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PMSG responsiveness during adult life after partial oogonia destruction with misulban in the rat embryo

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Summary. Pregnant rats were given one of the following doses of misulban on day 15 of pregnancy : 0.25, 0.36, 0.52, 0.75, 0.82, 0.88 or 0.95 mg/kg. In the three-month old offspring it was observed that :

1) The number of cyclic rats decreased according to the dose of misulban given to the mothers ;
2) Cyclic rats were given an injection of 20 IU of PMSG at metœstrus followed by an injection of 150 IU of HCG the evening the presumed œstrus. The following observations were made after such a treatment : *a*) there was no difference in ovulation rate between rats born from mothers treated with misulban (0.25-0.75 mg/kg) and untreated mothers ; *b*) there was no difference in ovulation rate between rats born from mothers treated with misulban (0.75-0.95 mg/kg) and controls receiving 10 IU PMSG and 150 IU HCG.
It is suggested that : *a*) the oogonia population is sensitive to increasing doses of misulban ; *b*) a sufficient number of primordial follicles is necessary at birth in order to maintain cyclicity in the adult ; *c*) the rate of superovulation is dependant on the number of growing and preantral follicles at the time of treatment with PMSG.

Introduction.

In the rat, some alkylating substances such as misulban administered during pregnancy lead to a reduced fertility in the female offspring (Hemsworth and Jackson, 1963). The loss is maximum when rats are treated on day 15 of pregnancy.

Male rats receiving a single injection of either 4 or 10 mg/kg body weight of misulban have reduced fertility. Following a single injection of 10 mg/kg, fertility does not return to normal level until rats are 14 weeks old (Jackson, Fox and Craig, 1959).

A single injection of 10 mg/kg of misulban given to pregnant rats on day 15 of pregnancy leads to a complete disparition of follicles in the ovaries of the female offspring.

Misulban has been shown to induce chromosomic anomalies (Chevremont *et al.*, 1959) and the cells most sensitive are those at prometaphase and metaphase. Oogonia are maximally sensitive to X-ray irradiation in the last period of division, i.e. about day 15 of pregnancy in the rat (Baker and Beaumont, 1967).

In the male, the rate of spermatogonial division determines the number of gametes (Courot *et al.*, 1970).

It is possible that the same situation occurs in the female and that the number of follicles left in the ovary of a young female depends on the dose of misulban administered to the mother during pregnancy.

It is not known if the reduced number of primordial follicles conditions the response of the ovary to the exogenous hormones. In the present study we report on PMSG responsiveness of females born from mothers treated with misulban on day 15 of pregnancy.

Material and methods.

Three-month old female rats were bred under standard conditions of light (14 h light — 10 h dark) and food and mated with fertile male rats. The rats which were presumed pregnant after examination of the vaginal plug were allotted to seven groups ; the rats of each group were given on the morning of day 15 of gestation, one single intraperitoneal injection of misulban (in a 0.5 p. 100 olive oil solution). The corresponding doses of each group were respectively 0.25-0.36-0.52, 0.75-0.81-0.88-0.95 mg/kg of live weight ; the rats were allotted as shown in table 1.

TABLE 1

Group number	1	2	3	4	5	6	7
Concentration of misulban (mg/kg)	0.25	0.36	0.52	0.75	0.811	0.878	0.949
Number of pregnant rats receiving misulban (1 injection at day 15 of pregnancy)..	5	5	7	14	6	8	8
Number of young females (at birth).....	16	19	27	54	35	37	28
Number of cyclic females (3 months old) receiving 20 IU PMSG at metoestrus and HCG at œstrus	9	12	18	18	10	3	10
p. 100	56	63	66	33	28	8	35

The cyclic females born from treated rats at 3 months after birth were given an intraperitoneal injection of 20 IU PMSG (in one ml of sterile serum) on the morning of metoestrus and a second intraperitoneal injection of 150 IU of HCG (in one ml of sterile serum) at 5 p. m. on the evening prior to the presumed oestrus.

