Amine Content of Vaginal Fluid from Untreated and Treated Patients with Nonspecific Vaginitis

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ABSTRACT We examined the vaginal washings from patients with nonspecific vaginitis (NSV) to seek biochemical markers and possible explanations for the signs and symptoms of this syndrome. Seven amines were identified including methylamine, isobutylamine, putrescine, cadaverine, histamine, tyramine, and phenethylamine. These amines may contribute to the symptoms of NSV and may contribute to the elevated pH of the vaginal discharge. They may also be partly responsible for the "fishy" odor that is characteristic of vaginal discharges from these patients. Among the seven amines, putrescine and cadaverine were the most abundant and were present in all vaginal discharges from each of ten patients before treatment. These amines are produced in vitro during growth of mixed vaginal bacteria in chemically defined medium, presumably by decarboxylation of the corresponding amino acids. We hypothesize that anaerobic vaginal organisms, previously shown to be quantitatively increased in NSV, are responsible for the amine production, because metronidazole inhibited the production of amines by vaginal bacteria in vitro, and *Haemophilus vaginalis* did not produce amines. *H. vaginalis* did release high concentrations of pyruvic acid and of amino acids during growth in peptone-starch-dextrose medium, whereas, other vaginal flora consumed both pyruvic acid and amino acids in the same medium during growth. These findings suggest that a symbiotic relationship may exist between *H. vaginalis* and other vaginal flora in patients with NSV.

INTRODUCTION

The vaginal fluid from women with nonspecific vaginitis (NSV)[1] has a prominent odor often described as a rotten-fish smell, particularly during and after sexual intercourse. This "fishy" odor can be intensified in vaginal fluid from women with NSV by the addition of 10% KOH to vaginal fluid from untreated patients as reported by Pheifer, et al. (1). The odor released by KOH suggested the presence of amine(s), and subsequent analyses of vaginal washings from untreated patients with NSV described in this report have identified seven amines, i.e., methylamine, isobutylamine, putrescine, cadaverine, histamine, tyramine, and phenethylamine. These amines appear to be the decarboxylated products of their corresponding amino acids. This report describes the identification and quantitation of these amines in vaginal washings obtained from patients before and after treatment of NSV. The biological roles of the putative putrefactive decarboxylases that may be responsible for the amine production, and the possible symbiotic relationship between *Haemophilus vaginalis* and other vaginal flora are also discussed.

METHODS

Study population. 10 women were selected for this study who had both symptoms and signs of NSV as described previously (1), with culture-proven *H. vaginalis* vaginal infection. Each was studied before and after antimicrobial treatment with metronidazole, ampicillin, and(or) erythromycin. Seven normal women who came for contraception examination and had no symptoms or signs of NSV were selected as controls.

Collection of vaginal washings. 2ml of sterile water was instilled into the vagina and a sterile cotton-tipped applicator was used to swab the adherent vaginal secretions into the pooled fluid. The diluted vaginal fluid was removed with a sterile pipette, transferred to a plastic tube, and the pH was measured with a pH meter with a combined pH electrode 9.5 × 200 mm (PHM 61, Radiometer Co., Copenhagen).

The collected fluid was centrifuged in a clinical centrifuge at 1,800 *g* for 2 min. To the supernate, which usually had a volume of 1.5 to 2.2 ml, was added 10 μl of concentrated HCl and this solution was then frozen at −20°C.

Analysis of vaginal washings by high-voltage electrophoresis at pH 2.1. The supernate of collected vaginal washings (15–25 μl) was spotted on a Whatman 3 MM paper (What-

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[1] *Abbreviations used in this paper*: NSV, nonspecific vaginitis; PSD, peptone-starch-dextrose.

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man, Inc., Clifton, N. J.). High-voltage electrophoresis at pH 2.1 was carried out for 12 min at 75 V/cm as described by Chen and Krause (2). After drying, the paper was stained with ninhydrin-cadmium (3). Amino acids (containing about 10 nmol each) and dansylsulfonic acid (5-dimethylaminonaphthalene-1-sulfonic acid) were run in parallel with the samples as markers (4).

