# RADIOBIOLOGICAL AND RADIOECOLOGICAL STUDIES WITH THE UNICELLULAR MARINE ALGAE *Acetabularia, Batophora* and *Dunaliella.*

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Unicellular marine algae are particularly useful for investigating the effects of ionizing radiations on living organisms as well as for studying the radioactive contamination of the aquatic ecosystem (1,2). *Acetabularia* (*A. crenulata, A. mediterranea, A. peniculus*) and *Batophora* (*B. oerstedii*) are giant unicellular uninucleate green algae,containing several million cytoplasmic organelles (chloroplasts and mitochondria). *Dunaliella* (*D. bioculata*) is a flagellated microalga, belonging to the Volvocales. These algae are being used in our laboratories for biological, radiobiological and radioecological studies. Due to the development of nuclear facilities, a detailed knowledge of the effects of radiations and of the biological behaviour of radioactive substances in the biosphere is urgently needed. This paper deals with the biological and the biochemical effects of X-rays (*Acetabularia, Batophora*) and with the incorporation of $^{3}H$ (*Acetabularia, Dunaliella*).

## MATERIALS AND METHODS

Most of the methods used have been previously reported (1,2,3). *Acetabularia* and *Batophora* cells were irradiated with increasing doses of X-rays (from 0 to 150 Kr) during their vegetative growth (stage 4) (4). Labeling experiments were performed with *Acetabularia* at stage 4 or with *Dunaliella* being in its stationary phase (about $2x10^{6}$ cells/ml). Tritiated organic molecules, obtained from CIS Association, were added to the culture medium of the algae during various periods of time. Radioactivity was measured in a liquid scintillation spectrometer. The intracellular concentration of radioactivity was calculated on the basis of the cells' fresh weight. *Acetabularia* chloroplasts were observed with Nomarski interference optics. The starch content was determined with the Boehringer hexokinase test after hydrolysis of the storage material with α-amyloglucosidase.

## RESULTS

### Radiobiological studies.

a) Biological effects of X-rays on *Acetabularia mediterranea*. X-rays interfere with the morphogenesis of *A. mediterranea*. The formation of the reproductive cap and of cysts (gametangia) are strongly reduced only at relatively high doses (> 50 Kr). Several types of morphological anomalies are observed in irradiated cells : 1) loss of the whorls; 2) enlargement of the first order articles of the whorls; 3) development of a new stalk from a hair of the whorls or from a branch of the rhizoid; 4) enlargement of the apex; 5) alteration of the cap symmetry; 6) irregular growth of the caps'

rays; 7) formation of irregularly shaped cysts in the caps' rays; 8) increase of cyst size; 9) cyst degeneration; 10) loss of or impaired compartition of the cytoplasm in the caps' rays prior to cyst formation. Light microscopical observations have shown the presence of elongated chloroplasts having large starch granules. Analyses performed with amyloglucosidase and hexokinase have revealed that starch accumulation in the chloroplasts increases with the radiation dose.

b) Biological effects of X-rays on *Acetabularia peniculus*. The morphogenetic processes of *A. peniculus* are affected by increasing doses of X-rays (Fig. 1A, B). Again, relatively high doses (> 50 Kr) are necessary to inhibit cap and cyst formation. Several types of morphological anomalies were found in irradiated cells : 1) loss of the apical whorl; 2) enlargement of the apical region of the stalk; 3) formation of irregularly shaped caps' rays; 4) reduction of the number and of the size of cap rays (see Fig. 1B); 5) increase of cyst size; 6) cyst degeneration; 7) absence of cyst formation in some caps' rays. Moreover, some irradiated cells turn dark green, suggesting a condensation of the cytoplasm and/or chlorophyll accumulation in the chloroplasts.

c) Biological effects of X-rays on *Batophora oerstedii*. In irradiated *B. oerstedii* cells the formation of the spherical compartments (sporangia), where later on cysts (gametangia) develop, is only delayed by the radiations (Fig. 1C, D). This finding shows that the morphological differentiation of *Batophora* is extremely radioresistant. X-rays, however, induce in *Batophora* several types of morphological anomalies; 1) reduction of the number of sporangia (see Fig. 1D); 2) formation of abnormal sporangia; 3) development of one or more sporangia along the first or second order articles of the whorls instead of at their tip; 4) enlargement of first order articles; 5) enlargement of the apical region of the stalk; 6) cyst formation in the first or second order articles of the whorls; 7) sporangia degeneration.

d) Biochemical effects of X-rays on *Acatebularia mediterranea*. Labeling experiments with thymidine-6-$^{3}$H, uridine-5-$^{3}$H and leucine-$^{3}$H have revealed that X-rays (50 Kr) provoke a strong reduction of DNA, RNA and protein synthesis in the chloroplasts. RNA synthesis was stimulated, however, for doses up to 25 Kr.

