

DICTYOSOME STRUCTURE IN *EUGLENA GRACILIS*

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INTRODUCTION

Dictyosomes are active in the segregation and/or synthesis of material for export from the cell (11). Product destined for export accumulates in vesicles attached to the cisternal tubules (12). These secretory vesicles may contain a variety of products, depending upon their immediate role in cellular metabolism. This heterogeneity is reflected in the appearance of the secretory vesicles and in the size, form, and density of the cisternae. Various products or inclusions have been found within forming secretory vesicles (for examples, see 1, 2, 6-8, 13, 15). In spite of this diversity of secretory product, very little morphological heterogeneity has been found in the central region of the dictyosome. This central region changes little (by present methods of observation) regardless of the functional state (11) of the dictyosome. For this reason, the centrally occurring cisternal modification described here seems of interest.

MATERIALS AND METHODS

The cells, *Euglena gracilis* (Klebs) "Z" strain, were grown axenically at 24°C in a modified Hunter medium (3) specified in Table I. Cultures were grown in cotton-stoppered 500-ml Erlenmeyer flasks con-

taining 100 ml of media, in the dark, and without shaking.

Cells were prepared for electron microscopy by standard procedures. They were prefixed in 0.03 M *s*-collidine-buffered acrolein (2%)-glutaraldehyde (2%), rinsed well in buffer, and postfixed in *s*-collidine-buffered OsO₄ (1%). They were dehydrated in ethanol followed by three acetone rinses, and then

TABLE I
Composition of 1 Liter of Culture Medium

L-glutamic acid	3.00 g
Glucose	10.00 "
DL-aspartic acid	2.00 "
DL-malic acid	1.00 "
Glycine	2.50 "
NH ₄ HCO ₃	0.62 "
Na ₂ succinate·6H ₂ O	0.10 "
EDTA (2Na)	0.50 "
KH ₂ PO ₄	0.30 "
MgSO ₄ ·7H ₂ O	0.5 "
NH ₄ Cl	0.5 "
CaCO ₃	20 mg
Thiamine HCl	1.0 "
Vitamin B ₁₂	0.005 "
"Metals 60A" (4)	0.18 g
pH	3.6-3.8

