CLINICAL MICROBIOLOGY REVIEWS, July 1992, p. 213–237
0893-8512/92/030213-25$02.00/0

Vol. 5, No. 3

Gardnerella vaginalis: Characteristics, Clinical Considerations, and Controversies

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INTRODUCTION

Different interpretations of the clinical significance of *Gardnerella vaginalis*, its Gram stain reaction, and its taxonomic position have generated considerable controversy. First recognized by Leopold (126), the organism was named *Haemophilus vaginalis* by Gardner and Dukes in 1955 (75) because it was a gram-negative rod successfully isolated on blood agar but not on other agar media and it was believed responsible for a characteristic vaginal discharge. The absence of requirements for X and V factors (hemin and NAD, respectively), which are needed for the growth of accepted *Haemophilus* species, the tendency to retain the crystal violet dye in the Gram reaction, and some corynebacterium-like features suggested that the organism might better be associated with the genus *Corynebacterium*. Hence, it was referred to as *Corynebacterium vaginale* by Zinnemann and Turner (249) and others (56, 243). Two large taxonomic studies published in 1980 (82, 174) analyzed data obtained by a variety of biochemical methods, DNA-DNA hybridization, and electron microscopy. The findings revealed that "*Haemophilus vaginalis*" forms a good taxospecies that displays little or no similarity to established gram-positive or gram-negative genera. The need for a new genus led Greenwood and Pickett (82) to propose the name *Gardnerella vaginalis*, a proposal supported by Piot et al. (174).

In this review I shall use the name *G. vaginalis* for the organism that was called *H. vaginalis* or *C. vaginale* in the earlier literature. Where relevant, I shall mention the type strain of the species, indicated by T, which was used as a reference in some studies. Greenwood and Pickett (82) designated strain 594 of Gardner and Dukes as the *G. vaginalis* type strain and deposited it in the American Type Culture Collection as ATCC 14018^T and in the National Collection of Type Cultures as NCTC 10287^T.

The *G. vaginalis*-associated vaginal syndrome was earlier called nonspecific vaginitis in recognition of the absence of recognized agents of vaginitis, such as *Trichomonas vaginalis* and *Candida* species. Until his death in 1982, Gardner staunchly maintained that the term nonspecific should include only those conditions without assignable etiology and that *G. vaginalis* vaginitis is a precisely defined, specific vaginal infection that accounts for most vaginitides previously classified as nonspecific (73, 74). Substitution of the term bacterial vaginosis was recommended because vaginitis suggests an inflammatory reaction of the vaginal epithelium, which is usually absent (46, 73, 91, 201). This syndrome has also been given more than 15 other names (96, 200, 235).

The pathogenic role of *G. vaginalis* has been controversial. Inadequate media and methods led to earlier failures to detect small numbers of the organism in cultures of blood and urogenital specimens. There is now a greater appreciation of *G. vaginalis* as a cause of extravaginal infections. Lacking results of anaerobic cultures, investigators were blind to the complex ecology of the vagina in health and disease. This deficiency has been corrected in recent studies of bacterial vaginosis which reveal a polymicrobic etiology that includes *G. vaginalis*, mycoplasmas, and various anaerobic bacteria (216). The role of *G. vaginalis* in disease has been well described in several reviews (88, 104, 216, 239, 240).

The intent of my review is to provide a critical evaluation of *G. vaginalis*, its differential and biological features, its pathogenic potential, and areas of fundamental research in need of further study.

IDENTIFICATION AND CHARACTERISTICS OF *G. VAGINALIS*

Appearance of Cells and Colonies

G. vaginalis cells are gram-negative to gram-variable, small, pleomorphic rods that are nonmotile and do not possess flagella, endospores, or typical capsules (82, 172). In vaginal fluid smears the Gram reaction of *G. vaginalis* may vary from positive to negative. The cellular morphology in a Papanicolaou preparation can be seen in noncrowded areas peripheral to the epithelial cells in Fig. 1.

The physiological state of the bacteria affects their morphology and staining reactions (107, 174). Both small coccobacilli and longer forms occur in 24-h cultures of *G. vaginalis* on blood agar. Their average dimensions are 0.4 by 1.0 to 1.5 μm (59). Although cells can be up to 2 to 3 μm in length, they do not elongate into filaments (82, 228). Smith (211) observed that cultures on vaginalis agar (V agar; see below) exhibited many short rods that were gram negative, whereas the cells were more pleomorphic, clumped, gram variable, and beaded on a medium containing starch, a fermentable compound. *G. vaginalis* growing in 48-h cultures of patients' blood specimens were reported as predominantly gram positive (124, 245). The organism was partially or entirely gram positive in the early exponential growth phase on the inspissated serum medium of Loeffler (174) or Roux (249).

The resemblance of *G. vaginalis* to coryneform (diphtheroid) bacteria (45) was described by many investigators (3, 13, 56, 59, 133, 164, 174, 208, 243, 246, 249). Angular and palisade (picket fence) arrangements of cells occur because of snapping that accompanies division. Metaphosphate (volutin) granules form in *G. vaginalis*, especially during cultivation in the presence of a fermentable compound (59) or sodium phosphate (243). These granules stain gram positive (56, 249) or are reddish purple (metachromatic) when stained with alkaline methylene blue.

G. vaginalis is beta-hemolytic on media containing human or rabbit blood but not on sheep blood agar (24, 59, 82, 172, 208, 243). Hemolysis is improved by anaerobic incubation (119). For media prepared with blood bank blood, hemolysis is clearest with blood which is "just time expired" (97). The importance of the composition of the basal medium to which blood is added is illustrated by the observation that the diameters of colonies on Columbia agar base with 5% sheep blood were at least twice as large as those on soybean-casein digest agar base with 5% sheep blood (78). Furthermore, hemolysis of human blood incorporated in Columbia agar base from BBL Microbiology Systems was superior to that obtained in the Difco Laboratories base of the same name (173).

On a medium prepared with 2% peptone, horse flesh digest, 1% maltose, and 4% human blood, Edmunds (59) observed that *G. vaginalis* colonies were only 0.5 mm in diameter after aerobic incubation for 3 days. *Streptococcus pyogenes* colonies were said to be "much larger" under the same conditions. Dunkelberg and McVeigh (55) tested commercial peptones and found that Proteose Peptone no. 3

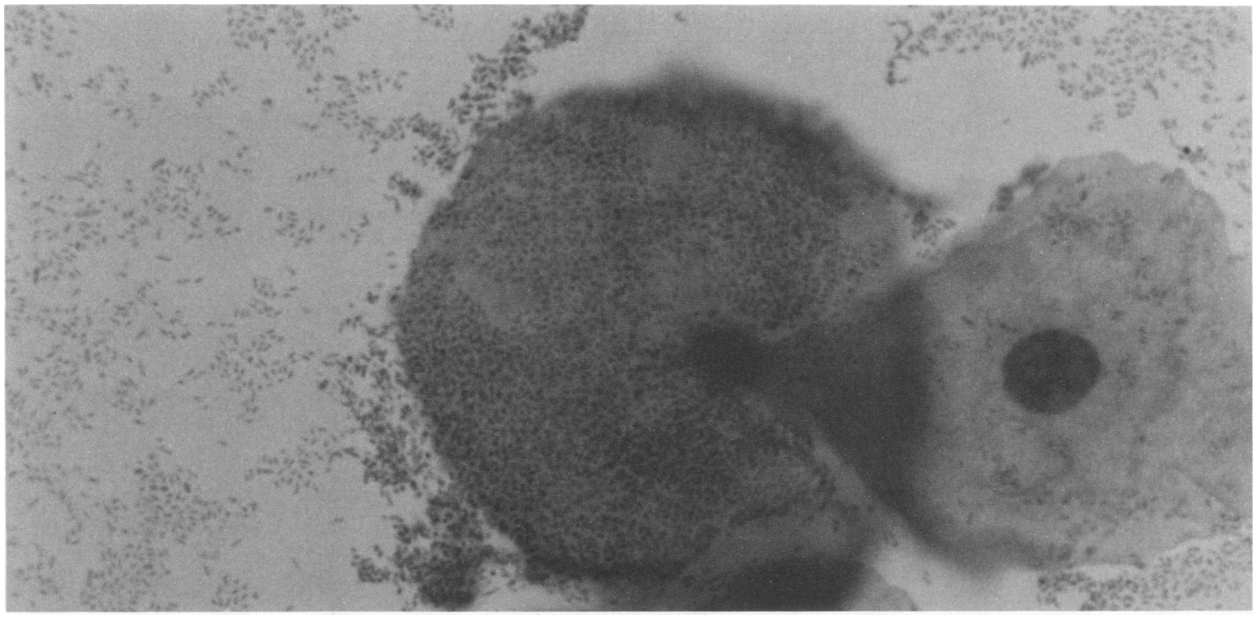

FIG. 1. Morphology of *G. vaginalis* shown unattached or attached in large numbers to one of the squamous epithelial cells (a clue cell) in a Papanicolaou preparation (magnification, ×1,200). (Photomicrograph provided by V. J. Schnadig.)

(Difco) or Myosate (BBL) gave better growth than four other peptones. On PSD agar, which contains Proteose Peptone no. 3, soluble starch, and dextrose, 48-h colonies of *G. vaginalis* are 0.5 to 2 mm in diameter with smooth edges and are domed and whitish (54). V agar is composed of Columbia agar base (BBL) supplemented with 1% Proteose Peptone and 5% human blood (81). Colonies on V agar are round, opaque, smooth, and 0.5 mm in diameter after incubation for 48 h at 37°C in a humidified atmosphere with 5% CO_2 (82).

Isolation Methods

Many different media have been used to isolate *G. vaginalis* from clinical specimens (78, 173). The most successful media possess both differential and selective features. The presence of human blood makes it possible to pick out beta-hemolytic *G. vaginalis* from among numerous nonhemolytic colonies. Goldberg and Washington (78) demonstrated the value of a semiselective medium containing colistin (10 μg/ml) and nalidixic acid (15 μg/ml) incorporated in Columbia agar base. The addition of 1% corn starch to a semiselective medium enabled Mickelsen et al. (144) to distinguish *G. vaginalis* colonies by their hydrolytic clearing of the opaque starch. Ison et al. (97) developed a human blood agar medium with gentamicin (4.0 μg/ml), nalidixic acid (30 μg/ml), and amphotericin B (2.0 μg/ml).

A *G. vaginalis* medium designated HBT (human blood-bilayer-Tween) was developed by Totten et al. (233). It consists of a bottom layer of Columbia colistin-nalidixic acid agar (BBL) supplemented with 1% Proteose Peptone no. 3 (Difco), amphotericin B (2.0 μg/ml), and 0.0075% Tween 80 (BBL) together with a top layer of the same composition except that 5% human blood is added. Zones of beta-hemolysis are clear with diffuse edges. Since zones are viewed through a shallower layer of blood-containing medium, hemolysis is easier to detect on HBT than on a single-layer medium. Furthermore, Tween 80 improves hemolysis (233) and enhances growth (133). Tests of 28 *G. vaginalis* strains that had been isolated on chocolatized sheep blood agar showed that 86% were beta-hemolytic on a single-layer medium compared with 100% on HBT. Significantly, *G. vaginalis* was isolated from vaginal fluids more often on HBT than on V agar ($P = 0.007$) (233). HBT medium was used successfully by Holst (92) to isolate *G. vaginalis* from anorectal specimens. Although at present HBT is the most satisfactory differential selective medium, it fails to inhibit a variety of gram-positive bacteria, such as lactobacilli, coryneforms, and streptococci (92, 173).

