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Intermale Aggression in Mice: Modification by Postneonatal Castration[1] (37805)

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Spontaneous aggression between previously isolated male house mice is androgen dependent. Thus postpubertal castration is associated with reduced aggressiveness among adults, while testosterone replacement tends to restore fighting behavior (1). Furthermore, circulating levels of testosterone encountered early during neonatal life in normal males appear to organize mechanisms requisite to aggressiveness among adults (2). A dual-action model for effects of testosterone on aggression in mice thus is probable in which testicular hormones (a) organize in the neonate a substrate associated with aggressiveness in adults, and (b) act in the adult upon the previously developed substrate to potentiate aggressiveness (3).

Recently, however, it has been shown (4) that onset of spontaneous intermale aggression in mice is associated with rising titers of total androgens in the peripheral circulation; concentration of plasma androgens increases between 21 and 55 days and declines subsequently. The present experiments were designed to assess the role which increasing circulating titers of testicular hormones during 21 to 55 days of age in mice may play in influencing androgen-dependent aggressive behavior.

Materials and Methods. Exp 1. Male mice derived from a wild strain (random bred, many generations removed from original stock) were weaned, castrated or sham-operated and caged singly at 21 days of age. All animals were from litters standardized to a maximum of nine young on the day following birth. Fourteen days prior to testing for aggressiveness at either 35 or 40 days of age, intact males received an implant of paraffin only (sc), while castrates received an implant of testosterone propionate (TP) in paraffin (15 mg%). The replacement level of TP used maintained seminal vesicle weights in 35- and 40-day-old castrated animals at a level approximating that occurring in intact 21-day-old males and above the seminal vesicle weights of castrates (Table I). In order to test whether this level of androgen replacement was adequate to maintain aggressiveness in adults, males which had been caged singly from weaning were castrated or sham-operated at 65 to 70 days of age, implanted 16 days later with TP or sham implants as above and tested for aggressiveness after 14 days.

Expt. 2. Male mice of the above strain were weaned and caged singly at 21 days of age. Animals then were castrated at 21, 35, 45 or 55 days of age or were sham-operated at 21 days of age. Controls were sham-oper-

TABLE I. Seminal Vesicle Weights (Mean ± SE) Among Intact or Castrated Male Mice.[a]

Age (days)	Treatment	Seminal vesicles (mg)
21	Intact	4.3 ± 0.5[b](20)
35	Castrated	2.6 ± 0.2 (10)
	Castrated + TP	5.9 ± 0.4 (20)
40	Castrated + TP	4.9 ± 0.3 (20)

[a]Sample size is shown in parentheses.
[b]Taken from McKinney and Desjardins, 1973 (4).

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ated only at 21 days of age since sham orchidectomy at this and older ages repeatedly has failed to influence significantly intermale aggression among adults (2). Fourteen days prior to aggression testing at 85 days of age, intact males received sham implants and castrates received TP implants as in Expt 1.

General. In both experiments, pair members were designated randomly and were placed for 15 min in a strange cage under white light between 8–10 P.M. Animals were maintained at 25° under a regime of 15 hr light–9 hr dark/24 hr and food (Rockland Mouse/Rat Diet) and water were available in excess except during pairing. Adjusted chisquare was incorporated to test for differences in incidence of aggression between treatments. Means for other indices of aggressiveness were compared using the Wilcoxon Rank-Sum test.

Results. Expt 1. Incidence of aggression was reduced among castrated males implanted with TP (Table II). Among 35-day-old animals, 75% of sham-operated controls fought, while fighting occurred in 10% of gonadectomized pairs ($P<0.005$). Similarly, incidence of aggression was 10% among 40-day-old castrates receiving TP, compared to 90% among controls ($P<0.005$). Incidence of fighting among pairs castrated as adults was unaffected by castration, but latency to first attack was increased 82% by gonadectomy ($P<0.05$), while total fights and accumulated attack time were reduced by 48 and 70%, respectively ($P<0.01$ in each case; Table III). Castrated animals receiving TP implants, however, did not differ significantly from controls in these indices of aggressiveness, indicating that replacement therapy was adequate to maintain a normal level of aggressiveness in males gonadectomized postpubertally.

TABLE II. Incidence of Fighting Among Males Castrated or Sham-Operated at 21 Days of Age and Receiving Testosterone Propionate for 14 Days Prior to Pairing.

Age at pairing (days)	Treatment	Incidence of fighting among pairs
35	Sham-operated	15/20
	Castrated + TP	1/10
40	Sham-operated	9/10
	Castrated + TP	1/10

Expt. 2. Age at castration influenced the incidence of spontaneous aggression in this experiment (Table IV). Compared to sham-operated males, frequency of fighting among pairs was reduced by approximately 60 and 90%, respectively, in animals castrated at 21 or 35 days of age ($P<0.005$ in each case). Conversely, no significant differences were observed in incidence of fighting among males castrated when 45 or 55 days old compared to controls ($P > 0.25$). Furthermore, fighting occurred among fewer pairs of males castrated prior to 40 days of age than among those gonadectomized beyond 40 days of age ($P<0.005$).

