilson) were used. These hepatomas have been studied in our laboratory from the point of view of their biological behaviour (Nash and Echave Llanos, 1971) and the circadian variation of their growth variables (Echave Llanos and Nash, 1970; Nash and Echave Llanos, 1971). Each of them was transplanted in the interscapular region of 6 healthy male mice, which were killed at 4 a.m., by decapitation and exsanguination, when the tumours attained a mean diameter of 1–3 cm. Four normal mice, killed at the same time, served as controls.

After killing the animals the skull was opened, the brain removed and the pituitary exposed by removal of the meningeal covers, within 2 minutes. Two or three drops of cold Millonig (1962) fixative were poured onto the sella turcica and, after a few seconds, the pituitary was removed and placed in a drop of cold fixative. Later on, the lateral wings were separated, fractionated into small pieces and transferred to fresh cold fixative, where they remained during 3 hours at 4° C. A few samples were fixed in glutaraldehyde (Sabatini, Bensch, and Barrnett, 1963) and postfixed in cold Millonig's fixative.

After fixation the samples were dehydrated in increasing concentrations of ethanol and embedded in Araldite (Luft, 1961). Sections were obtained on glass knives in an Ultratome III LKB ultramicrotome, mounted on copper grids and double stained with permanganate (Lawn, 1960) and lead citrate (Reynolds, 1963). The specimens were studied in an Elmiskop 1 electron microscope at 60 kV.

Observations

The changes described in the following paragraphs appear in animals bearing the fast growing as well as the slow growing hepatomas. They were not observed in control mice killed at the same time. No differences were observed between both experimental groups; all animals bearing tumour transplants presented extensive changes in STH cells. STH cells, easily recognizable, with granules ranging from 350 to 400 mμ, were abundant in the selected zones of the lateral wings.

The great majority of these STH cells were not rounded or ovoid, as classically described, but very irregular in shape and size. Bizarre nuclei, like the one illustrated in Fig. 1, were a common finding. The plasma membrane appeared very convoluted (Fig. 2). The endoplasmic reticulum was abundant, arranged as usually described for this cell type in parallel rows or sacculae (Fig. 4) or, quite frequently, as large, dilated vesicles, containing material of variable electron density, and with abundant ribosomes attached to their external surface (Fig. 3). Mitochondria were abundant, large, and very irregular in shape. It was not infrequent to find branching mitochondria with two or three arms (Fig. 3). The Golgi complex was hypertrophic and contained a great amount of vesicles and smooth surfaced and coated microvesicles (Fig. 5).

Images of granule-depleted STH cells (Fig. 4, 8) were commonly observed. The most striking difference between the STH cells of hepatoma bearing mice and those of normal mice, was the large amount of lysosome-like bodies in the former (Figs. 5–7). They varied in size and shape being of the autophagic vacuole type (Fig. 5) dense-body type (Fig. 7), multivesicular type and multilamellar type (Fig. 9). There was an inverse proportion between lysosomes and granules. In many STH cells an increased number of fat droplets was seen (Fig. 7). They were mostly close to the lysosomes, although they were also found interspersed among secretory granules, without lysosomes nearby.

