JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1989, p. 251–256
0095-1137/89/020251-06$02.00/0

Vol. 27, No. 2

Prevalence of Hydrogen Peroxide-Producing *Lactobacillus* Species in Normal Women and Women with Bacterial Vaginosis

DAVID A. ESCHENBACH,[1]* PAMELLA R. DAVICK,[1] BETSY L. WILLIAMS,[2] SEYMOUR J. KLEBANOFF,[3] KAREN YOUNG-SMITH,[1] CATHY M. CRITCHLOW,[3] AND KING K. HOLMES[3,4]

Departments of Microbiology,[2] Medicine,[3] Epidemiology,[4] and Obstetrics and Gynecology,[1] University of Washington, Seattle, Washington 98195

Received 20 June 1988/Accepted 25 October 1988

A predominance of *Lactobacillus* species in the vaginal flora is considered normal. In women with bacterial vaginosis, the prevalence and concentrations of intravaginal *Gardnerella vaginalis* and anaerobes are increased, whereas the prevalence of intravaginal *Lactobacillus* species is decreased. Because some lactobacilli are known to produce hydrogen peroxide (H_2O_2), which can be toxic to organisms that produce little or no H_2O_2-scavenging enzymes (e.g., catalase), we postulated that an absence of H_2O_2-producing *Lactobacillus* species could allow an overgrowth of catalase-negative organisms, such as those found among women with bacterial vaginosis. In this study, H_2O_2-producing facultative *Lactobacillus* species were found in the vaginas of 27 (96%) of 28 normal women and 4 (6%) of 67 women with bacterial vaginosis ($P < 0.001$). Anaerobic *Lactobacillus* species (which do not produce hydrogen peroxide) were isolated from 24 (36%) of 67 women with bacterial vaginosis and 1 (4%) of 28 normal women ($P < 0.001$). The production of H_2O_2 by *Lactobacillus* species may represent a nonspecific antimicrobial defense mechanism of the normal vaginal ecosystem.

The inhibition of the growth of one bacterial species by the H_2O_2 generated by another species is a well-recognized mechanism of bacterial antagonism (6, 29, 35). Lactobacilli, as well as other lactic-acid-producing bacteria, lack heme and thus do not utilize the cytochrome system (which reduces oxygen to water) for terminal oxidation. Lactobacilli utilize flavoproteins, which generally convert oxygen to H_2O_2. This mechanism, together with the absence of the heme protein catalase, generally results in the formation of H_2O_2 in amounts which are in excess of the capacity of the organism to degrade it. The H_2O_2 formed may inhibit or kill other members of the microbiota (6, 35), particularly those which lack or have low levels of H_2O_2-scavenging enzymes, such as catalase peroxidase. The microbicidal activity of H_2O_2 is considerably increased by the enzyme peroxidase in the presence of a halide ion (14). Among the peroxidases which can function in this way are those of milk and saliva (lactoperoxidase) (24), neutrophils and monocytes (myeloperoxidase) (14, 16), eosinophils (eosinophil peroxidase) (13), and genital tract secretions (rat uterine fluid and human cervical mucus) (3, 18). The peroxidase and the halide ion greatly enhance the H_2O_2-dependent microbial antagonism between two bacterial species (15), between bacteria and viruses (17), between bacteria and spermatozoa (19), and between bacteria and mammalian cells (4). Lactobacilli and other lactic-acid-producing bacteria are among the organisms which can generate the H_2O_2 required for peroxidase-catalyzed microbial antagonism (6, 16, 35).

Lactobacillus species are usually predominant in the vaginas of normal women and are believed to regulate the growth of other vaginal flora (9). It was originally postulated by Gardner and Dukes that *Haemophilus vaginalis*, now known as *Gardnerella vaginalis*, was the sole cause of bacterial vaginosis, because *G. vaginalis* was recovered from the vaginas of 92% of patients with bacterial vaginosis and from no women without bacterial vaginosis (8). However, the role of *G. vaginalis* in bacterial vaginosis became less clear as investigators began to report a 30 to 40% prevalence of *G. vaginalis* among normal women (23, 34). More recently is has been reported that the prevalence and concentration of certain vaginal anaerobic bacteria, as well as of *G. vaginalis*, are higher among women with bacterial vaginosis than among those without it (2, 27). Gardner and Dukes reported the lactobacilli were notably absent on vaginal smears for women with bacterial vaginosis (8), and other investigators isolated *Lactobacillus* species less often from women with bacterial vaginosis than from normal controls (27). We postulate that lactobacilli play an important role in the homeostasis of the normal vaginal flora by producing H_2O_2. An absence of H_2O_2-producing *Lactobacillus* species thus could allow an overgrowth of catalase-negative organisms, such as those found in high concentrations among women with bacterial vaginosis. Lactobacilli can be facultative or anaerobic and H_2O_2 generating or not; aerotolerance and H_2O_2 production by isolates in bacterial vaginosis have not been reported. In this paper, we compare the prevalence of H_2O_2-producing lactobacilli in women with or without bacterial vaginosis. Our results are compatible with a role of H_2O_2-producing lactobacilli in the prevention of bacterial vaginosis through the inhibition of the intravaginal growth of the causative microorganisms.

