ical studies. But it should be clear from the work reported here that much remains to be learned about the actual process and mechanisms by which ginsenosides induce redifferentiation of Morris hepatoma cells.

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The effects of actinomycin D and chloramphenicol on the rat preimplantation embryos

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Summary. Actinomycin D and chloramphenicol, injected in the rat on day 3 or 4 of gestation, induce embryolethality and embryotoxicity. These effects are revealed on day 5 of pregnancy by reduced number of blastocysts and by decrease of mean blastomeres number.

Although it is well known that drugs and other common environmental chemicals pass from the general circulation in the uterine fluid and readily penetrate the embryo in preimplantation stages[1,2], there is little information about the effects of chemicals on the conceptus during the earlier stages of pregnancy.

The flat-mount technique used by Lutwak-Mann[3] in order to study the embryotoxic and dismorphogenetic effects of thalidomide on the rabbit blastocyst is a useful method in this field, but not appliable to rodents. The purpose of the present investigation was to find a ready and efficient routine method for detecting the effects of exogenous agents on the preimplantation development.

In order to evaluate the techniques effectiveness, we tested 2 antibiotics: Actinomycin D (AcD), known to impair the mouse blastocyst development in vitro[3-5], and Chloramphenicol (CAF), teratogen in the rat at very high dosage (more than 1000 mg/kg) but probably not harmful on the preimplantation stages[6].

Materials and methods. Nulliparous Sprague-Dawley rats (Charles River), 200±20 g body weight, were paired overnight with males and the morning on which a sperm-positive vaginal smear was observed, was considered to be day 1 of pregnancy.

The mated females were treated with 300 µg/kg i.p. of AcD on day 3 or 4 of gestation (groups A and B). The females of the groups C and D were treated during the same days with 250 mg/kg i.p. of CAF and the females of group E were treated with 1 ml/kg i.p. of saline on days 3 and 4. Females were killed at 15.00 h on day 5 of pregnancy. The blastocysts were collected in watch glasses by flushing the uterine horns with buffered saline (0.5 ml/horn) and their number was recorded. The blastocysts were afterwards placed in 0.6% sodium citrate for 15 min at room temperature. With the aid of a micropipette, every blastocyst was drawn from this solution, placed in the middle of a slide and fixed with few drops of acetic alcohol (ethyl alcohol + glacial acetic acid 3:1)[7]. The air-dried slides were stained with toluidine blue (2% aqueous solution, for 3 min). This preparation makes it possible to count the scattered blastomeres (figures 1 and 2).

Results and discussion. The results of the investigation are summarized in the table. The CAF administration does not change the average number of collected blastocysts inde-

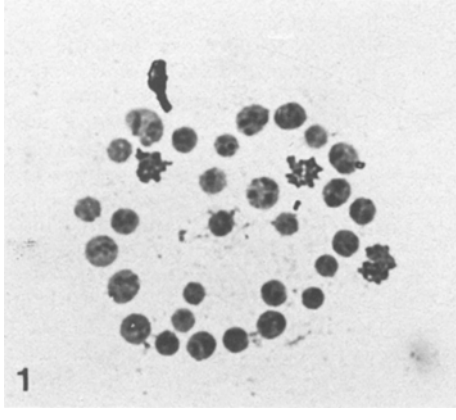

Fig. 1: Blastocyst of control prepared with the air-drying method. 32 blastomeres (86×).

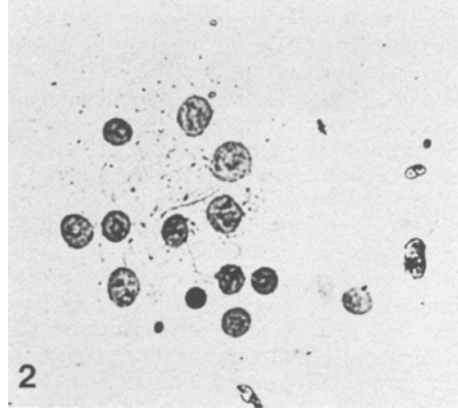

Fig. 2: Blastocyst from female treated with AcD. 13 blastomeres (86×).

