Heterogeneous Ultrastructure of the Granules of *Drosophila* Salivary Glands

Relatively few papers concerning the ultrastructural studies of cytoplasmic mucoproteic granules of salivary gland cells in larva of *Drosophila* have been published. They report differing appearances. GAY[1] demonstrated the secretion of the granules in areas delimited by concentric plates of endoplasmic reticulum. SWIFT[2] observed that some granules are contained in part by a flat sheet of a Golgi type. Neither GAY nor SWIFT discussed the structure of the granule. It was found in *Drosophila hydei*[3,4] that in the distal region of the gland some granules show 'peripheral hollows' that, when seen with a membrane, form a vacuole, while others show granular structure, attributed by the author to the accumulation of small granules. RITZKI[5] in *Drosophila melanogaster* found that the granules are delimited by a membrane and have a fibrillary structure. This paper reports the finding that these granules have a heterogeneous ultrastructure not previously described.

Methods. Salivary glands in the second half of third instar larva of wild type *Drosophila melanogaster* were extracted in Millonig buffer and fixed in 2.5% glutaraldehyde in Millonig buffer for 24 h. After washing in distilled water for 30 min they were stained with ammoniacal silver carbonate for 30 min, washed again and put in 1% formaldehyde for 30 min[6]. They were dehydrated and embedded in Epon. The Siemens Elmiskop IA electron microscope was used.

Results. The mucoprotein granules are heterogeneous (Figures 1 and 3). This morphological heterogeneity correlates with the different disposition of the metal of the stain. Two types of areas are seen, arbitrarely called area I and area II. In area I (Figure 1) the silver is seen on a filamentous structure, forming small and medium sized grains which are usually massed together forming dense accumulations (large size grains). In area II (Figures 1 and 4) silver grains are clearly seen when magnifications of $\times 15{,}000$ or greater are used; the metal is then seen as fine grains which appear to be joined to a structure apparently arranged in linear fashion. Furthermore, the electronic density of the substrate of area II is different from that of the substrate of area I (Figure 4). The ratio of the 2 areas and their disposition allow us to speak of 2 kinds of secretory granules. In the first kind, R granule (Figure 3), area I contains islets of area II. These small islets are seldom marginal, they are frequently separated from the area I by a space of less electronic density.

In the second kind of secretory granules, S granule (Figure 1), the area II regions are larger and fewer in number than in the previous kind of granules. They are usually marginal (Figures 1 and 3). The number of these areas per granule is small and one frequently finds granules with only 1 or 2 of these areas. In some granules the area II was seen to cross from one border to the other (Figure 1).

One frequently observes granules in different stages of disintegration near the lumen (Figure 2). The lumen seems to be occupied by the same type of material that forms the granules (Figure 2).

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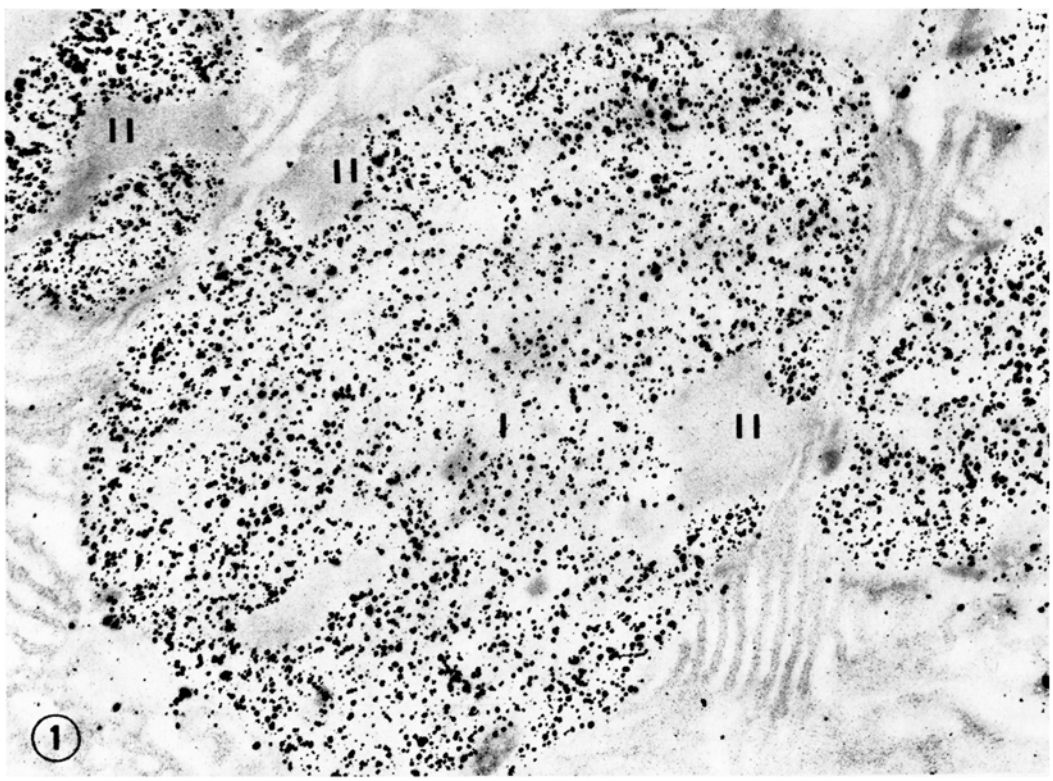

Fig. 1. In the center S granule, there are 2 marginal areas II (II). In the upper left portion of the photo, another S granule shows the area II crossing from one border to the other. $\times 19{,}000$.

