Musth and urinary testosterone concentrations in the African elephant (*Loxodonta africana*)*

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Summary. Urine samples were obtained from free-ranging African elephants that were considered to be in and out of musth. Testosterone concentrations, measured by radioimmunoassay were significantly greater in males that were in or around the time of behavioural musth. This study supports a correlation between the observed behavioural characteristics of musth and urinary testosterone levels.

Introduction

The phenomenon of musth in the male Asian elephant (*Elephas maximus*) has long been recognized (Darwin, 1874; Evans, 1901) and has now been identified in the African genus, *Loxodonta* (Poole & Moss, 1981). Musth, which has been likened to rutting behaviour in ungulates (Eisenberg, McKay & Jainudeen, 1971), refers to a set of visible physical and behavioural characteristics displayed periodically by adult male elephants (Poole & Moss, 1981). The most obvious manifestations in Asian and African elephants are a sharp rise in aggressive behaviour, copious secretions from and enlargement of the temporal glands, and the frequent discharge of urine (Jainudeen, McKay & Eisenberg, 1972a; Poole & Moss, 1981). During musth, free-living male elephants search out and associate with females (Eisenberg *et al.*, 1971; Poole & Moss, 1981).

Amongst free-ranging African elephants, the phenomenon of musth is central to understanding the mating system of elephants: males in musth experience a temporary rise in dominance rank; musth males are more successful at guarding and mating oestrous females than are non-musth males; high-ranking musth males appear to be able to inhibit musth in lower ranking individuals; oestrous females attempt to maintain proximity to musth males but never to non-musth males (Poole, 1982).

In the Asiatic elephant, changes in plasma testosterone levels with musth have been described (Jainudeen, Katongole & Short, 1972b). Although it is possible to collect blood samples from domesticated animals (Jainudeen *et al.*, 1972b), the collection of a large number of blood samples from free-ranging elephants is not feasible. In the present study, carried out in Amboseli National Park, Kenya, we therefore attempted to assess changes in urinary testosterone levels with observed musth behaviour.

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Materials and Methods

Study area, subjects and urine collection

The Amboseli National Park in Kenya covers an area of 390 km² and consists of semi-arid, wooded, bushed, and open grasslands interspersed with a series of permanent swamps. The surrounding area, making up an ecosystem of about 3000 km², is semi-arid savannah in which water availability is seasonal (Western, 1975). This ecosystem is inhabited by a free-ranging population of elephants numbering 600 individuals, which have been under observation by J. H. Poole and C. J. Moss since September 1975. This population includes 162 sexually mature males. All adults of both sexes and most juveniles are individually known.

Urine samples were collected from 26 adult males estimated to be at least 25 years of age. Male elephants reach puberty between 14 and 17 years of age (Laws, Parker & Johnston, 1975). Elephants continue to grow throughout life and age can be estimated from shoulder height (Laws, 1966). Hind footprint length has been found to be a good predictor of shoulder height (Western, Moss & Georgiadis, 1983) and elephants in this study were aged by this method.

The animals were observed closely during the year that urine samples were collected (June 1980–July 1981) and a detailed account was kept of each animal's behaviour, associations and sexual state. Individuals that periodically come into musth have been termed "socially mature" males. Younger adult males in the Amboseli population do not come into musth although they do have sexually active periods and can mate successfully (Poole, 1982). These males have been termed "socially non-mature."

The urine was collected by aspiration from the ground into a 20-ml syringe. Collection usually took place between 07:00 and 09:00 h. The time urine took to soak into the ground varied with soil type, but samples were usually collected within 1 min after urination, and filtered to remove debris whenever possible. The samples were stored in 20-ml glass containers and usually frozen within 4 h of collection. The samples were shipped on solid CO₂ to the endocrine laboratory at the San Diego Zoo for analysis.

The samples collected were from animals that were placed into one of five categories: (A) males showing physical signs of musth; (B) males that were not showing signs of musth but were in association with females; (C) males that periodically showed musth but were not showing signs of musth and were in association with other males; (D) mature males that were neither in musth nor in association with female groups at the time of collection, but came into musth within 1 month; and (E) mature, sexually active males, not in musth at the time of sample collection, in association with females, had already been seen in musth during that particular sexually active period and came into musth again within 1 week from the date of sample collection. In all, 52 urine samples were collected from 26 different males.

