tive to treatment compared to what happens in the subsequent stages. This hypothesis might be explained by possible structural variations in the enzymatic proteins or by the relative PLP concentration in the crude extract because of the difference in the hydratation of unripe and ripe materials.

During storage, CP and CV germination is similar and remains fairly constant for the 1st 4 years: on the other hand, the differences in the GAD activity are very pronounced, the level of activity being higher in CP embryos than in CV embryos. When both seeds are unable to germinate, this enzymatic activity also ceases: therefore this activity appears to be directly correlated to the germination capacity of seeds and their functional activity. This pattern also tallies with that of *T. durum* during seed ageing[10], even if the lifespan of *durum* wheat is possibly longer.

The addition of PLP improves the GAD activity of CP embryos more than that of CV embryos, thus pointing to a possible difference in the reactivation of the GAD of CP and CV embryos during storage. PLP treatment, however, is incapable of modifying the harmful effects of ageing also to be seen in the loss of germination capacity when seeds are older.

The pattern of the α-amylase activity both during seed ripening and during ageing makes it possible to differentiate between CP and CV seeds. Moreover, the differences to be seen in this enzymatic activity do not appear to have any effect as to germination during ripening and seed ageing. The increase in the amylolytic activity which occurs in CP endosperm is not accompanied, during ripening, by an improvement in germination energy. This confirms the finding that germination energy in ripening seeds is dependent upon growth regulators[18] more than upon the fact that storage products are made available to the embryo by the amylolytic activity. Similar conclusions may be drawn from the values of α-amylase in the endosperm during ageing. CP seeds, which loose their germinability in the 5th year of storage, show the same α-amylase activity when in dry conditions as well-germinating younger seeds do, and the α-amylase activity decreases in CV independently of the vigour of the seeds involved.

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Methods of metal incorporation into intracellular granules[1]

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Summary. The hepatopancreas of the garden snail *(Helix aspersa)* contains basophil cells which produce intracellular granules of $CaMgP_2O_7$. A variety of metals are incorporated into these granules either by direct substitution or by the synthesis of new pyrophosphate material.

Recent studies have shown that in virtually every group of invertebrate animals there are cells capable of producing intracellular inorganic granules[2-4]. These granules frequently contain a wide variety of environmentally available metals and therefore have often been interpreted as part of a cellular detoxification process[5].

In the common garden snail *(Helix aspersa)* pollutant metals become concentrated in the hepatopancreas tissue[6,7] and subsequent studies have identified the basophil cell as the site of this activity[8]. A variety of metals are taken up by these cells[9] and incorporated into granules which have been shown to be largely $CaMgP_2O_7$[10]. Pyrophosphates are in many ways ideally suited for such a cellular detoxification system for they are produced in large quantities by most anabolic processes and form extremely insoluble salts with a wide variety of metal ions[10]. The mechanisms whereby metals are deposited in these granules are not known but virtually all known examples of these concretions have been shown to be amorphous to X-rays. It is known that Mn^{2+} is incorporated into these granules extremely rapidly[9] and it was therefore decided to study the mechanism of metal incorporation by using this ion as a probe.

The snail *H. aspersa* was collected locally and used throughout this work. The animals were maintained in the laboratory on a diet of carrots, lettuce, cabbage and $CaCO_3$ powder for at least 2 weeks before use. Starved animals were left without food for 2-3 days. Three intravascular injections of 200 µl of 100 mM $MnCl_2$ in snail saline were given over a period of 30 h via a cannulated optic tentacle[9]. Granules were extracted from the hepatopancreas of the snail 24 h after injection by homogenizing the tissue and centrifuging the material in distilled water until a clean white deposit was obtained. The granules were spread on a grid and examined in a JEOL 100S electron microscope with ASID 5 using SEM. X-ray microprobe studies were performed on the same instrument using a Kevex detector and a Link analyser.

