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Pinocytotic Uptake of Protein from the Reservoir in *Euglena*

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Abstract. Alveolate vesicles in the anterior regions of *Euglena gracilis* are shown to function in pinocytosis, being capable of incorporating exogenous horseradish peroxidase through the reservoir wall. It is thought that this represents a mechanism for recovering large molecules otherwise lost during contractile vacuole discharge.

Key words: Contractile Vacuole — *Euglena gracilis* — Pinocytosis — Protein Uptake.

The contractile vacuole region of several algae has been found to contain distinctive alveolate-patterned 1000 Å vesicles, described by various authors as "alveolate vesicles", on "coated vesicles" (Manton, 1964; Leedale *et al.*, 1965; Leedale, 1967). These lie free in the cytoplasm or are fused in varying degrees with the walls of the contractile vacuole (CV), accessory vacuoles (AV), or reservoir; they are reportedly especially numerous immediately after CV discharge ("systole") (Leedale *et al.*, 1965).

Leedale *et al.* (1965) have postulated that these bodies serve an osmoregulatory function, probably by transporting materials into the CV, AV, and reservoir. Other suggestions as to their function have been the pinocytotic uptake of valuable materials from the effluent fluid (Porter, cited in Leedale *et al.*, 1965), and the pinocytotic uptake of macromolecules for chemo-reception (Leedale, 1967). Recently, it has been suggested that similar vesicles in *Amoeba* function in the recycling of excess membrane brought to the surface by fusion and discharge of the CV (McKanna, 1973).

We show here that, in *Euglena gracilis*, these vesicles are engaged in pinocytosis, and are able to take up large protein macromolecules from the external medium through the floor of the reservoir.

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Non-Standard Abbreviations Used. CV = contractile vacuole; AV = accessory vacuole; DAB = diaminobenzidine; MF = major flagellum; F = minor flagellum.

Methods and Materials

Cultures of *Euglena gracilis* strain Z were grown under heterotrophic conditions and resuspended in a non-nutrient "resting medium" for 3 days prior to use, as described previously (Kivic and Vesk, 1972). The pinocytotic uptake of peroxidase into *Euglena* was demonstrated by the method of Locke and Collins (1968) (slightly modified): the oxidation reaction catalyzed by the peroxidase causes the deposition of a dense, brown osmium-staining diaminobenzidine (DAB) reaction product around the enzyme.

A suspension of 6×10 cells per ml in the non-nutrient medium was incubated with purified horseradish peroxidase (Sigma Corp.) 10 mg per ml, for 15 min at 25° C with continuous agitation. The cells were then fixed in 1.7 % glutaraldehyde (final concentration) in 0.025 M phosphate buffer, pH 6.8, overnight at 10° C. After washing for 24 h in buffer to remove the glutaraldehyde, the fixed cells were incubated with 0.03 diaminobenzidine (K & K Laboratories) in the same buffer, for 10 min, with continuous agitation. Hydrogen peroxide 0.03 % (final concentration) was then added for a further 6 min incubation, at this point the cells turned dark brown. The cells were then washed several times in buffer, post fixed in 1 % OsO_4 and processed for electron microscopy as previously described (Kivic and Vesk, 1972). The location of the peroxidase within the specimens was identified in thin sections by the diaminobenzidine precipitate, which is very osmiophilic.

Results and Discussion

As described previously (Leedale *et al.*, 1965; Leedale, 1967) numerous alveolate vesicles are seen fused with, or in the vicinity of, the reservoir, CV, and AV. They may be distinguished from other similar-sized vesicles in the same vicinity (evidently emanating from the Golgi complex) by their "alveolate" or "basketlike" wall.

The location of peroxidase in these preparations is clearly established by the presence of intensely-staining granular DAB deposits. Copious amounts of this material are found in the reservoir and in the alveolate vesicles near, or fused with, the reservoir, as shown in Fig.1. The CV and AV, which are not connected to the exterior, contain no peroxidase, nor do the alveolate vesicles associated with them. It is clear that material is passing from the reservoir (and, presumably, the CV and AV) to the small vesicles, and not vice versa.

Fig.1. a Transverse section through the anterior region of an *Euglena gracilis* cell, demonstrating the uptake of horseradish peroxidase through the reservoir wall. Large deposits of DAB (*x*) are found in the reservoir; a black layer of DAB is also seen coating the reservoir wall and the major and minor flagella (*MF*, *F*). The alveolate vesicles (white arrows) originating from the reservoir wall also contain the DAB deposit; the alveolate vesicles (black arrows) originating from the contractile vacuole (*CV*) and accessory vacuole (*AV*) walls do not. Magnification: × 22000. b Detailed picture of the alveolate vesicles originating from the reservoir (*R*) and accessory vacuoles (*AV*). The reservoir-associated vesicles (white arrows) bear a thick coating of DAB (*x*) on the inner surface; vesicles originating from the accessory vacuoles (black arrows) contain no deposits of DAB. Magnification: × 55000

