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The Prepupal Salivary Glands of *Drosophila melanogaster*

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Evidence is presented in support of the concept that the larval salivary gland of Drosophila melanogaster *continues to function as an important secretory organ throughout prepupal stages and after pupation. Just after puparium formation, and at other later periods, the glands appear to be in the process of disintegration, but each time they recover until after pupation. Nuclear blebbing occurs through the time of survival of the glands, but is shown not to involve transport of RNA out of the nucleus. Transport in and out of the nucleus is clearly rapid and in a steady state as compared to the massive and intermittent export of cytoplasmic substance into the lumen of the gland.*

KEY WORDS: salivary gland; nuclear transport; RNA transport; blebs; histolysis.

INTRODUCTION

Results of our recent studies on puff-related protein synthesis (Tissieres *et al.*, 1974) and on chromosomal proteins (Mitchell and Lipps, 1975) in prepupal salivary glands of *Drosophila melanogaster* show that the glands are highly active in protein synthesis throughout and beyond the prepupal period. This observation is in accord with the demonstration by Ashburner (1967) that regular puffing patterns continue in prepupal salivary gland chromosomes to at least the time of pupation at 12 hr after puparium formation.

These results were somewhat surprising in view of earlier suggestions (Ross, 1939; Bodenstein, 1950) that the larval salivary glands begin histolysis very soon after puparium formation. Other investigators were preoccupied

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with lysosomes and gland self-destruction, and some element of this viewpoint remains in the more recent report of Lane *et al.* (1972).

It is the purpose of this article to present further evidence that the larval salivary gland does indeed serve as a significant secretory organ throughout the prepupal period and after pupation until histolysis occurs suddenly a few hours after pupation rather than gradually over the whole prepupal period.

MATERIALS AND METHODS

Animals and Stage Selection

The Oregon R wild-type strain of *Drosophila melanogaster* was grown in mass culture (Mitchell and Mitchell, 1964), and most staging was done by selection of white but sclerotized prepupae from the several thousand animals in one collection box. By such means, one can collect within a few minutes the 30–40 white prepupae needed for one experiment. In some cases, 5–5.5 hr prepupae were obtained by the flotation method (Mitchell and Mitchell, 1964).

Preparation of Glands

Salivary glands were dissected in medium A (Tissieres *et al.*, 1974), and for each time point glands were removed from at least five animals in order to make sure the one selected was representative of the state of synchrony.

Sample Preparation

Whole gland mounts for photography were made in medium A using a coverslip supported at each side by a No. 0 coverslip. Samples for sectioning and light and electron microscope observations were fixed and embedded in Epon as described earlier (Mitchell *et al.*, 1967).

Labeled glands ([^{3}H]uridine) for electron microscope autoradiography were prepared as in our recent report (Tissieres *et al.*, 1974). For autoradiography at the electron microscope level, thin sections from an Epon block were placed on parlodion-coated grids which were attached to glass slides with double-coated Scotch Tape. Ilford 4 emulsion was diluted 1 : 3 with water, and a small drop was placed directly on the grid. Exposure time was 8 days at 4 C. Development was carried out as described by Revel and Hay (1961).

RESULTS

Gross Morphology

As illustrated in Fig. 1, the larval salivary gland of *D. melanogaster* undergoes a number of rather drastic changes in gross morphology during the

