Hormone Levels and Anogenital Swelling of Female Chimpanzees as a Function of Estrogen Dosage in a Combined Oral Contraceptive (43482)

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Abstract. **A combined oral contraceptive consisting of ethinyl estradiol (EE_2) in three dosages (50, 100, and 400 μg) and norethindrone (0.5 mg) was given to female chimpanzees to determine the effect on endogenous sex hormone levels and anogenital swelling. Serum levels of EE_2 increased with increasing dosages of EE_2, estradiol decreased, and luteinizing hormone, progesterone and testosterone were maintained at approximately midfollicular phase levels. Urinary levels of EE_2 glucuronide increased with the increasing dosages of EE_2, whereas estrone and pregnanediol glucuronide were essentially undetectable. The cyclic increase in female anogenital swelling was abolished when the norethindrone was combined with 50 μg of EE_2 and relatively constant and low levels of swelling were recorded. Relatively constant but successively higher levels of swelling were recorded when the norethindrone was combined with the higher dosages of EE_2. These effects of oral contraceptives on female genital tissues are relevant to our laboratory studies of sexual behavior in chimpanzees given oral contraceptives and could also have some implications for women taking oral contraceptives.** [P.S.E.B.M. 1992, Vol 201]

Despite nearly 40 years of experience with oral contraceptives and their use by over 55 million women worldwide (1), there is still considerable uncertainty regarding the effects of oral contraceptives on the sexuality of women (2). Part of the problem relates to the different formulations of oral contraceptives that have been used and part to the difficulties in assessing the sexuality of humans. The chimpanzee, on the other hand, bears a close biological relationship to the human and is free of the self-consciousness (3) that contributes to the difficulty in studying human sexual behavior. The menstrual cycle of the chimpanzee closely resembles that of the human with respect to the cyclic pattern of circulating sex hormones (4, 5) and urinary hormone metabolites (6, 7). Its sexual behavior, while clearly influenced by female hormone levels, also exhibits considerable variability and responsiveness to social influences (3, 8). For these several reasons, the chimpanzee is a particularly useful model for the human in studies of reproductive endocrinology and sexual behavior (3, 9). Therefore, we studied the effects of oral contraceptives on the hormone levels and anogenital swelling of female chimpanzees as part of a larger study that included the effects of oral contraceptives on the sexual behavior of chimpanzees during laboratory pair-tests (10). This paper presents the data on female hormone levels and anogenital swelling during natural cycles and during cycles in which the females were given oral contraceptives.

The anogenital swelling of the female chimpanzee was monitored for two reasons related to the action of oral contraceptives. It is a visual cue to the male regarding the sexual status of the female; hence, it is relevant to interpreting the results of our study on sexual behavior (10). The genital tissues of the female chimpanzee are also homologous to the genital tissues of women (11, 12), but are considerably more respon-

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sive to changes in sex hormone levels (9, 13). As such, the chimpanzee's anogenital swelling may be regarded as a sensitive index of the peripheral effects of hormonal changes resulting from oral contraceptive (OC) use, which may have relevance for women who use oral contraceptives.

Materials and Methods

Animals. Nine sexually mature female chimpanzees (*Pan troglodytes*) were used, with an average age (±SD) of 25.6 ± 11.9 years (range, 11–44 years) and an average body weight (±SD) of 46.8 ± 5.7 kg (range, 39.4–56.4 kg). Seven of the females were multiparous and two were nulliparous. They had intrauterine devices (Saf-T-Coil, Nullip 25-SX; Schmid Laboratories, Inc., Little Falls, NJ) inserted to prevent pregnancy in the behavioral tests conducted during the natural cycles. They were maintained in two compartment cages consisting of an indoor temperature- and light-controlled compartment that measured 2.4 × 2.4 × 2.0 m high and an outdoor compartment that measured 2.4 × 4.0 × 2.4 m high, interconnected by a guillotine door. They were fed laboratory chow and fresh oranges twice per day at 0900 and 1600 hr; water was available *ad libitum*.