Incubation at 35 to 37°C for 48 h in a humidified atmosphere of air plus 5 to 10% CO_2 or in a candle flame extinction jar is satisfactory for practical purposes (81, 172, 227, 233). Obligately anaerobic *G. vaginalis* isolates have been identified (133, 173), but are not common (6, 139). Nevertheless, anaerobic incubation of medium inoculated with clinical specimens was preferred by some authors (4, 42, 67, 120, 144, 145, 243). A 72-h period of incubation leads to slight increases in isolation percentages depending on the medium. For example, 5 (10%) of 50 chocolate agar cultures that were negative after 48 h exhibited growth after 72 h, but incubation of 67 HBT cultures for 72 h yielded only one isolate not seen earlier (233). In considering the appropriate time of incubation, it is important to recognize that *G. vaginalis* cells sampled from unfavorable environments, such as acidic urine (119), are likely to exhibit atypically long lag periods which prolong the time required for colony formation.

A plastic envelope method for cultivation of *G. vaginalis* was described by Ching et al. (37). A semiselective Proteose Peptone broth contained in the envelope is inoculated with vaginal fluid and incubated at 35°C for 18 h with CO_2 generated by a self-contained unit. The resulting mixed culture is used in 2-h biochemical tests for provisional identification.

Media that contain the anticoagulant sodium polyanetholesulfonate are inhibitory for *G. vaginalis* (184) unless an additive such as gelatin is used to overcome the toxicity (186). Failure to detect bacteremias (77, 122) may be due to

TABLE 1. Characteristics of *G. vaginalis* and unclassified catalase-negative coryneform (UCNC) bacteria

Test or substrate	% Positive strains						
	G. vaginalis				UCNC		
	Study 1 (n = 175)[a]	Study 2 (n = 29)[b]	Study 3 (n = 78)[c]	Study 4 (n = 57)[d]	Study 1 (n = 70)[a]	Study 2 (n = 19)[b]	Study 4 (n = 5)[d]
Beta-hemolysis (human blood, bilayer medium)	99	—[e]	—	95	40	—	0
Beta-hemolysis (human blood, single-layer medium)	88	—	96	—	10	—	—
Alpha-hemolysis (human blood, bilayer medium)	0	—	—	—	37	—	—
Hemolysis (sheep blood, single-layer medium)	—	—	0	—	—	—	—
Catalase	0	0	0	0	0	0	0
α-Glucosidase	100	100	—	100	94	84	100
β-Glucosidase	0	0	—	0	57	21	0
Starch hydrolysis	100	97	100	—	71	95	—
Hippurate hydrolysis	90	86	92	100	58	44	100
β-Galactosidase	45	66	53	26	55	21	20
Lipase (egg yolk medium)	64	62	43	—	24	16	—
Acid phosphatase	—	93	—	100	—	63	60
Gelatin hydrolysis	—	3	0	—	—	5	—
Esculin hydrolysis	—	3	0	—	—	0	—
Urease	—	3	0	—	—	0	—
Growth on medium containing 2% NaCl	—	10	—	0	—	95	100
Acid (no gas) from							
L-Arabinose	38	45	9	31	13	53	30
Dextrin	—	—	100	—	—	—	—
Fructose	—	79	81	95	—	89	40
Galactose	—	38	96	33	—	16	0
Glucose	100	100	97	100	100	100	100
Maltose	100	97	100	93	98	100	80
Mannitol	0	0	0	0	20	0	0
Mannose	—	10	82	40	—	26	50
Raffinose	—	0	0	0	—	0	0
Rhamnose	—	0	0	0	—	16	0
Ribose	—	—	99	100	—	—	100
Salicin	—	0	0	0	—	32	0
Sorbitol	—	3	0	0	—	16	0
Sorbose	—	6	—	0	—	16	20
Starch	100	97	100	—	98	95	—
Sucrose	85	17	10	62	81	74	50
Trehalose	72	24	4	—	52	21	—
Xylose	44	10	15	10	22	63	50

[a] Results reported by Piot et al. (176).
[b] Results reported by Piot et al. (174). These strains were included in the later study (176).
[c] Results reported by Greenwood and Pickett (81).
[d] Results reported by Taylor and Phillips (227). The small number of UCNC strains tested and difficulty in reproducing results were responsible for anomalous results, such as 30 or 50% positive reactions for five strains.
[e] —, no percentage given.

inhibition by this anticoagulant. The incorporation of patient's blood in pour plate medium serves to limit contact with anticoagulant (183, 241) and permits counts of colonies. La Scolea et al. (121) recovered *G. vaginalis* from heparinized blood specimens that were pipetted on the surfaces of agar media; in contrast, duplicate blood specimens gave negative results in the conventional BACTEC radiometric system. Legrand et al. (124) reported a case of bacteremia in which *G. vaginalis* was recovered in a Hemoline bottle but not in BACTEC broth.

Differential Tests

Presumptive identification. The characteristics of colonies surrounded by zones of clear hemolysis with diffuse edges on HBT medium, the typical appearance of Gram-stained cells, and a negative catalase test provide a presumptive identification of *G. vaginalis* (172, 173, 201). Other workers add further tests, such as hippurate hydrolysis (107). These minimal criteria may be sufficient for strains isolated from vaginal fluid, but it is essential to confirm the provisional identification of strains isolated from extravaginal sites.

Confirmation. *G. vaginalis* is best identified by a typical profile revealed by the results of multiple tests. As seen in Table 1, a limited number of biochemical reactions could fail to give a definitive identification. The most valuable characteristics are the presence of α-glucosidase, absence of β-glucosidase, hydrolysis of starch and hippurate, and hemolysis of human blood but absence of hemolysis of sheep blood. Additional useful characteristics are negative mannitol fermentation and zones of inhibition on PSD cultures seen around disks containing 50 μg of metronidazole (6, 107, 201, 233, 243), 5 μg of trimethoprim, or 10% bile (172). Vaginal lactobacilli and unclassified, catalase-negative coryneform (UCNC) bacteria are substantially more resistant to these agents than *G. vaginalis* (172, 227). However, the diameters of inhibition zones vary between strains in each group (176) and are affected by the inoculum density.

Confusing catalase-negative bacteria. Isolates from blood or genitourinary specimens often resemble commensal skin

corynebacteria (3, 67, 111, 164, 246), and many test reactions (Table 1) do not adequately discriminate between UCNC bacteria and *G. vaginalis*. Most corynebacteria produce catalase, but catalase-negative reactions and fastidious growth (e.g., stimulation by serum or Tween 80) are characteristic of Centers for Disease Control coryneform groups E and 2. Also, the species *Actinomyces pyogenes* and *Arcanobacterium haemolyticum* that were formerly included with corynebacteria are catalase negative (45).

Various characteristics, in addition to those cited above, have been considered of differential value. Nutrient agar supports the growth of *G. vaginalis* only rarely (59, 227), whereas most strains of UCNC, vaginal lactobacilli, and streptococci can grow on it (174). Differences between nutrient agar preparations, however, limit the reliability of this test. Sodium chloride tolerance is possibly more reliable. *G. vaginalis* is inhibited by NaCl concentrations as low as 0.5% (56), 2% (Table 1), or 3% (81), whereas many strains of UCNC and streptococci are NaCl tolerant (174, 227). Hydrogen peroxide inhibits *G. vaginalis* (4, 6, 56, 107, 108, 116, 174) and also most UCNC strains (174), as seen by the absence of growth in a zone surrounding a drop of 3% H_2O_2 on a blood-free agar culture. The low concentration of bacitracin in Taxo A disks inhibits *G. vaginalis* (6, 81, 107, 211, 243).

The UCNC vaginal isolates studied by Piot and Van Dyke (173) produced smaller colonies than *G. vaginalis*, and the zones of beta-hemolysis, if any, were narrower. The lower percentage of beta-hemolytic UCNC strains on the thicker, single-layer medium than on bilayer medium (Table 1) is a reflection of their tendency for weaker hemolysis.

Methods: some yield inconsistent results. Inadequate growth in test media and the use of variant methods and media contributed to differing results reported for *G. vaginalis* in the early literature. Fermentation results varied greatly, especially acid production from glycogen, lactose, mannose, sucrose, and xylose (60, 174, 228). Piot et al. (176) emphasized the importance of using large inocula from cultures no older that 24 h. Detection of acid production during growth may require incubation of cultures for up to 5 days. Sterilization of carbohydrate solutions by filtration was recommended by Dunkelberg (54).

Growth media used for the fermentation test results reported in Table 1 contained 2% Proteose Peptone no. 3 (Difco), phenol red, and 1% carbohydrate either with the addition of 0.5% agar (81, 227) or without agar but supplemented with 5% sterile horse serum (176). For the rapid fermentation microtest method used by Piot et al. (174), a buffered salt solution containing phenol red and carbohydrate (5.6% for most carbohydrates) was inoculated with a dense suspension of an overnight culture on blood agar. Results were read after incubation at 37°C for 4, 8, and 24 h.

The hydrolysis of starch by anaerobic cultures on Mueller-Hinton agar (BBL) supplemented with 5% sterile horse serum was detected after incubation for 3 days by the absence of a blue color after addition of iodine solution (176). Alternatively, hydrolysis was detected by a clearing of the opacity of corn starch (81).

Lipase activity was detected by the appearance of an oily, iridescent sheen on an enriched egg yolk medium after anaerobic incubation. A more recent lipase test method makes use of an oleate substrate (24).

Tests for hydrolysis of hippurate (*N*-benzoylglycine) detected the formation of benzoic acid (174, 227) or glycine (176). Piot et al. (176) found that the pH of the hippurate test solution affected the results; pH 6.4 was optimum. Lin et al. (130) discussed these tests for hippurate hydrolysis and described a two-dimensional thin-layer chromatographic technique that was more specific for glycine than the standard ninhydrin reaction. They found 18 of 20 *G. vaginalis* isolates positive for hippurate hydrolysis by this method.

Other characteristics. Arginine, lysine, and ornithine decarboxylases are not produced. Tests for oxidase, indole, H_2S, nitrate reduction, β-glucuronidase, and tributyrin hydrolysis are negative. Acid is not produced from adonitol, arbutin, cellobiose, glycerol, inositol, melibiose, or any of 12 alcohols. Alkali is not produced in tests with any of 25 organic salts and amines. The methyl red test is positive if sufficient growth occurs in glucose broth; acetylmethylcarbinol is not produced. Acetic acid is the major volatile acid produced during growth in PSD broth. Growth is inhibited on MacConkey agar and Thayer-Martin medium. The foregoing test results for *G. vaginalis* were compiled from references 56, 81, 174, 227, and 228.

Growth inhibition by sodium polyanetholesulfonate or by alpha-hemolytic streptococci was included among tests for the presumptive identification of *G. vaginalis* in some studies (164, 185, 198, 228), but zone diameters vary under different test conditions (185).

Rapid detection. Pure cultures of catalase-negative, gram-negative to gram-variable bacteria with a typical morphology could be identified as *G. vaginalis* within 4 h. Janda et al. (101) correctly identified 24 (96%) of 25 isolates by use of the *Haemophilus-Neisseria* identification panel of 18 tests (American MicroScan, Sacramento, Calif.). Lien and Hillier (129) evaluated the enhanced rapid identification method (Austin Biological Laboratories), a system based on starch and raffinose fermentation and hippurate hydrolysis (247). Results for 96 (91%) of 105 strains agreed with those obtained with standard biochemical methods. However, the API-ZYM system for enzyme detection (227) and the API-20A and Minitek systems (6) were considered unreliable for the identification of *G. vaginalis* in the diagnostic laboratory.

Specific detection of *G. vaginalis* in patients' specimens has been achieved by use of a DNA probe-based hybridization method (161), an enzyme-linked immunosorbent microassay (23), and the direct (84) and indirect (30) methods of immunofluorescence microscopy.

