Studies on the Process of Mouse Meiosis and Ovulation with Daunomycin

Daunomycin[1], a flourescent glycosidic antibiotic of the anthracycline group, was studied for its effect on mouse ovum meiosis and ovulation. It was selected because of its mitotic effects in rat[2] and its similarity to Actinomycin D[3–5], in an effort to obtain additional information on agents potentially capable of altering the mammalian genetic pool.

Materials and methods. Experiments in vivo and in vitro were conducted using prepubertal or adult random bred Swiss/Webster[6] female mice. The in vitro experiments were done using a modification of the method of EDWARDS[7] with harvests of first metaphase cells at 5 h and second metaphase/polar body I cells at 15 h. Cytologic preparations were made using a modification of the method of TARKOWSKI[8] and examination of well-spread, intact cells was made with bright light and phase optics of a Zeiss photomicroscope after staining with toluidine blue and restaining with lacto-aceto-orecin. Scoring of number and structure was completed on chromosomes of both stages. The final concentrations of Daunomycin studied are tabulated in Table I. The number of ova normally completing meiosis to the desired stage averages 89% of the total added. The pH was maintained at 7.2.

Table I. In vitro effects of Daunomycin on female mouse meiosis

Concentration of Daunomycin (μg/ml of media)	No. of mice	Total No. of ova	No. non-divided	No. abnormal	Abnormal (%)
First metaphase					
0	8	86	5	0	0
0.00001	8	87	9	0	0
0.0001	8	76	5	0	0
0.001	8	81	7	0	0
0.01	8	86	5	64	74
0.1	8	75	4	71	94
1.0	8	83	8	75	90
Second metaphase					
0	8	80	4	0	0
0.00001	8	79	7	0	0
0.0001	8	72	6	0	0
0.001	8	86	7	0	0
0.01	8	69	4	65	94
0.1	8	81	5	76	93
1.0	8	79	7	72	93

Table II. In vitro meiotic cytologic effects observed at different intervals after intraperitoneal doses of Daunomycin

Dose (μg/gbw./i.p.)	Time after dose (h)	Abnormal Total eggs First metaphase	Abnormal Total eggs Second metaphase
0	18	0/51	0/43
	24	0/27	0/26
	48	0/13	0/24
	72	0/33	0/42
5	12	0/21	0/23
	18	0/32	0/19
	24	1/27[a]	0/16
	48	0/27	1/22[b]
	72	0/34	0/21
10	24	0/30	0/24
	48	0/16	0/27
	72	0/16	0/19

[a] Structural rearrangement. [b] Satellite association.

Three types of in vivo experiments were done. In the first of these, adult female mice were given a single dose of Daunomycin i.p. and samples of ova were removed from donor ovaries at intervals of 12–72 h later, placed in the incubation system and studied for first and second metaphase development. Concentrations of the drug were selected for comparison with the data on the in vivo effects on rat bone marrow previously studied. The second series of experiments utilized the classical superovulation technique[9] wherein prepubertal female mice 21–24 days of age were given pregnant mare's serum (PMS) followed 48 h later by an ovulating dose of human chorionic gonadotropin (HCG). On each day of this interval the mice were also given a single dose of 5μg/G/BW of Daunomycin s.c. Harvests of ovulated ova were sought in the oviducts at 15 h after the HCG and cytologic preparations were made. If ovulation failed to occur, ova were removed from the ovarian follicles and prepared for cytologic examination of chromosomes. The third series of in vivo experiments were designed to test the effects of Daunomycin on the superovulation phenomenon by giving the drug only at the time of the administration of the HCG or at 1, 3 or 5 h thereafter. Ova were again sought at 15 h after the administration of the HCG and examined as above.

Results. As noted in Table I and demonstrated in Figure 1, at concentrations of 0.01 μg/cm³ the bivalents of the first metaphase are broken and rearranged and the second metaphase chromosomes are agglutinated. At 0.1 μg/cm² both stages were agglutinated suggesting that the damage had been done by the time of the first metaphase harvest and the second metaphase harvest merely represented persistence from the earlier time.

