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The Vagina

Morphological, Functional and Ecological aspects

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Umeå University
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Morfological, functional and ecological aspects

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Abstract

The vagina is one organ of the body which has not been studied exhaustively. Moreover, most of the studies found in the contemporary literature have been performed on women affected by a variety of genital diseases.

In the present study the vaginal epithelium was examined with a histological method, morphometry, whereby cyclical changes related to hormonal variation during the menstrual cycle were demonstrated. Determination of the quantity of estrogen receptors in the vaginal epithelium on two occasions during the menstrual cycle revealed a significantly greater number in the follicular than in the luteal phase. The results of these studies indicate the presence of a menstrual variation in the vaginal epithelium comparable to that in the endometrium.

Phenoxymethylpenicillin (pcV) was used as a marker substance to study the dynamics of the transport mechanisms into the vagina. PcV was found to accumulate in the vaginal fluid and high concentrations persisted for a long period of time. In hysterectomized women, the appearance of pcV in the vaginal fluid followed the same pattern. Consequently, the substance is transported through the vaginal wall and need not enter with the secretions from the internal genitalia. The greatest concentration of pcV was in the distal portion of the vagina, possibly due to the specific internal circulation of fluid within the vagina.

Bacterial vaginosis as an example of an 'ecological disease' has been studied with regard to the formation of endotoxin, a constituent of the cell wall of Gram-negative bacteria. Large amounts of endotoxin were found and the clinical implication of this finding has been pointed out.

Furthermore, the influence of pcV on the vaginal microbial flora of healthy women has been investigated. A change from a situation with predominance of lactobacilli to the appearance of Gram-negative rods was observed. In one of the women the lactobacilli disappeared completely and were replaced by *E. coli* and high levels of endotoxin in the vaginal fluid were found. This study demonstrates the complexity of the ecological balance of the vaginal microbial flora and illustrates the difficulty of defining a 'normal' vaginal condition. Is there any unquestionable state of 'normality' even in a healthy woman free from symptoms of genital disease?

Key words: vagina, vaginal epithelium, vaginal fluid, morphometry, bacterial ecology, endotoxins, vaginosis.

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Morphological, Functional and Ecological Aspects

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*To Ruth,
my mother.*

ORIGINAL PAPERS

This thesis is based on the following papers, which will be referred in the text by their roman numerals.

- I. Sjöberg I, Cajander S, Rylander E. Morphometric characteristics of the vaginal epithelium during the menstrual cycle. *Gynecol Obstet Invest* 1988; 26: 136–44.
- II. Sjöberg I, Rylander E, von Schoultz B. Menstrual variation of estrogen receptor content in vaginal tissue. *Gynecol Obstet Invest* 1989; 27: 48–51.
- III. Sjöberg I, Håkansson S, Holm S. Accumulation of penicillin in vaginal fluid. *Obstet Gynecol* 1990; 75: 18–21.
- IV. Sjöberg I, Håkansson S. Excretion of phenoxymethylpenicillin in the vaginal fluid of hysterectomized women. *Acta Obstet Gynecol Scand* 1991 (in press).
- V. Sjöberg I, Håkansson S. Endotoxin in vaginal fluid of women with bacterial vaginosis. *Obstet Gynecol* 1991; 77: 265–6.
- VI. Sjöberg I, Grahn E, Håkansson S, Holm S. Influence of phenoxymethylpenicillin on the vaginal ecosystem. Manuscript.

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Key words: vagina, vaginal epithelium, vaginal fluid, morphometry, bacterial ecology, endotoxins, vaginosis.

INTRODUCTION

In contrast to most organs of the body, the vagina has not been studied exhaustively. It is the channel of birth and a local for sexual activity, but is seldom considered as an 'organ' in isolation. Probably for this very reason, not even the histology of the vaginal wall has been studied in depth. Perhaps this can be explained by the fact that it seems rather simple and obvious: ordinary, not cornified squamous epithelium.

Early this century, many histological studies were performed, but the results were equivocal, since most of the observations were disparate and not systematically related to the phases of the menstrual cycle. Furthermore, the histological techniques used permitted only a subjective assessment of the appearance of the epithelium (1–5). This kind of research was rendered obsolete by the introduction of cytological techniques. It was then demonstrated with certainty that the histology of the vaginal wall undergoes a cyclical variation (6).

