GENERAL GYNECOLOGY

Contemporary perspectives on vaginal pH and lactobacilli

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The concept of pH as a measure of hydrogen ion concentration, and thus the acidity or alkalinity of a solution, was introduced by the Danish biochemist, Sorensen, in 1899. The term pH may be derived from the Latin, pondus hydroeni; the French potential hydrogen; or the British potential of hydrogen. Examples of pH values of various solutions are shown in Table 1.

Vaginal pH has been an enduring topic of interest in the areas of vaginal physiology and microbiology. Two historical factors combined to create this focus. First, the early beginnings of scientific research on vaginal physiology were in the antiseptic era of the early 20th century, so it was natural to presume that some antiseptic substance regulated the vaginal microflora. Second, the new availability of pH paper evoked an interest in pH testing of clinical specimens such as vaginal secretions. Scizly and Friedenthal, as described by Szabadvary, were the first to introduce colorimetric determination of pH with test papers in 1903. Early researchers presumed lactic acid produced by Lactobacillus was the likely antiseptic. It was theorized that the mildly acidic vaginal pH of around 4.5 provided this regulatory action.

At the same time, vaginal flora was being studied through microscopic examination, and the early work of Doderlein led to the belief that vaginal flora associated with asymptomatic reproductive-age women was composed of a single microbial entity, which became eponymically known as Doderlein’s bacillus. A corollary of the microscopic observations of Doderlein was that a pure vaginal culture sent was unhealthy. This observation was prophetic because in recent decades a morphologic scoring system (Nugent scoring) based on Lactobacillus morphotypes for vaginal flora has emerged, and its correlation with vaginal pH and abnormal flora has been widely reported. In essence, the Reinheitsgrad of Doderlein became the Nugent score of the contemporary practitioner.

The science of microbiology at its early stage had not evolved to the level at which the full extent of normal vaginal colonization with other acid-producing microbes was appreciated. Because the earliest view of vaginal flora was based simply on the microscopic observation of vaginal secretions, asymptomatic women were deemed to be populated with a single organism, based on Gram stain smears. The concept that vaginal health was due to a pure culture of Lactobacillus was intellectually appealing and logical and became firmly entrenched in the minds of physicians.

Microbiology research, particularly when the ability to grow bacteria on artificial media and isolate different species became possible, subsequently identified multiple species coexisting as components of healthy vaginal flora. Anaerobic culture technology provided the next innovation and revealed that previously unknown anaerobic organisms were also present in the healthy vagina.

The past decade has witnessed another expansion in vaginal flora microbiology as gene amplification methods to detect microbial DNA have identified difficult-to-culture organisms as components of the flora. Whereas information about the physiology (including substrates consumed and end products released into spent culture medium) can be studied for cultivable organisms, the contributions of the as-yet-uncultivated members of the bacterial flora remain to be determined.

Although lactic acid is the primary acid in normal vaginal secretions, other
vaginal fluid. Hydrogen peroxide may be detected in a laboratory culture of *Lactobacillus*, but the vaginal microenvironment ostensibly would mitigate against significant accumulation of $\text{H}_2\text{O}_2$. This is only compounded by the presence of catalase, a peroxide-degrading enzyme elaborated by several bacterial species typically present in the vagina and by peroxide-degrading systems in host epithelial cells. Thus, a significant amount of hydrogen peroxide is not likely to be produced in the vagina and, if it were to be produced in significant quantity, would not be expected to accumulate in the normal vaginal environment. A recent study has cast strong doubt on hydrogen peroxide having any in vivo physiological function in the vagina.\(^7\) It is often forgotten that a substantial body of work was done in the early decades of the 20th century in an attempt to elucidate the connection between vaginal pH and vaginal microbiology. A series of studies by Weinstein and coworkers\(^8\) explored vaginal pH in monkeys, adult humans, infants, and postmenopausal women. They discovered that the presence of estrogen, and not *Lactobacillus*, was related to the acidic vaginal pH.\(^9\) In addition, the cervical canal has a nearly neutral pH and yet has a flora that is characterized by *Lactobacillus*, much as in the lower portions of the vagina.

