ULTRASTRUCTURE OF THE SECRETORY INCLUSIONS OF THE SALIVARY GLAND CELL IN *DROSOPHILA*

T. M. RIZKI. From the Department of Zoology, The University of Michigan, Ann Arbor

The salivary gland cells of dipteran larvae, particularly the various species of *Drosophila*, have been of unusual interest to studies in genetics as well as investigations in cell differentiation. The giant polytene chromosomes in the nuclei of these cells have favored extensive cytogenetic analyses, and more recently the phenomenon of regional puffing of the salivary gland chromosomes has been equated to visualization of gene activity (1). The specific cytoplasmic phenotype of these cells in fly larvae may be described in terms of mucoprotein inclusions which are continuously elaborated and accumulate during the period of larval development. One of the known functions of the salivary glands of these larvae is the formation of a mucoprotein which at a specific stage of development is discharged into the lumen of the gland. At the time of puparium formation this mucoprotein is expectorated and serves as a glue (2) for adhesion of the puparium to its substratum. A preliminary

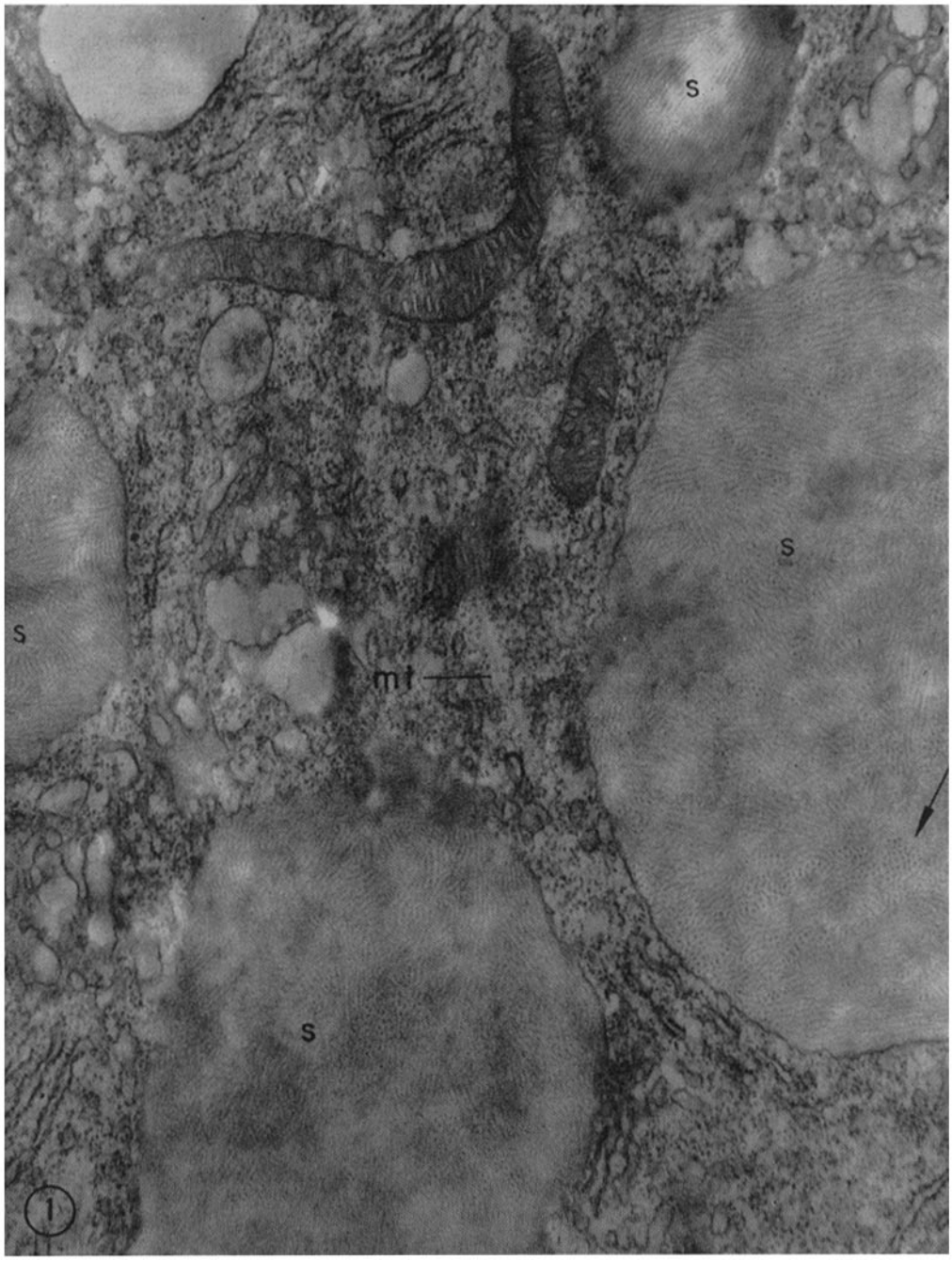

FIGURE 1 Secretory globules in the salivary gland cells of the larva of *D. melanogaster* showing the ordered array of electron-opaque material in both longitudinal (*s*) and cross-section (arrow). Note the microtubule (*mt*) in comparison to the size of the longitudinal strands in the secretory globules. An enlargement of the negative, including the area marked by the arrow, is presented in Fig. 2. × 20,000.

