tremely early in the history of the solar system, this preservation has not been complete and future work promises to teach us more concerning the subsequent histories of these objects and their parent bodies.

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Copulation in Castrated Male Rats following Combined Treatment with Estradiol and Dihydrotestosterone

Abstract. *Castrated male rats injected daily with 2 micrograms of estradiol benzoate (EB) combined with 200 micrograms of dihydrotestosterone propionate (DHTP) displayed masculine mating behavior which was indistinguishable from that of other castrates treated with 200 micrograms of testosterone propionate (TP). Significantly less copulation was seen in rats treated with either 4 micrograms of TP plus 200 micrograms of DHTP or 2 micrograms of EB. Mating in male rats may depend on the action of both estrogenic and 5α-dihydro metabolites of testosterone.*

Castration of the adult male rat invariably results in the disappearance of intromission and ejaculation behavior (*1*). Either of the testicular androgens, testosterone or androstenedione, is highly effective in maintaining copulation when administered systemically immediately after castration, and both restore mating when given many months after castration (*2*). This behavioral facilitation is thought to result from the action of a hormone on the hypothalamic and spinal neurons that control copulation (*3*); however, it is not known which hormone—testosterone, androstenedione, or one or several of their metabolites—is active in this respect. It has been shown that 5α-androstan-17β-ol-3-one (dihydrotestosterone, DHT), a metabolite which apparently mediates many of the effects of androgen on the accessory sex organs including the penis (*4*), can be formed in the male rat hypothalamus (*5*). Even so, administration of DHT does not prevent the postcastration decline in mating behavior (*6*) and is unable to duplicate the stimulatory effect of TP on penile reflexes in castrated rats with transected spinal cords (*7*). Estrogens constitute another group of androgen metabolites whose production has also been demonstrated in the male rat hypothalamus (*8*), leading to speculation that many of the effects of androgens on the rat brain, including the facilitation of masculine sex behavior, depend on their conversion to estrogens. However, administration of estradiol to castrated adult rats fails to stimulate intromission and ejaculation to the degree possible with testosterone (*9, 10*). We present evidence which suggests that the activational effects of testicular androgens on the mating behavior of male rats may depend on the formation and subsequent action of both estrogenic and 5α-dihydro derivatives of these androgens.

Thirty-four male hooded rats, born in the breeding facility of Erasmus University, Rotterdam, and caged in groups of four, were castrated when they were 49 to 55 days old. The animals were not sexually experienced. The rats lived in a room where the lights were off between noon and 8 p.m., during which period all behavioral tests were conducted. Between 26 and 30 days after castration all males were given three tests for masculine behavior and a single test for receptive behavior. In the tests of masculine behavior an animal was placed in a plastic cage identical to its home cage with two females whose ovaries had been removed and who had been made receptive with injections of EB and progesterone. Simple mounts, mounts with pelvic thrusting with and without intromission, and ejaculation responses were scored separately in each male. The tests lasted 15 minutes but were extended to 30 minutes whenever a mount with pelvic thrusting occurred. If an intromission was observed during this time, the test was extended to 1 hour or until the rat ejaculated, in which case the time elapsed between the initial intromission and the ejaculation (ejaculation latency) was noted. Following an ejaculation all tests were extended until postejaculatory interval of sexual inactivity had passed and the male had resumed intromitting. Tests of receptive behavior involved placing the individual males in a cage and recording all of their lordotic responses to mounts with pelvic thrusting performed by either of two stud males. Tests were stopped after ten mounts, and each animal's lordosis-to-mount ratio (lordosis quotient, L.Q.) was calculated.

Beginning 31 days after castration the animals, which weighed approximately 300 g, received daily subcutaneous injections of different steroids dissolved in 0.1 ml of sesame oil. One group received 200 μg of TP, a treatment which has been shown to stimulate high levels of copulation in sexually inexperienced, castrated rats (*10*). A second group received 2 μg of EB plus 200 μg of DHTP. This dose of EB was administered because in a previous experiment we found that daily injections of less than 2 μg of EB in

Table 1. Mating behavior and penile papillae in castrated male rats following hormone treatments. Rats were given six receptivity and six masculine behavior tests (see text). Comparisons with the group receiving 200 μg of TP were made by using Mann-Whitney U tests, provided a significant overall treatment effect had first been found with the Kruskal-Wallis test. For ejaculation at least once in six tests, $\chi^2 = 15.72$, degrees of freedom = 3, $P < .01$. The median number (med. No.) of tests with ejaculation is based only on results for animals which ejaculated. Median values for intromissions before ejaculation, ejaculation latency, and postejaculatory interval are based on median scores calculated for each rat. Abbreviations are TP, testosterone propionate; EB, estradiol benzoate; DHTP, dihydrotestosterone propionate; *N*, number of rats; L.Q., lordosis quotient.