Experiments. Two experiments were conducted. (i) In Experiment 1, both serum and urinary levels of the ovarian steroids and luteinizing hormone (LH) were measured throughout the menstrual cycle during the combined administration of ethinyl estradiol ([EE_2] at dosages of 50 μg, 100 μg, and 400 μg) and norethindrone ([NET] at a single dosage of 0.5 mg); two females were used for each of the three dosages of EE_2. (ii) In Experiment 2, urinary hormone levels and female anogenital swelling were measured throughout two natural cycles (for each of eight females) and two cycles with the combined administration of EE_2 and NET; a different set of three females received one of each of the three dosages of EE_2. The research was conducted during approximately the same months in three consecutive years, with the 50-μg dosage of EE_2 used the first year and the successively higher dosages used during the second and third years. Data collection for natural cycles and cycles with OC administration was balanced for order. At least two cycles intervened between cycles with OC treatment and the natural cycles that were used for comparison, during which ovulatory peaks in LH were confirmed. For the OC cycle studies, at least two cycles of OC administration preceded the collection of body fluids for hormone assay.

The lowest dosage of EE_2 (50 μg) was chosen because it is a dosage in combined oral contraceptives commonly used by women (14). When it was found that 50 μg of EE_2 combined with 0.5 mg of NET inhibited female anogenital swelling, the successively higher dosages of EE_2 were given to produce successively greater stimulation of the genital tissues and an appearance more similar to that of the swollen phase in the natural cycle. The EE_2 and NET were dissolved in 1 ml of absolute ethyl alcohol to ensure uniform bioavailability and were either mixed with 30 ml of orange juice or injected into pieces of fruit or sweet potato. The OC was given to the females between 1550 and 1605 hr daily, beginning on the day after the first day of menses, and continued for 27 days, approximately three fourths of the 36-day cycle, similar to the schedule used by women, e.g., 21 days of the 28-day cycle (14). During the OC cycles, serum and urine were obtained 3 days per week. During natural cycles, urine was obtained at least 3 days per week and more frequently during the midcycle, periovulatory phase. Blood (20 ml) was obtained from the femoral vein under ketamine HCl anesthesia at approximately 0930 hr. Urine was collected in metabolism cages over a 17-hr period from 1630 to 0930 hr or as an early morning spot sample and tested with commercial test strips for occult blood. Any dried or fresh blood on the labia minora or in the cage was also noted. Urine and serum samples were stored at −20°C until assayed. Anogenital swelling was assessed twice a day on weekdays and once a day on weekends using a procedure developed in this laboratory (13).

Hormone Assays. NET, EE_2, LH, progesterone, estradiol, and testosterone were measured in serum; NET glucuronide (NETG), EE_2 glucuronide (EE_2G), LH, estrone glucuronide (E_1G), pregnanediol glucuronide (PDG), and creatinine were measured in urine. All urinary hormone levels were expressed per mg of creatinine to correct for collection variations (15). Serum and urinary levels of LH were measured in a double-antibody radioimmunoassay (RIA), as described previously (16). Results were expressed in ng/ml of serum of the human pituitary gonadotropin standard LER-907, or in ng/ml of urine of the first international standard (WHO 70/45) for human urinary gonadotropins. Dilutions of the chimpanzee serum and urine extracts ran parallel to LER-907 in the hLH RIA. Intra- and interassay coefficients of variation for the hLH RIA were 14.1% and 19.8%, respectively, at $B/B_o = 0.51$. The mean recovery (±SD) of first international standard added to duplicate aliquots of early follicular phase urine was 80% ± 10% ($n = 4$). The curve for serum LH measured 2–30 ng and urinary LH measured 1.8–36 ng. Standard samples of 9 ng and 10 ng, respectively, were used to measure variation. During natural cycles in Experiment 2, the urinary LH peak was determined by RIA and the OvuSTICK test (Monoclonal Antibodies, Inc., Mountain View, CA) in Years 1 and 2, and by the OvuSTICK test alone in Year 3. The OvuSTICK test is an enzyme immunoassay dipstick test for the semiquantitative assessment of LH which we have verified in chimpanzees by RIA (13).