The study by Jainudeen *et al.* (1972b) showed that plasma testosterone levels increase dramatically with the onset of musth in Asian elephants and so one-tailed tests of statistical significance were considered justified.

Testosterone assay

Method. Urinary testosterone concentrations were measured by a method similar to that described by Czekala, Hodges, Gause & Lasley (1980). All samples were analysed for creatinine concentration (Tausky, 1954) to allow for the correction of the variations in concentrations of the urine samples. Urine samples containing <0.1 mg creatinine/ml (N = 2) were considered too dilute for further evaluation. Enzymic hydrolysis of urine samples (0.2 ml) was accomplished by first diluting the urine with an equal volume of assay buffer (0.1 M-NaPO₄, 0.9% (w/v) NaCl, 0.1% NaN₄, 0.1% gelatin, pH 5), adding 0.02 ml β -glucuronidase-arylsulphatase (Boehringer-Mannheim, West Germany) and incubating for 24 h at 37°C. Methodological loss was monitored and accounted for by adding 2000 c.p.m. repurified [³H]testosterone before extraction with 8.0 ml

freshly opened diethyl ether (Mallinckrodt, St Louis, Missouri). The aqueous phase was separated by snap-freezing in methanol–solid CO₂ and the ether extracts were reduced to 0.5 ml under a nitrogen stream at 40°C. Iso-octane (1.0 ml) was added at this time to limit the amount of adherence of steroid to the vial, and the volume was reduced to remove the residual ether (Ramsay, Lasley & Stabenfeldt, 1981).

Chromatography on celite columns was performed on each extractant as previously described (Czekala *et al.*, 1980) and aliquots of each eluate were taken for recovery and radioimmunoassay using the Abraham S-791 No. 7 antiserum. A total volume of 0.7 ml including 9000 c.p.m. [1,2,6,7-³H]testosterone (sp. act. 94 Ci/mmol; New England Nuclear, Boston, Massachusetts), in assay buffer, pH 7, was incubated overnight at 15°C.

Separation of antibody-bound from free steroid was achieved by the addition of 0.2 ml charcoal–dextran (0.65% and 0.065%, respectively) in assay buffer (pH 7). The sensitivity of all assays was 5 pg/tube because this was the smallest amount of pure standard that gave values more than two s.d. less than the mean of the zero tubes. Water blanks were <5 pg/tube.

The intra-assay precision, as expressed by coefficient of variation (CV), was obtained by evaluating 8 replicate urine samples in the same assay giving a value of 3.1% over the range 48 to 51% bound. The interassay CV for controls in the middle of the range was 9% ($n = 5$). The recovery of tritiated testosterone after celite chromatography averaged $79 \pm 0.3\%$ (s.e.m.; $n = 60$).

Validation. Specificity of the antiserum for determining urinary testosterone was determined empirically since it may not be possible to predict the potential contributory components in elephant urine. Serial dilutions of hydrolysed elephant urine were assayed for testosterone and showed a parallel dose–response to the pure testosterone standards. Celite co-chromatography of [³H]testosterone and hydrolysed elephant urine is shown in Text-fig. 1. Parallelism of the pure radiolabelled testosterone and immunoreactivity indicates that this separation procedure is able to separate urinary testosterone from other immunoreactive components and that testosterone is the major immunoreactive component.

Text-fig. 1. Celite co-chromatography of male elephant urine extract showing recovery of added [³H]testosterone and concentrations of testosterone as measured by RIA. Arrows indicate the characterized elution pattern of pure testosterone.

Co-chromatography by high-pressure liquid chromatography (HPLC) was performed by hydrolysis of 0.5 ml of urine, addition of 2000 c.p.m. each [³H]testosterone and [³H]dihydrotestosterone and extraction as described previously. The dried sample was reconstituted in 0.055 ml methanol (Mallinckrodt) before injection onto a 4.6 × 250 mm Lichrosorb C₁₈ column (Altex Scientific, Berkeley, California) using acetonitrile : water (40 : 60 v/v) as the isocratic system. Eluate fractions were collected at 0.5-min intervals for 15 min then at 0.3-min intervals for an additional 21 min (flow rate consistently at 1.0 ml per min). The fractions were evaporated under nitrogen, reconstituted in 1.0 ml assay buffer (pH 7) and divided into aliquots; 0.4 ml for testosterone assay,

and 0.4 ml for monitoring recovery of labelled hormone. The results are shown in Text-fig. 2 and substantiate the indication that testosterone was, in fact, the major component. A second peak which co-eluted with [^3H]dihydrotestosterone, was present and would be the only contaminating factor to the testosterone measurements if celite chromatography were not employed.