Infections with human papilloma virus have attracted renewed interest in the morphology of the vaginal epithelium. This virus causes one of the most common of sexually transmitted diseases, condylomata. The diagnosis is established by colposcopy and histological examination of biopsy material (7). Therefore, a thorough knowledge of the normal morphology of the vaginal epithelium is fundamental. More sophisticated techniques to determine viral DNA now exist, but they will not be used in clinical routine for many years to come.

The presence of estrogen receptors in the vagina is another subject of interest that has not been studied until recently. Estrogen receptors and their high concentrations in the lower genital tract have been known for 30 years (8), but not until 10 years ago were they determined for the first time in the vaginal epithelium (9). Only a few studies on this issue have yet been performed. Some authors were unable to localize estrogen receptors in all biopsy samples (10), while others demonstrated a more widespread presence (9,11). There has also been disagreement in the past as to whether the number of receptors varies during the menstrual cycle (12,13). These receptor studies have been performed in patients with various diseases and without a prospective design regarding the menstrual phase. Since they have been based on radioligand-binding assays, interference with endogenous, unspecific binding of estrogen has not been excluded (8). Also, regarding the estrogen receptors, it is conceivable that there is a menstrual variation in relation to the amount of circulating estrogen as demonstrated in the endometrium.

The vagina is a passive receptacle of the secretions from the fallopian tubes, the endometrium and the cervix. The most distal parts may also be influenced by the secretions of the glands of Skene and Bartolin. The bulk of the vaginal fluid is a transudate through the vaginal wall via the intercellular spaces (14). Almost all the fluid is reabsorbed most distally (15), but in infectious conditions (e.g. salpingitis, endometritis, cervicitis and vaginitis) the amount of secretion may exceed the reabsorption capacity and will be noticed by those affected as a vaginal discharge.

The passage of antibiotics into the vaginal lumen is well known because of their adverse clinical effect in the form of vaginitis. This may be caused by an imbalance of the microbial flora. Such a disturbed ecology may also have an indirect growth-promoting effect, for example on *Candida albicans* (16). On the other hand, despite

prolonged treatment with penicillin, group-B-streptococci may persist in the vaginal flora (17), even though these are highly sensitive to penicillin *in vitro* (18).

The following questions arise: how high for example will the concentration of phenoxymethylpenicillin (pcV) be in the vaginal fluid during ordinary peroral treatment? Is the excretion dependent on the hormonal state? What are the effects on the vaginal microflora?

In the vaginal fluid, bactericidal concentrations of trimethoprim—but not of sulphamethoxazole—were found in a study performed by Stamey and Condry (19). Excretion was protracted—at least 36 hours. Thin et al. detected high concentrations of oxytetracycline in vaginal discharge (20). Both these studies were performed on patients with a variety of infections. Furthermore, the patient materials have been heterogenous regarding age, hormonal state and contraceptive use and there have only been sporadic observations during the treatment. To our knowledge, no study based on an investigation in healthy individuals is to be found in the literature.

The vaginal fluid is a modified plasma transudate (14) that can pass very quickly into the lumen (21), most likely via the intercellular spaces (22). It is conceivable that antibiotics and other substances may also pass this way. Stamey and Condry also drew this conclusion, since they found trimethoprim in the vaginal fluid in three hysterectomized women (19).

Moreover, it has never been established whether there are concentration differences along the length of the vagina. Hitherto, techniques have been used whereby the whole vagina was rinsed out and the concentration in the exchange was measured (19). Such an approach excludes the possibility of detecting variations in concentration. Along the vagina, there are in fact indications that this may be the case:

- a. Vaginal fluid flows in the distal direction and reabsorption takes place most distally (15).
- b. The embryological origin is different, the distal part of the vagina being entodermal, while the upper part together with the urethra is mesodermal, and in consequence the vascular supply originates from different sources. For the upper part it stems from the uterine artery, while the lower part is supplied by branches of the internal pudendal artery (23).

