less antigenic if unadsorbed. Since mineral carriers are believed to exert their antibody enhancing effects by increasing the particle size of the antigen given in vaccines, the most likely explanation for the present findings would seem to be that use of the mineral carrier achieved a near optimal particle size with undegraded HA antigens(9). Since the amount of $AlPO_4$ used in the present investigation adsorbed 50% or less of the hemagglutinating activity of the vaccines, a search for more efficient adsorbants might prove rewarding.

The fact that HA vaccines behaved as weaker antigens in mice but strongly in rabbits and humans remains unexplained. In considering this discrepancy it is noteworthy that in the separate experiments with the different HA vaccines a very large dose was used for the primary and booster inoculations of rabbits and that a large dose was given to adult human subjects who as a class generally exhibit booster responses (1–4). In contrast, the dosage of vaccines given to mice was deliberately lowered in order to determine antigenic extinction titers. The differences noted, therefore, may be dose dependent. However, the possibility that different species might respond differently to HA vaccines cannot be ignored. Clearly further studies are needed if the results of testing HA vaccines in animals are to be used as standards for predicting the performance of such products in man.

Summary. Virus vaccines with $AlPO_4$, HA vaccines and HA vaccines with $AlPO_4$ were prepared as monovalent suspensions using 4 strains of influenza A and 2 of influenza B. In all cases, dilutions of the HA vaccines induced lower antibody levels and lower levels of immunity than their virus counterparts. Addition of $AlPO_4$ restored the antigenicity of HA vaccines to levels almost equal to those of whole virus vaccines. The significance of these findings is briefly discussed.

1. Davenport, F. M., Hennessy, A. V., Brandon, F. B., Webster, R. G., Barrett, C. D., and Lease, G. O., J. Lab. Clin. Med. **63**, 5 (1964).

2. Hennessy, A. V. and Davenport, F. M., J. Immunol. **97**, 235 (1966).

3. Brandon, F. B., Cox, F., Lease, G. O., Timm, E. A., Quinn, E., and McLean, I. W., Jr., J. Immunol. **98**, 800 (1967).

4. Webster, R. G. and Laver, W. G., J. Immunol. **96**, 596 (1966).

5. Drescher, J., Hennessy, A. V., and Davenport, F. M., J. Immunol. **89**, 794 (1962).

6. Horsfall, F. L., Jr., J. Exptl. Med. **70**, 209 (1939).

7. Committee on Standard Serological Procedures in Influenza Studies, J. Immunol. **65**, 347 (1950).

8. Davenport, F. M., Hennessy, A. V., Drescher, J., and Webster, R. G., "CIBA Found. Symp. Cellular Biol. Myxovirus Infections," p. 272. Little, Brown, Boston, Massachusetts, 1964.

9. Edsall, G., "Intern. Conf. Vaccines Against Viral and Rickettsial Diseases of Man, 1st," p. 560. Scientific Pub. No. 147, PAHO/WHO, 1967.

Received Oct. 12, 1967. P.S.E.B.M., 1968, Vol. 127.

Critical Exposure Time for Androgenization of the Rat Hypothalamus Determined by Antiandrogen Injection* (32749)

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It is well established that there is a postnatal steroid sensitive period during which testicular or exogenous androgen may induce a permanent masculinization of the hypothalamic mechanisms which regulate pituitary gonadotrophin secretion in the rat, i.e., androgenization (1–3). Recent studies indicate that pentobarbital can protect the 5-day-old female from this action of androgen, provided the barbiturate is administered within ap-

* Supported in part by USPHS Grant HD-01182, and a Training Grant from the Ford Foundation.

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TABLE I. Critical Exposure Time for Androgenization of the 5-Day-Old Rat as Determined by Antiandrogen Injection.

Androgen (μg of TP) at time zero on day 5	Time of CA[a] injection (hours after androgen)	Incidence of sterility (No. anovulatory/No. injected)			
		at 45 days of age	%	at 90 days of age	%
30	none[b]	10/13	83.3	11/12	91.6
30	zero	3/28[c]	10.3	7/20	30.0
30	6	11/18	58.8	16/18	88.8
30	12	10/14	71.4	11/14	78.5
30	24	10/13	77.5	12/13	92.3
none[d]	zero	1/11	9.0	1/11	9.0

[a] Cyproterone acetate, 0.6 mg/0.05 ml of castor oil–benzyl benzoate vehicle.
[b] Given 0.05 ml of castor oil vehicle.
[c] Eight of these animals sacrificed at 45 days of age.
[d] Given 0.02 ml of peanut oil.

proximately 12 hours of the androgen injection[2]. It seems likely that the developmental changes in neurons in response to the inductive stimulation of androgen may be triggered very rapidly and perhaps be completed within a short period of time.