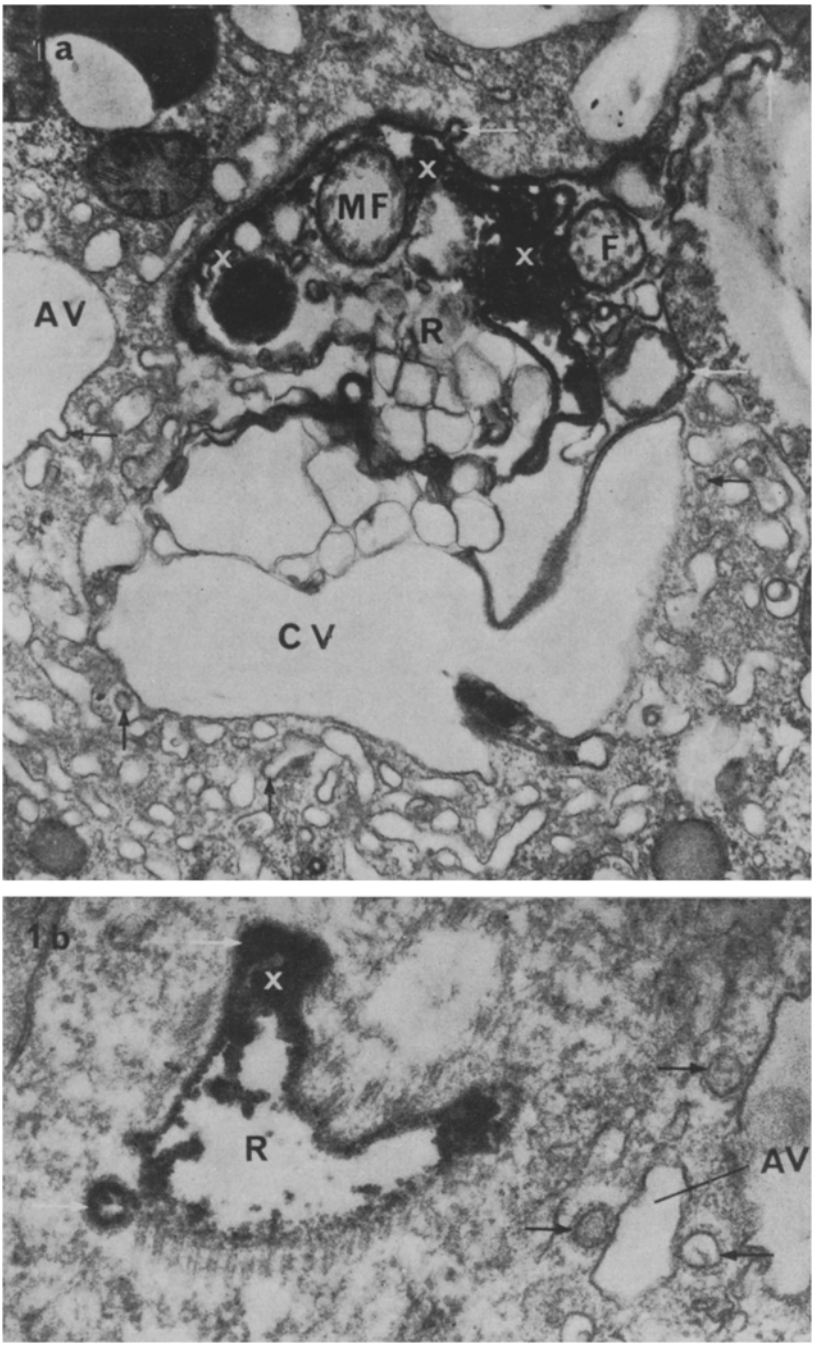

Fig. 1 a and b

The vesicles will of course carry back into the cell some of the excess CV and AV membranes. From the number of vesicles present, however, it seems questionable that this could be their major role. Indeed, judging from the amount of membranous debris always present within the CV and reservoir, it would appear that in *Euglena* the main part of the redundant AV and CV membrane is sloughed, not recycled.

It thus appears that the alveolate vesicles are pinocytotic vesicles, as suggested by their morphological similarity to the known pinocytotic vesicles of various animal tissues (for example, Kanaseki and Kadota, 1969; Leedale *et al.*, 1965), and that they transport materials from the reservoir, CV and AV fluid back into the cell.

The purpose of this pinocytotic activity is not certain. It is unlikely that chemoreception (Leedale, 1967) is the major function, as this would not account for the involvement of the CV and AV; the same argument would also rule out the uptake of foodstuffs as a major role. They obviously cannot serve to transport wastes to the CV, AV, and reservoir (Leedale, 1967). It appears most likely that the pinocytotic vesicles act as a salvage mechanism, recovering useful materials from the effluent about to be discharged (see Porter, cited in Leedale *et al.*, 1965).

The nature of the material resorbed is also open to speculation. This work demonstrates that relatively large protein molecules can be taken up; other metabolites could also be involved, however. In the mammalian renal tubules, plasma proteins are thought to be similarly salvaged from the filtrate by a pinocytosis mechanism, and it is likely that pinocytotic vesicles of this general type are adaptations for recovering proteins and other large molecules from relatively dilute solutions (see Fawcett, 1965).

The Schmidt-Nielson and Schrauger (1963) hypothesis of contractile vacuole function postulates the internal deposition of a concentrated osmoticum within small vesicles (probably the Golgi-derived vesicles, see Schnepf, 1968) which then take in water, swell, fuse, and discharge to the exterior.

However, as the CV fluid is known to be hypo-osmotic to the cell, the osmoticum must be resorbed into the cell prior to discharge of the fluid (Schmidt-Nielson and Schrauger, 1963). Assuming this hypothesis is correct, the alveolate vesicles could well be the mechanism by which the osmoticum is recovered.

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