The vaginal bacterial flora is composed of a large number of organisms of diverse genera and species. Their coexistence and interaction create an ecological system of the utmost complexity (24). This complicates studies on ecological changes and different experimental study designs are needed in order to understand the transition from normal to pathological conditions. For example, in bacterial vaginosis, age, extent of sexual activity, and use of an IUD are all factors associated with the emergence of the disease (25,26), but the exact mechanism which triggers the ecological change has not been defined. In a recent review article, Sobel eloquently summarizes bacterial vaginosis as an 'ecological mystery' (27).

Bacterial vaginosis was long considered to be a disagreeable but harmless disorder. However, during the 1980s several studies have shown a possible connection between bacterial vaginosis and deep gynecological and post-hysterectomy infections (28,29) as well as urinary infections (30). An even stronger relation to postpartum infections—and above all to prematurity—has also been found (31). The ecological shift from a lactobacillus-dominated flora to a situation with predominance of Gram-negative bacteria may result in the production of endotoxins which in turn can activate the synthesis of prostaglandins (32). Endotoxins are biologically potent

lipopolysaccharides, produced by Gram-negative bacteria as a constituent of the outer membrane of the cell wall. The production of this substance could constitute a possible link between bacterial vaginosis and premature labour.

The excretion of antibiotics via the vaginal fluid must arguably influence its bacterial composition. Bacterial vaginosis has actually become commonplace during the same era as antibiotics have revolutionized the treatment of infections (31,33).

A study of the influence of antibiotics on the vaginal flora calls for a definition of 'normal' conditions. The difficulty connected with such an attempt is underlined in the excellent review by Redondo-Lopez et al (34). These authors find reasons to criticise most earlier studies on the normal vaginal flora. Yet the following facts cannot be disputed: there must be a predominance of lactobacilli, no prominent presence of Gram-negative bacteria, and absence of fungi.

AIMS OF THE STUDY

The aims of the study were to investigate:

- the changes occurring in the vaginal epithelium during the menstrual cycle, by undertaking a longitudinal and prospective study in healthy, regularly menstruating women.
- the pattern of appearance in the vaginal fluid of a marker substance, phenoxymethylpenicillin, in healthy women with normal ovarian function.
- whether endotoxin is produced in the vaginal fluid of women with bacterial vaginosis.
- the ecological changes in the vaginal flora caused by the presence of phenoxymethylpenicillin in healthy subjects.

PRESENT INVESTIGATIONS

Morphological structure

Specimens were taken every third day during a menstrual cycle from 5 healthy, regularly menstruating women between 28 and 47 years of age. No subject had used hormonal contraceptives within at least one year preceding the study. The biopsy samples were taken under colposcopic guidance from the fornices, clockwise around the cervix, fixed in buffered formalin and embedded in glycol methacrylate. Three sections were obtained from each sample and stained with hematoxylin and eosin.

A total of 159 sections from 53 biopsies were analysed by morphometry. The thickness of the epithelium is variable and is sometimes cut tangentially. Efforts were therefore made to measure the thinnest part with the basement membrane parallel to one side of the square that was being measured. This corresponded to approximately 150 cells. The area of each cell nucleus was measured and the mean nuclear area for each day was calculated.

The mean nuclear area was correlated to the caryopyknotic index. Determination of menstrual phase was performed by measuring of estradiol and progesterone concentrations in serum.

By using this technique, a cyclical variation was found in 3 of the subjects with higher peak values during the follicular phase. This variation was also present (though less conspicuous) in the fourth subject, while in the fifth there was no variation at all. The caryopyknotic index showed a normal variation in all but the fifth subject. The hormonal assay revealed a normal pattern of ovulation in all 5 women.

Estrogen receptor content

Multiple epithelial biopsies to a total volume of approximately 0.5 cm^3 were obtained from the lateral vaginal fornices of 12 regularly menstruating women on cycle days 10 and 21. The women were between 25 and 35 years of age and had no history of gynecological disorders. The specimens were immediately placed in store at -70°C until use and all were run in the same assay.

Estrogen receptors (ER) were quantified using monoclonal antibodies in a solid-phase enzyme immunoassay based on the 'sandwich' principle. The sensitivity of this method is approximately $1.5 \text{ fmol ER/mg cytosol protein}$. The menstrual phase was determined by measuring estradiol and progesterone concentrations in serum.