**Vaginal pH and the microbial inhabitants of the vagina**

Although the pH of the vagina undoubtedly influences the composition of the microbial ecosystem at that site, the magnitude of an acidic vaginal pH in effecting protection from invasion by pathogenic microorganisms, especially those that are sexually transmitted, remains an open question. Clinical vaginal infections by *Candida* species readily occur at a pH of 4.5 or less. It is also not generally acknowledged that sexually transmitted pathogens are acquired by transfer of the male ejaculate and its microbial contents into the vagina. Seminal fluid also contains enzymes that inactivate hydrogen peroxide.

The stomach may be considered as an example of acid pH so harsh that there is little doubt that bacteria reaching the stomach are inhibited on the basis of pH. But *Helicobacter pylori* is able to survive stomach acid, not by neutralizing the entire stomach but by creating a zone of ammonia around the bacterium that protects it from the larger environment.\(^10\) Thus, it is critical to understand the concept of microenvironments that may represent islands of hospitality for particular microbial species in a sea of inimical conditions.

**Vaginal mucosa vs *Lactobacillus* as the source of acidic pH**

The vaginal mucosa of reproductive-age women is known to be an important source of lactic acid in the vaginal lumen. With its limited blood supply, the human epithelium must depend on diffusion of glucose, oxygen, and various essential nutrients from the underlying submucosal tissues. This metabolically precarious situation results in a relatively anaerobic environment within the vaginal mucosa. Thus, lactic acid is a significant metabolic byproduct of obligate anaerobic glucose metabolism within the vaginal mucosa. Consistent with this state of relative metabolic jeopardy, epithelial cells are among the few cells in the body that do not require insulin for glucose uptake.\(^11\) Epithelial cells have insulin receptors that influence metabolism, but glucose has been shown to diffuse into epithelial cells in the absence of insulin.\(^12\)

Vaginal mucosa undergoes recurring cycles of proliferation at the basal layer, maturation, and desquamation into the vaginal lumen, with a turnover time of about 96 hours. Normal epithelium is highly metabolically active and may produce hundreds of different substances.\(^13\) Energy for this process is provided by glucose metabolism. Glucose is stored in vaginal mucosal cells as glycogen. When cells need energy, the glycogen is converted to glucose. Under anaerobic conditions, glucose is metabolized to pyruvic acid and energy equivalents in the form of adenosine diphosphate reduction by hydrogen transfer and then to lactate.\(^14\) Lactic acid diffuses outside the

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**TABLE 1**

**Examples of pH values**

<table>
<thead>
<tr>
<th>Solution</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric acid</td>
<td>1.5-2.0</td>
</tr>
<tr>
<td>Vinegar</td>
<td>2.9</td>
</tr>
<tr>
<td>Orange juice</td>
<td>3.5</td>
</tr>
<tr>
<td>Beer</td>
<td>4.5</td>
</tr>
<tr>
<td>Vaginal fluid(^a)</td>
<td>4.5</td>
</tr>
<tr>
<td>Skin surface moisture</td>
<td>4.0-5.5</td>
</tr>
<tr>
<td>Milk</td>
<td>6.5</td>
</tr>
<tr>
<td>Pure water</td>
<td>7.0</td>
</tr>
<tr>
<td>Saliva</td>
<td>6.5-7.4</td>
</tr>
<tr>
<td>Semen</td>
<td>7.2-8.0</td>
</tr>
<tr>
<td>Blood</td>
<td>7.3-7.5</td>
</tr>
<tr>
<td>Seawater</td>
<td>7.7-8.3</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>8.4</td>
</tr>
<tr>
<td>Hand soap solution</td>
<td>9.0-10.0</td>
</tr>
<tr>
<td>Bleach</td>
<td>12.5</td>
</tr>
</tbody>
</table>

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\(^a\) In reproductive-age women.

cells and accumulates in the extracellular milieu.

