URINARY EXCRETION OF ANDROSTERONE THROUGHOUT THE MENSTRUAL CYCLE IN THE RHESUS MONKEY (*MACACA MULATTA*)

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Summary. Urinary excretion of androsterone was studied in four rhesus monkeys during six menstrual cycles. Androsterone excretion ranged from 28 to 388 $\mu\text{g}/24 \text{ hr}$. In two menstrual cycles, the excretion of androsterone in the mid-luteal phase was approximately 60% greater than that in the follicular phase. In the remaining four cycles, enhanced excretion of androsterone in the luteal phase was not observed and therefore urinary androsterone does not appear to provide a reliable index of corpus luteum function in this macaque.

Studies with $[4\text{-}^{14}\text{C}]$ progesterone have shown that androsterone is the major urinary metabolite of progesterone in the rhesus monkey (Plant, James & Michael, 1971; Reddy, Balin & Nes, 1971). It was therefore of interest to study the endogenous excretion of this urinary 17-oxosteroid throughout the menstrual cycle.

Four adult female rhesus monkeys (5.0 to 7.1 kg body wt) were housed individually in metabolism cages in a quiet room. Twenty-four-hour urine samples were stored at -18°C until assay. Androsterone was estimated only in the glucuronide fraction since little is excreted as the sulphate conjugate in the rhesus monkey (O'Malley & Lipsett, 1966; Pal, 1971). In order to determine procedural losses, $[7\text{-}^3\text{H}]$ androsterone glucuronide (11,250 d/min), isolated from urine following the metabolism *in vivo* of $[7\text{-}^3\text{H}]$ androstenedione (sp. act., 17.5 mCi/mg) in an ovariectomized rhesus monkey, was added to urine before the preparation of a conjugate extract. Following incubation with β -glucuronidase (Ketodase: W. R. Warner and Co. Ltd), androsterone was estimated by the Zimmermann reaction (James & DeJong, 1961) after paper chromatography in two systems as described by James (1961). The radiochemical purity of the conjugate preparation used for determining recoveries was characterized by paper chromatography before and after hydrolysis with β -glucuronidase: approximately 90% of the tritium was accounted for by androsterone glucuronide, and an appropriate correction factor was applied when calculating procedural losses. Estimates were performed on one-half of a 24-hr urine collection and were usually in duplicate. Plasma progesterone was estimated using the competitive protein-binding method of Neill, Johansson & Knobil (1967).

The mean recovery of $[7\text{-}^3\text{H}]$ androsterone was 65.5 ± 8.2 (S.D.) % and water blanks taken through the entire procedure gave values equivalent to an androsterone excretion of less than $2.0 \mu\text{g}/24 \text{ hr}$. The method was precise (coefficient of variation, 5.2%) and minimum rates of urinary androsterone excretion exceeded the sensitivity of the method (15 to $20 \mu\text{g}/24 \text{ hr}$). Evidence obtained by gas-liquid chromatography and from absorption spectrometry of the Zimmermann chromogens indicated that the fractionation procedure was sufficiently rigorous in eliminating non-specific Zimmermann-positive material (see Vande Wiele, Herrmann & van Wagenen, 1957), and that androsterone was the main steroid component in the eluate of the final chromatogram.

In forty-five determinations on urine collected from four females during six menstrual cycles, the mean excretion rate of androsterone was 115 ± 72 (S.D.) $\mu\text{g}/24 \text{ hr}$ (range, 28 to $388 \mu\text{g}/24 \text{ hr}$). Inter-animal variation in the excretion of this urinary metabolite was marked and, generally, was greater than the day-to-day changes in excretion shown by individual females. For example, the mean androsterone excretion rate of one female was $50 \pm 14 \mu\text{g}/24 \text{ hr}$ while the value for a second female was $201 \pm 26 \mu\text{g}/24 \text{ hr}$. It is generally recognized that ovulation in the rhesus monkey occurs between Days 9 and 16 of the menstrual cycle (Hartman, 1944; Kelly, Marston & Eckstein, 1969). For the purpose of the present study, Day 15 before the next menstruation was taken as the expected time of ovulation so that a comparison could be made of androsterone excretion in the follicular and luteal phases of the menstrual cycle. In two of the six cycles (Nos 2 and 3), urinary androsterone excretion during the mid-luteal phase was approximately two-thirds greater than in the follicular phase. In the remaining four cycles, however, the excretion of this 17-oxosteroid did not increase after the expected time of ovulation despite rises in plasma progesterone in two cycles (Table 1). In one of the cycles that showed an enhanced androsterone excretion during the luteal phase (No. 3), sufficient data were obtained to allow more detailed analysis (Text-fig. 1). The mean androsterone excretion rates during the follicular and luteal phases were $41 \pm 3 \mu\text{g}/24 \text{ hr}$ and $62 \pm 12 \mu\text{g}/24 \text{ hr}$ respectively, and the difference between these values was significant (Student's *t* test, $P < 0.01$). These changes in the urinary excretion of androsterone appeared to be related to changes in plasma levels of progesterone. The rise in progesterone on Day 14 before menstruation was followed on Day 13 by a rise in urinary androsterone. High values were observed for both plasma progesterone and urinary androsterone during the mid-luteal phase, and they declined together immediately before menstruation. An increase in urinary androsterone during the luteal phase of the menstrual cycle has also been reported for the orang-utan (Collins, Graham & Preedy, 1973). The similarity between the two species in this respect is of interest because data for the chimpanzee (Romanoff, Grace, Sugarman & Pincus, 1963) have indicated that the metabolism of progesterone in certain great apes resembles that of man rather than macaques.

