EVIDENCE FOR GRANULOLYSIS IN THE RETINULA CELLS OF A STOMATOPOD CRUSTACEAN, SQUILLA MANTIS

ALAIN PERRELET, LELIO ORCI, and FRITZ BAUMANN. From the Institutes of Histology and Physiology, University of Geneva School of Medicine, Geneva, Switzerland

INTRODUCTION

In different endocrine cells of the rat, Smith and Farquhar (1) and Orci et al. (2) described the incorporation of secretory granules within lysosomal bodies. This process, called granulolysis (2) or crinophagy,1 occurs through fusion of the granule's membrane with that of the lysosome and has been interpreted as a cellular mechanism for disposing of excess secretory product under certain experimental (1) or metabolic conditions (2, 3). Granulolysis, at a low rate, is also present under normal conditions, as a means for the catabolism of superfluous secretory granules (1).

FIGURE 1 Thick section of a retina of *Squilla mantis*, stained with toluidine blue and seen in the light microscope. Each ommatidium is formed of seven retinula cells (RC) surrounding the central rhabdome (rh). The cytoplasm of the retinula cells appears filled with small dark granules (accessory pigment). Some cells show a lobulated nucleus (n). Between the ommatidia, sleeves of elongated pigment cells (PC) containing large black granules can be seen. × 600.

FIGURE 2 Part of a retinula cell cytoplasm showing a large number of accessory pigment granules (PG), as well as numerous bodies (Ly) containing granule cores. One also sees vacuoles with a pale and flocculent content (arrows). Glycogen particles are scattered throughout the cytoplasm. × 22,700.
numerous accessory (nonvisual or screening) pigment granules present in the retinula cells were involved in a process closely resembling granulolysis or crinophagy in endocrine cells. Granulolysis in retinula cells could represent the normal pathway for catabolizing the accessory pigment granules. The formation of accessory pigment granules has been studied by Shoup (4) in the eye of Drosophila and it has been shown that most granules first appear within Golgi vesicles. The cellular site where destruction of pigment granules possibly occurs has not been investigated so far.

MATERIALS AND METHODS
Living Squilla mantis were kept at room temperature in aerated artificial seawater. The stalked eyes were removed and sliced, then fixed overnight in a cold formaldehyde-glutaraldehyde mixture in 0.1 M phosphate buffer, pH 7.6, containing NaCl and sucrose (5). After brief washing in phosphate buffer, the slices were postfixed for 2 hr in 2% phosphate-buffered osmium tetroxide, pH 7.6 (6), then dehydrated in alcohol and embedded in Epon (7). Ultrathin sections contrasted with lead citrate (8) were examined in a Philips EM300 electron microscope. Thick sections stained with toluidine blue in 1% Borax (9) were photographed on Agfa negative plates in a Zeiss Ultraphot microscope (Carl Zeiss, Oberkochen, Germany). The acid phosphatase was demonstrated with the method of Gomori (10). For this purpose fixation was carried out as described above for 3 hr but with Na-cacodylate 0.1 M, pH 7.4, as buffer. After an overnight washing in 0.1 M cacodylate buffer containing 7% sucrose, the eye slices were cut into tiny fragments with a razor blade and the fragments were incubated in Gomori's medium for 1 hr at room temperature. The blocks were then briefly rinsed in 2% acetic acid, washed several times in cacodylate buffer, pH 7.4, and postfixed for 45 min in 2% osmium tetroxide buffered with phosphate.

Controls consisted of blocks incubated in Gomori's medium without glycerophosphate. The blocks were dehydrated and embedded as described above. Thin
sections were examined either unstained or after contrasting with lead citrate.

RESULTS AND DISCUSSION

The retinula cells of *Squilla mantis* contain a large number of accessory pigment granules dispersed throughout the cytoplasm (Figs. 1 and 2). Most of the granules are of small diameter, averaging 0.2µ. Some granules, however, are of larger size, up to 0.5 µ. In cell areas not occupied by pigment granules (periphery of the retinula cell, opposite the rhabdome) numerous cisternae of granular endoplasmic reticulum and Golgi stacks can be seen. The cytoplasm of the retinula cells also contains abundant glycogen particles (Fig. 2). Besides the single, scattered pigment granules described above, several granules were found forming clusters enclosed within a membrane. These bodies are dispersed within the retinula cell cytoplasm without apparent preferential localization. They contain from two to several dozen pigment granules, embedded in a matrix of variable electron opacity (Figs. 2, 3, and 4). In areas of large accumulation of bodies containing pigment granules, several vacuoles of varying diameter are seen (Fig. 3); the vacuoles are lined by a single membrane and show a pale and flocculent content which is suggestive of that of primary lysosomes (11). Single membrane-bounded granules sometimes appear in contact with these vacuoles (inset Fig. 3); the fusion of their respective membranes could lead to the incorporation of the granule within the vacuole (as a granule core, the limiting membrane of the granule being lost during incorporation). The successive incorporation of several granules might produce the typical bodies containing five to seven granule cores. These bodies could later fuse with others and give rise to giant inclusions as seen in Fig. 4. Morphological evidence suggests that the pigment granules undergo progressive degradation within the inclusion (Figs. 4 and 5) which finally resembles a residual body (Figs. 6 and 7). The digestion of the granules could be aided by the incorporation into the vacuole of microvesicles (Fig. 5), as these were shown to be involved in the transport of lytic enzymes (12). In blocks incubated for the demonstration of acid phosphatase, activity was observed in the vacuoles with a pale content (Figs. 8 and 11), as well as in the bodies containing granule cores (Figs. 9 and 10). This localization of acid phosphatase supports the morphological evidence presented above and which suggested that both the vacuoles and the bodies containing pigment granules are of lysosomal nature. The process of incorporation of pigment granules by fusion with lysosomes appears quite different from that reported by Fahrenbach in *Limulus* ommatidium (5) and consisting of the sequestration of pigment granule together with other cytoplasmic components within autophagic vacuoles. At present we ignore the mechanisms capable of initiating or modifying granulolysis in *Squilla* retinula cells.

We thank Mrs. M. Perrelet-Bridges for the revision of the English manuscript.

This work was supported by a grant from the Fonds National Suisse de la Recherche Scientifique.

Received for publication 8 July 1970, and in revised form 26 October 1970.

REFERENCES