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Teratogenic Effect of Chlormadinone Acetate in Mice and Rabbits. (30803)

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Among steroids, glucocorticoids(1,2) and estrogens(3,4) are known to induce fetal abnormality, such as cleft palate and open eye, when administered to pregnant animals in large amounts. For progestogens such teratogenicity has not been reported, though some of them are known to cause masculinization of the female fetuses when administered during pregnancy(5-8). However, it is noteworthy that progesterone showed an antimitotic effect on the cultured cells or mammalian eggs(9-11). Hence, a study was undertaken to ascertain whether or not progestogens, remedies commonly used for the therapy of threatened abortions or as oral contraceptives, administered at various stages of pregnancy produce any fetal malformation other than female pseudohermaphroditism.

Materials and methods. We used for our experiments 3 groups of colony bred stock animals: Japanese ddS mice from Aburahi Farm, Shionogi & Co., Ltd., Aburahi, Shiga Prefecture; CF#1 mice maintained at this laboratory; and Japanese albino rabbits purchased from Funabashi Farm, Funabashi, Chiba Prefecture. Animals were kept on a diet of pellets: ddS mice on those prepared by Aburahi Farm (crude protein, 23.7%; crude fat, 6.7%; carbohydrate, 49.6%; total ash, 3.3%); CF#1 mice on N.M.F. pellets supplied by Oriental Co., Tokyo (crude protein, 26.5%; crude fat, 6.1%; crude fiber, 4.1%; N.F.E., 49.8%; total ash, 6.5%); and albino rabbits on those supplied by Funabashi Farm (crude protein, 24.9%; crude fat, 4.1%; crude fiber, 17.0%; N.F.E., 43.3%; total ash, 5.6%). In addition, fresh tap water was given *ad libitum*.

Suspension of chlormadinone acetate (CA), of norethisterone (NE), and of norethynodrel (ND) in 0.5% sodium carboxymethyl cellulose (CMC) aqueous solution was prepared at various concentrations.† In mice nulliparous females at 12 to 16 weeks of age were used and the day when the vaginal plug was found was designated as day 0 of gestation. The suspension was given at 10 ml/kg of body weight by an esophageal tube once a day during day 8-15, 14-17 or 8-17 of gestation. In rabbits females at 8 to 10 months of age were used and the day when copulation was observed was described as day 0. The suspension was given at 1 ml/kg by a gastric tube daily from day 8 to day 20 of gestation. Sodium carboxymethyl cellulose solution without the tested compound was given to the control animals in the same way as in the respective experiment. Mice were sacri-

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† NE and ND contain mestranol as an additive at the rate of 1 and 2% respectively. The CMC aqueous solution contains Tween 80 at 0.5%.

TABLE I. Effect of Progestogens Administered Orally to Pregnant Mice or Rabbits upon Their Offspring. Chlormadinone acetate (CA), norethisterone with 1% mestranol (NE), and norethynodrel with 2% mestranol (ND) were used.

Group	Drug	Daily dose (mg/kg)	Day of treatment	No. of mothers	No. of fetuses	Dead or resorbed (%)	Survivors malformed (%)	Type and No. of malformations†
				Japanese ddS mice				
1a	CA	50	8-15	19	145	68.9*	75.5*	CP:34*
1b	CA	50	14-17	24	185	17.3*	18.3*	CP:27,* CF:2
1c	CA	10	8-15	23	151	33.1*	53.5*	CP:53*
1d	CA	10	14-17	17	128	5.5	12.4*	CP:15*
1e	CA	3	8-17	19	143	11.2	12.6*	CP:15,* CF:1
1f	CA	1	8-17	18	139	7.2	9.3*	CP:12*
1g	NE	10	8-15	24	193	29.5*	2.9	CF:2, CP:1, PD:1
1h	NE	10	14-17	19	141	9.9	4.7	CF:6
1i	NE	3	8-17	21	170	16.5	2.8	CP:4
1j	NE	1	8-17	20	161	16.8	.7	CF:1
1k	ND	10	8-15	24	187	98.9*	100.0	CP:2
1l	ND	10	14-17	21	171	4.7	4.9	CP:8
1m	Placebo		8-15	35	268	13.8	3.0	CF:4, CP:2, PD:1
1n	"		14-17	39	302	7.6	2.9	CF:7, PD:1
1o	"		8-17	33	260	12.2	2.6	CF:4, CP:1, PD:1
				CF #1 mice				
2a	CA	10	8-17	22	209	12.4	9.3	CP:13,* CF:3, PD:1
2b	CA	1	8-17	20	198	5.1	2.6	CF:3, CP:1, PD:1
2c	Placebo		8-17	16	136	12.5	3.4	PD:4
				Japanese albino rabbits				
3a	CA	10	8-20	9	82	45.1*	60.0*	CW:14,* DA:12,* CP:10,* EC:4
3b	CA	3	8-20	10	79	13.9	13.2	CW:5, CP:4, CY:1, OD:1
3c	CA	1	8-20	11	96	7.3	1.1	OD:1
3d	NE	10	8-20	5	(All animals died from anorexia)			
3e	NE	3	8-20	6	34	79.4*	.0	—
3f	NE	1	8-20	6	58	13.8	6.0	AC:1, OE:1, SB:1
3g	Placebo		8-20	12	118	17.0	4.1	CW:1, OD:1, SB:1, ST:1

* Significantly higher than the respective control at $P < .01$.