Glycogen metabolism and the enzyme glycogen synthetase are greatest in the intermediate vaginal epithelium cell layer. Development of the vaginal intermediate cell layer and its metabolic activity is estrogen dependent. The metabolically active intermediate layer presumably is the chief source of mucosal lactate as well as the production site for the hundreds of metabolic substances that are produced within the mucosa.

_Lactobacillus_ species that predominate in the vagina of reproductive-age women also metabolize extracellular glycogen into lactic acid by anaerobic glycolysis. Actually any microorganism that is capable of anaerobic glycolysis would release lactic acid into the vaginal lumen. A wide range of non-Lactobacillus microorganisms have been identified that presumably contribute to vaginal acid production. Fermentation patterns may have lactate as a predominant endproduct (as in homolactic fermentation); however, there are many organisms that engage in a so-called mixed acid fermentation in which other organic acids are produced along with lactic acid. Thus, hydronium ions may be contributed by various organic acids and not just lactic acid.

The pH range of the normal vagina actually may not be ideal for _Lactobacillus_ anaerobic metabolism. For example, _L. salivarius_ grows best at a pH of 6.5. Similarly, a study of _L. plantarum_ showed the greatest growth at pH 6 and substantially restricted growth at pH 4. The pH optimum for growth and lactic acid production by all typical vaginal strains remains to be evaluated but may not be the generally accepted vaginal optimal pH of 4.5.

A mucosal source of vaginal lactate has been known since the 1930s. The final vaginal pH is determined by the sum total of lactic acid production by epithelial cells and the acid contribution from the endogenous bacterial flora. The relative contribution of vaginal mucocellular and bacterial microflora to vaginal pH is controlled by unique circumstances in each female. When the vagina is not colonized with _Lactobacillus_, the vaginal pH may still be in the acidic pH range.

The newborn infant demonstrates that _Lactobacillus_ is not essential for an acid pH. Prior to colonization with any microorganisms, a vaginal pH around 5 is found in the newborn. Vaginal mucosal anaerobic metabolism declines as the influence of maternal estrogen is lost. Simultaneously the vaginal pH of the infant rises to the neutral range by 6 weeks of life. After puberty, estrogen again stimulates glycogen-dependent mucosal metabolism and _Lactobacillus_ and other acid-producing bacteria colonize the vagina. The more neutral menopausal vaginal pH is associated with diminished glucose metabolism in the intermediate cell layer of the mucosa as well as with the absence of _Lactobacillus_. Similarly, the loss of vaginal _Lactobacillus_ also is accompanied by a marked elevation in vaginal pH.

Epithelial cells produce only the L-isomer of lactate. Bacteria may produce both D- and L-isomers. In a study of vaginal lactate production in 11 women, Boskey et al concluded that the greater portion of D-lactate over L-lactate in vaginal secretions of most of the study participants supported the belief of a primary role of _Lactobacillus_ in the production of vaginal lactic acid. In this small study, the proportion of microbial D-lactate in vaginal secretions ranged from 6% to 75%, with a mean of 55%. This wide range does not clearly show a dominant microbial source of lactate in all women. Boskey et al further suggested that active mucosal transport of protons into the vaginal lumen would be necessary to overcome this D- to L-lactate imbalance for the vaginal epithelium to be the primary source of hydrogen ions.