Starved snails will clear their blood of an injection of ^{54}Mn within 6-12 h. Most of this material can be recovered from the hepatopancreas of these animals and X-ray microprobe

Ratios of Ca/P and Mn/P in various regions of the granules of a) starved and b) fed snails following the intravascular injection of Mn^{2+} ions. Data are based on peak-background counts for granules from starved snails using sectioned material. These results show that both ions are evenly distributed across the diameters of these granules. Data for fed snails were obtained from intact granules and show that towards the outer edge and in the irregular surface layers Mn replaces Ca as the major cation. All readings are means ± SD for 6 sets of analyses

Starved snails	Centre of granule	Middle region	Outside region
Ca/P	0.52 ± 0.11	0.57 ± 0.07	0.52 ± 0.06
Mn/P ($\times 10^{-1}$)	0.95 ± 0.55	0.73 ± 0.38	0.95 ± 0.68
Fed snails	**Centre of granule**	**Edge**	**Irregular layer**
	0.49 ± 0.08	0.49 ± 0.16	0.23 ± 0.11
	0.58 ± 0.41	2.62 ± 1.06	3.75 ± 0.62

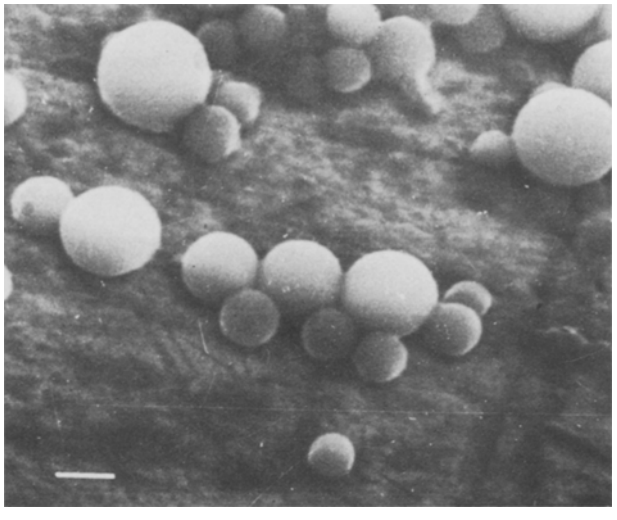

Figure 1. Spherical granules isolated from the hepatopancreas of *H. aspersa* after injection of Mn^{2+} into a starving animal. Bar = 2 µm.

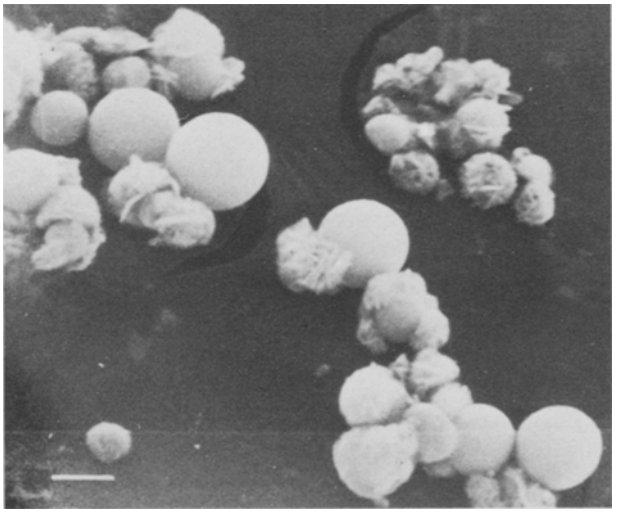

Figure 2. Irregularly shaped granule isolated from the hepatopancreas of *H. aspersa* after injection of Mn^{2+} into a fed animal. Bar = 2 µm.

analyses of the spherical granules from this tissue (fig. 1) show a clear Mn peak in the energy spectrum (fig. 3). It was expected that this Mn would be deposited as a distinct layer on the outside of these granules but it is impossible to decide this on these intact spheres because of the difficulty in determining the depth of beam penetration into the mineral. Granules were therefore embedded in Taab resin and despite their extreme toughness, it was possible to

