In vivo assessment of human vaginal oxygen and carbon dioxide levels during and post menses

Donna R. Hill,1 Marianne E. Brunner,1 Deborah C. Schmitz,1 Catherine C. Davis,2 Janine A. Flood,3 Patrick M. Schlievert,4 Sherry Z. Wang-Weigand,2 and Thomas W. Osborn1

1FemCare Product Development, 2FemCare Product Safety and Regulatory Affairs, 3Corporate Microbiology, Central Product Safety Division, The Procter and Gamble Company, Cincinnati, Ohio; and 4University of Minnesota, Minneapolis, Minnesota

Submitted 27 December 2004; accepted in final form 23 May 2005

In vivo assessment of human vaginal oxygen and carbon dioxide levels during and post menses. J Appl Physiol 99: 1582–1591, 2005. First published June 2, 2005; doi:10.1152/japplphysiol.01422.2004.—Previous in vitro and in vivo animal studies showed that O2 and CO2 concentrations can affect virulence of pathogenic bacteria such as Staphylococcus aureus. The objective of this work was to measure O2 and CO2 levels in the vaginal environment during tampon wear using newly available sensor technology. Measurements by two vaginal sensors showed a decrease in vaginal O2 levels after tampon insertion. These decreases were independent of the type of tampons used and the time of measurement (mid-cycle or during menstruation). These results are not in agreement with a previous study that concluded that oxygenation of the vaginal environment during tampon use occurred via delivery of a bolus of O2 during the insertion process. Our measurements of gas levels in women showed the presence of both O2 and CO2 in women. The tampons inserted into the vagina contained O2 and CO2 levels consistent with atmospheric conditions. Over time during tampon use, levels of O2 in the tampon decreased and levels of CO2 increased. Tampon absorbent capacity, menses loading, and wear time influenced the kinetics of these changes. Colonization with S. aureus had no effect on the gas profiles during menstruation. Taken collectively, these findings have important implications on the current understanding of gaseous changes in the vaginal environment during menstruation and the potential role(s) they may play in affecting bacterial virulence factor production.

Staphylococcus aureus; menstruation; gaseous changes

TOXIC SHOCK SYNDROME (TSS) was first described by Todd et al. (39) in 1978 as a multi-system illness characterized by the rapid onset of fever, hypotension, and multi-organ involvement, followed by desquamation upon recovery. Menstrual TSS (mTSS) has been associated with tampon use during menstruation. Despite the low incidence of mTSS, the illness remains of interest because tampons are widely used, and although rare, mTSS can be life threatening.

In a descriptive research study, Czerwinski (11) reported that ~80% of study participants (women from California under the age of 41) used tampons during menstruation. It has also been reported that about 70% of women in the USA, Canada, and much of Western Europe use tampons during menstruation (25). TSS is thought to be caused by colonization with Staphylococcus aureus capable of producing the superantigen, toxic shock syndrome toxin-1 (TSST-1) that penetrates through the mucosal surface (12) in a person who lacks neutralizing antibodies to TSST-1. S. aureus has previously been determined to colonize the nares, axillae, vagina, vulva, anus, pharynx, or damaged skin of 30–50% of healthy adults (8, 18). This figure could be much higher based on results of recent analyses using culture-independent methods to identify S. aureus (40). Although S. aureus is capable of producing several superantigens, TSST-1 is considered to be the cause of nearly all cases of mTSS and at least 50% of nonmenstrual cases (2).

The development and progression of TSS may depend on host susceptibility and factors that regulate TSST-1 production by S. aureus. A large percentage of the population develops specific antibodies to TSST-1 during the first decade of life (41). Lack of anti-TSST-1 neutralizing antibodies has been associated with cases of mTSS (5, 32). In addition, standard culture-based methods have shown that only about 10–20% of vaginal S. aureus isolates produce TSST-1 (14, 20, 21). Toxin production has been reported to be higher during menses, possibly due to the altered levels of iron, O2, CO2, pH, hormones, and osmolarity, which could affect the total number of bacteria present, the number of colonizing species (7, 36, 42), and/or gene expression (45).

Tampax tampons were introduced in the United States in 1936. One of the first clinical studies indicating the safe use of tampons was published in 1942 (22). Their continued safe use has been subsequently documented (35). It has been shown, however, that there is an increased risk for mTSS attributed to tampon use (13, 27, 30, 33). Epidemiological studies have implicated five other risk factors for mTSS, including young user age, tampon absorbency, continuous usage of tampons without use of pads, tampon composition, and oxygen entrapped in the tampon, although none has been definitively confirmed (3, 17, 28).

Numerous in vitro studies have demonstrated the importance of O2 tension in the regulation of TSST-1 production by S. aureus (29, 38, 44, 45). Results of early work in this area prompted the evaluation of O2 as a risk factor in later epidemiological studies (17, 28). A study by Wagner et al. (43),
Presence of *S. aureus* not done. Gas analysis, vaginal gas mapping analysis (experimental questions. Do the concentrations of O$_2$ and CO$_2$ as well as inside the tampon during wear. Simultaneous monitoring of O$_2$ and CO$_2$ tensions in the vagina, tampon surface may have actually measured the gas tensions at the surface of the tampon, rather than in the vaginal environment. Recent advances in sensor technology now allow the simultaneous monitoring of O$_2$ and CO$_2$ tensions in the vagina, as well as inside the tampon during wear.

Umbilical microsensors were used to address several experimental questions. Do the concentrations of O$_2$ and CO$_2$ change in the vagina during and post menses? Does tampon use, as well as tampon absorbency, affect vaginal O$_2$ and CO$_2$ concentrations during and post menses? Can menses be a source of oxygen for the vaginal environment? Does vaginal colonization with *S. aureus* affect the vaginal gas profiles measured during tampon use? Results from this translational research have generated new discoveries through basic scientific inquiry by the process of applying ideas, novel techniques, and discoveries that have the potential to lead to new insights into diseases of the human female urogenital system.

**MATERIALS AND METHODS**

**Study participants.** Participants were recruited and gave written consent before participation. The study protocol and informed consent documents were approved by The Procter and Gamble Corporate Institutional Review Board. All participants were at least 21 years of age, in good general health (self-reported), had menstrual cycles between 21 and 35 days with menstruation lasting at least 3 days, had no previous or current gynecological complaints, typically used tampons for normal menstrual protection, and agreed to abide by the study requirements and restrictions. Participants were excluded if they reported a history of TSS or symptoms consistent with TSS, streptococcal infections within the last 3 mo, difficult wearing tampons, or body piercing in the vulvar area, or if they were currently pregnant or not done. Gas analysis, vaginal gas mapping analysis (n = 5). Fluid analysis, menstrual fluid analysis (n = 8). FISH/PCR analysis of tampon: M, menstrual; NM, nonmenstrual condition. NA, not applicable.