Structure, Composition, and Toxic Products

Cell wall. The Gram reaction indicates fundamental differences between the compositions and structures of eubacterial walls. Gram-negative walls (as represented by *Escherichia coli*) have an outer membrane consisting of lipopolysaccharide, protein, and phospholipid that is seen as two electron-dense layers with an electron-translucent middle layer. This trilaminar membrane overlies a thin layer containing peptidoglycan. On the other hand, typical gram-positive walls (as represented by *Bacillus subtilis*) are relatively thick, amorphous matrices of intertwined peptidoglycan and secondary polymers, such as teichoic acid (14). Beveridge (14) analyzed the Gram reaction and suggested that the amount of peptidoglycan in the wall primarily determines whether a cell stains gram negative or gram positive. Strains characterized as gram variable may appear gram positive during the exponential growth phase but gram negative as the culture ages because the peptidoglycan layer becomes too thin to retain the crystal violet-iodine aggregates (14, 197).

Reyn et al. (188) provided the first ultrastructural evidence of the gram-positive character of *G. vaginalis* (Fig. 2). The

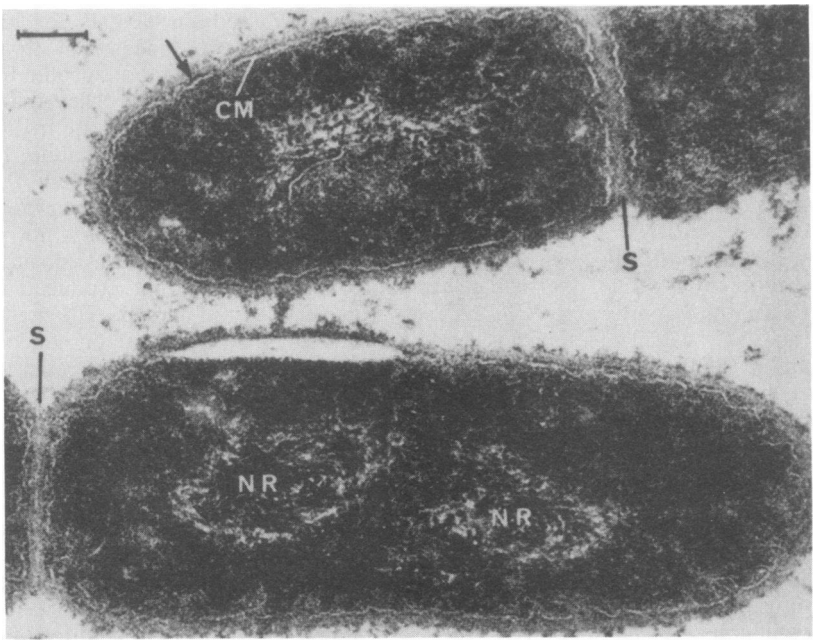

FIG. 2. Ultrastructure of *G. vaginalis* 594 (ATCC 14018^T) shown in a longitudinal section of two dividing cells; this electron micrograph, published in 1966, first showed the gram-positive character of the cell wall. Cells from an 11-h culture were prefixed with osmic acid, fixed in buffered 1% OsO_4, and then treated with uranyl acetate; the thin section was poststained with lead hydroxide. Arrow points to asymmetric area of the cytoplasmic membrane (CM). NR, nuclear region; S, septum. Bar = 0.1 μm. From Reyn et al. (188) with permission of the publisher.

wall is relatively thin compared with the walls of reference strains of *Corynebacterium diphtheriae*, *Butyribacterium rettgeri*, and *Lactobacillus acidophilus*. Beneath the wall and in close association with it lies the cytoplasmic membrane. Figure 2 shows that the wall, like a "rigid corset," remained intact in an area where the electron-translucent layer split during sectioning. Well-defined septa typical of gram-positive cells develop prior to division. Younes (248) made use of electron microscopy to study the effect on *G. vaginalis* of growth in a subinhibitory concentration (0.75 μg/ml) of furazolidone, a nitrofuran. Cellular lysis and extrusion of cytoplasmic material could be seen within 6 h, leaving a rigid, laminated membrane apparently intact.

Criswell et al. (48, 49) compared *G. vaginalis* ATCC 14018^T with *E. coli* and found that both possess a multiple-layered cell wall and both have a low peptidoglycan content, which represent 20 and 23%, respectively, of the total wall weight. Greenwood and Pickett (82) also examined the type strain and observed multiple laminations. This wall was interpreted as more closely resembling the wall of a gram-negative than a gram-positive organism, although it was recognized as not "typical." Greenwood (80) pointed out that the unique laminations might explain why *G. vaginalis* is more difficult to lyse than either typical gram-positive or gram-negative bacteria.

A reexamination of the wall ultrastructure and composition of strain ATCC 14018^T and *G. vaginalis* clinical isolates led Sadhu et al. (197) to conclude that the walls show gram-positive organization. The thinness of the walls accounts for their gram-negative staining tendency and for the deceptive lamellar appearance seen when the cell envelope is not sectioned at exactly a right angle. Furthermore, cell wall extracts did not contain 2-keto-3-deoxy-D-manno-2-octonoic acid. Diaminopimelic acid and hydroxy fatty acids were not found, and only a low concentration of endotoxin was detected by the *Limulus* amebocyte lysate assay (50, 86, 102, 157, 197). This evidence indicated that *G. vaginalis* does not contain classical lipopolysaccharide. The cell wall contains major amounts of alanine, glutamic acid, glycine, and lysine; glucose, galactose, and 6-deoxytalose, a diagnostic wall sugar, are also found (56, 86, 157).

Exopolysaccharide layer. A flocculent (82, 188) or fibrillar (49) exterior layer was visualized by electron microscopy (Fig. 2), and ruthenium red staining indicated that it has a polysaccharide component (80). This microcapsular material, described as electron-dense, dehydration-condensed, fibrillar exopolysaccharide (197), can be seen as weblike strands occasionally connecting cells lying close together (236). Its role in adherence of *G. vaginalis* to epithelial cells of the vagina has been demonstrated (203, 214, 236). I suggest that this microcapsular material may also be responsible in part for the clustering of cells that occurs in broth cultures (18, 37, 59).

Pili. Pili (fimbriae) radiating from the surfaces of *G. vaginalis* cells have been observed by electron microscopy (21, 105, 203). The diameters of the pili range between 3.0 and 7.5 nm. Boustouller et al. (21) found that pili were carried by the vaginal isolates from women with bacterial vaginosis and also by urethral isolates from men with nongonoccal urethritis. Clinical isolates displayed more heavily piliated cells and fewer sparsely piliated or nonpiliated cells than laboratory strains that had been subcultured frequently (21, 105).

Lipids. O'Donnell et al. (157) determined the fatty acid and polar lipid compositions of strain ATCC 14018^T and four other *G. vaginalis* strains. Straight-chain saturated and unsaturated nonhydroxylated fatty acids were present; the major components were hexadecanoic acid (16:0), octade-

cenoic acid (18:1), and octadecanoic acid (18:0). Other analyses of *G. vaginalis* strains have been reported (50, 82, 102, 151). Polar lipid patterns were very characteristic and consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, five glycolipids, and an unidentified phospholipid (157).

Proteins. Ison et al. (100) found that the specificities of *G. vaginalis* serotyping antibodies were directed to proteins of differing molecular weights. Boustouller et al. (20) observed differences between the polypeptide profiles of whole-cell extracts of *G. vaginalis* analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Furthermore, antigenic differences were revealed by Western blot (immunoblot) analyses of the separated polypeptides. In spite of their antigenic heterogeneity, all strains tested displayed a common immunodominant molecule with an apparent molecular mass of 41 kDa.

DNA. The guanine-plus-cytosine (G+C) contents of DNA preparations from *G. vaginalis* ATCC 14018^T were determined in three laboratories. The value found by the thermal denaturation midpoint method was 42 mol% (234). The cesium chloride buoyant density gradient centrifugation method gave values of 43.1 (82) and 41.8 (174) mol%. The G+C contents of three other *G. vaginalis* strains were 43.5 (82), 42.1 (174), and 43.0 (174) mol%.

Investigations have been limited by difficulties experienced in lysing *G. vaginalis* (80, 82, 174). A procedure described by Nath et al. (154) for isolating DNA should facilitate restriction endonuclease analyses of chromosomal DNA from clinical isolates and exploration for extrachromosomal plasmids (52).

Toxic products. Rottini et al. (196) described a cytolytic, extracellular toxin released by *G. vaginalis* during growth in a supplemented peptone broth containing added starch and Tween 80. The purified cytolysin is an amphiphilic protein with a molecular mass of 63 or 61 kDa, as determined by two methods. The toxin undergoes a spontaneous decay of activity that is minimized by ammonium sulfate and nonionic detergents and by binding to erythrocyte membranes. Tests of 30 ng of toxin did not detect phospholipase C activity. Interestingly, this hemolysin has a marked target specificity: 50% lysis of human erythrocytes was obtained with 0.75 ng of toxin, whereas 100-times-higher concentrations failed to lyse horse, rabbit, sheep, or guinea pig erythrocytes. Human polymorphonuclear leukocytes and human endothelial cells also were susceptible to lysis, although at doses higher than those required for hemolysis of human erythrocytes. Moran et al. (150) studied the electrical properties of ionic channels formed upon introduction of the cytolytic toxin into artificial membranes. The successful incorporation into liposomes indicated that the channel-forming conformation of the protein is independent of the erythrocyte target specificity of the cytotoxin.

The phospholipase activities of a variety of bacteria isolated from the female genital tract were reported in two large surveys. Unfortunately, neither report provided information about the characteristics of the bacteria or the criteria for their identification. A high specific activity of phospholipase A_2 was detected in sonicated cells from one strain of *G. vaginalis* (11). The report (143) of phospholipase C production by two of seven *G. vaginalis* isolates is difficult to evaluate because of unanswered questions about the identity of the seven strains and the adequacy of culture conditions (growth for 18 h in tryptose minimal growth medium).

Surface Adhesion

Adhesion has been of interest ever since Gardner and Dukes (75) recognized the value of vaginal clue cells for the diagnosis of bacterial vaginosis (see below). Clue cells are squamous epithelial cells whose surfaces are heavily coated with bacteria resembling *G. vaginalis* (Fig. 1). They are most commonly found in vaginal specimens but are also seen in urine aspirated from the bladders of women (67). Furthermore, clue cells have been observed in semen (99), urethral discharge (38), and endourethral swab samples (29, 123) from men. Adhesion to urogenital epithelial cells allows *G. vaginalis* to colonize, thereby minimizing contact of the bacteria with potentially deleterious extracellular enzymes and local antibodies and reducing their chances of being flushed away in vaginal fluid or urine.

Vaginal epithelial cells (VECs) scraped from the vaginal wall were used in studies of in vitro adhesion (135, 166, 203, 214, 215). Mårdh and Weström (135) investigated the adherence to VECs of several species of bacteria isolated from the vagina. Fewer *L. acidophilus* cells than *G. vaginalis* cells adhered during incubation for 30 min, but the numbers of adherent bacteria per cell observed on 50 VECs varied widely for each of the species studied. Such variation, also recorded by others (215), could be partly due to the heterogeneity of the VECs in vaginal scrapings. Making use of a human vaginal tissue culture system and scanning electron microscopy, Sobel et al. (214) found that adherence of *G. vaginalis* to mature cells, many in the process of desquamation, was greater than adherence to actively proliferating epithelial cells. An in vivo study by van der Meijden et al. (236) showed that the vitality of VECs is a significant factor in adherence. The majority of clue cells collected from the vaginal discharge of patients with bacterial vaginosis excluded trypan blue, an indication of vitality. In the absence of vitality (trypan blue retained), VECs were 10-fold less likely than vital VECs to be clue cells.

