tance of the hydrase reactions lies in (i) detoxification of carcinogenic compounds to less harmful ones, (ii) possible alteration of this detoxification potential by environmental factors thus leading to the malignant state, and (iii) potential manipulation of this metabolism as a therapeutic tool. Generally, the hydrase enzymes have been associated with the mixed-function oxidases —the latter being responsible for the activation of certain hydrocarbons to their more carcinogenic oxides. Efforts to manipulate the enzymes responsible for detoxification of the oxides are complicated by unwanted effects upon the enzymes responsible for formation of the oxides. In the case of CAE hydrase, the substrate is of natural origin and is formed photochemically; the fact that CAE levels appear to be directly related only to irradiation and CAE hydrase activity suggests that mixed-function oxidases are not involved. Thus the paradox faced by others in the manipulation of enzyme systems related to both carcinogen activation and detoxification is not pertinent to the CAE hydrase system. This might allow alteration of those enzymes involved in detoxification of CAE in a way that could be of preventive value.

JARVIS T. CHAN
HOMER S. BLACK
Department of Dermatology, Baylor College of Medicine, and *Veterans Administration Hospital, Houston, Texas 77025*

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Human Vaginal Secretions: Volatile Fatty Acid Content

Abstract. *Vaginal samples (682) were collected by a tampon method from 50 healthy young women. Samples were analyzed by gas chromatography. The volatile aliphatic acids increased during the late follicular phase of the menstrual cycle and declined progressively during the luteal phase. Women on oral contraceptives had lower amounts of volatile acids and did not show any rhythmic changes in acid content during the menstrual cycle. These same substances possess sex-attractant properties in other primate species.*

Volatile aliphatic acids (acetic, propanoic, methylpropanoic, butanoic, methylbutanoic, methylpentanoic) have been demonstrated in the vaginal secretions of the rhesus monkey (*Macaca mulata*), anubis baboon (*Papio anubis*), patas monkey (*Erythrocebus patas*), pigtail monkey (*M. nemestrina*), crab-eating monkey (*M. fascicularis*), and squirrel monkey (*Saimiri sciureus*); thus, they are present in a wide range of primate species (*1, 2*). These acids possess sex-attractant properties in the rhesus monkey and stimulate the sexual activity of males via olfactory pathways (*3*). Preliminary studies have also demonstrated their occurrence in women (cooperative wives of colleagues) (*4*). 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 (*5*). Samples were collected by lavage, and only 2 of the 28 contained acids in concentrations similar to those observed in the earlier study. Women presenting themselves at a clinic for gynecological examination sometimes take such extreme steps to prepare themselves by washing and douching that it is impossible even to collect vaginal smears for cytology and, because the negative results from the clinic population contrasted with our preliminary findings, it was considered essential to investigate a population of normal, healthy women.

The subjects were recruited for study as follows. 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 (i) offered a gynecological examination, (ii) provided with a box containing tampons and bottles, and (iii) 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 to the project and who distributed and collected the boxes. The biochemists conducting assays were aware only of code numbers. When assay of all 682 samples had been completed, data were grouped in relation to the stage of the menstrual cycle, and subjects were separated into oral contraceptive users and nonusers.

Self-collection of vaginal secretions by aspiration with a pipette containing distilled water (pipettes were used successfully in infrahuman primates) 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 hours, 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 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 of methanol. Subjects were requested to wear each tampon in the usual way for 6 to 8 hours 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 to 8 hours out of each 48 hours, any disturbance to the normal bacterial flora was minimized. Immediate immersion in methanol after removal both prevented bacterial action (*2*) 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 μl of 0.1*N* sodium hydroxide, and evaporated to dryness. Residues were taken up in 1 ml of water, washed with 4 ml of ether (to remove basic and neutral components), and the aqueous layers were acidified and extracted with 4 ml of ether containing *n*-pentanol as a concentration marker before gas-liquid chromatography (*6*).