Gorodeski et al subsequently showed that under the influence of estrogen, superficial vaginal mucocellular cells actually do secrete hydrogen ions into the vaginal lumen in a manner similar to gastric chief cells. At the time of ovulation, there is a transient decline in the vaginal pH. It is unlikely, although not impossible, that this transient fall in pH is due to acute changes in _Lactobacilli_ metabolism or growth. Vaginal lactic acid concentration is greatest from 48 hours prior to the luteinizing hormone surge until 24 hours afterward. The pH decline at this time is most likely hormone mediated and could be related to increased mucosal metabolic activity, rather than to changes in the lactobacillus population. The observation that the pH at the vaginal fornix is lower than in the midportion of the vagina, whereas the _Lactobacilli_ concentration is uniform throughout the vaginal canal suggests that the vaginal mucosal metabolism may be more consistently dominant in determining the final pH.

Vaginal mucosal immune interaction with microbes

In recent years, research has disclosed significant immune capability of the female genital tract to regulate vaginal microorganisms. PubMed lists more than 3000 research papers that have been published in recent years associated with the topic of vaginal immunology. Although immunology of the female genital tract remains a complex subject, comprehensive reviews have been published. Recent research has led to the identification of innate immune system antimicrobial components such as Toll-like receptors, mannose-binding lectin, secretory leukocyte protease inhibitor, surfactant protein A, cytokines, and lactoferrin that are present in the vagina.

The lower female genital tract, particularly the endocervix, is also capable of producing antimicrobial immunoglobulin A antibodies that diffuse into vaginal secretions. The major elements of vaginal antimicrobial innate immune system defense are listed in Table 2. The vaginal mucosal immune response is also influenced by estrogen. Thus, it is clear that an acidic vaginal pH and the presence of _Lactobacilli_ are components of a multifaceted antimicrobial defense system operative in vaginal fluid during the reproductive years.

Influence of pH on the vaginal epithelium

As a consequence of a relatively anaerobic environment within the mucosa, a mildly acidic intraepithelial pH reflects normal skin metabolic activity, including the production of various substances that are important for vaginal immune
defense. In keratinized epithelium, stratum corneum acidity within the epithelium is known to be essential for a normal inflammatory response and optimal skin barrier function.

This observation most likely also applies to vaginal mucosal epithelium. It is not surprising that the normal surface pH, or “acid mantle of the skin,” is reflected by a pH in the range of 4-5.5 that is similar to normal vaginal pH. The rise in vaginal pH after menopause is associated with a loss of natural skin defenses and an increased rate of colonization with microbial pathogens in the vagina and urinary tract.

Topical application of estrogen to the menopausal vagina restores a normal low surface pH and lowers the risk for infection. A neutral surface pH is harmful to the epithelium. Application of neutral pH buffers to the skin compromises stratum corneum integrity and cohesion. Consistent with this observation, the menopausal rise in pH results in defective vulvar skin enzyme function and intraepithelial ceramide deficiency. This leads to an increased vulvar susceptibility to contact dermatitis. It is reasonable to presume that the vaginal mucosa is similarly compromised by the neutral pH of vaginal fluid after the menopause.

### Clinical value of vaginal pH testing

Whatever the mechanism for its occurrence, measurement of vaginal pH can be of clinical value as an initial screening tool in assessing altered vaginal physiology. A change to an elevated vaginal pH in an individual woman in the absence of recent exposure to semen, menstruation, or exogenous products indicates an alteration of her vaginal ecosystem. This might signify a transition to a bacterial vaginosis (BV)-like flora, the presence of aerobic vaginitis, or a sexually transmitted infection such as Chlamydia trachomatis, Neisseria gonorrhoeae, or Trichomonas vaginalis. Conversely, the presence of a vaginal discharge, pain and/or pruritus in the presence of a normal vaginal pH might indicate a Candida infection or a noninfectious etiology.

In settings in which vaginal microscopic examination or microbial culture is not available, vaginal pH determinations may be especially valuable in the initial assessment of possible causes of clinical symptoms. An elevation in vaginal pH during pregnancy may indicate bacterial vaginositis or a Trichomonas infection, risk factors for preterm birth. The intriguing work of Hoyme and Saling demonstrated that self-detection of an elevated vaginal pH by pregnant women, followed by a prompt follow-up consultation and treatment by her physician if the vaginal flora is altered, effectively lowered the rate of premature birth.

