

HORMONAL STEROIDS AND SEXUAL COMMUNICATION IN PRIMATES

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SUMMARY

Under laboratory conditions, pairs of rhesus monkeys show at least four different patterns of sexual behavior in relation to the stage of the menstrual cycle. The most interesting of these is characterized by a distinct mid-cycle peak followed by a secondary pre-menstrual rise. High doses of progesterone administered to ovariectomized females receiving estrogen replacement treatment resulted in a marked reduction of the sexual behavior of the pair either because the female refused the male or because the male made fewer mounting attempts. Males were shown by operant conditioning and nose-blocking procedures to detect the hormonal status of their female partners by olfactory mechanisms. Shortchain fatty acids in the vaginal secretions of females were found to be responsible for this effect. The same acids have been shown to be normal constituents of human vaginal secretions, and levels were highest in the late follicular phase but declined progressively during the luteal phase of the menstrual cycle. In women taking oral contraceptives, levels of volatile acids in the secretions were lower than normal and rhythmic production during the menstrual cycle was abolished.

INTRODUCTION

It is only recently that sustained efforts have been made to understand the neuroendocrine factors that influence and, in some cases, determine the behavioral patterns of primates. These new developments are possible because techniques are now available that permit reliable quantification of the behavior of certain primate species under laboratory conditions, and because of recent advances in the radioimmunoassay of hormones in plasma. It is worthwhile, at the outset, to emphasize that such studies are, by their nature, interdisciplinary ones, the results of the team-work of a group for which the present writer is merely a spokesman.

There are certain differences between the organization of experiments on behavior in primates and those in lower mammals such as the rat. First, in contrast to highly in-bred strains of laboratory rodents, very great individual differences occur in the sexual behavior of, for instance, different pairs of rhesus monkeys. Our animals were all born and reared in the jungle, we have no knowledge of their past life histories, and indeed, it would even be quite difficult accurately to determine their ages. A given male or female will show quite striking preferences for particular partners, and these individual "likes" and "dislikes" are far less obvious in the behavior of lower mammals. This implies that the pair must be the unit for study, and that it should serve as its own control in detailed, longitudinal investigations. These individual differences also make it difficult to pool results from different pairs, particularly when the number of animals in an experiment is necessarily quite small: not even the large primate laboratories in the U.S.A. can afford to use 40-50 animals in a study in the way it is practical to do with rats. Nevertheless, reliably repeatable results, with acceptable levels of statis-

tical significance, can be obtained if due consideration is given to the experimental design. The extent to which such data can be generalized to the species as a whole must remain problematical until numbers are sufficient for results to be placed on a population basis. There are certain additional problems. Primate sexual behavior is less precisely dependent on hormonal factors than similar behavior in lower mammals. Rhesus monkeys may continue to permit copulation for quite long periods after bilateral ovariectomy. Furthermore, the great development of neocortical mechanisms in these highly evolved forms is reflected in the fact that immediate past experience and memory play a more conspicuous part in ongoing behavior—which is much less stereotyped and repetitive than in lower mammals; experiments must be designed with this in mind. It is unlikely that the investigation of the neuroendocrine mechanisms involved in primate behavior will be successful if its greater complexity is ignored.

THE INFLUENCE OF THE MENSTRUAL CYCLE ON BEHAVIOR

Catarrhine monkeys and apes, alone among infra-human mammals, have a true, external menstruation and, in the case of the rhesus monkey, the cycle is approximately 28 days. Copulation in the rhesus monkey is very suitable for quantitative analysis since it consists of a series of separate mounts, each normally (but not necessarily) associated with an intromission and a variable number of thrusts. The mounting series is terminated by a final mount in which ejaculation occurs. There is then usually a period of mutual grooming and a second, and possibly a third, series of mounts may occur during a standard 1-h test session. There is evidence from both

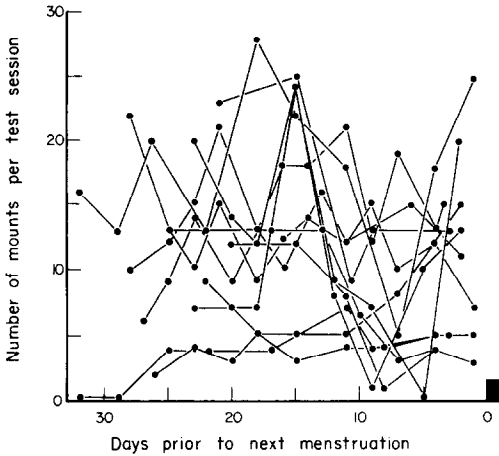


Fig. 1. When attempts are made to relate the mounting activity of males to the stage of their partners' menstrual cycle, individual variations are so great that the picture is a confusing one. Test sessions = 1 h. Solid black bar indicates menstruation.

field and laboratory studies that the females of several species of anthropoid primates have periods of heightened sexual receptivity, usually near mid-cycle, at which time they are more ready to permit males to copulate. However, when detailed studies on individual pairs of rhesus monkeys are conducted under well-controlled laboratory conditions, this observation is difficult to identify and document. Figure 1 shows the apparent disorder one encounters when an attempt is made to relate the number of mounts made by males during 1-h tests to the stage of the menstrual cycles of their female partners. However, when these data are separated so that the behavior of individual

pairs can be examined, the changes occurring in the number of mounts per test on successive days of the female's menstrual cycle fall clearly into four main patterns (Fig. 2): A, those with well-defined maxima near mid-cycle, sharp declines early in the luteal phase and a secondary rise immediately before menstruation; B, those with sustained high levels of activity throughout the follicular phase, again sharp declines early in the luteal phase, and with low levels persisting until the next menstruation; C, cycles without evidence of rhythmic changes but with fairly high levels of mounting throughout the cycle; D, again cycles without any rhythmic changes and with generally low levels of interaction throughout. Approximately 50% of the pairs and cycles studied showed clear evidence of rhythmicity (Types A and B). Type C seemed to occur with certain old and dominant males that generally showed high levels of sexual activity with all partners, irrespective of the latter's hormonal status, and Type D was characteristic of pairs that were sexually disinterested in each other, where levels of interaction were very low. Pairs belonging to Types A and B were most interesting because of an indication that the male of the pair was responding to the hormonal state of the female partner. These data alone demonstrate very convincingly the individual variations these primates show and stand in marked contrast to results in lower mammals where receptivity is confined to short periods of heat, often of only a few hours duration, when the female is receptive to the male.