G. vaginalis adherence to VECs increased with increasing acidity of the test medium, being greatest at pH 4 in phosphate-buffered saline (215) and at pH 5 to 6 in citrate-acetate-phosphate buffer (166). It is known that both VECs and bacteria carry net negative charges that create an electrostatic repulsive force. This is reduced at a lower pH, with the result that binding is increased. Adherence in the vaginal microenvironment doubtless also is influenced by pH.

The influence of subinhibitory concentrations of two therapeutic agents on the adherence of *G. vaginalis* to VECs was studied by Peeters and Piot (166). Metronidazole concentrations of one-eighth and one-fourth the MIC significantly reduced the adherence to VECs of three strains but not a fourth. On the other hand, ampicillin at subinhibitory concentrations did not reduce the capacity for adhesion to VECs of any of the four strains.

Hemagglutination of one or more species of erythrocytes that occurred at 5°C and was reversed upon warming was described by Edmunds (60). Ison and Easmon (98) studied variables that affect the adhesion of ^{3}H-labeled *G. vaginalis* to human erythrocytes. Scott et al. (203–205) further characterized hemagglutination and compared it with adhesion to McCoy cells, an epithelial tissue culture cell line. Tests of 105 clinical isolates showed that the isolates differed with respect to their adhesion indexes, measured as the percentage of McCoy cells displaying adherent *G. vaginalis* (205). Some strains attached heavily to all McCoy cells, whereas other strains exhibited low adhesion indexes. A correlation

TABLE 2. *G. vaginalis* type strain susceptibilities under various test conditions

Authors (reference)	Agent[a]	Medium[b]	CFU/ml in inoculum	Incubation		MIC (μg/ml)
				Atmosphere	Time (h)	
Smith and Dunkelberg (212)	Metronidazole	PS broth	NS[c]	Air + CO_2	24	12.5
				Air + CO_2	48	50.0
Ralph et al. (180)	Metronidazole	PSD broth	10^4	Air + CO_2	24	1.0
			10^4	Air + CO_2	48	8.0
			10^4	Anaerobic	24	≤0.25
			10^6	Air + CO_2	24	16.0
			10^6	Anaerobic	24	4.0
	Tetracycline	PSD broth	10^4	Air + CO_2	24	≤0.25
			10^6	Air + CO_2	24	1.0
Ralph and Amatnieks (179)	Metronidazole	PSD broth	10^6	Anaerobic	48	4.0
	Hydroxy M		10^6	Anaerobic	48	2.0
Balsdon and Jackson (7)	Metronidazole	CCA	NS	Anaerobic	48	10.0
	Hydroxy M		NS	Anaerobic	48	2.5
Shanker and Munro (207)	Metronidazole	Agar	10^6	Anaerobic	48	4.0
	Hydroxy M		10^6	Anaerobic	48	2.0
	Tinidazole		10^6	Anaerobic	48	2.0
	Hydroxy T		10^6	Anaerobic	48	0.5
Ison et al. (97)	Vancomycin	PSD agar	10^7[d]	Air + CO_2	48	1.0
	Gentamicin		10^7	Air + CO_2	48	4.0
	Colistin		10^7	Air + CO_2	48	>8.0
	Nalidixic acid		10^7	Air + CO_2	48	128.0

[a] Hydroxy M, hydroxy metabolite of metronidazole; hydroxy T, hydroxy metabolite of tinidazole.
[b] PS, peptone-starch; PSD, peptone-starch-dextrose; CCA, Columbia chocolate agar.
[c] NS, not stated.
[d] Final inoculum was 10^4 CFU delivered with a multipoint inoculator.

was observed between the McCoy cell adhesion index of an isolate and the percentage of clue cells in the vaginal fluid from which the *G. vaginalis* strain was isolated. In contrast, hemagglutination did not correlate with the McCoy cell adhesion index (205).

Another study (204) demonstrated that hemagglutinating strains were more hydrophobic than McCoy cell-adherent strains. Also, marked differences were found between the characteristics of *G. vaginalis*-erythrocyte adherence and *G. vaginalis*-McCoy cell adherence after various chemical and physical treatments. The proposition that different *G. vaginalis* adhesins are responsible for adhesion to the receptors on the two kinds of cells (204) was verified by electron microscopy (203). Adhesion to McCoy cells involved principally the outer fibrillar coat, whereas thin sections of agglutinated erythrocytes revealed that attachment of *G. vaginalis* occurred by means of thin pili.

Susceptibility to Chemotherapeutic Agents

The effectiveness of various treatment regimens for bacterial vaginosis was evaluated by Lossick (131, 132) and Piot (171). Metronidazole is commonly used and gives initial cure rates of approximately 90% or better (8, 17, 79, 108, 146, 167, 170, 226, 237). Metronidazole becomes widely distributed in the body and undergoes oxidative metabolism in the liver, with the formation of several metabolites (132). The most common of these is the hydroxy metabolite, which can attain concentrations in plasma and urine that are likely to inhibit *G. vaginalis* (58, 178). Since metronidazole treatment does not adversely affect lactobacilli, they are able to recolonize the vagina, thereby reducing the risk of late-stage relapse (132, 167, 219).

5-Nitroimidazoles and imidazoles. Metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole] was originally thought to have a spectrum limited to obligate anaerobes, such as *Bacteroides* spp. and *T. vaginalis*, because they alone have electron transfer proteins of sufficiently low redox potential to reduce the nitrogroup (61). However, in 1977, Smith and Dunkelberg (212) showed that *G. vaginalis* is susceptible to metronidazole, and in 1978, Pheifer et al. (170) demonstrated its value for therapy of *G. vaginalis*-associated vaginosis. Recognizing that in vitro test results with metronidazole did not reflect the excellent therapeutic results, Ralph et al. (179, 180) studied the influence of various test conditions on the activities of metronidazole and the hydroxy metabolite of metronidazole [1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole] (Table 2).

Inocula of 10^4 CFU/ml and anaerobic incubation for 24 h result in MICs of metronidazole that can be 12 times lower than those found in tests of 10^6 CFU/ml incubated aerobically for 48 h (180). Therefore, anaerobic conditions were used by many workers for tests of metronidazole activity (8, 9, 58, 108, 127, 145, 170, 209, 242). The hydroxy metabolite of metronidazole is at least two to four times more active than the parent metronidazole for most *G. vaginalis* strains (108, 171, 178, 209, 222; Table 2). For example, in a study of 510 *G. vaginalis* clinical isolates, Bannatyne et al. (9) found that the range of metronidazole MICs was 1 to 16 μg/ml and that the MIC for 50% of the strains (MIC_{50}) was 3 μg/ml. The range of its hydroxy metabolite was 0.125 to 8 μg/ml (MIC_{50}, 0.7 μg/ml). These tests made use of Diagnostic Sensitivity

Test agar (Oxoid, Ltd.) supplemented with 5% lysed horse blood and anaerobic incubation for 48 h. Time-kill tests performed by Ralph (178) showed that the hydroxy metabolite of metronidazole has a more rapid and complete bactericidal effect than metronidazole at two- to four-times-higher concentrations.

A characteristic useful for identifying *G. vaginalis* is the presence of a zone of inhibition around a disk containing 50 μg of metronidazole (172). A more definitive disk test could take advantage of the differing susceptibilities to metronidazole and its hydroxy metabolite. The basis for this suggestion is illustrated by the results of quantitative tests of 11 strains incubated anaerobically. Eight strains were 4 to 64 times more susceptible to the hydroxy metabolite than to the parent metronidazole, as expected. In contrast, three strains were resistant to both drugs at concentrations of ≥128 μg/ml (108), which suggests to me that these strains were not typical *G. vaginalis*.

A rapid reduction of the antibacterial activity of metronidazole occurs in broth cultures of *Bacteroides fragilis* and other susceptible obligate anaerobes presumably as a result of bacterial uptake and intracellular reduction of the nitro group (61, 152, 181). Although the rate of inactivation is slower, large inocula of *G. vaginalis* can inactivate both metronidazole and its hydroxy metabolite (178). Ralph et al. (180) suggested that such inactivation might be responsible for the unexpected finding that the MICs of metronidazole are higher after incubation for 48 h than after incubation for 24 h (Table 2).

Bacterial adhesion resulting in clue cell formation is an important feature of bacterial vaginosis. Peeters and Piot (166) demonstrated that the capacity for adhesion to VECs was reduced by growth in subinhibitory concentrations of metronidazole. Bacteria cultivated in air plus CO_2 were used in these adhesion assays. Further assays performed under anaerobic conditions might well show that metronidazole and its hydroxy metabolite exert substantially greater effects on adhesion of *G. vaginalis* than detected in aerobic assays. The observation by Easmon et al. (58) that some samples of plasma from metronidazole-treated volunteers elicited agglutination of *G. vaginalis* suggests that cellular surface properties may be affected by metronidazole and its hydroxy metabolite.

Scanning electron microscopy was used by Skarin and Mårdh (209) to observe the morphological effects of growth in the presence of subinhibitory concentrations of metronidazole and tinidazole. *G. vaginalis* populations became heterogeneous, with some cells appearing swollen and bumpy but not elongated. In contrast, *B. fragilis* cells became long and snakelike. Cellular elongation under the influence of certain antibacterial agents occurs commonly with gram-negative bacteria (33). The absence of filamentation by *G. vaginalis* is consistent with its lack of a typical gram-negative cell wall.

Other clinically useful 5-nitroimidazoles are tinidazole and nimorazole (145, 152). Tinidazole and metronidazole were about equally active in tests of 510 *G. vaginalis* strains (9), and both drugs show enhanced in vitro activities under anaerobic conditions (209). As expected, tinidazole is less active than its hydroxy metabolite (207).

Three imidazole derivatives are interesting because they possess both antifungal and antibacterial activities. Tests of *G. vaginalis* susceptibility by Jones et al. (109) showed that the MICs of fenticonazole, clotrimazole, and miconazole all fall within the range ≤0.03 to 0.25 μg/ml. However, no available data support their use as effective agents for the treatment of bacterial vaginosis.

β-Lactams and carbapenems. High levels of susceptibility to β-lactam drugs are typical of most *G. vaginalis* strains. Thus, King and Phillips (115) found that ampicillin MICs ranged between 0.008 and 0.5 μg/ml (MIC_{50}, 0.016 μg/ml). McCarthy et al. (139) recorded MICs of <0.06 to 0.25 μg/ml and 0.035 to 0.15 μg/ml for ampicillin and penicillin G, respectively. A survey of 206 clinical isolates gave the range of ampicillin MICs as 0.016 to >4.0 μg/ml, with 90% of the strains inhibited by 0.4 μg/ml (22). Verification of the identities of the few strains characterized by high MICs is needed, however, before they are accepted as ampicillin-resistant *G. vaginalis*.

King et al. (113) reported that piperacillin MICs were 0.016 to 0.25 μg/ml (MIC_{50}, 0.06 μg/ml). Two carbapenems, imipenem and meropenem, were highly active against *G. vaginalis* with MICs of ≤0.12 μg/ml.

Amoxicillin MICs were given as 0.03 to 1 μg/ml (MIC_{50}, 0.06 μg/ml) for 52 *G. vaginalis* vaginal isolates (171) and 0.001 to 0.015 μg/ml (MIC_{50}, 0.003 μg/ml) for 32 isolates from urine (222). Agar dilution methods with inocula of 10^5 CFU and incubation in air plus CO_2 were used in both studies, but neither included a reference strain needed for comparisons between the results. Accordingly, their disagreement cannot be attributed with certainty either to methodological differences or to the more interesting possibility of strain differences.

