Ultrastructural Localization of Acid Phosphatase in the Yeast *Cryptococcus neoformans*

Cryptococcus neoformans is a large-sized (30–70 μ) yeast, enveloped by a polysaccharide capsule, which reproduces by budding[1]. Although generally saprophytic, *C. neoformans* is capable of infecting humans or animals suffering from a debilitating disease; hence, host defence mechanisms against the yeast are being studied in various laboratories[2–4]. Recently, the ultrastructure of *C. neoformans* was described in a study by EDWARDS et al.[5], in which the following organelles were identified: the nucleus with its envelope, mitochondria, smooth endoplasmic reticulum, ribosomes, storage granules and vacuoles; lysosomes, however, were not described in this paper. Since there is an increasing interest in lysosomes, and in the localization of hydrolytic enzymes in plants and yeasts[6], it was decided to apply cytochemical methods on the ultrastructural level in studying these questions in the yeast *C. neoformans*.

C. neoformans was grown in a LITTMAN and TSUBURA[7] medium for 6 h at 28 °C followed by 18 h incubation at 37 °C. The yeast cells were centrifuged, and fixed for 15 min with 2% glutaraldehyde in 0.1 *M* cacodylate buffer at pH 7.2; they were then washed in 0.25 *M* buffered sucrose solution and suspended in a modified Gomori incubation medium for β-glycerophosphatase activity[8]. The cells were incubated for 10–60 min at room temperature, then washed, post-fixed in OsO_4 and embedded in Araldite. Ultrathin sections were viewed either unstained or stained with uranyl acetate through an EMU 3G RCA electron microscope. In control experiments, sodium fluoride (0.01 *M*) was added to the incubation medium.

Although grown in a different medium, the cryptococci showed the same morphological features which have been described by EDWARDS et al.[5]. However, the vacuoles were not well preserved – a problem already known from the literature[9].

When tested for demonstration of β-glycerophosphatase, yeasts with a well-preserved cell membrane showed negative or only slightly positive activity (Figure 1). On the other hand, deposits of the reaction product were found in the vacuoles, mainly adjacent to the periphery, in all those cryptococci which showed signs of damage in the cell membrane (Figure 2). These findings suggest that a well-preserved plasma membrane prevents penetration of the substrate, and the eventual depositing of lead[9]. No reaction was observed in organelles other than the vacuoles. In control experiments, the reaction was inhibited by sodium fluoride.

Lysosomal-like structures in plant cells and yeasts constitute a subject widely discussed in the literature[6]. In recent work, vacuoles of yeast and plant cells are increasingly coming to be regarded as organelles corresponding to lysosomes of animal cells. Using histochemical methods, POUX[10,11] has shown that vacuoles of wheat

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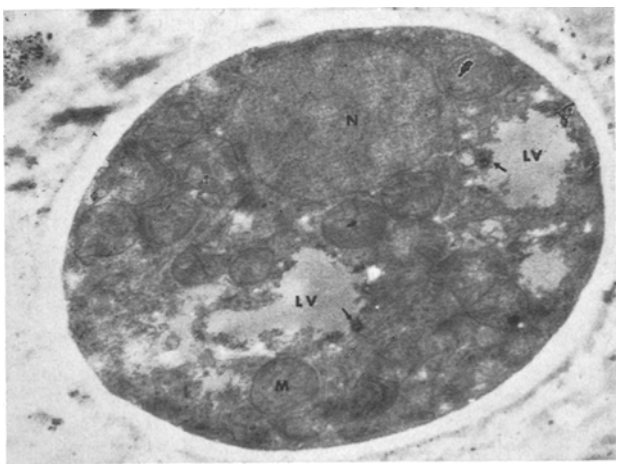

Fig. 1. Intact *Cryptococcus neoformans* incubated for 30 min in Gomori medium to demonstrate β-glycerophosphatase activity. A very small deposit of lead (arrows) is seen in the lysosomal vacuole (LV). N, nucleus; M, mitochondria. × 25,000.

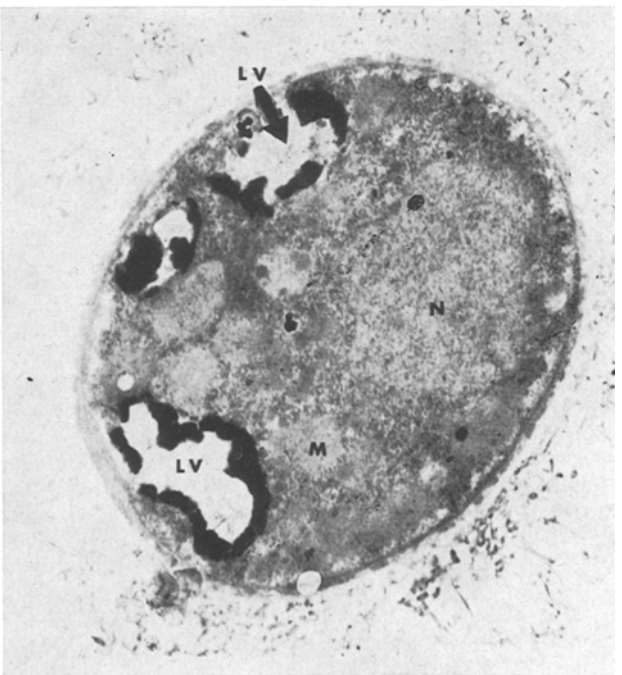

Fig. 2. β-glycerophosphatase activity in lysosomal vacuoles (LV) of *Cryptococcus neoformans* incubated for 30 min in the Gomori medium. Signs of damage to the cell membrane are visible. Uranyl-acetate stained preparation. × 20,000.