Two questions immediately present themselves (i) what is the characteristic species-typical behavior and to what extent is the behavior observed in the labor-

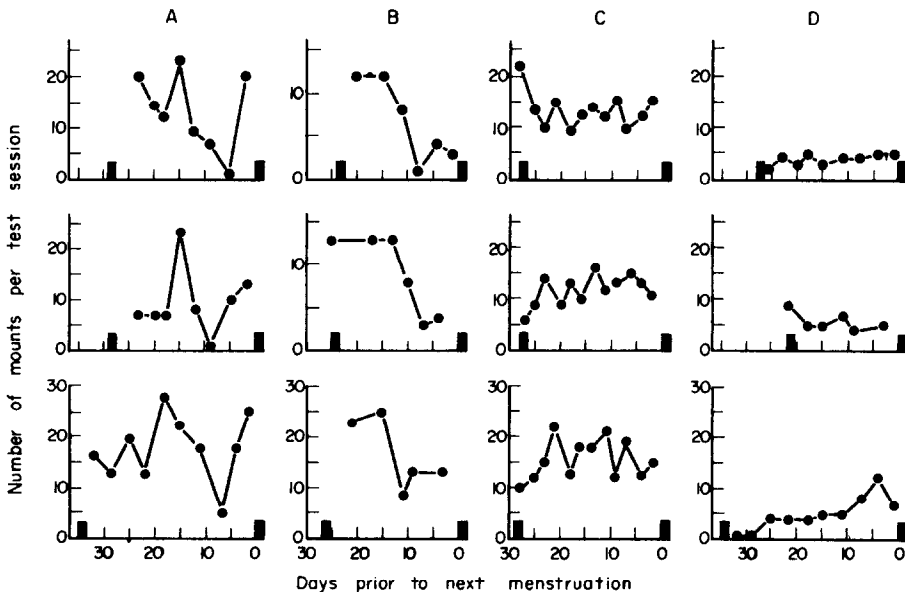


Fig. 2. Same data as in Fig. 1, plotted on an individual pair basis. The twelve pairs show four main types of changes in mounting activity in relation to the menstrual cycles of partners: (A) maxima near mid-cycle and secondary rises before menstruation; (B) high levels of mounting during follicular phases and low levels during luteal phases; (C) high levels of mounting throughout the cycles; (D) low levels of interaction throughout the cycles. Types A and B show rhythmicity. Solid rectangles indicate menstruation.

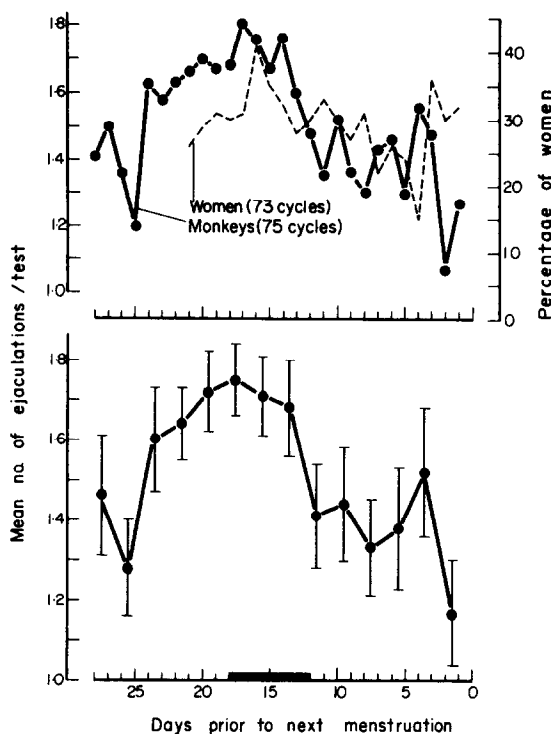


Fig. 3. Upper part: comparison of the copulatory activity of rhesus monkeys and man in relation to the menstrual cycle. ●—●, mean number of ejaculations per test (32 pairs of rhesus monkeys); ----, percentage of women reporting sexual intercourse (40 women).

Lower part: rhesus monkey data smoothed by plotting means of 2 consecutive days. Vertical bars = standard errors of means. Horizontal bar gives expected time of ovulation [23]. (From Michael and Zumpe, 1970a) [3].

atory representative of that occurring in the wild, and (ii) the more complex question of how the changing state of the female during different phases of her menstrual cycle actually results in changes in the behavior of her male partner.

(i) *The species-typical pattern*

Striking individual variations and partner preferences are encountered: thus, a given male may show consistently more mounting activity and a greater number of ejaculations with one female partner than with another and, conversely, the level of sexual activity of two males may differ consistently when they are paired with the same female on successive days throughout a menstrual cycle. Both the male [1] and the female [2] contribute to the variation, and it is hardly surprising that results from different laboratories are not always entirely consistent with each other. Nevertheless, it is possible to make some generalizations about the rhythmic changes in sexual activity in a population of rhesus monkeys when one considers changes in the frequency of ejaculation occurring in a large group of animals. Figure 3 shows data from 75 menstrual cycles observed in 32 pairs of animals during a six year period [3]. These data are shown in the upper part of the figure where a comparison is made with some human data [4]; each