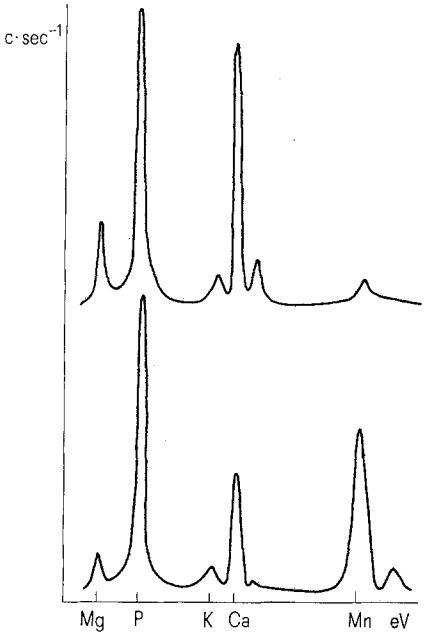

Figure 3. X-ray microanalyses from the centre of a spherical granule (upper) or from the irregular region of a granule isolated from a fed animal (lower). All analyses were carried out at 60 kV with a spot size of 80 nm for 100 sec counting time. Both animals had received an injection of Mn^{2+}. Note the large concentration of this metal in the lower trace.

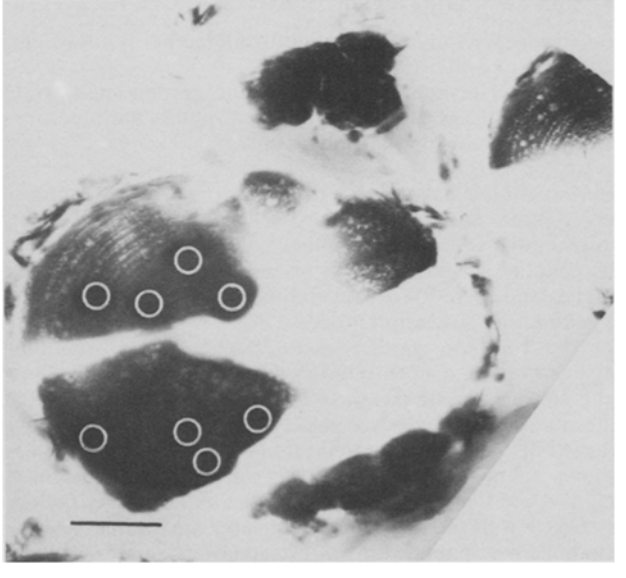

Figure 4. Section of one of the granules shown in figure 1. The granules have a concentrically layered structure and analyses were only undertaken on slices where this arrangement was visible i.e. where both the upper and lower surfaces of the granule had been removed. Circled areas indicate sites of analyses. Bar = 0.5 µm.

section them with a diamond knife. Spot analyses from the centre to the outside of these granules (fig. 4) showed no detectable difference in Ca/P or Mn/P ratios (table). It appears therefore that metals may be incorporated into these granules by permeation through the amorphous inorganic structure rather than by the synthesis of new material upon the outside of the granule.

Using fed snails produced a different set of results. Granules from these animals were frequently no longer spheres but possessed rough outer surfaces (fig. 2). X-ray analysis of this material showed that the main cation in these outer regions was Mn (table) which appeared to be associated with newly synthesized pyrophosphate (fig. 3).

It appears therefore that intracellular granules may be capable of accumulating metal ions by 2 distinct processes. Metals may penetrate the amorphous structure and become bound to pre-existing anionic groups or new material may be synthesized and added to the outside of these deposits. This latter process appears to be facilitated by feeding and this would be in keeping with the formation of pyrophosphate ions by anabolic processes in the cell. It is therefore likely that there is a correlation between feeding and metal detoxification systems in these invertebrates. This interpretation is supported by the fact that faecal strands from these snails show both types of intracellular granules demonstrating that they are exocytosed from the cells and thereby provide a route for the removal of metals from the body of the snail.