Serum levels of the steroid hormones were meas-

Table I. Serum Levels of NET, EE_2, LH, Progesterone, E_2, and Testosterone in Female Chimpanzees during Daily Administration of NET Combined with One of Three Dosages of EE_2[a]

	NET (ng/ml)	EE_2 (pg/ml)	LH (ng/ml)	Progesterone (ng/ml)	E_2 (pg/ml)	Testosterone (ng/ml)
$Flora_{50}$	0.28 ± 0.09	UND	18.5 ± 0.50	0.25 ± 0.01	132.8 ± 8.9	0.328 ± 0.058
$Anna_{50}$	1.05 ± 0.10	UND	40.0 ± 3.7	0.34 ± 0.02	116.3 ± 13.0	0.321 ± 0.068
$Flora_{100}$	0.41 ± 0.06	67.0 ± 11.7	UND	0.82 ± 0.05	56.5 ± 5.8	0.140 ± 0.011
$Anna_{100}$	0.64 ± 0.14	18.8 ± 7.6	40.0 ± 7.7	0.78 ± 0.05	71.1 ± 6.1	0.138 ± 0.007
$Jacq_{400}$	0.40 ± 0.22	117.1 ± 20.2	LS	LS	UND	0.343 ± 0.028
$Anna_{400}$	0.95 ± 0.18	209.4 ± 32.4	LS	LS	UND	0.239 ± 0.010

[a] The daily dosage of NET was 0.5 mg and the three dosages of EE_2 were 50, 100, and 400 μg. For the 400-μg dosage of EE_2, Flora was replaced by Jacq due to illness. Dosages of EE_2 in μg are indicated as subscript after female's name. UND, at or below the lower limits of detectability of the assay; LS, sample lost. Data are mean ± SE.

Table II. Urinary Levels of NETG, EE_2G, LH, E_1G, and PDG, Indexed by Creatinine, in Female Chimpanzees during Daily Administration of NET Combined with Three Different Dosages of EE_2[a]

	NETG (ng/mg Cr)	EE_2G (ng/mg Cr)	LH (ng/mg Cr)	PDG (ng/mg Cr)	E_1G (ng/mg Cr)
$Flora_{50}$	51.3 ± 9.1	4.7 ± 0.4	77.2 ± 9.8	UND	3.4 ± 0.3
$Anna_{50}$	13.1 ± 1.3	8.5 ± 0.7	54.2 ± 3.1	UND	3.1 ± 0.2
$Flora_{100}$	42.9 ± 1.9	65.7 ± 6.2	32.4 ± 2.9	UND	UND
$Anna_{100}$	62.5 ± 5.2	72.8 ± 9.1	69.8 ± 5.4	UND	UND
$Jacq_{400}$	20.5 ± 2.7	145.8 ± 24.1	LS	UND	UND
$Anna_{400}$	36.1 ± 3.1	118.2 ± 10.3	LS	UND	UND

[a] The daily dosage of NET was 0.5 mg and the three dosages of EE_2 were 50, 100, and 400 μg. For the 400-μg dosage of EE_2, Flora was replaced by Jacq due to illness. Dosages of EE_2 in μg are indicated as subscript after female's name. UND, at or below the lower limits of detectability of the assay; LS, sample lost. Data are mean ± SE.