point is a mean of between 24 and 30 pairs. There is a maximum incidence of ejaculation 17 days before the first day of the next menstruation with a progressive decline throughout the luteal phase, a low point being reached two days before menstruation. The lower part of Fig. 3 shows the same data, but smoothed by plotting means of two consecutive days. The mean number of ejaculations from the first to the fifth reverse cycle days was significantly lower than from the 13th to the 24th reverse cycle days (t test, $P < 0.001$). Since these 75 cycles showed wide variations in length and included many without any obvious rhythmicity, it can be concluded that higher levels of sexual activity near mid-cycle are a characteristic feature of the sexual interactions of rhesus monkeys. The marked similarity between the time-course of changes in the behavior of rhesus monkeys and in the incidence of sexual intercourse in the human population (percentage of women mating on each day of the cycle), points to the possibility that similar basic underlying neuroendocrine mechanisms may be responsible for the behavioral changes in the two species. Observations in other laboratories and those from field studies [5] (reviewed by Michael and Zumpe, 1971) confirm the phasic nature of mating activity in rhesus monkeys. Although it is difficult to determine the exact stage of the cycle under field conditions, the consensus of opinion is that heightened activity during the middle part of the menstrual cycle is the rule.

(ii) *Sexual communication between the male and female*

Communication systems have developed in the course of evolution by which rhesus monkeys convey information about their behavioral state to their partners. These take the form of certain gestures and behavioral patterns, called Displays, and a good deal of work has been done by ethologists, both in identifying the behavioral patterns themselves, and in attempting to clarify their information content [6–11]. If the frequency with which a particular gesture is made increases, does this tell us something about the animal's motivational state? For instance, if a female draws away from a male as he attempts to mount her (a refusal reaction), does this mean her motivation for sexual interaction is low? If the female stops making refusal reactions when given estradiol, what, if anything, can we infer from this? Unfortunately, the most obvious and direct conclusion is not always the correct one, and this is due to the complex nature of primate behavior. Because of this, it has been found from experience that it is necessary to use several different measures of behavior simultaneously [12–14], and then see how they vary in response, for example, to the administration of hormones. It would not be appropriate to enter into details of all the behavioral indices employed here. But we have already seen that male rhesus monkeys make fewer mounts upon the female, and ejaculate less frequently, when the partner is in the luteal phase

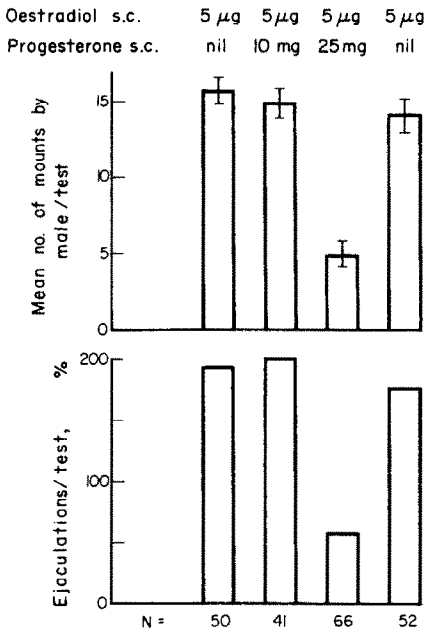


Fig. 4. The suppression of mounting activity and of ejaculation in male rhesus monkeys by the administration of progesterone to their ovariectomized, estrogen-treated female partners. N = number of tests (six pairs). (When values exceed 100% more than one ejaculation occurred per test.) Vertical bars = standard errors of means. (From Michael, Saayman and Zumpe, 1968)[15].

of the menstrual cycle, and this can be shown to be due to the behavioral effects of progesterone.

When ovariectomized females receiving estradiol were given, in addition, large doses of progesterone, both the number of mounts and of ejaculations made by their male partners were depressed [15] (Fig. 4). The effect on the behavior of the male of treating the female with progesterone is extremely interesting because two mechanisms appear to be responsible for its action in depressing sexual activity [15]. Figure 5 shows that animal pairs fall into two groups (A and B) which can be distinguished by the effect of progesterone on the number of mounting attempts made by the males (lower set of histograms). In group A, mounting attempts remained relatively constant across treatments whereas in group B, mounting attempts declined conspicuously when 25 mg of progesterone per day was administered to the females (209 tests). In group A pairs, the decline in the number of mounts made by males (upper set of histograms) with females on high doses of progesterone was almost entirely due to the marked increase in female refusals (middle set of histograms): thus, in these pairs, treating the female with progesterone resulted in a decline in her receptivity. However, the males' sexual interest in these females, as expressed by the number of male mounting attempts, remained unchanged. In group B pairs, the situation was quite different. Here, the high doses of progesterone resulted in a very marked decline in the number of male mounting attempts, but only in a small, non-significant increase in the number of refusals made

by females. This lack of interest of males in progesterone-treated females was shown by an almost complete cessation of male mounting activity. Thus, we have a situation in which treating females with progesterone depresses male sexual activity in all cases (decreases mounts, decreases ejaculations), but two quite different mechanisms can underlie the effect: which mechanism operates depends upon the identity of the pairs under consideration. We can readily understand how "refusal behavior" conveys information to the male, but a totally different mechanism must be responsible for the loss of male interest in females that are not at the time making refusals. Clearly, another communication system is involved, and it is with this that I will deal now.