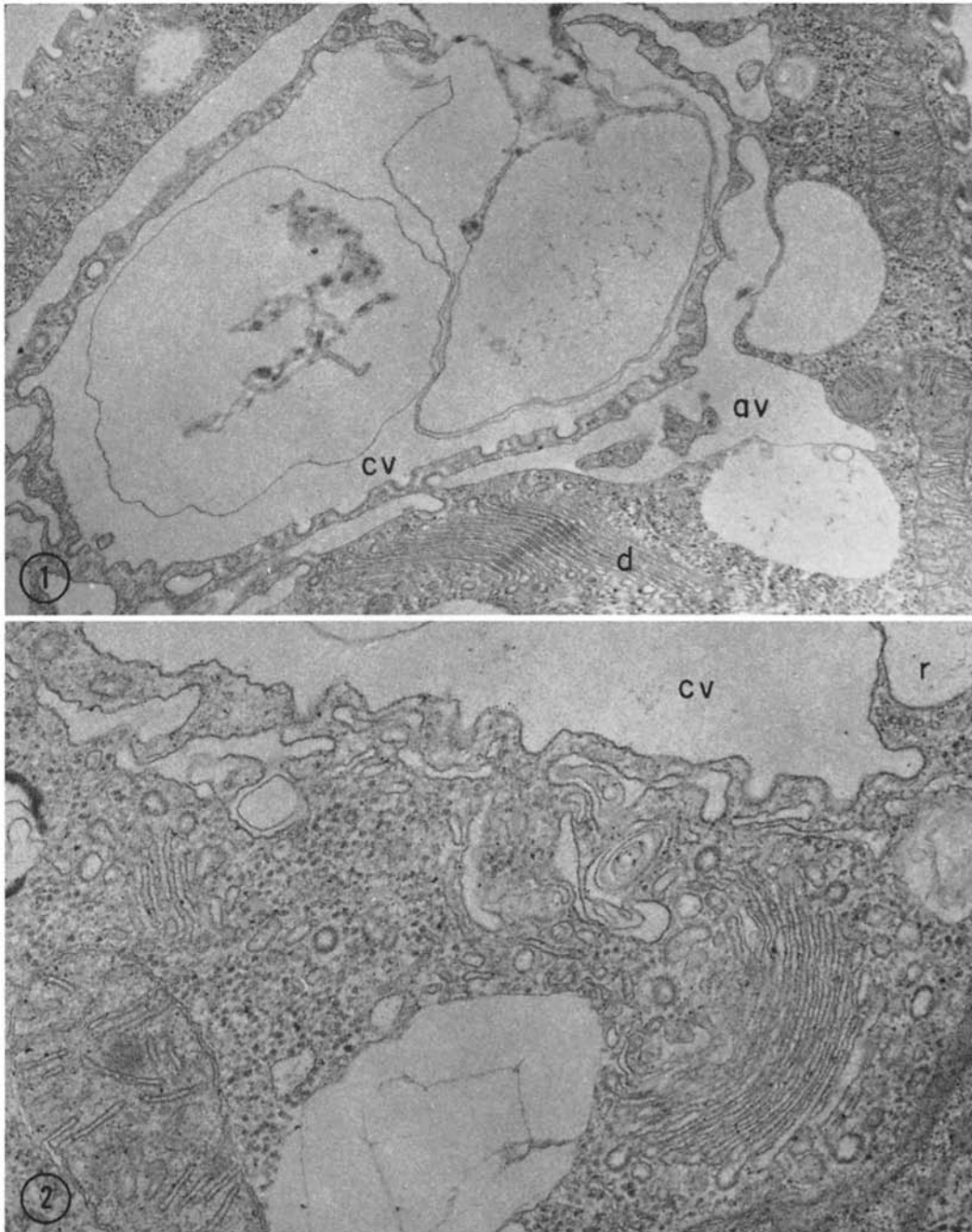


FIGURE 1 A longitudinal section through the anterior end of a cell showing the contractile (*cv*) and accessory (*av*) vacuoles, a dictyosome (*d*), and portions of several mitochondria. Numerous coated vesicles are associated with the contractile vacuole, particularly on the side closest to the dictyosome. Coated vesicles are also associated with the dictyosome (see also Figs. 2 and 3 *a*) though these differ in size from those associated with the contractile vacuole. The dictyosome form illustrated in this figure is representative of most of the dictyosomes of the cell. $\times 30,000$.

FIGURE 2 This figure further illustrates the various forms of coated vesicles and the relationship between the contractile vacuole and adjacent dictyosomes. Coated vesicles, similar to those illustrated here, seem to be a characteristic identifying feature of all plant and animal dictyosomes. Contractile vacuole (*cv*), reservoir (*r*). $\times 45,000$.

embedded in an Epon-Araldite epoxy resin mixture as described previously (10).

RESULTS AND DISCUSSION

Euglena have numerous dictyosomes, each consisting of a stack of 15–30 cisternae (Figs. 1–3 *a* and reference 5). Dictyosomes are distributed throughout the cell, although at least one appears always in the immediate vicinity of the contractile vacuole (Figs. 1, 2 and reference 5). Coated vesicles are associated with the periphery of the dictyosome cisternae (Figs. 1–3 *a*) and with the contractile vacuole (Figs. 1, 2). The coated vesicles of the contractile vacuole, however, are characteristically larger than those of the dictyosome (Figs. 1, 2). No large secretory vesicles (of the type usually associated with transport of large molecules out of the cell) are present, and the dictyosomes are presumably not active in secretion.

Near the center of each dictyosome, a dense structure is present within the lumen of the cisternae (Figs. 1, 3 *a*, 3 *b*). In sections tangential

to the dictyosome cisternae, this region of cisternal modification appears circular (Fig. 4); whereas in sections transverse to the plane of the cisternae, the outline of the modified region is more nearly elliptical or cone-shaped (Figs. 1, 3 *a*). Occasionally, there appear to be several modified regions within a single dictyosome.

No exactly comparable dictyosome form is presently known by the authors. However, Manton and her associates have shown that in the formation of scales an intracisternal structure (the forming scale) may be located within the cisternae, in regions presumed to be forming secretory vesicles (6–8). Also, Stoermer et al. (14) have shown a product accumulation (“dense bodies”) in the central portion of the dictyosome. However, whether these dictyosomes are related to those described here is not clear. It seems more likely that the intracisternal modification shown here is a form of the “osmiophilic conical substance” seen in dictyosomes of *Distigma* (see Plate II of Mignot [reference 9]). A more rudimentary form,