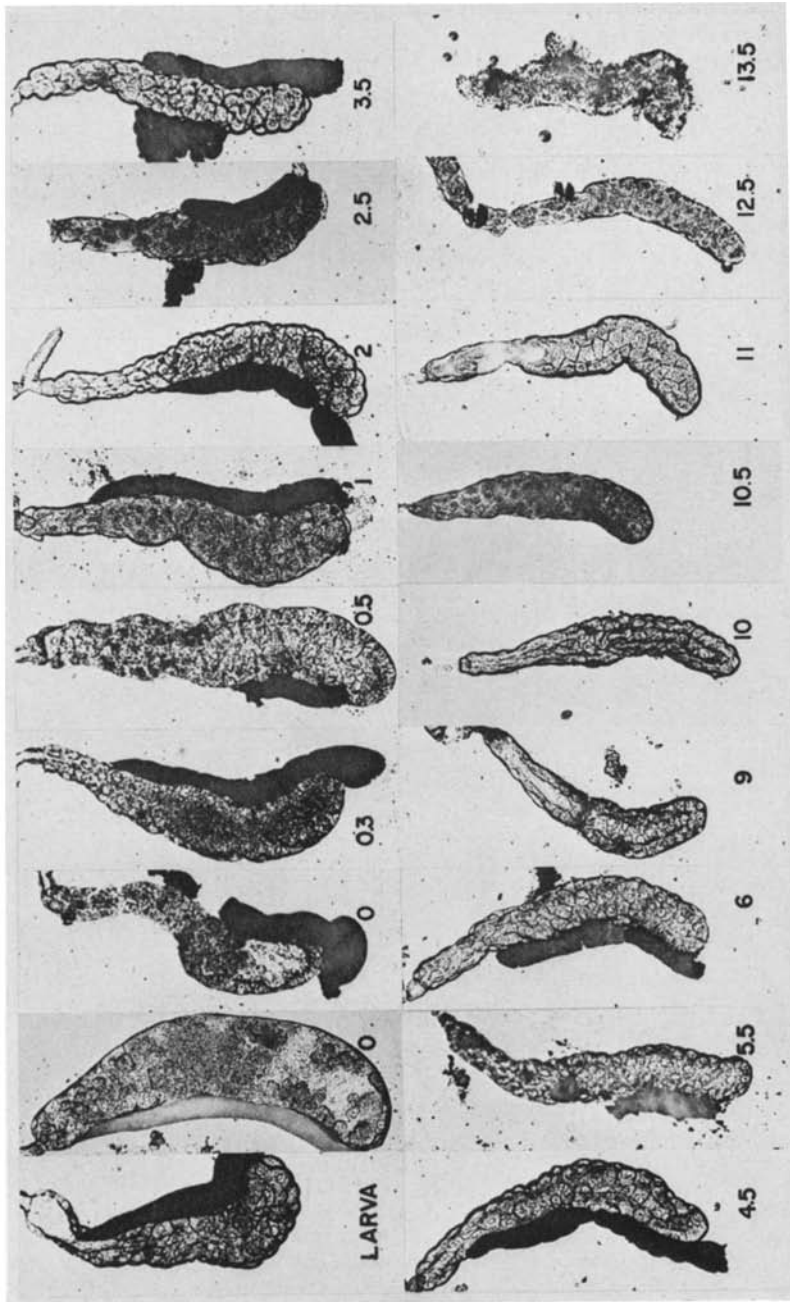

Fig. 1. Gross morphology of salivary glands. The numbers given are hours after puparium formation. The larval sample was about 2 hr before puparium formation. Under our culture conditions (25 C and 65% humidity), pupation was between 11 and 12 hr.

prepupal stages. The situation commonly encountered during preparation of chromosome smears is shown in the first three pictures (larva, 0, 0). The one labeled "larva" (see also Fig. 2, −2 hr) is typical of late larvae, with transparent, rounded cells which contain large numbers of secretion granules. The next two pictures (0 hr) came from the same animal just at puparium formation and show the extreme bloated condition with a full lumen and thin stretched cells followed by a gland in which both cells and lumen are virtually emptied. In the latter condition, the glands are opaque, fragile, and apparently histolyzed since normal cell structures are rare in thin sections as observed by both light and electron microscopy. However, the cells recover,