PRIMATE PHEROMONES AND FEMALE SEXUAL ATTRACTIVENESS

Olfactory mechanisms are known to be important in the control of reproductive processes in lower mammals, particularly in laboratory mice, and substances with the properties of pheromones are involved [16]. Among primates, the prosimians provide many examples of communication between the sexes by olfaction, and many possess specialized apocrine scent glands used for territorial marking, for self-marking and marking each other. Use was made of an operant conditioning situation to assess the in-

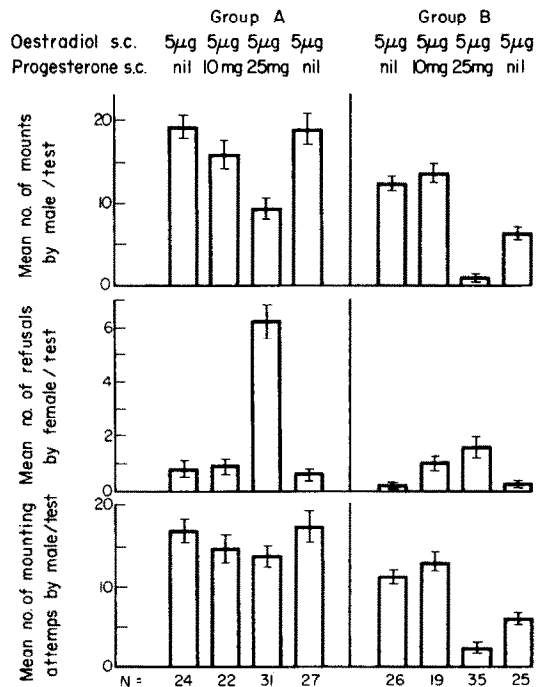


Fig. 5. Two mechanisms underlying the suppression of male mounting activity by progesterone in rhesus monkeys: group A, three pairs with a marked increase in female refusals (refusal reaction); group B, three pairs with a marked decrease in the number of male mounting attempts (loss of female attractiveness). In both cases, a decline in the number of mounts results (upper part). N = number of tests. Vertical bars = standard errors of means. (From Michael, Saayman and Zumpe, 1968)[15].

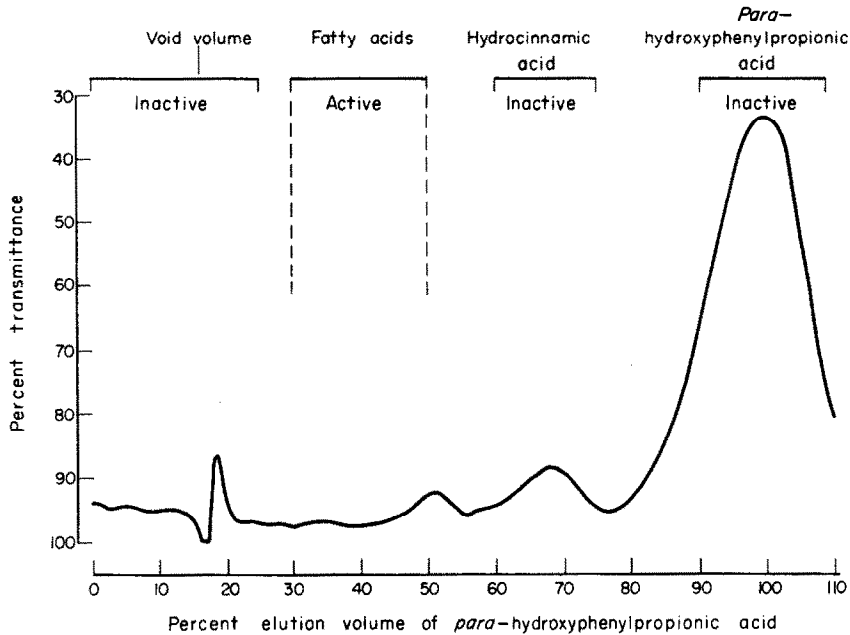


Fig. 6. Elution profile at 280 nm on DEAE-cellulose of alkaline re-extracts of vaginal secretions from ovariectomized, estrogen-treated rhesus monkeys. The fractions indicated above were tested for behavioral activity and only that containing fatty acids was found to be active. This fraction was subjected to further analysis by gas chromatography and mass spectrometry. (From Curtis *et al.*, 1971)[22].

fluence of olfactory mechanisms on the behavior of male rhesus monkeys. Males were trained to press a lever 250 times in order to operate a lifting partition between two compartments of a cage and thereby gain access to a female. Males would not work the lever consistently to obtain access to an ovariectomized, untreated female, but high rates of lever-pressing were regularly obtained when the female was treated with estradiol. If the male was made anosmic by inserting plugs into the nasal olfactory area, he was unable to detect any changes in the endocrine status of his female partner, and it appeared from these experiments that the male was responding to an olfactory cue emitted by the female when under the influence of estrogen [17-19].

The sites of production of sex-attractant pheromones have been studied by collecting vaginal secretions from ovariectomized, estrogen-treated rhesus monkeys and transferring these secretions to long-ovariectomized, untreated females: this bioassay system has been used to help determine the chemical nature of the substances in vaginal secretions with sex attractant properties. It was found that ether extracts of vaginal secretions contained effective sex-attractant pheromones [20]. We demonstrated the acidic nature of the behaviorally active constituents by applying to the sexual skin of ovariectomized females a solution prepared by treating the ether extract with 2 ml 0.01 N sodium hydroxide, acidifying the alkaline layer, and re-extracting into ether: this extract retained behavioral activity. Ether-soluble neutral and basic components of vaginal secretions, notably cholesterol, were removed by this procedure. The behaviorally active constituents were further

examined by ion-exchange chromatography. A pool of 40 vaginal secretion extracts was washed and eluted through a column of DEAE-cellulose with 50 mM (pH 7.4) sodium phosphate buffer. The elution profile is shown in Fig. 6. The elution volume of the fraction corresponding to that of short-chain fatty acids contained the behaviorally active material and this was further examined by analytical gas chromatography. After preliminary screening with different gas-liquid chromatographic media, Carbowax 20M-terephthalic acid columns and temperature gradients between 50 and 200°C were used to study further the acidic, behaviorally active components [21, 22]. Chromatograms of extracts of individual secretions collected from 5 ovariectomized, untreated females showed, in each case, that the amounts of volatile components were low (Fig. 7a). Chromatograms of extracts of individual secretions collected from 5 ovariectomized females during treatment with estradiol showed that the amounts of volatile components (peaks 1-6) were at least 8 times greater (Fig. 7b). To determine whether the increased production of these constituents by females during treatment with estradiol was responsible for the changes in the behavioral properties in the secretions, we used an effluent splitting device to trap peaks 1-5 from the gas chromatograph into ice-cold ether. The material from this trapping procedure was obtained from a pool of 40 vaginal washings collected from estrogen-treated females. The trapped fractions, when applied daily to the sexual skin of ovariectomized females, was shown powerfully to stimulate the mounting attempts of male rhesus monkeys that were tested with them (Fig. 8). To ascertain whether the five ali-