embryos and aleurone vacuoles contain acid phosphatase. Similar results were shown by BRANDES and BERTINI[12] in autophagic vacuoles of starving Euglena. Fractionation of vacuoles from yeast protoplasts have shown a very high specific activity of lysosomal enzymes[6,13]. The latter results indicate that the vacuoles are the only source of lysosomal enzymes within the yeast cells. The results given in the present communication also favour the notion that the vacuoles are the site of lysosomal enzyme activity.

Résumé. La localisation de la phosphatase-acide dans la levure *Cryptococcus neoformans* est étudiée ici par des méthodes cytochimiques au niveau ultrastructural. On a trouvé que l'enzyme se concentrait exclusivement dans les vacuoles, ce qui s'accorde avec les résultats que d'autres auteurs ont obtenus sur la localisation de l'enzyme dans les cellules végétales.

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Anti-Gastrin Immunofluorescence in the Skin of *Hyla crepitans* and the Cytochemistry of the Cells Involved

In 1966 ERSPAMER et al.[1] reported on the presence of a polypeptide in the skin of Australian and South American tree frogs, especially *Hyla caerulea*. They suggested the name caerulein for the new product and described its effects as well as its chemical structure. A most important observation, by the same group of workers[2], was the recognition that the amino-acid sequence of a portion of the new decapeptide was identical with that of a corresponding portion of the gastrin molecule. Confirmation of this observation has come from the immunochemical studies of MCGUIGAN[3].

Because of our interest in polypeptide-secreting endocrine cells, especially those belonging to the APUD series[4–7], our attention was drawn to this new discovery. We report here the results of immunofluorescence studies on the skin of a South American tree-frog *Hyla crepitans*, and on the chief cytochemical characteristics of the cells giving positive immunofluorescence with anti-gastrin sera.

Skin from the ventral and dorsal surface of *Hyla crepitans* was studied. The material was processed as follows: (1) Small portions of skin were quenched in melting Arcton 22 (Freon 22) for (a) cryostat sections for immunofluorescence and enzyme cytochemistry, and (b) freeze-drying and subsequent treatment with formaldehyde vapour. (2) Other small portions of skin were fixed in glutaraldehyde or in glutaraldehyde-picric acid[8] for investigation of masked metachromasia. (3) Small blocks were fixed in glutaraldehyde and embedded in Epon for electron microscopy.

Antibodies to human synthetic gastrin I (I.C.I.) were produced by repeated immunization of white rabbits by the procedure of BOYD and PEART[9]. Antiserum obtained 14 days after the last immunization was passed through a DEAE Sephadex column to obtain a pure IgG fraction. Normal rabbit serum treated in the same way was used as a control. Cryostat sections (4 μ), unfixed and post-fixed in cold formol-calcium, were used for indirect immunofluorescence with labelled goat anti-rabbit globulin (Microbiological Association Inc.).

The controls used were: (1) fluorescent goat anti-rabbit IgG, alone: (2) normal rabbit IgG followed by fluorescent anti-rabbit IgG; (3) rabbit anti-human gastrin serum after absorption with purified human gastrin I.

Cytochemical reactions characteristically positive in cells of the endocrine polypeptide (APUD) series were also applied. They were: phosphoglycerate dehydrogenase, non-specific cholinesterase, non-specific esterase, masked

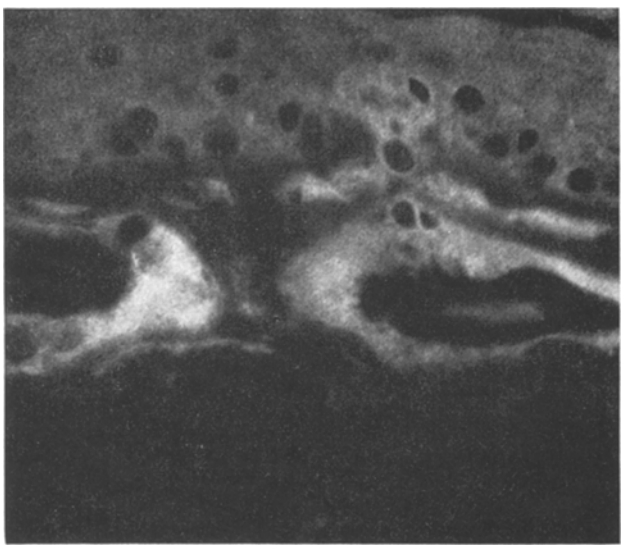

Fig. 1. Skin of *Hyla*, fresh-frozen cryostat section. Anti-gastrin immunofluorescence. Indirect method using rabbit anti-human gastrin I and fluorescein-labelled goat anti-rabbit γ-globulin. × 620.

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