

Urinary oestrogen excretion in the female cotton-topped tamarin (*Saguinus oedipus oedipus*)

Heather M. Brand

*Institute of Zoology, Department of Reproduction, Zoological Society of London,
Regent's Park, London NW1 4RY, U.K.*

Summary. Daily urinary oestrogen excretion was measured by radioimmunoassay in 6 adult female cotton-topped tamarins. Four females showed obvious cyclicity in oestrogen excretion and the mean cycle length for 10 complete cycles was 22.7 ± 1.7 days with a range of 19–25 days. In 3 of the 6 females a dip-and-read test for blood in the urine gave positive readings which were distributed during trough and mid-cycle periods although females of this species are believed not to menstruate.

Introduction

Growing interest in primates of the Family Callitrichidae as research animals and the present emphasis on the captive breeding of non-human primates for research purposes lends weight to studies of the reproductive biology and endocrinology of such animals.

The relevant published material has mainly concerned the common marmoset, *Callithrix jacchus* (Hearn & Lunn, 1975; Abbott & Hearn, 1978; Chambers & Hearn, 1979). The reproductive biology and endocrinology of the cotton-topped tamarin, *Saguinus oedipus oedipus*, in spite of its important position as an endangered species and in cancer research (Wolfe & Dienhardt, 1978), has received little attention (Hampton & Taylor, 1971; Preslock, Hampton & Hampton, 1973; Hampton, 1975). Preslock *et al.* (1973), in the only endocrinological study of the cotton-topped tamarin reported to date, showed that the ovarian cycle length was 15.5 ± 1.5 days. This result was determined from the mean interval between peaks of plasma progestagens which were measured by a non-specific assay in samples collected 3 times a week. As the routine trapping and sedating of these animals in order to collect blood samples is disruptive to both animal and colony, a different method of sample collection is obviously preferable in colonies of breeding tamarins.

The collection of daily urine samples and the measurement of relevant hormone metabolites provide a means for following certain reproductive hormonal events without causing stress in the colony. The usefulness of this method for gathering endocrinological information on non-human primates has been known for some time (Hopper, Tullner & Gray, 1968; Townsley, 1974; Martin, Seaton & Lusty, 1975; Ando, Nigi, Tanaka & Ohsawa, 1976), but only recently has it been applied to the New World primates (Lunn, 1978; Bonney, Dixson & Fleming, 1979).

This paper presents the results obtained from the oestrogen radioimmunoassay of daily urine samples collected from captive adult female cotton-topped tamarins.

Materials and Methods

Animals and housing

Six wild-caught adult female cotton-topped tamarins (*Saguinus oedipus oedipus*) (average adult weight 450 g) which had been in the colony at the Institute of Zoology for at least 1 year

were studied. Initially, all were housed individually in cages measuring 50.8 × 50.8 × 76.2 cm (Type 1) which were interspersed amongst the other cages of animals in the colony. These cages were equipped with nest-boxes and perches and had wire mesh floors (mesh size 1.27 × 1.27 cm) under which were positioned the cage waste trays. After approximately 40 days and for purposes of a wider study, the females were each moved to a different cage measuring 101.6 × 66 × 91.4 cm in total (Type 2) with a removable vertical wire-mesh central divider (mesh size 2.54 × 2.54 cm). Females were again housed singly but this time a male was housed in the other half of the divided cage. These cages were fitted with nest-boxes, perches and custom-built stainless-steel funnel-like waste trays positioned just below the cage floor grids and occupying the entire floor space in each half of the divided cage. For 1 h every day the cage divider was removed and the male and female allowed uninterrupted access to each other. No female in the study was housed next to another female.

The room in which the animals were housed was maintained within the range 24–27°C and illuminated by artificial lighting (08:00–20:00 h) and natural lighting from large skylights.