ured by extracting serum samples three times with 1 ml of diethyl ether. The ether extract was evaporated *in vacuo* and the recovery was measured for each sample using ^{3}H-standard, as described previously in our laboratory (17). Aliquots of the ether extract were measured for the steroids by RIA using specific antisera. In each assay, the intra- and interassay coefficients were <6% and <10%, respectively. Our procedures for RIA of 17β estradiol, progesterone, and testosterone were described previously (6, 17, 18). EE_2 and NET were measured using specific antisera obtained from Dr. E. Cook, Research Triangle Park, NC. The sensitivity of the EE_2 assay was 10 pg/tube and the curve measured from 10 pg to 250 pg. Standard samples of 25 pg and 100 pg were used to measure the coefficients of variation. The NET assay was sensitive to 20 pg/tube and the curve measured from 20 pg to 450 pg. Standard samples of 50 pg and 200 pg were used to measure variation. E_1G and PDG were measured by RIA using specific antisera, as described previously in our laboratory (19). The intra- and interassay coefficients of variation were <6% and <10%, respectively, for each assay. EE_2G and NETG were measured as the free compounds after hydrolysis with β-glucuronidase. Usually, 1 ml of urine was diluted with 1 ml of acetate buffer and incubated overnight with 2000 units of β-glucuronidase at 37°C. Radioactive EE_2G or NETG (2000 dpm) was added to determine the efficiency of the hydrolysis and extraction. The incubation mixture was extracted three times with equal volumes of diethyl ether, evaporated *in vacuo*, and resuspended in phosphate-buffered saline (pH 7.0). Aliquots were taken for recovery and RIA. The sensitivity of the assay for EE_2G was 22 pg/tube and the curve measured 22–450 pg. Standard samples of 50 and 200 pg were used to measure variation. For NETG, the sensitivity was 40 pg/tube and the curve measured 40 – 1200 pg. Standard samples of 200 pg and 800 pg were used to measure variation.

Analysis of Anogenital Swelling. The anogenital scores were calculated on an analytical scale of 0 to 12 (13) and were then converted to a more traditional 5-point descriptive scale (0 to 4) to facilitate comparison with earlier data (5 to 7). The conversion from analytical (A) to descriptive (D) scores followed the form: if A = 0 or 1, D = 0; if A = 2, 3, 4, or 5, D = 1; if A = 6 or 7, D = 2; if A = 8, 9, or 10, D = 3; and if A = 11 or 12, D = 4.

Statistical Analyses. The different anogenital swelling scores and urinary levels of EE_2G during the OC cycles at the different dosages of EE_2 were tested for significance using two-way analysis of variance (dosage × cycle day) and orthogonal comparisons (20). Analysis of the anogenital scores was conducted on 30

