It has been noticed that in some of the oocytes of *T. c. carnifex*, while the majority of the loops were retracted, one loop remained inserted on one of the small bivalents of the complement; this loop is comparable to the giant fusing loops described by CALLAN and LLOYD[8] on the bivalents X and XI (Figure 5). As maturation proceeds, it has also been observed that the spheres tend to detach themselves from the chromosomal axes. Thus, in addition to oocytes with spheres still attached to their respective chromosomes, we have also noticed oocytes in which some or all of the spheres were absent. The detachment of the spheres could not be synchronous in the two homologues (Figure 4).

In the maturing oocytes the nucleoli appear larger in size, round and rich in vacuoli, which are sometimes fused into a single large central vacuole (Figure 4). As regards the nucleolus-organizing regions, in *T. c. carnifex* we have observed nucleoli inserted at the level of these loci, whether in chromosomes which still mantained the typical lampbrush morphology[12] or in chromosomes which were shortened and almost without loops (Figure 6).

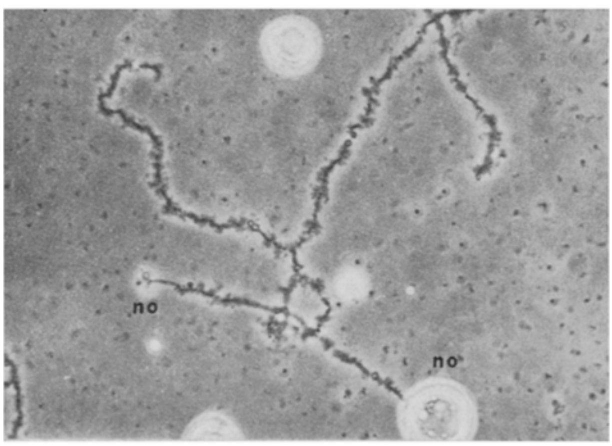

Fig. 6. Bivalent VI of *T. c. carnifex* with nucleoli attached to the nucleolus-organizing regions (no). 5 h from the beginning of incubation in progesterone. ×545.

For the most part, the results produced agree with the observations made on *Triturus viridescens*[5,6]. Thus, a method is available for inducing the in vitro oocyte maturation by progesterone also in the urodele amphibians. Consequentely investigations on the modifications of the lampbrush chromosomes in the period between the hormonal induction and the formation of the first meiotic spindle, become possible. Such observations could be relevant in relation to the problems of structure, organization, and physiology of the chromosomes and of the hormonal influences on the genoma. It appears to be of future interest to verify the biosynthetic activities of the residuous structures on the lampbrush chromosomes during the maturing period and, eventually, in relation to the successive stages of the embryonic development.

Riassunto. È stata indotta maturazione in vitro mediante progesterone in ovociti isolati pienamente accresciuti di *Triturus cristatus carnifex* e *Triturus vulgaris meridionalis*. È stata osservata la sequenza delle modificazioni morfologiche, successive all'induzione ormonale, interessanti i cromosomi lampbrush.

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Zinc Toxicity in Irradiated *Bacillus megaterium*

Combination treatments of metal ions with γ-rays change the radiation effect, but there is little information concerning the role of metal salts in modifying the radiobiological action[1–7]. In a previous paper it was reported that toxic concentrations of zinc chloride combined with γ-radiation had a synergistic inhibitory effect on the ability of *B. megaterium* to form colonies[8].

In the present study we further investigate the radiosensitivity of bacteria after a pre-treatment with a toxic concentration of zinc chloride and the sensitivity of the irradiated cells to the bactericidal action of the metal.

Cultures of *B. megaterium* (strain Elstre) were grown in nutrient broth (Difco) at 35 °C for 24 h. At that time, the culture was in the logarithmic phase with an optical density of 0.120.

Samples containing approximately 8×10^5 cells/ml were used for the following experiments: 1. Irradiation with γ-rays in a 3000 Ci cobalt-60 source at room temperature. The dose rate was 4,000 rads/min. 2. Irradiation as above of bacteria treated for 1 h with zinc chloride at a toxic concentration ($4 \times 10^{-5} M$). The chemical remained in contact with the bacteria during irradiation. 3. Exposure to various concentrations of zinc-chloride and determination of the lethal effect in irradiated and non-irradiated bacteria.

Viable counts of all experimental samples were made by the plating method and the results are presented in tabu-

Percentage of survivors

Time (min)	Zinc chloride ($4 \times 10^{-5} M$)	Combination of irradiation and $ZnCl_2$		γ-irradiation	Dose (rads)
		in pre-treatment	in post-treatment		
0	100	100	100	100	0
60	65 ± 3	38 ± 6	18 ± 6	65 ± 3	7,500
60	65 ± 3	27 ± 4	9 ± 3	48 ± 5	12,000
60	65 ± 3	11 ± 2.5	2 ± 0.6	27 ± 2	20,000
60	65 ± 3	7 ± 1.5		20 ± 1.5	26,000

lar form or as plot. The lower curves in both figures are normalized to 100% so that the slopes in each figure are directly comparable.