Tests of a mixture of amoxicillin plus clavulanic acid yielded MICs that ranged between 0.016 and 0.25 μg/ml (MIC_{50}, 0.03 μg/ml) (114). Clavulanic acid is an example of an active-site-directed, irreversible inhibitor of β-lactamase. This so-called suicide inhibitor serves to protect the β-lactam ring of amoxicillin from enzymatic cleavage. The activity of the amoxicillin-clavulanic acid mixture against β-lactamase-producing bacteria, such as *Branhamella catarrhalis* (35), is well documented. However, since *G. vaginalis* is not known to produce a β-lactamase, clavulanic acid would not be expected to have much influence on in vitro tests of amoxicillin. Nevertheless, Augmentin (amoxicillin-clavulanic acid) was used successfully for the treatment of women suffering from bacterial vaginosis, whereas amoxicillin alone was less effective (226). Ampicillin treatment also failed to eradicate *G. vaginalis* or bring about the clinical improvement of most infected women (131, 170). These failures presumably are due to β-lactamase inactivation of the drugs by other species of bacteria in the vagina (171, 219).

The cephalosporins available in the late 1970s were not very active against *G. vaginalis* (139). However, King et al. (113, 114) found higher activities in tests of 20 strains with cefotaxime (MICs, 0.03 to 1 μg/ml), cefpirome (MICs, 0.06 to 1 μg/ml), and cefepime (MICs, 0.03 to 0.5 μg/ml).

Erythromycins. *G. vaginalis* is highly susceptible to erythromycin and the related azithromycin (110, 139). Erythromycin MICs ranged between 0.015 and 0.12 μg/ml (MIC_{50}, 0.03 μg/ml) (171) and between 0.007 and 2 μg/ml (MIC_{50}, 0.015 μg/ml) (242). Nevertheless, erythromycin therapy was successful in only 3 (23%) of 13 women with bacterial vaginosis (131), and treatment did not eliminate the amines found in vaginal fluids of infected women (91). Holmes (200) attributed therapeutic failures to reduced activity of erythromycin at the low pH of vaginal fluids.

Tetracyclines. Great diversity is seen in the levels of *G. vaginalis* susceptibility to tetracycline. Tetracycline MICs of 0.5 μg/ml (139) or lower (62, 109, 231; Table 2) were found. In contrast, for other strains MICs were as high as ≥32 (231),

>50 (127), 64 (139), and >128 (62) μg/ml. Therapeutic trials of tetracycline and doxycycline in patients with bacterial vaginosis gave some cures and many failures (8, 131, 170, 171).

A bimodal distribution of MICs was noted by McCarthy et al. (139) for 56 *G. vaginalis* strains isolated in the United States before 1979. Tetracycline concentrations of ≤4.0 μg/ml inhibited 57% of the isolates, whereas concentrations between 16 and 64 μg/ml were required to inhibit the remaining 43%. Roberts et al. (191) examined 66 *G. vaginalis* vaginal isolates from women in Seattle: 20 (51%) of 39 strains isolated in 1978 and 21 (78%) of 27 isolates in 1984 exhibited tetracycline MICs of ≥16 μg/ml. Resistance was less common among vaginal isolates in Sheffield, United Kingdom, where in 1988, Eley and Clarry (62) found tetracycline MICs of >16 μg/ml for only 7 (19%) of 36 strains.

The Tet M determinant is responsible for tetracycline resistance in *G. vaginalis* (62, 190, 191). First recognized in streptococci, Tet M is often associated with a conjugative transposon located in the streptococcal chromosome. Roberts et al. (191) and Eley and Clarry (62) found that DNA from all tetracycline-resistant *G. vaginalis* strains but from no susceptible strains hybridized with a Tet M probe. Tet M apparently has a chromosomal location in *G. vaginalis*. Plasmid DNA was not detected (62, 191). Six *G. vaginalis* strains failed to transfer Tet M, which suggested that they carried only an incomplete Tet M transposon that could not function in conjugation (189).

Clindamycin. *G. vaginalis* strains were inhibited by clindamycin concentrations of ≤0.24 (79, 139, 171, 222) or ≤2 (242) μg/ml. The clindamycin MIC_{50}, determined on agar, has been given as 0.003 (222), 0.03 (171), <0.06 (139), and 0.06 (242) μg/ml. In contrast, a clindamycin MIC_{50} of 8 μg/ml was obtained in tests of 57 *G. vaginalis* endometrial isolates performed by a microtube dilution method (BBL Sceptor, Towson, Md.), and the MIC_{90} was 16 μg/ml (136). Clindamycin administered orally (79) or intravaginally as a 2% cream (89) was effective in the treatment of bacterial vaginosis.

Quinolones. Ciprofloxacin MICs ranged between 0.5 and 2 μg/ml (115) or between 1 and 4 μg/ml (128, 231) for *G. vaginalis*. The ranges of MICs of other quinolones were 0.025 to 16 (22) and 2 to 8 (128) μg/ml for difloxacin, 2 to 8 μg/ml for pefloxacin (115), and 8 to 32 μg/ml for norfloxacin (115). The MICs of ofloxacin were determined by two dilution methods. In small volumes of broth the MIC_{90} was 2 μg/ml (136), whereas in agar the MIC_{90} was 32 and the MICs ranged between 8 and 32 μg/ml (128).

Other antibacterial agents. Streptomycin (MICs, 0.5 to 4 μg/ml) was the most active of the five aminoglycosides tested by McCarthy et al. (139). The ranges of gentamicin MICs were 1.0 to 8 (97, 139) and 4 to 32 (113) μg/ml. The MICs of spectinomycin, an aminocyclitol, lay between 0.25 and 32 μg/ml (171).

Good activity against *G. vaginalis* was reported for two newer and two older agents. The MICs (in micrograms per milliliter) were as follows: pristinamycin, a member of the streptogramin group, 0.015 to 0.5 (MIC_{50}, 0.06) (242); ramoplanin, a lipoglycopeptide, 0.06 to 0.25 (MIC_{50}, 0.12) (158); vancomycin, 0.25 to 0.5 (139, 158); and chloramphenicol, 0.25 to 2 (MIC_{50}, 0.5) (139).

Sulfonamides are ineffective against *G. vaginalis*; the MICs were >128 μg/ml (97, 139, 171), although the presence of blood in test media may have increased the apparent resistance. Unaccountably, a product (Sultrin cream) that contains sulfathiazole, sulfacetamide, and sulfabenzamide is approved by the Food and Drug Administration for treatment of *G. vaginalis* vaginitis.

Trimethoprim MICs as determined in three studies ranged between 0.03 and 32 μg/ml (97, 127, 171) but were 4 to 64 μg/ml in a fourth study (139). The MICs of a combination of trimethoprim with sulfamethoxazole (1:20 ratio) ranged between 2 and 64 μg/ml (139).

High resistance to both colistin and nalidixic acid (MICs, >128 μg/ml) was recorded for 56 *G. vaginalis* strains (139), confirming earlier findings of resistance (164). It is important to note, however, that Ison et al. (97) found two clinical isolates that displayed colistin MICs of 2 μg/ml. Such atypical susceptibility could result in inhibition of growth on colistin-nalidixic acid medium.

Growth Requirements

Investigations by Dunkelberg and McVeigh (55) of the growth-stimulating effects on *G. vaginalis* of various additives to peptone-based media led to the development of PSD medium and the demonstration that the organism does not require either hemin or NAD (X and V factors, respectively). Since requirements for these compounds traditionally define *Haemophilus* spp., it was evident that the name "*Haemophilus vaginalis*" given by Gardner and Dukes (75) was inappropriate even before this was confirmed by genetic methods.

A semidefined fluid medium that permitted good growth of two *G. vaginalis* strains, 594^T and 317, was developed (55). The components were a vitamin-free enzymatic digest of casein, five vitamins, six nucleic acid bases, maltose, glucose, and a mixture of inorganic salts. The medium failed to support growth when prepared with acid-hydrolyzed casein or some lots of enzymatically hydrolyzed casein. Tryptophan improved the growth of strains on medium containing one lot of casein hydrolysate but not another. The latter medium may have contained trace amounts of toxic fatty acids, which are known to inhibit multiplication of some bacteria (34), possibly including *G. vaginalis* (55).

Interestingly, adenine was identified as a factor required for growth of strain 594^T but not strain 317. Both strains failed to grow in the medium devoid of purine and pyrimidine bases, but the omission of any one base (except adenine) from the medium containing the remaining five did not reduce growth (55). It would be useful to investigate the requirements of *G. vaginalis* strains in various completely defined media that contain or lack certain amino acids, nucleosides, bases, and vitamins.

Epidemiological Typing

Piot et al. (175) divided *G. vaginalis* strains isolated from vaginal fluids into eight biotypes on the basis of β-galactosidase, lipase, and hippurate hydrolysis reactions. The commonest biotypes in Antwerp, Belgium (175), Bombay, India (160), and Sheffield, England (62), were 1 (all three reactions positive), 2 (lacking β-galactosidase but positive lipase and hippurate hydrolysis), and 5 (lacking both β-galactosidase and lipase but positive hippurate hydrolysis). Biotypes 2 and 5 predominated in London, England (100), and biotype 1 predominated in Dublin, Ireland (205), Madrid, Spain (12), and Nairobi, Kenya (175). Isolates from women in Seattle, Wash., were biotyped in two studies: the first (175) showed that the commonest biotypes were 1 (38% of 67 isolates) and 5 (31%), whereas the second (24) found that biotype 5

predominated (41% of 261 isolates) and that each of the other biotypes represented not more than 15% of the strains.

The lipase-positive biotypes 1, 2, 3, and 4 were recovered more frequently from women with bacterial vaginosis than from women with normal vaginal flora in one study (24) but not in another (175). A possible explanation for the conflicting results is the greater specificity of the oleate substrate assay (24) compared with the egg yolk substrate assay (175). Concurrent colonization of the vagina by multiple *G. vaginalis* biotypes was detected in 48% of women studied by Briselden and Hillier (24), which raises a question about the interpretation of some of their findings. For example, they concluded that a patient had acquired a new strain if her biotypes changed between primary and follow-up visits; instead, there might have been a shift in the proportion of resident *G. vaginalis* strains characterized by different biotypes.

Seven serological groups were established by Edmunds (60) on the basis of results of precipitin tests of 50 *G. vaginalis* strains with 13 antisera. Ison et al. (100) devised a dot blot technique in which complexes of antigen and rabbit antibody were visualized by use of anti-rabbit immunoglobulin linked to alkaline phosphatase. Tests of 79 *G. vaginalis* strains with nine rabbit antisera led to the development of a scheme that could discriminate 20 serotypes.

DNA restriction endonuclease analysis is a successful method of typing used for *Branhamella catarrhalis* (35) and other organisms. Defalco et al. (52) found that nine restriction endonucleases cleaved *G. vaginalis* DNA to fragments that gave satisfactory banding patterns visualized by agarose gel electrophoresis. Nath et al. (154) concluded that DNA restriction profiles alone were not satisfactory for typing, but that hybridization of these bands with specific *G. vaginalis* DNA fragment probes was a potentially useful typing method.

Restriction endonuclease banding profile data were interpreted as suggesting that strains ATCC 14018^T and ATCC 14019 might be "genotypically identical" (52). However, a phenotypic difference is seen in the nutritional requirement for adenine displayed by strain 594 (ATCC 14018^T) but not by strain 317 (ATCC 14019) (55). The existence of adenine auxotrophy and other possibly different natural requirements suggests that a system of typing could be developed for *G. vaginalis* on the basis of multiple nutritional requirements detected by use of chemically defined media, analogous to auxotyping of gonococci (34).