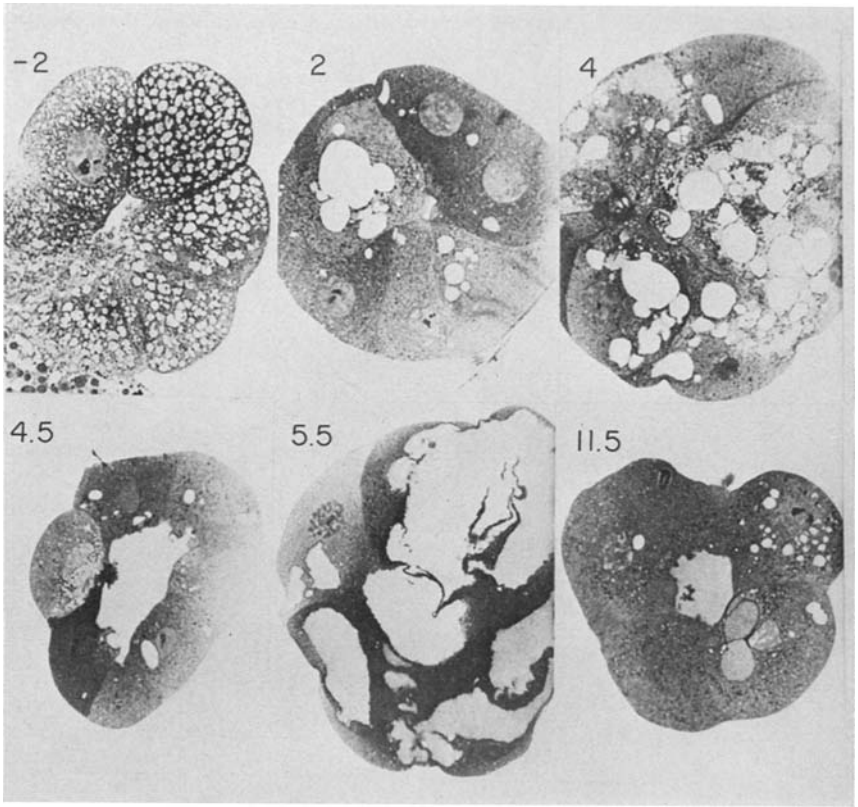

Fig. 2. Center cross sections of salivary glands. The numbers are hours before or after puparium formation as in Fig. 1. The apparent holes at −2 show well-oriented fibrous material at higher magnification (secretion granules), but no order is evident in materials contained in the larger vesicles at later stages. Note the relative sizes of the lumen where it shows at −2, 2, 4, and 11.5.

and, as shown, they are quite normal (similar to those in late larvae) at 2, 3.5, 4.5, 6, and 11 hr. Probably they are normal at about 0.4 hr also, and they are again bloated at 0.5 hr, but changes are too fast in this period to give a clear picture. In any case, it seems certain that in addition to the sudden expulsion of a very large amount of secretion at the time of puparium formation, similar secondary events occur in the intervals 0.5–1, 2–2.5, 4.5–5.5, 9–10.5, and 11–12.5 hr. Although less drastic than the initial event, these secondary events leave the gland cells in relatively poor condition, as illustrated in Fig. 2 at 4–5.5 hr. The other center cross-sections in Fig. 2 at 2, 4.5, and 11.5 hr show rather normal cells and a considerable accumulation in the lumen at 4.5 and 11.5 hr. We have done longitudinal as well as center cross-sections for full time series as in Fig. 1 with essentially the same results. Except for a few cells at the anterior tip, failure of cell synchrony within a gland was not more extreme than shown here.

It should be noted at this point that transport of secretions into the lumen may occur continuously, but repeated periods of relatively massive transport do occur. Furthermore, it should be emphasized that after puparium formation, secretions are retained within the pupal case and probably within the prepupal cuticle after 4 hr.

Nucleus and Cytoplasm

Since massive secretion and extrusion of materials are evident in salivary glands, massive interchange of materials between the nucleus and cytoplasm of the gland cells must also occur. Visual evidence of this was noted long ago by Gay (1956) and Kaufmann and Gay (1958) in terms of "blebs" at the nuclear membrane. These have also been described more recently by Lane *et al.* (1972). With a view toward gaining additional information on the nuclear transport question, we have prepared glands from several time series such as that in Fig. 1 in order to see how blebbing varies with development and if the dense bodies in blebs contain RNA. First, as shown in Fig. 3, there are, in addition to the blebs previously described (see picture 1), several variations in the phenomenon. Picture 2 shows blebs containing two dense bodies instead of one, but these, like the singles, appear to be formed from the inclusions within endoplasmic reticulum and at the site of at least one nuclear pore. In contrast, the bleb shown at 3 and the inclusion in the cytoplasm at the right in 4 clearly involve the nuclear membrane as well as the endoplasmic reticulum. This type of bleb is relatively rare, but it appears to be real and not an artifact from a convoluted nuclear surface. The double bleb, one inside and one outside of the nucleus, as in picture 4, is also relatively rare, but several examples have been observed.Several examples of inclusions within the nucleus like that in picture 5 have also been observed. Here blebbing within a