Of the original 50 women, data from 3 were excluded because of irregular bleeding or unreliable menstruation data. The remaining 47 subjects [mean age 20.4 ± 2.3 (S.D.) years; mean cycle length, 29.2 ± 3.8 (S.D.) days] gave data for 86 menstrual cycles (635 samples); they were grouped into 32 subjects not taking oral contraceptives (61 cycles, 449 samples) and 15 subjects using oral contraceptives (25 cycles, 186 samples). Figure 1 shows the total volatile aliphatic acid content of vaginal secretions of women not taking ("nonpill" cycles) and taking ("pill" cycles) oral contraceptives, arranged according to successive 3-day periods of the menstrual cycle (day 1 being the first day of menstruation). Women not taking oral contraceptives 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 concentration was completely absent in women taking the pill. The mean acid content of all "nonpill" samples was 105.6 ± 7.8 (S.E.) μg per sample ($N = 449$), that of all "pill" samples was 76.3 ± 4.3 (S.E.) μg per sample ($N = 186$). The difference between these means was highly significant ($P < .001$) using a logarithmic transformation to eliminate skew and Student's *t*-test (one-tailed). To assess the significance of the increases in acid content near midcycle, a two-way analysis of variance for nonorthogonal data was used (*7*). Increases were significant for the "nonpill" cycles ($P < .02$), but there were no significant differences during the "pill" cycles.

All women produced acetic acid in some of their samples, and 34 percent produced acetic acid only. A further 30 percent of women produced acetic acid and amounts greater than 10 μg per sample of other volatile acids in addition. To facilitate comparison with an infrahuman primate, the acid content (mean ± S.E.) in the vaginal secretions of this latter group of women was compared with that of similarly selected secretions from the rhesus monkey, analyzed earlier (*2*) (data shown in parentheses): acetic 225.8 ± 22.1 (monkey, 35.9 ± 4.1), propanoic 65.3 ± 19.2 (monkey, 12.1 ± 0.7), methylpropanoic 5.9 ± 1.3 (monkey, 2.6 ± 0.3), butanoic 27.1 ± 7.6 (monkey, 36.6 ± 2.6), methylbutanoic 15.3 ± 3.5 (monkey, 6.2 ± 0.6), methylpentanoic 2.1 ± 1.6 (monkey, 4.9 ± 0.6), total 341.5 ± 51.8 (monkey, 98.0 ± 6.7). The proportions of the different acids in the two species are rather similar except for the higher levels of acetic and propanoic acids in human samples. A striking example of cyclic variations in acid content during three menstrual cycles from one woman not using oral contraceptives, and who was one of the highest producers, is given in Fig. 2.

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 midcycle as they do in infrahuman pri-