Dansylation of amino acids, amines, and vaginal washings. The procedure for dansylation of amino acids, amines, and vaginal washings was essentially the same as described by Gray (5). 10 μl of dansyl chloride (5-dimethylaminonaphthalene-1-sulfonyl chloride, 2.5 mg/ml acetone) was added to a mixture that consisted of 5 μl of vaginal washings (or 5 μl of 2 mg/ml of amino acid or amine) and 5 μl of 1 M $NaHCO_3$. The reaction was carried out at 37°C for 1 h. The sources of amines were described in a separate report.[2]

Separation of dansyl derivatives by thin-layer chromatography on polyamide sheets. The dansylated mixture from vaginal washings was centrifuged, and the supernate was dried under vacuum over concentrated H_2SO_4 and NaOH pellets (in separate beakers) in a vacuum desiccator. The dried products were dissolved in 5 μl of 50% pyridine. 1 μl of the dansylated mixture was spotted on both sides of a polyamide sheet (Chen-Chin polyamide layer sheet, Accurate Chemical & Scientific Corp., Hicksville, N. Y.) at the same position and a dansyl(5-dimethylaminonaphthalene-1-sulfonyl)-derivative mixture of known amino acids and amines was spotted at the same position on the back side of the sheet as markers. The concentration of each dansyl-derivative marker was preadjusted to be barely seen under the ultra violet light (a 10–100 times dilution of the 20 μl-reaction mixture with water was usually made). Thin-layer chromatography was carried out by the procedure described by Woods and Wang (6) except that the second-dimensional chromatography was run to only two-thirds the length of the sheet. The positions of the amines in vaginal washings on the polyamide sheet after two-dimensional chromatography were identified by comparing the positions of dansyl derivatives in vaginal washings on the front side of the sheet to the corresponding intensified spots at the positions of the known amines on the opposite side. Dansyl derivatives of tyramine and histamine co-chromatographed to the same position in the system described above, but separated well after chromatography in the third solvent system (*n*-heptane: *n*-butanol: glacial acetic acid = 40:30:9) in the same dimension to full length.

Conversion of amino acids to amines in vitro by microorganisms from patients with NSV. Whereas *H. vaginalis*, a facultative bacterium, is generally the predominant organism isolated from the vaginal discharges of women with NSV, obligate anaerobic bacteria often are also present in larger concentrations in vaginal discharges from women with NSV than from normal women (1). To determine the putative microbial source of the amines in vaginal washings from patients with NSV, the following experiments were performed. During speculum examination of patients with typical symptoms and signs of NSV, a cotton swab was used to inoculate vaginal discharge onto chocolate agar (BBL GC Base with 5% chocolated sheep blood and 1% IsoVitaleX enrichment BBL Microbiology Systems, Becton, Dickinson & Co., Cockeysville, Md.) for incubation in a candle extinction jar to isolate *H. vaginalis*, and onto prereduced blood agar (BBL Brucella agar with 5% sheep blood) for anaerobic incubation in Gas Pak jars (Ferguson Industries, Dallas, Tex.) (7). All cultures were incubated at 35°C.

Chocolate agar plates were examined after 48 h for growth of *H. vaginalis*, which was identified as described by Pheifer et al. (1). Pure subcultures of five separate isolates of *H. vaginalis* from chocolate agar plates were inoculated with a wire loop into sterile tubes that contained 5 ml of peptone-starch-dextrose (PSD) medium (8). Inoculated PSD medium and uninoculated medium controls were incubated anaerobically at 35°C in a Gas Pak jar. After 3 d of incubation, aliquots of the spent test media were subcultured to chocolate agar to check purity of the cultures.

