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SHORT NOTE

Loss of Fertility in X-Irradiated *Acetabularia*

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ABSTRACT: In X-irradiated *Acetabularia* cells, cysts fail to germinate. However, meiosis of the primary nucleus and mitosis of secondary nuclei take place. The observed loss of fertility could result from a 'late' death effect or from deleterious interaction between nuclei of opposite sign inside multinucleate cysts.

Algae are known to be more resistant to ionizing radiation than higher plants or animals (Chipman, 1972). The unicellular marine alga *Acetabularia* shows a high tolerance to both X- and gamma rays (Bacq et al., 1955; Hämmerling, 1956; Six, 1958; Bonotto et al., 1970). However, early investigations on *A. Wettsteinii (= A. parvula)* and *A. mediterranea (= A. acetabulum)*• revealed that X-rays reduce fertility already at doses which have little effect on cell growth and morphogenesis (Hämmerling, 1956; Six, 1958). Since the process leading to loss of fertility is not yet understood, our investigations on the effect of X-rays focused on the reproductive phase of *A. acetabulum (= A. mediterranea)*.

Cells at Stage 4 (Bonotto and Kirchmann, 1970) were irradiated with single X-ray doses of 50 and 100 Kr and their morphological differentiation was continuously monitored. Table 1 lists representative data on the percentage of cells which had developed a cap (reproductive organ comparable to a sporangium) and cysts (gametangia) in the cap rays. The formation of cysts in 58 % and 11 % of cells, which had received a dose of 50 and 100 Kr respectively, indicates that meiosis of the primary nucleus in the rhizoid and subsequent mitotic divisions occurred, as well as the migration of the haploid secondary nuclei into the cap ray cytoplasm (for a recent review on meiosis and mitosis in *Acetabularia*, see Koop, 1979).

Upon microscopical inspection of cyst-bearing caps, morphological alterations became obvious (Fig. 1). In contrast to the rather uniform size and ovoid shape of cysts in control cells (Fig. 1 a), cysts in irradiated cells are highly variable in size and morphology (Fig. 1 b). Moreover, photographic recording of cyst development revealed the formation of multinucleate cytoplasm portions rather than uninucleate ones as in control cells (Lüttke and Bonotto, in preparation). Since the number of secondary nuclei visible in completed cysts exceeds that initially observed during constriction of the cytoplasm, further mitotic divisions must have taken place.

For the normal sequence of meiosis and subsequent mitoses before and after cyst formation, despite the morphological alterations, no prediction on gamete formation and germination could be made. Hence cysts from single caps were collected in separate test vials and their germination capacity checked after a preceeding dark period (Koop, 1975). The experimental protocol is outlined in Table 2 together with the developmental stage of the cells. In three experiments, carried out with cells of different stock cultures, germination occurred only once (1 out of 22 caps; this corresponds to 5 %).

A second attempt was made with single giant cysts – isolated, subjected to dark treatment, and then returned to light in order to induce germination. They equally failed to germinate. In either case most of the cysts degenerated and turned pale after a few days in the light.

Table 1. *Acetabularia acetabulum.* Cap and cyst formation in X-irradiated individuals

Dose (Kr)	No of caps•		%	No of caps with cysts•		%
0	33	(35)	94	27	(33)	82
50	48	(51)	94	28	(48)	58
100	46	(56)	85	5	(43)	11

• Numbers in parentheses state sample size

• Under the International Code of Botanical Nomenclature the correct name of *Acetabularia mediterranea* Lamouroux is now *Acetabularia acetabulum* (L.) Silva (Silva, 1952)

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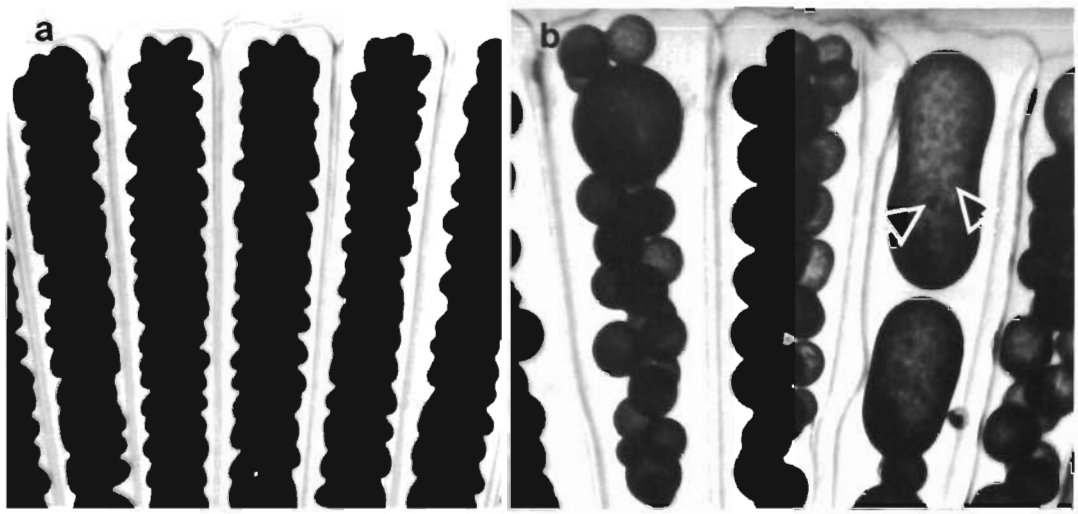

Fig. 1. *Acetabularia acetabulum*. *In situ* cysts of control and irradiated cells 44 d after exposure to a single dose of 50 Kr. (a) Control, 6 mm = 200 µm; (b) irradiated, 9.2 mm = 200 µm; arrows: secondary nuclei

Table 2. Germination of cysts from *Acetabularia acetabulum* after X-irradiation. Note: Scheme not on scale

Irradiation (date)	Cyst isolation (date)	Dark (months)	Light (date)	Germination* Controls	50 Kr
12/12/78	28/2/78	≈ 3	25/ 5/79	lost by infection	1 out of 22
13/12/79	29/2/80	≈ 4.5	14/ 7/80	5 out of 5	no out of 5**
8/ 7/80	23/9/80	≈ 2.5	4/12/80	9 out of 10	no out of 9

2n 1n 1n

* Each test vial contained cysts from 1 cap
** 6 cells developed (the potential germination per cap might exceed 10^6 cells: Schulze, 1939)

Considering recent observations on the cell cycle of *Acetabularia* (Koop, 1979) and our results, it seems safe to state that meiosis and subsequent mitoses occur even in cells irradiated with very high radiation doses. Though we have not, at present, a satisfactory explanation for the lack of cyst germination, we suggest two possibilities: (1) Lethality of cysts formed by X-irradiated cells is manifested only after several mitoses have elapsed; this 'late' death (Chipman, 1972) is evidenced by the failure to produce viable germlings and by cyst degeneration. (2) Since most of the cysts in irradiated cells are already multinucleate prior to the second burst of mitotic divisions, a completely abnormal situation arises during gamete formation; theoretically, gametes of opposite mating type would be produced in the same cyst (compartment); this situation might be incompatible with the formation and/or viability of gametes.

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