Radioecological studies.

a) Experiments with tritiated water (HTO). When *Acetabularia* cells (*A. crenulata, A. mediterranea*) are grown in the presence of HTO (0-5 µCi/ml), a significant amount of $^{3}$H is incorporated in the total nucleic acid and protein fraction (2). However, $^{3}$H supplied in the form of tritiated water is not accumulated by the algae.

b) Experiments with tritiated organic molecules. Since recent work suggested that $^{3}$H may be accumulated when this element is bound to organic molecules (see ref. 3), we have studied the uptake of 10 different tritiated organic molecules by *Acetabularia mediterranea* and by *Dunaliella bioculata* : 1) thymidine-methyl-$^{3}$H; 2) adenine-2-$^{3}$H; 3) uridine-5-$^{3}$H; 4) L-leucine-4-$^{3}$H; 5) glycine-2-$^{3}$H; 6) L-arginine-3.4-$^{3}$H; 7) L-aspartic acid-2.3-$^{3}$H; 8) L-phenylalanine-

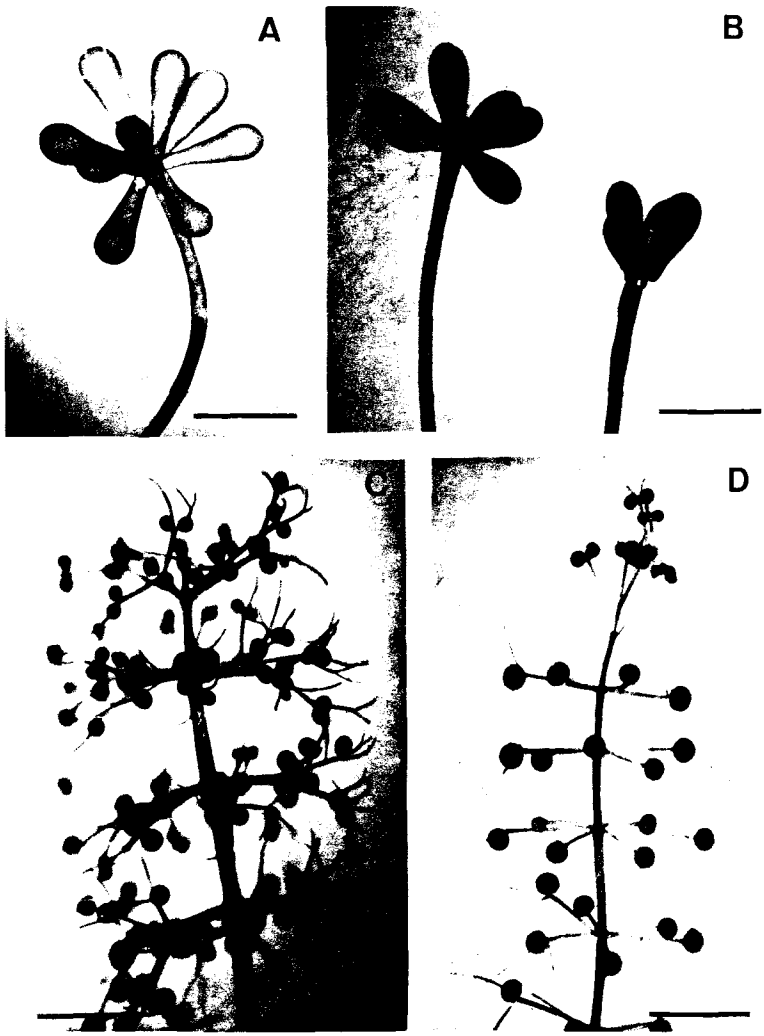


Figure 1. Morphological effects of X-rays on *Acetabularia* and *Batophora*. A : control cells of *A. peniculus* bearing a normal reproductive cap with 9 rays; B : 2 *A. peniculus* cells, 17 days after the irradiation with a dose of 150 Kr, showing a reduced number of caps' rays. C : Control cell of *B. oerstedii* showing the typical spherical sporangia, where later on cysts (gametangia) develop; D : *B. oerstedii* cell, 30 days after the irradiation with a dose of 50 Kr, having a reduced number of sporangia. Scale = 2 mm.

2.3-$^{3}$H; 9) D-glucose-1-$^{3}$H; 10) D-glucose-6-$^{3}$H. After a short incubation (30 min.), the intracellular concentration of the tritiated molecules can reach that of the external medium. However, *Acetabularia* accumulates adenine, arginine and glucose (respective concentration factors : 4.6; 5.1; 5.7), and *Dunaliella* is capable of concentrating adenine and leucine (respective concentration factors : 122.7; 11.4).

## DISCUSSION

Our radiobiological studies show that the main morphogenetic processes of *A. mediterranea*, *A. peniculus* and *B. oerstedii* are affected by the radiations. Certainly, the sequence of events during the cells' developmental cycle is only realized under a well co-ordinated co-operation between the nucleus and the organelles. Most probably, X-rays interfere with this intergenomic co-operation, provoking different types of morphological anomalies. The radiations inhibit the syntheses of DNA, RNA and proteins in the chloroplasts of *Acetabularia* cells. Chloroplasts, which transform solar energy for the benefit of the cell, may play an important role for the realization of morphogenesis.

Experiments with tritiated water have revealed that *Acetabularia* cells are unable to concentrate $^{3}$H. However, a significant amount of this radionuclide is incorporated into the genetic material of the cells (3). When organically bound $^{3}$H is supplied to *Acetabularia* or to *Dunaliella*, a selective accumulation of some substances is observed. Our results contribute to a better understanding of the impact of radiations on living organisms and of the biological behaviour of $^{3}$H in the aquatic system.

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