Separate anaerobic cultures of vaginal discharge from four patients were incubated 4 d on the prereduced blood agar, after which the mixed growth was removed from the agar plates with a sterile cotton swab and suspended in sterile broth. The suspension was mixed thoroughly and a sterile capillary pipette was used to inoculate ≅0.1 ml into 5 ml of three types of amine production test broth media: (*a*) the mixed anaerobic growth from one patient was inoculated into PSD medium, (*b*) the mixed anaerobic growth from three of these patients was separately inoculated into a chemically defined medium (9) adjusted to pH 5.5, and (*c*) the mixed growth from one of these patients was also inoculated into the same defined medium containing 0.1 mM metronidazole. The inoculated media and the uninoculated media controls were incubated at 35°C in a Gas Pak jar, and after 3 d of incubation, bacteria were removed from the test broth media by centrifugation at 10,000 *g* for 10 min and supernates of the blanks and all spent test media were then analyzed for concentrations of amino acids and amines.

Amino acid and amine analyses by amino acid analyzer. The supernate of vaginal washings (40–240 μl) or media (20 μl), after centrifugation as described above, was loaded with pH 2.2 buffer to the columns of the amino acid analyzer for amino acid and amine analyses. All analyses were performed on an amino acid analyzer equipped with a 10-mm light path from the Japan Electron Optical Laboratories, Tokyo, No. JLC-6AH. The procedure for the basic amino acid and amine analyses was reported in a separate paper.[2]

Determination of total keto acids and pyruvic acid in media. Total keto acids and pyruvic acid concentrations of PSD media before and after organism growth were determined according to the methods of Friedemann and Haugen (10) with sodium pyruvate (Sigma Chemical Co., St. Louis, Mo.) as a standard.

RESULTS

Detection of amines in vaginal washings by high-voltage electrophoresis. As shown in Fig. 1, the vaginal washings from 10 patients before or after treatment with erythromycin, which is ineffective in most cases of NSV,[3] always showed ninhydrin-positive substances that moved faster than the basic amino acids, lysine, arginine, and histidine, whereas the washings from the same patients who no longer had symptoms or signs of NSV at the end of the subsequent treatment either with metronidazole or ampicillin, failed to show the fast-moving, ninhydrin-positive band. Electrophoretograms of vaginal washings from seven normal women who had no symptoms or signs of NSV re-

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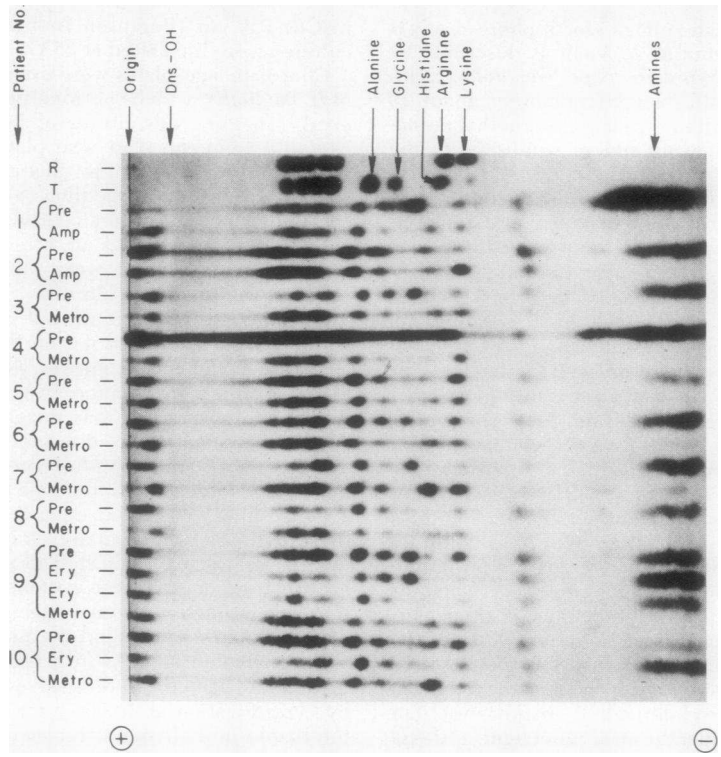

