released in active form after the penetration of conjugates into histiocytes[23].

Note added in proof: While this paper was in the press it came to our knowledge an article by TROUET et al., (Nature new Biol., *239*, 110, 1972) in which similar concepts were exposed and the effect of a complex daunorubicin-DNA on mouse L 1210 leukemia was tested with encouraging results.

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Riassunto. Viene descritta la particolare sensibilità dei macrofagi al coniugato amanitina-albumina. È inoltre prospettata e discussa la possibile attività antineoplastica di coniugati dell'albumina con sostanze inibenti la mitosi o la sintesi del DNA.

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Induction of in vitro Maturation in Oocytes of *Triturus* (Amphibia Urodela)

In the last few years, numerous studies have been conducted on the induction of in vitro oocyte maturation by progesterone in the anuran amphibians[1-4], while analogous research on the urodele has not, for the most part, produced satisfactory results[1].

Recently, it has been made possible to induce in vitro oocyte maturation by progesterone in the urodele *Triturus viridescens*[5,6]. The urodeles represent the animal group in which the morphology and the structure of the lampbrush chromosomes have been studied most extensively[7-9]. For this reason, we thought it would be interesting to verify the possibility of inducing in vitro oocyte maturation in other species of urodeles, with the particular aim of investigating the morphological changes of the lampbrush chromosomes during the maturing period.

Material and methods. The study was conducted on the ovarian oocytes of *Triturus cristatus carnifex* (Laurenti 1768) and of *Triturus vulgaris meridionalis* (Boulenger 1882). The morphology of the lampbrush chromosomes in these species is well known[8,10]. The specimens used came from the outskirts of Pisa and Naples. A total of 9 experiments, using 8 females of *T. c. carnifex*, and of 6 experiments, using 5 females of *T. v. meridionalis*, were performed in a period from December to April.

The females were pretreated with a gonadotrophic hormone ('Pregnyl', Organon). Each female received 3 injections, on alternate days, of 100 units each for *T. v. meridionalis* and of 200 units each for *T. c. carnifex*. One or both of the ovaries were then removed, and the larger oocytes were isolated by dissection in Ringer's solution for amphibians. The diameters of these oocytes measured between 1.5 mm and 1.75 mm in *T. c. carnifex*, and between 0.96 mm and 1.4 mm in *T. v. meridionalis*. Some of the oocytes were left in Ringer's and used as a control (67 oocytes for *T. c. carnifex* and 26 for *T. v. meridionalis*), and some were incubated for 1 h in Ringer's solution containing progesterone (Schering) at a concentration of 10 μg/ml, and then once again transferred to Ringer's (173 oocytes for *T. c. carnifex* and 49 for *T. v. meridionalis*).

The maturation process could be observed in the intact oocytes since the germinal vesicle, almost central in the immature oocytes, migrates toward the animal pole during maturation and becomes visible from the outside; at the end of the maturation, a small light area near the animal pole indicates the zone of formation of the second meiotic spindle and of expulsion of the first polar body. The germinal vesicle breakdown was also ascertained by dissection of the oocytes.

At various times, from the beginning of the period of incubation, some of the oocytes were fixed in Bouin's or in Goldsmith's solution and used for histological preparations; preparations of lampbrush chromosomes were made from other oocytes[11]. The control oocytes were processed analogously.

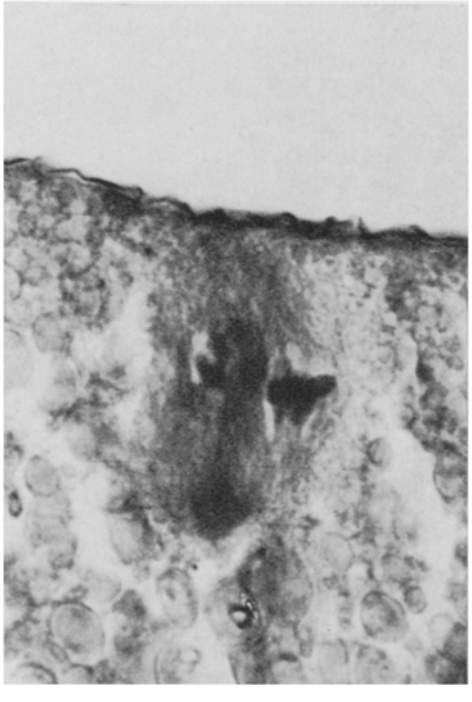

Fig. 1. First meiotic spindle in an oocyte of *T. c. carnifex*. 3 h from the time of incubation in progesterone. ×1520.