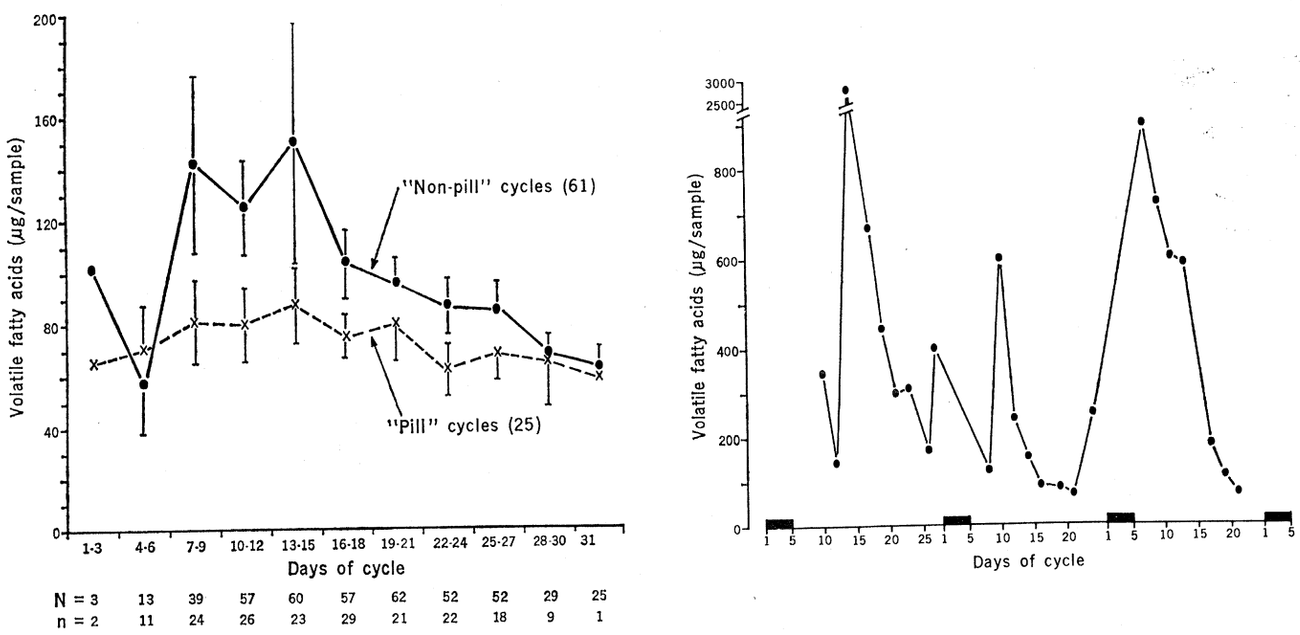

Fig. 1 (left). Volatile fatty acid content (mean ± S.E.) of the vaginal secretions of 47 women (86 cycles) during successive 3-day periods of the menstrual cycle. The rise before midcycle found in normal women was abolished in those taking oral contraceptives ("pill" cycles). *N*, number of "nonpill" samples; *n*, number of "pill" samples. Fig. 2 (right). Volatile fatty acids in samples of vaginal secretions during three menstrual cycles (28, 26, and 25 days) from a woman who was one of the highest producers. There are consistent declines in the acid content during the luteal phases of each cycle. More than 42 percent of the total acids was due to those other than acetic. Horizontal bars indicate menstruation.

mates (*2*). However, in women taking oral contraceptives, the increase was abolished and amounts of acids were significantly lower. The precise role of olfactory mechanisms in human sexual behavior needs to be clarified. We have scant information on the physiological importance of these volatile constituents in the human, although it is of interest that the same substances (copulins) (*1*) possess sex-attractant properties in other primates. Furthermore, human vaginal secretions have been demonstrated to possess this activity in cross-taxa experiments with rhesus monkeys (*4*). Constant vaginal douching results in the destruction of the normal bacterial flora. The current vogue this practice enjoys is based on widely felt anxieties about genital odors which may, in fact, be wholly unnecessary and quite misplaced.

RICHARD P. MICHAEL, R. W. BONSALL
PATRICIA WARNER
Department of Psychiatry,
Emory University School of Medicine,
Atlanta, Georgia 30322, and
Georgia Mental Health Institute,
Atlanta 30322

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6. Extracts were concentrated to 50 μl and analyzed on 10 percent FFAP columns in a Perkin-Elmer gas chromatograph programmed from 50° to 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 percent (acetic acid) to 67 percent (methylpentanoic acid). Overall coefficients of variation ranged from 6 percent (butanoic acid) to 18 percent (acetic acid). Control tampons contained 12.8 ± 1.7 μg ($N = 31$) of acetic acid only. This blank was not subtracted.
7. Biomedical Computer Program Series—10 V. Data for the first 7 days were excluded because samples were not collected reliably during the menstrual flow. The remaining days of each cycle were divided into five equal periods. Data from six subjects could not be used for statistical analysis because of empty cells (missed samples). Preliminary tests showed a lack of subject-time interaction. Pairwise comparisons by the Scheffé test showed period 2 (late follicular) to be significantly higher than period 4 (midluteal) ($P < .05$).
8. We thank Dr. Michael Kutner, Department of Biometry, Emory University, for the analyses in (*7*). This work was supported by NIMH grant MH19506. We thank the Grant Foundation for providing essential equipment, and we thank also the women who participated in this study.