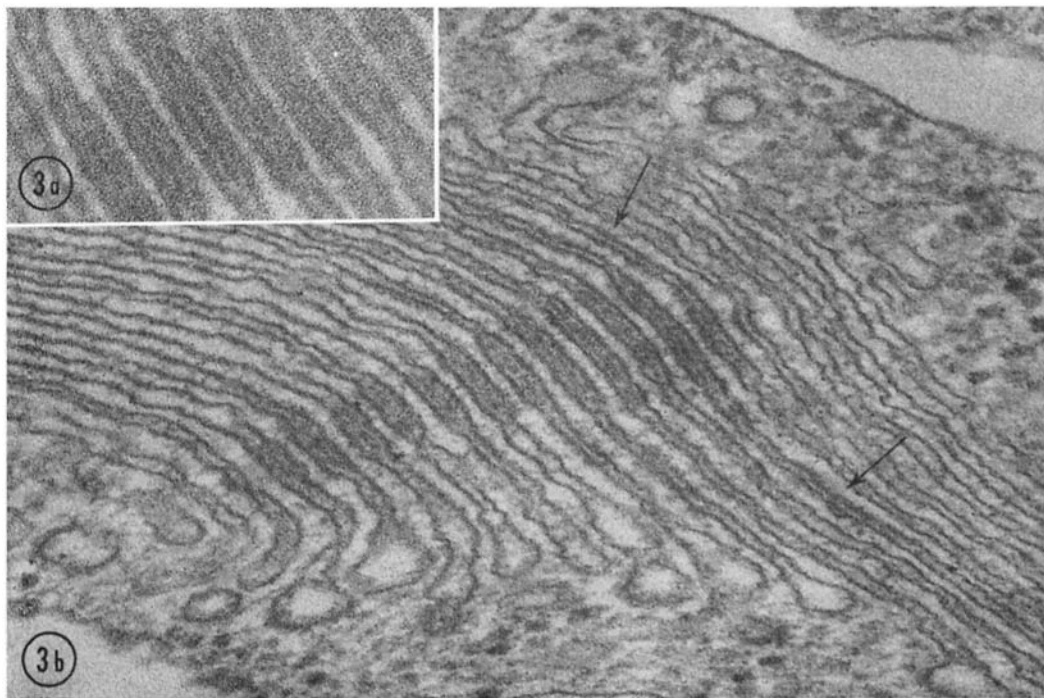


FIGURE 3 *a* and *b* Transverse sections through two dictyosomes showing the form of the intracisternal structure. This structure appears as a dense line located in the cisternal lumen midway between the bounding membranes. The dense line is structurally different from, and does not appear continuous with, the membranes of the cisternae. The arrows point to two regions of the cisternae which are only partially modified. Fig. 3 *a*, $\times 140,000$; Fig. 3 *b*, $\times 200,000$.

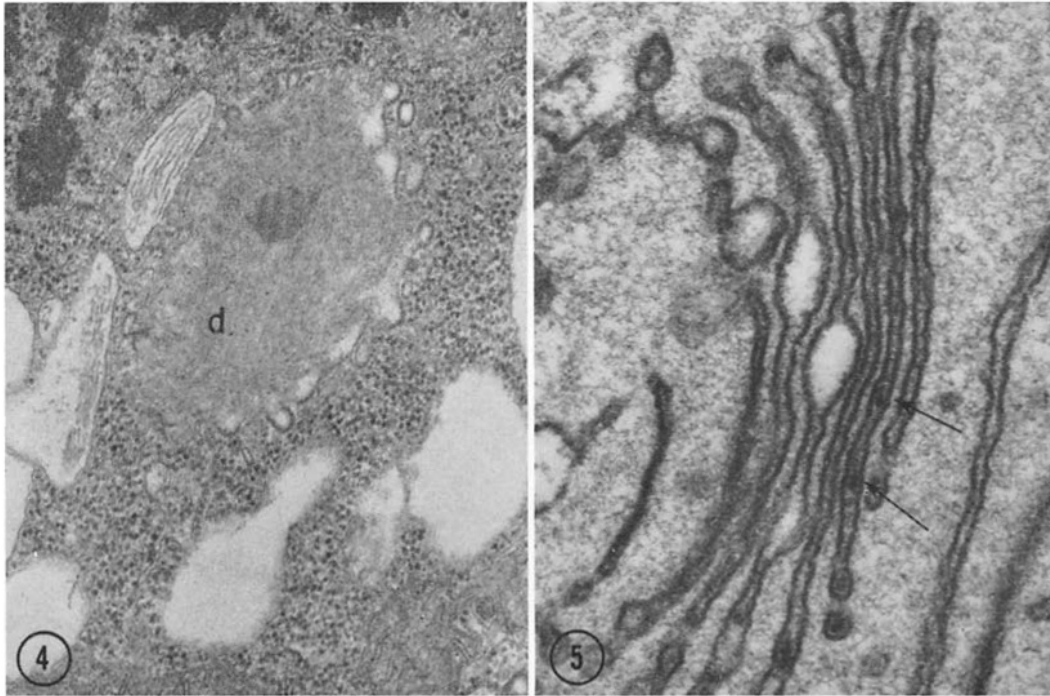


FIGURE 4 A nearly tangential section through a dictyosome (*d*), showing the circular form of the intracisternal structure. $\times 35,000$.