Effects of AcD and CAF administration in pregnant rats on day 3 or 4 of gestation

Groups	Treatment	Number pregnant females	Number blastocysts recovered	Mean number blastocysts per female ± SD	Mean number cells per blastocyst ± SD
A	AcD 300 µg/kg day 3	13	64	4.9 ± 3.40*	29.41 ± 6.60*
B	AcD 300 µg/kg day 4	7	35	5.0 ± 2.94*	36.45 ± 4.80
C	CAF 250 mg/kg day 3	7	59	8.4 ± 2.81	29.38 ± 3.87*
D	CAF 250 mg/kg day 4	7	62	8.8 ± 2.03	34.98 ± 5.90
E	Saline 1 ml/kg days 3 and 4	7	60	8.6 ± 2.99	36.95 ± 5.60

* Significantly different from controls at $p < 0.05$.

pendently from the day of treatment. However the CAF administered on day 3 of pregnancy reduces significantly the average number of blastomeres in the rat blastocyst. The same effect is detected also in the blastocysts from females treated with AcD, but this drug induces a remarkable embryolethality, as can be seen by the dramatic decrease of the collected blastocysts in the A and B groups. The antibiotics used in this experiment show different effects on the preimplantation embryo: the AcD induces embryolethality and embryotoxicity, while CAF shows exclusively a remarkable embryotoxic effect revealed by the reduction of the mean blastomeres number when administered on the day 3 of gestation.

Our results agree with those obtained by Wilson[8], who described, in rats treated with 300 µg/kg on the day 4 of pregnancy, a remarkable preimplantation loss, deduced by a low number of implantation sites at term; and with results obtained by Fritz and Hess[6], who did not find decrease of implantation rate in females treated with CAF from day 1 to day 6 of gestation.

The reduced number of blastomeres observed in our investigation is a sign of a harmful effect of the drugs on the conceptus and, therefore, could be used as an index of a probable teratogenic effect.

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Oxygen permeability of the chorion in relation to diapause termination in *Bombyx* eggs[1]

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Summary. Oxygen permeability of the chorion of the silkworm, *Bombyx mori*, was measured in relation to embryonic diapause. It did not change appreciably when the eggs were freed from diapause by being kept under long chilling. This finding suggests that the increase in oxygen permeability of the chorion is not a pre-requisite for the termination of the diapause and resynthesis of glycogen from 2 polyols.

Chino[3,4] has shown that almost all glycogen present in the eggs of *Bombyx* silkworm is rapidly converted to 2 polyols (i.e. sorbitol and glycerol) at the onset of the diapause, and glycogen is resynthesized from these polyols at the termination of the diapause. It has also been demonstrated that the conversion of glycogen to 2 polyols resulted from anaerobic metabolism in the non-diapause eggs[5–9]. From the experiments of dechorionation and measurement of water loss from the silkworm eggs, Okada[5] has proposed a hypothesis that the formation of an oxygen barrier in the chorion causes an oxygen-deficiency, which induces diapause, and recovery of oxygen permeability of the chorion to the initial level after long chilling causes the termination of diapause and resynthesis of glycogen from 2 polyols. However, our recent result with the direct determination of the oxygen permeability of the chorion does not support Okada's hypothesis, at least as to the formation of an oxygen barrier in the chorion at the onset of the diapause[9]. The purpose of the present study is to test whether or not the oxygen permeability of the chorion changes at the termination of the diapause after long chilling.

Materials and methods. Eggs of the bivoltine race (Nichi 106 × Daizo) of the silkworm, *Bombyx mori*, were used. For the purpose of artificially terminating diapause, diapause eggs kept at 25 °C for 2 days after oviposition were placed at 5 °C for about 100 days. Glycogen content was determined using the anthrone method[10]. For measurement of oxygen permeability of the chorion, the apparatus especially designed for this purpose[9] was used with minor modifications. The quantity of oxygen was measured using high speed gas chromatography (Yanagimoto HSG-1). A stainless steel micro column, 60 cm long and 1 mm inner diameter packed with Molecular Sieve 5 A, 200/250 mesh (Gasukuro Kogyo Co.) was used. The column was operated at 40 °C. The pressure of hydrogen gas as a carrier gas was 1.5 kg/cm^2.

Results and discussion. To know the period of termination of the diapause by chilling, the glycogen content of the eggs