

## EFFECT OF COLCHICINE ON PRE-IMPLANTATION BLASTOCYSTS AND 10-DAY-OLD EMBRYOS OF THE GOLDEN HAMSTER\*

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Orsini and Panski (1952) used colchicine in their investigations on the proliferative growth of hamster tissue. They found that injected doses of colchicine varying from 0.1 up to 2 mg per 100 g body weight did not cause any significant variation from the normal number of mitoses, and the presence of anaphase and telophase stages with normal spindles in normal ratio suggested that these doses were ineffective. They stated that effective doses in other rodents (mice, rats, guinea-pigs, and rabbits) are usually below the lethal level. In an attempt to establish the effective dose, they injected colchicine intraperitoneally at concentrations ranging from 0.13 to 7 mg per 100 g body weight into a series of hamsters, rats, mice, and a rabbit. At these levels colchicine was lethal for the latter three series of animals, but was not lethal for hamsters, even up to a concentration of 10 mg per 100 g body weight. They concluded that the hamster possesses a natural resistance to this compound.

The present study has been made to find out the effect of colchicine on different stages of development of golden hamster embryos.

### *Material and Methods*

Hamsters were maintained in a colony receiving artificial lighting from 6 a.m. to 6 p.m. each day. From other evidence (Austin and Braden 1956), it was presumed that ovulation occurred in the colony between 1 and 3 a.m. The stage of the cycle was determined by daily examination using the method described by Orsini (1964). Mating was carried out by penning females overnight, singly, with fertile males.

The occurrence of mating was checked by examination of vaginal smears for the presence of spermatozoa. The day of presumed ovulation is referred to here as day 1. In an attempt to increase the number of eggs shed some females received a subcutaneous injection of 25 i.u. pregnant mare serum gonadotropin (PMSG) 4 days before oestrus.

In order to increase the number of mitotic figures available for examination in embryos studied before implantation, females were injected intraperitoneally with 0.25 ml of 0.03% (w/v) solution of colcemid (CIBA) 77–84 hr after ovulation.

Eggs were recovered at autopsy at various times after mating. After removal of the ovaries and genital tract, counts were made of the corpora lutea in each ovary and the contents of the uterine horns were flushed onto a watch glass with warm (37°C) saline. Stained preparations of air-dried whole egg mounts were prepared by modification of the method described by Tarkowky (1966) as follows. The eggs were first treated by exposure to a hypotonic (1%) solution of sodium citrate, swelling of the eggs being observed visually under a dissecting microscope. The eggs were then pipetted onto clean dry slides. After removal of excess hypotonic solution the eggs were fixed by cautious addition to the slide of a drop of acetic acid-methanol (1 : 3). Fixation was completed by evaporation of the fixative, the process being observed by microscopical examination.

Embryos (10 days old) recovered after implantation were incubated for 90 min at 37°C in a medium of the following composition: medium 199 (Wellcome)+10% calf serum, 9.5 ml; colchicine (0.01%), 0.5 ml. At the end of 90 min, the medium was replaced with 10 ml of 1%

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sodium citrate and incubated for further 20 min at 37°C using the procedure described by Vickers (1969) for air-dried preparations. Whole embryos were fixed by this method, after the removal of the sodium citrate by the addition of 3–5 ml acetic acid–methanol (1 : 3). After fixation for 30 min at 4°C the fixative was replaced by 3 ml of 45% acetic acid. After 5 min this was removed and replaced with fresh 1–2 ml of 45% acetic acid, and the embryo was broken down by gentle pipetting. Smears were prepared by pipetting drops of the cell suspension onto clean dry slides held on a hot plate at 54°C and dried.

For squash preparations, embryos were cultured and treated with hypotonic citrate solution as above; they were then transferred intact into aceto-carmine (Darlington and La Cour 1960). After 24 hr the embryos were broken up by pipetting and a small fragment of tissue was placed on an albuminized slide and covered by a siliconized coverslip. Squashes were made by applying constant pressure to the coverslip with the thumb. After freezing the coverslip (ethyl chloride spray) it was removed using a knife blade and the smears were dried and stained by aceto-orcein for 30 min, dehydrated in ethanol, and mounted in DPX.

### Results

No embryos were recovered from hamsters autopsied after 2 p.m. on day 4, i.e. more than 85 hr after the estimated time of ovulation. Of a total of 24 PMSG-treated hamsters examined 81–85 hr after ovulation, six yielded eggs, all of them less than 83 hr after ovulation. Of 31 untreated hamsters, 12 yielded eggs. Of these, 11 were examined not later than 83 hr after the estimated time of ovulation. Of 79 eggs found to have undergone cleavage, none had developed beyond the 32-cell stage and only 24 had developed beyond the 16-cell stage. These findings show that implantation had apparently taken place on the afternoon of day 4 when the embryos would not have developed beyond the 16-cell stage. For this reason alone the hamster blastocyst was found to be an unsatisfactory source of model material for cytological analysis.

A further disadvantage was the absence of any effect of colchicine-treated mothers. Of 22 examined, six showed mitotic figures. No preparations suitable for analysis were made from pre-implantation embryos.

The failure of colchicine to arrest mitotic activity in implanted hamster embryos is in agreement with the observation of Orsini and Panski (1952) regarding the natural resistance of hamster tissue to the anti-mitotic effect of this agent.

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