Measurable quantities of estrogen receptor were found in all the women. In 8 women in whom the hormone measurement confirmed an ovulatory menstrual cycle, there was a significant fall in estrogen receptor content from the follicular to the luteal phase. The statistical method used was Wilcoxon's rank sum test for paired observations.

Accumulation of penicillin in the vaginal fluid

Ten healthy, regularly menstruating women between 25 and 36 years of age were enrolled in the study. None of the subjects took any medication during the study, nor

did any subject use hormonal contraceptives or an IUD. During the study period, all subjects refrained from sexual intercourse.

In the first part of the investigation, 5 of the women were given pcV (1g) on two occasions during a menstrual cycle (days 10 and 21) and the excretion of pcV was measured at regular intervals during 3 h. A final measurement was made after 15 h. Excretion of penicillin in the vagina was correlated to that in saliva and the concentration in serum was also determined.

In the second part of the study, the 5 remaining women were given 1g of pcV twice a day for 10 days. The excretion of pcV was measured twice during the first day and once around noon on days 3, 5, 7, 9, 11, 12, 13. The serum concentration was measured on the morning of day 9.

The specimens were collected on paper discs, 5 mm in diameter which absorbed the vaginal fluid. Each disc was prepared in advance, inserted into a small plastic tube which was sealed, and weighed. The discs were placed in the posterior fornix for a period of 15 min. During part one of the study, the discs were returned to the tube, again weighed, and immediately assayed for penicillin content. During the second part, the discs were stored in their respective tubes at -70°C . After thawing and equilibration at room temperature, these tubes were weighed and the discs assayed. Thus, for each disc the amount of absorbed material was determined, ranging from 5–25 mg/disc.

The amount of penicillin in the fluid absorbed by the paper disc was determined by a microbiologic agar diffusion method, with a strain of *Bacillus stearothermophilus* as a test organism incorporated in the agar.

After ingestion of a single dose of 1g pcV, the concentration of penicillin slowly increased during the period to reach a mean value of 0.32 mg/l at 3 h. After 15 h the concentration was markedly higher, close to 1.2 mg/l. The concentration in serum and saliva reached a maximum value after 1.5 h. After 15 h no trace of the substance could be detected in either serum or saliva. The menstrual phase did not affect the excretion of penicillin into the vaginal fluid.

During the long-term treatment, high concentrations of penicillin were found in vaginal fluid after 6 h and were maintained throughout the medication period (mean range 2–3 mg/l). After the medication was stopped, considerable amounts of penicillin were still detected on day 11, but on days 12 and 13 no detectable amounts of penicillin were present in the vagina.

Excretion of penicillin in the vaginal fluid of hysterectomized women

A total of 19 hysterectomized women were examined. Eight had undergone a total and 11 a subtotal operation. They were given 1g pcV at 7 o'clock a.m. while fasting, and one hour later the serum penicillin concentration was determined, followed 6 h later by the determination of penicillin excretion in the vaginal fluid. Filter papers were placed in the fornix and in the folds of Shaw, near the urethra. The penicillin concentration was measured as described earlier.

Penicillin was excreted in the same range as reported earlier for the normally menstruating women. There was a significant difference between the amounts excreted proximally in the vagina and those most distally. The concentration in the

distal part of the vagina was 3–6-fold higher than that of the fornix. Statistical evaluation of the concentration differences obtained for the upper and lower parts of the vagina was performed with Wilcoxon's signed rank sum test for paired observations.

Endotoxin in bacterial vaginosis.

Vaginal fluid was obtained from 19 women with complaints of pathological discharge who fulfilled the criteria of bacterial vaginosis (i.e. homogenous grey-white discharge, positive amine test, clue cells in the wet smear and pH >4.8) and from 9 healthy controls. The specimens were collected with the same type of sterile paper disc as previously described and analysed for the content of endotoxin with a test that uses the capacity of endotoxins to activate an enzyme system in the circulating amoebocytes of the horseshoe crab (paper V). Briefly, 100 µl of the suspension was incubated with 100 µl of the limulus amoebocyte lysate reagent for 10 min at 37°C, then mixed with 200 µl of a chromogenic substrate and incubated for 3 min at 37°C. The reaction was stopped by adding of 200 µl acetic acid. The colour developed was read on a spectrophotometer at 405 nM, expressing the results as endotoxin units (EU) per millilitre. The results were correlated to a standard curve and the amount of endotoxin per milligram of absorbed vaginal fluid was calculated. For statistical evaluation, the two-tailed Wilcoxon rank sum test was used.