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Differential action of penicillin and UV-light on endosymbionts of the ciliate *Euplotes crassus**

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Summary. In this work the action of penicillin and UV-light on the cytoplasmic endosymbionts present in 6 different stocks of *Euplotes crassus* is investigated and their different levels of sensitivity to these forms of treatment are analyzed. The loss of endosymbionts does not appear to hamper the survival of *Euplotes* or to reduce their fission rate.

Endosymbionts are assumed to be bacteria of common occurrence in specimens of the stocks of the marine ciliate *Euplotes crassus*[1-3]. Cytoplasmic endosymbionts are morphologically distinguishable in 4 types (A, B, C, D) according to Rosati et al.[3]. In addition these authors subdivided the type B into at least 3 kinds according to the properties they confer on the host cell, i.e. mate-killer, killer, or the absence of any easily recognizable characteristic. These subtypes have been called B_1, B_2 and B_3 respectively by Rosati and Verni[4].

Endosymbiont-free cells, giving rise to sensitive clones, were obtained from killer stocks of *E. crassus* by means of penicillin or UV light treatment[5]. Fauré-Frèmiet[6] had shown that penicillin removes endosymbionts from the cytoplasm of *E. eurystomus* and *E. patella,* and the endosymbiont-free cells become unable to originate viable clones. A similar phenomenon has been thoroughly analyzed by Heckmann in *E. aediculatus*[7].

In this work the action of penicillin and UV light on the cytoplasmic endosymbionts present in 6 different stocks of *E. crassus* is investigated, and their varying sensitivity to the treatments is analyzed.

Materials and methods. The stocks of *Euplotes crassus* used in this investigation were collected along the Somalian coast (C_8, M, W_5, G_{III} and G_{VI}) and the Tuscan coast near Leghorn (21A7). All the stocks were grown with *Dunaliella salina* inoculated in Erd-Shreiber medium. They were kept at the constant temperature of 22 ± 1 °C in our laboratory reproducing at a rate of about 2 divisions per day.

Antibiotic treatment. 100 cells were put in a depression slide in presence of food and penicillin (250 or 500 units/ml). After 2 days, 20 cells were picked up and transferred singly into fresh food with penicillin. These cells were allowed to divide so that 20 lines were obtained. After 2 more days 1 cell was isolated from each line. This isolation procedure was repeated for each stock every alternate day 3 times (7 days of treatment) and 7 times (15 days of treatment).

UV-treatment. Cells were put in a glass depression slide containing 0.5 cm^3 of artificial sea water, corresponding to a depth of 3 mm, then irradiated at a distance of 10 cm from a 15 W germicidal Champion G15T8 lamp providing 253 nm UV-light. The cells received approximately 22 erg/sec/mm^2.

EM-techniques. Cells were fixed in 1% OsO_4 in phosphate buffer (0.1 M, pH 7.4) dehydrated in alcohol and embedded in Epon Araldite mixture. Sections were observed using a Siemens Elmiscope 101 EM, after staining with Reynolds solution.

20 cells of each stock were fixed after 7 or 15 days of penicillin treatment. 5, randomly chosen, cells for each of the kinds of treatment were cut and 8-9 grids, containing 10 sections on the average, were observed per cell. Only 1 section, chosen among the largest on each grid, was photographed. As 8-9 photographs correspond by and large to 10 surface units (1 unit = 100 μm^2), about 5000 μm^2 of each stock were examined after 7 or 15 days of penicillin treatment. The UV-treated cells were similarly analyzed under the EM.

Results. The effect of penicillin and UV-treatment was measured by counting the number of endosymbionts found per surface unit, compared with the number present in the control cells. The results are reported in the table.

If we assume that type A endosymbionts are identical irrespective of the host harbouring them, these endosym-