Anovulatory menstrual cycles were reported to be most frequent during May and June in rhesus monkeys maintained in the laboratory (Hartman, 1931). In the present study, data for one cycle (No. 1) were obtained from urine collected at this time of year, and the failure to observe increased excretion of

Table 1. Excretion of androsterone in two 24-hr urine samples, and levels of plasma progesterone for the same days, collected in the follicular and luteal phases of six menstrual cycles for four adult rhesus monkeys

Animal no.	Cycle no.	Follicular phase			Luteal phase			Cycle length (days)	Month when urine was collected
		Reverse cycle day	Urinary androsterone ($\mu\text{g}/24 \text{ hr}$)	Plasma progesterone (ng/ml)	Reverse cycle day	Urinary androsterone ($\mu\text{g}/24 \text{ hr}$)	Plasma progesterone (ng/ml)		
MM62	1	29 21	110.8 143.8	— —	10 8	134.3 137.9	— —	31	May to June
MM62	2	27 25	200.4 200.7	0.0 0.2	11 9	388.1 298.5	2.8 2.6	32	Dec to Jan
MM69	3	23 21	42.9 40.9	0.2 —	9 7	63.7 70.2	6.9 7.1	28	Dec to Jan
MM69	4	26 22	42.1 45.7	— —	11 8	42.3 28.1	— —	27	Oct to Nov
MM99	5	26 24	134.0 88.2	0.2 0.2	12 8	119.4 80.8	4.6 4.2	31	Dec to Jan
MM101	6	25 23	223.6 212.5	0.3 0.3	12 8	163.5 203.4	2.9 6.2	31	Dec to Jan

TEXT-FIG. 1. Androsterone excretion in urine (open columns) and progesterone concentrations in plasma (hatched columns) of a rhesus monkey during one menstrual cycle. The solid bar shows the expected time of ovulation (see text).

androsterone in the luteal phase in this case might have been due to the absence of ovulation. In two cycles in which rises in plasma progesterone in the luteal phases occurred, however, there were no increases in urinary androsterone excretion. The relative contributions from the ovary and adrenal cortex to the urinary output of androsterone are unknown, but the latter's secretion may mask the ovarian rhythm. In any case, in contrast to urinary pregnanediol in women, urinary androsterone is unlikely to provide a consistently reliable index of ovarian function in the rhesus monkey.

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REFERENCES

- COLLINS, D. C., GRAHAM, C. E. & PREEDY, J. R. K. (1973) Urinary levels of estrogens, pregnanediol and androsterone during the menstrual cycle of the orangutan. *Biol. Reprod.* **9**, 107.
 HARTMAN, C. G. (1931) The breeding seasons in monkeys, with special reference to *Pithecus* (*Macacus*) *rhesus*. *J. Mammal.* **12**, 129.
 HARTMAN, C. G. (1944) Recovery of primate eggs and embryos. Methods and data on the time of ovulation. *West. J. Surg. Obstet. Gynec.* **52**, 41.
 JAMES, V. H. T. (1961) The excretion of individual 17-ketosteroids by normal females. *J. Endocr.* **22**, 195.
 JAMES, V. H. T. & DE JONG, M. (1961) The use of tetramethylammonium hydroxide in the Zimmermann reaction. *J. clin. Path.* **14**, 425.

- KELLY, W. A., MARSTON, J. H. & ECKSTEIN, P. (1969) Effect of an intra-uterine device on conception and ovulation in the rhesus monkey. *J. Reprod. Fert.* **19**, 143.
- NEILL, J. D., JOHANSSON, E. D. B. & KNOBIL, E. (1967). Levels of progesterone in peripheral plasma during the menstrual cycle of the rhesus monkey. *Endocrinology*, **81**, 1161.
- O'MALLEY, B. W. & LIPSETT, M. B. (1966) Urinary ketosteroids in the rhesus monkey. *Steroids*, **8**, 711.
- PAL, S. B. (1971) The pattern of urinary excretion of individual 17-ketosteroids in rhesus monkeys. *Acta endocr., Copenh.* **67**, 711.
- PLANT, T. M., JAMES, V. H. T. & MICHAEL, R. P. (1971) Conversion of [4-¹⁴C]progesterone to androsterone by female rhesus monkeys (*Macaca mulatta*). *J. Endocr.* **51**, 751.
- REDDY, V. V., BALIN, H. & NES, W. R. (1971) The metabolism of progesterone in the rhesus monkey (*Macaca mulatta*). *Steroids*, **17**, 49.
- ROMANOFF, L. P., GRACE, M. P., SUGARMAN, E. M. & PINCUS, G. (1963) Metabolism of progesterone-4-¹⁴C in immature chimpanzees. *Gen. & compar. Endocr.* **3**, 649.
- VANDE WIELE, R., HERRMANN, W. & VAN WAGENEN, G. (1957) Excretion of 17-ketosteroids in the rhesus monkey. *Proc. Soc. exp. Biol. Med.* **95**, 555.