**Culture protocol.** Vaginal swabs were prepared for analysis within 24 h of collection. Swabs were streaked on mannitol salt agar (Difco, Detroit, MI) and incubated aerobically overnight at 37°C. Identities of presumptive *S. aureus* isolates were confirmed via Staphaurex Slide Coagulation Test (Murex Diagnostics, Dartford, UK) and gram stained (Deaconess Hospital, Cincinnati, OH).

**Sensors used in the study.** The Neotrend sensors (Diametrics Medical, St. Paul, MN) used in this study were initially developed for intra-arterial monitoring of critically ill neonates (24, 37). This is the first application of these devices to the measurement of gas tensions in the vaginal environment. Each sensor is composed of four sensing units, three fiber optic-based units for simultaneously monitoring dissolved O$_2$, CO$_2$, pH, and a thermocouple for measuring temperature. For perspective, these sensors (<0.5 mm in diameter and 23 mm in length) are considerably smaller in diameter than the Clark electrodes (24 and 10 mm) used in the previous study (43). On the basis of their application to intra-arterial blood gas monitoring, the response time of the sensor unit is expected to be less than 15 s at 37°C. The detection ranges for PO$_2$ and PCO$_2$ in blood gases are expected to be 20–500 mmHg and 10–160 mmHg, respectively, and drift in the signal is expected to be less than 0.5%/h of operation. The sensors used in this study were calibrated and deployed according to the manufacturer’s general instructions for use. In addition, assessments were made to evaluate the ability of these sensors to measure PO$_2$ and PCO$_2$ in menstrual fluid.

**Tampons used in this study.** Two types of tampons were used in this study. Tampax regular absorbency (Lot #27N909049, #0165243038, #0165243028, The Procter & Gamble Company, Cincinnati, OH) and Kotex Super absorbency (Lot #VP 9306, Kimberly-Clark, Neenah, WI). These two tampons were selected to represent the range of entrapped air found in commercially available tampons. Tampax regular tampons contain a blend of cotton and rayon fibers with an absorbency rating of between 6 and 9 g. Tampons such as these have been found to contain the least amount of entrapped air (19). Kotex Super tampons are made of rayon fibers with an absorbency rating of between 9 and 12 g. Tampons such as these have been found to contain the most entrapped air (19). These findings were independently verified in our laboratory for the actual products used in this study (data not shown).

The tampons were modified to monitor the environment inside the tampon during use. The tampon-sensor assembly was made by producing a small hole (<2 mm in diameter) in the center axis of the tampon. A cutoff radial artery catheter (Arrow International, Reading, MA) was used to make the hole. After making the hole, the tampon was secured in the sensor using an O-ring. The sensor was placed in the tampon in an aseptic manner. The tampon-sensor assembly was then placed inside a Tissu-Tight ##27000-3 (Life Science Products, Emeryville, CA).

**Table 1. Demographics of participants and participation matrix**

<table>
<thead>
<tr>
<th>Participant</th>
<th>Race</th>
<th>Age</th>
<th>Deliveries</th>
<th>Flow</th>
<th><em>S. aureus</em></th>
<th>Gas Analysis</th>
<th>Fluid Analysis</th>
<th>FISH/PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cau</td>
<td>44</td>
<td>0/1</td>
<td>Light</td>
<td>(−)</td>
<td>Yes</td>
<td>No</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>Cau</td>
<td>36</td>
<td>2/0</td>
<td>Light</td>
<td>(−)</td>
<td>Yes</td>
<td>No</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>Cau</td>
<td>38</td>
<td>3/0</td>
<td>Moderate</td>
<td>(−)</td>
<td>No</td>
<td>Yes</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>Cau</td>
<td>45</td>
<td>3/0</td>
<td>Moderate</td>
<td>(−)</td>
<td>No</td>
<td>Yes</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>Cau</td>
<td>45</td>
<td>3/0</td>
<td>Heavy</td>
<td>(−)</td>
<td>Yes</td>
<td>Yes</td>
<td>NA</td>
</tr>
<tr>
<td>6</td>
<td>Cau</td>
<td>42</td>
<td>3/0</td>
<td>Moderate</td>
<td>(+)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes (M + NM)</td>
</tr>
<tr>
<td>7</td>
<td>Afr-A</td>
<td>42</td>
<td>0/2</td>
<td>Heavy</td>
<td>(+)</td>
<td>No</td>
<td>No</td>
<td>NA</td>
</tr>
<tr>
<td>8</td>
<td>As-A</td>
<td>23</td>
<td>0/0</td>
<td>Light</td>
<td>(+)</td>
<td>No</td>
<td>No</td>
<td>NA</td>
</tr>
<tr>
<td>9</td>
<td>Afr-A</td>
<td>40</td>
<td>2/0</td>
<td>Heavy</td>
<td>(+)</td>
<td>No</td>
<td>Yes</td>
<td>NA</td>
</tr>
<tr>
<td>10</td>
<td>Cau</td>
<td>43</td>
<td>2/0</td>
<td>Heavy</td>
<td>(+)</td>
<td>No</td>
<td>Yes</td>
<td>NA</td>
</tr>
<tr>
<td>11</td>
<td>Cau</td>
<td>42</td>
<td>2/0</td>
<td>Moderate</td>
<td>ND</td>
<td>No</td>
<td>Yes</td>
<td>NA</td>
</tr>
</tbody>
</table>

Cau, Caucasian; Afr-A, African-American; As-A, Asian American. Deliveries is number vaginal/number of caesarian deliveries. Flow is self-reported. Presence of *S. aureus* determined by culture method, not taken in parallel with gas mapping study, (+) *S. aureus* detected; (−) *S. aureus* not detected; ND, not done. Gas analysis, vaginal gas mapping analysis (n = 5). Fluid analysis, menstrual fluid analysis (n = 8). FISH/PCR analysis of tampon: M, menstrual; NM, nonmenstrual condition. NA, not applicable.