Genetic Relationships

Hybridization experiments with radioisotope-labeled DNA from ATCC 14018^T and DNAs from a variety of other bacteria were reported by Greenwood and Pickett (82), Piot et al. (174), and Tønjum et al. (232). Eight *G. vaginalis* strains displayed high proportions of nucleotide sequences shared with the type strain (82, 174). DNA from another *G. vaginalis* strain, which showed only 47% homology with ATCC 14018^T DNA at 60°C, hybridized with the radiolabeled DNA from UCNC strain ITG 75 to the same extent (45% homology). The hybridization of unlabeled ITG 75 DNA with labeled ATCC 14018^T DNA indicated 55% homology (174). Two other UCNC strains showed lower levels of sequence relationship with ATCC 14018^T (29 and 22% homology), and three other UCNC strains gave ≤4% homology. Thus, these six UCNC strains belonged to three genetically different groups as determined by reciprocal hybridization. Too few UCNC strains were used in hybridization experiments to permit Piot et al. (174) to decide whether any of them should be proposed as a new species within the genus *Gardnerella*.

DNA-DNA hybridization analyses failed to indicate a relationship between *G. vaginalis* and organisms in any of the following species: *Haemophilus influenzae*, *Haemophilus aphrophilus*, *Pasteurella multocida*, *Actinobacillus actinomycetemcomitans*, *Corynebacterium diphtheriae*, *Corynebacterium haemolyticum*, *Corynebacterium pyogenes*, *Corynebacterium xerosis*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus fermentum*, *Propionibacterium jensenii*, *Streptococcus pneumoniae*, *Bifidobacterium* spp., *Oligella urethralis*, *Eikenella corrodens*, *Cardiobacterium hominis*, and various species of *Moraxella*, *Neisseria*, *Branhamella*, and *Kingella* (82, 174, 232).

Hybridization of DNA with rRNA, which reveals relationships that are too distant to be detected by DNA-DNA hybridization, should prove valuable in taxonomic studies of *G. vaginalis* (234).

ANIMAL MODELS FOR STUDIES OF *GARDNERELLA* SPP.

Intravenous inoculation of mice with 2×10^7 CFU of viable *G. vaginalis* produced no ill effects (20). Four primate species were investigated in attempts to develop an animal model for studies of vaginosis. Following intravaginal inoculations with *G. vaginalis*, pigtailed macaques were colonized for up to 39 days, but clue cells were not found. Tamarins and chimpanzees were not colonized (106). The grivet monkey was used in another exploratory study (134). Preinoculation vaginal samples contained various anaerobic bacteria but no *G. vaginalis* and few lactobacilli. Intravaginal inoculation of *G. vaginalis* did not lead to an increased vaginal secretion or colonization. However, inoculation of *G. vaginalis* mixed with a strain of *Mobiluncus* (described as the long, curved rod type, but species not named) gave rise after 5 days to a profuse vaginal discharge from which both organisms could be recovered for 12 days; the *Mobiluncus* strain was isolated alone for at least an additional 25 days. Clue cells were not observed.

Efforts to develop an equine model might prove rewarding. Salmon et al. (198) detected the presence of *Gardnerella* strains in the genital flora of mares, the first observation of natural infection in animals. The characteristics used to identify equine isolates as *G. vaginalis* included the appearance of Gram-stained cells, catalase-negative reaction, hippurate hydrolysis, beta-hemolysis on HBT medium, absence of sheep blood hemolysis, fatty acid profiles, and ultrastructure observed in electron micrographs. Genetic studies and further phenotypic analyses are needed to clarify the relations between equine and human strains. However, the natural infection of mares opens the way for the development of a model of potential value in investigations of *G. vaginalis* pathogenesis.

SIGNIFICANCE OF *G. VAGINALIS* IN BACTERIAL VAGINOSIS

Bacterial vaginosis, earlier designated nonspecific vaginosis because of the absence of recognized pathogens, afflicts as many as one-third of women attending sexually transmitted disease clinics (65) and primary health care units (95). Although a mild disease (5, 8), it is a risk factor for obstetric infections, various adverse outcomes of pregnancy, and pelvic inflammatory disease (65, 87, 90, 136, 193, 216, 241).

To appreciate the significance of *G. vaginalis* in bacterial vaginosis, it is necessary to consider the character of vaginal fluids and their bacterial populations. The differing clinical diagnostic criteria used in early studies led to conflicting conclusions that emphasized the need for standardization.

Diagnostic Criteria

Vaginal discharges significantly associated with bacterial vaginosis typically have the following characteristics (5, 36, 65, 75, 95): (i) a nonviscous, homogeneous consistency; (ii) an elevated pH (>4.5 or ≥4.7, according to different investigators); (iii) a fishy trimethylamine odor detected after alkalinization with 10% potassium hydroxide; (iv) the presence of clue cells which may represent 10 to 20% or more of vaginal epithelial cells; (v) replacement of the *Lactobacillus*-dominated flora by large populations of *G. vaginalis*, various species of anaerobes, and *Mycoplasma hominis*; (vi) an increased concentration of succinate relative to lactate, with a ratio of succinate peak height to lactate peak height of ≥0.4; and (vii) the presence of the diamines putrescine and cadaverine.

Amsel et al. (5) proposed that at least three of the first four characteristics (i to iv) should be used as criteria for the clinical diagnosis of bacterial vaginosis. These criteria excluded patient symptoms, such as malodorous discharge and other complaints listed by Eschenbach et al. (65), which various authors (5, 46, 88, 230) regard as unreliable indicators, too dependent on subjective factors. The application of standard clinical criteria allowed for the recognition of so-called asymptomatic bacterial vaginosis, which afflicts many women (5).

Endogenous Vaginal Flora

The healthy vaginas of premenopausal women are colonized by a variety of aerobic and obligate anaerobic bacteria (70, 88, 127, 137, 159, 199). These bacteria constitute the endogenous (88, 140) or indigenous (28) flora. These terms are preferred to "normal flora" because they are compatible with the current view that quantitative rather than qualitative changes in the spectrum of species occur in many cases of clinically significant bacterial vaginosis.

Conflicting reports of the percentages of healthy women colonized by *G. vaginalis* result in part from methodological differences. Investigators isolated *G. vaginalis* from 30% (140), 21% (182), 11.5% (28), or none (4, 75) of vaginal specimens cultured on nonselective media. Various semiselective media, on the other hand, yielded recoveries of 69% (233), 58% (88), 55% (65), 54% (70), 40% (5), 18% (137), or 14% (46) of healthy women.

Sautter and Brown (199) investigated the bacterial populations in vaginal samples collected two to three times a week for 1 month from seven sexually active healthy women. *Lactobacillus* spp. and *G. vaginalis* were present in 79 and 58%, respectively, of 65 serial cultures; 37 species were isolated. *G. vaginalis* was recovered from all vaginal samples from one woman but from only 1 of 11 samples from another; however, it was isolated from all seven women at some time during the month. Various aerobic and anaerobic species fluctuated between detectable and undetectable levels. This study provided valuable insight into the temporal variation of endogenous populations of bacteria in the vagina.

Influences on Endogenous Vaginal Flora

The compositions of endogenous floras are influenced by interactions, as yet incompletely understood, among the host, the bacteria, and exogenous factors. Estrogen produced by the host increases glycogen in the vaginal epithelium. The glycogen is metabolized to glucose and then to lactic acid; the lowered pH favors multiplication of the lactic acid-producing lactobacilli and other acid-tolerant bacteria (88, 159). The number of lactobacilli ranged between 1×10^7 and 5×10^9 CFU/ml of vaginal fluid from healthy women (64, 127, 137). Treatment with certain antibiotics reduces the lactobacillus population, which leads to an elevated pH and effects on other vaginal bacteria. An intrauterine device is considered by Amsel et al. (5) and Holmes (200) to be a risk factor for bacterial vaginosis, estimated as a fivefold increase in risk (201). A positive correlation was observed between the use of intrauterine devices and heavy colonization with *G. vaginalis* (15, 84) and gram-negative anaerobic bacteria (5, 88, 159).

Much remains to be learned about the interactions among members of the vaginal microflora. For example, what are the factors responsible for the striking inverse relationship between numbers of lactobacilli and numbers of *G. vaginalis* and anaerobes? Skarin and Sylwan (210) found that vaginal strains of *L. acidophilus* and *L. casei* can inhibit growth on solid media of *G. vaginalis* and species of *Mobiluncus*, *Bacteroides*, and anaerobic cocci. They considered that the acid produced by lactobacilli was responsible for these inhibitions.

Another possible antibacterial agent is hydrogen peroxide. Interestingly, H_2O_2-producing lactobacilli were isolated from higher percentages of samples of vaginal fluid from healthy women than from those with bacterial vaginosis (64, 153). The inhibitory interactions between vaginal isolates were examined by two methods. Nagy et al. (153) inoculated 10^6 CFU of indicator strains on Columbia agar supplemented with 5% horse serum, added drops of potentially inhibitory bacteria, and looked for zones of inhibition of growth after anaerobic incubation for 3 days. Several *G. vaginalis* strains exhibited antibacterial activities in tests of three lactobacillus indicator strains. On the other hand, 18 *G. vaginalis* strains were not inhibited by any of 46 lactobacillus isolates (20 of which produced H_2O_2). Klebanoff et al. (117) used a more definitive method for the study of bactericidal activities. Suspensions of lactobacilli and *G. vaginalis* cells prepared in a 0.1 M Na_2SO_4 solution were mixed, incubated at 37°C for 60 min, and plated on HBT agar. The numbers of colonies of the two species were determined by taking advantage of their differences of colony morphology and beta-hemolysis. An H_2O_2-producing *L. acidophilus* strain killed *G. vaginalis* ATCC 14018^T and *Bacteroides bivius*. This bactericidal action was inhibited by catalase but not by inactivated catalase and was not exhibited by non-H_2O_2-producing lactobacilli. Vaginal peroxidase plus a halide (e.g., chloride) would be expected to enhance the lethal action of H_2O_2 formed by lactobacilli, and this may contribute to the control of the vaginal flora (117).

Flora Associated with Bacterial Vaginosis

The development of bacterial vaginosis is accompanied by a transition of the *Lactobacillus*-dominated, principally aerobic microflora typical of the acidic vaginal fluid to one characterized by an increased variety and quantity of mainly anaerobic bacteria, fewer lactobacilli, and a pH of ≥4.7 (88,

91, 95, 137, 210). The rise in pH decreases the oxidation-reduction potential, which results in a more anaerobic microenvironment (216, 220). Amines that are produced by anaerobes also raise the vaginal pH (36, 206). Although the causes of this microbial transition remain obscure, the outcome can be detected by cultures, microscopic examinations, and biochemical analyses of vaginal discharges.

Cultural findings. *G. vaginalis* occurred in numbers exceeding 10^6 CFU/ml in 77 of 78 clue cell-positive vaginal discharges (17). Further, between 93 and 100% of women suffering from bacterial vaginosis were heavily colonized by *G. vaginalis* (8, 18, 65, 89, 92, 94, 167, 170, 226), and the numbers of CFU in samples from diseased vaginas were much higher than in healthy vaginas according to most (5, 182, 213, 219, 233), but not all (25, 137), authors. As expected, the finding of a negative *G. vaginalis* culture had a 97% predictive value for exclusion of bacterial vaginosis (5, 46).