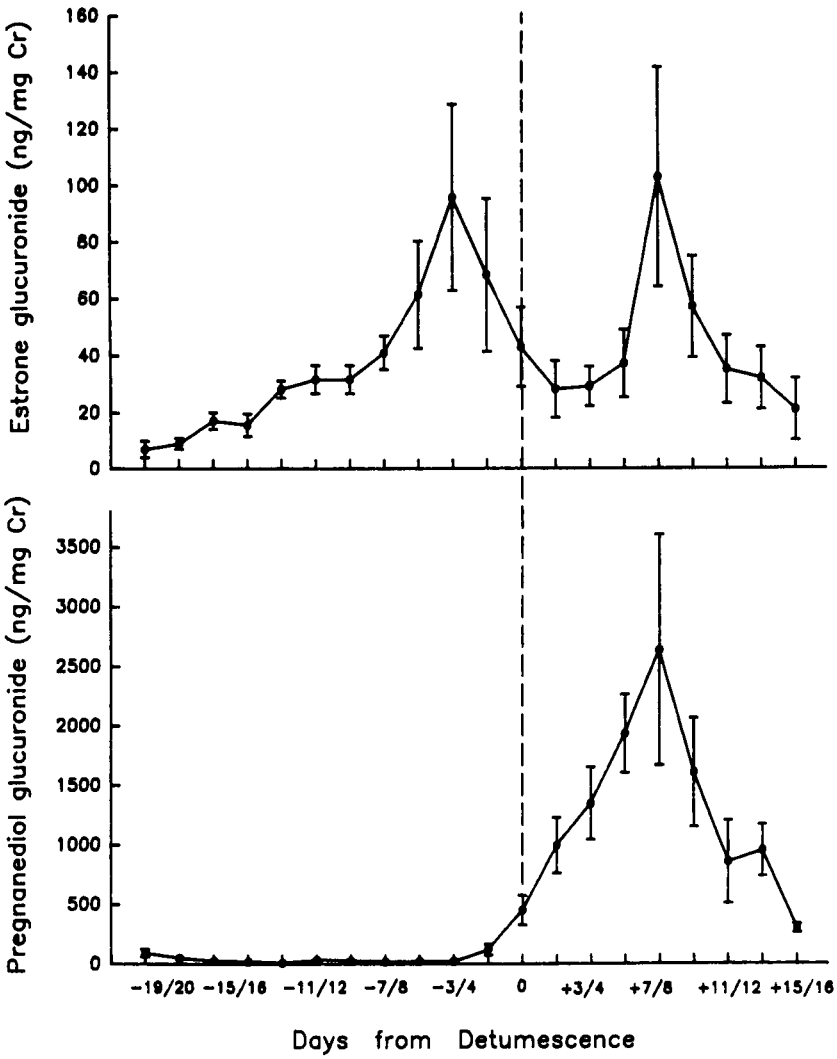

Figure 1. Mean urinary levels (±SE) of estrone glucuronide and pregnanediol glucuronide, indexed by creatinine, during 16 natural menstrual cycles of eight female chimpanzees (two cycles per female); two conception cycles of one female are excluded). The hormonal data, presented as 2-day averages, are normalized to the first day of detumescence from maximal anogenital swelling (Day 0).

daily averages of three females, two cycles per female; a different set of three females was used for each dosage of EE_2. The analysis of EE_2G levels was conducted on 16 2-day averages of three females, two cycles per female; a different set of three females was used for each dosage of EE_2.

Results

Experiment 1. *Serum and urinary hormone levels.* Cyclicity in serum and urinary levels of endogenous hormones was abolished by administration of the OC. Daily administration of EE_2 at dosages of 50, 100, and 400 μg combined with 0.5 mg of NET resulted in serum levels of NET of less than 1 ng/ml and increasing levels of EE_2 from less than 30 pg/ml at 50 μg of EE_2/day to over 100 pg/ml at 400 μg of EE_2/day (Table I). Serum levels of LH, progesterone, and testosterone were maintained within a range found during the normal follicular phase (4, 5). Estradiol (E_2), on the other hand, decreased with the increasing dosages of EE_2, from approximately midfollicular phase levels at a dosage of 50 μg of EE_2/day to nearly undetectable levels (<20 pg/ml) at a dosage of 400 μg of EE_2/day. The result of this interaction between EE_2 and E_2 was that the sum of EE_2 and E_2 levels did not increase commensurately with increasing dosages of EE_2. Urinary levels of NETG varied within a range of 5–85 ng/mg of creatinine (Cr), whereas EE_2G increased with the increasing dosages of EE_2, from an average of less than 10 ng/mg of Cr at 50 μg of EE_2 to over 100 ng/mg of Cr at 400 μg of EE_2 (Table II). Urinary LH levels remained at low, nonperiovulatory values, whereas levels of E_1G and PDG were essentially undetectable at all dosages of EE_2.