FIGURE 1 High-voltage electrophoretic analyses at pH 2.1 of vaginal washings from patients with NSV. Pre refers to specimens from patients who had not been treated. Amp and metro refer to specimens from patients treated successfully with ampicillin or metronidazole, as described previously (1). Ery refers to specimens from patients treated with erythromycin estolate, 500 mg four times daily for 7 d. The electrophoretic mobility was toward the cathode. R and T were amino acid markers as described (4) Dansyl sulfonic acid is not charged at pH 2.1 and was used as the neutral marker. The electrophoretic conditions were as described in Methods.

sembled those of NSV patients after treatment with metronidazole (results not shown).

The ninhydrin-positive bands that moved faster than lysine with preparative electrophoresis at pH 2.1 (11) were eluted with 0.01 N HCl and dried in a vacuum desiccator under vacuum. Upon addition of 2 N NaOH to the dried eluent, a fishy odor appeared. This suggested the presence of volatile amine(s) in the vaginal fluid of the untreated women.

Identification of amines in vaginal washings by dansylation and thin-layer chromatography of dansyl derivatives. As a result of smearing of the ninhydrin-positive components (presumably caused by high electrolyte concentration, Fig. 1), efforts to identify the individual amine components of vaginal washings by high-voltage electrophoresis at 2.1, 3.5, or 6.5 pH or by paper chromatography (2) were unsuccessful. Dansyl chloride was therefore used to dansylate the vaginal washings and the dansyl derivatives were separated by high resolution thin-layer chromatography on polyamide sheets. As shown in Fig. 2, seven dansyl derivatives of vaginal washings from the untreated patients were found to cochromatograph with the dansyl

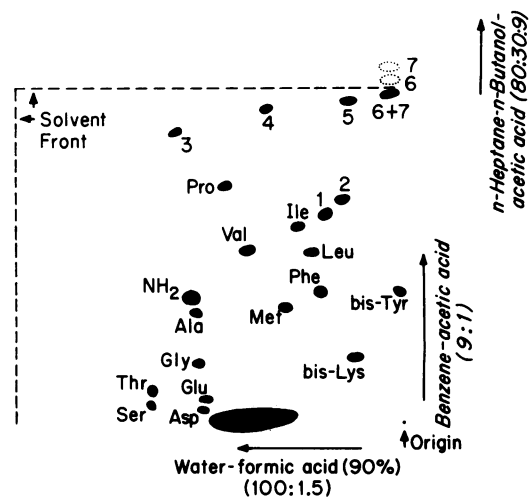

FIGURE 2 Separation of dansyl amino acids and amines by two-dimensional, thin-layer chromatography on polyamide sheets. 1, putrescine; 2, cadaverine; 3, methylamine; 4, isobutylamine; 5, phenethylamine; 6, tyramine; 7, histamine. The dansyl derivative mixture of vaginal washings from untreated patients with nonspecific vaginitis cochromatographed with some or all these amines.

derivatives of putrescine, cadaverine, methylamine, isobutylamine, phenethylamine, histamine, and tyramine. Among these identified amines, putrescine, and cadaverine were the most abundant and were identified in all vaginal washings from the untreated patients or patients treated with the ineffective drug, erythromycin.

Quantitative analyses of amines in vaginal washings from patients. Table I shows the amine concentrations in vaginal washings from 10 patients before and after treatment. The patient numbers shown in Table I correspond to the numbers in Fig. 1. Again, putrescine and cadaverine were the most abundant and were present in all vaginal washings from the untreated patients or patients treated with erythromycin. Cadaverine was present at low concentration in fluids from one patient given ampicillin and one given metronidazole. Histamine, tyramine, and methylamine were also frequently present in the untreated patients or in erythromycin treated patients. Isobutylamine and phenethylamine were present in four and two untreated patients, respectively, at low concentrations.

pH values of vaginal washings from patients. As shown in Table I, the total amine base concentration of the vaginal washings from the untreated or erythromycin treated patients ranged from 0.05 mM to 4.13 mM. This is reflected in the pH value of the vaginal washings of the 10 patients before and after treatment, as shown in Fig. 3. The pH of the vaginal washings of the 10 patients dropped an average of 1.4 U after final treatment. The pH of ampicillin treated vaginal washings was higher than 4.3, whereas the pH of metronidazole treated washings was always below 4.3. The average pH of vaginal washings of the seven normal women was 4.04.