The mean value of endotoxin in the bacterial vaginosis specimens was about 40 times that of the controls ($p < 0.001$). Among the vaginosis specimens there was a considerable variation. This was not found in the controls.

Ecological study

From 14 healthy, regularly menstruating women a vaginal culture and a wet smear were obtained. Vaginal pH was also determined. If the vaginal flora was normal (i.e. predominance of lactobacilli, absence of Enterobacteriaceae and fungi), as well as the wet smear and pH, the same procedure was repeated late in the same menstrual cycle and once again early in the following menstrual cycle.

Six out of 14 subjects fulfilled the criteria of normality at the three first examinations and they began peroral intake of pcV (1g twice daily) for 10 days. On the last day of medication the study was completed with a fourth vaginal examination. On the third and fourth occasions, samples of vaginal fluid were also obtained for determination of endotoxin and pcV.

Vaginal cultures were obtained with a cotton swab which was placed in 1 ml of anaerobic culture broth. This specimen was serially diluted and from each dilution, 100 µl was plated in duplicate on blood agar plates containing pcV, and on Rogosa and Sabouraud agar plates.

Bacteria were quantitated on the basis of colony morphology and expressed as colony forming units (CFU)/ml in the undiluted medium. Bacteria with fewer than 10^3 CFU/ml were not processed further. All other organisms were Gram-stained and isolated in aerobic, anaerobic, and microaerophilic atmospheres at 37°C.

Lactobacillus strains recovered from the third and fourth vaginal cultures were tested for the production of hydrogen peroxide. The *in vitro* growth interference with isolated strains of *Enterobacteriaceae* sp. was investigated with an agar overlay tech-

nique under anaerobic and microaerophilic conditions. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of pcV on the *Lactobacillus* isolates were determined by conventional technique using a dilution series of pcV in MRS-medium. PcV and endotoxin were determined as previously described.

In 4 of the 6 subjects, Gram-negative rods (*E.coli* or *K. oxytoca*) appeared in significant numbers in the vaginal flora. In one women heavy growth of *C. albicans* was also induced. In 2 subjects, no changes in the flora were observed. The number of lactobacilli was not reduced in 5 of the subjects, though in one other person, they disappeared completely and an overgrowth of *E. coli* was found.

In all subjects, vaginal pH was normal on the three consecutive occasions before intake of pcV. On the fourth occasion, a pathological pH of 5.3 or more was found in 4 subjects, whereas the other 2 were still normal. The concentration of pcV in vaginal fluid was in all cases >1mg/l.

The endotoxin content of vaginal fluid was low in all subjects before pcV intake. In 5 persons the levels were unchanged after medication. However, in the one subject with heavy growth of *E.coli*, there was a marked increase in the endotoxin concentration.

Hydrogen peroxide was produced in seven of the 15 isolates of *Lactobacillus*. Two isolates, recovered from the same subject, effectively inhibited the growth of all four isolates of Enterobacteriaceae obtained in CO₂-atmosphere. Under anaerobic conditions, however, no inhibition was found. These isolates proved negative for production of hydrogen peroxide.

The MIC values of pcV of the *Lactobacillus* isolates varied between 0.05 and 0.2 mg/ml. There was no tendency to increasing resistance to pcV after exposure of the drug. From 2 subjects, three isolates showed a paradoxical response to pcV, i.e. regained growth at increasing pcV concentrations after initial inhibition. This phenomenon was the same before and after pcV influence. One isolate was tolerant to pcV.

