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Induction of Female Behavior in Male *Anolis carolinensis* with Testosterone Propionate.*

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In the course of an earlier experiment,[1] dealing with the effects of testosterone propionate upon the urogenital system and sexual behavior of the lizard, *Anolis carolinensis,* we observed a single case of an immature, testosterone-treated, castrate male (38 mm in body length) which displayed female as well as male mating behavior. The purpose of the present report is to describe the effects of testosterone propionate upon the sexual behavior of a large series of immature male *Anolis.*

Experimental Procedure. Fifty-two immature male *Anolis carolinensis* (average length 48.4 mm from snout to anus) were divided into 3 experimental groups. Thirty-one were subjected to partial hypophysectomy, 12 were castrated and 9 served as an unoperated control group. Hypophysectomy was attempted in order to secure morphological data, which will be reported separately. Castration was performed to eliminate normal production of testicular hormone.

Pituitary insult was not followed by obvious postoperative shock. Thirteen to 15 days after operation, 15 partially hypophysectomized males received subcutaneous implants of testosterone propionate pellets.† The remaining 16 lizards of this group received no hormone therapy. Recovery appeared complete 3 days after castration and 6 castrated males were then implanted with testosterone propionate pellets, while 6 other castrates were kept as untreated controls. Five unoperated males were given similar implants and 4 intact animals were untreated. The pellets were removed, dried and reweighed at the end of the experiment. At insertion, the pellets

* Aided by grants from the Committee for Research in Problems of Sex, National Research Council, and from the Josiah Macy, Jr., Foundation.

The experiment was planned together with and completed under the direction of the senior author. Manuscript preparation and final interpretation of results are the responsibility of the junior author.

1 Noble, G. K., and Greenberg, B., Proc. Soc. Exp. Biol. and Med., 1940, **44**, 460.

† We are indebted to the Ciba Pharmaceutical Products, Inc., for the testosterone propionate (perandren) utilized in this study.

ranged in weight from 1.11 to 3.71 mg, the average weight being 2.62 mg. The amounts apparently absorbed after 26-29 days of treatment varied from 0.63 to 1.54 mg with an average of 1.02 mg. However, there were some losses of hormone in handling and in attachment to the skin.

The 52 lizards were kept in 5 similarly arranged cages (35 inches by 33 inches by 42 inches). Each cage contained implanted and non-implanted males which had received the same operative treatment. Large Spider Lilies *(Crinum)* and crossed tree branches provided climbing and hiding places. The cages were located in a sunlit greenhouse with a constant temperature of 28°C. Diet consisted of flies and mealworms, and water was provided by sprinkling the cages 3 times daily. Behavior was observed constantly from 9 a.m. to 4 p.m. on 5 week days and occasionally on Saturdays and Sundays.‡ The observation period extended from October 28, 1940 through November 17, 1940.

Results and Discussion. Two forms of social behavior are observed in normal *Anolis:* fighting and sexual activity.§ In the former, one lizard is dominant over all other individuals in the group. Dominance is manifested by a very clear postural attitude; subordination by a series of characteristic head bobs. In mating, the active male, bobbing rapidly, displays his dewlap (a colored gular fan), struts toward the female and attempts to secure a neck grip. The female in full estrus submits to copulation without struggle or running away, and flexes her neck as the male approaches. The complete copulation position consists of the male's neck grip, body of the male arched over that of the female and his pelvic region, tail and legs arranged in a characteristic grip around the female's tail base. In the discussion which follows, the terms "dominant" and "subordinate" will be used to describe fight relationships, while "male" and "female behavior" will refer to mating.

Many of our testosterone propionate-treated males, whether partially hypophysectomized, gonadectomized or unoperated, reacted sexually both in typical male and in typical female fashion, while their respective untreated controls were completely inactive.

Although each cage contained an equal number of control and implanted lizards, the controls were never observed to exhibit mas-

‡ Mr. H. Bloom and Miss S. Dushkind assisted in observing and recording behavior. The personnel of Works Progress Administration, Official Project No. 165-1-97-8 (WP-10) aided in animal maintenance and preparation of materials.