MATERIALS AND METHODS

Study population. The patient population consisted of 67 women with bacterial vaginosis, three-quarters of whom were examined at the University of Washington Student Health Center Women's Clinic and one-quarter of whom were examined at the Harborview Medical Center Vaginitis Clinic. The 21 control women were randomly selected from a group of women who had no complaints of vaginal discharge and attended the Student Health Center for contraceptive counseling. All subjects gave written informed consent. As previously described (1), the diagnosis of bacterial vaginosis was based upon the presence of a vaginal discharge with three of the four following characteristics: a

* Corresponding author.

homogeneous appearance: a pH of >4.5; the presence of a fishy amine odor upon the addition of 10% potassium hydroxide (KOH); and the presence of clue cells. The vaginal discharge of control women had no more than one of these four characteristics, and none contained clue cells. None of the women with bacterial vaginosis and none of the control women had *Trichomonas vaginalis* or *Candida albicans* observed on the wet mount examination or recovered by culturing.

Women were excluded from the study if they had received systemic antibiotic therapy or local vaginal antimicrobial therapy within the preceding month; were pregnant, postmenopausal, premenarcheal, or menstruating at the time of the first study examination; or were using an intrauterine contraceptive device.

Clinical evaluation. At the time of entry into the study, a detailed demographic, medical, contraceptive, and sexual history was obtained. A clean unlubricated speculum was then placed into the vagina. The lateral vaginal fornices were swabbed with three cotton-tipped applicators. The swabs were used to make two smears for Gram staining and to inoculate media for the isolation of *Mycoplasma hominis* (25), *Ureaplasma urealyticum* (25), *T. vaginalis* (7), and *C. albicans* (22) and to measure the vaginal pH with Color pHast (EM Reagents) indicator sticks. Endocervical material was obtained with separate calcium alginate swabs for the isolation of *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.

Reduced saline was then introduced into the vagina. Briefly, the sterile reduced saline contained 0.85% sodium chloride, 0.02% dithiothreitol (Sigma Chemical Co., St. Louis, Mo.), and 0.0001% resazurin in distilled water (final pH, 6.9); it was pipetted into autoclaved 7-ml red-top VACUTAINER tubes (Becton Dickinson Vacutainer Systems, Rutherford, N.J.) inside an anaerobe chamber (Coy Laboratory Products, Ann Arbor, Mich.) containing 85% nitrogen, 10% hydrogen, and 5% carbon dioxide. A 3.5-ml volume of the reduced saline was removed from a VACUTAINER tube with a sterile syringe and injected into the vagina. A sterile cotton swab was used to bring vaginal material into the vaginal pool, avoiding contact with the cervix. The saline-vaginal material mixture was aspirated with a 5-ml syringe attached to a sterile pipette. Air was removed from the syringe, and 1 ml of the specimen was injected through a 20-gauge needle into a prereduced VACUTAINER tube for the isolation of anaerobic bacteria.

A drop of vaginal fluid was placed on a glass slide containing a drop of normal saline and examined for the presence of clue cells and for trichomonads. Another drop was placed on a glass slide containing 10% KOH and examined for the presence of a fishy amine odor (1) and microscopically for yeast forms. A 0.01-ml volume of vaginal fluid was serially diluted into sterile saline with an Eppendorf pipette to obtain serial 10-fold dilutions of 10^{-3} to 10^{-12} ml of vaginal fluid for inoculation into human bilayer agar with Tween (30), MacConkey medium, and mannitol-salt medium. The remaining fluid was frozen at −20°C for biochemical analysis.

Microbiologic methods. Qualitative and quantitative culturing of vaginal flora was performed. Collection tubes for all vaginal wash samples were prepared as described above. Samples were plated in either an anaerobic or a 3% CO_2 atmosphere at 37°C within 2 h after collection. The methods used to isolate *G. vaginalis* and other facultative and anaerobic bacteria have been previously described (27).