Text-fig. 2. High-performance liquid co-chromatography of male elephant urine extract not separated by celite chromatography. Arrows indicate the characterized elution patterns of the pure hormones, testosterone (T) and dihydrotestosterone (DHT).

Results

In 5 of the 26 animals studied (Nos 78, 80, 97, 99 & 107), samples were collected when the elephants were in musth and not in musth. All 5 individuals showed markedly higher levels ($P = 0.004$, non-paired t -test) of urinary testosterone (ng/mg creatinine) during musth (range 164–393) than during non-musth periods (range 39–153).

The results for all the samples are shown in Table 1. Groups were statistically compared using a non-paired group t test. Musth males (Group A) had statistically higher testosterone levels than did those in Groups B and C (non-musth) ($P = 0.04$ and 0.001 , respectively).

Non-musth, sexually active males (Group B) had statistically higher levels than did non-musth, sexually inactive males (Group C; $P = 0.01$). The males in Groups D and E had statistically higher testosterone levels than did those in Group C ($P = 0.0001$ and 0.003 , respectively).

Discussion

The study by Jainudeen *et al.* (1972b) demonstrated that musth in the Asian elephant is associated with, and possibly caused by a marked increase in testicular secretion. They also suggested that musth may be induced by exposure to oestrous females. Studies on African elephants show that individual testicular testosterone concentrations are highly variable (3–490 ng/100 g testicular tissue; Short, Mann & Hay, 1967; Buss & Johnson, 1967). However, in both these studies there was a large age range amongst the subjects. In addition, their past histories were unknown and the phenomenon of musth in these elephants had not been recognized. Although no relations between steroid concentration and behavioural state were established, the results did suggest that the variations could reflect inherent cyclicity in individual reproductive activity.

Table 1. Testosterone concentrations (ng/mg creatinine) in urine collected from male African elephants in musth (Group A), sexually active but not in musth (Group B), sexually inactive and not in musth (Group C), pre-musth (Group D), and those mature, sexually active, not in musth at the time of collection, in association with females, had already been seen in musth during that sexually active period and came into musth again 1 week from the date of sample collection (Group E)

Group A		Group B		Group C		Group D		Group E	
Male	Testosterone	Male	Testosterone	Male	Testosterone	Male	Testosterone	Male	Testosterone
99	164.7	149	76.0	107	39.2	44	176.2	46	91.9
78	181.4	97	95.6	51	41.1	107	181.7	57	142.3
13	193.5	91	141.8	22	41.5	57	303.6	150	223.0
97	194.0	147	205.5	28	47.1	114	361.3	80	248.5
107	226.0	132	214.6	99	58.0				
114	264.2			41	67.3				
119	313.6			154	69.9				
80	393.3			80	73.7				
				44	77.9				
				126	80.0				
				45	99.2				
				78	153.0				
				10	162.4				
Mean	241.3		146.7		77.7		225.7		176.4
s.d.	78.4		62.6		39.7		91.7		72.3
s.e.m.	27.7		38.0		11.0		45.9		36.1

In the present study the physical and behavioural characteristics associated with musth in African elephants have been related to levels of urinary testosterone. Behavioural (Poole, 1982) and hormonal data indicate that adult male elephants over the age of 25 have sexually active periods, but that some individuals experience heightened periods of sexual activity, i.e. musth. Males that were considered to be 'pre-musth' (Group D) exhibited changes in behaviour (i.e. postural, agonistic and habitat use; Poole, 1982) and had high levels of urinary testosterone. These findings are in agreement with those of Jainudeen *et al.* (1972b) who reported elevated plasma testosterone concentrations and behavioural changes in Asian elephants several weeks before the onset of musth. Sexually active 'inter-musth' males (Group E) also showed high urinary testosterone values. Poole (1982) suggested that musth in these males was being inhibited by the presence of higher ranking males in musth.

These data suggest that the physical manifestations of musth may be predicted by means of regular urine analysis. In view of the fact that few males are kept in captivity because of management problems, and the need for breeding programmes to perpetuate the species, endocrine monitoring could help to indicate when males should be controlled physically, or possibly by chemical means, before musth was observed.

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