GENERAL DISCUSSION

Much of our knowledge of the vagina is based on assumptions and pragmatism rather than on scientifically proven facts. There has been a tacit assumption of a cyclic menstrual variation in the vaginal epithelium, without taking into consideration that the changes thereby evoked may influence the histological appearance. The influence of antibiotics on the vaginal content has also long been known, but very little data has been systematically collected about the mechanisms involved and the nature and extent of the changes. Finally the infectious diseases affecting the vagina have been regarded as harmless disorders, of no particular interest for the physician to take care of. Until recently we have not understood that the preservation of the ecological balance helps to prevent both urinary infections (35) and ascending infections in the genitalia (29). Along with the accumulating evidence of the role of bacterial vaginosis as a contributory cause of premature labour, premature rupture of the membranes as well as ascending gynecological infections (29), a consciousness of the importance of the 'ecological balance' has arisen. However, this calls for detailed knowledge of the normal state and how the healthy vaginal environment is maintained.

Three of the 5 women in the study on the histology of the vagina had similar 'profiles' in the morphometrical measurements. There was also a correlation to caryopyknotic index. The epithelium is thicker and contains more glycogen in the preovulatory state, while it is thinner with a pronounced tendency to desquamation in the luteal phase. At the ovulation there is a characteristic appearance, reminding one of the histological picture in a HPV-infected epithelium. The consequence of this will be that information regarding the last period is essential for the interpretation of the morphology of the vaginal wall. Otherwise the risk for overdiagnosing HPV-infection is obvious. Another characteristic of the vaginal epithelium around the time of ovulation is that it becomes granular, which can also be observed by colposcopy.

A menstrual variation has also been demonstrated regarding the presence of estrogen receptors, with numbers decreasing from the follicular to the luteal phase. In this study it was possible to find receptors in *all* specimens *and* to quantify them, using a sensitive ELISA-method. Furthermore, the women examined were healthy, regularly menstruating and not using contraceptives, in contrast to those in other studies. It has unfortunately been customary to enrol patients with various diseases and with undefined hormonal state. These earlier studies have also lacked a prospective design regarding menstrual phase cyclicity (9,10,11,12,13,36).

Different hormonal states can influence the adherence of microorganisms. During the premenstrual phase the epithelium is thinner, with the outer layer desquamated, which may affect the ability of microorganisms to attach (37). For example, candida infections are more common during the premenstrual phase (38), whereas vaginal colonization with group-B-streptococci is most frequently found in the follicular phase (39). Moreover, the composition of the normal flora changes during the menstrual cycle (34).

It is well known that women develop vaginitis during treatment with antibiotics, an indirect proof that antibiotics are also present in the vaginal fluid. In the study regarding excretion of pcV in vaginal fluid, unexpectedly high concentrations of pcV were demonstrated for a prolonged period of the time, during which the concentra-

tions in serum and saliva had again reached zero. The high concentration in vaginal fluid has been demonstrated in hysterectomized women, proving that large amounts of penicillin are transported through the vaginal wall. Thus it is not primarily transported with the secretions from the internal genitalia. It was also found that the concentrations of penicillin were highest in the most distal part of the vagina.

Penicillin passes the brain barrier and the mechanism is a simple diffusion (40). It is plausible that it passes the vaginal wall in the same way via the intercellular spaces (22). Effective pathways exist for the lubrication fluid and it is possible that the penicillin uses these (22). The biological activity of penicillin is not substantially impeded at a moderately acidic pH (41). Thus pcV should be active in the normal vaginal environment.

The high penicillin concentration may be further augmented by the counter-current vascular supply around the upper genitalia. Bendz *et al.* (42) injected Krypton-85 into the utero-ovarian vein and registered an increase in radioactivity in the ovary, which speaks for a counter-current exchange mechanism in the vessels of the human uterine adnexa. This may also be the factor behind the high concentration of progesterone and estradiol in oviductal tissue shown by Batra *et al.* (43). They measured the concentrations of these hormones in the tissue of the Fallopian tube and found a considerably higher steroid level in the tissue than in the plasma. It has also been demonstrated in smokers, that nicotine concentrates strongly in the cervical mucus, compared with plasma (44). These pieces of evidence constitute a possible background to the finding of increased concentrations of various substances in the vaginal fluid.

The concentration of pcV is highest most distally. Probably this is due to the fluid circulation within the vagina. Thirty years ago Odeblad showed that there is a flow in the distal direction, with reabsorption of fluid in the folds of Shaw, besides the urethra (15). A consequence of this may be that the most pronounced disturbances in the ecological balance take place in this portion of the vagina, close to the perineum. These circumstances could generate an ascending disturbance of the ecology and militate against the spontaneous restoration to normality.

