Localization of concanavalin-A binding sites in *Tetrahymena* by scanning electron microscopy[1]

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Summary. Binding sites of concanavalin-A were detected in *Tetrahymena* on the body ciliature, while the cell membrane itself and the oral ciliature failed to bind the lectine.

It is known that unicellulars - although they do not themselves possess a hormonal regulation - are capable of binding certain exogenous hormones. Evidence of this has emerged not only from the often specific response[2-6] of the unicellular to the given hormone but also from demonstration of hormone binding sites[7-10] in such organisms. The unicellular alga, *Acetabularia mediterranea*, possesses binding sites for insulin[7], the protozoon *Tetrahymena* for insulin[8], histamine[9], serotonin[9] and thyroxin[8]. The plant lectines, noted for nonspecific binding to cellular surface receptors, may also become bound by unicellulars, as has been shown with *Tetrahymena*[11] and *Amoeba*[12]. Scanning electron microscopic detection of the localization of the plant lectine concanavalin-A (con-A) in *Tetrahymena* is reported in this paper.

Binding of con-A is not visible by electron microscope unless the lectine is bound to a conspicuous tracer. Colloidal gold proved to be the tracer of choice, at 50 nm particle diameter - as the cilia of *Tetrahymena pyriformis* are 50-100 nm wide - and it was conjugated with con-A as proposed by Horisberger et al.[13].

The *Tetrahymena* cells were collected from the nutrient medium by centrifugation at 500 × g, followed by 3 washes in Losina-Losinsky[14] solution. The last supernatant was

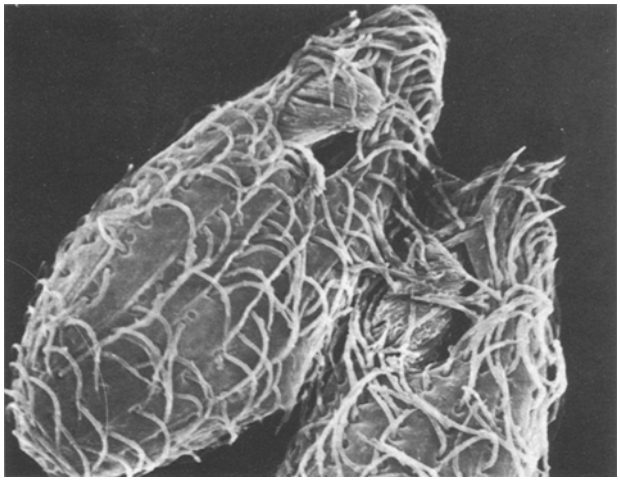

Fig. 1. Untreated (control) *Tetrahymena*. × 3000.

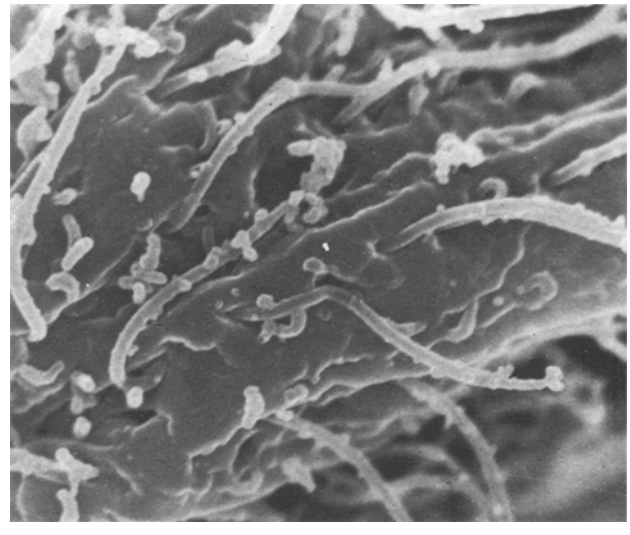

Fig. 3. *Tetrahymena* treated with con-A + colloidal gold conjugate. Note absence of particles along the cell membrane, and their abundant presence along the cilia. × 8000.

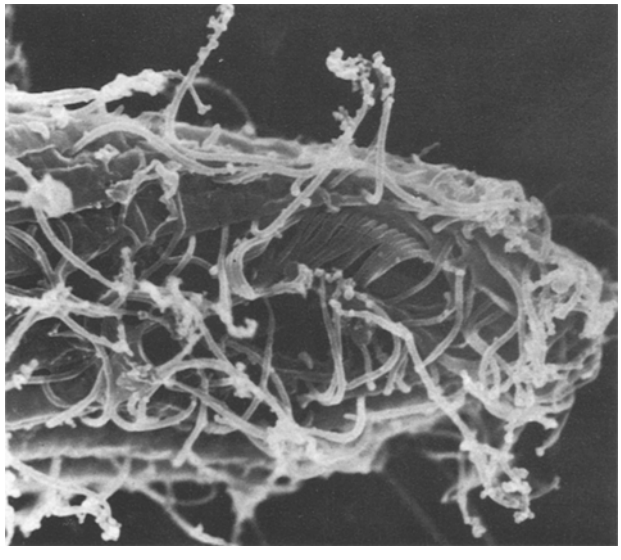

Fig. 2. *Tetrahymena* treated with con-A + colloidal gold conjugate. Note absence of particles on the oral ciliature, while they are abundantly present on the cilia of the body, especially at their tips. × 5000.