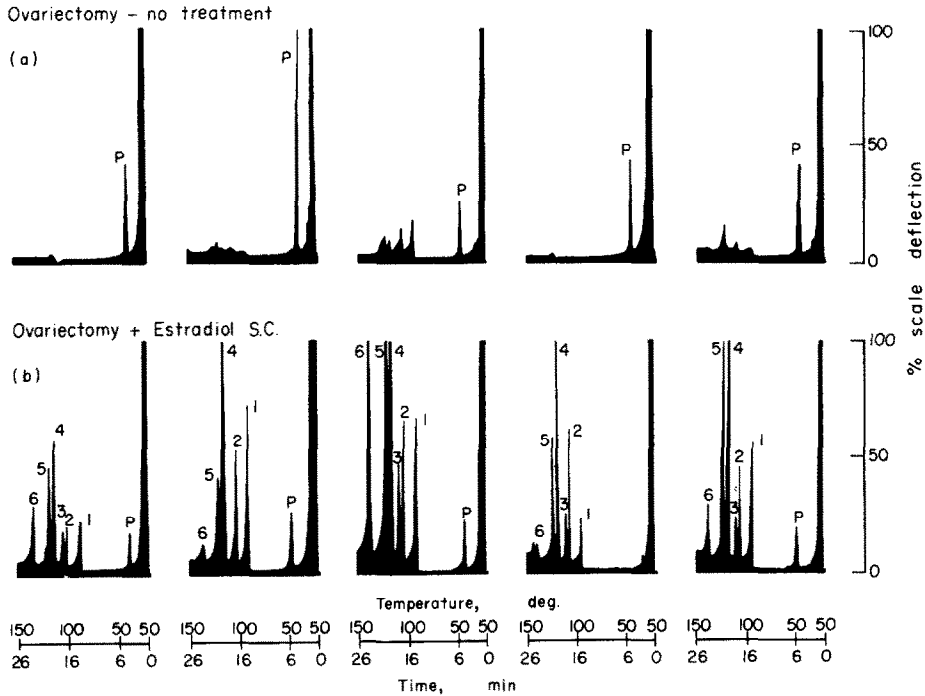


Fig. 7. Gas chromatograms of extracts of rhesus monkey vaginal secretions. (a) Chromatograms of secretions collected from five ovariectomized, untreated females. The volatile acid content of the secretions was low ($0\text{--}3.3\ \mu\text{g}$ per collection), and the four, from recipient females, that were tested (four at left) were without any behavioral activity (Fig. 8, pretreatment). (b) Chromatograms of secretions collected from five ovariectomized females during treatment with estradiol. The volatile acids in the secretions showed an 8-fold increase ($26.0\text{--}53.0\ \mu\text{g}$ per collection), and the four from donor females that were tested (four at left) showed marked behavioral activity (Fig. 8, treatment). The right-hand chromatograms (a and b) are from the same animal before and during treatment with estradiol. Peak P, after the solvent front, is authentic *n*-pentanol added to the extracting ether as a marker. (From Michael, Keverne and Bonsall, 1971)[21].

phatic acids in the behaviorally active fraction were indeed responsible for its sex-attractant properties, a mixture of authentic acids was made up to match the concentrations present in a pool of 24 washings collected from ovariectomized, estrogen-treated female rhesus monkeys. This synthetic mixture contained $9.2\ \mu\text{g}$ of acetic, $8.8\ \mu\text{g}$ of propionic, $4.2\ \mu\text{g}$ of isobutyric, $12.8\ \mu\text{g}$ of *n*-butyric, and $8.3\ \mu\text{g}$ of isovaleric acids per milliliter of ether. When a sample of this mixture was applied to the sexual skin of an ovariectomized female there was a marked stimulatory effect on the mounting activity of the male partner; this is illustrated in Fig. 9. It appeared from these data that the powerful sex-attractant properties of estrogen-stimulated vaginal secretions depended on their content of volatile fatty acids (copulins).

To ascertain if the production of aliphatic acids in vaginal secretions was an isolated phenomenon in the rhesus monkey, we turned our attention to other primate species and conducted similar gas chromatographic studies. Figure 10 shows that the same aliphatic acids were found in much the same proportions in the vaginal secretions of both New and Old World monkeys, in other species of macaques and also in the anubis baboon: it will be observed that the profiles or patterns of acid production seem to differ for different species.

Although these aliphatic acids are readily recovered from the vagina by lavage, it is not known if their source is primarily the cervical or vaginal glands, the glands about the introitus or a transudate through the vaginal wall. There is, however, clear evidence that microbial action is responsible for their production. Amounts of volatile acids in vaginal secretions increased when they were incubated at 37°C *in vitro*, and autoclaving or the addition of penicillin to the media inhibited their production. It seems probable, therefore, that the production of these acidic pheromones depends upon the bacterial flora of the vagina, and that one means by which ovarian steroids may regulate acid production *in vivo* is by determining the availability of substrate; a rather novel symbiotic relationship.