Quantitative isolation, characterization, and identification of *Lactobacillus* species. Suspensions containing 10^{-3}, 10^{-5}, and 10^{-7} dilutions of vaginal discharge in reduced saline were plated onto prereduced bifidobacterium medium in the anaerobe chamber (12) and incubated anaerobically for 3 to 5 days for the recovery of *Lactobacillus* species. *Lactobacillus* species were also recovered from brucella agar medium which had been inoculated with 10^{-4}, 10^{-5}, and 10^{-7} dilutions and incubated anaerobically for 7 days. Catalase-negative, gram-positive bacteria were confirmed as a aerotolerant species by their growth upon incubation for 2 to 3 days in a candle jar.

Lactobacillus isolates were further characterized and identified by the criteria outlined in the *Anaerobe Laboratory Manual* (10). The optical rotation of lactic acid produced in peptone-yeast extract medium with glucose was determined on 72 h cultures. The basal medium for fermentation and biochemical reactions was peptone-yeast extract supplemented with Tween 80 (5). Inocula (2 ml) from 24-h cultures in this basal medium were suspended in 100 ml of the same medium immediately after the latter medium had been autoclaved and cooled to room temperature. Samples (2 ml) of the suspended inocula were aseptically dispensed into previously autoclaved culture tubes containing 0.2 ml of the 10-fold-concentrated substrate in distilled water. A 0.5-ml amount of inoculum was also added to 5 ml each of milk and gelatin media. Culture tubes with loose-fitting plastic caps were incubated anaerobically in an atmosphere of 10% hydrogen–10% carbon dioxide–80% nitrogen achieved by evacuation and replacement. Residual oxygen was removed with a palladium catalyst. Acid production was measured with a pH meter following an incubation period of 6 days at 35°C. Assays for indole production, reduction of nitrate and nitrite, and deamination of urea, arginine, lysine, and ornithine were performed as described by Cowan (5). The hydrolysis of esculin, gelatin, and starch and the reactions in milk were assayed by the methods of Holdeman et al. (10).

Detection of hydrogen peroxide production. Tetramethylbenzidine (TMB) medium was developed for the detection of hydrogen peroxide-producing organisms. Briefly, it consisted of 4.3 g of brucella agar base (Difco Laboratories, Detroit, Mich.), 25 mg of TMB (Sigma) as a substitute for benzidine (21), 1 mg of horseradish peroxidase (Organon Teknika, Malvern, Pa.), 0.5 mg of hemin (bovine crystalline; Sigma; 1 ml of a stock solution prepared by dissolving 50 mg in 1.0 ml of 1.0 N sodium hydroxide and diluting the mixture to 100 ml with water), 0.1 mg of vitamin K_1 (Sigma; 0.02 ml of a stock solution containing 25 μl of vitamin K_1 in 5 ml of 95% ethanol), and 1 g of starch for each 100 ml of medium.

Each *Lactobacillus* colony type on the primary plate was picked and identified. Each *Lactobacillus* species identified was also tested for the production of H_2O_2. Each *Lactobacillus* isolate was inoculated into TMB medium and incubated under anaerobic conditions in an anaerobic glove box at 37°C for 2 to 3 days, after which the isolate was exposed to ambient air. The horseradish peroxidase in this medium is known to oxidize TMB in the presence of H_2O_2 to form a blue pigment in the H_2O_2-producing colony. All plates were used within 1 week of preparation because the peroxidase was unstable 10 days beyond preparation.

Hemin was added to TMB medium to stimulate the growth of heme-requiring anaerobic bacteria. Whittenburg (36) observed that some strains of lactobacilli were capable of splitting H_2O_2 in the presence of hematin, thus resulting in a false-negative result when testing for the production of H_2O_2. Although it was postulated that these organisms were able to form catalase when grown on media containing

TABLE 1. H_2O_2 production by facultative and anaerobic lactobacilli isolated from the vaginal fluid of normal control women and patients with bacterial vaginosis