Collection of samples

Urine was collected by 2 different methods depending on whether the animals were housed in Type 1 or Type 2 cages. In Type 1 cages urine was collected with disposable glass pipettes from sheets of polythene which had been cut to size and placed in the cage waste trays and left for 24 h. The urine was collected once a day between 14:50 and 15:50 h just after the animals had been fed, and due to evaporation and increase in urination after feeding, was nearly always recently voided. After collection the urine was placed in 3 ml glass bottles, tested for the presence of haemoglobin by a dip-and-read test ('Hemastix': Ames Co., Slough, U.K.), and then frozen at –20°C. Every day after sample collection the soiled polythene sheets were replaced with clean ones and the grid cleaned of food and faeces. Urine was collected thus for approximately 40 days from females in Type 1 cages.

When the females were transferred to the Type 2 caging, the urine collection system was designed to collect 24-h samples for comparison of the two methods, but in effect a full 24-h sample was never collected. Glass bottles (20 ml) containing 0.5 ml 1% NaN₃ to preserve the sample were placed under the central opening of the funnel-like waste tray. Every day between 14:50 and 15:50 h the urine which had collected in this bottle was measured, mixed, tested for haemoglobin, separated into 3-ml aliquots and frozen at –20°C. The cage divider was then removed and the male and female were allowed access to each other for 1 h. When returned to their respective cages the funnel waste trays were thoroughly washed and dried and clean urine collecting bottles positioned. Urine was collected for approximately 40 days whilst animals were housed in Type 2 cages.

Radioimmunoassay

The frozen urine samples were thawed and 50 µl of each daily sample were transferred to a corresponding series of 12 × 75 mm plastic tubes (Luckham Ltd, Burgess Hill, U.K.). The urine (50 µl) was hydrolysed with 0.4 i.u. β-glucuronidase ('Pasteur': Uniscience, Cambridge, U.K.) in 50 µl phosphate-buffered saline (containing 0.2% NaCl and a total phosphate concentration of 0.02 M), pH 7.0, for 1 h at 37°C. Each hydrolysed sample was then diluted with a standard volume (1:50) of assay buffer (phosphate-buffered saline, pH 7.0, containing 0.1% thimerosal, 1.0% (w/v) gelatin, 0.9% (w/v) NaCl and a total phosphate concentration of 0.1 M) and frozen until assayed.

The samples were directly assayed in duplicate (without prior extraction or chromatography). Each assay tube contained 100 µl of sample (diluted hydrolysed urine), 100 µl (~50 pg) [2,4,5,7(n)-³H]oestradiol (sp. act. 108 Ci/mmol: Radiochemical Centre, Amersham, U.K.)

and 100 μ l antiserum 461/9 (raised in a goat to oestradiol-17 β -succinyl-bovine serum albumin by Dr B. J. A. Furr, ICI Pharmaceutical Division, Macclesfield, U.K.). The antiserum was diluted to give approximately 20% binding of [³H]oestradiol-17 β in the absence of unlabelled steroid and had cross-reactions of 50.3% with oestradiol-17 α ; 27.9% with oestrone; 2.8% with oestriol; 0.007% with androstenedione; 0.009% with testosterone; and 0.014% with progesterone. Each tube was mixed, incubated for 1 h at 37°C and cooled in an ice-bath for 15 min after which 200 μ l of a dextran-coated charcoal suspension (0.1% dextran; 1.0% charcoal in pH 7.0 assay buffer) were added to all tubes which were then mixed, incubated for 4 min in an ice-bath and centrifuged at 4°C and 1750 g for 3 min. Removal of the separated antibody-bound steroid from the unbound steroid took place after centrifugation when 300 μ l supernatant were transferred from each tube to mini-scintillation vials to which 1.0 ml scintillant ('Pico-Fluor': Packard Instruments Ltd, Caversham, U.K.) was added. The vials were capped, shaken and counted for 3 min in a scintillation spectrometer (Model 3255 Packard Tri-carb). For every series of samples analysed samples containing 0.03–10 ng oestradiol-17 β /ml were treated in the same way to provide the standard curve. The 50% inhibition level (\pm s.d.) was at 0.665 ± 0.103 ng/ml ($n = 20$). Biological standards derived from a hydrolysed urine pool were included in the processing of every batch of samples and treated in the same way as the samples.