The control animals were three-month old cyclic rats born from mothers which were not treated with misulban ; 24 and 22 control rats were given on the morning of metoestrus morning respectively 20 and 10 IU of PMSG and 150 IU of HCG at 5 p.m. on the evening prior to oestrus. Eggs were recovered by dissecting the oviducts on the following morning.

Results.

1) *Proportion of three-month old females having regular cycles.* — Normal 4-day cycles were observed in 63 p. 100 of control rats. The number of treated rats with 3

normal consecutive cycles decreased to approximately 24 p. 100 with increasing doses of misulban (fig. 1, table 1).

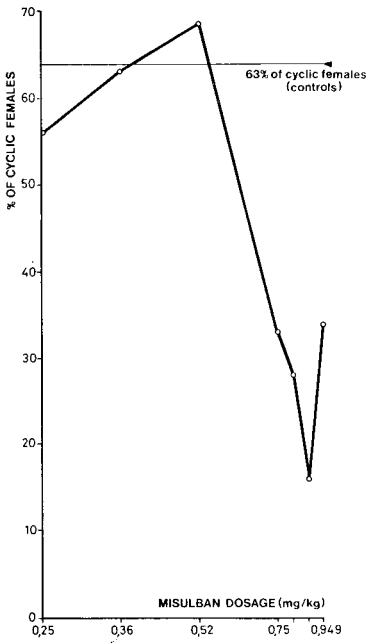

FIG. 1. — *Percent of three-month cyclic rats born from mothers treated with misulban during pregnancy*

2) *Ovarian response expressed by ovulation rates.* — The number of eggs recovered from control rats was 36.5 to 52.08 after 20 IU PMSG and 17,3 to 27,2 after 10 IU PMSG. The mean number of ovulations of cyclic rats treated *in utero* with a dose of misulban lower than 0.75 mg/kg was slightly lower than in control rats treated with 20 IU of PMSG (fig. 2) ; the mean ovulation rate of 3-month old cyclic rats treated *in utero* with a dose of misulban higher than 0.75 mg/kg after 20 IU of PMSG was greatly reduced and was similar to that of control rats receiving only 10 IU of PMSG (fig.2).

Discussion.

De Rooij and Kramer (1970) showed how increasing dosages of misulban gradually destroyed different types of spermatogonia in the rat, mouse and golden hamster.

The destruction of spermatogonia A1, A2, A3, A4, B is only partial when a dose lower than 5 mg/kg is administered but destruction is complete with a dose of 10 mg/kg. Above this dose only partial destruction occurs for pachytene stage ; with a larger dose the destruction of spermatogenesis is complete.

It is possible that misulban given in sufficient quantities partially or completely destroys oogonia population.

It seems that oogonia are as sensitive as spermatogonia since one dose of 10 mg/kg eliminates most of the follicle as soon as birth (0 to 1 p. 100 of the total population — Vanhems, 1975).

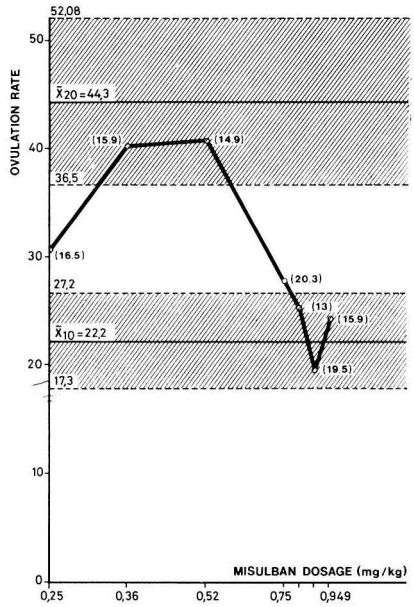

FIG. 2. — *Ovulation rate of cyclic rats born from mothers treated with misulban during pregnancy and receiving at three months of age* 20 UI PMSG and 150 UI HCG. The upper area corresponds to the confidence limit of the mean response of controls receiving 20 UI PMSG. The lower area corresponds to the confidence band of the mean response of controls receiving 10 UI PMSG. The numbers in parenthesis are the standard errors of the ovulation rates for each dose of misulban.