The role of lactobacilli in maintaining bacterial homeostasis in the vagina is still obscure. The acidic environment has long been considered to be fundamental to defence against infections and the lactic acid produced by the lactobacilli was believed to be the principle compound responsible for this (34). There are other bacterial compounds with acidic properties, however. Fatty acids may be a more important source and the vaginal fluid is already acidic at birth despite the sterility of the vagina (34). It has also been shown that lactobacilli interfere with the colonization of uropathogens by steric hindrance (45). They can also produce bacteriocins (35) as well as antibiotic-like substances (45). Finally, hydrogen peroxide production may inhibit the growth of other catalase-negative bacteria, i.e. Enterobacteriaceae and anaerobes (46).

In the vagina, penicillin may influence the number and function of lactobacilli which usually are moderately sensitive to penicillin (47,48). Likewise, microbes that either are resistant to penicillin or produce penicillinases could be favoured. This could mean the selection of Gram-negative, facultative anaerobic, or strictly anaerobic rods and fungi. The synthesis of soluble beta-lactamases from different microorganisms in the vagina could also protect bacteria locally, e.g. group-B-streptococci, which are highly susceptible to penicillin *in vitro* (18). It has been shown that vaginal colo-

nization of group-B-streptococci may persist in spite of treatment with penicillin (17). A similar phenomenon has been demonstrated in the oral flora where group-A-streptococci may be protected from the action of penicillin by beta-lactamases produced by Gram-negative anaerobes (49,50).

The effects of penicillin on the normal microflora have also been elucidated. Of 14 healthy women, with no complaints of genital disease and not using contraceptives, 8 had to be excluded because of divergence from the criteria of normality:

In 4 of the 6 'normal' women Enterobacteriaceae appeared in the vaginal flora after penicillin treatment. In one of these women, all lactobacilli disappeared and there was a heavy growth of *E. coli*. In 4 of the 6 women the acidity diminished significantly.

Accumulating evidence indicates a connection between bacterial vaginosis and prematurity (29). Endotoxins produced by Gram-negative bacteria are present in vaginal discharge in women with bacterial vaginosis and have also been demonstrated in amniotic fluid in women with premature labour (32). Endotoxins are biologically potent compounds capable of triggering various important cascade systems of the body (e.g. coagulation, fibrinolysis, complement and indirectly also prostaglandin activation) (51). Possibly this is the link between the disrupted ecology in the vagina and premature labour. In a disturbed vaginal flora, bacteria may also be the source of phospholipase A2 (52) which may activate the synthesis of prostaglandins from arachidonic acid. Locally high concentrations of endotoxin could also induce the production of cytokines, i.e. tumour necrosis factor and interleukins, thus indirectly promoting prostaglandin synthesis. To this end, Romero et al. recently demonstrated that interleukin-1 is produced by human decidua in response to endotoxin stimulation (53).

In an experimental approach using monkeys, infected with *E. coli* in the genito-urinary tract, half of the animals regained the normal flora when they were treated with live lactobacilli. However, after receiving vaginal fluid from healthy donors, the cure rate was 100% (54). This study amply demonstrates the complexity of the vaginal environment where the constant action and interaction of different bacteria and their products constitute a dynamic eco system.

CONCLUSIONS

- There is a morphologically demonstrable cyclicity in the vaginal epithelium. This is confirmed by the presence of estrogen receptors, which diminish in number from the follicular to the luteal phase.
- Phenoxymethylpenicillin, taken orally, is excreted in high concentrations and over a long period of time in the vaginal fluid.
- Phenoxymethylpenicillin passes through the vaginal epithelium into the vagina, with the highest concentrations most distally.
- Large amounts of endotoxin are released in the vaginal discharge in women with bacterial vaginosis.
- Phenoxymethylpenicillin may interfere with the normal vaginal flora and may provide an environment suitable for Enterobacteriaceae and fungi. When there is an extensive overgrowth of Gram-negative bacteria, markedly elevated concentrations of endotoxins may be found in the vaginal fluid.

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