Intra- and inter-assay coefficients of variation were 6.8 and 11.8% respectively ($n = 10$), determined by the repeated assay of biological standards within a single assay and in separate assays. Accuracy was tested by measuring known concentrations of oestradiol (2.5, 1.25, 0.625 and 0.312 ng/ml) in hydrolysed urine from a male tamarin (diluted 1:5000, the usual dilution for female urine). A linear regression was calculated for recovered oestradiol against added oestradiol ($r = 0.999$; $y = 0.984x + 0.030$), the slope of which was not significantly different from 1.

Creatinine assay

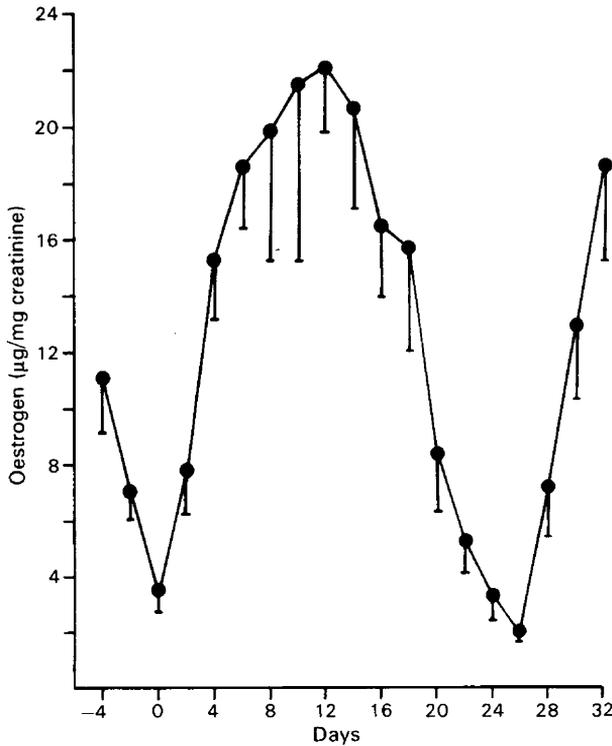
In order to control for urine concentration the creatinine value was obtained for every urine sample assayed. Frozen samples were thawed and 25 μ l were transferred in duplicate to 1 cm light-path plastic cuvettes (Hughes & Hughes Ltd, Romford, U.K.) to which were added 3.0 ml of an alkaline triton and picric acid solution (1 vol. 5% triton, 0.075 N-NaOH to 1 vol. saturated picric acid solution and 4 vols deionized distilled water). Samples were left undisturbed for 1½–2 h away from direct light after which the absorbance of each sample was determined at a wavelength of 520 nm (SP15 Concentration Colorimeter, Pye-Unicam Ltd, Cambridge, U.K.). A standard curve ranging from 0.5 to 3 mg creatinine/ml was provided for every series of samples assayed. Biological standards obtained from urine pools of high and low creatinine concentration were also included and treated the same as samples.

Coefficients of variation were established by repeated assay of biological standards within a single assay and in separate assays. Intra-assay coefficients of variation were 1.3% for high and 1.9% for low concentrations ($n = 10$) and inter-assay coefficients of variation for high and low concentrations were 8.6 and 4.2% respectively ($n = 12$).

Results

Of the 6 females in the study, 4 displayed unambiguous cyclicity in oestrogen excretion, 1 showed an irregular cyclical pattern and the other female appeared acyclic. The composite pattern of oestrogen excretion in 10 complete cycles from 4 females is shown in Text-fig. 1. The larger standard deviations apparent at Days 8 and 10 can be halved by the removal of just one of the 10 cycles. The oestrogen cycle is characterized by an immediate rise in concentrations from Days 1–2 with peak values at Days 11–12 and then a rapid decline towards base levels from Day 18. The mean oestrogen cycle length for 10 complete cycles (4 animals) was $22.7 \pm$

1.7 days with a range of 19–25 days. The oestrogen levels in the apparently acyclic female ranged between 6 and 29 $\mu\text{g}/\text{mg}$ creatinine.