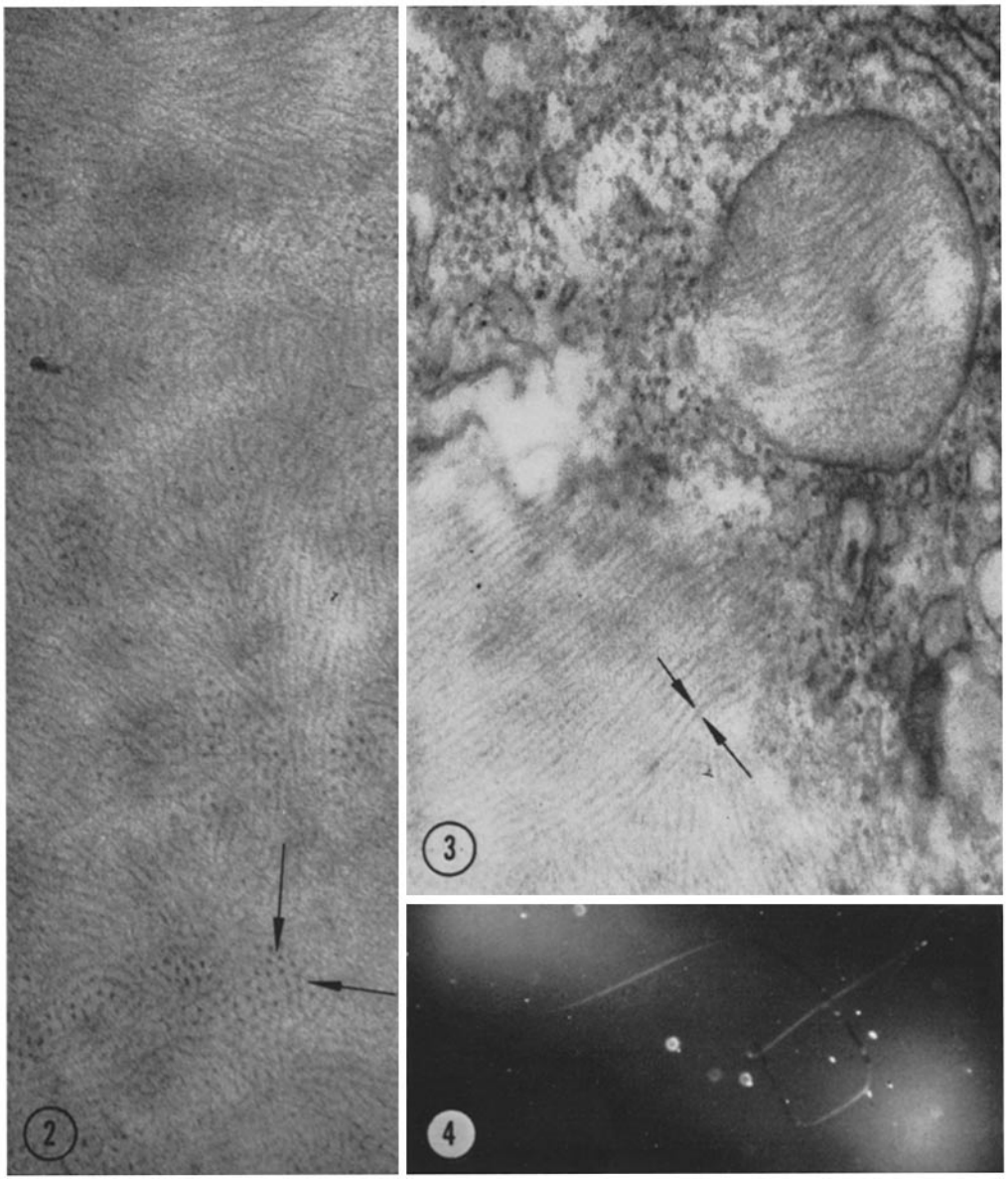

FIGURE 2 In cross-section the electron-opaque strands appear to be interconnected by finer elements (indicated at arrows). × 39,000.

