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Cyproterone Acetate Exposure During Gestation in Mice Retards Fetal Growth

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VOM SAAL, F. S. *Cyproterone acetate exposure during gestation in mice retards fetal growth.* PHYSIOL. BEHAV. 21(4) 515–517, 1978.—Pregnant mice were injected with one of seven doses of cyproterone acetate (CA) from Day 12 through Day 17 of pregnancy. Exposure to doses of CA which blocked masculinization (as indicated by anogenital distance) severely retarded fetal growth. CA also increased the incidence of fetal deaths. CA is thus not recommended for use as an antiandrogen during gestation in mice.

Cyproterone acetate Antiandrogen Mice Gestation Fetal growth

CYPROTERONE acetate (CA) is an antiandrogen which is used in clinical studies to arrest precocious puberty in children [5] and hirsutism in women [4]. Cyproterone acetate also has been used extensively in animal research as a tool for revealing the normal action of testicular androgen. For example, CA administered to pregnant rats during late gestation yields male offspring that are indistinguishable from females at birth on external examination [2].

The present experiment was designed to determine an effective dose of CA which would block masculinization of male mouse fetuses when administered during the last trimester of gestation (when male fetuses produce androgen; [1]). The objective was to utilize such non-masculinized males for behavioral studies during later life.

METHOD

Thirty nulliparous female Rockland-Swiss (R-S) mice, 60 days of age, were timed-mated, with Day 0 of pregnancy being defined as the date a vaginal plug was observed. All females then were isolated and assigned to one of seven treatment groups identified by the dose of CA administered per injection. The doses of CA administered per injection were: 0 (oil), 50 μg, 100 μg, 250 μg, 500 μg, 1 mg, and 2 mg. The CA was suspended in sesame oil and administered via a 0.02 cc subcutaneous injection each evening at 2100 hr from Day 12 through Day 17 of gestation. All pups were delivered surgically between 2000 hr and 2400 hr on Day 18 of gestation, just prior to the onset of normal parturition in untreated mothers.

The number of fetuses being resorbed was noted at delivery. After each pup was cleaned, the anogenital distance (the distance between the genital papilla and the anus) was measured under a dissecting microscope using calipers (Univar) accurate to 0.05 mm. The pups then were weighed using a Mettler p1200 balance and were killed to verify sex (as revealed by the presence of testes or ovaries). The data were analyzed by analysis of variance, and a posteriori comparisons were made using Duncan's New Multiple Range Test ($p<0.05$).

RESULTS AND DISCUSSION

Twenty-six fetuses were being resorbed at term in the 18 litters treated with 250 μg, 500 μg, 1 mg and 2 mg of CA. On the other hand, there were only two resorbtions observed in the 12 litters treated with oil, 50 μg and 100 μg of CA. The total number of pups carried to term did not vary significantly as a function of dose, however. Ellendorf, Rover and Smidt [3] also reported that administering 5 mg of CA to pregnant mice caused an increase in the number of fetuses dead at term; however, they did not report any morphological data.

Body weight of both males and females varied as a function of dose of CA administered as indicated by a significant Dose effect for males, $F(6,115)=25$, $p<0.001$, and for females, $F(6,144)=24$, $p<0.001$ (see Fig. 1). Males and females did not differ in body weight. The pups exposed to the 50 μg dose of CA weighed significantly less than oil-treated pups but were significantly heavier than the pups exposed to the higher doses of CA. The pups exposed to the 100 μg through 2 mg doses of CA did not differ from each other and were approximately three-fourths normal weight at delivery.