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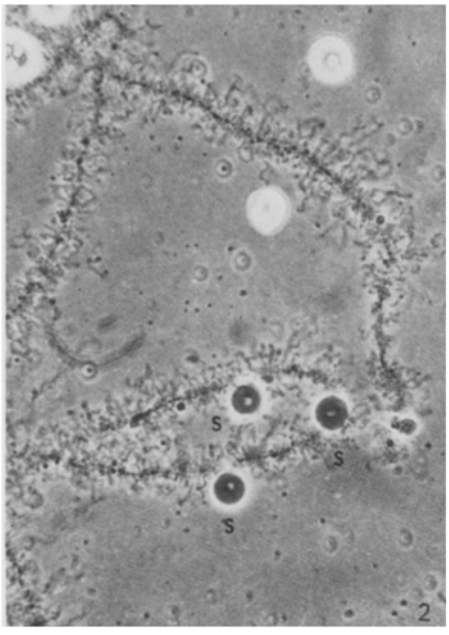
[8] H. G. CALLAN and L. LLOYD, Phil. Trans. R. Soc. B, *243*, 135 (1960).
[9] I. NARDI, M. RAGGHIANTI and G. MANCINO, Chromosoma *37*, 1 (1972).
[10] G. BARSACCHI, L. BUSSOTTI and G. MANCINO, Chromosoma *31*, 255 (1970).



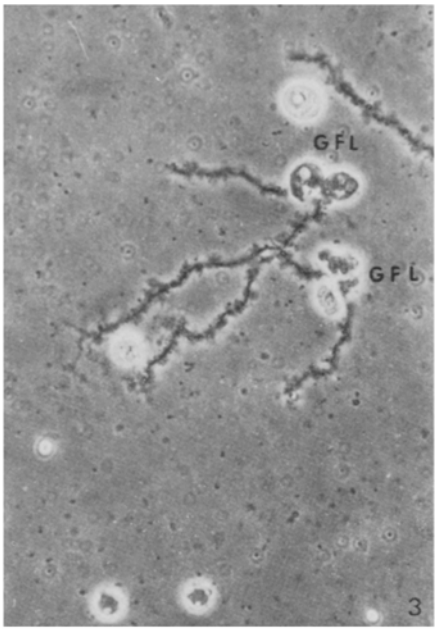
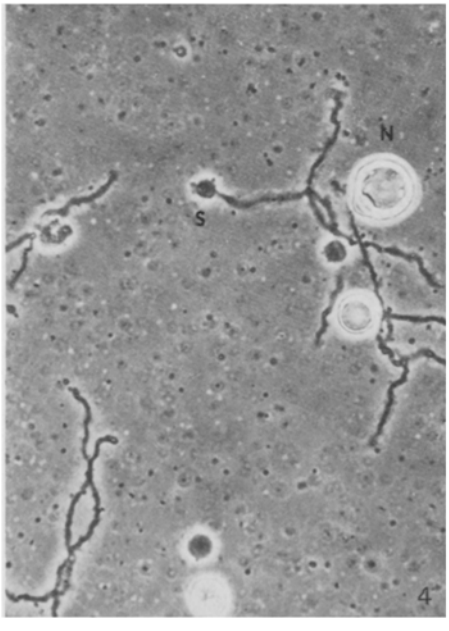


