MEIOSIS AND INHIBITION OF OVULATION IN MOUSE EGGS TREATED WITH ACTINOMYCIN D

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INTRODUCTION

Barros and Austin (1) have reported on the inhibition of ovulation in the golden hamster by parenteral actinomycin D (AMD). During studies aimed at characterising the meiotic chromosome damage induced by various inhibitors of DNA, RNA, and protein, the author has made some observations in vitro and in vivo on the effects of AMD on female mouse meiosis. These data extend the information of Barros and Austin (1) on hamster and of Pool and Lipner (2) on rabbit, particularly with reference to cytogenetic changes in the inhibited eggs.

TABLE I

Effect of Actinomycin D on Mouse Egg Chromosomes Incubated for 4 or 14 hr

AMD	No. ova with damage/total no.	No. ova with damage/total no.
μg/ml	*4 hr*	*14 hr*
0.05	0/32	0/51
0.10	0/24	0/44
0.20	31/35	50/51
0.50	36/37	27/27
1.0	27/30	40/40
2.5	26/26	49/52
5.0	13/13	29/29

MATERIALS AND METHODS

Random-bred CFLP albino mice were used throughout. Adult, 40 to 60-day-old, dioestrous females provided ova for in vitro studies. 15 to 20 eggs were incubated each time in minimum essential media with various concentrations of AMD at 37°C in a 5% CO_2/air atmosphere (Table I) and harvested at 4 hr for first metaphase and 14 hr for second metaphase. Cytological preparations were made by a modification of the method of Tarkowski (3) and examined with a Zeiss photomicroscope using phase and bright light microscopy after staining with lacto-aceto orcein and permanent mounting.

The in vivo studies were conducted with 21-day-old female mice with use of the superovulation technique (4). 5 IU of pregnant mare serum (PMS) were given intraperitoneally, and 48 hr later 2.5 IU of human chorionic gonadotrophin (HCG) were given. Doses of AMD from 0.1 to 10 μg per g body weight per day were given subcutaneously during the time of hormonal stimulation, the last dose being given with the HCG. Single equivalent doses were also given at intervals of 1–8 hr after HCG. Cytological preparations were made, in the same fashion, of either ovulated or intrafollicular ova.

RESULTS

In vitro: Concentrations of 0.2–1.0 μg/ml of actinomycin D (AMD) damaged the chromosomes of about 90% of first metaphase (M^I) eggs with the production of breaks, fragments, and marked fuzziness of chromosome borders (Table 1). With concentrations above 1 μg/ml, the chromosomes were severely distorted. Harvests for second metaphase (M^{II}) at 14 hr revealed progression of the damage induced by 0.2–1.0 μg/ml at 4 hr to marked fragmentation and rearrangement of most of the complement with retention of first metaphase relationships and configurations in 60–75% of ova (Fig. 1). In the remainder of the ova, the chromosome complements, though badly damaged, were divided into "quasi" second metaphase and first polar body (PB^I) sets with formation of the latter within the zona pellucida.

TABLE II

Distribution of Meiotic Stages in Blocked Intrafollicular Ova

AMD (1 μg/gBW)	M^I	M^{II}
hours after HCG		
1	3	18
3	2	41
4.5	9	36
Without AMD	0	360*

* Oviductal ova from superovulation.

In vivo: AMD 1 μg/g body weight (BW) given subcutaneously (SC) in three doses on the days of hormonal treatment prevented ovulation in 100% of animals. When given intraperitoneally (IP) or SC at 6 hr after HCG this dose did not prevent