Experiment 2. *Intermenstrual intervals and urinary hormone levels.* The mean intermenstrual interval (± SE) during 16 natural menstrual cycles (two cycles for each of eight females) was 36.6 ± 0.9 days (range, 31–46 days; two conception cycles of one female that

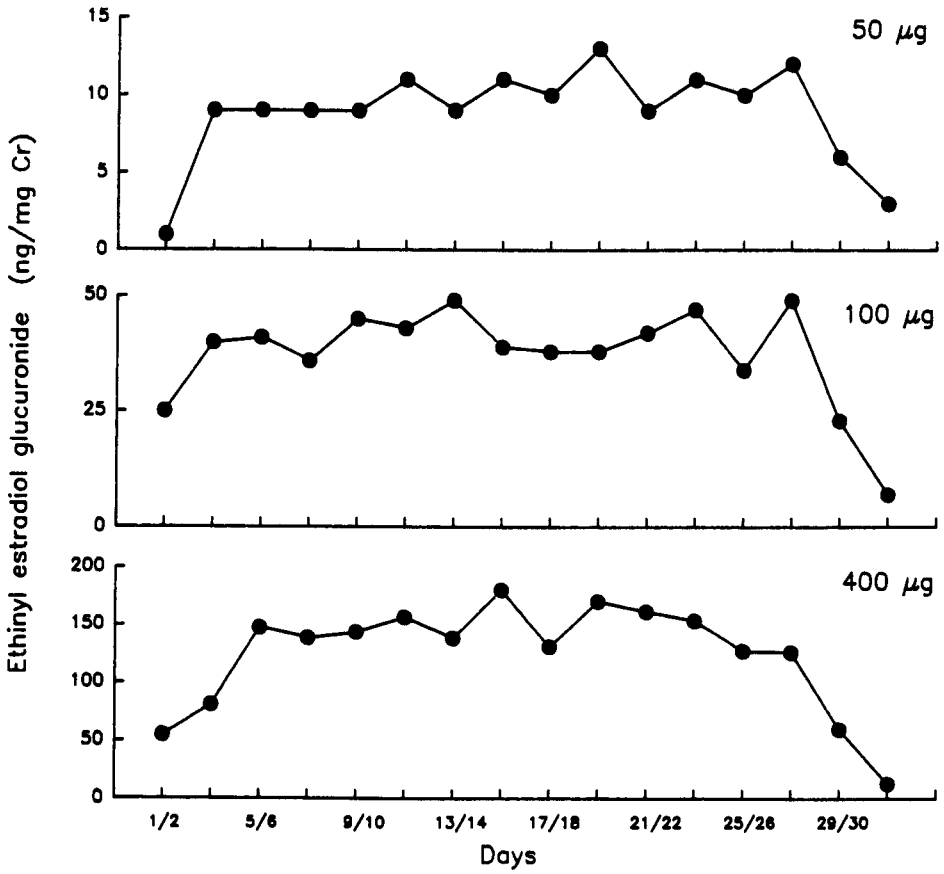

Figure 2. Mean level of EE_2G in the urine of nine female chimpanzees during cycles when an OC was given consisting of norethindrone (0.5 mg) and one of three dosages EE_2 (50, 100, or 400 μg; $n = 3$; a different set of three females per dosage, two cycles per female). The dosage of EE_2 given is indicated in the upper right-hand corner of the panel for the corresponding level of EE_2G. The data, presented as 2-day averages, are normalized to the day after the first day of menses when OC administration was initiated (Day 1). $OC_{50} < OC_{100}$, $P < 0.01$; $OC_{50} + OC_{100} < OC_{400}$, $P < 0.01$.

conceived twice, in the first cycle, despite an intrauterine device, are not included). The mean intermenstrual interval during 18 cycles with daily administration of the OC (two cycles for each of nine females) was 30.3 ± 0.4 days (range, 28–34 days). The shorter intermenstrual interval during the OC cycles resulted from the relatively rapid onset of menstruation that occurred approximately 3 days after the last day of OC treatment on Day 27, regardless of the dosage of EE_2 in the OC.