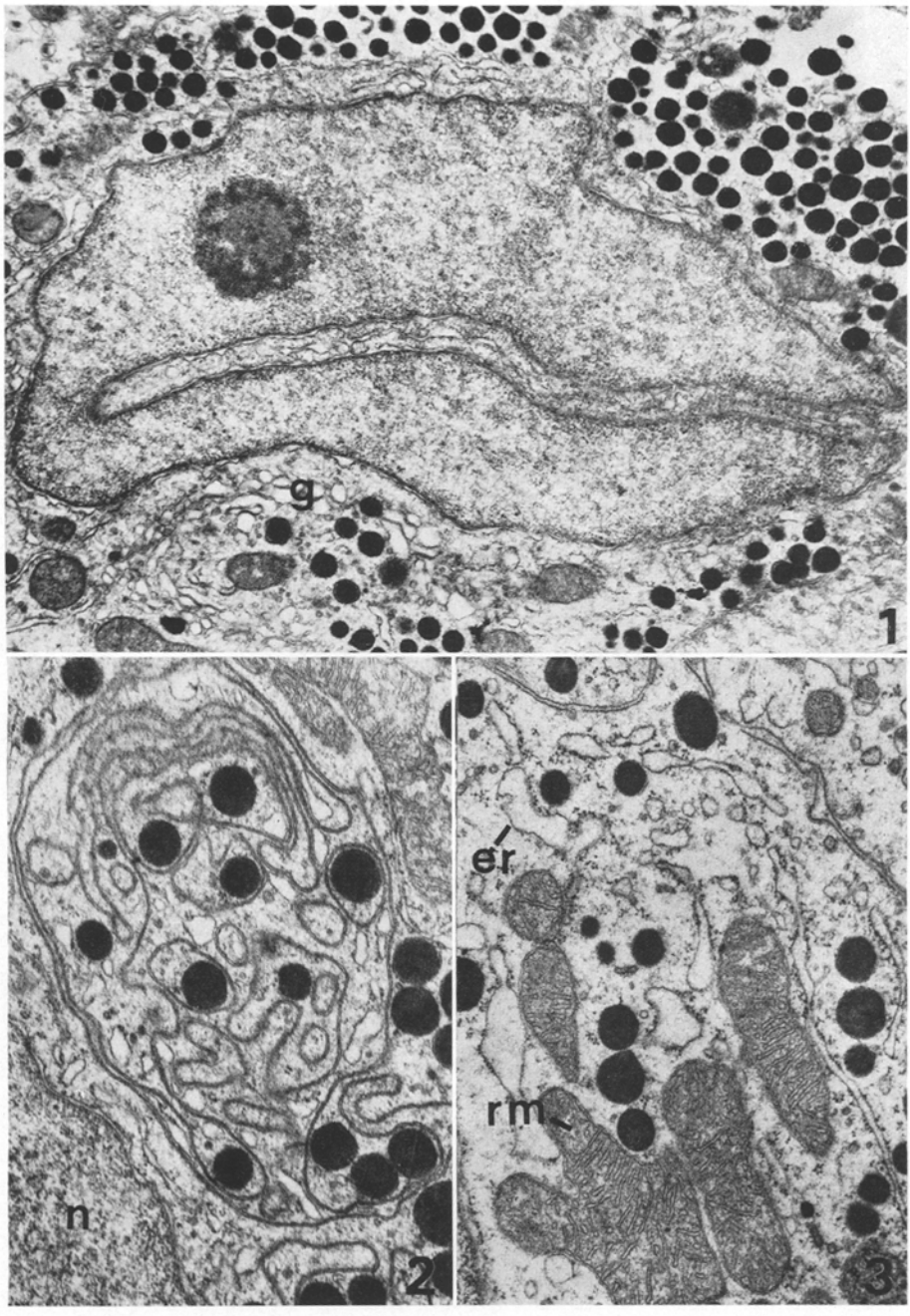

Fig. 1. STH cell from a mouse bearing SS1H hepatoma. Bizarre nucleus with irregular outline showing a deep infolding of the nuclear envelope. *g* Golgi complex. × 22000

Fig. 2. Deep and complicated infoldings surrounding granules, in the plasma membrane of an STH cell of a mouse bearing SS1H hepatoma. × 22000

Fig. 3. Portion of the cytoplasm of an STH cell showing dilated endoplasmic reticulum (*er*) with intracisternal electron lucent material. Several mitochondria one of them hypertrophic and ramified (*rm*) can be seen. Mouse with SS1H hepatoma. × 23000