Daily treatment	*N*	Rats ejaculating at least once (%)	Tests with ejaculation (med. No.)	Intromissions before ejaculation (med. No.)	Median ejaculation latency (min)	Median postejaculatory interval (min)	Penile papillae (med. No.)	Median L.Q., six-test total
TP (200 μg)	8	100	4.0	14.50	12.0	6.9	54.5	.00
EB (2 μg) + DHTP (200 μg)	10	100	4.5	14.75	9.8	6.9	60.5*	.01†
TP (4 μg) + DHTP (200 μg)	8	37	1.0*	13.00	16.1	7.7	56.0	.00
EB (2 μg)	8	37	1.0*	30.00	27.4*	8.6	0.0‡	.06‡

* $P < .05$ (two-tailed). † $P = .05$ (one-tailed). ‡ $P < .01$ (two-tailed).

combination with 200 μg of DHTP failed to elicit copulation in the majority of a group of castrated rats (*11*). A third group received 4 μg of TP plus 200 μg of DHTP. At the time of behavioral testing the rats in the two groups treated with 2 μg of EB weighed significantly less than the animals in the remaining groups. Therefore, instead of 2 μg of TP, a dose of 4 μg of TP was compared to 2 μg of EB combined with 200 μg of DHTP in order to compensate liberally for this body weight difference. A fourth group of rats received 2 μg of EB. All males were given six receptivity and six masculine behavior tests on alternate afternoons beginning 16 days after the first hormone injection. Each animal was killed and autopsied after the last behavior test, and the glans penis was prepared for histological examination. The number of cornified papillae in one 10-μm section was subsequently determined for each rat.

In tests given before the hormone treatment castrated males displayed only a few simple mounts and never showed lordosis. Table 1 shows that after the administration of hormones rats treated with 2 μg of EB plus 200 μg of DHTP ejaculated as frequently as animals which received 200 μg of TP. Also, the number and timing of the intromission responses leading to ejaculation and the degree of sexual arousal, as reflected by the length of the postejaculatory interval, were the same in these groups. Injections of 4 μg of TP plus 200 μg of DHTP supported ejaculation in a significantly lower percentage of rats than in the previous two treatments, and rats of this group which ejaculated did so only during the final two tests. Even so, the pattern of mating associated with ejaculation in the three responding males of this group was similar to that seen in males treated with either 200 μg of TP or 2 μg of EB plus 200 μg of DHTP, perhaps because penile development, including the growth of cornified papillae which may provide sensory input during copulation (*12*), had been stimulated in all three groups. This conclusion is strengthened by observations of the copulatory pattern of the three rats which ejaculated after treatment with 2 μg of EB. These males, whose penes had no cornified papillae, tended to require more intromissions and needed more time to ejaculate. Yet despite the absence of penile papillae, males receiving 2 μg of EB ejaculated as frequently as males treated with 4 μg of TP plus 200 μg of DHTP, and a higher percentage (87 compared to 37 percent) of the males receiving EB displayed intromission in at least one test.

The results reported here together with the results of Larsson *et al.* (*13*), who reported high levels of copulation in castrated male rats after injections of 5 μg of EB plus 1 mg of DHT, suggest that mating behavior in the male rat may depend on the combined action of the estrogenic and 5α-dihydro metabolites of circulating testosterone. The primary role of estrogen appears to be facilitation of activity in neural tissues controlling mounts with pelvic thrusting and intromission. Low concentrations of estradiol-17β have been detected in the plasma of intact male rats by a radioimmunoassay (*14*). Perhaps because the circulating level of estrogen is very low, intact male rats rarely display lordosis (*15*). However, we found that 2 μg of EB, while facilitating intromission in a high percentage of castrated animals, also stimulated a low, but significant, amount of lordotic behavior (Table 1). This result implies that in normal rats the estrogen needed to facilitate mounts with pelvic thrusting and intromission is not obtained from the circulation, but may instead be formed from androgen taken up by the neural tissues which control these particular behaviors. Animals which intromitted were almost sure to ejaculate provided that penile development, including the growth of cornified papillae, had been stimulated by treatment with either DHTP or TP. It seems likely that DHT mediates the effects of androgen on the penis (*4*), and thereby contributes to the occurrence of ejaculation. Finally, our findings raise the possibility that, in the presence of estrogen, DHT acquires the ability to facilitate further the activity of neural tissues controlling mounts with pelvic thrusting. Rats receiving 2 μg of EB showed mounts with pelvic thrusting, with or without intromission, in a median of 2.0 tests, whereas males treated with 2 μg of EB plus 200 μg of DHTP displayed these responses in 4.5 tests (Mann-Whitney $U = 11.5$, two-tailed; $P < .05$). This difference is not easily explained by the absence of penile papillae and a resultant sensory deficiency in animals treated only with 2 μg of EB, since anesthetization (*16*) or removal of the penis (*17*) does not diminish mounts with pelvic thrusting.