γ-irradiation of *B. megaterium* caused a significant decrease of survivors exponentially related to the dose. Exposure for 1 h to $ZnCl_2$ at a concentration of $4 \times 10^{-5} M$ decreased the survivors to 65%.

When bacteria treated with zinc chloride were irradiated in the presence of zinc, the number of survivors was further reduced to 38, 27, 11 and 7%. The irradiation doses were 7.500, 12,000, 20,000 and 26,000 rads respectively.

The results clearly suggest a synergistic effect of the metal and the irradiation for doses higher than 12,000 rads. After irradiation with doses equal or below 12,000 rads, the observed values are the expected ones from combined treatment of metal and irradiation.

The synergistic effect is more intense in a post-treatment with $ZnCl_2$ than in a pre-treatment. Even with 7,500 rads when there was no synergism in pre-treatment (38%) the survivors were reduced to 18% in a post-treatment (Figure 1 and Table).

The irradiated survivors are considerably more sensitive to the deleterious effect of zinc chloride than are the non-irradiated ones. The sensitivity to zinc depends on the irradiation dose.

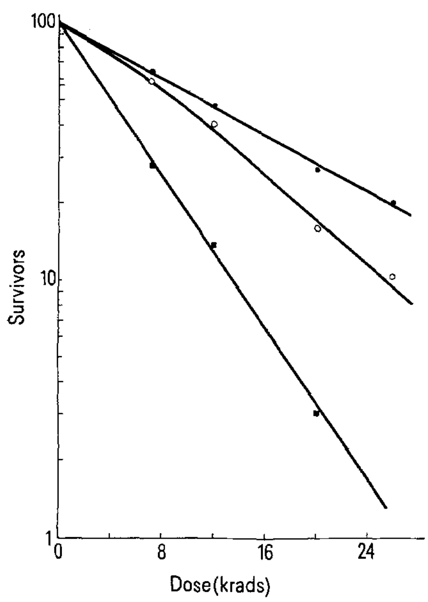

Fig. 1. Survival curves of *B. megaterium*, irradiated with γ-rays (upper curve) pretreated with $ZnCl_2$ (middle curve) and post-treated (lower curve).

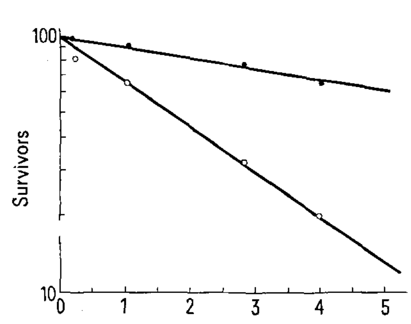

Fig. 2. Zinc toxicity on different concentrations in non-irradiated (upper curve) and irradiated (lower curve) cultures of *B. megaterium* (dose of irradiation sufficient to reduce the population initially to 25% and after 1 h to 10%).

Non-irradiated bacteria, immerged to the above concentration of $ZnCl_2$, showed 65% survivors. When bacteria were first irradiated with 7,500, 12,000 and 20,000 rads, then treated with the same concentration of zinc, the survivors were 18, 9 and 2% respectively. The sensitivity of irradiated bacteria towards $ZnCl_2$ varies also with the concentration of this substance.

Cultures irradiated with 20,000 rads contained 25% survivors. The same bacteria left for one hour in the irradiated medium were further reduced to 10%. If $ZnCl_2$ was added and left in contact with the irradiated cultures for the same period of time, in concentrations as high as $2 \times 10^{-6} M$, $1 \times 10^{-5} M$, $2{,}8 \times 10^{-5} M$ and $4 \times 10^{-5} M$ the resulting reductions were 8, 6.5, 3.2 and 2 % respectively (Figure 2).

The synergistic inhibitory effect exerted by $ZnCl_2$ and γ-irradiation on *B. megaterium* when zinc was present during irradiation, could be attributed to a radiosensitization brought up by zinc. This apparent sensitization may also result from toxic injuries to the cells, induced by the metal and the radiation.

Irradiated cultures left for some time without any further treatment already show a decrease of survivors. Toxic substances produced by irradiation possibly continue their lethal effect as long as the cells remain in contact with the irradiated medium. Zinc added to irradiated cultures for the same period of time enhances this lethal effect. The last reduction of survivors is due to the toxicity of the metal.

Zinc toxicity markedly increases in irradiated cells as compared to non-irradiated ones. This enhancement of toxicity is related both to the irradiation dose and to zinc chloride concentration. Irradiation eventually sensitizes the bacteria towards zinc. It is also possible that irradiation, by modifying membrane permeability, allows more zinc to enter into the cells, as is suggested by the uptake of the metal by irradiated bacteria[8].

Résumé. Des cultures de *B. megaterium* traitées pendant 1 h avec du $ZnCl_2$ et irradiées aux rayons gamma en présence du zinc subissent une action létale synergique de ces deux facteurs. Ces effets sont plus intenses lors d'un post-traitement au zinc que lors d'un pré-traitement. Les survivants irradiés se montrent bien plus sensibles à l'action nocive du chlorure de zinc que les non irradiés. La sensibilité des bactéries irradiées vis-à-vis du métal augmente avec la dose d'irradiation et la concentration du zinc.

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