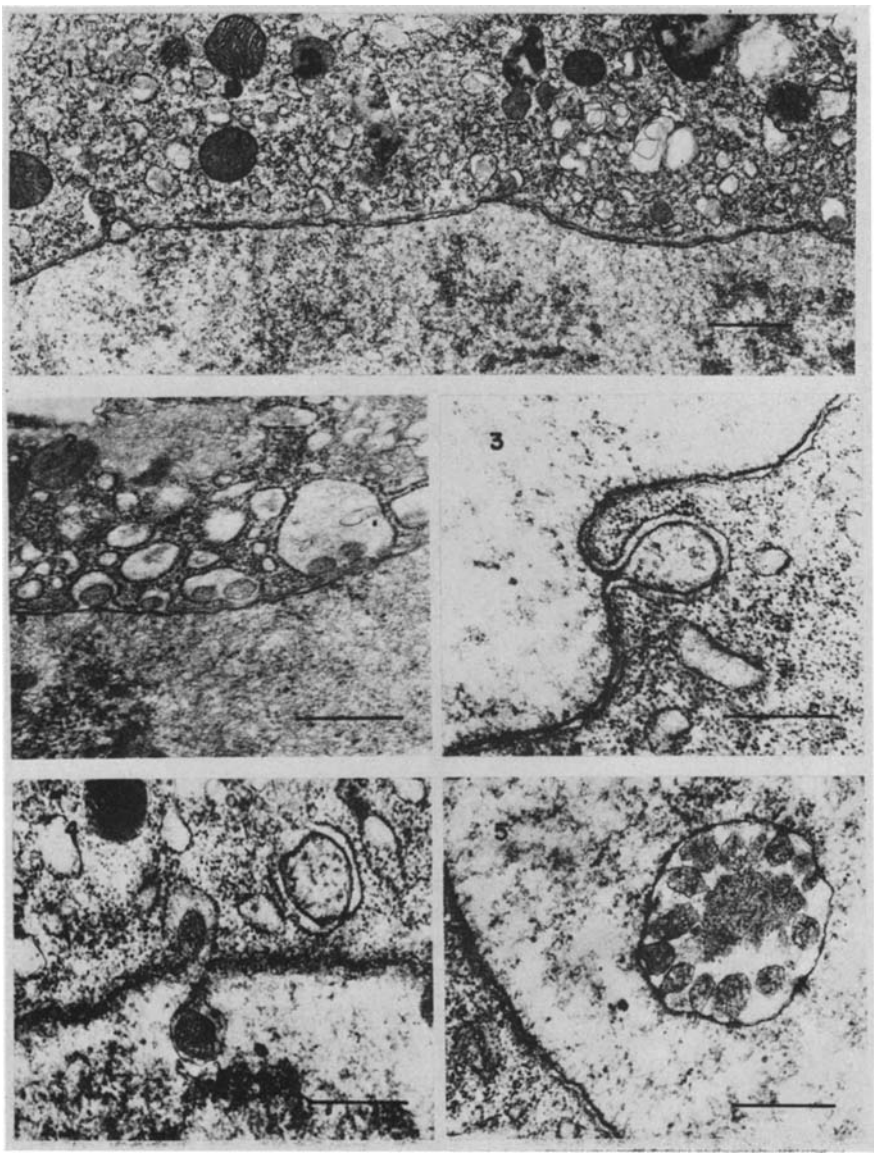

Fig. 3. Electron microscope pictures showing a variety of bleb types. 1: A portion of one nucleus (below) showing five blebs in the cytoplasm (upper part). This is about an average frequency. Marker, 0.5 μm. 2: Blebs with more than one dense body. 3: An invagination into the cytoplasm involving both membranes. 4: A double bleb going both into and out of the nucleus. 5: An inclusion in the nucleus with a kind of blebbing in itself.