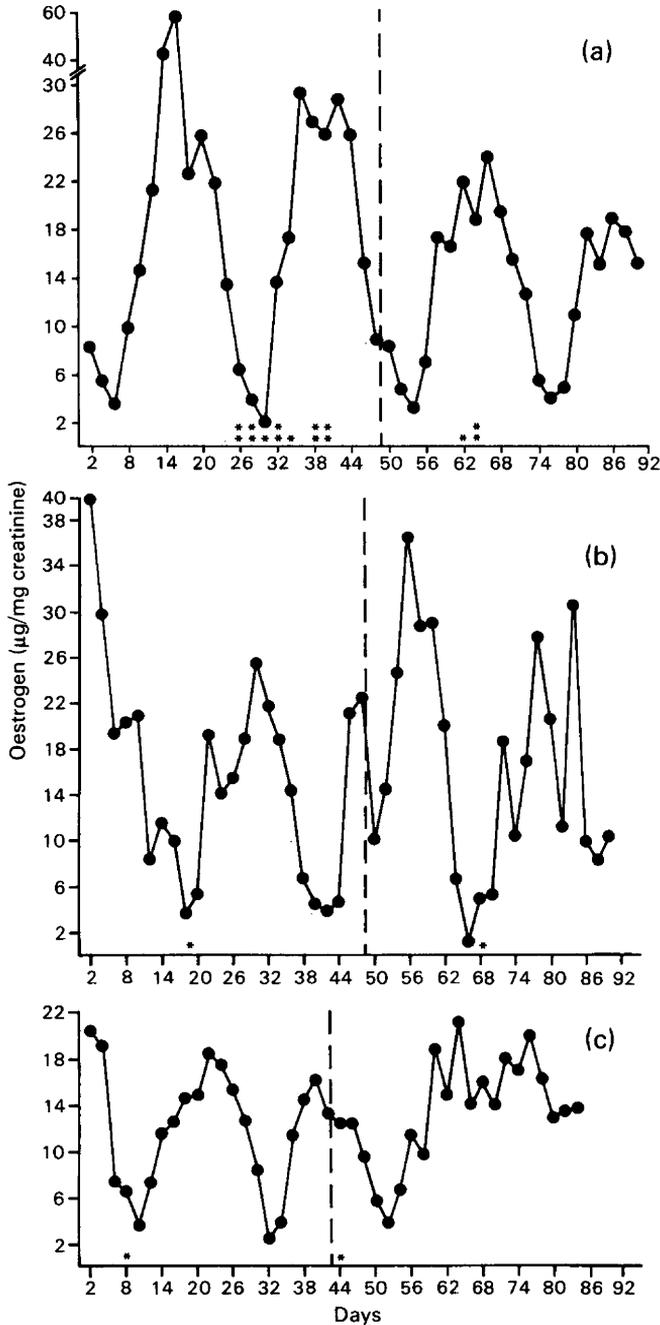
Text-fig. 1. Composite graph of urinary oestrogen levels in 10 complete cycles of 4 female cotton-topped tamarins. Points represent mean values of samples for 2 consecutive days. Day 0 indicates the mean of the lowest oestrogen level and the value preceding it.

The results from the dip-and-read test for blood in the urine were not consistent in all the animals. Positive readings were given by urine from only 3 of the 6 females and could be associated with the lowest levels of oestrogen excretion (4 instances: Text-figs 2a, b and c) or with the mid-cycle period (3 instances: Text-figs 2a and c). In 1 of the 3 females blood was present in the urine for consecutive days at both the trough and mid-cycle periods (see Text-fig. 2a). Moving a female from one cage to another (or from an isolated condition to one in close proximity to a male) appeared to affect slightly the level of oestrogen excreted in only 1 female (Text-fig. 2b). The collection of single urine samples at the same time every day revealed patterns of oestrogen cyclicity similar to those obtained from daily pooled urine.