TABLE I
Amine Concentration in Vaginal Washings from Patients with NSV

Patient	Treatment status*	Concentration in vaginal washings							Total base‡
		Meth	Isob	Putr	Cada	Hist	Tyra	Phen	
		mM							
1	Pre	0.06	0	0.27	0.96	0	0	0	2.52
	Amp	0	0	0	0.04	0	0	0	0.08
2	Pre	0	0.02	0.17	0.21	0.01	0.07	0.02	0.89
	Amp	0	0	0	0	0	0	0	0
3	Pre	0	0.02	0.10	0.09	0.01	0.06	0.02	0.50
	Metro	0	0	0	0	0	0	0	0
4	Pre	0.13	0.10	0.73	1.14	0.08	0	0	4.13
	Metro	0	0	0	0	0	0	0	0
5	Pre	0	0	0.01	0.02	0	0	0	0.06
	Metro	0	0	0	0	0	0	0	0
6	Pre	0	0	0.06	0.05	0.01	0.03	0	0.27
	Metro	0	0	0	0	0	0	0	0
7	Pre	0	0.02	0.08	0.07	0.01	0	0	0.34
	Metro	0	0	0	0.03	0	0	0	0.06
8	Pre	0	0	0.02	0.02	0	0.01	0	0.09
	Metro	0	0	0	0	0	0	0	0
9	Pre	0	0	0.02	0.02	0.01	0.01	0	0.11
	Ery	0.01	0	0.18	0.16	0.02	0.07	0	0.80
	Ery	0	0	0.03	0.03	0.01	0.02	0	0.16
	Metro	0	0	0	0	0	0	0	0
10	Pre	0	0	0.01	0.01	0	0.01	0	0.05
	Ery	0	0	0.05	0.05	0	0.02	0	0.22
	Metro	0	0	0	0	0	0	0	0

Abbreviations used in this table: cada, cadaverine; hist, histamine; isob, isobutylamine; meth, methylamine; phen, phenethylamine; putr, putrescine; tyra, tyramine.
* Pre, amp, ery, and metro are defined in legend for Fig. 1.
‡ Total base concentration is the sum of the basic group concentrations from amines in the vaginal washings.

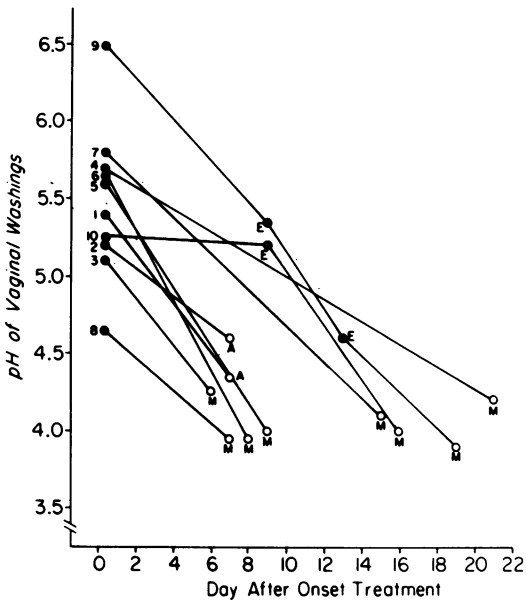

FIGURE 3 Relationship between pH of diluted vaginal fluid (vaginal washings) and treatment status of patients with NSV. Arabic numbers 1–10 correspond to the patient numbers as described in Fig. 1 and Table I. A, E, and M were specimens from ampicillin-, erythromycin-, and metronidazole-treated patients; solid circle, ●, indicates amines present, open circle, O, represents amines absent or reduced to ≤0.04 mM. (Table I).