§ A full account of the social behavior of this species is in preparation by the junior author.

culine sexual reactions. Thirty-nine matings occurred in cages containing partially hypophysectomized animals. Implanted lizards played the masculine rôle in all of these encounters, and in 37 of the 39 instances testosterone-treated animals served as the submissive partner. In the cage containing castrated males 10 copulations were recorded. In each case only implanted lizards were involved. The 9 matings observed in the cage containing unoperated males were all between testosterone-treated lizards. Nonimplanted lizards participated in only 2 of the 58 copulations recorded. In both of these instances the masculine rôle was taken by a treated male.

The estrous type of neck bend, characteristic of female sexual behavior was observed 10 times in the testosterone-treated, partially hypophysectomized males (4 individuals, one reacting 5 times); and one treated castrate displayed this behavior 4 times. No neck bends were observed on the part of unoperated testosterone-treated males. However, in a further experiment at present in progress, 2 intact males (37 and 39 mm in size) have been observed to bend their necks after implantation with testosterone propionate pellets. No neck bends were observed in any of the control groups.

To illustrate the dual nature of the response to testosterone, we quote directly from experimental records. Male 3 reacted to 2 other testosterone-treated males by alternately displaying male and female sexual behavior.

1:15—Cage G—Male 57 approaches male 3 and male 3 bends neck (female behavior). Male 57 courts male 3 who stands with neck bent. Male 57 moves away.

2:30—Cage G—Male 57 courts male 10. Male 3 is a foot away, bobs subordinately and approaches half the distance. Male 10 moves close to both other males. Male 3 courts and shows dewlap (male behavior). Male 57 shows a combination of fight and courtship toward male 3 who bobs subordinately and bends neck (female behavior). Male 57 does not mate. Male 10 moves past male 3 who again courts male 10 and approaches him in typical strut (male behavior). Male 57 comes over in fight position, approaching male 3 very closely. Male 3 bobs subordinately and bends neck (female behavior).

The following day male 57 mated male 9, another testosterone propionate-treated male. After the mating the following behavior was observed:

12:45—Cage G—Male 3 approaches male 57. The latter displays in fight challenge and male 3 bobs subordinately. Male 57 approaches in fight attitude and again male 3 bobs subordinately. Male

57 rests his chin on male 3 and male 3 stands with neck bent (female behavior). Male 57 displays in fight challenge and as male 3 remains with neck bent, moves away.

Male 3 jumps at male 10 and grips at neck (male behavior). Male 10 struggles at first, then stops. Male 57 approaches and attacks the pair in tense fight posture. Males 3 and 10 fall from the leaf, emerging again from under a lower leaf. Male 57 attacks and nips at the pair, biting male 3 across the head. This breaks the pair. At 1:10, male 3 courts male 2 (a control) who escapes (male behavior).

This case clearly shows that a dominance relationship existed between male 57 and male 3, and although 3 was sexually active in the masculine direction, his reaction to 57 was of the estrous female type.

The implanted males were observed to fight much more frequently than the controls. Ninety-nine fights occurred in cages containing partially hypophysectomized lizards. Implanted animals won 97 of these encounters; untreated males won twice. Eight fights occurred in the cage of castrated males, and in each case the winner was a testosterone propionate-treated lizard. One hundred and one of 107 fights occurring among intact males were won by hormone-treated animals, and in 6 cases the victor was a control lizard. The loser in various fights was sometimes a treated and sometimes a control male. Although no statistical data are available it is probable that in the majority of cases the loser as well as the victor was a hormone-treated male. This conclusion is indicated by the observation that the aggressive nature of implanted lizards tended to involve such animals in the majority of encounters. Controls were apt to avoid conflict by abrupt withdrawal before any real attack occurred.

In each of the 5 cages, one or 2 of the testosterone-treated males fought and won most of the encounters. In all but one of these cases the dominant male also mated most frequently. Table I indicates the correlation between dominance and frequency of masculine copulatory pattern.