VOLATILE FATTY ACIDS IN THE VAGINAL SECRETIONS OF WOMEN

These studies embody the results of unpublished data obtained in collaboration with Dr. R. W. Bonsall and Mrs. P. Warner. The demonstration that a series of short-chain aliphatic acids are responsible for the sex-attractant activity of vaginal secretions of rhesus monkeys, and their presence in other primate species, has stimulated a search for copulins in the secretions of women. A preliminary study on the cooperative

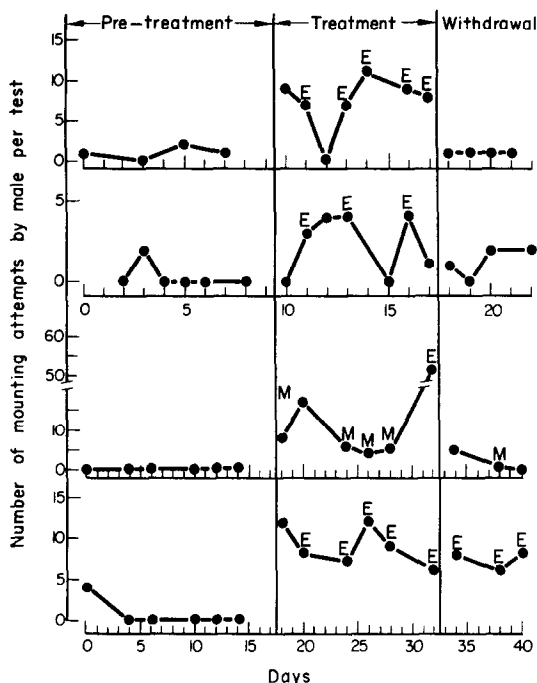


Fig. 8. Sexual stimulation of male rhesus monkeys by components of vaginal secretions fractionated by gas chromatography. In tests with four males each paired with a different ovariectomized (recipient) female, the application to the latter's sexual skin of material collected by trapping from the gas chromatograph resulted in a marked stimulation of the sexual behavior of their male partners. E = ejaculation; M = masturbation to ejaculation. Time scale of lower two pairs which were tested on alternate days is half that of upper two pairs which were tested daily. (From Michael, Keverne and Bonsall, 1971)[21].

wives of colleagues demonstrated their occurrence in the human female also [19]. In an effort to obtain quantitative data on a larger human sample we collected secretions from 28 women attending an infertility clinic at the Samaritan Hospital for Women, London: only two of the twenty eight produced acids in similar concentrations to those found in the preliminary study. Women presenting themselves at a gynecological clinic for examination often take extreme steps to prepare themselves by washing and douching and, because these negative results from a clinic population contrasted with our preliminary findings, we felt it necessary to investigate a population of healthy women with normal menstrual cycles.

Subjects and methods

The way in which subjects were recruited was important. University women were simply informed in a leaflet that a study on the physiology of women was being undertaken that involved measures on the biochemical composition of body fluids, and that this would require participants to wear vaginal tampons. Those women indicating their willingness to cooperate were then contacted individually by a mature female psychologist who helped them complete a questionnaire designed to exclude those with gynecological problems. After participants signed a Consent

Form protecting their Human Rights and Welfare, they were (1) offered a gynecological examination, (2) were provided with a box containing tampons and bottles, and (3) given detailed instructions on their use (see below). Identities were protected by code numbers. A month after their distribution, boxes were collected under conditions preserving confidentiality, and a second questionnaire was completed. Efforts were made to recheck menstruation dates, but attention was also given to the use of oral or vaginal contraceptives and feminine hygiene products. Subjects were not aware of the specific purpose of the study or of the type of assays to be conducted. There was no direct contact between laboratory and subjects, the only link being the psychologist who recruited women and distributed and collected the boxes. The biochemists conducting assays were aware only of code numbers, so the procedure was double-blind. Only when assay of all 682 samples had been completed, were data grouped in relation to the stage of the menstrual cycle and subjects separated into oral contraceptive users and non-users.

Self-collection of vaginal secretions by aspiration with a pipette containing distilled water proved quite distasteful to the majority of women. Accordingly, the following acceptable method was developed by which women could conveniently and reliably take a sample of their own vaginal secretions. A commercial tampon was reduced to about 1 cm. in length, washed with hot methanol in a Soxhlet extractor for 2 h, dried at 110°C, and hermetically sealed in a polyethylene bag. This procedure removed waxes and other extractable matter that would later interfere with gas

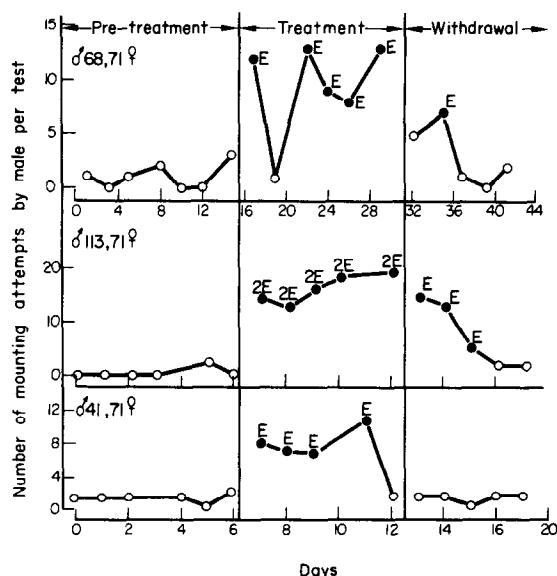


Fig. 9. The sexual stimulation of three male rhesus monkeys by the application of a synthetic mixture of authentic fatty acids to the sexual skin of an ovariectomized, recipient female: a significant increase in mounting activity and in ejaculations occurred. ●E = one ejaculation in the test; ●2E = two ejaculations in the test; ○ = test without ejaculation. (From Michael, 1972)[19].