All absorbency ratings for tampons used were based on release criteria set by in vitro absorbent capacity measurements using the syngyna test as specified in 21 CFR 801.430(f)(2).

---

1 All absorbency ratings for tampons used were based on release criteria set by in vitro absorbent capacity measurements using the syngyna test as specified in 21 CFR 801.430(f)(2).
PA) was sutured to the tampon, using sterile suture thread (Ethibond Excel, Ethicon, Sommerville, NJ), to support the sensor as it was deployed into the tampon. The collar acted as the anchor point of the catheter as it was sutured gently to the base of the tampon to hold the catheter in place.

Collection of menstrual fluid. Samples of menstrual fluid were collected and analyzed for the levels of O2 and CO2 using several analytical approaches. Eight participants provided a total of 17 samples of menstrual fluid for analysis. One participant provided a total of seven samples over four consecutive menstrual cycles.

Samples of menses were collected using a modified INSTEAD 12 Hour Feminine Protection Cup (Ultrafem, Missoula, MT) to minimize disruption of the vaginal environment and exposure of menses to atmospheric conditions before analysis. The INSTEAD Cup is a nonabsorbent, flexible cup designed and marketed to collect menses. When inserted according to the manufacturer’s instructions, the cup fits just below the cervix. The cups were individually modified in the laboratory, as described below, to allow the collection, detection, and sampling of menses for subsequent analysis of blood gases. Water vapor transmission analysis showed that the INSTEAD Cup has a low water vapor transmission (0.32 mg m⁻² h⁻¹ at 23°C and 50% relative humidity); therefore, we expected very low permeability to CO2 and O2 during the course of menses collection (30–50 min).

Three holes were made in the plastic film of an INSTEAD Cup. The first hole was 0.1 cm from the outer rim. The second hole was 1 mm from the first hole, and a third hole was ~2 cm from the center of the film. The tip of an umbilical artery catheter (Diamic Medical, St. Paul, MN) was inserted through the first hole. Suture material (Ethibond Excel green braided polyester surgical suture thread, Ethicon, Sommerville, NJ) was used to secure the plastic of the cup to the catheter by wrapping and tying the suture thread around the extended plastic and catheter. A Neotrend sensor (Diamic Medical) was attached and deployed through catheters in the first two holes. The plastic film was bunched around the catheters and secured by an elastic orthodontic band. This improved pooling of menses in the cup and reduced potential leakage from the cup.

The blue butterfly end of Vacutainer tubing (23 gauge; 3/4 in; 12 in length; Becton-Dickinson, Franklin Lakes, NJ) was inserted through the third hole, allowing the tubing to extend on the inner surface of the cup. The tubing for each device was secured by twisting an orthodontic elastic band around the end of the tubing on the inside of the cup. The tubing was then retracted until the band rested against the inner surface of the cup. The needle from the white port of the Vacutainer tubing was removed and discarded and the end was capped. Participants inserted the modified cup for the collection of menstrual fluid. After ~15–30 min of wear, two Neotrend sensors were inserted into the catheters and deployed into the cup. Sensor outputs were allowed to stabilize for 15–20 min. When the sensor output (PO2, PCO2) indicated a change from the initial reading, menses was withdrawn from the cup using a 1-ml non-heparinized syringe attached to the luer adaptor of the tubing.

Analysis of menstrual fluid. Immediately after collection, menses was analyzed using the Immediate Response Mobile Analysis (IRMA) SL Blood Analysis system (Diametrics Medical) according to the manufacturer’s instructions for blood samples. In addition, the IRMA system was evaluated for its suitability for this work by comparing blood gas values obtained from standard samples (Defibrinated Sheep Blood, Cleveland Scientific, Cleveland, OH) with results obtained using a standard blood gas analyzer (Corning model 388) at Deaconess Hospital (Cincinnati, OH).

Vaginal mapping. Vaginal mapping evaluations were designed to evaluate participants before and after the insertion of a tampon under menstrual and nonmenstrual conditions (Table 2). The purpose was to allow participants to act as self-controls. Initial mapping evaluations during menstruation were typically performed on the second day (i.e., 24–30 h post initiation of menstrual flow) because it typically demonstrates the highest average menstrual flow (34) and mTSS has been reported to be more prevalent during the first 2 to 3 days after onset of menstruation (13, 33). In several cases, additional evaluations were performed on the third day of menstruation (Table 2). In addition to menstrual samples, nonmenstrual evaluations were conducted during mid-cycle (days 10–20) to evaluate the vaginal environment in the absence of menses.

For all evaluations, participants were in a supine position during the entire monitoring period. Neotrend sensors were positioned at two different vaginal sites to obtain baseline O2 and CO2 levels (Fig. 1A). To deploy the vaginal sensors in the desired positions (Fig. 1A), the umbilical artery catheters used to deploy the sensors were precut to lengths that differed by 2 cm. The two catheters were placed side-by-side and sutured to secure a spacing of 2 cm from the end of one catheter to the other catheter. The catheter assembly was premarked to

Table 2. Participants, sensors, and tampons used in vaginal mapping studies

<table>
<thead>
<tr>
<th>Participant</th>
<th>Sequence</th>
<th>Nonmenstrual</th>
<th>Menstrual</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preinsertion</td>
<td>Postinsertion</td>
<td>Preinsertion</td>
</tr>
<tr>
<td>1</td>
<td>A (day 2)</td>
<td>M (Tampax)</td>
<td>C, M, T (Tampax)</td>
</tr>
<tr>
<td>2</td>
<td>A (mid-cycle)</td>
<td>M (Tampax)</td>
<td>C, M, T (Tampax)</td>
</tr>
<tr>
<td>3</td>
<td>B (day 2)</td>
<td>M (Tampax)</td>
<td>C, M, T (Tampax)</td>
</tr>
<tr>
<td>4</td>
<td>A (cycle 1; day 2)</td>
<td>M (Tampax)</td>
<td>C, M, T (Tampax)</td>
</tr>
<tr>
<td>5</td>
<td>B (cycle 1; day 3)</td>
<td>M (Tampax)</td>
<td>C, M, T (Tampax)</td>
</tr>
<tr>
<td>6</td>
<td>C (cycle 2; day 2)</td>
<td>M (Tampax)</td>
<td>C, M, T (Tampax)</td>
</tr>
<tr>
<td>7</td>
<td>D (cycle 2; day 3)</td>
<td>M (Tampax)</td>
<td>C, M, T (Tampax)</td>
</tr>
<tr>
<td>8</td>
<td>E (mid-cycle)</td>
<td>M (Tampax)</td>
<td>C, M, T (Tampax)</td>
</tr>
<tr>
<td>9</td>
<td>F (mid-cycle)</td>
<td>M (Tampax)</td>
<td>C, M, T (Tampax)</td>
</tr>
<tr>
<td>10</td>
<td>G (mid-cycle)</td>
<td>M (Tampax)</td>
<td>C, M, T (Tampax)</td>
</tr>
<tr>
<td>11</td>
<td>H (mid-cycle)</td>
<td>M (Tampax)</td>
<td>C, M, T (Tampax)</td>
</tr>
<tr>
<td>12</td>
<td>I (mid-cycle)</td>
<td>M (Tampax)</td>
<td>C, M, T (Tampax)</td>
</tr>
<tr>
<td>13</td>
<td>J (mid-cycle)</td>
<td>M (Tampax)</td>
<td>C, M, T (Tampax)</td>
</tr>
</tbody>
</table>