During natural cycles, urinary levels of E_1G increased during the follicular phase to a peak that occurred approximately 2 days before the first day of detumescence of the anogenital swelling from maximal swelling (approximately 3 days before the loss of labial occlusion (13)), and then decreased before rising to a second peak during the midluteal phase (Fig. 1). The LH peak occurred on the day of the preovulatory peak in E_1G or the day after. The levels of PDG remained low during the follicular phase, began increasing in association with the first day of detumescence, and remained elevated for essentially the entire luteal phase. During OC treatment, urinary hormone levels were relatively constant throughout the cycle, with the level of EE_2G in urine related to the amount of EE_2 in the OC (Fig. 2). There were significant differences in the mean level of EE_2G (±SE) for the different dosages of EE_2 ($P < 0.01$); orthogonal comparisons indicated that the mean level of EE_2G for 50 μg of EE_2 (8.9 ± 0.8 ng/mg of Cr) differed significantly from the mean level for 100 μg of EE_2 (37.3 ± 2.7 ng/mg of Cr) ($P < 0.01$) and the average of the mean levels for the two lower dosages of EE_2 (23.1 ± 1.7 ng/mg of Cr) differed significantly from the mean level for the 400-μg dosage (123.7 ± 11.7 ng/mg of Cr) ($P < 0.01$).

Anogenital swelling. During the natural cycle, female anogenital swelling exhibited the cyclic pattern characteristic of the species, including an extended midcycle period of maximal or near maximal genital scores (Fig. 3). During OC treatment, the cyclic increase in anogenital swelling was abolished. Swelling scores were relatively constant throughout the cycle, with the degree of swelling related to the dosage of EE_2 administered. An analysis of variance indicated that there was a significant overall difference among the mean swelling scores for the three treatment groups ($P < 0.01$). Tests of orthogonal comparisons indicated there was a small,

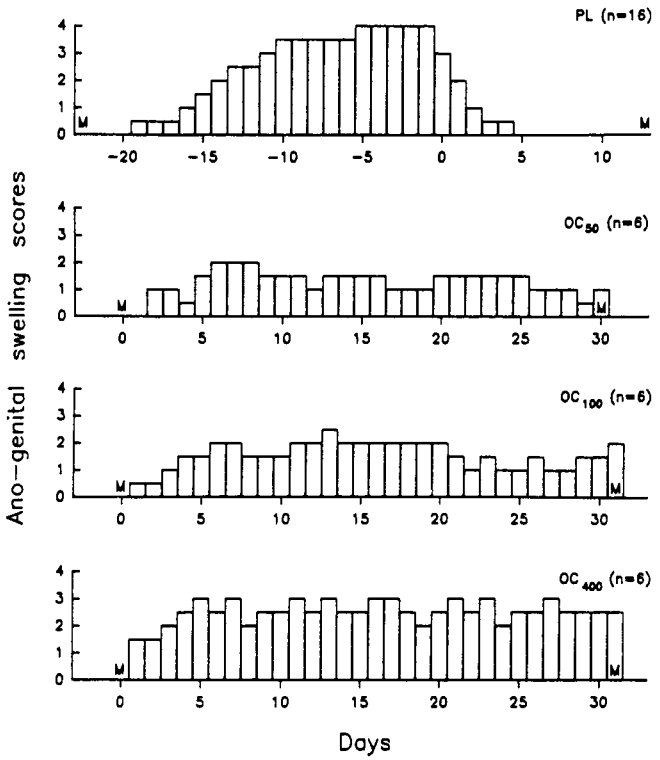

Figure 3. Mean anogenital swelling scores of female chimpanzees during the natural menstrual cycle (PL; $n = 8$ females, two cycles each) and during cycles when an OC was given consisting of norethindrone (0.5 mg) and one of three dosages of EE_2 (50, 100, or 400 μg; $n = 3$; a different set of three females per dosage, two cycles per female). The dosage of EE_2 given is indicated as a subscript in the OC panels. The data for the natural cycles are normalized to the first day of detumescence from maximal anogenital swelling (Day 0); the data for the OC cycles are normalized to the day after the first day of menses, when OC administration was initiated (Day 1). $OC_{50} < OC_{100}$, $P < 0.05$; $OC_{50} + OC_{100} < OC_{400}$, $P < 0.01$. M = menses.

but significant, difference between the mean swelling score (±SE) during administration of 50 μg of EE_2 (1.3 ± 0.1) and 100 μg of EE_2 (1.5 ± 0.1) ($P < 0.05$), and a larger difference between the average of the mean scores for those two dosages (1.4 ± 0.1) and the mean score for 400 μg of EE_2 (2.5 ± 0.1) ($P < 0.01$).