Anaerobic culture findings have greatly contributed to knowledge of the etiology of bacterial vaginosis. Studies by Pheifer et al. (170), Spiegel et al. (217), and Blackwell et al. (17) were important in focusing attention on the joint roles of anaerobes and *G. vaginalis* in the disease process, and their findings have been amply confirmed (16, 26, 168, 216). The anaerobes recovered from high percentages of women with vaginosis and found in high numbers in quantitative cultures of vaginal secretions included species of *Prevotella*, *Bacteroides*, *Peptostreptococcus*, and *Fusobacterium* (17, 88, 89, 95, 137, 159, 167, 201, 210, 219). In addition, anaerobic, motile, curved rods (*Mobiluncus* spp. [220]) were detected in 33% (192), 38% (17), 42% (89), and 85% (95) of vaginal samples from women with vaginosis but from fewer than 5% of healthy women (46, 92, 95). *G. vaginalis* and *Mobiluncus* spp. were found together in cultures from one-third of women with vaginosis (46, 94).

Routine cultures of vaginal specimens for the detection of *G. vaginalis* and the diagnosis of bacterial vaginosis are not currently recommended, since cultures are costly and do not provide decisive evidence (16, 17, 27, 46, 132, 201, 202, 216).

Microscopic findings. The importance of microscopic examinations of vaginal fluids was emphasized by Gardner and Dukes (75), who regarded the appearance of exfoliated, squamous epithelial cells in a wet mount as a valuable clue to the presence of *G. vaginalis*. Typical clue cells have indistinct margins and a granular appearance resulting from the adherence of numerous, small bacteria. Many investigators observed clue cells in the vaginal discharges from most or all patients with bacterial vaginosis (5, 17, 95, 162, 218). and this led to the conclusion that clue cells are the most reliable indicator of bacterial vaginosis (168, 230, 235). The results of some other studies, however, were not in agreement (4, 40, 97, 127, 202).

Another controversy concerns the best means of detecting the clue cells, whether by inspection of Gram-stained smears (4, 172, 229), Papanicolaou-stained preparations (202), or wet mounts (46, 65, 75). Petersen and Pelz (168) add 0.1% methylene blue to wet preparations and find that the blue-stained bacteria on clue cells are easily seen and *G. vaginalis* can be distinguished from lactobacilli. The customary heat fixation of dried smears to be Gram stained can affect the appearance of clue cells. Fixation in absolute methanol for 5 to 10 min better preserves the morphology of epithelial cells and leukocytes (32, 243).

To identify the bacteria on clue cells, Cook et al. (44) made use of an indirect immunofluorescence technique with polyclonal antisera specific for whole-cell bacterial preparations. *G. vaginalis* was observed in 82% of clue cell-positive vaginal smears, and *Bacteroides* (*Prevotella*) *melaninogenicus* was detected in 44%. Antibodies directed to a mixture of *Mobiluncus mulieris* and *Mobiluncus curtisii* antigens identified adherent bacteria as members of the genus *Mobiluncus* in 22% of clue cell-positive smears. *Fusobacterium nucleatum* was found in 11% of the smears.

An investigation by Schnadig et al. (202) of bacteria in 157 ectocervical smears stained by the Papanicolaou method and examined at a magnification of ×1,000 revealed three bacterial patterns: (i) large bacillus pattern (typical of lactobacilli) seen in 73 smears (46%), (ii) the so-called anaerobic pattern found in 77 smears (49%), and (iii) scanty bacteria seen in 7 smears. The anaerobic pattern was characterized by mixed populations consisting of small, slender, pleomorphic bacilli (consistent with *G. vaginalis*) and various percentages of coccobacilli, filamentous or fusiform bacteria, and cocci, but with few or no rods resembling lactobacilli. Curved (comma-shaped) bacilli were observed in 34 smears, 13 of which contained so-called comma cells, described as squamous epithelial cells covered predominantly by curved bacilli, which often hang lengthwise beyond the edges of the cells, creating a shag rug appearance. Cultures prepared from the ectocervical samples yielded *G. vaginalis* in 89% of the cases in which smears revealed anaerobic patterns. Also, *Mobiluncus* spp. (species not identified) were cultured from 83% of the samples that exhibited anaerobic patterns with curved bacilli in Papanicolaou smears (202).

Figure 3 shows examples of squamous epithelial cells with adherent bacteria that were observed in four Papanicolaou smears. These are a cell with attached bacteria that resemble lactobacilli (the Lactobacillus morphotype) (Fig. 3A), clue cells with adherent bacteria of mixed morphotypes (Fig. 3B) and the Gardnerella morphotype (Fig. 3C), and a comma cell with adherent, overhanging bacteria of the Mobiluncus morphotype (Fig. 3D). The mixture of bacterial morphotypes representative of the anaerobic pattern is seen in the backgrounds of Fig. 3B and D.

Five studies demonstrated the feasibility of basing a laboratory diagnosis of bacterial vaginosis on an evaluation of morphotypes in Gram-stained smears of vaginal fluids. The Kopeloff modification of the Gram stain and the use of basic fuchsin as the counterstain were recommended as a means of preventing overdecolorization (216, 218). Spiegel et al. (218) and Rotimi et al. (195) made semiquantitative assessments of five morphotypes: (i) Lactobacillus, (ii) Gardnerella, (iii) gram-negative bacilli, (iv) curved gram-negative rods, and (v) gram-positive cocci. To simplify the assessments, morphotypes ii and iii were combined and v was disregarded in the studies by Mazzulli et al. (138), Nugent et al. (156), and Joesoef et al. (103). The proportion of each morphotype was estimated in terms of 0 and 1+ to 4+, and standardized scoring systems were developed for analysis of the data. Eschenbach et al. (65) found that, if bacterial vaginosis is defined by composite clinical criteria, the independent diagnosis of bacterial vaginosis by Gram stain by the criteria of Spiegel et al. (218) has a sensitivity of 97%, a specificity of 79%, and a positive predictive value of 69%.

Biochemical findings. The vaginal discharge associated with this syndrome is notorious for a disagreeable odor (75). A fishy, trimethylamine odor detected after alkalinization of the discharge with 10% KOH (170) is one diagnostic criterion. The presence of amines in vaginal fluid from women with bacterial vaginosis but not in normal vaginal fluid was demonstrated by electrophoresis (91). Thin-layer chroma-

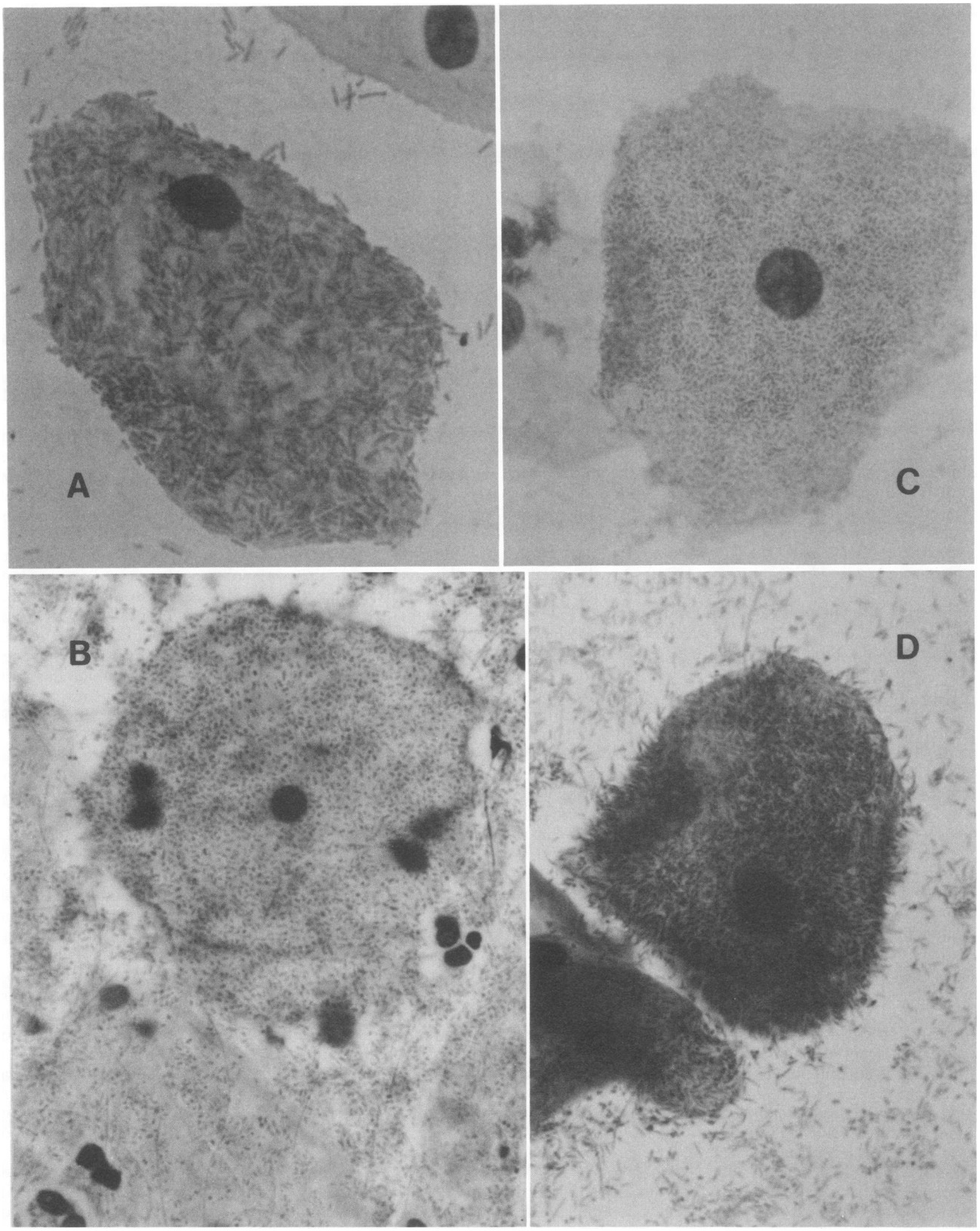

FIG. 3. Morphotypes of bacteria unattached or attached to squamous epithelial cells in Papanicolaou preparations. Magnification, ×1,200. (A) Lactobacillus morphotype; (B) mixed morphotypes typical of bacterial vaginosis and clue cell with attached bacteria; (C) Gardnerella morphotype; (D) Mobiluncus morphotype. (Photomicrographs provided by V. J. Schnadig.)

tography of dansylated fluids enabled Chen et al. (36) to identify the diamines putrescine and cadaverine and also γ-amino-*n*-butyric acid in broth cultures of mixed anaerobes isolated from a patient with bacterial vaginosis. It is important to note that these compounds were not detected in pure cultures of *G. vaginalis* isolated from women with bacterial vaginosis. Gas-liquid chromatography was used to determine changes in the concentrations of short-chain organic acid metabolites in vaginal fluids (218). Lactic acid predominated in normal fluid, whereas succinate, acetate, butyrate, and propionate were increased and lactate decreased in discharges from patients with bacterial vaginosis.

Bacterial vaginosis has a polymicrobic etiology. The cultural, microscopic, and biochemical findings taken together show that *G. vaginalis* does not act alone to produce the various clinical features of bacterial vaginosis. There is now widespread agreement that this disease is caused by *G. vaginalis* in concert with certain nonsporeforming anaerobic bacteria and mycoplasmas (17, 26, 36, 65, 70, 94, 95, 116, 132, 167, 202, 206, 216, 219). Additional support for this conclusion was obtained in a study of the grivet monkey as a model for bacterial vaginosis. Mårdh et al. (134) found that intravaginal inoculation with both *G. vaginalis* and a *Mobiluncus* sp. (the long, curved rod species) gave rise to a profuse vaginal discharge from which both organisms could be isolated, whereas inoculation with either organism alone gave rise to little or no discharge.