Lactobacillus species isolated	No. (%) of the following women with lactobacilli:		*P*
	Control (n = 28)	With bacterial vaginosis (n = 67)	
Total	27 (96)	35 (52)	<0.001
Facultative only			
H_2O_2 producers only	20 (71)	3 (4)	<0.001
Non-H_2O_2 producers only	0 (0)	8 (12)	0.1
H_2O_2 producers and nonproducers	6 (21)	0 (0)	<0.001
Total	26 (93)	11 (16)	<0.001
Facultative and anaerobic[a]			
Facultative H_2O_2 producers	1 (4)	1 (1)	0.5
Facultative non-H_2O_2 producers	0 (0)	6 (9)	0.2
Total (facultative H_2O_2 producers)	27 (96)	4 (6)	<0.001
Anaerobic only[a]			
H_2O_2 producers only	0 (0)	0 (0)[b]	
Non-H_2O_2 producers only	0 (0)	13 (21)[b]	0.008
Total	0 (0)	17 (27)[b]	<0.001

[a] No anaerobic lactobacilli produced H_2O_2.
[b] Excludes four patients with anaerobic lactobacilli for which H_2O_2 production could not be determined.

hematin (36), we found no disparity in our results upon retesting all viable lactobacilli for the production of H_2O_2, using duplicate plates twice with hemin and once without hemin. Vitamin K_1 is required for the growth of some vaginal microorganisms, and starch was added as a preferred carbon source for these organisms.

Statistical methods. All tests of two proportions were done with the Fisher exact test. Lactobacillus concentrations were compared with Student's t test. All P values were from two-tailed tests.

RESULTS

H_2O_2 production by *Lactobacillus* species. The distribution and characteristics of *Lactobacillus* species in the vaginas of normal women and patients with bacterial vaginosis are shown in Table 1. *Lactobacillus* species were isolated on either bifidobacterium medium or brucella agar from 27 (96%) of 28 normal control women and from 35 (52%) of 67 women with bacterial vaginosis ($P < 0.001$, Table 1). Facultative *Lactobacillus* species only, without anaerobic lactobacilli, were isolated from 26 (93%) of 28 control women and from 11 (16%) of 67 women with bacterial vaginosis ($P < 0.001$).

Among women with facultative lactobacilli, an additional control woman and seven women with bacterial vaginosis also had anaerobic lactobacilli. Facultative H_2O_2-producing lactobacilli were isolated from a total of 27 (96%) of 28 normal control women (all of the women with lactobacilli) and from only 4 (6%) of 67 women with bacterial vaginosis (4 of the 35 women with lactobacilli) ($P < 0.001$). Among women from whom facultative lactobacilli were isolated, H_2O_2-producing species were isolated from 27 of 27 normal women and 4 (22%) of 18 women with bacterial vaginosis ($P < 0.001$). Facultative lactobacilli which did not produce H_2O_2 were isolated from 6 (21%) of 28 normal control women and 14 (22%) of 63 women with bacterial vaginosis. Thus, the difference in the prevalences of vaginal facultative lactobacilli among women with and without bacterial vaginosis was solely attributable to the striking differences in the prevalence of H_2O_2-producing facultative lactobacilli.

TABLE 2. Concentrations of H_2O_2-producing and non-H_2O_2-producing lactobacilli among normal control women and patients with bacterial vaginosis[a]

Lactobacillus species	Viable count (geometric mean)/ml in the following women:		*P*
	Control (n = 28)	With bacterial vaginosis (n = 67)	
Facultative only	2.4×10^7	5.6×10^7	NS[b]
Anaerobic only		2.6×10^8	
Facultative and anaerobic	3.9×10^6	1.1×10^8	NS
Facultative H_2O_2 producing	8.4×10^6	3×10^7	0.03
Mean	1.2×10^7	1.4×10^8	0.18

[a] The number of women in each cell can be determined from the data in Table 1.
[b] NS, Not significant.

In contrast, anaerobic *Lactobacillus* species were isolated from 1 (4%) of the 28 normal women and from 24 (36%) of the 67 women with bacterial vaginosis ($P < 0.001$). H_2O_2-producing facultative lactobacilli were simultaneously isolated from only 2 of the 25 women harboring anaerobic lactobacilli. Although four of the anaerobic *Lactobacillus* species that were isolated on the primary plate lost viability with storage, H_2O_2 was not produced by any of the remaining 20 viable anaerobic lactobacilli.

The mean age, race, marital status, socioeconomic status, birth control method, and number of lifetime sexual partners of patients and controls were similar. Patients had had more partners in the prior 2 weeks (0.9 ± 0.4) than controls (0.7 ± 0.4) ($P < 0.02$) and more partners in the past 6 months (1.9 ± 1.7) than controls (1.2 ± 0.8) ($P < 0.02$). However, within the patient and control groups there was no relationship between the number of sexual partners at either the 2-week or the 6-month interval and H_2O_2-producing lactobacilli.