Discussion

The patterns of E_1G and PDG during the natural menstrual cycle and their relationship to female anogenital swelling are comparable to data on circulating hormone levels and anogenital swelling of chimpanzees (4, 5). The current data are also comparable to earlier data on urinary hormone metabolites for which urine was collected over 24-hr periods (6, 7). The results, thereby, further support the reporting of urinary hormone levels indexed to creatinine levels. The advantage of this approach is that it may be applied to relatively short periods of urine collection (13, 21).

The suppression of cyclicity in serum and urinary levels of sex hormones by the oral contraceptives is consistent with results on the effects of oral contraceptives in females of other species, including women (22–24). In agreement with such studies, NET combined with the lowest dosage of EE_2 (50 μg) resulted in endogenous hormone levels that approximated those of the midfollicular phase in the natural cycle, albeit with some differences in levels among individuals. The major effects of increasing the dosage of EE_2 were increasing levels of serum EE_2 and urinary EE_2G and decreasing levels of serum E_2. The onset of menses approximately 3 days after cessation of OC administration appears to be more rapid than that observed in women (14).

The inhibition of the cyclic increase in female anogenital swelling by NET combined with the 50-μg dosage of EE_2 and the increased degree of swelling with the two higher dosages of EE_2 (100 and 400 μg) are readily interpretable as resulting from an initial suppression of endogenous E_2 and subsequent elevation in serum levels of EE_2. This interpretation is consistent with the results of an earlier study that demonstrated that exogenous estrogen stimulated female anogenital swelling in amenorrheic and ovariectomized chimpanzees (6). We cannot rule out, however, a confounding of the effect of the dosage of EE_2 with the year of administration. The apparent effect of the OC on anogenital tissues of intact females, associated with the different dosages of EE_2, has important implications for interpreting the influence of oral contraceptives in our study on the sexual behavior of chimpanzees (10). Because the anogenital swelling of the female chimpanzee is a conspicuous cue to the male regarding the female's sexual responsiveness (3, 8), alteration of the swelling by the OC likely accounts for our finding of reduced attractiveness of the females to the males during OC administration.

The effect of oral contraceptives on genital tissues of female chimpanzees may also have implications for women taking oral contraceptives, given the homologies that exist between the genital tissues of chimpanzees and women. The genital tissues of the female chimpanzee that undergo swelling, in particular, are homologous to the labia minora of women (11, 12). The effect of oral contraceptives on the genital tissues of the chimpanzee is clearly more apparent than the comparable effect in women, given the greater responsiveness of the chimpanzee's genitals to hormonal fluctuations, generally. It is well recognized, however, that the loss of ovarian steroids in postmenopausal and ovariectomized women results in reduced lubrication of the labia, vaginal atrophy, and variable degrees of discomfort during sexual intercourse (25–27). Although the reduction in sex steroid levels produced by oral contraceptives is less extreme than that found in these other conditions, similar symptoms are reported by some women taking oral contraceptives, e.g., atrophic vaginitis and dyspareunia (28). The current emphasis

on low-estrogen oral contraceptives, moreover, could exacerbate the genital problems associated with OC use in women because there is less EE_2 in these oral contraceptives than the lowest dosage (50 μg) we used. The effect in the chimpanzee serves to focus attention on what appears to be a homologous response to oral contraceptives in women, and on the need to consider the possible relevance of such genital changes in women to their sexuality and overall well-being.

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