The results of the first type of in vivo experiment are tabulated in Table II. One example of structural rearrangement at first metaphase was found at 24 h and one of satellite association at metaphase II as noted by ROHRBORN[10] (Figure 2). Five μg/G/BW of Daunomycin blocked superovulation in all cases. Examination of the cytologic characteristics of the ova remaining in the large follicles revealed clumped and degenerate second metaphase chromosomes (Figure 2), and polar body chromosomes. A few cells had not proceeded beyond the germinal vesicle stage. The modification of the superovulation technique revealed that a single dose of 5 μg/G/BW of Daunomycin given simultaneously with the HCG or 1 h afterwards, did not prevent ovulation, but the oviductal ova were fragmented at second metaphase (Figure 2). Doses given at 3 or 5 h after HCG did not prevent superovulation and the ovulated ova were cytogenetically normal.

Discussion. Daunomycin can now be added to the other anti-tumor substances isolated from *Streptomyces*

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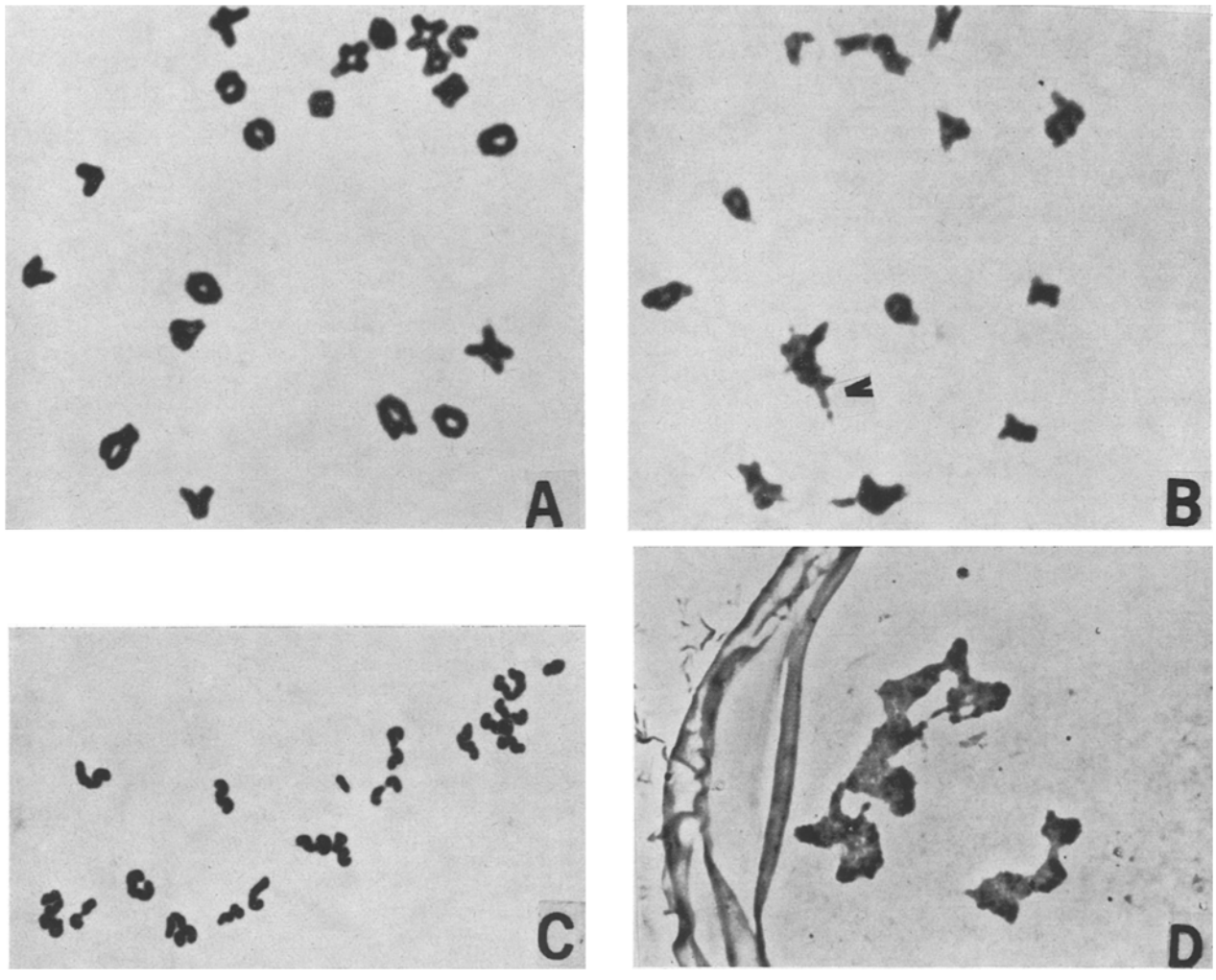