FIGURE 3 A micrograph representing higher resolution of the parallel array of electron-opaque elements, one of which is indicated by two arrows. × 38,000.

FIGURE 4 The secreted glue from the salivary gland drawn into a fiber and viewed in polarized light. Fiber thickness, 10μ.

analysis of the amino acid content of this mucoprotein has been reported by Blumel and Kirby (3), and, in addition to the identification of 15 amino acids, Kodani (4) identified a ninhydrin-positive material as glucosamine in this secretion from the salivary glands of *Drosophila*. Histochemical studies on the nature of the secretory product were undertaken by Lesher (5) who employed the periodic acid–Schiff reaction for localization of mucoprotein. Several excellent studies on the ultrastructure of the salivary gland cells of the *Drosophila* larva are available (6, 7), and the secretory inclusions in the salivary gland cells of *Drosophila hydei* have been described as inhomogeneous in structure (8). Therefore, the present report is limited to observations on the ultrastructure of the secretory inclusions in the salivary gland cells of *D. melanogaster* where they occur as highly organized, clearly patterned structures.

Larvae of *D. melanogaster* were raised on cream of wheat–molasses medium at 24°C. The present study utilized larvae at an age of 66–68 hr after hatching (mid third instar). The salivary glands were fixed at room temperature (23° ± 1°C) in 2.3% nascent formalin in phosphate buffer at pH 7.15 according to Pease (9). The material was washed with this same buffer to remove excess formalin, postfixed with Veronal-buffered osmium tetroxide at pH 7.15, dehydrated, and embedded in Epon. Sections were stained with uranyl acetate (pH 4.3) followed by lead hydroxide.

Fig. 1 indicates the general quality of fixation of mitochondria, endoplasmic reticulum, and other fine structures of the cytoplasm together with the large mucoprotein inclusions. The latter structures are delimited by a membrane, and the fine structure within these inclusions is arranged in the form of parallel rows of electron-opaque material. In several areas these microstructures can be seen in cross-section. It is here that one can discern a cross-linkage of the parallel stacking (Fig. 2). Fig. 3 is a small segment of the secretory inclusion, and here further structural details of the electron-opaque microstructures can be seen.

Estimates indicate that a single electron-opaque strand measures approximately 31 A; this value, of course, includes the error of estimate as well as shrinkage due to fixation, and other variable factors involving fluctuations of electron microscope operation. Since previous investigations have established that these structures contain the periodic acid–Schiff positive material which is a mucoprotein, it is likely that the electron-opaque areas are occupied by this mucoprotein. The cross-strands which we have noted may, or may not, comprise the same chemical entity as the array of longitudinal strands, for these are narrower in diameter and seem to run a distance of approximately 245 A.

The secretory product of the salivary gland is a semiliquid and the cytoplasmic inclusions are not rigidly limited structures. These inclusions increase in size as development proceeds. Regularity of arrangement of the structural elements in the inclusions suggests that the secretory product of the salivary gland or its precursor exists in a paracrystalline state in these structures. The extruded glue can be drawn into fibers (Fig. 4), and these fibers show anisotropy of molecules in polarized light. It is hopeful that X-ray diffraction analysis may provide further information on the nature of this mucoprotein and the ultrastructure of the secretory inclusions of the salivary gland cells.

This study was supported by research grants (No. GB 1909 and GB 4581) from the National Science Foundation. I would also like to acknowledge with gratitude the valuable technical assistance of Miss Sandra K. Erickson.

Received for publication 1 September 1966.

REFERENCES

1. CLEVER, U. 1965. *Brookhaven Symp. Biol.* **18**:242.
2. FRAENKEL, G., and V. J. BROOKES. 1953. *Biol. Bull.* **105**:442.
3. BLUMEL, J., and H. KIRBY. 1948. *Proc. Natl. Acad. Sci. U. S.* **34**:561.
4. KODANI, M. 1948. *Proc. Natl. Acad. Sci. U. S.* **34**: 131.
5. LESHER, S. 1952. *Anat. Rec.* **114**:633.
6. GAY, H. 1956. *Cold Spring Harbor Symp. Quant. Biol.* **21**:257.
7. SWIFT, H. 1962. *In* Symposium on the Molecular Control of Cellular Activity. J. M. Allen, editor. McGraw-Hill Book Co., New York. 73.
8. BERENDES, H. D., and W. C. DE BRUYN. 1963. *Zeit. Zellforsch. Mikr. Anat. Abt. Histochem.* **59**: 142.
9. PEASE, D. C. 1964. *In* Histological Techniques for Electron Microscopy. 2nd ed. Academic Press Inc., New York. 51.