Among women from whom any lactobacilli were recovered, the geometric mean concentration determined by viable counts was 10-fold higher for women with bacterial vaginosis than for control women, but the difference was not significant, owing to a large standard deviation in both groups (Table 2). Similarly, there was no difference between the two groups of women in the mean concentration of facultative lactobacilli among those who were culture positive for such organisms. Surprisingly, the mean concentration of H_2O_2-producing facultative lactobacilli was higher among women with bacterial vaginosis who harbored such organisms (3×10^7/ml) than among normal control women (8.4×10^6) ($P = 0.03$), although only four women with bacterial vaginosis had H_2O_2-producing facultative lactobacilli.

***Lactobacillus* species identification.** The results of *Lactobacillus* species identification are shown in Table 3. A total of 14 isolates were *L. acidophilus*, 12 were *L. jensenii*, 3 were *L. catenaforme*, 5 were *L. delbrueckii*, 4 were *L. brevis*, 4 were *L. fermentum*, 3 were *L. helveticus*, 3 were *L. leichmannii* and 1 was *L. salivarius*; 9 were viable strains which could not be placed in any known species. Sixteen isolates were tested for H_2O_2 production, but no attempt was made at complete identification. Four lost viability and could not

TABLE 3. *Lactobacillus* species found among normal women and women with bacterial vaginosis

Lactobacillus species	H_2O_2 production	No. of the following women with the indicated species:	
		Normal (n = 28)	With bacterial vaginosis (n = 67)
L. acidophilus	+	3	0
L. jensenii	+	3	2
L. acidophilus and *L. jensenii*	+	4	0
L. catenaforme	+	3	0
L. acidophilus and *L. leichmannii*	+	1	0
Lactobacillus sp. unknown	+	2	0
Lactobacillus sp. not further identified	+	7	1
L. jensenii and *L. brevis*	+ and −	1	0
L. jensenii and *Lactobacillus* sp. unknown	+ and −	1	0
L. acidophilus and *Lactobacillus* sp. unknown	− and +	1	0
L. fermentum and *Lactobacillus* sp. unknown	− and +	1	0
L. jensenii and *L. delbrueckii*	+ and −	0	1
L. acidophilus	−	0	3
L. acidophilus and *L. delbrueckii*	− and −	0	1
L. acidophilus and *Lactobacillus* sp. unknown	− and −	0	1
L. delbrueckii	−	0	3
L. brevis	−	0	1
L. fermentum	−	0	1
L. helveticus	−	0	3
L. leichmannii	−	0	1
L. salivarius and *Lactobacillus* sp. unknown	− and −	0	1
L. fermentum and *L. brevis*	−	0	2
Lactobacillus sp. unknown	−	0	2
Lactobacillus sp. not further identified	−	0	8
Nonviable for identification	ND[a]	0	4
No *Lactobacillus*		1	32

[a] ND, Not determined.

be tested for H_2O_2 production or identified to the species level. While it was possible to identify the lactobacilli to the genus level in all cases, any further identification of the lactobacilli was not successful for 9 (16%) of 57 isolates. All H_2O_2 producers were identified as *L. acidophilus*, *L. jensenii*, or *L. catenaforme*; with the exception of one *L. leichmannii* isolate which was present together with *L. acidophilus* in a normal woman. H_2O_2 was produced by 20 of 21 isolates of these three species from normal control women versus 3 of 8 isolates of these three species from women with bacterial vaginosis (P = 0.003). When species identification was performed, these three species accounted for 21 of 24 *Lactobacillus* species from control women versus 8 of 24 *Lactobacillus* species from women with bacterial vaginosis (P = 0.0003).

DISCUSSION

Lactobacillus species have been isolated from a large number of premenopausal women without apparent vaginal infections in a series of studies recently reviewed (9). In studies in which the differentiation was stated, facultative *Lactobacillus* species were isolated from 45 to 88% and anaerobic *Lactobacillus* species were isolated from 10 to 45% of normal women (9). It has been postulated that lactobacilli are responsible for the presence of a low vaginal pH (3.5 to 4.5) by producing lactic acid from glycogen (37), with the glycogen first being broken down to glucose, either by the vaginal epithelial cells or by bacteria other than lactobacilli, before it is metabolized by *Lactobacillus* species (28). Low pH would be expected to inhibit the growth of nonacidophilic organisms while stimulating the growth of acidophilic organisms such as lactobacilli. We postulate that H_2O_2 production represents an additional mechanism by which lactobacilli regulate the growth of other organisms in the vagina.