† AC: Acephaly; CF: Clubfoot; CP: Cleft palate; CW: Contracture of the wrist joint; CY: Cyclopia; DA: Defect of the abdominal wall; EC: Exencephaly; OD: Oligodactyly; OE: Open eyelids; PD: Polydactyly; SB: Spina bifida; ST: Short tail.

ficed on day 18 and rabbits on day 29 of gestation, when the fetuses were examined for intrauterine resorption or death and for external deformities. Body weight and anogenital distance of the surviving fetuses were also examined.

Results and discussion. The results are presented in Table I. In ddS mice, the mortality of the fetuses in Group *1a, 1b, 1c, 1g* and *1k* in the Table was significantly higher than that in the respective control *1m* or *1n* ($P<0.01$). The treatment beginning from day 14 was less lethal than that from day 8. Among the tested compounds ND showed the highest lethal effect in accordance with the result in the rats(12). Surprising was the fact that CA did induce malformations in the surviving fetuses even at 1 mg/kg of body weight and their incidence increased according to the dosage. Most of the malformations were cleft palate. It is interesting that the administration of CA beginning on day 14 induced cleft palate, though the effect was less severe than the treatment begun on day 8, as in the case of cortisone(13). In ddS mice cleft palate occurs in rare cases spontaneously, as shown in the control groups *1m* and *1o*, and this stock might thus be considered to be fairly susceptible to various teratogenic agents. CF#1 mice, in which no spontaneous occurrence of cleft palate had been observed in our laboratory, were more resistant to 1 mg/kg of CA but were susceptible to 10 mg/kg of the agent. Body weight of the surviving fetuses was not significantly affected nor was masculinization of the female fetuses observed in any group of the experiments or in the controls.

Chlormadinone also produced a teratogenic effect on the offspring of the rabbits in Group

3a. Types of the malformations induced in this group were more varied than those in mice, for example, contracture of the wrist joint, defect of the abdominal wall and cleft palate were observed at a significantly high frequency in comparison with that in the control. On the other hand, lethal effect of NE on the rabbit fetuses was far higher than that of CA. NE, however, did not show teratogenicity even in doses large enough to kill most of the fetuses. Though not shown in the Table, CA generally suppressed the growth of the surviving rabbit fetuses, including the malformed ones: mean body weight of Group *3a, 3b* and *3c* was 26.4, 38.7 and 42.1 g respectively, each of which was significantly lower than 45.5 g in the control *3g*. Masculinization of the female fetuses was recognized in neither CA groups nor NE groups. Maternal food intake was never suppressed by CA in contrast to NE, which killed the pregnant animals at 10 mg/kg, probably owing to severe anorexia, and even at 1 mg/kg significantly decreased the food intake. In order to see whether or not the tested compounds had exerted a corticoid action, the adrenal gland of the mother rabbits at day 29 was weighed and examined histologically in comparison with the control. No involution of the cortex was found in any experimental group.

It is concluded that a progestogen, CA, which has scarcely any corticoid-like or estrogen-like effect(14), exerts a teratogenic effect other than masculinization of the female when administered in a large dose to pregnant mice and rabbits. However, from the above results, it should not be concluded that CA will show similar adverse effects on human fetuses when administered to women at therapeutic doses in gynecological practice(15-17). In attempting to extrapolate these animal data to man, it must be kept in mind that a considerable dosage difference exists. Effective doses of CA determined by Clauberg assay, based on inhibition of ovulation in the adult rabbits or pregnancy maintenance in the ovariectomized rabbits, are reported to be about or less than 0.05 mg/kg(18-20), which is much lower than the teratogenic dose in our experiments.

Summary. Oral administration of a large amount of chlormadinone acetate into pregnant mice and rabbits produced a large number of fetal malformations, such as cleft palate and others, while norethisterone with 1% mestranol showed no such teratogenic activity though its lethal effect on the fetuses was stronger than that of chlormadinone acetate. Norethynodrel with 2% mestranol showed the strongest lethal effect of the progestogens tested in mouse embryos, and cleft palate was induced in the surviving fetuses at 10 mg/kg. The dosage of the drugs which showed teratogenicity was generally much higher than human therapeutic or pharmacological doses in rabbits on the basis of maternal body weight.

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