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Periodicity of Susceptibility to Pneumococcal Infection: Influence of Light and Adrenocortical Secretions

Abstract. *Circadian periodicity of susceptibility to pneumococcal infection was altered but not abolished in blind or adrenalectomized mice. Serum corticosterone concentrations 24 hours after pneumococcal challenge were greatest in animals challenged at 0400 hours, a time when circulatory corticosterone is lowest; the smallest absolute increase in serum corticosterone followed challenge at 1600 hours.*

A circadian rhythmicity of susceptibility to pneumococcal infection has been documented in mice (*1*). We have elucidated several factors that modulate this rhythmicity and have documented that survival patterns could be altered by environmental lighting conditions (*2*). In contrast, survival periodicity was not altered by changes in feeding or activity of the mice or by administration of penicillin (*2*). Serum corticosterone concentrations of infected mice at 6 and 12 hours after challenge were greatest when infection was initiated at 0400 hours and lowest after infection at 1600 hours (*2*). In this report we describe the effects of blinding on the periodicity of susceptibility to infection, as well as additional attempts to ascertain the role of adrenocortical secretions in modulating periodicity of morality.

Normal or adrenalectomized male mice (16 ± 2 g) of the CD-1 strain (Charles River Laboratories, Wilmington, Massachusetts) were used for all experiments. Normal mice were blinded by bilateral optic enucleation. Blinded and normal mice were conditioned for 21 days in an animal chamber maintained at 24.4°C and lighted from 0600 to 1800 hours. Animals had free access to Wayne Mouse Breeder Blox and water. Adrenalectomized mice were conditioned similarly but were maintained with 0.9 percent sodium chloride for drinking water.

In all experiments, groups of 30 mice were inoculated subcutaneously, the first group at 0800 hours and the others at successive 4-hour intervals ending 24 hours later. *Diplococcus pneumoniae*, type I, A5, was the challenge organism, and either 10^3 organisms of modified virulence or 10^3 fully virulent organisms were inoculated. Virulence of the challenge organism and its preparation by serial dilution have been described (*1*). Counts of viable bacteria before and after injection at each time interval showed a maximum variation of 5 percent in the number of challenge organisms. The inoculum was suspended in 0.5 ml of tryptose phosphate broth, *p*H 7.4. Groups of ten normal or adrenalectomized control mice were inoculated with sterile broth at each challenge time.

In each experiment, mice were checked and deaths were recorded hourly beginning at the time of challenge; mice were observed for 14 days or until all had died. In experiments in which corticosterone concentrations were assessed, mice were killed by cardiac puncture 24 hours after challenge, and the blood obtained was used for measurement of corticosterone. A modification (*3*) of the ultramicromethod for measurement of corticoids as described by Murphy (*4*) was used for corticosterone evaluation.

Rectal temperatures were obtained before inoculation on groups of five mice at each challenge time. Temperatures were obtained by means of a veterinary electronic thermometer (Diagnostic V, Diagnostic, Inc., Indianapolis, Indiana). Temperatures were also recorded on groups of five control mice at each challenge time. These controls were normal mice that had not been blinded or adrenalectomized.

Statistical analysis was performed by the division of biostatistics, Washington University School of Medicine. Survival time was expressed in logarithmic form to facilitate use of mathematical assumptions necessary for analysis of variance. The model equation was

$$X_{ik} = u + t_i + e_{ik}$$

where X_{ik} denotes the logarithm of survival of the kth animal in the ith groups, u is the general mean, t_i is the effect of time, and e_{ik} is the experimental error. For each experiment, individual differences between the groups inoculated at various times were investigated by the multiple range test. The proportion of animals surviving challenge was analyzed separately by chi-square statistics, and the significance of differences in number of survivors in each experimental group was compared by the method of Kimball (*5*).

Results after challenge of blind mice with 10^3 *D. pneumoniae* of modified virulence were evaluated by analysis of variance. No significant differences in survival time were noted, but significant differences in the number of animals surviving challenge were found. The number of survivors after challenge at 0400 hours was significantly less ($P < .05$) than that after challenge at 0800, 1200, 1600, or 2000 hours. The number of survivors at 2400 hours was significantly less ($P < .05$) than at 0800 hours. Mean rectal temperatures of blind mice infected at 1600 hours were significantly less ($P < .01$) than those of any other group.

Analysis of variance revealed a significant difference ($P < .05$) in the survival pattern after challenge of adrenalectomized mice. The mean survival time of animals challenged at 2400 hours was significantly less ($P < .05$) than that for animals challenged at 1200 hours. In addition, mean survival time of the group challenged at 2000 hours was significantly less than that for animals challenged at 0800, 1200, or 1600 hours ($P < .05$). No significant differences in rectal temperatures were noted among the groups of animals.

The mean corticosterone concentra-