H_2O_2-producing facultative *Lactobacillus* species were isolated more frequently from normal control women (96%) than from women with bacterial vaginosis (6%), whereas the reverse was true for anaerobic lactobacilli (4% from normal women and 67% from women with bacterial vaginosis). The difference between women with and without bacterial vaginosis in the prevalence of H_2O_2-producing lactobacilli is greater than the differences that we have observed in the rates of colonization by any other vaginal organism, including *G. vaginalis*, *Mobiluncus* sp., *Bacteroides* sp., or *M. hominis*. In the present study, the prevalence of *G. vaginalis*, *Bacteroides* sp., and *M. hominis* was significantly higher in patients with bacterial vaginosis than in controls, consistent with previous reports (2, 27). These results will be presented in detail in a separate report. It is possible that the absence of H_2O_2-producing *Lactobacillus* species constitutes a primary defect which increases susceptibility to overgrowth by certain organisms, particularly anaerobes, resulting in the syndrome of bacterial vaginosis. Alternatively, the absence of H_2O_2-producing *Lactobacillus* species could be a consequence of bacterial vaginosis (i.e., an epiphenomenon). In this regard, we recently monitored a cohort of pregnant women without bacterial vaginosis and determined that the initial absence of H_2O_2-producing *Lactobacillus* species at the time of enrollment was a significant risk factor for the subsequent development of bacterial vaginosis later in pregnancy (S. Hiller and D. A. Eschenbach, unpublished data).

Although we were not able to detect H_2O_2 production in any of the anaerobic lactobacilli in this study, anaerobic lactobacilli may transiently form small amounts of H_2O_2. Whittenburg (36) demonstrated that there was no correlation between H_2O_2 production and a preference for anaerobic or aerobic growth conditions. He suggested that all organisms contain flavoprotein oxidases which produce H_2O_2 but that certain organisms also possess enzymes which reduce the concentration of H_2O_2 to undetectable levels. He found that some lactic-acid-producing bacteria such as *Lactobacillus* species could form catalase in the presence of hematin or heated blood. However, under our conditions, heme did not induce the presence of catalase, and there were no differences in our results when duplicate plates with and without heme were used. A non-heme-containing pseudocatalase also has been demonstrated in lactic acid bacteria (19, 36), and strains of *Lactobacillus plantarum* which possessed pseudocatalase did not accumulate H_2O_2 (20). However, pseudocatalase-containing organisms did not have a growth advantage over catalase-negative organisms, which accumulated H_2O_2 (20). The physiological significance of pseudocatalase is unknown at this time. Thus, anaerobic *Lactobacillus* species in this study produced no H_2O_2, produced H_2O_2 in amounts that were not detectable by our methods, or produced H_2O_2 which was rapidly degraded to undetectable

amounts by flavoprotein peroxidase, pseudocatalase, or catalase. Whatever the mechanism for the lack of H_2O_2 detection with some *Lactobacillus* species, there remains a preponderance of H_2O_2-generating lactobacilli in the normal vagina and a paucity of such organisms in bacterial vaginosis. It is therefore reasonable to hypothesize that the microbial flora of the vagina can be influenced by the H_2O_2 generated by indigenous lactobacilli and that the absence of H_2O_2-generating lactobacilli can contribute to disease.

It cannot be answered at the present time whether peroxidase in the vagina influences the antimicrobial activity of the H_2O_2 generated by lactobacilli. The uterine fluid of estrogen-primed rats contains a peroxidase which can utilize the H_2O_2 generated by *L. acidophilus* to kill *Escherichia coli* (18) or spermatozoa (19) in the presence of a halide ion. Peroxidase activity has been demonstrated in human cervical mucus (3) and in endometrium and endocervical epithelium (11, 31, 32). The peroxidase of human cervical mucus has been partially purified (26). It is present in the soluble fraction of cervical mucus and has a pH optimum (with 2, 2′-azinobis[3-ethylbenzothiazoline-6-sulfonic acid] as an electron donor) of 4.45, which is within the usual pH range of normal vaginal fluid (1). It is of interest that the pH of vaginal fluid of women with bacterial vaginosis is typically greater than 4.5 (1). Variations in cervical mucus peroxidase activity in the menstrual cycle have been reported, with a fall midcycle (32). The source of the peroxidase of cervical mucus (e.g., granulocytes, endocervical epithelium, and endometrium) is not known. Radioactive halide and thiocyanate ions injected intravenously into normal women were detected in the cervical mucus, with the iodide reaching 10 times the level of serum (33). Chloride ions in cervical mucus also would be anticipated. Thus, the components of the peroxidase-mediated antimicrobial system, with H_2O_2 supplied by lactobacilli, are present and may contribute to the control of the microbial flora in the endometrium, cervix, and vagina. Catalase-negative organisms would be expected to be particularly sensitive to H_2O_2-dependent antimicrobial systems, although it should be emphasized that H_2O_2 can be scavenged by a number of mechanisms in cells. The study reported here suggests that nonenzymatic or peroxidase-mediated control of the microbial flora by H_2O_2 generated by *Lactobacillus* species may prevent the development of bacterial vaginosis in some women.