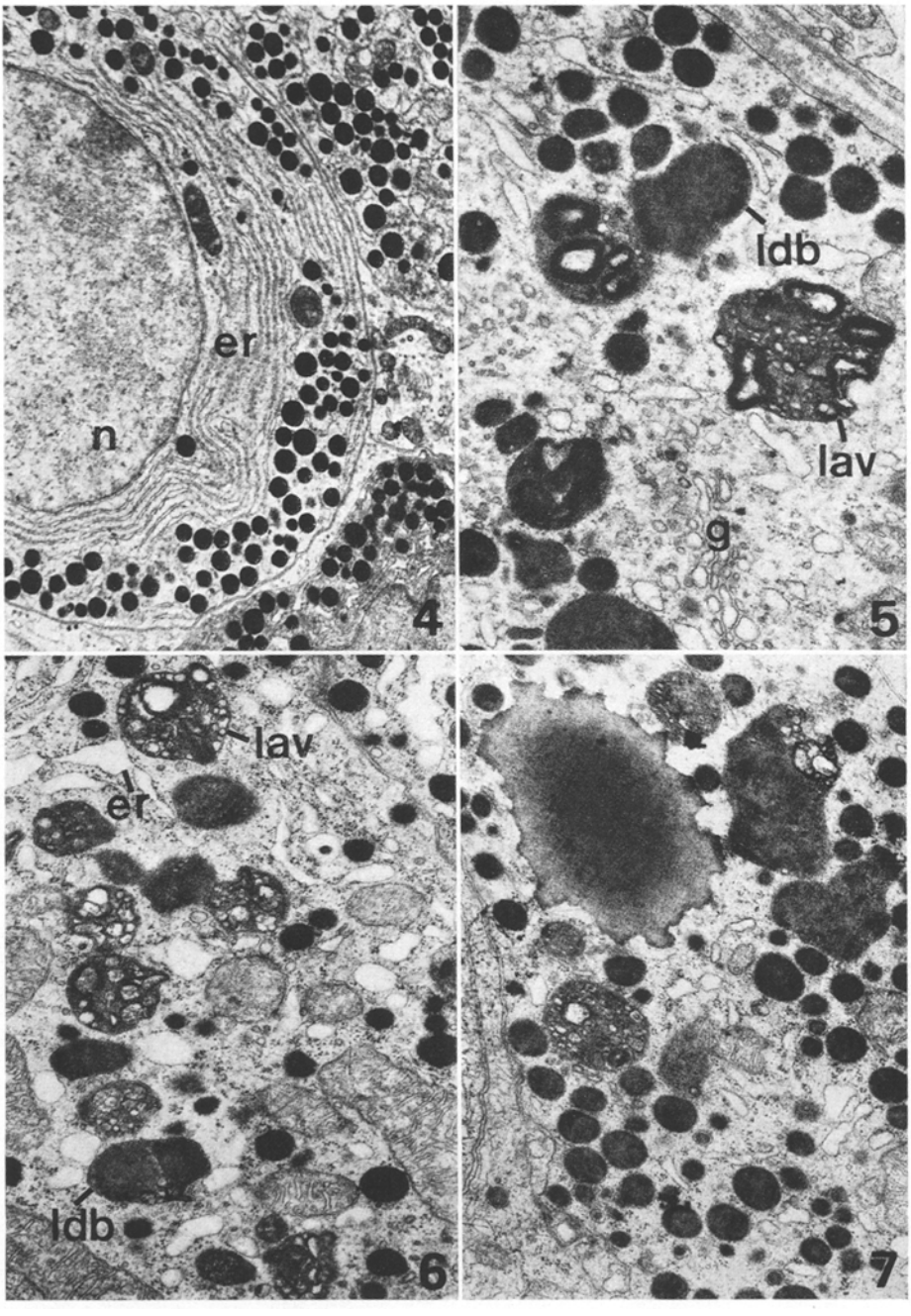

Fig. 4. STH cell with peripheral location of reduced number of secretory granules and well developed endoplasmic reticulum (*er*) close to nucleus (*n*). At top right a probable zone of granule release in tangential section of cell. Mouse bearing SS1K hepatoma. × 8000