Sequence is experimental mapping evaluations for each participant, where letters indicate sequence of evaluations followed by the cycle and day of cycle information. Evaluations continued before and after tampon insertion either during nonmenstrual or menstrual conditions. Following were used in the evaluations: C, cervix sensor; M, mid-zone sensor; T, tampon-sensor assembly. Parentheses indicate the type of tampon inserted, where Tampax = Tampax Regular absorbency and Kotex = Kotex Super absorbency. Except where indicated, pre-tampon insertion evaluations were conducted using 2 vaginal sensors (see Fig. 1A) and post-tampon insertion experiments were conducted using 2 vaginal sensors and 1 sensor internal to the tampon (see Fig. 1B).
Applicator. Once the tampon-sensor assembly was in place, the vagina was inserted either digitally or using a Kotex Super absorbency tampon placed in the umbilical artery catheters. The tampon-sensor assembly was then removed and the vaginal sensors were retracted into the vagina. To prevent damage during insertion of the hyperterminal into an Excel spreadsheet.

Data points were collected every 30 s via an RS232 port and downloaded via the hyperterminal into an Excel spreadsheet.

Indicate the depth to deploy the sensors for proper placement in the vagina.

The vaginal-sensor assembly was then digitally inserted into the predetermined depth into the vaginal lumen by a medical professional. The sensors were deployed by extending the sensors from the arterial catheter and allowed to equilibrate for 10–15 min. Readings from both sensors were acquired for up to 45 min to establish baseline vaginal levels of CO$_2$ and O$_2$ from both vaginal sites. Data points were collected every 30 s via an RS232 port and downloaded via the hyperterminal into an Excel spreadsheet.

After the baseline response was recorded, the vaginal-sensor assembly remained in the vagina. To prevent damage during insertion of the tampon-sensor assembly, the vaginal sensors were retracted into the umbilical artery catheters. The tampon-sensor assembly was then inserted either digitally or using a Kotex Super absorbency tampon applicator. Once the tampon-sensor assembly was in place, the vagina sensors were carefully redeployed from the catheters (Fig. 1B). Partial pressures of O$_2$ and CO$_2$ were then monitored from all three sensors. Data collection continued, as described previously, for an additional 6–8 h.

Tampon loading evaluations. Tampons were weighed before assembly of the tampon-sensor device as well as on completion of menstrual mapping evaluation. Tampon menses load was determined by weight difference. Tampon menses load values obtained were either less than or equal to 3.5 g or greater than 6.0 g. For the purposes of this study, tampons with 3.5 g or less of menses were considered “low load.” Tampons containing over 6 g of menses were considered “high load.”

Tampon selection and subsample preparation. Tampons from study participants were removed and frozen at −70°C after mapping studies and stored for analyses of the presence and location of S. aureus on the tampons pending vaginal culture results. Subsequently, tampons from the colonized subject worn during and post menses were analyzed via PCR and fluorescent in situ hybridization (FISH). To prepare samples for PCR and FISH analyses, each of the frozen tampons was aseptically cut into 12 pieces using autoclaved razor blades and flame sterilized forceps. Four sections of each tampon (tip, upper middle, lower middle, and base) were each cut into three zones (outer, bulk, and core). Each piece was placed into a sterile Whirl-Pak bag (WVR Scientific, West Chester, PA) with 3 ml of sterile water and stomached for 3 min to extract bacterial cells. Fluid was removed, aliquoted, and frozen. The stomached fibers were air dried at 37°C until the weight remained stable for 2 consecutive days.

Analysis of S. aureus in tampons. Tampons from the participant who was colonized with S. aureus were analyzed with culture-independent methods (see Table 1). The presence of S. aureus was confirmed using PCR (6), and the concentration of S. aureus in each subsample was determined using FISH analysis (40). FISH has been shown to be more sensitive for the detection of S. aureus than culture-based methods (9, 40).

Fluid from the stomached aliquots was spotted into separate wells on 5-mm well microscope slides (Erie Scientific, Portsmouth, NH), as were negative and positive control cultures (S. epidermidis and S. aureus, respectively). The spotted fluids were allowed to dry, then covered with 100% ethanol and air-dried again. Cells in each well were then fixed by covering with fresh 4% paraformaldehyde for 1 h at 4°C, then rinsed with distilled water (dH$_2$O), being careful to prevent run-off from any well passing over other wells. After air-drying, fixed cells were permeabilized by covering the cells for 45–60 min at 37°C with a permeabilization solution: 0.1 mg/ml lipase (Sigma, L-0382) and 3 mg/ml lysozyme (Sigma, L-7651), in 25 mM Tris, pH 8.0. After the permeabilization treatment, the wells were rinsed with distilled water and slides were frozen at −20°C until required for FISH analysis. FISH for the specific detection of S. aureus was conducted according to published procedures (40) using probes Saur327 and Saur72. All FISH-positive cells were enumerated by scanning the whole wells microscopically. Any counts from the negative control wells were subtracted as background. These background-subtracted counts were normalized to dry weight of the tampon subsample. Negative controls used for each sample were S. epidermidis culture with S. aureus-specific probes, S. epidermidis with no probes, and the sample with no probes. The positive control well included S. aureus with S. aureus-specific probes. The presence of S. aureus was confirmed using PCR (data not shown). Genomic DNA was extracted and isolated from each stomached fluid aliquot using a Blood Spin kit (Mo Bio Labs, Carlsbad, CA). Extracted DNA was amplified by PCR using S. aureus specific primers (nuc gene) (6), and the product was examined on agarose gel to confirm the presence of S. aureus.