In the one exception, male 3 in cage F fought most often (a total of 56 times), and courted most often (11 of 24 courtships in this cage), but was never observed to mate. Although obviously stimulated to court and fight, he did not complete the pattern even though other treated males stood before him with necks flexed. This lizard repeatedly broke up the mating of other residents in his cage by

TABLE I.
Relation Between Dominance in Fighting and Copulation in Testosterone-treated Immature Male *Anolis carolinensis*.

Group	Cage	Male No.	Fights as Dominant	Total fights (all individuals)	%	Matings as masculine partner	Total matings (all individuals)	%
Partially	F	3	56	65	86	0	5	—
hypophysec-	G	57	19	22	86	8	16	50
tomized males	*H	9	5			5		
		10	6			8		
Total	H		11	12	92	13	18	72
Castrate males	J	4	6	8	75	7	10	70
Intact males	D	1	87	107	81	6	9	67
Totals		6	179	214	83	34	58	59

*Two males were successively dominant in this cage and are therefore considered together.

fight attacks upon pairs in preliminary neck-grip position. His behavior markedly reduced courtship activity in Cage F. One factor underlying this dominance was his size, 54 mm, the largest male in the cage. On one occasion, however, male 3 assumed the feminine rôle in a copulation with a smaller treated male (48 mm in body length). The behavior of this individual indicates the importance of other factors in producing the complete copulatory pattern, such as possible differences in threshold of response of the central nervous system to testosterone propionate.

As indicated in Table I and by the excerpts from our experimental records, male behavior in copulation appears to be correlated with dominance in fighting, and this supports our previous conclusion[1] with reference to females: that in any one testosterone propionate-treated individual, the assumption of male or female sex behavior is correlated with position as dominant or subordinate in fights among the group.

Prolonged testosterone propionate treatment appeared to have an effect upon the male copulatory pattern. In 20 matings (15 copulations by 8 partially hypophysectomized males and 5 copulations by 3 castrated males), it was observed, upon assumption of the complete mating posture, that this posture was maintained for periods of 10 minutes to an hour if the partner managed to pull away during the copulation. A male showing this behavior could be handled and examined without breaking the tension of his body, and in running away he might even maintain the bent tail-region. Normal males do not continue in mating position if disturbed. Evidently testosterone reinforces the reflexes involved in the copulation posture.

Summary. Testosterone propionate, introduced in pellet form into intact, castrated or partially hypophysectomized immature male *Anolis carolinensis,* elicits both male and female sexual behavior, the latter including submission to copulation and display of a distinctive neck reflex. Testosterone-treated males fight more actively than controls, and the assumption of male or female behavior is apparently related to position as dominant or subordinate in fights among the group. Testosterone affects the reflexes involved in the copulation posture so that they are maintained in exaggerated form after the escape of the partner.

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Bile Acids and the Pulmonary Tumor Incidence in A Strain Mice.

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The principal bile acids of the higher vertebrates are hydroxy derivatives of cholanic acid, a compound which can be obtained *in vitro* from sterols by degradation. The bile acids as well as sterols have condensed carbon ring molecules to which are attached side-chains in a position such that a new 6-membered ring can be formed so as to give the carcinogenic 1:2-benzanthracene ring system without any molecular rearrangement or migration of groups within the molecule. In 1934 it was shown[1] that such a ring closure to the 1:2-benzanthracene system had occurred in the formation of dehydronorcholene from desoxycholic acid. Upon dehydrogenation of this product methylcholanthrene, a highly carcinogenic substance, was formed. Later[2] it was shown that methylcholanthrene could be obtained from cholic acid, the chief acid of human bile.

These discoveries led to tests for carcinogenic properties of the bile acids with conflicting results. Application of desoxycholic acid in alcohol or benzene to the skin in a large series of mice gave completely negative results. Injections *sub cutem* of 70 mg desoxycholic acid in sesame oil produced sarcomata in 3 of 10 mice at a late date.[3] This experiment was repeated using the C3H strain of

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1 Cook, J. W., and Haslewood, G. A. D., *J. Chem. Soc.,* 1934, 428.

2 Fieser, L. F., and Newman, M. S., *J. Am. Chem. Soc.,* 1935, **57**, 961.

3 Cook, J. W., Kennaway, E. L., and Kennaway, N. M., *Nature,* 1940, **145**, 627.