The anogenital distance of a mouse at birth serves as a bioassay for androgen exposure during gestation. Anogenital distance of both males and females was found to vary significantly as a function of dose of CA (males: $F(6,115)=162$, $p<0.001$; females: $F(6,144)=8$, $p<0.001$). However, the CA

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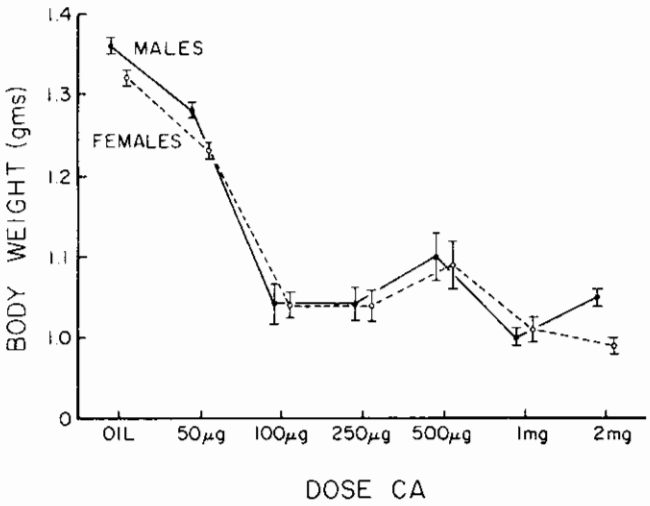

FIG. 1. Mean body weight of males and females at birth as a function of the dose of cyproterone acetate (CA) administered during gestation.

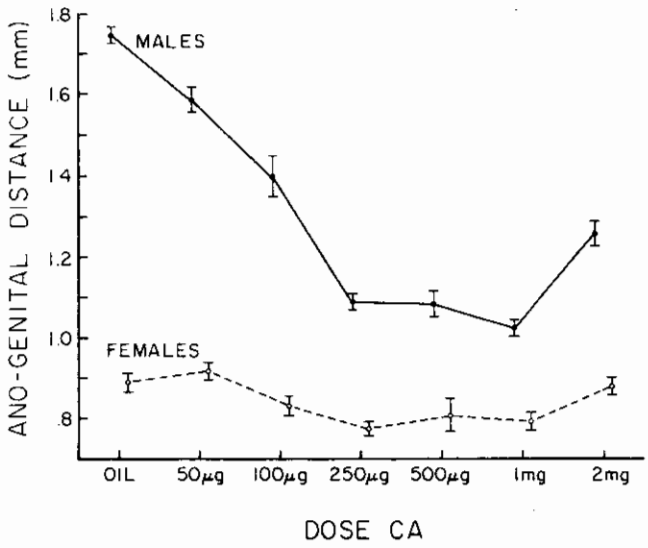

FIG. 2. Adjusted mean anogenital distance of males and females at birth as a function of the dose of CA administered during gestation. Means are adjusted for the effect of cyproterone acetate (CA) on body weight.

had a greater effect on the males than the females as a function of increasing dosage (Dose×Sex interaction, $F(6,259)=59$, $p<0.001$). Contrary to the data in rats, at no dose were the males indistinguishable from the females. In all cases, positive identification of sex was possible based on the anogenital distance measure. The Duncan's Test revealed that for males, the mean anogenital distance decreased significantly with each increment in dose up to 250 μg, while the means for the 250 μg, 500 μg and 1 mg groups did not differ. The mean anogenital distance of the male pups in the 2 mg group was significantly larger than the means for the 250 μg, 500 μg and 1 mg groups but significantly smaller than the mean for the 100 μg group.

CA had a similar dose related effect on the anogenital distance of female pups. As was the case for the males, the 250 μg, 500μg and 1 mg doses of CA produced the largest decrease in mean anogenital distance for the female pups. The oil, 50 μg and 2 mg treated females did not differ from each other but were significantly larger than the means for the 250 μg, 500 μg and 1 mg groups. The mean anogenital distance of females exposed to the 100 μg dose was intermediate and did not differ significantly from the mean of any other group.

