Effect of Testosterone Propionate on the Urogenital Organs of Immature Crocodile *Crocodylus palustris* Lesson[1]

Effect of androgens on the reproductive organs of immature lizards[2,3] and chelonians[4] has been reported. Of the order Loricata, only the American alligator[5,6] has been studied. No account of the effect of sex hormones on the urogenital structures of any crocodile is available. This may be attributed to the difficulty in procuring suitable material for experiments and the present note is intended to fill this lacuna.

Two-year-old litter-mate crocodiles of both sexes were taken for our experiments. The animals were obtained from the local zoological gardens (Jaipur, India), where crocodiles are occasionally bred. The average weight of the specimens at this age is 950 g and they measure 600 mm from snout to tail tip. Testosterone propionate (Perandren, Ciba) in oil was injected intramuscularly in doses of 5 mg every alternate day and the total dose administered to each animal was 25 mg. The experimentals were autopsied ten days after the last injection. Suitable litter-mate controls were maintained.

The testes (Figure 1, t) of these immature crocodiles are small white rod-like bodies. Histologically, the testis is composed of seminiferous tubules (Figure 2) and the interstitial tissue, both held together by the thick tunica albuginea. In sections, the seminiferous tubules appear as composed of a single layer of cells surrounding a lumen which is filled with a fibrous network (Figure 4). Sertoli and spermatogonial cells line the lumen of the seminiferous tubule. Spermatocytes, spermatids and spermatozoa are absent. There is no indication of spermatogenesis and the spermatogonia do not show cell division. The interstitial tissue is by comparison massive and, besides blood vessels and fibroblasts, contains numerous large and oval Leydig cells (Lc); these cells are present in clusters of three or four.

On gross examination, the testes of the testosterone-treated crocodile have increased in length and girth (Figure 1). The histology is also revealing. In section, the diameter of the seminiferous tubules has considerably increased (Figure 3). Spermatogenesis is induced and the lumen of the tubules is filled with germ cells in various stages of proliferation. The spermatogonia have divided to give rise to primary and these to secondary spermatocytes and these in turn to spermatids; certain tubules even show the presence of spermatozoa (Figure 5). There is a consequent reduction in the interstitial tissue and the cells of Leydig (Lc) appear now as closely packed ones in between the seminiferous tubules. They do not show any change in size on receiving the androgen and appear the same as in control animals. FORBES[6] described that in seventeen-month old alligators, a 10 mg dose of testosterone had no effect on the gonads; in the ovaries, probably the medulla had regressed, but he does not mention any effect of the androgen on the gonads in the three-month old alligators which he investigated[5]. However, in the immature males of the lizard *Sceloporus*[2] and juvenile terrapin *Malaclemmys*[4], testes were slightly stimulated by testosterone propionate. Similarly, we have noticed that testosterone propionate stimulates spermatogenesis in the testes of immature *Varanus*[7].

The Wolffian ducts of the testosterone-injected crocodile have slightly increased in width, but the androgen had no effect on the Wolffian ducts of three-month-old alligators examined by FORBES[5]. However, testosterone had a stimulatory effect on the Wolffian ducts of immature lizards[2,3] and juvenile terrapin[4].

The median penis (Figure 1, p) of the treated crocodile developed enormously both in length and breadth, and at the time of autopsy, on pressing the area around the tail, it spontaneously protruded from the cloaca, while that of the control, which was minute in size, did not respond. In two- to two-and-a-half-month-old *Malaclemmys*[4], testosterone propionate brought about the hypertrophy

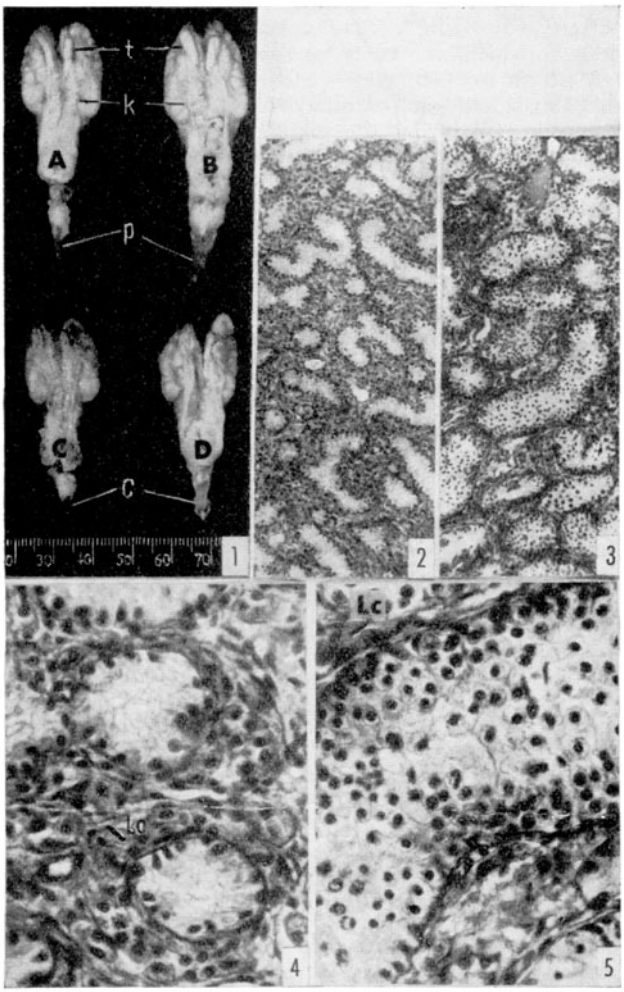

Fig. 1. Kidneys and reproductive tracts of crocodile, control male (A), injected male (B), control female (C), and injected female (D).

