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Effects of Cadmium, Lead and Copper on Rat Preimplantation Embryos

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The development of preimplantation mammalian embryos may be impaired by environmental chemicals (BLACKBURN & CLEGG 1979,GIAVINI et al. 1979,LTWAK-MANN & HAY 1969). Metals and their salts are known to induce congenital malformations,resorptions,intrauterine death and developmental retardations in the laboratory species on which have been tested (FERM 1972).
The effects of these chemicals on preimplantation mammalian embryos have been little investigated;copper inserted into the uterine lumen prevents pregnancy in the rat,rabbit,and hamster (CHANG et al. 1970,ZIPPER et al. 1969) and copper salts are lethal for mouse blastocysts cultured in vitro (BRINSTER & CROSS 1972, NOESLUND 1972).Inorganic lead causes a delay of the first divisions in the 48h mouse embryos (JACQUET et al. 1976) and interferes with the viability and the outgrowth of mouse blastocysts cultured in vitro (WIDE 1978).
The present study was undertaken to investigate the effects of Cd, Pb, and Cu on the preimplantation development of the rat embryo.

MATERIALS AND METHODS

Virgin female Sprague Dawley rats (Charles River, Italy),200±20 g body weight,were caged overnight with males of the same strain and the morning on which a sperm positive vaginal smear was found,was designated the first day of pregnancy.The mated females were randomly divided into 4 experimental groups and treated intraperitoneally with 1 ml/animal saline,3mg/kg $CdCl_2$, 50mg/kg $Pb(NO_3)_2$ and 7.5mg/kg $CuSO_4$ respectively,on day 3 of gestation.The dose levels were nearly equivalent to teratogenic dose 50 of the different salts on the rat (McCLAIN & BECKER 1975,BARR 1973, MAROIS & BUVET 1972).All animals received an injection of colchicine (1mg/kg) 1h before being killed. Females were sacrificed at 15.00h on day 5 of gestation.The blastocysts collected by flushing the uterine horns with buffered saline (1ml/horn) were observed for morphological alterations and their number

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was recorded.Afterwards they were placed in 0.5% sodium citrate for 15 min.,set in the middle of a slide and fixed with acetic alcohol.The scattered blastomeres were counted after coloration with toluidine blue,according to the previously described technique (GIAVINI et al. 1979).Data were analyzed by Student's t-test.

RESULTS AND DISCUSSION

The results of this investigation are shown in table 1.The metals administration does not reduce the mean number of collected blastocysts in comparison with the control.This means that Cd and Pb have not embryolethal effects on the preimplantation rat embryos.The Cu-treated blastocysts,on the contrary, showed sometimes serious morphological alterations and signs of degeneration (absence of the blastocoele; little,vescicolous and irregular blastomeres;fig.1 b, c,d).

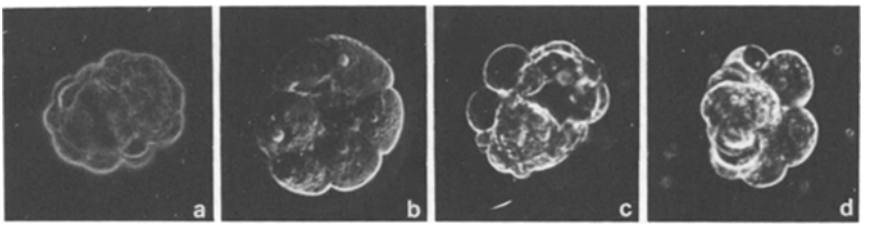

Fig.1 : a. 5-days control blastocyst.
b,c,d. Abnormal 5-days blastocysts from females treated with $CuSO_4$.

This result is in according with the observations of BRINSTER & CROSS (1972) on the mouse blastocysts cultured in medium containing $CuCl_2$. It is likely that $CuSO_4$ have an embryolethal effect resulting in a reduction of the number of blastocysts able to implant later into the uterus.
The average number of blastomeres was significantly reduced in all metal-treated groups.Therefore the three administered metals induce on the preimplantation rat embryos an evident toxic effect,particularly dramatic in the groups treated with Pb and Cu. These data agree with the results of NOESLUND (1972), JACQUET et al. (1976) and WIDE (1978) on the mouse. Cd,Pb,and Cu are therefore able to induce remarkable alterations on the rat embryos in the stages of the

TABLE 1 - Effects of $CdCl_2$, $Pb(NO_3)$ and $CuSO_4$ administration in pregnant rat on day 3 of gestation.

Treatment	Number Pregnant Females	Number Blastocysts Recovered	Mean Number Blastocysts per Female ± SD	Mean Number Blastomeres per Blastocyst ± SD	Number Abnormal Blastocysts
Saline (1ml/animal)	15	152	10.1±3.73	40.96±3.52	-
$CdCl_2$ (3mg/kg)	10	120	12.0±1.56	36.92±3.31*	-
$Pb(NO_3)_2$ (50mg/kg)	10	103	10.3±3.91	33.92±4.90**	-
$CuSO_4$ (7.5mg/kg)	10	96	9.6±2.27	33.01±4.47***	10

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

preimplantation development.It is likely that the toxic effects that we observed in this investigation as a reduction of the average number of blastomeres,is correlated with the teratogenic,embryotoxic and embryolethal effects observed by other authors who administered these metals during the organogenetic periods.

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