Statistical analysis and modeling. Before statistical analysis, the raw experimental data were examined in detail and some data were excluded from further analysis for specific reasons. The following data were excluded from the statistical analysis: 1) data recorded before equilibration of the sensors or while the sensors were withdrawn into catheters; 2) data from tampon sensors that showed spikes due to interferences associated with direct contact between blood and the sensor tip (37); and 3) results that showed evidence of obvious sensor malfunctioning during the course of the evaluation.

For each gas/experiment/vaginal site value, basic time units were 5-min intervals from the time of tampon insertion. Within each time interval, gas (CO$_2$ or O$_2$) levels were averaged. Primary statistical significance was declared at the two-sided 0.05 significance level. PC

Fig. 1. Placement of vaginal sensors in vaginal canal pre (A)- and post (B)-tampon insertion.

Fig. 2. Diagram of frozen tampon dissection into 12 pieces.
SAS Release 8.1 (SAS/STAT User’s Guide, Version 8, 1999; SAS Institute, Cary, NC) was used to analyze all data.

A mixed model analysis of covariance (ANCOVA) was conducted to compare gas levels from the two vaginal sensors (cervix and midzone). For each menstrual/nonmenstrual condition and tampon insertion phase, the fixed terms in the ANCOVA model were tampon type, vaginal site, and tampon type by site interaction. The random term was “participant,” and covariate was “menses load level.” Because of insufficient data (i.e., no Super Kotex cervix nonmenstrual data), the comparison between cervix and midzone was not estimable for some cases using this model. Thus another ANCOVA model was also conducted for each menstrual/nonmenstrual condition and tampon insertion phase, but included only “vaginal site” as a fixed factor. Again the random term was “participant,” and covariate was “menses load level.”

A mixed model ANCOVA was also used to compare pre-tampon average gas levels to post-tampon average gas levels. For each vaginal source and menstrual/nonmenstrual condition, the fixed terms in the ANCOVA model were tampon type, pre- or post-tampon insertion phase, and tampon type by insertion phase interaction. The random term was participant, and the covariate was menses load level. (For some combinations of vaginal source and menstrual/nonmenstrual condition, the ANCOVA could not be conducted because of insufficient data.) ANCOVA results indicated that tampon type data could be combined. Thus a series of paired tests was conducted to use all available data.

For each experiment/vaginal site, the average pre- and post-tampon gas levels were calculated. The post-tampon minus pre-tampon deltas then were derived per experiment/vaginal site. For each load category, an average delta per subject/vaginal site also was calculated. Both the deltas per experiment/vaginal site and average deltas per subject/vaginal site were analyzed for each load category, both separately and combined, via the UNIVARIATE procedure in SAS. High and low load data from subjects who had both load categories under the same menstrual/nonmenstrual condition in separate experiments were treated as independent. If the deltas were normally distributed, then paired t-tests were conducted; otherwise, signed-rank tests were conducted. The Shapiro-Wilk normality tests were conducted at the 0.05 significance level. The above statistical tests were performed by both including and excluding nonmenstrual data from an experiment on one subject, where only two valid pre-tampon insertion measurements were made per vaginal site. Results were found to be similar with and without the inclusion of these data.

To evaluate the effect of tampon menses load on O2 and CO2 values within tampons during menstruation, a mixed model ANOVA was conducted. For each gas/experiment, the following parameters were derived from the tampon menstrual measurements: delta (first – last value) for O2 and CO2 profiles, maximum in CO2-O2 differences and maximum in O2/CO2. These parameters were analyzed via the MIXED procedure in SAS. The fixed terms in the ANOVA model were tampon type, load category, and tampon type by menses load category. The random term was participant.

For these analyses, ANOVA with menses load category (light, moderate, and heavy) as a class factor was used, because the focus was on distinguishing the effects of specific load levels. This objective differs from that of the ANCOVA models with menses load as a continuous covariate. ANCOVA was run to provide precise as possible estimates of the effect of tampon type (Tampax vs. Kotex), tampon phases (pre- vs. post-tampon insertion), and vaginal site (cervix vs. midzone) on gas levels.

RESULTS

Assessment of blood gas analysis approaches. Results showed that the IRMA system was capable of measuring mean PO2 and PCO2 in standard samples with accuracy and reproducibility similar to that obtained with the Corning BGA. Good agreement was observed between measured values for mean PO2 (Corning BCA 140.20; IRMA 138.86 mmHg) and PCO2 (Corning BGA 56.40; IRMA 57.24 mmHg), as indicated by the small differences in the measured mean values (1%). In addition, the relative standard deviation (RSD) in the mean values, a measure of the variability in results between multiple samples, was found to be very similar for both approaches (Corning BGA 5%; IRMA 6%).

The IRMA system was then used to analyze samples of menstrual fluid from study participants. The mean PO2 and PCO2 values obtained for seven samples of menstrual fluid taken over four menstrual cycles from participants 9 were found to be 41.6 ± 6.9 and 54.2 ± 3.4 mmHg, respectively. Compared with standard samples, the results showed an increase in the variability of the measured PO2 (from 6 to 17%), as indicated by an increase in the RSD of the mean. This increase in variability appears to reflect the normal fluctuation of PO2 within an individual. In addition, the IRMA system was also used to analyze 17 menstrual fluid samples from eight study participants. Means were obtained for each subject before calculation of the overall mean. The overall mean PO2 and PCO2 values were found to be 41.8 ± 16.3 and 43.5 ± 9.2, respectively. Results from this larger sample set showed a further increase in the variability in the mean PO2 and PCO2 (RSD; 17–39% and 6–21%, respectively). Finally, experiments were conducted to assess the ability of the Neotrend sensors to measure PO2 and PCO2 in menstrual fluid. This was done by comparing results obtained for nine samples from six study participants using Neotrend sensors with results obtained using the IRMA analyzer. Results indicated that Neotrend sensors were capable of measuring PO2 and PCO2 in menstrual fluid with accuracy and reproducibility similar to that obtained with the IRMA analyzer. Agreement in the mean PO2 and PCO2 values between the two measuring approaches for these samples was high (IRMA 37.77 ± 11.53, 48.96 ± 8.64; Neotrend 35.44 ± 11.25, 49.68 ± 8.27 mmHg). In addition, the RSD in the mean values was found to be very similar for both O2 and CO2 using both approaches (IRMA 31%; 18% Neotrend 32%; 17%).