Discussion. While the appearance of the granules shown by BERENDES[3,4] differs from ours, the 'peripheral hollows' or 'vacuoles' could represent our area II. In the picture of RITZKI[5] there is a more electron dense area that the author does not discuss, that also resembles the area II. We attribute the clarity of our images to the fixer used and to the use of silver in alkaline solution, which, without other stains used for contrast, allows one to observe subcellular regions with less artefacts. The different disposition of the silver – small, medium and large size grains – is interpreted as a consequence of regional spirilization of the substrate. The R and S granules could be different in origin or could represent different sections of the same type of granules.

The chemical nature of the granules (high content of lysine and arginine and no tryptophane[7]) points to a composition similar to the basic proteins. HORN and WARD[8] suggest that there are histones in the cytoplasm of these cells because of the strongly positive alkaline fast-green and Sakaguchi reactions. BLACK et al.[9,10] have proved that the formaldehyde-ammoniacal silver-formaldehyde technique stains histones at optical level. From the previous work just discussed and our present findings, it is not illogical to infer that the silver seen in the granules is, in part if not exclusively, attached to the basic proteins[11–13].

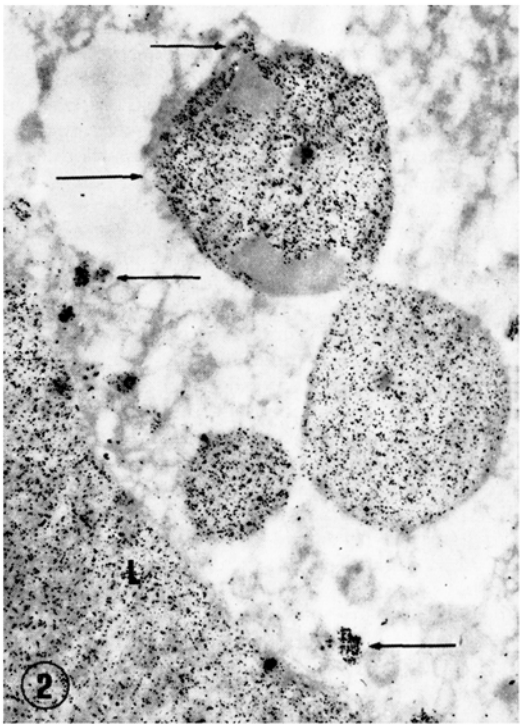

Fig. 2. Arrows point to disintegrating granules near the lumen (L). ×13,000.

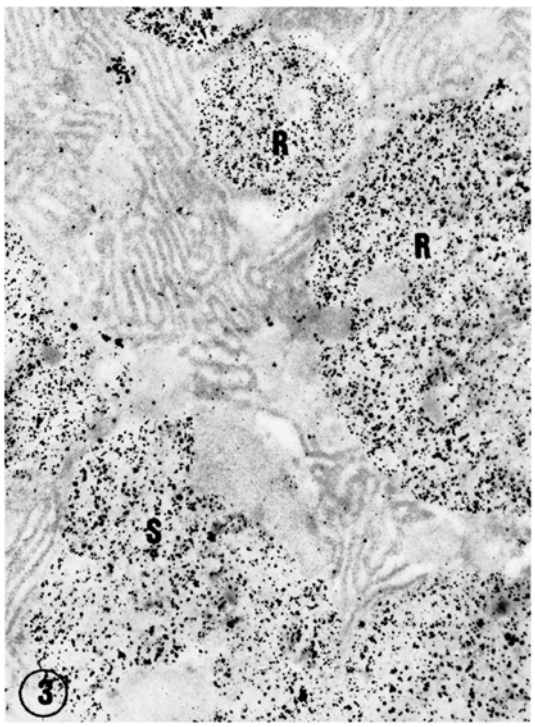

Fig. 3. 2 R granules (R) and 1 S granule (S). ×13,000.

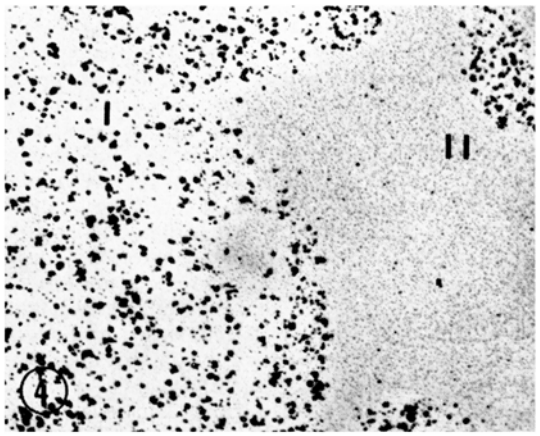

Fig. 4. Area I (I) and area II (II) of an S granule. Note the different electron density between the substrate of both areas. Area II shows the fine silver grains in a definite linear order with both straight and curved variants. Area I acts as a control for area II. ×40,000.

Resumen. Aplicando la tecnica de glutaraldehido-carbonato de plata amoniacal-formaldehido, se ha demostrado la heterogenea ultraestructura de los granulos citoplasmaticos en las celulas de la glandula salival de larvas de *Drosophila melanogaster*. Los granulos contienen dos areas que difieren en densidad electronica y en la disposicion del metal del colorante usado. Se sugiere la posibilidad que la plata se une a las proteinas basicas.

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[13] This work was partialy supported by grants of National Research Council of Argentina and University of Buenos Aires. The authors wish to thank Prof. Dr. J. H. HUNZIKER for their helpful criticism of the manuscript.