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Biologia. — *Inhibition of cell sponge reaggregation by bonellin.* Nota di MARINA DE NICOLA GIUDICI (*), presentata (**) dal Socio G. MONTALENTI.

RIASSUNTO. — È stato dimostrato che la *bonellina*, il pigmento verde della *Bonellina viridis*, provoca la disaggregazione delle cellule della spugna marina *Axinella verrucosa* e impedisce la riaggregazione delle cellule separate in acqua di mare priva di ioni bivalenti. L'azione della *bonellina* si manifesta solo in presenza della luce. Viene discussa l'ipotesi che gli effetti osservati siano provocati da un'alterazione definitiva della membrana cellulare.

INTRODUCTION

Previous work has shown that *bonellin* [1], a natural chlorin, extracted from *Bonellia viridis*, irreversibly affects embryonic cells of echinoderm [2, 3] and human erythrocytes [4] by inducing modifications of plasmatic membrane.

Since studies on the reaggregation process of dissociated sponge cells [5] have led to new insights into the problem of cellular recognition, I have tested *bonellin* on the marine sponge *Axinella verrucosa.*

The results presented here show that *bonellin* causes sponge dissociation also in presence of divalent cations and irreversibly inhibits the reagregation of dissociated single cells.

MATERIALS AND METHODS

The marine sponge *Axinella verrucosa* was used freshly collected. Small pieces of blotted tissue were fragmented and submerged in calcium and magnesium-free artificial sea water (CaMgF–SW) at pH 7 and 0 °C and soaked for 30 minutes. The resulting cell suspension was filtered through cloth, sedimented for 2 minutes at 200 rpm and the isolated cells were resuspended in fresh cold CaMgF–SW.

For reaggregation experiments, the cell suspension was sedimented, resuspended in Millipore filtered sea water (SW) at concentration of 40×10^6 cells/ml and placed in rotating flasks at 22 °C.

Bonellin was purified according the method previously described [4]. The *bonellin* stock solution in SW was prepared by dissolving the pigment (0.2 mg/ml) in 0.1 M Na_2CO_3 in filtered SW. The final concentration of *bonellin* used in the experiments was 9.5×10^{-70}.

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Two different treatments were used:

a) dissociation of sponge tissue was performed as previously described using instead of CaMgF–SW, the bonellin solution under light irradiation for 10 minutes (60 Watt tungsten lamp).

b) single cells, obtained by dissociation of the tissue in CaMgF–SW, were suspended in bonellin solution and irradiated for 10 minutes, washed twice and resuspended in Millipore filtered SW as the control for reaggregation experiments.

Cell suspension controls were examined under the light microscope.

Results

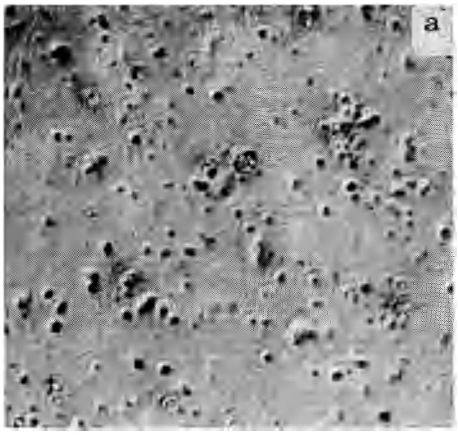
The dissociation of *Axinella verrucosa* tissue in single cells takes place after 30 minutes of soaking at 4 °C in CaMgF–SW (Plate I, *a*) or in bonellin solution (Plate I, *d*). When dissociated cells were placed in SW they began to adhere to each other and the aggregates thus became larger. The aggregates attained the maximum size after about 6 hr (Plate I, *b* and *c*) at 22 °C and about 12 hr at 4 °C. No aggregation occurs with cells dissociated in bonellin solution (Plate I, *e*) or with cells dissociated in CaMgF–SW and subsequently treated with bonellin. If bonellin treatments were performed in the dark, the cells aggregate like the control.