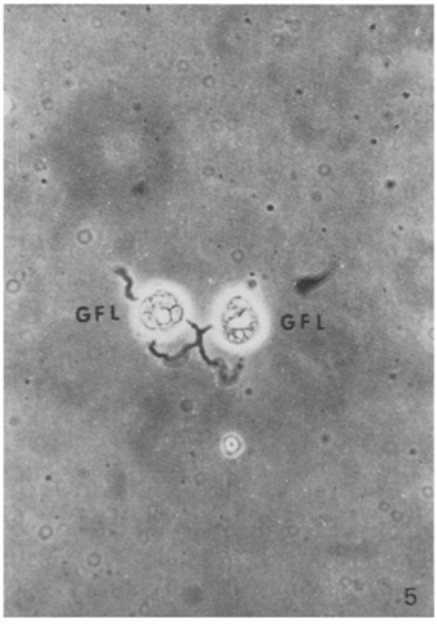
Fig. 2. Lampbrush chromosomes of *T. c. carnifex* with well expanded loops. 3 spheres (S) are attached to the bivalent V. × 545.

Fig. 3. Lampbrush bivalent of *T. c. carnifex* in which retraction of the loops has begun. GFL, giant fusing loops. 5 h from the beginning of incubation in progesterone. × 545.

Fig. 4. Bivalents of *T. c. carnifex* with retracted loops. One sphere (S) is attached to a single homologue. Numerous globules and granules are free in the nuclear sap. The free nucleoli (N) are spherical and vacuolized. 5 h from the beginning of incubation in progesterone. × 545.

Fig. 5. Very contracted bivalent of *T. c. carnifex*. Of the loops, only the GFL remain. 8 h from the beginning of incubation in progesterone. × 545.





Results and discussion. In *Triturus cristatus carnifex* approximately 42% (73 oocytes out of 173) and in *Triturus vulgaris meridionalis* approximately 51% (25 oocytes out of 49) of the oocytes incubated in the presence of progesterone, underwent the in vitro maturation process. It has been observed that only the fully grown oocytes are capable of maturing. Except for a minimal percentage (2 out of 67 in T.*c. carnifex* and 2 out of 26 in *T. v. meridionalis*), the control oocytes did not undergo maturation.

The histological study of the maturing oocytes has shown that the maturation occurs normally and that there are no apparent cytological anomalies. Generally maturation is completed after approximately 20 h from the beginning of incubation, although the time can vary for different oocytes, even if they are from the same ovary. The first meiotic spindle usually appears after about 12 h, although some oocytes reach this stage in a shorter period of time (Figure 1). The latter occurrence could depend upon the fact that some oocytes had already initiated the maturation process in the ovary, as even the few control oocytes which reached maturation may indicate.

The observations made at various times on the lampbrush chromosomes of the oocytes incubated in the presence of progesterone have shown, above all, a gradual disappearance of the majority of the loops, which withdraw into the chromosomic axis, and a progressive shortening and thickening of the chromosomes, accompanied by a loss of the characteristic chromomeric structure. Contemporaneously, the number of granules and globules increase in the nuclear sap, probably because of a discharge of material from the withdrawing loops (Figures 2–5).

[11] J. G. GALL, in *Methods in Cell Physiology* (Ed. D. PRESCOTT; Academic Press, New York 1965), vol. 2, p. 37.

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).

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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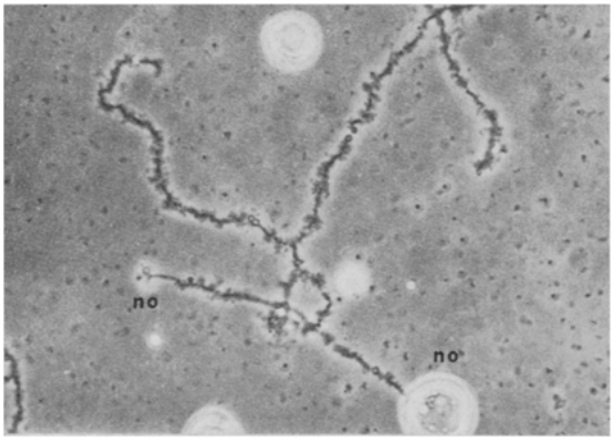

Fig. 6. Bivalent VI of *T. c. carnifex* with nucleoli attached to the nucleolus-organizing regions (no). 5 h from the beginning of incubation in progesterone. ×545.

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[13] Research financially supported by C.N.R., Roma.

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