### Future directions

Whereas an alteration in vaginal pH to less acidic values is strongly associated with vaginal flora changes it remains unclear in some situations whether the pH change is a primary event or a secondary consequence of an altered microbial ecosystem. *T. vaginalis*, *C. trachomatis*, and *N. gonorrhoeae* infections induce host cell changes (cervical bleeding or transmucosal water loss) and thereby secondarily raise the vaginal pH. Vaginal infections by *Candida* species do not affect the underlying vaginal epithelium and do not result in an altered vaginal pH.

A perplexing unresolved condition is the alteration in vaginal flora known as BV. In BV, there is a loss or diminution in vaginal Lactobacilli and a concomitant large increase in the concentration of anaerobic bacilli, *Gardnerella vaginalis*, and mycoplasmas. This is accompanied by an increase in vaginal pH to a value greater than 5.0. Whether the elevated pH in BV is a consequence of an altered flora that negatively influences epithelial cell glycogen metabolism or solely due to the loss of Lactobacilli remains undetermined.

The physiological factors that result in specific organisms colonizing the vagina and the associated consequences remain to be elucidated. A recent comprehensive study has demonstrated that the vaginal pH of healthy reproductive-age women may also vary by ethnicity. The mean vaginal pH of Hispanic and black women was greater than 4.5, whereas that of white and Asian women was less than 4.5.

A complex biological system is operative in the vagina: an epithelium that produces lactic acid, bacteria that produce lactic acid and other acids, individual differences in strains of colonizing lactobacilli and other components of the vaginal flora, variations in genetic background that influence levels of immune mediators and metabolic enzymes and individual environmental and dietary exposures.

Additional observations are still needed to adequately define the breadth of variables that exist in the vaginal ecosystem. For example, it would be of interest to evaluate associations between genetic polymorphisms in the genes responsible for lactic acid production and vaginal pH in women with comparable vaginal Lactobacilli species and concentrations.

Viewing the vaginal tract as a single environment also ignores the possibility that it may contain microniches, functionally different from what one might predict by in vitro cultures of bacteria in artificial media at various pH values. Further research on the individual components of the vaginal ecosystem and microbial adaptations to, and effects on, vaginal pH and their influence on reproductive health remain to be determined.

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**TABLE 2**

Major elements of the antimicrobial innate immune system in the vagina

<table>
<thead>
<tr>
<th>Element</th>
<th></th>
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<tbody>
<tr>
<td>1. Secretory leukocyte protease inhibitor</td>
<td></td>
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<tr>
<td>2. Mannose-binding lectin</td>
<td></td>
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<tr>
<td>3. Lactoferrin</td>
<td></td>
</tr>
<tr>
<td>4. Complement</td>
<td></td>
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<tr>
<td>5. Surfactant protein A</td>
<td></td>
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<tr>
<td>6. β-Defensins</td>
<td></td>
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<tr>
<td>7. Nitric oxide</td>
<td></td>
</tr>
<tr>
<td>8. Toll-like receptors</td>
<td></td>
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<tr>
<td>9. Heat shock proteins</td>
<td></td>
</tr>
</tbody>
</table>

See Nasu and Narahara for details and primary references.

Summary

History presents Lactobacillus as the prominent determinant of vaginal pH as well as a major regulatory factor of microbial composition of the vaginal flora. This reductionist thinking negates the complexities of mucosal metabolism, immune defenses, and microbial colonization. Contemporary research suggests that classic ideas about pH and vaginal flora are not entirely correct. Acid pH may be influenced by estrogen-regulated mucosal metabolism to a greater extent than glycogen-mediated microbial metabolism. Vaginal acidity may actually be more important for normal mucosal function than for inhibition of potential pathogens or regulation of normal flora. The complex vaginal immune system may be the chief regulator of microflora.

REFERENCES