It is not known whether the oogonia population is heterogeneous in respect to the duration of the cellular cycle or if some oogonia subpopulations are more resistant to misulban, depending on the dose administered.

The present study does not permit a choice between the hypothesis of a homogeneous oogonia population gradually being destroyed by increasing dosages of misulban or that of a heterogeneous population including certain types of oogonia which are more resistant than others to a given dosage of misulban.

It is possible that the germ cell destruction is only partial with doses lower than 1 mg/kg. A dose of 1 mg/kg induces complete destruction of oogonia and complete absence of follicles (Vanhems, 1975).

Mechanisms insuring ovulation and ovarian responsiveness to exogenous gonadotrophins are maintained and we have not reached the threshold where the number of follicles left interferes with these mechanisms (Peters, 1969).

But among rats there is a large variation in the oogonia depletion in response to a given dose of misulban. De Rooij and Kramer (1970) have observed the same phenomenon in spermatogonial destruction. It is possible that follicular destruction is too extensive after a single dose of misulban to assess cyclicity of some treated rats.

We suggest that two levels of sensitivity exist. The first is concerned with the sensitivity of the oogonial population to misulban, and the second may implicate a threshold expressed as the minimum number of follicles existing at birth and making cyclicity possible ; below this threshold it may be impossible to allow for the renewal of growing and antral follicles up to three months of age and consequently for feed-back mechanisms insuring cyclicity would be impossible.

The results of this experiment do not support the idea that the ovulation rate is determined by the number of primordial follicles since the ability of a primordial follicle to move from the pool and grow is a determining element for the number of growing and antral follicles (Mariana, 1978).

PMSG acts on this population by reducing the number of atretic follicles (Peters *et al.* 1975), increasing the growth rate (Mariana, 1976) and changing the existing correlation between the number of antral follicles and the number of growing follicles (Mariana, 1976).

The reduced response to PMSG of rats treated with misulban suggests that the number of growing follicles existing in the ovary at three months of age is reduced compared to that present in the control ovary, and that the number of growing follicles present at the time of PMSG treatment is one of the factors determining variability in the number of ovulated eggs.

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Résumé. Des rattes gestantes ont reçu au 15e jour de leur gestation une injection unique de misulban. Les différentes doses injectées étaient 0,25, 0,36, 0,52, 0,75, 0,81, 0,88, 0,95 mg/kg. On observe la descendance de ces rattes, parvenue à l'âge de 3 mois.

1) Le pourcentage de rattes qui sont cycliques à 3 mois décroît en fonction de la dose qui a été injectée aux mères ;

2) Les rattes provenant de mères traitées et devenues cycliques reçoivent à trois mois une injection de 20 UI de PMSG au métœstrus suivie d'une injection de 150 UI d'HCG, la veille de l'œstrus présumé. On observe chez ces animaux : *a*) un taux d'ovulation qui est maintenu quand les mères ont reçu de 0,25 à 0,75 mg/kg de misulban. Ce taux d'ovulation est comparable à celui des témoins qui ont reçu 20 UI de PMS et 150 UI d'HCG ; *b*) un taux d'ovulation qui est équivalent à celui des témoins qui ont reçu 10 UI de PMSG quand les mères ont reçu une dose de misulban supérieure à 0,75 mg/kg.

On fait les hypothèses : *a*) que la population des ovogonies est sensible à des doses croissantes de misulban, *b*) qu'un nombre suffisant de follicules primordiaux est nécessaire à la naissance pour permettre les mécanismes de la cyclicité à 3 mois.

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