Electrophoretic mobilities at pH 2.1 of amines found in vaginal washings from NSV patients. The electrophoretic mobilities relative to lysine of seven amines listed in Table I and two polyamines, spermidine and spermine are shown in Table II. Spermine and spermidine were presumably from semen, because they were found only in some vaginal washings from untreated patients, and were absent in washings from patients who were advised not to have intercourse during treatment. Based on electrophoretic mobility at pH 2.1, spermine, spermidine, cadaverine, histamine, putrescine, and methylamine would be included on the "amine" region shown in Fig. 1.

Amine production in vitro by mixed vaginal flora from NSV patients. To examine whether isolates from patients with NSV were capable of amine production in vitro from medium that contained amino acids, the vaginal discharges from four patients before treatment were inoculated separately onto prereduced blood agar plates and incubated anaerobically. Aliquots of the mixed bacterial growth from this medium were then transferred to PSD medium, chemically defined medium (pH 5.5), or the same chemically defined medium supplemented with 0.1 mM metronidazole, and grown anaerobically for 3 d at 35°C (see Methods). Media without organisms were also incubated under the same conditions. After 3 d, the bacteria were removed by centrifugation at 10,000 *g* for 10 min, and the supernates of the spent media were used for amino acid and amine analyses.

After anaerobic incubation of mixed isolates from four patients in PSD medium, defined medium, or defined medium plus metronidazole, only facultative organisms survived in the medium that contained metronidazole, whereas both anaerobes and facultative organisms grew in the media without metronidazole.

The concentrations of each amine and its parent amino acid in one of the uninoculated and incubated media, or in spent media (inoculated and incubated) with or without 0.1 mM metronidazole are shown in Table III. Putrescine can be produced either from ornithine by decarboxylation or from arginine by decarboxylation and hydrolysis or vice versa (12). Putrescine and ornithine were undetectable in uninoculated defined medium. Growth of mixed vaginal organisms in chemically defined medium resulted in disappearance of arginine accompanied by appearance of putrescine in a concentration representing 87% of the arginine originally present. Addition of 0.1 mM metronidazole reduced the amount of putrescine formed by over 10-fold. Similarly, increases in cadaverine and tyramine concentrations were accompanied by reduction in lysine and tyrosine concentrations in the inoculated defined medium. The concentrations of putrescine and cadaverine also increased in PSD medium

TABLE II
Electrophoretic Mobilities at pH 2.1 of Amines Found in Vaginal Washings from Patients with NSV

Amine	Tyra	Phen	Isob	Spm	Spmd	Cada	Hist	Putr	Meth
Mobility*	0.78	0.88	1.06	1.56	1.72	1.72	1.77	1.86	1.88

Abbreviations used in this table: cada, cadaverine; hist, histamine; isob, isobutylamine; meth, methylamine; phen, phenethylamine; putr, putrescine; spm, spermine; spmd, spermidine; tyra, tyramine.
* Electrophoretic mobility is relative to lysine at pH 2.1 (the distance between dansylsulfonic acid and Lys = 1.0). The electrophoretic conditions were described in Methods.