O2 and CO2 in the vaginal environment. In general, the design objectives for this study were met, with only two exceptions (Table 2, participants 1 and 9). Statistical analysis of absolute vaginal gas levels showed no significant differences between the values measured by the sensors placed in the cervix or midzone regions throughout the course of these experiments (data not shown). Differences were observed in the changes in vaginal gas levels before and after tampon insertion. Similar trends were observed regardless of the sensor location (cervix or midzone), the type of tampon inserted (Tampax Regular or Kotex Super), or the time during the menstrual cycle that the experiments were conducted (day 2, day 3, or mid-cycle). In all cases, results showed a decrease in the calculated mean PO2 measured in the vaginal canal after insertion of a tampon. On the other hand, the calculated mean PCO2 measured in the vaginal canal after tampon insertion either increased slightly or remained the same.

The data for the two types of tampons were combined to increase the power of the statistical analysis and to compare our findings with previous work (43). This was possible because statistical analysis showed that the changes in mean O2
and CO₂ levels were independent of the type of tampon inserted (see Statistical analysis).

Combined tampon results obtained for mid-cycle and days 2 and 3 are shown in Figs. 3 and 4, respectively. As shown in Fig. 3, insertion of a tampon during mid-cycle decreased the mean partial pressure of O₂ in the vaginal environment, as measured by sensors in both the cervix (P = 0.108) and mid-zone (P = 0.043) locations. The decrease in mean O₂ level measured by the mid-zone sensor was found to be significant at the two-sided 0.05 significance level (P = 0.043).

Conversely, the combined data set showed a significant increase in mean PCO₂ in the vaginal environment (cervix sensor P = 0.008; mid-zone P = 0.031) on insertion of a tampon.

Similar trends were observed in the results for vaginal O₂ and CO₂ obtained during menstruation (Fig. 4). Insertion of a tampon during days 2 and 3 of menstruation decreased the mean PO₂ in the vaginal environment, as measured by sensors in both the cervix (P = 0.63) and mid-zone (P = 0.031) locations. The decrease in mean O₂ level measured by the mid-zone sensor was found to be significant at the two-sided 0.05 significance level (P = 0.031). The findings for CO₂ showed only a slight increase after tampon insertion, as measured by the cervix sensors (P = 0.101), and no change in CO₂ level as measured by the mid-zone sensor (P = 0.862).

It is also important to note that the mean vaginal PO₂ measured before tampon insertion (mid-cycle or days 2 and 3) were in the range of 15–35 mmHg, much lower than atmospheric levels (100–140 mmHg). Conversely, the mean values of PCO₂ before tampon insertion ranged from 35 to 55 mmHg, much higher than levels expected for atmospheric conditions (5 mmHg).

O₂ and CO₂ levels in the tampon environment. The initial response of the tampon sensor after insertion of the tampon-sensor assembly into the vaginal canal was similar regardless of the type of tampon (Tampax Regular or Kotex Super) or the time during the menstrual cycle that the experiments were conducted (days 2 and 3 or mid-cycle). In all cases, the tampon sensor initially indicated readings consistent with atmospheric conditions (O₂ between 100 and 140 mmHg and CO₂ <20 mmHg). Over the course of the evaluation, the response of the tampon sensor was observed to conform to one of two general profiles (Figs. 5–7).

The O₂ and CO₂ profiles obtained from tampon sensors used during nonmenstrual conditions and from tampon sensors used during menstruation that subsequently were found to have low menses loads (≈3.5 g) were found to be similar in general characteristics (Figs. 5 and 6). In most cases, the measured PO₂ in tampons was observed to decline slowly from an initial high value but remained high (>100 mmHg) during the course of the evaluation and did not approach the low PO₂ measured in the vagina (Figs. 3 and 4). On the other hand, PCO₂ in tampons was observed to increase rapidly from initial low values and
The measured PO2 was found to drop to below 60 mmHg and the participant B:
statistical analyses to determine whether the trends observed
measured PCO2 was observed to rise to levels above those
from tampons subsequently found to be highly loaded with
an intersection between O2 and CO2 profiles.
variation in these trends, profiles under these conditions show
mmHg). Although the profiles shown in Fig. 7 indicate some
approach the partial pressure measured in the vagina (50–65
mmHg).

Appreciably different O2 and CO2 profiles were observed from
tampons subsequently found to be highly loaded with
menses (>6.0 g), as shown in Fig. 7. In these cases, the
measured PO2 was found to drop to below 60 mmHg and the
measured PCO2 was observed to rise to levels above those
typically measured in the vagina during menstruation (50–65
mmHg). Although the profiles shown in Fig. 7 indicate some
variation in these trends, profiles under these conditions show
an intersection between O2 and CO2 profiles.

The data from these complex curves were subjected to
statistical analyses to determine whether the trends observed
were real. Multiple statistical models were used to evaluate the
data, and all models provided similar conclusions. The model
reported in Table 3 compared the changes in PO2 and PCO2
values at the beginning of the experiment to determine
whether the changes were real. The results of the statistical
analysis demonstrated that the PO2 and PCO2 levels in tampons
were significantly different between low load and high-load
tampons. The results allow us to discuss the implications of
these data.

Of particular interest are tampon O2 and CO2 profiles ob-
tained from a single participant on 2 consecutive days during
menstruation wearing different tampon products (see Fig. 8).
Intersections were observed in both cases, but the times at
which the intersections occurred varied from ~1 to 3 h after
the tampon-sensor device was inserted.

Table 3. Statistical modeling of tampon O2 and CO2 profiles

<table>
<thead>
<tr>
<th>Statistical Model</th>
<th>Product</th>
<th>Low Load</th>
<th>High load</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta in O2*</td>
<td>Both Types</td>
<td>18.610</td>
<td>87.891</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Tampax Regular</td>
<td>21.318</td>
<td>68.303</td>
<td>0.067</td>
</tr>
<tr>
<td></td>
<td>Kotex Super</td>
<td>15.903</td>
<td>107.478</td>
<td>0.022</td>
</tr>
<tr>
<td>Delta in CO2†</td>
<td>Both Types</td>
<td>-30.013</td>
<td>-61.305</td>
<td>0.037</td>
</tr>
<tr>
<td></td>
<td>Tampax Regular</td>
<td>-23.737</td>
<td>-54.549</td>
<td>0.065</td>
</tr>
<tr>
<td></td>
<td>Kotex Super</td>
<td>-36.290</td>
<td>-68.062</td>
<td>0.155</td>
</tr>
</tbody>
</table>

Values are adjusted means. Adjusted means were obtained by applying the
model (see METHODS for full description of model). P values ≤ 0.05 indicate
significant differences at the 95% confidence level. *Response is defined as
first value minus last value in O2 profile. †Response is defined as first value
minus last value in the CO2 profile.