It has been reported that cyproterone acetate[3] (CA), a synthetic antiandrogenic steroid, counteracts the effect of exogenous androgen in adult male (4) or prenatal female (5) rats, and prevents androgen induced differentiation of the male reproductive system (6–8). Prenatal and neonatal treatment with CA prevents normal masculinization of the male hypothalamus (9,10). The present experiments were undertaken to demonstrate[4] the protective action of CA against androgen sterilization in female rats, and to investigate further the minimal exposure to androgen needed for androgenization.

Materials and Methods. Sprague-Dawley female rats were raised in our vivarium and were treated with steroid(s) on day 5 of age (the day of birth was considered day one). A single subcutaneous injection of 30 μg of testosterone propionate (TP) was given in 0.02 ml of peanut oil. At a different subcutaneous site, 0.6 mg of CA was injected in 0.05 ml of castor oil–benzyl benzoate mixture [as previously described (5)] at various intervals following TP injection: 0, 6, 12, or 24 hours. At 45 days of age, these females were either laparotomized or sacrificed to check for the presence or absence of corpora lutea in their ovaries. At 90 days, the surviving animals were autopsied and body and organ weights were recorded.

Results. A single injection of 30 μg of TP given to female rats on day 5 resulted in anovulatory sterility in 83.3 and 91.6% of the animals at 45 and 90 days of age, respectively (Table I). When CA was given at the same time with TP, only 10.3% of the rats were anovulatory at 45 days of age, and the incidence of sterility[5] (IS) remained low at autopsy (30.0). However, when TP was allowed to act for 6 hours before the injection of CA, the IS was increased to 58.8 at 45 days of age, and 88.8% of the rats were anovulatory at 90 days of age. Five of the seven animals of this group which had ovulated during their postpubertal period developed the anovulatory persistent estrus syndrome by 90 days of age. This rate of 5/18 represents a moderate incidence of the Delayed Anovulation Syndrome.[6]

When TP was allowed to act for 12 hours

[2] Arai, Y., Gorski, R. A., *Endocrinology,* in press.
[3] 6-chloro-Δ^6 -1,2 α-methylene-17-hydroxyprogesterone acetate.
[4] During preparation of this manuscript Wollman and Hamilton (11) published data which demonstrate that 25–100 μg of CA can protect against the injection of 10 μg of TP on day 5.
[5] Number anovulatory rats/number injected rats, times 100.
[6] Gorski, R. A., Endocrinology, in press.

TABLE II. Data Obtained at Autopsy of 90-Day-Old Female Rats Injected with TP and/or Antiandrogen at 5 Days of Age.

Injections	Time of injection of CA (hours after TP)	Condition of ovaries	No. of rats	Body wt. (gm)	Ovarian wt. (mg)	Uterine wt. (mg)
30 μg TP	—	Normal	1	253	98.2	486
		Polyfollicular	11	242 ± 7[a]	40.2 ± 4.2	462 ± 21
30 μg TP + CA	zero	Normal	13	263 ± 7	103.6 ± 8.5	527 ± 22
		Polyfollicular	7	264 ± 8	58.2 ± 4.2	456 ± 23
	6	Normal	2	279	89.2	487
		Polyfollicular	6	250 ± 5	42.6 ± 2.2	438 ± 16
	12	Normal	3	268	76.7	382
		Polyfollicular	11	256 ±11	45.5 ± 6.5	438 ± 44
	24	Normal	1	255	124.5	514
		Polyfollicular	12	243 ± 5	51.5 ± 1.8	492 ± 41
CA	—	Normal	11	232 ±10	95.0 ± 3.5	476 ± 16
		Polyfollicular	1	241	41.2	428

[a] Mean ± SE.

or more prior to antiandrogen injection, CA was completely ineffective. The CA alone was essentially without effect, although 1 out of 11 rats did become anovulatory. Autopsy data for androgen and CA injected rats are presented in Table II. Both ovarian and uterine weight in animals apparently protected by CA are greater than in the anovulatory females. This is due to the absence of corpora lutea and normal cyclic ovarian secretory activity in the latter. The dramatic influence of the interinjection interval on the effectiveness of CA is illustrated in Fig. 1.

Discussion. These data confirm the observation of Wollman and Hamilton (11) that CA can protect neonatal female rats from androgen induced masculinization of the hypothalamus. Both studies are consistent with the ability of CA to prevent normal hypothalamic development by counteracting the influence of endogenous androgen in the male (9, 10). However, the present data also indicate that exposure of the hypothalamus of the 5-day-old female to androgen from an injection of 30 μg of TP for about 6 hours, is sufficient time to render CA ineffective as a protector against androgenization. Although 41.2% (7 of 18) of the animals given CA 6 hours after TP do ovulate postpubertally, by 90 days of age, five of these seven females were anovulatory and possessed polyfollicular ovaries. This development of the Delayed Anovulation Syndrome[6] in these five animals suggests that CA is at best, capable of exerting only partial protection against TP, when given 6 hours after the androgen injection.