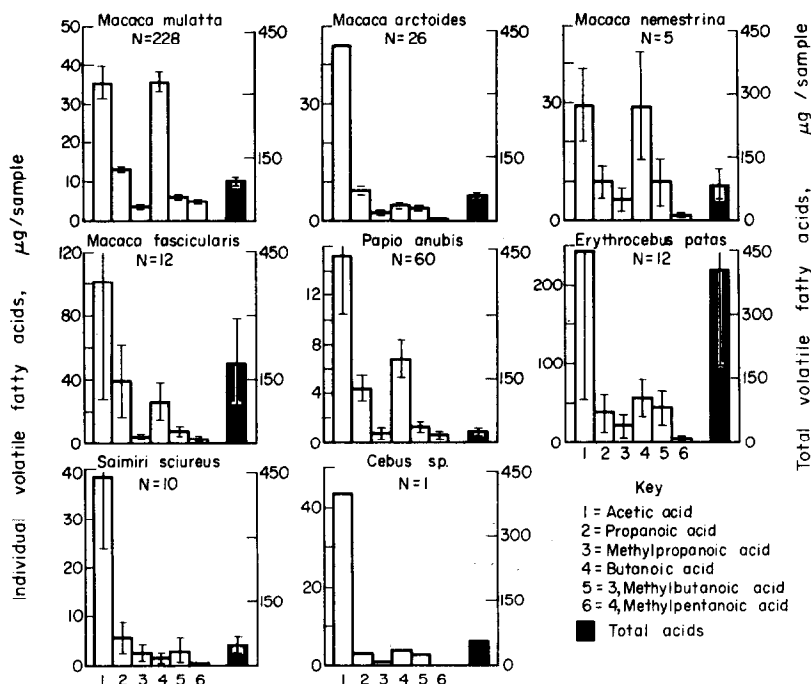


Fig. 10. Content of volatile fatty acids in vaginal secretions obtained from six species of Old World primates and two species of New World primates. For each species, the total acid content is drawn to the same scale to facilitate comparisons between species. Vertical bars give standard errors. N = number of samples.

chromatography. Each subject was provided with a convenient box containing 16 tampons (1 a control blank) and 15 numbered, snap-cap bottles each containing 20 ml methanol. Subjects were requested to wear each tampon in the usual way for 6–8 h, and on removal, to drop it immediately in the bottle provided. Tampons could be worn by day or by night, whichever was most convenient, and a day or night was then missed before inserting the next one. By using very small tampons for only 6–8 h out of each forty-eight hours, any disturbance to the normal bacterial flora was minimized. Immediate immersion in methanol after removal both prevented bacterial action outside the body and initiated the first stage of the extraction process. Tampons were assayed by packing them into columns and washing with methanol in chromatographic fashion. Eluates were combined with the methanol from the sample bottles, mixed with 100 μ l N/10 sodium hydroxide and evaporated to dryness. Residues were taken up in 1 ml water, washed with 4 ml ether (to remove basic and neutral components), and the aqueous layers were acidified and extracted with 4 ml ether containing *n*-pentanol as a concentration marker. Extracts were concentrated to 50 μ l and analysed on 10% FFAP columns in a Perkin Elmer gas chromatograph programmed from 50–220°C. The area of the pentanol peak and calibration constants (determined with authentic compounds) were used to calculate amounts of acids present. Recoveries varied from 45% (acetic acid) to 67% (methylpentanoic acid). Overall coefficients of variation ranged from 6% (butanoic acid)

to 18% (acetic acid). Control tampons contained low levels of acetic acid only ($12.8 \pm 1.7 \mu$ g, N = 31); this blank was not subtracted.

RESULTS

Three of the 50 women were excluded because of irregular bleeding or unreliable menstruation data. The remaining 47 subjects (mean age 20.4 ± 2.3 years, \pm S.D.) provided 635 samples during 86 menstrual cycles (mean cycle length, 29.2 ± 3.8 days, \pm S.D.). Figure 11 shows the total volatile aliphatic acid content of the vaginal secretions of women arranged according to successive 3-day periods of the menstrual cycle (Day 1 being the 1st day of menstruation). Secretions showed high levels of volatile acids in the late follicular phase, and a progressive decline during the luteal phase of the menstrual cycle. The significance of the increase in acid content near mid-cycle was assessed by a two-way Analysis of Variance for non-orthogonal data, preliminary tests having shown a lack of subject-time interaction. Data for the first 7 days were excluded because samples were not collected reliably during the menstrual flow, and the remaining days of each cycle were divided into 5 equal periods. A logarithmic transformation was used to eliminate skew. The analysis showed a significant variance between these periods ($P < 0.02$), and the late follicular period was significantly higher than the mid-luteal period; variance between subjects was highly significant ($P < 0.001$).

It transpired that of the 47 subjects, 32 were not

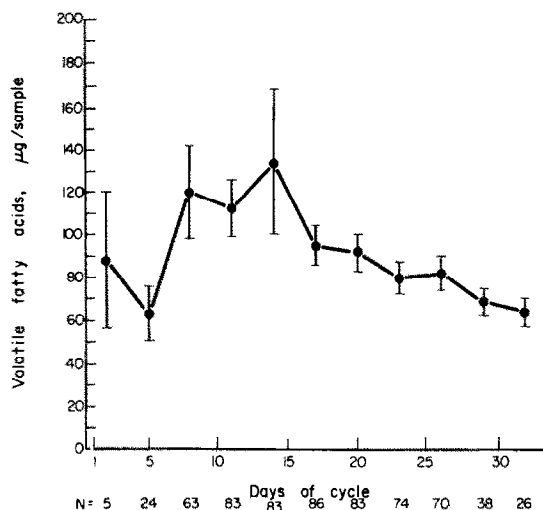


Fig. 11. Volatile fatty acids (mean \pm S.E.'s) in the vaginal secretions of 47 women (86 menstrual cycles) during successive 3-day periods of the menstrual cycle. The increase before mid-cycle and the decrease in the luteal phase was statistically significant ($P < 0.02$). N = number of samples. (Unpublished results—Michael, Bonsall and Warner).

taking oral contraceptives (61 cycles, 449 samples) and 15 subjects were using oral contraceptives (25 cycles, 186 samples). The mean acid content for the samples from oral contraceptive users was 76.3 ± 4.3 (\pm S.E.) μg per sample compared with 105.6 ± 7.8 μg per sample for subjects not using oral contraceptives. The difference between these means was highly significant ($P < 0.001$), using a logarithmic transformation to eliminate skew and Student's t test (one-tailed).