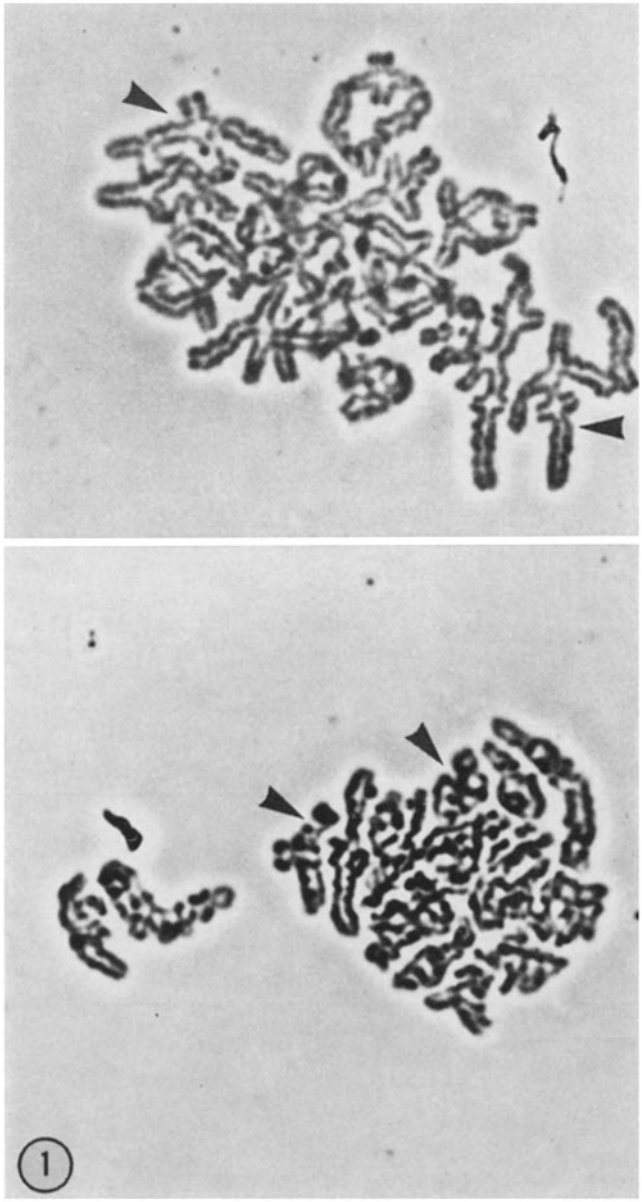

FIGURE 1 First metaphase configurations recovered after 14 hr of incubation with AMD. Note multiple breaks and rearrangements (arrows). × 500.

ovulation, but when given at 1, 3, or 4.5 hr after HCG it did prevent ovulation. Blocked follicles were visibly enlarged and often hemorrhagic. Microscopic examination of intrafollicular ova showed that some ova were still in M^{I} (Table II) and that the remainder were in M^{II} with PB^{I}. The chromosomes were not damaged at either stage of meiosis at this dose (Fig. 2), although the M^{I} was commencing to show coiling changes more characteristic of M^{II}.

DISCUSSION

The dose of AMD required to block ovulation in the CFLP strain of mouse was slightly greater than that required for the golden hamster, and the effective time was 4.5 hr after HCG as compared with 5.5 hrs for the hamster. When given at 6 hrs AMD was ineffective in mouse, whereas at 7.5 hr the effective dose did not block ovulation in the hamster. The minimum effective dose