Fig. 5. STH cell with large pleomorphic lysosomes. *lav* lysosome of the autophagic vacuole type. *ldb* lysosome of the dense body type. *g* hypertrophic Golgi complex with smooth surfaced and coated microvesicles. Mouse bearing SS1K hepatoma. × 23000

Fig. 6. Abundant lysosomes of the autophagic vacuole type (*lav*) and dense body type (*ldb*), alternating with dilated endoplasmic reticulum (*er*), mitochondria, and granules in an STH cell of mouse bearing SS1H hepatoma. × 20000

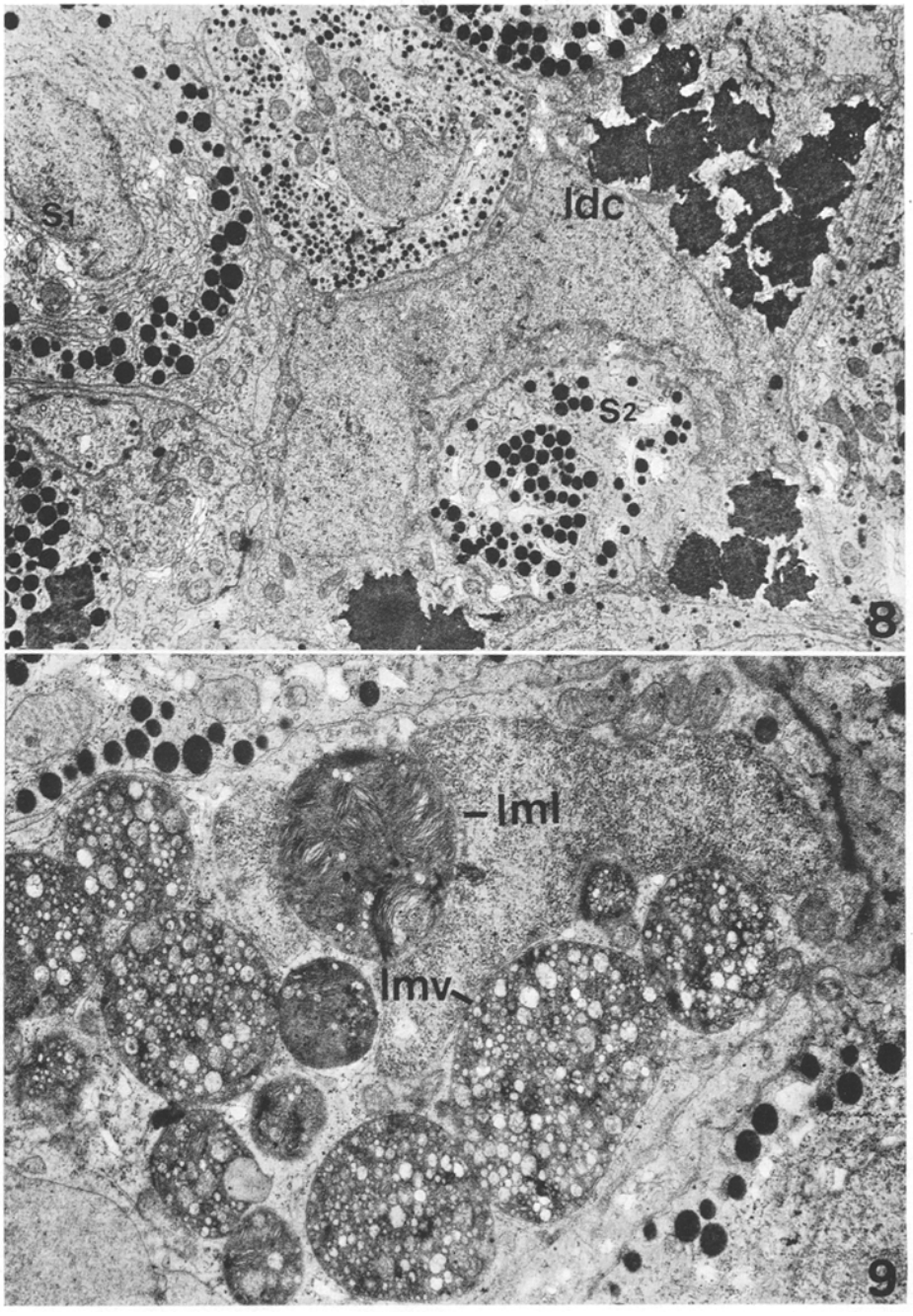

Fig. 8. A large and irregularly shaped cell (*ldc*) with many lipid droplets in the cytoplasm. *S1 and S2* granule depleted somatotrophs. Mouse bearing SS1H hepatoma. × 9000

Fig. 9. Cell with scarce cytoplasm and numerous large lysosomes of the multivesicular (*lmv*) and multilamellar (*lml*) types. Mouse bearing SS1H hepatoma. × 15000

Fig. 7. Portion of an STH cell with lysosomes next to a large lipid droplet. Mouse bearing SS1H hepatoma. × 22000

Unidentifiable cells with scarce cytoplasm, completely filled with fat droplets (Fig. 8) or large lysosomes (Fig. 9) and having very few organelles, were a common feature in the present material.

Discussion

The morphological changes in STH cells reported here, strongly suggest that these cells are under an increased stimulation that leads to an enhanced hormone release and synthesis. Increased synthesis and granule elaboration is indicated by the dilated endoplasmic reticulum (Fig. 3), containing intracisternal material and by the hypertrophy of the Golgi complex (Fig. 5). These two features have been described in STH cells of mice with regenerating liver (Echave Llanos, Gomez Dumm and Nessi, 1971). The hypertrophy of the Golgi complex is in contrast with the poor development in STH cells of normal mice killed at midnight (Gomez Dumm and Echave Llanos, 1970).