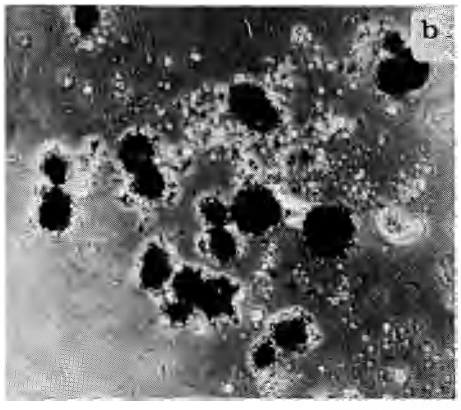
Discussion

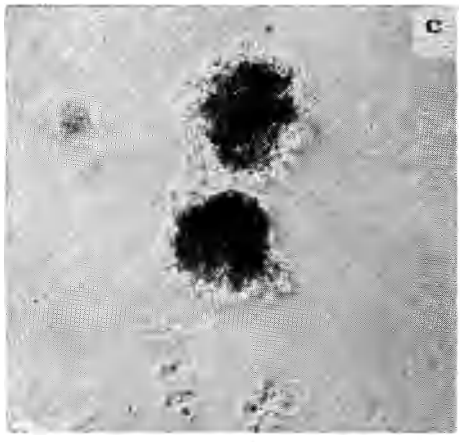
It is known [6] that the adhesive process in sponge cells is mediated by specific aggregation factors (AF) which, in the presence of Ca^{2+} act as a ligand binding the cells through cellular membrane receptors [7]. In the absence of divalent cations, the AF is released into the medium and the tissue cells undergo dissociation. The AF is species–specific and the reaggregation inhibition occurring in the presence of heterologous factors (HAF) has been interpreted as a competitive saturation of cell membrane receptors by HAF molecules [8].

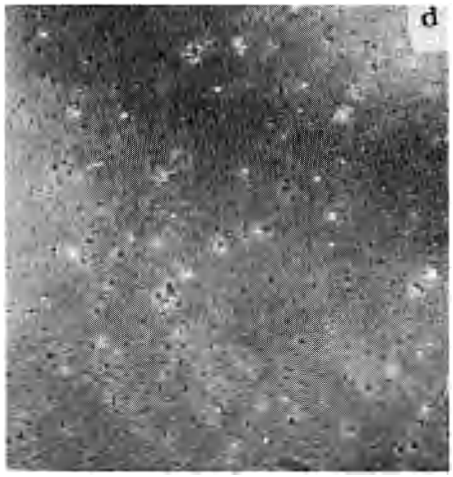
Since bonellin inhibits the aggregation of cells from dissociated tissue of *Axinella verrucosa*, it could be hypothesized that the chlorin affects the production of AF factor or its binding to the cell surface. However the fact that the action of bonellin is irreversible and occurs in the presence of Ca^{2+} under conditions of light and that it causes also dissociation of tissue sponge suggests that these effects can be ascribed to a definite modification of the cellular membrane. In agreement with this hypothesis recent results [4] show that bonellin, involving a photoactivated process, causes cross-linking of membrane protein and formation of cholesterol peroxidase in human erythrocytes and in echinoderm eggs.

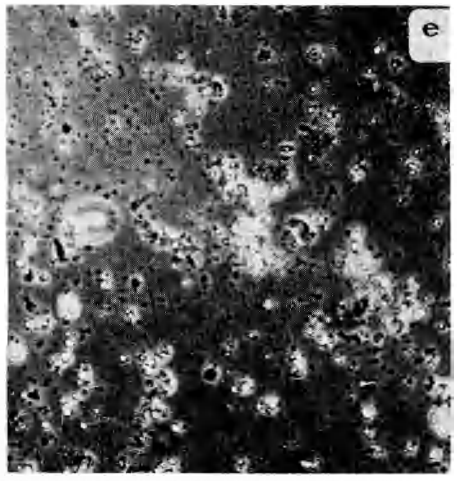
Last, it should be pointed that bonellin acts at different levels of biolological organization, i.e. in human cells, in echinoderm cells and in sponge cells.











a) Dissociated cells in CaMgF–SW. (448).

b), *c*) Reaggregated cells after 3 hr and 12 hr at 22 °C in SW. (×448).

d) Dissociated cells in bonellin solution. (×448).

e) Bonellin treated cells in SW after 12 hr at 22 °C. (×448).

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