Fig. 6. Typical O2 (solid lines) and CO2 (dashed lines) profiles obtained from
a sensor embedded within a tampon for 4 participants during menstrual
conditions (days 2 and 3). Subsequent to these evaluations, menses loading for
these tampons was found to be low (<3.5 g). Data shown include A:
participant 5, expt A (day 2), Tampax Regular, menses load = 2.172 g; B:
participant 5, expt D (day 3), Kotex Super, menses load = 2.89 g; C:
participant 7, expt B (day 3), Tampax Regular, menses load = 1.328 g; D:
participant 2, expt B (day 2), Tampax Regular, menses load = 3.509 g.

Fig. 7. Typical O2 (solid lines) and CO2 (dashed lines) profiles obtained from
a sensor embedded within a tampon for 4 participants during menstrual
conditions (days 2 and 3). Subsequent to these evaluations, menses loading for
these tampons was found to be high (>6.0 g) and to exceed the absorbency rating of
the tampon (Tampax Regular 6–9 g; Kotex Super 9–12 g). Data shown include A:
participant 5, expt C (day 2), Tampax Regular, menses loading = 12.645 g;
B: participant 9, expt B (day 3), Kotex Super, menses loading = 15.009 g; C:
participant 7, expt A (day 2), Kotex Super, menses loading = 16.842 g; D:
participant 1, expt A (day 2), Tampax Regular, menses loading = 10.752 g.

Fig. 8. O2 (solid lines) and CO2 (dashed lines) profiles obtained from partici-
patent 9 on 2 consecutive days during menstruation wearing different tampon
products. A: day 2 wearing Tampax Regular tampon found to contain 11.972
g of menses (absorbency rating 6–9 g); B: day 3 wearing Kotex Super found
to contain 15.009 g of menses (absorbency rating 9–12 g).

Downloaded from http://jap.physiology.org/ on July 12, 2017 by 10.220.3.6.
DISCUSSION

The microsensors used in this seminal work allowed simultaneous in vivo measurements of the PO$_2$ and the PCO$_2$ in the vagina and within tampons. Findings show the vaginal environment is anaerobic before tampon insertion, which is consistent with expectations and Wagner’s earlier work (43). In contrast to his findings, the process of tampon insertion was not observed to introduce a bolus of oxygen into the vaginal environment. In fact, surprisingly, measurements by two independent vaginal sensors showed decreases in the vaginal PO$_2$ on tampon insertion. The observed decrease in PO$_2$ was independent of tampon types used (Tampax Regular and Kotex Super) and the time of the cycle when the measurements were made (mid-cycle or days 2 and 3). Insertion of a tampon either increased or had little impact on the PCO$_2$ in the vaginal environment.

The mean values for PO$_2$ and PCO$_2$ in menstrual fluid measured in this study were similar (41.8 and 43.5 mmHg, respectively) and tended to be in the range expected for venous blood. The range of values observed across the entire sample base was greater than the range of either venous or arterial blood. These findings suggest that sensors can provide a source of oxygen to the vaginal environment. These findings may be important for genital diseases that affect women more frequently during menses such as pelvic inflammatory disease, cervical Chlamydia trachomatis, human immunodeficiency virus, and bacterial vaginosis as well as menstrual vaginal TSS (15, 16, 23, 33).

Despite differences in methods used for collection and analysis of menses, as well as differences in participant demographics, our findings are in general agreement with those reported by Wagner (42).

In later in vivo work, Wagner (43) concluded that tampon insertion resulted in the introduction of a bolus of oxygen and this was a major source of oxygen to the vaginal environment. Our in vivo results do not show tampon insertion to be a source of vaginal oxygen. Insertion of a tampon was found to decrease the PO$_2$ in the vagina.

Before tampon insertion, vaginal gas levels ranged from 15 to 35 mmHg for PO$_2$ and 35 to 55 mmHg for PCO$_2$. These values are lower and higher, respectively, than atmospheric levels (PO$_2$ = 100–140 and PCO$_2$ = 5 mmHg). Compared with Wagner’s (43) results, the absolute values reported here are higher for PO$_2$ (15–35 vs. 3 mmHg) and lower for PCO$_2$ (35–55 vs. 64 mmHg). The higher PO$_2$ could be due to the fact that the vast majority of the participants in this study had been pregnant, whereas none of the participants in Wagner’s study had been pregnant. Prior pregnancies could allow higher levels of O$_2$ to enter the introitus, which could account for the apparent higher PO$_2$ measured here. Nevertheless, these findings are generally consistent with expected anaerobic conditions in the vagina.

On insertion of a tampon, our results showed a decrease in the PO$_2$ in the vaginal environment. Insertion of a tampon either increased or had little impact on the PCO$_2$ in the vaginal environment. These findings are not in agreement with Wagner’s results (43). Wagner reported an increase in PO$_2$ from a mean value of 3–112 mmHg and a decrease in PCO$_2$ from 64 to 50 mmHg. Whereas several factors such as differences in participant demographics, measurement devices, and types of tampons evaluated could contribute to the conflicting findings, our analysis suggests that very small sensors that remain exclusively inside the vaginal environment are capable of monitoring changes in vaginal environment independent of changes at or near the tampon. The tampon sensor used in Wagner’s study is described as a tampon (Tampax Regular, o.b. normal, or Playtex Regular) with a Clark electrode attached. Wagner used the best technology available at the time, a Clark electrode, with a diameter of 24 mm, which is at least twice the diameter of the tampons used in that study (~10 mm). The electrode faces (i.e., measurement surfaces) were placed toward the tampon material and then inserted into the vagina. As a result, Wagner’s post-tampon insertion findings are more likely to correlate with findings from the tampon-sensor assembly used in this study.