The abundance of granule-depleted cells (Fig. 4, 8) and the irregular outlines of the plasma (Fig. 2) and nuclear (Fig. 1) membranes, as well as the abundance of irregular mitochondria (Fig. 3) are also indicating an increased cellular function, as was the case for STH cells of hepatectomized animals (Echave Llanos, Gomez Dumm, and Nessi, 1971).

The increase in the number of lysosomes has also been related to increased cell function in LTH cells (Smith and Farquhar, 1966), as well as STH cells (Coates, Ashby, Krulich, Dhariwal, and McCann, 1970; Echave Llanos, Gomez Dumm, and Nessi, 1971). These lysosomes are probably engaged in the management of the excess membranes remaining after hormone release. Another possible explanation is based in their regulatory role, suggested by Smith and Farquhar (1966), in the elimination of an excess of granule material resulting from an increased synthetic stimulation.

We have not found many images of granule extrusion or exocytosis (Farquhar, 1961). Some pictures like that of the top right of Fig. 4, could be interpreted as a tangential section of a part of a cell surface, with much simultaneous release of individual and multiple secretory granules. As exocytosis appears to be a very fast process and other mechanisms of release have been suggested (Echave Llanos, Gomez Dumm and Nessi, 1971; Echave Llanos and Gomez Dumm, 1971), the scarcity of these images in the present material do not appear to represent a contradiction.

The presence of lipid droplets (Fig. 7) could be explained, as Smith and Farquhar do (1966) by suggesting that they are segregated from lytic bodies as lipid residues which, later on, aggregate and form larger droplets. The abundance of cells rich in lipid droplets (Fig. 8) or lysosomes (Fig. 9), together with the changes described in STH cells, suggests that they are the final step of these changing cells. Nevertheless this possibility remains to be demonstrated.

References

Coates, P. W., Ashby, E. A., Krulich, L., Dhariwal, A. P. S., McCann, S. M.: Morphologic alterations in somatotrophs of the rat adenohypophysis following administration of hypothalamic extracts. Amer. J. Anat. **128**, 389–412 (1970).