ACKNOWLEDGMENTS

This study was supported by Public Health Service program project grants AI-12192 and AI-07763 from the National Institutes of Health.

LITERATURE CITED

1. **Amsel, R., P. A. Totten, C. A. Spiegel, K. C. S. Chen, D. Eschenbach, and K. K. Holmes.** 1983. Nonspecific vaginitis: diagnostic criteria and microbial and epidemiological association. Am. J. Med. **74**:14–22.
2. **Blackwell, A. L., A. R. Fox, I. Phillips, and D. Barlow.** 1983. Anaerobic vaginosis (nonspecific vaginitis): clinical, microbiological and therapeutic findings. Lancet **ii**:1379–1382.
3. **Blain, J. A., P. J. Heald, A. E. Mack, and C. E. Shaw.** 1975. Peroxidase in human cervical mucus during the menstrual cycle. Contraception **11**:677–680.
4. **Clark, R. A., S. J. Klebanoff, A. B. Einstein, and A. Fefer.** 1975. Peroxidase-H_2O_2-halide system: cytotoxic effects on mammalian tumor cells. Blood **45**:161–170.
5. **Cowan, S. T. (ed.).** 1974. Cowan & Steel's manual for the identification of medical bacteria. Cambridge University Press, London.
6. **Dahiya, R. S., and M. L. Speck.** 1968. Hydrogen peroxide formation by lactobacilli and its effect on *Staphylococcus aureus*. J. Dairy Sci. **51**:1568–1572.
7. **Diamond, L. S., D. R. Harlow, and C. C. Cunnick.** 1978. A new medium for the anexic cultivation of *Entamoeba histolytica* and other *Entamoeba*. Trans. R. Soc. Trop. Med. Hyg. **72**:431–432.
8. **Gardner, H. L., and C. D. Dukes.** 1955. *Haemophilus vaginalis* vaginitis. Am. J. Obstet. Gynecol. **69**:962–976.
9. **Hill, G. B., D. A. Eschenbach, and K. K. Holmes.** 1985. Bacteriology of the vagina. Scand. J. Urol. Nephrol. Suppl. **86**:23–39.
10. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1974. Anaerobe laboratory manual. Virginia Polytechnic Institute and State University, Blacksburg.
11. **Holinka, C. F., and E. Gurpide.** 1980. Peroxidase activity in glands and stroma of human endometrium. Am. J. Obstet. Gynecol. **138**:599–603.
12. **Johnston, M. A., and E. A. Delwiche.** 1962. Catalase of the *Lactobacillaceae*. J. Bacteriol. **83**:936–938.
13. **Jong, E. C., W. R. Henderson, and S. J. Klebanoff.** 1980. Bacteridical activity of eosinophil peroxidase. J. Immunol. **124**:1378–1382.
14. **Klebanoff, S. J.** 1967. Iodination of bacteria: a bactericidal mechanism. J. Exp. Med. **126**:1063–1076.
15. **Klebanoff, S. J.** 1968. Myeloperoxidase-halide-hydrogen peroxide antibacterial system. J. Bacteriol. **95**:2131–2138.
16. **Klebanoff, S. J.** 1970. Myeloperoxidase-mediated antimicrobial systems and their role in leukocyte function, p. 89–110. *In* J. Schultz (ed.), Biochemistry of the phagocytic process. North-Holland Publishing Co., Amsterdam.
17. **Klebanoff, S. J., and M. E. Belding.** 1974. Virucidal activity of H_2O_2-generating bacteria: requirement of peroxidase and a halide. J. Infect. Dis. **129**:345–348.
18. **Klebanoff, S. J., and D. C. Smith.** 1970. Peroxidase-mediated antimicrobial activity of rat uterine fluid. Gynecol. Invest. **1**:21–30.
19. **Klebanoff, S. J., and D. C. Smith.** 1970. The source of H_2O_2 for the uterine fluid-mediated sperm-inhibitory system. Biol. Reprod. **3**:236–242.
20. **Kono, Y., and I. Fridovich.** 1983. Isolation and characterization of the pseudocatalase of *Lactobacillus plantarum*: a new manganese-containing enzyme. J. Biol. Chem. **258**:6015–6019.
21. **Liem, H. H., F. Cardenas, M. Tavassoli, M. B. Poh-Fitzpatrick, and U. Muller-Eberhard.** 1979. Quantitative determination of hemoglobin and cytochemical staining for peroxidase using 3, 3′, 5, 5′-tetramethylbenzidine dihydrochloride, a safe substitute for benzidene. Anal. Biochem. **98**:388–393.
22. **Mackenzie, D. W. R.** 1962. Serum tube identification of *Candida albicans*. J. Clin. Pathol. **15**:563–565.
23. **McCormack, W. M., C. H. Hayes, B. Rosner, J. R. Eurard, V. A. Crockett, S. Alpert, and S. H. Zinner.** 1977. Vaginal colonization with *Corynebacterium vaginale* (*Hemophilus vaginalis*). J. Infect. Dis. **136**:740–745.
24. **Reiter, B., and J. D. Oram.** 1967. Bacterial inhibitors in milk and other biological fluids. Nature (London) **216**:328–330.
25. **Shepard, M. C., and R. S. Combs.** 1979. Enhancement of *Ureaplasma urealyticum* growth on a differential agar medium (A7B) by a polyamine, putrescine. J. Clin. Microbiol. **10**: 931–933.
26. **Shindler, J. S., R. E. Childs, and W. G. Bardsley.** 1976. Peroxidase from human cervical mucus: the isolation and characterization. Eur. J. Biochem. **65**:325–331.
27. **Spiegel, C. A., R. A. Amsel, D. Eschenbach, F. Schoenknecht, and K. K. Holmes.** 1980. Anaerobic bacteria in nonspecific vaginitis. N. Engl. J. Med. **303**:601–607.
28. **Steward-Tule, D. E. S.** 1964. Evidence that vaginal lactobacilli do not ferment glycogen. Am. J. Obstet. Gynecol. **88**:676–679.
29. **Thompson, R., and A. Johnston.** 1950. The inhibitory action of saliva on the diphtheria bacillus: hydrogen peroxide, the inhibitory agent produced by salivary streptococci. J. Infect. Dis. **88**:81–85.
30. **Totten, P. A., R. Amsel, J. Hale, P. Piot, and K. K. Holmes.** 1982. Selective differential human blood bilayer media for