After insertion into the vagina, tampon sensors used in this study showed initial PO$_2$ and PCO$_2$ to be consistent with atmospheric conditions. As the evaluation continued, the PCO$_2$ measured in the tampon generally increased, and the PO$_2$ in the tampon tended to decrease. In cases where the menses load was found to be extremely high, the individual gas profiles for O$_2$.
and CO₂ were found to intersect each other for both tampons evaluated in this study (Tampax Regular and Kotex Super). In other cases (nonmenstrual and low load), the individual profiles were not observed to intersect within the timeframe of these experiments. The wear times at which the intersections occurred were observed to vary from ~1 to 3 h and appeared to be related to the tampon absorbency and mensologies loading.

As hypothesized, the tampon sensor findings reported here are in some aspects consistent with post-tampon insertion results reported by Wagner (43). Wagner reported changes in gas profiles on tampon insertion similar to those observed here from tampon sensors; however, Wagner did not report intersections between O₂ and CO₂ profiles. This may be attributed to the relatively short duration of the majority of Wagner’s evaluations (90 min) and the narrow demographic profile of his participants (nursing students 22–24 yr of age, nulliparous, and exclusively of Northern European descent).

FISH/PCR analysis of selected tampons used in this study showed S. aureus to be present and evenly distributed throughout tampons whether worn menstrually or nonmenstrually. It is important to note that this approach identifies the presence of S. aureus, but cannot discriminate between toxigenic and nontoxigenic S. aureus strains. Vaginal colonization with S. aureus (both toxigenic and nontoxigenic) in healthy women has been reported infrequently. Vaginal carriage of these strains in 495 healthy menstruating women was found to be low (2.6% toxigenic vs. 4.0–5.2% nontoxigenic; Ref. 10). The majority of healthy women who are colonized with these strains also demonstrate measurable antitoxin titers (4).

As noted above, tampon sensors showed Pₒ₂ in the tampon tended to decrease and the P_cₒ₂ tended to increase over the course of the vaginal mapping experiments. Importantly, we did not measure a corresponding increase in Pₒ₂ in the vaginal environment; therefore, this consumption of O₂ and subsequent production of CO₂ measured within the tampons suggests that respiration may be occurring within the tampon. It has been demonstrated that vaginal bacteria can colonize the tampon during wear. It has also been shown that other microbes can colonize tampons during menstruation, and the microbial population usually reflects that which is normally cultured in the vagina (26). Vaginal colonization with S. aureus did not affect vaginal gas profiles in the subject who was culture positive for this organism. We did not observe intersections in the O₂ and CO₂ profiles for tampons with low menses load or for tampons worn nonmenstrually. It appears that near saturation of tampons with menses may be associated with increased bacterial respiration resulting in the intersection of the O₂ and CO₂ profiles within the timeframe of these experiments. The nonmenstrual O₂ and CO₂ profiles showed a trend that may suggest similar profiles after excessive wear times. There are multiple hypotheses as to why the respiration would be altered with tampon loading and/or longer wear times. For example, as the bacteria increase over time, a subsequent reduction in Pₒ₂ with a commensurate increase in P_cₒ₂ would be expected. As the vital nutrients are consumed due to bacterial growth, the respiration rate would lessen, explaining the shape of these curves. Superimposed on this would be the complex effects of the aerobes. We speculate that the accelerated rate of change in the O₂ and CO₂ profiles associated with saturated tampons is related to the enhanced nutrient medium that menses provides.

It is recognized that toxin production is enhanced when the organism becomes stressed due to nutrient depletion as well as enhanced when CO₂ levels rise. Although these data potentially provide theoretical underpinning for appropriate selection of wear time criteria, the association of mTSS with wear time has never been clearly elucidated in epidemiological studies (27).

The specific environmental conditions that promote the in vivo production of TSST-1 by toxigenic S. aureus are not known. Numerous in vitro studies have demonstrated the importance of Pₒ₂ in the regulation of TSST-1 production (29, 38, 44). It has also been shown that an increase in the P_cₒ₂ in the presence of O₂ can increase the rate of TSST-1 production under in vitro conditions (45). Other studies have shown that a neutral pH environment is necessary for TSST-1 production (31), which is the pH of menses (6.8–7.0; Ref. 1). Production of TSST-1 requires a nexus of tampon conditions, the absence of antibodies to TSST-1, and the presence of the appropriate S. aureus strain. It has been found that most women colonized with toxigenic S. aureus also have measurable antibody titers (4).

There are other factors known to affect gene regulation and expression that these results do not address. Nevertheless, it is possible to propose a putative model based on these findings that may help to understand the association of mTSS with menstruation and tampon usage. Before menstruation, the vaginal environment is anaerobic, and with a pH of about 4.5, neither condition is conducive to TSST-1 production. The presence of menses increases the pH of the vaginal environment and introduces a source of O₂ and CO₂. If toxigenic strains of S. aureus are present, conditions could be favorable for TSST-1 production. This might explain the association of mTSS with menstruation in the absence of tampon usage.

The insertion of the tampon introduces a new ecological niche within the vagina. Bacteria colonize the tampon and, as menses enter the tampon, the bacteria are provided with an O₂ and nutrient-rich environment. This environment, with a near neutral pH and increasing levels of CO₂ due to bacterial respiration, can lead to TSST-1 production. This putative model integrates the results of our in vivo studies with previous in vitro and epidemiological studies.

ACKNOWLEDGMENTS

We thank the following individuals for their important technical assistance: Michaele Jones (P&G) for the design and execution of the statistical analyses, Kathy Kerr (P&G) for the PCR work, and Bill Hood (P&G) for the vapor transmission analyses. We also thank Mark Anderson (P&G), Melanie Hansmann (P&G), and Anne Hochwait (P&G) for helpful comments. We also thank ALG Technical Communications for assistance in the preparation of this manuscript.

S. Z. Wang-Weigand is currently affiliated with Takeda Pharmaceuticals North America, Lincolnshire, IL. J. A. Flood is currently affiliated with The Procter & Gamble Company, Microscopy Section, Corporate Analytical Department, Corporate Research & Chemical Technologies Division, Cincinnati, OH 45252.

DISCLOSURES

This work was sponsored by The Procter & Gamble Company (P&G).

All authors of this manuscript, with the exception of Dr. Schlievert, are currently or have been employees of the Procter & Gamble Company. Dr. Schlievert declares a financial relationship with The Procter & Gamble Company.
Schlievert consulted with the group regarding the historical knowledge of human vaginal gas tensions, design of the studies, and development of the manuscript. He did not receive compensation for the work associated with this study.

REFERENCES