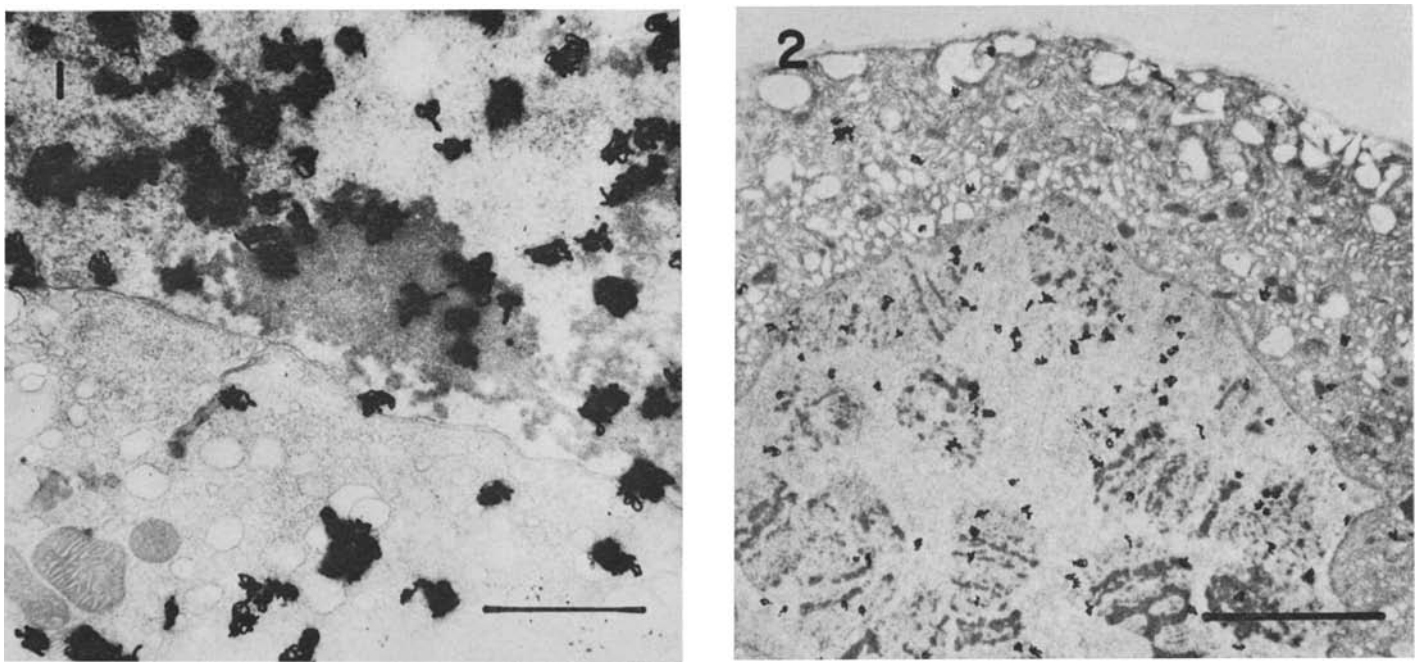

Fig. 4. Electron microscope autoradiographs showing results of RNA labeling. Both samples were labeled for 3 min with [^{3}H]uridine followed by a chase with a hundred-fold excess of cold uridine. Acid-soluble label was extracted before embedding. Exposure time, 8 days. 1: Chase, 30 min. Marker, 1 μm. 2: Chase, 5 min. Marker, 5 μm.