isolation of *Gardnerella (Haemophilus) vaginalis*. J. Clin. Microbiol. **15**:141–147.
31. **Tsibris, J. C. M., J. L. Thomason, A. Kunigk, F. S. Khan-Dawood, C. V. Kirschner, and W. N. Spellacy.** 1982. Guaiacol peroxidase levels in human cervical mucus: a possible predictor of ovulation. Contraception **25**:59–67.
32. **Tsibris, J. C. M., Y. P. Trujillo, B. B. Fernandez, W. A. Bardawil, A. Kunigk, and W. N. Spellacy.** 1982. Distribution of guaiacol peroxidase in human endometrium and endocervical epithelium during the menstrual cycle. J. Clin. Endocrinol. Metab. **54**:991–997.
33. **Von Kaulla, K. N., J. K. Aikawa, P. D. Bruns, W. T. Wikle, and V. E. Drosec.** 1957. Secretory function of the human uterine cervix. Studies with radioisotopes. Fertil. Steril. **8**:444–454.
34. **Vontver, L. A., and D. A. Eschenbach.** 1981. The role of *Gardnerella vaginalis* in nonspecific vaginitis. Clin. Obstet. Gynecol. **24**:439–460.
35. **Wheater, D. M., A. Hirsch, and A. T. R. Mattick.** 1952. Possible identity of 'lactobacillin' with hydrogen peroxide produced by lactobacilli. Nature (London) **170**:623–624.
36. **Whittenburg, R.** 1964. Hydrogen peroxide formation and catalase activity in the lactic acid bacteria. J. Gen. Microbiol. **35**:13–26.
37. **Wylie, J. G., and A. Henderson.** 1969. Identity and glycogen-fermenting activity of lactobacilli isolated from the vagina of pregnant women. J. Med. Microbiol. **2**:363–366.