TABLE III

Concentrations of Total Keto Acids, Pyruvate, Amines, and their Parent Amino Acids in Uninoculated and Inoculated Chemically Defined or PSD Media

Medium*, final pH	Concentration‡										
	TKA	Pyru	Putr	(Orn)	(Arg)	Cada	(Lys)	Hist	(His)	Tyra	(Tyr)
						mM					
Defined, uninoculated, pH 5.5	ND	ND	0	0	1.66	0	0.49	0	0.25	0	0.77
Defined + mixed organisms, pH 5.2§	ND	ND	1.45	0	0	0.11	0.12	0	0	0.74	0
Defined (0.1 mM metronidazole) + mixed organisms, pH 4.9§	ND	ND	0.13	0.73	0	0	0.37	0	0.17	0.63	0
PSD, uninoculated, pH 6.7	0.10	0.05	0.06	0.11	0.28	0.08	0.38	0.03	0.13	0.06	0.71
PSD + mixed organisms, pH 7.6‖	0.09	0.01	1.12	0	0	2.91	0.02	0	0	0.06	0
PSD + *H. vaginalis* pH 4.7¶	1.06	0.92	0.06	0.16	1.58	0.09	1.34	0.03	0.58	0.06	1.47

Abbreviations used in this table: cada, cadaverine; hist, histamine; ND, not determined; putr, putrescine; pyru, pyruvate; TKA, total keto acids; tyra, tyramine.

* Chemically defined medium was prepared as described (9) and was used as ×2 concentrate. PSD medium was prepared as described (8). Mixed organisms were originally from anaerobic cultures on blood agar as described in Methods. All incubations were carried out for 3 d anaerobically at 35°C.

‡ Total keto acids and pyruvate were determined as described (10). The total keto acids concentration was expressed as equivalent to pyruvate concentration.

§ A culture of mixed vaginal anaerobic isolates from one patient with NSV was employed in the defined medium, with and without metronidazole, in the experiment shown in the table. Cultures of mixed anaerobic isolates from two other patients were also grown in the defined medium without metronidazole and gave similar results.

‖ A culture of mixed vaginal anaerobic isolates from an additional patient was employed in PSD medium.

¶ Five separate isolates of *H. vaginalis* were tested in PSD medium and the results shown from one isolate are representative.

inoculated with mixed organisms but not in PSD medium inoculated with *H. vaginalis*. No increase in concentration of histamine occurred in any medium, and the tyramine concentration did not increase in PSD medium with mixed organisms or *H. vaginalis*. The amines produced in defined medium that contained 21 amino acids (9) were almost certainly from their parent amino acids. Tyramine concentrations in the spent media with or without metronidazole were about the same. This suggested that metronidazole resistant facultative organisms were responsible for the tyramine production, whereas putrescine and cadaverine were likely produced by metronidazole sensitive anaerobes. *H. vaginalis* failed to grow in chemically defined medium at pH 5.5 anaerobically. Therefore it was not possible to use this chemically defined medium under these conditions to test the role of *H. vaginalis* in amine production.

TABLE IV

Concentrations of Amino Acids in Uninoculated and Inoculated PSD Media

Medium*, final pH	Amino acid concentration‡												
	Asp	Thr + Asn + Gln	Ser	Glu	Pro	Gly	Ala	½Cys	Val	Met	Ile	Leu	Phe
							mM						
PSD, uninoculated, pH 6.7	0.83	0.63	0.71	1.57	0.36	0.98	1.63	0.29	0.84	0.62	0.43	2.60	1.39
PSD + mixed organisms, pH 7.6§	0.71	0.12	0.04	0.08	0.64	1.68	6.55	0.48	0.60	0	0.15	0.06	0
PSD + *H. vaginalis*, pH 4.7§	1.27	1.72	1.83	2.62	0.67	2.20	3.83	0.44	2.55	1.00	2.03	4.71	2.02

* PSD medium was prepared as described (8). Mixed organisms were inoculated into the medium as described in Methods. All incubations were carried out for three days anaerobically at 35°C.

‡ Tyrosine and basic amino acid concentrations are shown in Table III. Thr, Asn, Gln emerged in the same peak on the chromatogram.

§ Isolates are the same as those described for PSD medium in Table III.