The gestation period in cotton-topped tamarins in this colony is more than 166 days, and the pattern of oestrogen excretion in 1 cyclic female when conception probably occurred is shown in Text-fig. 2c. The times of ovulation and conception cannot be indicated because pregnanediol-3- α -glucuronide was undetectable in the urine of these female cotton-topped tamarins and no other appropriate measurement was available.

Discussion

Published data on ovarian cycle lengths of callitrichid primates are available for only two



Text-fig. 2. Urinary oestrogen excretion levels in successive cycles in female cotton-topped tamarins. *Indicates the occurrence of blood in the urine; the vertical broken line indicates the time when the female was moved from isolation into limited contact with a male. The female represented in (c) gave birth 192 days after being moved.

species, the common marmoset with a cycle length of 16.4 ± 1.7 days (Hearn & Lunn, 1975) and the cotton-topped tamarin, 15.5 ± 1.5 days (Preslock *et al.*, 1973). The cotton-topped tamarin oestrogen cycle of 22.7 ± 1.7 days presented in this paper, however, is closer to that of

the larger-bodied capuchin monkey (Family Cebidae) with its cycle length of 21.0 ± 1.1 days and range of 18–24 days (Nagle *et al.*, 1979). The discrepancy between the cycle length found by Preslock *et al.* (1973) and the findings in this paper might be due to the different methods of sample collection, because the method used in the present study did not involve physical contact with the animals. In view of the intractable and excitable nature of these animals, it is possible that the degree of handling used by Preslock *et al.* (1973) could have affected the cycle length. Nevertheless, whichever cycle length reflects the natural state more closely, they both fall within the range emerging for the New World monkeys in which the mean cycle length is typically shorter than that found for the Old World monkeys.

Although all Old World primates have menstrual cycles, not all of the New World monkey species appear to menstruate. Based on the examination of vaginal cytology, the absence of menstrual bleeding has been noted in the common marmoset (Hearn & Lunn, 1975), the squirrel monkey (Jarosz, Kuehl & Dukelow, 1977) and the owl monkey (Bonney *et al.*, 1980). In the light of these reports and as the vaginal cytology of the cotton-topped tamarin cycle was not followed in this study, the occasional presence of blood in the urine collected was considered a possible bacterial artefact. However, the distribution pattern of this blood in relation to the oestrogen cycles presented in Text-fig. 2 indicates a need for vaginal cytology studies. Of particular interest is the presence of blood in the urine at the mid-cycle period in 3 cycles. Inter-menstrual bleeding has been reported to occur in the langur (David & Ramaswami, 1969), rhesus macaque (Hartman, 1932), Japanese macaque (Nigi, 1977) and possibly the chimpanzee (Tinklepaugh, 1933), and is considered to be associated with ovulation (Jeffcoate, 1975).

This investigation was funded by the Zoological Society of London, the Council for Scientific and Industrial Research, South Africa, and the Central Research Fund of the University of London. I am grateful to Dr R. D. Martin for supervision of the investigation, Dr R. C. Bonney for advice on radioimmunoassay techniques, Mr D. M. Jones for veterinary assistance and Mrs B. Murrill for care of the animals.