Fig. 1. In vitro effects of Daunomycin on meiosis in mice ova. A) Control first metaphase. B) Metaphase I treated with 0.01 μg/ml. Note breaks. C) Control second metaphase. D) Metaphase II treated with 0.1 μg/ml. Agglutinated nucleus. Magnification: ×1,200.

which are effective in damaging mammalian chromosomes during mitosis and female meiosis. Like Streptonigrin and Phleomycin, breaks and rearrangements can be detected in first metaphase after 5 h incubation with test drugs, but a 10-fold difference in threshold between Phleomycin (0.001 μg/ml), Daunomycin (0.01 μg/ml) and Streptonigrin (0.1 μg/ml) existed among the 3 derivatives for the induction of such bivalent damage. In each case, as with in vivo studies with somatic tissue, longer exposure time resulted in more extensive chromosome damage.

Comparison of the meiotic abnormalities which could be detected at intervals after a dose of Daunomycin with those seen in rat bone marrow and peripheral blood at a comparable dose demonstrated considerable resistance of the germ cells to the drug. In the rat chromatid and chromosome aberrations in bone marrow preparations rose from 27% of all cells examined at 12 h after treatment to a maximum of 59% after 24 h with a decline to 0.7% at 72 h. In the present experiments the finding of 1 first metaphase ovum among 27 with a rearrangement studied 24 h after treatment and 1 second metaphase out of 22 ova with a configuration resembling the 'satellite associations' seen by ROHRBORN[10] would seem to indicate considerable resistance of the ovum to the effects of a compound which affects hematopoietic tissue in the bone marrow with great efficiency.

In vivo effects of Daunomycin on ova during the active meiotic stages of superovulation were very similar to those obtained with Steptonigrin at a dose of 1.25 μg/g of body weight and Phleomycin at 6 μg/G/BW. All 3 compounds effectively block ovulation coincident with destruction of the second metaphase and polar body I. It would appear that these compounds, though inhibiting the process of ovulation and damaging the chromosomes severely, do not prevent abstriction of the polar body. This would seem to indicate an independence of the process of disjunction on the meiotic spindle and the physical separation of the polar body I from the need for chromosomal integrity.

Further light is shed on the process of mammalian ovulation by a comparison of the effect of Daunomycin given with the ovulating stimulus (HCG) or at intervals thereafter with the effects of Actinomycin D, a known inhibitor of DNA-dependent RNA synthesis. When an appropriate dose of Actinomycin D was given at 1, 3, or 4–5 h after the HCG, ovulation was blocked[11], but the chromosomes of the intrafollicular ova were microscopically intact. When given at 6 h after the HCG, ovulation

