INVESTIGATIONS ON THE MODE OF ACTION OF D-6-METHYL-8-CYANOMETHYLERGOLINE IN SUPPRESSING PREGNANCY IN THE MOUSE

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Pregnancy in the rat can be prevented by a single intragastric administration of D-6-methyl-8-cyanomethylergoline (CME), a new synthetic ergoline derivative, during the first 7 days after mating (Režabek, Semonský & Kucharczyk, 1969). This substance is structurally closely related to the naturally occurring alkaloid, agroclavine, which has been shown to prevent implantation in mice and rats (Mantle, 1969; Edwardson & MacGregor, 1969).

In a preliminary experiment, following the technique (Režabek *et al.*, 1969) successfully used in rats, CME (250 μ g in 250 μ l of 1% tartaric acid) was administered to five mice by a single intragastric injection on each of the first 7 days of pregnancy. Eighteen out of thirty-five treated mice remained pregnant in comparison with twenty-three out of thirty-five untreated control mice. Thus, as there was no consistent evidence that implantation had been suppressed, it was concluded that the mouse was possibly less sensitive to a single intragastric administration of CME.

By contrast, daily administration of food (5g) containing 250 μ g CME to groups of five mice during 3-day periods after mating (Days 1, 2 and 3; Days 3, 4 and 5; Days 5, 6 and 7) completely prevented pregnancy. The effect on the uterus in the immediate implantation period (Days 3, 4 and 5) was similar to that previously observed in response to treatment with agroclavine (Mantle, 1969; Finn & Mantle, 1969). In subsequent studies, therefore, CME was administered in the food.

A single day's treatment with CME on Day 1 did not affect the cellular changes which take place in the uterus just before implantation (Finn & Martin, 1967) and pregnancy was not prevented (Tables 1 and 2). If, however, CME treatment was continued for two days, this resulted, as in those mice treated on Days 1, 2 and 3, in a failure of the uterine cellular changes characteristic of Day 4 of pregnancy. In spite of returning the CME-treated mice to a normal diet on Day 3, the effect of the alkaloid was not diminished and implantation did not take place.

Two days' treatment with CME postimplantation, starting on Day 6, elicited no macroscopic effect on pregnant uteri by Day 7, but resorption of all foetuses was evident in several animals by Day 9 (Table 1). Similar treatment beginning on Day 7 had no effect on pregnancy.

TABLE 1
EFFECT OF CME ON EARLY PREGNANCY IN MICE

<i>CME meal given*</i>	<i>Mice killed</i>	<i>No. of animals</i>	<i>Pregnant</i>	<i>Condition of pregnant uteri</i>
Day 1	Parturition	4	3	Normal
Days 1 to 2	Parturition	4	0	—
None	Parturition	4	4	Normal
Day 6	Day 7	6	6	Normal
None	Day 7	5	4	Normal
Days 6 to 7	Day 8	5	4	Three normal; one early resorption
Days 6 to 7	Day 9	5	5	One normal; four resorption
Days 6 to 7	Parturition	6	2	Normal
None	Day 9	5	4	Normal
Days 7 to 8	Parturition	7	7	Normal
None	Parturition	6	6	Normal

* 5 mg CME/100 g meal.(5 g/mouse/day up to Day 5, 6 g/mouse/day from Day 6.)

TABLE 2
EFFECT OF CME, WITH OR WITHOUT PROLACTIN, ON CELL DIVISION IN THE UTERUS ON THE 4TH DAY OF PREGNANCY

<i>CME meal given*</i>	<i>No. of animals†</i>	<i>Mean no. of mitoses ± S.E.</i>		
		<i>Epithelium</i>		<i>Stroma</i>
		<i>Lumen</i>	<i>Glands</i>	
Day 1	4	0	1 ± 1	33.5 ± 4.3
Days 1 and 2	6	33 ± 17.5	28.3 ± 9.0	10.6 ± 4.7
Days 1, 2 and 3	4	53.2 ± 23.7	7.5 ± 3.1	9.7 ± 1.4
Days 1 and 2 +prolactin† Days 2, 3 and 4	4	0	0	23.0 ± 5.6

* 5 g of 5 mg CME/100 g meal/day/mouse.

† 12.5 i.u. subcutaneously morning and evening (NIH P-S-G ovine prolactin).

‡ Killed 2 hr after subcutaneous injection of Colcemid (0.1 mg) (Finn & Martin, 1967).

TABLE 3
EFFECT ON PREGNANCY OF PROLACTIN REPLACEMENT THERAPY FOLLOWING PREGNANCY-SUPPRESSING TREATMENT WITH CME

<i>CME meal given*</i>	<i>Prolactin given†</i>	<i>Mice killed</i>	<i>No. of animals</i>	<i>Pregnant</i>
Days 1 and 2	Days 2 to 5	Day 6	4	4
Days 1 and 2	None	Day 8	5	0
Days 1 and 2	Days 2 to 5	Day 10	4	4
Days 1 and 2	Days 2 to 10	Postparturition	4	4
None	Days 2 to 10	Postparturition	5	4
None	None	Postparturition	9	9

* 5 g of 5 mg CME/100 g meal/day/mouse.

† 12.5 i.u. subcutaneously morning and evening.

The cellular changes in the uterus just before implantation are influenced by progesterone which is secreted from the corpora lutea under the control of pituitary prolactin. The pituitary is essential until about Day 7 of pregnancy when the placenta starts secreting a luteotrophic substance. Thus, the effects observed following CME treatment could be mediated through a block in the release of pituitary prolactin. To investigate this, the effect of exogenous ovine prolactin was tested (Tables 2 and 3). The total suppression of pregnancy by CME treatment on Days 1 and 2 was completely reversed by prolactin so that the usual preparation of the uterus for implantation occurred (Table 2) and normal pregnancy was established (Table 3). The ineffectiveness of CME treatment on Days 7 and 8 suggests that there is no effect on luteotrophin synthesis in the placenta but that the suppression of pregnancy at an earlier stage is mediated at the pituitary or hypothalamic level.

The effect of CME on the induction of pseudopregnancy was also investigated. Thirteen female mice were fed on the standard CME diet (250 µg CME/mouse/day) for 2 days after mating with a vasectomized male. Eleven mice returned to oestrus on Day 4 or 5. The immediate postcoital administration of CME apparently prevented pseudopregnancy, which in ten out of ten untreated control mice lasted for 10 to 12 days; a result in accord with the suppression of pituitary prolactin.

Recently, Seda, Režabek, Marhan & Semonský (1971) have shown a gonadotrophic effect of CME on ovarian hypertrophy after unilateral ovariectomy and suggested that CME stimulates the hypothalamus to release FSH- and LH-releasing factors. The present results, by indicating the suppression of prolactin secretion, also support the possibility of a general hypothalamic stimulation. If there is a general stimulatory effect on the hypothalamus, the complete restoration of normal pregnancy by exogenous prolactin implies that the pregnancy-inhibiting effect of CME is mediated principally by an increase in prolactin-inhibiting factor.

In the mouse, CME appeared to have a more persistent action than agroclavine (Mantle, 1969). It is also less toxic and, being a dihydro-ergoline derivative, is more stable than agroclavine. It is suggested that, if the central effects of CME can be confirmed by more direct methods, this substance could become a useful tool in the study of the rôle of prolactin.

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