References

- Abbott, D.H. & Hearn, J. P.** (1978) Physical, hormonal and behavioural aspects of sexual development in the marmoset monkey, *Callithrix jacchus*. *J. Reprod. Fert.* **53**, 155–166.
- Ando, A., Nigi, H., Tanaka, T. & Ohsawa, N.** (1976) Routine measurement of urinary total estrogens of the female Japanese monkey as an index for estimating the time of ovulation. *Primates* **17**, 89–94.
- Bonney, R.C., Dixon, A.F. & Fleming, D.** (1979) Cyclic changes in the circulating and urinary levels of ovarian steroids in the adult female owl monkey (*Aotus trivirgatus*). *J. Reprod. Fert.* **56**, 271–280.
- Bonney, R.C., Dixon, A.F. & Fleming, D.** (1980) Plasma concentrations of oestradiol-17 β , oestrone, progesterone and testosterone during the ovarian cycle of the owl monkey (*Aotus trivirgatus*). *J. Reprod. Fert.* **60**, 101–107.
- Chambers, P.L. & Hearn, J.P.** (1979) Peripheral plasma levels of progesterone, oestradiol-17 β , oestrone, testosterone, androstenedione and chorionic gonadotrophin during pregnancy in the marmoset monkey, *Callithrix jacchus*. *J. Reprod. Fert.* **56**, 23–32.
- David, G.F. & Ramaswami, L.S.** (1969) Studies on menstrual cycles and other related phenomena in the langur (*Presbytis entellus entellus*). *Folia Primat.* **11**, 300–316.
- Hampton, S.H.** (1975) Placental development of the marmoset. In *Contemporary Primatology*, pp. 106–114. Eds S. Kondo, M. Kawai & A. Ehara. Karger, Basel.
- Hampton, S.H. & Taylor, A.C.** (1971) Gonadal development in marmosets. *Proc. 3rd Int. Congr. Primat. Zurich* **1**, 246–259.
- Hartman, C.G.** (1932) Studies on the reproduction of the monkey *Macacus (Pithecus) rhesus*, with special reference to menstruation and pregnancy. *Contrib. Embryol. Carneg. Instn* **134**, 1–161.
- Hearn, J.P. & Lunn, S.F.** (1975) The reproductive biology of the marmoset monkey. In *Breeding Simians for Developmental Biology* (Lab. Anim. Handbk No. 6), pp. 191–204. Eds F. T. Perkins & P. N. O'Donoghue. Laboratory Animals, London.
- Hopper, B.R., Tullner, W.W. & Gray, C.W.** (1968) Urinary estrogen excretion during pregnancy in a gorilla (*Gorilla gorilla*). *Proc. Soc. exp. Biol. Med.* **129**, 213–214.
- Jarosz, D.J., Kuehl, T.J. & Dukelow, W.R.** (1977) Vaginal cytology, induced ovulation and gestation in the squirrel monkey (*Saimiri sciureus*). *Biol. Reprod.* **16**, 97–103.
- Jeffcoate, N.** (1975) *Principles of Gynaecology*, 4th edn. Butterworth, London.

- Lunn, S.F.** (1978) Urinary oestrogen excretion in the common marmoset, *Callithrix jacchus*. In *Biology and Behaviour of Marmosets*, pp. 67–73. Eds H. Rothe, H.-J. Wolters & J. P. Hearn. Eigenverlag Hartmut Rothe, Göttingen.
- Martin, R.D., Seaton, B. & Lusty, J.A.** (1975) Application of urinary hormone determinations in the management of gorillas. *Jersey Wildlife Preservation Trust*, 12th Annual Report, pp. 61–70.
- Nagle, C.A., Denari, J.H., Quiroga, S., Riarte, A., Merlo, A., Germino, N.I., Gomez-Argaña, F. & Rosner, J.M.** (1979) The plasma pattern of ovarian steroids during the menstrual cycle in capuchin monkeys (*Cebus apella*). *Biol. Reprod.* **21**, 979–983.
- Nigi, H.** (1977) Laparoscopic observations of ovaries before and after ovulation in the Japanese monkey (*Macaca fuscata*). *Primates* **18**, 243–259.
- Preslock, J.P., Hampton, S.H. & Hampton, J.K., Jr** (1973) Cyclic variations of serum progestins and immunoreactive estrogens in marmosets. *Endocrinology* **92**, 1096–1101.
- Tinklepaugh, O.L.** (1933) Sex cycles and other cyclic phenomena in a chimpanzee during adolescence, maturity and pregnancy. *J. Morph. & Physiol.* **54**, 521–547.
- Townsley, J.D.** (1974) Estrogen excretion of the pregnant baboon (*Papio papio*). *Endocrinology* **95**, 1759–1762.
- Wolfe, L.G. & Deinhardt, F.** (1978) Overview of viral oncology studies in *Saguinus* and *Callithrix* species. In *Marmosets in Experimental Medicine*, pp. 96–118. Eds N. Gengozian & F. Deinhardt. Karger, Basel.

Received 26 September 1980