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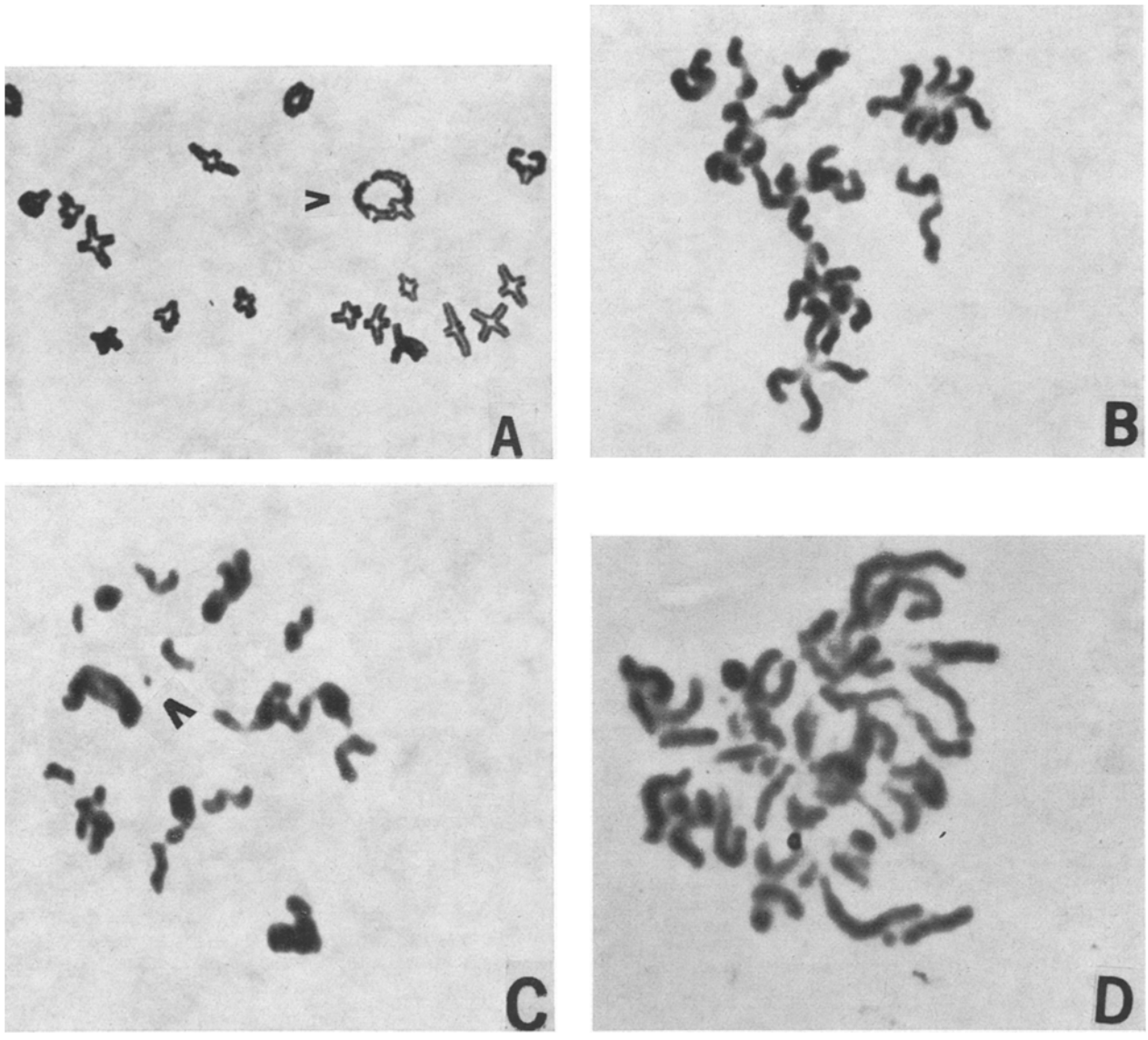

Fig. 2. In vivo effects of Daunomycin on meiosis in mouse ova. A) Structural rearrangement at first metaphase. Arrow indicates location. B) Satellite association at second metaphase. Arrow indicates one set. C) Fragmented oviductal second metaphase. Arrow indicates pieces. D) Degenerate second metaphase from blocked intrafollicular ovum. Magnification: ×1,200.

occurred and ova were in normal second metaphase. In contrast, the superovulation blocking dose of Daunomycin (5 μg/G/BW) did not block ovulation when given with the HCG or at intervals of 1, 3 or 5 h thereafter, yet the ova recovered from the oviducts when the Daunomycin was given with the HCG or 1 h thereafter contained damaged second metaphase chromosomes. Those ova recovered from animals treated 3 or 5 h after HCG contained normal second metaphases. This further chemical dissection of the process of ovulation has demonstrated that Actinomycin D affects a mechanism concerned with the ovulatory process without damaging the ova chromosomes at second metaphase/PB[1] while Daunomycin given at times specifically related to the ovulatory stimulus affects the ovum chromosomes only when given with the ovulating stimulus or just after. Once the process of entry into first metaphase is well underway, Daunomycin cannot affect either the integrity of the chromosomes, their progression to the reduced state nor the mechanical process of ovum expulsion from the follicle.

Zusammenfassung. Der Effekt von Daunomycin auf weibliche Keimzellen wurde untersucht und mit bereits bekannten Befunden an somatischen Zellen verglichen. Es finden sich Unterschiede, die für das Wissen über die Effekte chemischer Mutagene auf den Säuger von Interesse sind.

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19 June 1972.

[12] Acknowledgment. The work described above was made possible by grants from The National Foundation – March of Dimes and the National Institutes of Health (Grant No. NICHD 5-RO1-HD-05862-01), USA.