Couch, E. F., Arimura, A., Schally, A. V., Saito, M., Swano, S.: Electron microscopic studies of somatotrops of rat pituitary after injection of purified growth hormone releasing factor (GRF). Endocrinology **85**, 1084–1091 (1969).

Virgilis, G. de, Meldolest, J., Clementi, F.: Ultrastructure of growth hormone producing cells of rat pituitary after injection of hypothalamic extract. Endocrinology **83**, 1278–1284 (1968).

Echave Llanos, J. M., Gomez Dumm, C. L.: Release of growth hormone from STH cells of hepatectomized mice without the participation of the Golgi complex. Experientia (Basel) **27**, 318–319 (1971).

— — Nessi, A. C.: Ultrastructure of STH cells of the pars distalis of hepatectomized mice. Z. Zellforsch. **113**, 20–38 (1971).

— Nash, R. E.: Mitotic circadian rhythm in a fast growing and a slow growing hepatoma: Mitotic rhythm in hepatomas. J. nat. Cancer Ins. **44**, 581–585 (1970).

Farquhar, M. G.: Origin and fate of secretory granules in cells of the anterior pituitary gland. Trans. N. Y. Acad. Sci. **23**, 346–351 (1961).

Girod, Ch., Dubois, P.: Recherche en microscopie optique et en microscopie électronique sur les cellules somatotropes antéhypophysaires du Hamster doré (*Mesocricetus auratus* Waterh). C. R. Acad. Sci. (Paris) **268**, 2361–2363 (1969).

Gomez Dumm, C. L., Echave Llanos, J. M.: Variations in the Golgi complex of mouse somatotrops at different times during a 24 hour period. Experientia (Basel) **26**, 177–178 (1970).

Halberg, F., Barnum, C. P., Silber, R. H., Bittner, J. J.: 24 hour rhythms at several levels of integration in mice with different lighting regimens. Proc. Soc. exp. Biol. (N.Y.) **91**, 897–900 (1958).

Herlant, M.: Quelques notions récentes sur l'histophysiologie de l'antéhypophyse. Biol. Medicale **51**, 205–222 (1962).

Lawn, A. M.: Use of potassium permanganate as an electron dense stain for sections of tissue embedded in epoxy resin. J. biophys. biochem. Cytol. **7**, 197–199 (1960).

Luft, J.: Improvements in epoxy resin embedding methods. J. biophys. biochem. Cytol. **9**, 409–414 (1961).

Millonig, G.: Further observations on a phosphate buffer for osmium solutions in fixation. In: S. S. Breeze (ed.), Fifth International Congr. for Electron Microscopy 2, 8. New York and London: Academic Press 1962.

Nash, R. E., Echave, Llanos, J. M.: Ritmo circadiano en el contenido gástrico de ratones intactos y hepatectomizados. Rev. Soc. argent. Biol. **45**, 181–186 (1969).

— — Circadian variation in DNA synthesis in a slow growing hepatoma (SS1H Wilson). DNA synthesis rhythm in hepatoma. J. nat. Cancer Inst. (unpublished) (1971).

— Moreno, F. R., Echave Llanos, J. M.: Caracteristicas morfologicas y biologicas de dos hepatomas injertados, de crecimiento lento (SS1H Wilson) y rápido (SS1K Wilson). Rev. Soc. argent. Biol. (in press) (1971).

Reynolds, E. S.: Use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. **17**, 208–212 (1963).

Sabatini, D. D., Bensch, K., Barrnett, R. J.: Cytochemistry and electron microscopy: The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J. Cell Biol. **17**, 19–58 (1963).

Smith, R. E., Farquhar, M. G.: Lysosome function in the regulation of the secretory process in cells of the anterior pituitary gland. J. Cell Biol. **31**, 319–347 (1966).

Vilchez, C. A., Echave Llanos, J. M.: Circardian rhythm in the feeding activity of *Oxymyxterus rutilans*. Role played by light and food availability. J. interdiscipl. Cycle Res. (in press) (1971).

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