Discussion. The importance of testicular androgens in neonatal organization of a presumed neural control system associated with malelike aggressiveness in the adult as well as in enhancing propensity to intermale aggression among adult house mice is well documented (2, 3). Present studies extend these observations and demonstrate that the rise in plasma titers of hormones of testicular origin which occurs in intact, singly caged, prepubertal animals (4) may influence the ontogeny of aggressive behavior. Prevention of the normal rise in circulating androgens (4) by castration plus TP replacement at low levels effectively prevented onset of aggressive behavior through 40 days of life. In apparent conflict with present findings, Peters, Bronson and Whitsett (2) reported normal incidence of aggression following androgen treatment among adult male mice which had been castrated on Days 6 and 40 of life. However, the large doses of hormone utilized may have obviated requirements for increased plasma titers of testicular hormones at an earlier age. Based on seminal vesicle weights, replacement therapy in the present study approximated an injection schedule (sc) of 2 μg of testosterone propionate daily (McKinney, unpublished data), less than 1% of the dosage indicated above (2). Importantly, the level of TP administered in the present study was adequate to maintain normal levels of aggression among males gonadectomized postpubertally, or late during puberty, while

TABLE III. Indices of Aggressiveness (mean ± SE) in Male Mice Castrated or Sham-Operated at 65 to 70 Days of Age and Paired 30 Days Later.

Treatment	Incidence of fighting	Latency to attack	Total fights	Accumulated attacking time (sec)
Castrate	13/14	296.7 ± 39.7	5.2 ± 0.8	27.3 ± 5.6
Castrate + TP	14/19	189.6 ± 47.3	8.1 ± 1.3	87.2 ± 22.4
Sham	16/17	163.0 ± 31.4	9.6 ± 1.0	91.1 ± 13.1

castration without replacement therapy reduced aggressiveness significantly. Reduced aggressiveness among males castrated at 40 days of age and earlier therefore cannot be attributed to inadequacy of replacement therapy per se. Although gonadectomy of sexually mature males in the present study resulted in reduced aggressiveness 30 days postsurgery, the incidence of fighting among pairs of castrates was unaffected. In contrast, previous data have indicated significant reduction in incidence of fighting among adult mice after a similar time lapse following gonadectomy (5, 6), suggesting possible genetic differences in effects of castration on intermale aggression.

Removal of the testes prior to approximately 40 days of age considerably reduced the capacity of TP administered during adulthood to enhance fighting behavior, while no effect of gonadectomy on the response to TP during adulthood was apparent when orchidectomy was affected on animals older than 40 days. These results indicate that rising plasma levels of testicular hormones prior to approximately 40 days of life may influence the response of some neural control system to testicular hormones during adulthood. It also has been shown that injections of testosterone may induce development of aggressiveness prior to 35 days of age in house mice (7, 8), thus supporting the concept that onset of intermale aggressiveness may be linked to increasing titers of circulating androgens in the normal, prepubertal male.

TABLE IV. Incidence of Aggression Among Males Castrated or Sham-Operated at 21 to 55 Days of Age and Receiving Testosterone Propionate as Adults.

Age (days) at castration or sham surgery	Incidence of fighting among pairs
Castrated	
21	2/10
35	0/8
45	5/7
55	6/10
Sham-operated	
21	17/19

Summary. Male mice were castrated between 21 and 55 days of age and received testosterone propionate replacement for 14 days through either 35–40 days or 85 days of age. At the above ages, animals were paired and aggressiveness was evaluated using incidence of fighting. Fighting was prevented in 35–40-day-old males by castration and androgen therapy at a level known to sustain aggressiveness among animals castrated postpubertally or late during puberty. Incidence of aggression also was considerably reduced among males castrated when 21 or 35 days old and receiving androgen replacement as adults. On the other hand, castration at 45 or 55 days of age failed to reduce androgen-induced aggressiveness among adults. It is postulated that normally rising plasma levels of testicular hormones in the prepubertal male house mouse influence development of neural systems associated with intermale aggression.

1. Bevan, W. D., Daves, W. F., and Levy, F. W., Anim. Behav. 8, 6 (1960).

2. Peters, P. J., Bronson, F. H., and Whitsett, J. M., Physiol. Behav. 8, 265 (1972).

3. Bronson, F. H., and Desjardins, C., *in* "The Physiology of Aggression" (B. E. Eleftheriou and J. P. Scott, eds.), p. 43. Plenum, New York (1971).

4. McKinney, T. D., and Desjardins, C., Biol. Reprod., 9, 279 [1973).

5. Beeman, E. A., Physiol. Zool. 20, 373 (1947).

6. Suchowsky, G. K., Pegrassi, L., and Bonsignori, A., *in* "Aggressive Behavior" (S. Garattini and E. B. Sigg, eds.), p. 164. Excerpta Medica Monograph, Amsterdam (1969).

7. Lagerspetz, K. M. J., and Talo, S., Rep. Psychol. Inst. Univ. Turku 28, 1 (1967).

8. Levy, I. V., and King, I. A., Anat. Rec. 117, 562 (1953).

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