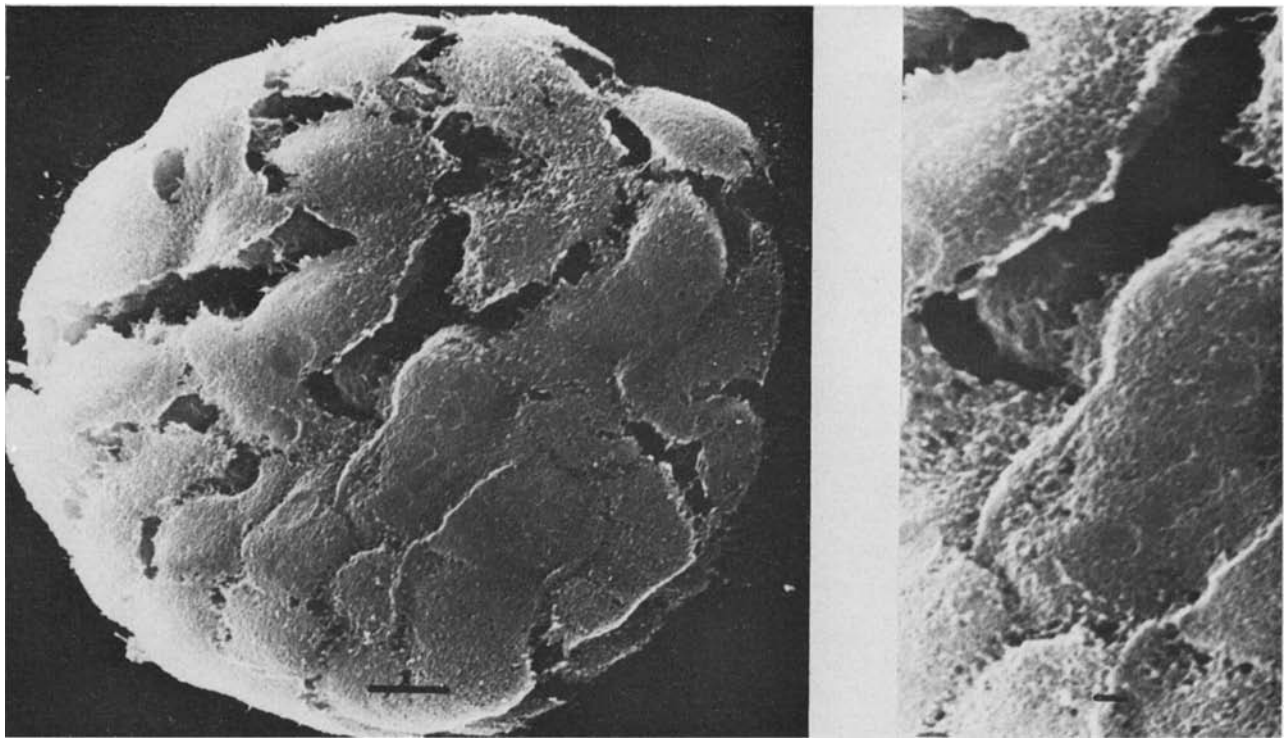

Fig. 5. The external appearance of a nucleus from a salivary gland. Blebs are of the size of the small holes in the higher-magnification inset at the right. Tightly-packed chromosomes are visible underlying the membrane and through the cracks which were formed during the preparation. Nuclei were isolated as described earlier (Mitchell and Lipps, 1975), critical-point-dried with Freon, and gold-coated for examination in the scanning electron microscope. Marker, 1 μm.

larger inclusion seems evident, but the relation to transport in or out of the nucleus is not clear.

With respect to blebbing at different developmental stages, we have made approximations of the number per nucleus from total cross-sections [Fig. 3(1) shows a portion of one]. Results indicate at least 2000 per nucleus with no significant difference in numbers between -2 and $+12$ hr. Errors are large, partly due to clustering, but the order of magnitude seems correct.

It appears that the most common bleb represents material moving out of the nucleus, and even though this is not certain, pulse-chase experiments were carried out to see if the blebs contain RNA. Results from one experiment with a 3-min pulse of tritiated uridine are shown in Fig. 4 (1, 2). Both pictures are from glands given the same pulse, but the chase with excess cold uridine was 30 min with 1 and 5 min with 2. As shown, much more of the label had moved into the cytoplasm in 1 than in 2, as would be expected if messenger RNA was in transit during this period. As shown, especially at the higher magnification in 1, the silver grain positions do not coincide with the bleb positions at the nuclear membrane. The same situation obtained with numerous other chase times and one other complete experiment.

