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-

Stocks	Phenotype	Controls Types of endosymbionts (No./100 μm²) A	B_1	B_2	B_3	C	D	Treatments (penicillin) Percent of endosymbionts removed by 2 dosages 250 units/ml 7 days of treatment		250 units/ml 15 days of treatment		500 units/ml 7 days of treatment		500 units/ml 15 days of treatment		Treatments (UV) Percent of endosymbionts removed 2'30" of exposition 7 days of treatment	
21A7	SK-SMK	1.02	-	-	-	-	-	1.96	-	44.10	-	28.43	-	100	-	60.78	-
C_8	MK	0.34	3.16	-	-	-	-	52.94	100	100	100	85.29	100	100	100	79.41	100
M	K	0.28	-	3.74	-	-	-	57.14	54.0	100	100	100	77.54	100	100	100	79.10
W_5	SK-SMK	0.37	-	-	3.28	-	-	45.94	10.0	100	45.73	100	65.51	100	100	98.47	100
G_{III}	SK	-	-	-	-	-	1.68	-	52.38	-	100	-	69.64	-	100	-	100
G_{VI}	SK-SMK	-	-	-	-	1.22	-	-	39.34	-	100	-	67.21	-	100	-	100

In the columns of the 2 panels on the right (treatments) the figures on the left refer to the type A endosymbionts. The percentages in the 2 panels on the right (treatments) refer to the corresponding figures in the left panel (controls), which are considered equal to 100%. K, killer; SK, sensitive to killing action; MK, mate-killer; SMK, sensitive to mate-killing action.

bionts appear to be more resistant when present alone in the cytoplasm of *Euplotes crassus.* When they coexist with type B endosymbionts, their resistance decreases in all cases. This phenomenon is particularly evident when the cells are treated for 7 days with 500 units/ml of penicillin; in fact, while in the stock 21A7 after this treatment only 28.43% of A endosymbionts are removed, in the stock M, for example, they completely disappear.

In the endosymbionts of group B different degrees of resistance are present: the B_1 is removed following any kind of treatment; B_2 shows only some resistance to the mild treatment (250 units/ml for 7 days); while a large percentage of B_3 is removed only by treatment with 500 units/ml. Types C and D both show the same resistance as type B_2.

Also in the case of the UV-treatment it can be noted that the maximum degree of resistance is shown by type A endosymbionts when they are the only type present in the cytoplasm of the host cell. Among the other types of endosymbionts only B_2 shows a little resistance to this treatment. From the results reported in the table it is evident that the host cells can get rid of their endosymbionts with both treatments.

Cells treated with penicillin for 14 days at 500 units/ml were followed for some weeks more, and found to remain free of endosymbionts. They continued to divide at the same rate as cells containing endosymbionts. Apparently the presence of endosymbionts is not essential to the life of the *E. crassus* stocks examined here.

Discussion. From the results it appears that all types of endosymbionts are more sensitive to UV-treatment than to penicillin. This may be due to the different types of action of the 2 agents: the former affecting DNA synthesis, and the latter the synthesis of the bacterial cell wall[8].

The fact that the type A endosymbionts show a different resistance whether they are alone or coexisting with type B endosymbionts is interesting. Type A endosymbionts probably established a relationship with *Euplotes crassus* cells before any other type of endosymbiont. This hypothesis is supported by the fact that type A endosymbionts are the most widespread, in fact they are present in all the non-autogamous stocks examined, which have been collected from different geographical regions[3]. It is possible that the presence of a 2nd type of endosymbiont causes a change in the relationships between the A endosymbionts and the host cell and the endosymbionts become more sensitive. The presence of 2 different types of endosymbionts has also been observed in the cytoplasm of *E. eurystomus*[9]. In this case, however, nothing is known about a possible effect on the reciprocal relationships with the host cell.

As regards B endosymbionts the results obtained confirm that, although they are morphologically indistinguishable, they differ from each other. The differences consist not only in the properties they confer on the cell[3] but also in the differential resistance to the treatments. Less resistance is shown by the B endosymbionts namely B_1 and B_2 responsible of the mate-killer and killer trait in *E. crassus.* The mate-killer and killer stocks appear to be scanty in natural populations; for example among stocks collected in different regions and examined during a study on the mating-type system only 1 mate-killer and 2 killer stocks were found (Dini and Luporini, personal comunication). It cannot be exluded that the rarity of these 2 traits is due to the fragility of the endosymbionts which are responsible for them. On the other hand, the relationships between mate-killer[10] and killer[5] cells and their endosymbionts are under nuclear control and only the stocks with a determined genotype are able to maintain these types of endosymbiont. In this regard it is possible that in these cases UV-light acts on the endosymbionts both directly and indirectly through its effects on the genes of the host cell.

The C and D endosymbionts appear to resemble those of type B in their morphology and degree of resistance. The morphological differences observed between them and the type B endosymbionts may be due to the reproductive isolation of the autogamous stocks in which they live[11].

Few cases are known where endosymbionts have become essential for the host cell. One of these is the *lambda* particles of *Paramecium aurelia*[12]; another case is represented by the endosymbionts of *Urostyla grandis*[13]. As mentioned above, some fresh water species of *Euplotes,* in contrast to the marine *E. crassus,* are also unable to survive and reproduce when completely deprived of endosymbionts[6,7]. It might be suggested that the endosymbionts settled in *E. crassus* cells more recently than in fresh water species.

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