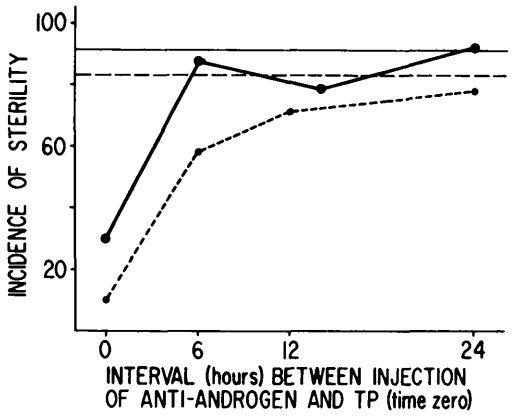

FIG. 1. Incidence of sterility (IS) at 45 and 90 days of age following injection of 30 μg of TP to the 5-day-old female rat alone, simultaneous with, or at various intervals prior to cyproterone acetate (CA) injection. —— IS at 90 days of age following injection of 30 μg of TP on day 5; horizontal - - - - IS of these rats at day 45.

This report and others (9,10,12) indicate that CA inhibits the action of androgen on the brain as well as on its peripheral target organs. However, the mechanism of CA–androgen antagonism is unknown. It has been suggested that CA competitively inhibits the action of androgen at the level of receptors within its target organs (4,6). On the basis of our previous study of the protective effect of barbiturates against androgenization,[2] it is possible to suggest that CA inhibits the uptake of androgen by hypothalamic neurons in the 5-day-old rat. In that study comparison of the protective effect of delayed injections of pentobarbital or phenobarbital suggested that the latter (or more likely, a low dose of barbiturate) specifically inhibited an early and brief phase of androgen action, presumably androgen uptake by the hypothalamus. The observation that CA can protect against the effect of TP only when given with the androgen, suggests that CA antagonizes only the early stages of androgenization. Furthermore, the observation that the Delayed Anovulation Syndrome is not of high incidence is consistent with an effect of CA on androgen entry into hypothalamic neurons or other active sites. If androgen cannot enter the neuron and/or initiate unknown biochemical processes, even partial masculinization of the hypothalamus cannot occur. The probable rapid action of androgen is supported by the observation that radioisotope labeled steroids reach maximum concentrations in the brain within 1 hour after injection (13).

Although only one dose of CA was utilized, this dose is 6–24 times greater than that previously reported as effective when given simultaneously with TP (11). It seems unlikely that higher doses of CA would be able to interrupt or prevent subsequent phases in androgenization, as does pentobarbital. Further analysis of the dynamics of the interaction between the developing brain, androgen, and specific or nonspecific blocking agents, may elucidate both the mechanism of action of CA, and the nature of the changes induced in the hypothalamus by the inductive stimulus of androgen.

Summary. The simultaneous injection of 0.6 mg cyproterone acetate (CA), an antiandrogen, protects the 5-day-old female rat against the hypothalamic masculinizing action of 30 μg testosterone propionate. When CA was administered 6 or more hours after androgen injection, however, its effectiveness was markedly reduced. It is concluded that a CA-labile component of the process of hypothalamic masculinization, which lasts for less than 6 hours, may consist of the uptake of androgen by hypothalamic neurons.

We wish to express our appreciation to B. Gibson for technical assistance, Dr. F. Neumann for the cyproterone acetate, and Dr. C. H. Sawyer for awarding Y. Arai a Ford Foundation Fellowship.

1. Barraclough, C. A., Recent Progr. Hormone Res. **22**, 503 (1966).

2. Gorski, R. A., J. Reprod. Fertil. Suppl. 1, 67 (1966).

3. Wagner, J. W., Erwin, W., and Critchlow, V., Endocrinology **79**, 1135 (1966).

4. Neumann, F. and von Berswordt-Wullrabe, R., J. Endocrinology **35**, 363 (1966).

5. Neumann, F. and Kramer, M., Endocrinology **75**, 428 (1964).

6. Junkmann, K. and Neumann, F., Acta Endocrinol., Suppl. 90, 139 (1964).

7. Neumann, F., Elger, W., and Kramer, M., Endocrinology **78**, 628 (1966).

8. Elger, W. and Neumann, F., Proc. Soc. Exptl. Biol. Med. **123**, 637 (1966).

9. Neumann, F. and Elger, W., Endokrinologie **50**, 209 (1966).

10. Neumann, F., Hahn, J. D., and Kramer, M., Acta Endocrinol. **54**, 227 (1967).

11. Wollman, A. L. and Hamilton, J. B., Endocrinology **81**, 350 (1967).

12. Bloch, G. J. and Davidson, J. M., Science **155**, 593 (1967).

13. Eisenfeld, A. J., Federation Proc. **26**, 365 (1967).

Received Oct. 13, 1967. P.S.E.B.M., 1968, Vol. 127.