Possible symbiotic relationship between H. vaginalis and other mixed organisms. As shown in Tables III and IV, when mixed organisms grew in PSD medium, amino acids were consumed by the organisms from the medium to a greater extent than they were proteolyzed from the medium or were biosynthesized by the organisms, except for proline, glycine, and alanine. Therefore, free amino acids in the medium could play an important role for growth. In contrast, when *H. vaginalis* grew in PSD medium, generation of free amino acids exceeded consumption. The final pH of the PSD spent medium was found to be 4.3–5.0 (pH of the uninoculated medium was 6.7) when pure cultures of *H. vaginalis* from five clinical isolates were grown in PSD medium for 3 d. This low pH may result from the production of a high concentration of pyruvic acid (Table III), which has a low pKa of 2.5 in addition to acetic acid (pKa = 4.74) which is known to be produced by *H. vaginalis* (13, 14). These acids may contribute to death of the organisms (15).

Thus, in patients with *H. vaginalis* vaginal infection, a symbiotic relationship may exist between *H. vaginalis* and other yet undefined anaerobic vaginal organisms, in which *H. vaginalis* generates amino acids and keto acids, especially pyruvic acid, which are utilized by anaerobic members of the vaginal flora, whereas anaerobic organisms relieve excess acidity by producing amines and by utilizing the generated keto acids.

DISCUSSION

As reported by Pheifer et al. (1), an amine-like odor is liberated when 10% KOH is added to vaginal discharges from patients with NSV. High-voltage electrophoresis of vaginal washings confirms the presence of amines in such secretions. This technique is rapid (12 min) and sensitive (1 nmol of diamines can be detected), and provides an objective method for evaluating response to anti-microbial treatment.

Quantitative analysis of amines in vaginal secretions may provide a molecular explanation for at least some of the symptoms of NSV. Cadaverine and phenethylamine are known to be skin irritants and possible sensitizers (16). Methylamine is also a skin irritant (16). Isobutylamine can cause erythema and blistering in skin (16). Histamine has many actions, including dilation and increased permeability of the microcirculation. These various amines might contribute to the epithelial cell shedding and the discharge of some patients as well as to the characteristic odor of NSV.

As shown in Fig. 3, the pH range of vaginal washings from untreated patients is from 4.7 to 6.5. Any amines present in this pH range exist in the protonated form (salt) and are not volatile. However, after addition of 10% KOH (1), the amines are converted to an unprotonated form (free base) and become volatile and thus odorous. During and after intercourse, the alkaline prostatic fluid may convert part of the amines to the unprotonated form and thus cause the characteristic fishy odor.

H. vaginalis has been reported as the most common organism among vaginal flora in patients with NSV (1, 17). It is not certain whether this organism represents simply a marker for the syndrome as an innocent bystander, or whether it contributes to the pathogenesis of NSV. It is possible that other organisms, by producing decarboxylases that convert amino acids to amines, and perhaps by other mechanisms such as further metabolizing acids produced by *H. vaginalis* raise the pH of vaginal secretions to a level that enhances growth of the *H. vaginalis*.

Putrescine is known to be an essential growth factor for some organisms (18–21), and may be essential for growth of some organisms in vaginal flora. As shown in Table III, putrescine was produced in vitro by anaerobe(s) in the vaginal flora of patients with NSV.

Gardner and Dukes (17) inoculated 15 patients proven free of *H. vaginalis* infection with fluid from the vaginal tract of patients with vaginitis associated with *H. vaginalis*. 11 of the 15 patients developed the symptoms of NSV and *H. vaginalis* was recovered subsequently as the predominant bacterium from each of these 11 volunteers. However, a much smaller percentage of healthy women (8%) inoculated with pure cultures of *H. vaginalis* developed *H. vaginalis* infection (17). Thus the presence of certain other vaginal organisms in NSV may enhance infectivity and survival of *H. vaginalis*. It is also possible that *H. vaginalis* somehow enhances the growth of those organisms that decarboxylate amino acids, thus increasing the concentrations of amines in vaginal secretions and contributing to the pathogenesis of NSV. The specific organism(s) responsible for decarboxylation of amino acids in vaginal secretions remains to be determined.

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