Since both body weight and anogenital distance varied as a function of dose of CA, it was necessary to determine if the effect of CA on anogenital distance was secondary to the effect of CA on body weight. Analysis of covariance revealed that when corrected for the effect of CA on body weight, anogenital distance still varied significantly as a function of dose for both males, $F(6,114)=79$, $p<0.001$, and for females, $F(6,143)=5$, $p<0.001$ (see Fig. 2). It should be noted that the analysis of covariance resulted in the same distribution of means as described above for the analysis of variance.

To determine if the effect of CA on growth was strain specific, 250 μg of CA was administered to pregnant CF-1 female mice, originally obtained from Charles River (Wilmington, MA), using the procedure previously described. The results were identical in all respects to those observed in the R–S fetuses exposed to the 250 μg dose of CA.

The fact that there was a dose related change in the anogenital distance measure for the female fetuses which was similar to that for the males suggested that CA was exerting an antiandrogenic effect on females as well as males. vom Saal and Bronson [8] have subsequently found that in utero contiguity to male fetuses masculinizes females morphologically and behaviorally during development and adulthood. More specifically, female mouse fetuses located between male fetuses have a larger anogenital distance both at birth and in adulthood and are masculinized in terms of the transmission of and response to reproductively-important social cues relative to females located next to other female fetuses during gestation. In a separate experiment, vom Saal [7] found that exposure to the 250 μg dose of CA, using the procedure reported above, eliminated the effect of contiguity to male fetuses on the anogenital distance of female mice at birth.

While the above finding concerning the effect of CA on the anogenital distance measure in females is intriguing, the profound effect that CA had on fetal growth necessitates the use of caution in interpreting the data. Even subsequent to birth mice remain sensitive to nonspecific effects of CA on development; Polackova, Viklicki and Vojtiskova [6] administered CA to mice from birth to Day 30 and also found that CA exposure severely retarded growth and produced morphological changes in some tissues.

Taken together, these findings indicate that exposure to CA during both prenatal and postnatal development can severely retard growth in mice. Thus, the use of CA as an androgen antagonist in mice is not recommended, at least prior to adulthood.

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REFERENCES

1. Block, E., M. Lew and M. Klein. Studies on the inhibition of androgen formation: testosterone synthesis by fetal and newborn mouse testes in vitro. *Endocrinology* **88:** 41–46, 1971.
2. Elger, W., F. Neumann and R. vonBerswordt-Wallrabe. The influence of androgen antagonists and progestagens on the sex differentiation of different mammalian species. In: *Hormones in Development*, edited by M. Hamburgh and E. Barrington. New York: Appleton-Century-Crofts, 1971, pp. 651–667.
3. Ellendorf, F., H. Rover and D. Smidt. The effect of cyrpoterone acetate on the duration of pregnancy and parturition in mice. *J. Reprod. Fert.* **32:** 299–303, 1973.
4. Ismail, A., D. Davidson, A. Souka, E. Barnes, W. Irvine, H. Kilmnik and Y. Vanderbeeken. The evaluation of the role of androgens in hirsutism and the use of a new anto-androgen "cyproterone acetate" for therapy. *J. clin. Endocr. Metab.* **39:** 81–95, 1974.
5. Kauli, R., R. Prager-Lewin, R. Keret and Z. Laron. The LH response to LH releasing hormone in children with true isosexual precocious puberty treated with cyproterone acetate. *Clin. Endocrinol.* **4:** 305–311, 1975.
6. Polackova, M., V. Viklicky and M. Vojtiskova. Retardation of development including immunogenic expression of histocompatability antigens in mice by post-natal administration of antiandrogenic steroid. *Folia Biol.* **21:** 20–26, 1975.
7. vom Saal, F. S. Prenatal exposure to androgen influences intraspecific aggression in male and female mice. Unpublished doctoral thesis, Rutgers University, 1976.
8. vom Saal, F. S. and F. H. Bronson. *In utero* proximity of female mouse fetuses to males: effect on reproductive performance during later life. *Biol. Reprod.* (in press).