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Microtubule-Macrotubule Transitions: Intermediates after Exposure to the Mitotic Inhibitor Vinblastine

Abstract. *Vinblastine treatment of microtubule protein or intact microtubules assembled in vitro produced bifilar rings and bifilar helices. Suspensions of rings and helices were demonstrated to bind [^3H]colchicine, a diagnostic property of microtubule protein. Macrotubules are suggested to consist of tightly coiled helices formed by longitudinal compacting of loosely coiled protofilament pair intermediates.*

After chemical and physical treatments which in general disrupt microtubules, larger diameter macrotubules may appear in the cytoplasm and nucleus of a variety of cell types (*1*). Prominent among the chemical agents which induce macrotubules is the *Vinca* alkaloid, vinblastine sulfate (VLB). Paracrystals induced by VLB (*2*) are reported to be the result of the lateral aggregation of macrotubules (*1*). Since VLB-induced paracrystals bind colchicine (*3*), and, since paracrystals and microtubules exhibit a high binding affinity for the same specific antibodies (*4, 5*), it seems probable that macrotubules consist of microtubule protein. VLB can also induce the formation of

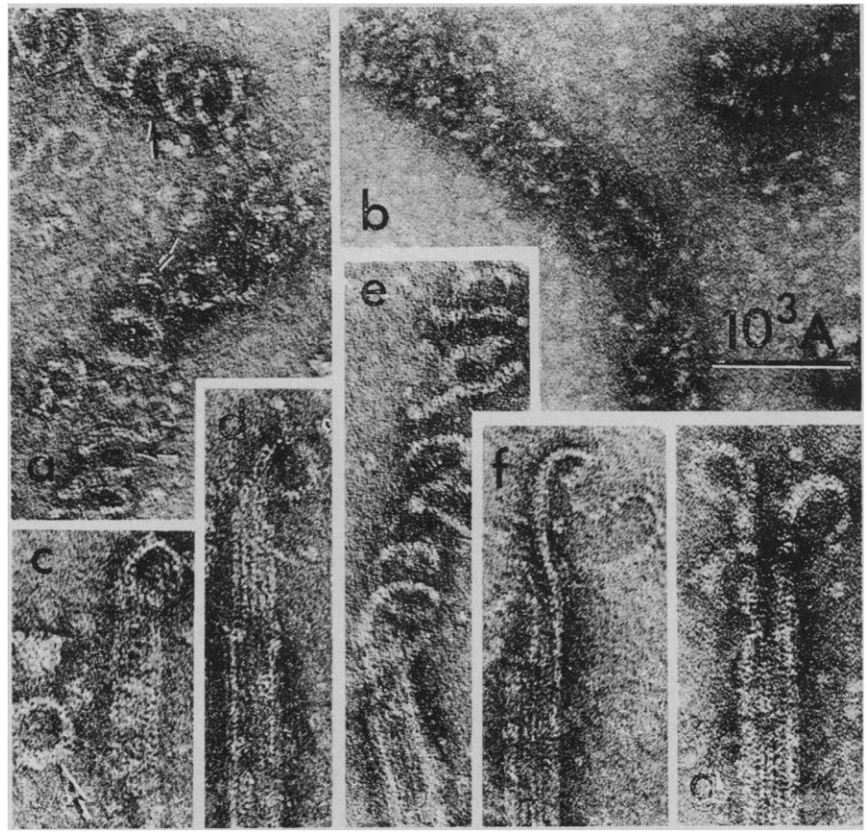

Fig. 1. MTP and microtubules after treatment with VLB. MTP extracted from porcine brain was incubated with $10^{-4}M$ VLB at 4°, 23°, and 37°C. Helices and rings were stained with 1 percent uranyl acetate and could be identified within 1 minute of VLB incubation. Microtubules were assembled by incubating MTP at 37°C for 20 to 30 minutes and cooling the suspension to 23°C. Assembled microtubules were subsequently treated with $10^{-4}M$ VLB. (a) Loosely coiled helices from VLB-treated MTP. Arrows point to regions where the bifilar nature of helices is evident. (b) Helices from VLB-treated MTP exhibiting compacting of coils. (c to g) Unraveling ends of assembled microtubules after VLB treatment. The apparent continuity between a microtubule protofilament pair and loosely coiled helix in (e) suggests the rationale of what is graphically illustrated in Fig. 2b. Samples of VLB-treated intact microtubules revealed: (i) no unraveling at 1 minute, (ii) unraveling ends and a few short free helices at 4 minutes, and (iii) unraveling ends and many free or apparently attached long helices at 10 minutes; the scale represents 1000 Å.