No gross distortions of nuclear membranes, like those of cell membranes of actively secreting cells (Fig. 2), were observed among thin sections prepared for electron microscopy. In order to examine this situation further, we have looked at a number of isolated nuclei in the scanning electron microscope with overall results as illustrated in Fig. 5. The picture at the left is that of a whole nucleus which was cracked in the upper portion during preparation. The compact portion in the lower center is typical of intact nuclei. Even here outlines of the large polytene chromosomes are visible, with cracks and ridges emphasizing the compactness of the underlying chromosome package. In this picture and in the higher magnification at the right, variable-sized pockmarks are visible on the smoother areas of the surface. The more uniform-sized small holes and protrusions on the smooth surfaces in the higher magnification picture are near the size of the blebs seen in thin sections. However, it is not clear at present which surface of the nucleus (nuclear membrane or endoplasmic reticulum) is what is visible here. In any case, we have not observed any more drastic distortions of the nuclear surface than seen in thin sections.

DISCUSSION

The observations described here on continued secretory function of the larval salivary gland of *D. melanogaster* through the prepupal period are consistent with several other observations but not with a number of earlier deductions. It is quite reasonable in relation to the fact of the continued puffing sequences

of the salivary gland chromosomes (Ashburner, 1967). Furthermore, we have observed that salivary gland total protein and salivary gland chromosomal proteins (Tissieres *et al.*, 1974; Mitchell and Lipps, 1975) become labeled at about the same rates at all stages from late larvae to past pupation time (at least to 13 hr). The observations here are not compatible with earlier descriptions which have gland and/or single cell degeneration beginning just after puparium formation (Ross, 1939; Bodenstein, 1950; Lane *et al.*, 1972). It is certainly true that the glands appear to be in the process of degeneration each time the cell secretions are massively transported into the lumen. However, such cells and glands do recover repeatedly prior to the complete loss a few hours after pupation.

It seems likely that the gross morphological changes observed for the salivary glands are related in the time sequence to the pattern of chromosomal puffs (Ashburner, 1967), but we have no direct evidence on this question. We have evaluated the degree of synchrony of development of prepupae by dissection of at least five animals at each point in time. In virtually all cases when great care was exercised to maintain identical conditions of temperature, humidity, feeding, and population density through larval stages, glands from all five animals showed the same characteristics. A greater degree of synchrony is easier to achieve in mass culture than in single small culture bottles.

In contrast to the rather violent processes of secretion of material from the cytoplasm of salivary cells into the lumen of the gland, the exchange of materials between the nucleus and cytoplasm is less disruptive. This is in spite of the fact that messenger transport related to puffs must be rapid (Tissieres *et al.*, 1974) and protein exchange is fast and extensive (Mitchell and Lipps, 1975). As shown here, the most visible evidence of exchange, the blebbing process, does not involve transport of RNA. In support of this conclusion, we showed earlier (Tissieres *et al.*, 1974) that chromosome puffs label with uridine and puff-related messenger translation occurs in the cytoplasm of salivary glands under the same conditions and temporal sequence as described here. Thus mRNA is being transported out but not associated with blebs.

It does seem very likely that blebs represent transport as postulated long ago by Gay (1956). It also seems likely that they represent extrusion of material from the nucleus to give ribosome-studded vesicles as noted by Lane *et al.* (1972). We have observed this also, but have no additional evidence as to the direction of movement in or out of the nucleus. The double bleb [Fig. 3(4)] is not common, but in any case reciprocal exchange is possible even with the more usual variety of blebs.

With respect to bleb number per nucleus, Gay, in response to a question of Poulson (Gay, 1956), suggested about 200. We feel the number should be

more like 2000 as a minimum. However, in the absence of information on bleb half-life, no real estimate of transport potential can be made. Just as a basis of thought for further consideration of this transport problem, a bleb half-life of 1 sec could result in movement of the volume of one nucleus in 10 hr.

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