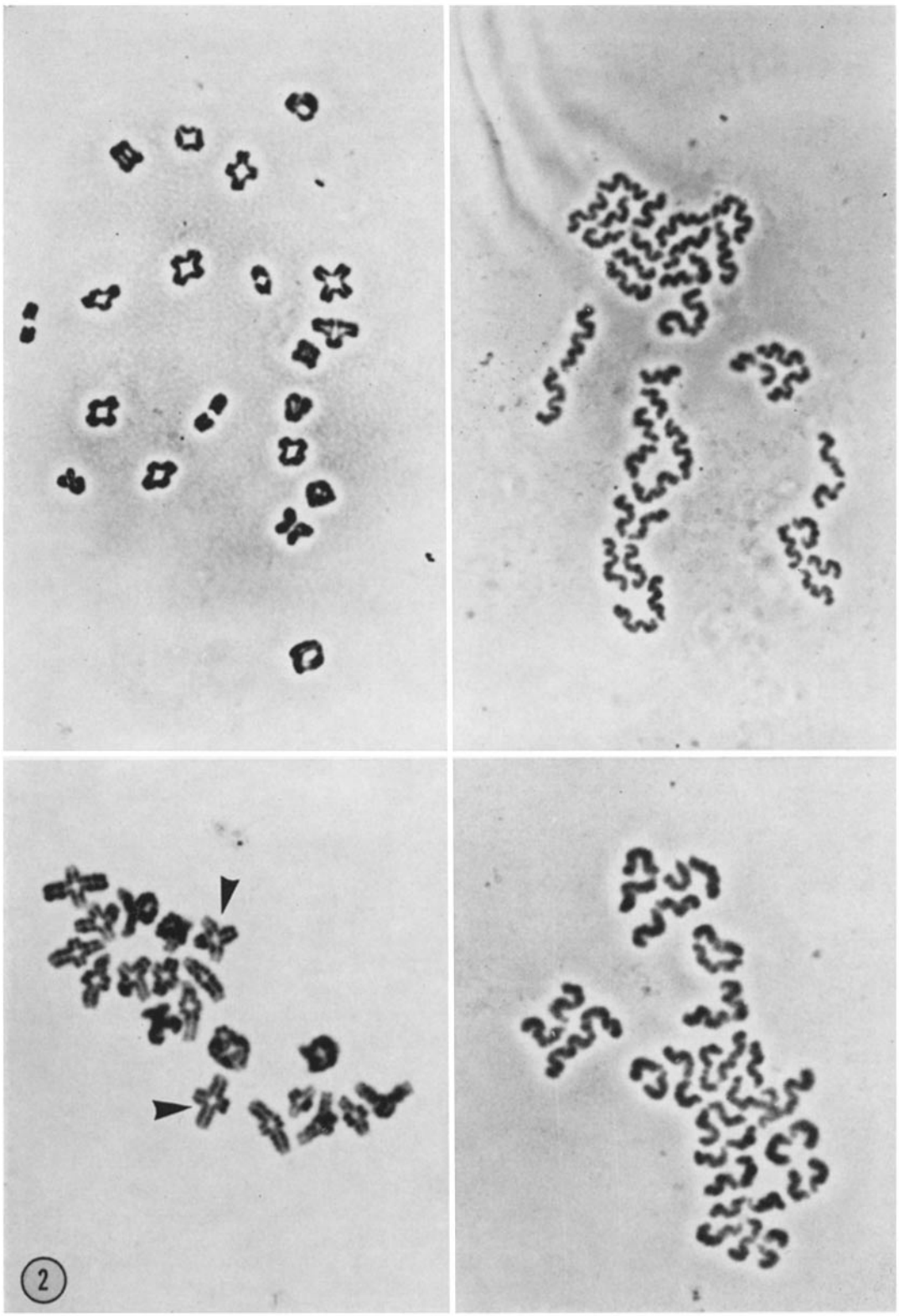

FIGURE 2 Control first and second metaphase (*a* and *b*) and first and second meiotic metaphases (*c* and *d*) from eggs removed from animals treated with AMD 1μg per g body weight. Note coiling changes (arrows), usually seen at second metaphase, in first metaphase bivalent. × 500.

in the immature mice was 100% inhibitory regardless of the interval after HCG, whereas in hamster the effectiveness declined with increasing interval in the adult and a few ovulations were noted in the superovulated immature animals. The inhibition in mouse was not relieved by 18 hr after HCG and would seem not to be reversible, as it was in rabbit where 0.001 μg of the drug was introduced directly into follicles of unknown volume. Extrapolating from our in vitro data which indicated a minimum effective dose of 0.20 μg/ml for cytologically detectable damage, the concentration of AMD in blocked mouse ova where chromosomes were intact must have been less than 0.2 μg/ml. A more specific comparison with rabbit is not possible.

Previous studies with mouse have indicated (5) that Phleomycin, an inhibitor of DNA synthesis and a chromosome-breaking agent, damaged meiotic chromosomes seriously but did not necessarily prohibit ovulation. If one can accept the demarcation of effects of this agent and AMD in this system, it would seem that AMD produced the opposite effect with no cytologic evidence for chromosome damage yet total inhibition of ovulation. These two situations would tend to support the idea that an RNA system, perhaps induced by the follicular cells or laid down in the ovum cytoplasm much earlier in oogenesis via the nucleus, directs the ovulatory process in mouse independent of events in the chromosomes at the time of preovulatory meiotic changes.

Unlike the findings in the immature golden hamster, the intrafollicular ova of mouse which had been prevented from ovulating were, in many cases, still in first metaphase. The incidence of first metaphase figures in the blocked intrafollicular ova and the high incidence of their persistence in the long in vitro studies suggest that AMD may also be affecting a synthetic system required for the formation of second metaphase and first polar body which is separate from the support of M^I formation, as suggested by Jain and Singh (6) for locust. The mechanical aspects of bivalent separation or peri-spindle mitochondria (7) may also be altered. A direct spindle effect is unacceptable, since it has been observed that, unlike in *Pectinaria*, AMD has no effect on the duration of Colcemid- and Vincristine-induced spindle inhibition of female mouse meiosis.[1]

[1] G. M. Jagiello. *Experentia.* In press.

SUMMARY

The effects of Actinomycin (AMD) on the cytogenetics of meiosis and ovulation in the female mouse have been studied in vitro and in vivo. Concentrations of 0.20–1.0 μg/ml in vitro damaged first metaphase (M^I) configurations, with further damage at second metaphase (M^{II}) and partial persistence of M^I relationships. In vivo, 1 μg/g body weight (BW) was the minimum effective dose for blocking ovulation. Inhibited intrafollicular ova contained normal first or second metaphase configurations. Comparisons with data from other species are made.

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