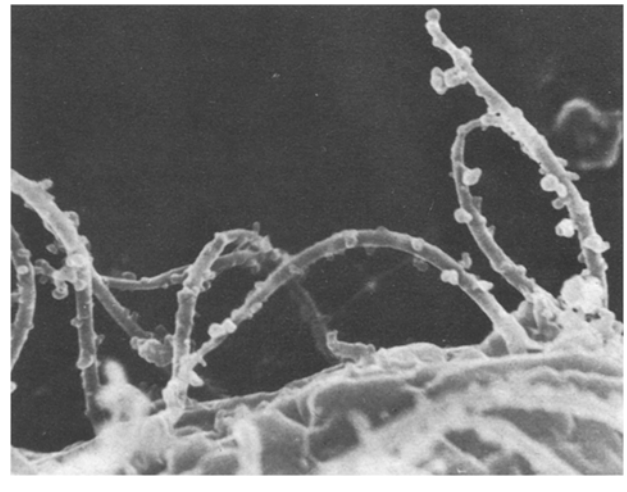

Fig. 4. *Tetrahymena* treated with con-A + colloidal gold conjugate. There are no particles on the cell membrane, but many along the cilia. × 15, 000.

decanted, and the cells were fixed in 2.5% buffered glutaraldehyde for 2 h at room temperature (Buffer solution: 0.15 M NaCl, 0.05 Tris, pH 7.0, 0,5 mg/ml Carbowax, passed through a 0.45 µm Millipore filter). After 3 washes of the fixed cells in buffer, 1.0 colloidal gold + con-A conjugate was added to 0.4 ml cell sediment and the system was incubated at room temperature for 10 h. Subsequent addition of 3 ml buffer, mild shaking and hand centrifugation was followed by 2 washes in buffer solution, dehydration in amylacetate by addition of CO_2 at the critical point, drying, and gold evaporation. The preparations were examined in a Jeol 100 C Temiscan electron microscope. The control preparations were dehydrated immediately after fixation, and processed further as above.

The binding of con-A by *Tetrahymena* was indicated by the presence of many colloidal gold particles on the body ciliature of conjugate-treated cells, contrasted to absence of the tracer in both membrane and ciliary regions of controls. Although in the treated preparations the colloidal particles could be seen along the entire length of the cilia, the bulk was obviously associated with the tips of cilia, and there was practically no indication of lectine binding by the cell membrane and oral ciliature.

This experimental observation has substantiated the former implication[11,12] that unicellulars, including *Tetrahymena*, do possess lectine binding sites, and it also throws light on the nonuniform distribution, i.e. absence of lectine-binding in certain cell regions. Absence of binding along the cell mebrane of the body, despite its presence along the cilia, suggests the specialization of certain cell membrane regions. The apparent lack of binding sites in the oral structures, contrasted with their abundance at the ciliary tips, supports the same conclusion. There is reason to postulate that, in unicellulars, certain sites of chemical recognition (receptors) are localized in the body ciliature predominantly on the tips of cilia, which seem to maintain functional superiority also in this respect. Evidence of cell membrane specialization at a relatively very low phylogenetic level may promote the understanding of receptor specialization at higher levels.

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Pinitol, a larval growth inhibitor for *Heliothis zea* in soybeans

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Summary. A search for insect growth inhibitors in methanol extracts of soybean leaves resulted in isolation of pinitol. Pinitol caused a 50% reduction in weight gain (ED_{50}) of *Heliothis zea* larvae at about 0.7% when added to a synthetic diet. Although myo-inositol is a normal component of the insect diet, it also caused growth inhibition at higher concentrations; ED_{50} 4%.

This paper reports work on soybean leaf (*Glycine max* [L] Merr) constituents as part of a systematic search for naturally occurring insect antifeeding or antigrowth substances in economically important crop plants[2]. Knowledge of these components would aid plant breeders in selecting for resistance to insect pests[3].

The bollworm (*Heliothis zea* [Boddie]) is an important pest on soybeans. The larvae eat the leaves or seed pods causing, in some cases, substantial plant damage[4]. Certain lines of soybeans, however, are relatively resistant to *H. zea*[5]. This paper reports a search for insect antifeeding or antigrowth substances in these resistant soybean lines using a feeding bioassay developed in this laboratory[6] as a guide in the isolation procedure. Test substances were mixed with *a*-cellulose and then incorporated in the wheat germ-casein diet. Newly hatched bollworm larvae were placed on diet pieces and were incubated at 26 °C for 12 days, whereupon the larvae were weighed and compared with a control group.

Extraction of freeze dried soybean leaves from the cultivar Davis or plant introduction PI 229358 with ethyl acetate, acetone or any solvent of lesser polarity gave, after removal of solvent, material which was inactive in an insect feeding bioassay using *H. zea* larvae on the artificial diet. On the other hand, extraction with acetone to remove inactive nonpolar substances followed by extraction with methanol and then water exhaustively in a Soxhlet extractor gave material in both latter extracts which cause a reduction in larval weight gain in the bioassay (table). This procedure was then used routinely to prepare active extracts. The results indicated that the active antigrowth material(s) was very polar and heat stable.

The methanol extracts were chromatographed on Sephadex LH-20 with methanol. Fractions were combined according to retention volumes and bioassayed. In this fashion, active material was concentrated and separated from much inactive material. The active fractions were then decolorized by treatment with Norite. The nearly colorless active syrupy residue showed no appreciable UV-absorption suggesting that the active material did not contain a UV-chromophore above 200 nm. This active fraction gave negative ninhydrin and Dragendorfs tests suggesting that it was free of nitro-