Fig. 2. Photomicrograph of the testis of control crocodile. × 80.

Fig. 3. Photomicrograph of the testis of injected crocodile. × 80.

Fig. 4. The same, as Figure 2, magnified × 360.

Fig. 5. The same, as Figure 3, magnified × 360.

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of the glans clitoris in the female and of the penis in the male. In six-month-old, testosterone-treated alligators[5,6], the penes hypertrophied so much that the tips of them protruded from the cloaca; similarly, we have observed that testosterone brings about a considerable hypertrophy of the penis of the pond tortoise *Lissemys punctata* (unpublished observation).

The juvenile crocodiles treated with testosterone propionate also show enlarged kidneys (Figure 1, k). In the alligator, FORBES[6] noted that testosterone had no effect on the mesonephroi, but no mention is made of the kidneys. The preterminal segment of the renal tubule of the kidney does not develop into a sexual segment in males of crocodiles and chelonians, and even a relatively heavy dose of the androgen has not brought about the development of this segment in the juvenile crocodile studied by us.

In the female crocodile, testosterone propionate had no appreciable effect on the ovaries and oviducts, which were well formed. There does not seem to be any reminiscent medulla in the gonad, as the exogenous androgen did not develop this into a testis-like structure. The gonads of testosterone-treated female alligators were also not affected, according to FORBES[5,6], but the oviducts showed considerable hypertrophy and their anterior ends were thrown into irregular folds. Similarly the oviducts are also stimulated by testosterone in the lizard[2,3].

The most pronounced effect of testosterone propionate in the immature female crocodile examined by us is the enormous hypertrophy of the clitoris (Figure 1, c). The clitoris of the injected specimen exceeds even the size of the penis of the control male and compares well with the penis of the injected male. In the six-month-old alligators, testosterone had no effect on the clitorides of the females[5], but in the seventeen-month-old female alligators, the clitorides were stimulated and the tips of these organs protruded out of the cloaca[6]. It is not clear why androgen had no effect on the clitoris of an earlier stage in the alligator; probably it was not sensitive to androgen. In the immature female terrapin[4], testosterone also caused the hypertrophy of the glans clitoris.

To sum up, testosterone propionate induces spermatogenesis in the testes of immature crocodiles and causes enormous development of the penis and clitoris. The Wolffian duct of the male shows slight enlargement, while the ovaries and the Mullerian ducts in the female are not affected on receiving the androgen. The preterminal segment of the renal tubule of the treated kidney did not develop into a sexual segment in the male crocodile, though the kidney itself showed enlargement.

Zusammenfassung. Nicht geschlechtsreifen, zweijährigen Krokodilen aus gleicher Brut wurden je 25 mg (Gesamtdosis) Testosteronpropionat verabreicht. Sektionsbefund 7 Tage nach der letzten Injektion: Spermatogenese und entwickelte Spermien, Rückbildung des interstitiellen Gewebes. Penis und Klitoris hypertrophisch. Nierenvergrösserung, Ovarien und Müllersche Gänge unverändert. Kontrollen ohne Spermatogenese, jedoch mit Vermehrung der Leydigschen Zellen.

L. S. RAMASWAMI and D. JACOB

Department of Zoology, University of Rajasthan, Jaipur (India), September 8, 1964.

Estrogen-Induced Uterine Metaplasia in Rats Given Oral Supplements of Vitamin A[1]

Many investigators[2-4] have noted an apparent antagonism between estrogen and vitamin A with a certain balance between these two substances being required to maintain normal reproductive tract morphology in female rats. A further extension of this concept which has gained some support is that estrogen stimulation of experimental animals may cause a hypovitaminotic A condition. Thus, MCCULLOUGH and DALLDORF[5] and CRAMER[6] speculated that estrogen-induced uterine metaplasia may be a result of a deficiency in vitamin A which develops after prolonged hormone stimulation. The present investigation was designed to test this hypothesis by studying the effect of two levels of excess vitamin A on uterine stratified squamous metaplasia (keratinizing metaplasia) resulting from exogenous estrogen treatment.

Materials and methods. Forty-eight Wistar strain rats were bilaterally ovariectomized at 20 days of age and maintained on a vitamin A-deficient diet thereafter. These animals were divided into five experimental groups as follows: (1) untreated, (2) estrogen treated (2.3 mg estradiol cyclopentylpropionate per week), (3) estrogen treated plus 250 international units (IU) of vitamin A per week, (4) estrogen treated plus 100,000 IU vitamin A per week, (5) 100,000 IU vitamin A weekly. All animals were sacrificed after 60 days (80 days of age). The uteri were embedded in paraffin and sectioned at 8 μ. Every fifth section was mounted and stained with Harris' hematoxylin and eosin.

Results. The data (Table) reveal that when exogenous estrogen is administered to rats deficient in vitamin A (group 2) the uterine lesions are numerous and extensive. The simultaneous administration of moderate amounts of vitamin A suppresses the incidence of the lesions (group 3). In contrast, high doses (group 4) apparently introduce other factors, at present unexplained, and afford very little protection against the adverse effects of prolonged estrogen injections.

Discussion. The high degree of metaplasia observed in the uteri of animals of group 2 was expected since the combination of hypovitaminosis A and excess estrogen stimulation (in amounts used in this investigation) is known to precipitate such lesions[7].

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