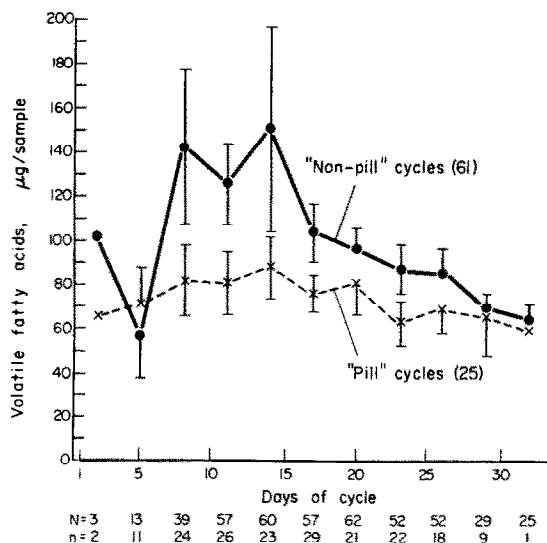


Fig. 12. Volatile fatty acids (mean \pm S.E.'s) in the vaginal secretions of 32 women (61 cycles, 449 samples) with normal menstrual cycles and 15 women (25 cycles, 186 samples) using oral contraceptives. Data plotted as in Fig. 11. The women divide into two sub-groups: those using oral contraceptives show no significant increases during the menstrual cycle. N = number of samples from normal cycles. n = number of samples from contraceptive pill users. (Unpublished results—Michael, Bonsall and Warner).

Table 1. Types of oral contraceptives used by the fifteen women in this study while they were providing samples of vaginal secretions.

NUMBER OF USERS	BRAND NAME	COMPOSITION
4	OVRAL	0.5 MG NORGESTREL, 0.05 MG ETHINYLESTRADIOL
3	ORTHO-NOVUM	1 MG NORETHINDRONE, 0.05 MG MESTRANOL
2	NORINYL	1 MG NORETHINDRONE, 0.05 MG MESTRANOL
2	OVALIN 21	1 MG ETHYNDIOL DIACETATE, 0.1 MG MESTRANOL
1	DEMULEN	1 MG ETHYNDIOL DIACETATE, 0.05 MG ETHINYLESTRADIOL
1	NORLESTRIN	1 MG NORETHINDRONE ACETATE, 0.05 MG ETHINYLESTRADIOL
1	LOESTRIN	1 MG NORETHINDRONE ACETATE, 0.02 MG ETHINYLESTRADIOL
1	ORACON	16 TABS 1 MG ESTRADIOL
		THEN 25 MG DIMETHISTERONE, 0.1 MG ETHINYLESTRADIOL

Women with normal menstrual cycles showed high levels of volatile acids in the late follicular phase, and a progressive decline during the luteal phase of the menstrual cycle. This rhythmic change in volatile acid content was completely absent in samples from women using oral contraceptives (Fig. 12). The type of contraceptive used by these women is given in Table 1. All women produced acetic acid in some of their samples. Thirty-four per cent produced acetic acid only, 36% produced acetic acid together with amounts below $10 \mu\text{g}$ per sample of the other volatile acids, and the remaining 30% of women produced acetic acid and amounts greater than $10 \mu\text{g}$ per sample of other volatile acids. A striking example of cyclic variations in acid content during three menstrual cycles from one woman not using oral contraceptives, and who was also one of the highest producers, is given in Fig. 13.

DISCUSSION

These data demonstrate that volatile fatty acids are normal, physiological constituents of the vaginal secretions of healthy young women with regular menstrual cycles. Amounts increased near mid-cycle as

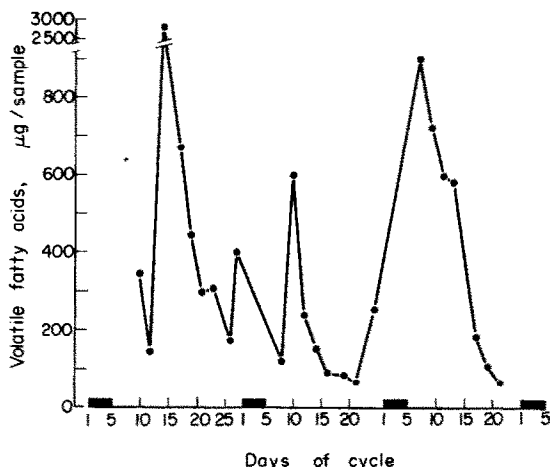


Fig. 13. Volatile fatty acids in vaginal secretions during three menstrual cycles (28, 26 and 25 days) from a woman who was one of the highest producers. Note consistent declines in acid content during the luteal phases of each cycle. More than 42% of the total acids was due to those other than acetic. Horizontal bars indicate menstruation. (Michael, Bonsall and Warner—unpublished results).

they do in infra-human primates. However, in women on various types of oral contraceptives, the mid-cycle increase was abolished and levels of acids were significantly lower. The precise role of olfactory mechanisms in human sexual behavior needs to be clarified. None of the data presented here provides proof that the volatile constituents in human vaginal secretions act as attractant pheromones. Nevertheless, it is clearly of interest that the same substances (copulins) possess sex-attractant properties in other primate species. Furthermore, human vaginal secretions have been shown to possess exactly this pheromonal activity in cross-taxa experiments with rhesus monkeys [19]. Constant vaginal douching results in the destruction of the normal bacterial flora upon which the production of copulins depends. The current vogue for this practice is based on widely felt anxieties about genital odors which may, in fact, be wholly unnecessary and quite misplaced.

CONCLUSIONS

Ethological and behavioral techniques when used sensibly in conjunction with plasma steroid assays and operant conditioning methods help us to understand the communication systems of higher primates. These interdisciplinary studies reveal that both the patterns of motor behavior (visual signals) and the production of pheromones (olfactory signals) are influenced by the gonadal steroids. It is fascinating to observe how comparative studies in a range of infra-human primate species have eventually directed attention to similar mechanisms in man.

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