FIGURE 5 More rudimentary forms of intracisternal structures (arrows) are also occasionally seen in dictyosomes from cells other than *Euglena*. One form from bean (*Phaseolus vulgaris*) root tip is illustrated in this figure. Fixation was done in 2% KMnO_4 followed by dehydration and embedding as described in reference 10. $\times 65,000$.

involving only single cisternae, is also occasionally seen in dictyosomes from other cells (see arrows, Fig. 5). The modified region of a cisterna is also characterized by an increased intracisternal spacing and by changes in the surface texture of the membranes (Figs. 3 *a-b*).

The intracisternal structure described here was common to most of the dictyosomes of the cell and was not restricted to those of a particular

region. Whether this dictyosome form is associated with a specialized activity or functional state (11) has not been determined. Very little is known about the nonsecretory Golgi apparatus, and additional work will have to be done before the functional significance of the structure becomes apparent.

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REFERENCES

1. CARDELL, R. R., S. BADENHAUSEN, and K. R. PORTER. 1967. Intestinal triglyceride absorption in the rat. An electron microscopical study. *J. Cell Biol.* 34:123.
2. GOURANTON, J., and P.-L. MAILLET. 1967. Origine et structure des brochosomes. *J. Microscop.* 6:53.
3. HUTNER, S. H., M. K. BACH, and G. I. M. ROSS. 1956. A sugar-containing basal medium for *B₁₂* assay with *Euglena*; application to body fluids. *J. Protozool.* 3:101.
4. HUTNER, S. H., A. C. ZAHALSKY, S. AARONSON, H. BAKER, and O. FRANK. 1966. In *Methods in Cell Physiology*. D. M. Prescott, editor. Academic Press Inc., New York and London. 2:217.
5. LEEDALE, G. F. 1966. *Euglena*: A new look with the electron microscope. *Advan. Sci.* 107:22.

6. MANTON, I. 1967. Further observations on the fine structure of *Chrysochromulina chiton* with special reference to the haptonema, "peculiar" Golgi structure and scale production. *J. Cell Sci.* **2**:265.
7. MANTON, I., and H. Ettl. 1965. Observations on the fine structure of *Mesostigma viride* Lanterborn. *J. Linnean Soc. London, Botany.* **59**:175.
8. MANTON, I., D. G. RAYNS, H. Ettl, and M. PARKE. 1965. Further observations on green flagellates with scaly flagella: The genus *Heteromastix* Korshikov. *J. Marine Biol. Assoc. U. K.* **45**:241.
9. MIGNOT, J.-P. 1965. Étude ultrastructural des Euglèniens: II. A, Dictyosomes et dictyocinèse chez *Distigma protens* EHRBG B, Mastigonèmes chez *Anisonema costatum* Christen. *Protistologica.* **1**:17.
10. MOLLENHAUER, H. H. 1964. Plastic embedding mixtures for use in electron microscopy. *Stain Technol.* **39**:111.
11. MOLLENHAUER, H. H., and D. J. MORRÉ. 1966. Golgi apparatus and plant secretion. *Ann. Rev. Plant Physiol.* **17**:27.
12. MOLLENHAUER, H. H., and D. J. MORRÉ. 1966. Tubular connections between dictyosomes and forming secretory vesicles in plant Golgi apparatus. *J. Cell Biol.* **29**:373.
13. SILVEIRA, M. 1967. Formation of structured secretory granules within the Golgi complex in an *Acoel turbellarian*. *J. Microscop.* **6**:95.
14. STOERMER, E. F., H. S. PANKRATZ, and C. C. BOWEN. 1965. Fine structure of the diatom *Amphipecton pellucida* II. Cytoplasmic fine structure and frustule formation. *Am. J. Botany.* **52**:1067.
15. WHALEY, W. G. 1966. Proposals concerning replication of the Golgi apparatus. In *Wiss. Konf. Ges. Duet. Naturforsch. Ärzte, 3., Probleme der biologischen Reduplikation*, Wien, 1965, 340-371, Springer-Verlag, Berlin.