Volunteer women were used in earlier efforts to demonstrate the pathogenicity of *G. vaginalis*. Many of these subjects, "known to be free" of *G. vaginalis* infection initially, developed vaginosis after intravaginal inoculations with 12-h cultures of *G. vaginalis* (47) or with material taken directly from the vaginas of infected patients (75). The conclusion that the resulting vaginal infections were due to a single etiological agent was not justified because the culture media were not adequate to detect low numbers of *G. vaginalis* in preinoculation cultures and the exclusive reliance on aerobic incubation blinded the investigators to a view of anaerobic components of the microflora in healthy and diseased vaginas.

The discharge associated with bacterial vaginosis has a homogeneous character that differs from the purulent discharge characteristic of gonorrhea, which is provoked by a strong chemotactic response of leukocytes. A possible reason for this difference came from a study of chemotaxis. Sturm (223) found that the migration of leukocytes through agarose in response to a bacterial chemoattractant was inhibited by culture filtrates of succinate-producing *Bacteroides ureolyticus* and *Bacteroides bivius* (now *Prevotella bivia*). In contrast, chemotaxis was not inhibited by *G. vaginalis*, which does not produce significant amounts of succinate (6, 133, 151, 223). Such findings indirectly incriminate succinate-producing anaerobes as participants in bacterial vaginosis.

Successful treatment of bacterial vaginosis with metronidazole is accompanied by decreased numbers of anaerobes and *G. vaginalis*, disappearance of the vaginal amines, and return of the *Lactobacillus*-dominated flora, with a consequent increase in lactic acid and reduction of the vaginal pH (36, 91, 167, 217, 219).

Transmission of Bacteria Associated with Vaginosis

Bacterial vaginosis is commonly considered a sexually transmitted disease (1, 54, 72, 75, 132, 170, 240). Thus, the prevalence of *G. vaginalis* was approximately 4% in women attending a university clinic but 33% in women attending a sexually transmitted diseases clinic (65). *G. vaginalis* was found in urethral samples from 58% (170), 11.4% (51), 10.5% (221), 7.2% (116), and 4.5% (93) of unselected men attending clinics for sexually transmitted diseases and 96% (75), 79% (170), 35% (1), and 5% (92) of the male sexual partners of women with *G. vaginalis*-associated vaginosis. Whether positive urethral cultures from asymptomatic men represent passive acquisition from infected women or a more permanent colonization remains unclear.

The importance of concurrent treatment of partners was illustrated by the elimination of relapses in a woman who had suffered repeated episodes of vaginosis presumably due to reinfection from her constant partner who carried *G. vaginalis* in his semen (63). On the other hand, the treatment of male sexual consorts of women with bacterial vaginosis did not significantly reduce the recurrence rates of vaginosis reported in two large studies (146, 237).

Vaginal cultures from 12 women with vaginosis and urethral cultures from their 12 male consorts were obtained within the same 24-h period. The *G. vaginalis* biotypes isolated from both partners were the same for 11 of the couples ($P = <0.005$), thus providing evidence of sexual transmission. Piot et al. (175) emphasized, however, that the findings did not imply that bacterial vaginosis is necessarily a sexually transmitted disease.

Evidence of the possible endogenous source of bacteria associated with vaginosis was provided by Holst et al. (92, 95). Specimens from 148 women with clinically proven bacterial vaginosis were obtained by passing swabs 3 cm into the anal canals, making use of lateral pressure to minimize contact with feces. *G. vaginalis* was found in 45% of anal canal cultures; *Mobiluncus mulieris*, in 56%; *Mobiluncus curtisii*, in 62%; and *Mycoplasma hominis*, in 54%. These four species were also isolated from anorectal specimens from between 10 and 14% of 69 healthy women and between 4 and 11% of 131 male sex partners of either healthy women or those suffering from vaginosis. The technical difficulty of detecting *G. vaginalis* may account for the unsuccessful attempts to isolate it from anorectal specimens from 57 (51) or 15 (120) men.

G. vaginalis or one of the other species mentioned above was recovered from the anal canals of between 5 and 9% of 22 sexually inexperienced children (92). The possible anal-vaginal transfer resolves the special problem inherent in reports of the presence of *G. vaginalis* in vaginal cultures from 5 (4.2%) of 119 nonabused children ranging in age from 1 to 11 years (10), 3 (6%) of 51 children who were 2 months to 10 years of age (83), and 9 (17%) of 52 adolescent girls believed to be virgins (27).

Thus, the recognition that *G. vaginalis* and other bacteria involved in bacterial vaginosis may be present in the anal canal adds a new dimension to the picture of transmission and epidemiology. The flora of the healthy adult vagina consists of many organisms, including some species associated with bacterial vaginosis. Because of the multifactoral etiology of this syndrome, the introduction of one or more critical species (not necessarily a particular one) by either penile-vaginal or anal-vaginal transmission has the potential of complementing the endogenous flora and initiating the transition to vaginosis. The disease can be symptomatic or asymptomatic depending on the particular interactions among members of the vaginal populations and between them and the host.

G. VAGINALIS IN OTHER SITES

Endometrium, Fetal Membranes, and the Newborn Infant

Maternal infections. *G. vaginalis* has been detected in intrauterine infections (118, 133, 147), intra-amniotic infections (77, 149), chorioamnionitis (31, 90, 122, 155), postabortal pelvic inflammatory disease (87), and postpartum endometritis following cesarean section (148). Large studies of postpartum endometritis in women who delivered at hospitals in Houston, Tex., and Seattle, Wash., identified *G. vaginalis*, often mixed with other bacteria, in endometrial cultures from 25% (136) and 38% (193, 241) of infected patients. An important technical feature was the use of an endometrial suction device (136) or a triple-lumen sampling device (193, 241) which protects the endometrial specimen from contamination by cervicovaginal flora. Therefore, the finding that endometrial cultures contained a mixture of bacteria corresponding to those typical of vaginosis could not be attributed to contamination during sampling. Instead, it is likely that early-onset postpartum endometritis is due to the introduction of cervicovaginal bacteria into the amniotic fluid and endometrial cavity during labor and delivery, especially by cesarean section (136, 241).

Vaginitis emphysematosa is an uncommon manifestation of infection caused by either *G. vaginalis* or *T. vaginalis* that is characterized by numerous gas-filled vesicles in the vaginal and cervical mucosa (73). Josey and Campbell (112) proposed that the disorder afflicts patients with impaired immunological competence, rendering the mucosa susceptible to superficial penetration by these vaginal organisms.

Neonatal infections. *G. vaginalis* was cultured from various specimens from newborn infants, including an oral swab (187), gastric aspirates (31, 71, 224), tracheal aspirate (71), conjunctival pus (41), pus from a scalp lesion (177), and an abscess that developed on an area of the right cheek abraded by delivery forceps (125).

Osteomyelitis of the right parietal bone was described by Nightingale et al. (155) as a complication of a cephalhematoma at the site of a scalp electrode insertion. On day 16 after delivery, bloody purulent material was aspirated from a raised, reddened, and devitalized area superimposed on the cephalhematoma. Gram-negative pleomorphic rods resembling *G. vaginalis* were observed but not recovered in culture. The placenta showed acute chorioamnionitis, and a culture of the fetal side of the placenta yielded *G. vaginalis*.

Monif and Baer (148) reported the recovery of *G. vaginalis* from cord blood of four neonates in cases of presumed chorioamnionitis. *G. vaginalis* bacteremia occurred in six neonatal infants, three of whom died (31, 177, 238). Meningitis in a 5-day-old infant who displayed fever (38°C), polypnea, and a pale greyish complexion was described by Berardi-Grassias et al. (13). The spinal fluid contained 4,500 leukocytes per mm^3 and gram-variable bacilli and yielded a pure culture of *G. vaginalis*. Growth was not detected in a blood culture.

Suppurative Lesions

G. vaginalis and *Peptostreptococcus asaccharolyticus* were isolated from purulent exudative material aspirated from a liver abscess that developed in a 23-year-old woman who had earlier delivered by cesarean section. *G. vaginalis* was recovered from an endocervical specimen obtained on admission, but blood and urine cultures were negative. Ezzell and Many (66) suggested that the abscess formed as a result of suppurative pylephlebitis, the origin of infection being postpartum endometritis.

A lung abscess, empyema, and bacteremia developed in a male alcohol abuser following inhalation pneumonia; *G. vaginalis* in pure culture was isolated from blood and mixed with other bacteria in cultures of a bronchoscopic aspirate and pleural drainage (124). A vaginal abscess, Bartholin gland abscesses, puerperal abscess, and abdominal incisions were the sources of *G. vaginalis* studied by Malone et al. (133). *G. vaginalis*, in some cases accompanied by anaerobic bacteria, was isolated from abdominal wound infections that developed after cesarean section (3, 222, 224, 241) or hysterectomy (59, 72). An episiotomy wound infection yielded *G. vaginalis* in pure culture (39).

Female Urinary Tract

G. vaginalis urinary tract infections afflict women more often than men (42, 67, 68, 104, 111, 120). A possible reason for this is the presence in postpubertal women of vaginal inclusion epithelium in the region of the bladder trigone. Similar areas of squamous epithelium are not seen in the male bladder. Fairley and Birch (67) suggested that the ability of *G. vaginalis* to attach to urogenital squamous epithelium might provide a means of migration from the vagina and thus determine the site of primary colonization within the bladder. They observed clue cell-like squamous epithelial cells in bladder urine collected by suprapubic aspiration from women infected with *G. vaginalis* (67). Also, clue cells were seen in numbers exceeding 10^4/ml in some midstream urine specimens (119).

McFadyen and Eykyn (142) championed percutaneous suprapubic aspiration as a means of obtaining urine free of urethral and vaginal contamination. By use of this method, they obtained bladder urine from 1,000 pregnant women in attendance at a routine antenatal clinic in London, United Kingdom, and identified *G. vaginalis* in cultures from 15.9% of these patients. A study by McDowall et al. (141) in Melbourne, Australia, found a corresponding percentage (18%) of *G. vaginalis* in cultures of bladder urine from healthy pregnant women. In contrast, 84% of 31 pregnant women with known or suspected renal disease harbored *G. vaginalis* in the bladder. Bladder washout localization tests performed by Lam et al. (120) showed that *G. vaginalis* was present in the kidneys of 11 of 15 women with renal disease. In another study (67), *G. vaginalis* was cultured from samples of bladder urine from 22 women, and kidney involvement was demonstrated in 12 of them.

A 9-year study in Melbourne of nonpregnant women who experienced acute urinary symptoms was reported by Fairley and Birch (68). Urine collected by suprapubic aspiration from 561 patients with bacteriuria yielded 28 bacterial species, many of which were isolated infrequently. *G. vaginalis* was recovered from 208 cultures, and 311 contained *Ureaplasma urealyticum*. Both organisms were isolated from 74 aspirated urines. The numbers of *G. vaginalis* CFU per milliliter of urine were between 10^2 and 10^4 in 42 cultures, between 10^4 and 10^5 in 48 cultures, and $>10^5$ in 118 cultures.

Wilkins et al. (244) studied interstitial cystitis in 19 women and 1 man. *G. vaginalis* was recovered from bladder biopsies and also cystoscopic and midstream urine specimens from five patients and from cystoscopic and/or midstream urine specimens from four others. Scanning electron microscopic examination of one biopsy specimen revealed bacteria embedded in the vesical mucosa, which indicated that *G. vaginalis* was not an innocent colonizer of the bladder urine.

Other studies revealed numbers of *G. vaginalis* exceeding 10^5 CFU/ml in a midstream urine specimen from an acutely dysuric woman (163) and in specimens from two women with spontaneous cystitis with pyuria (222). Pyuria is not necessarily present in *G. vaginalis* urinary infections, however (42, 67, 68, 111, 141).

The presence of *G. vaginalis* in cultures of voided or catheterized urine specimens was surveyed in three large studies. Clarke et al. (42) recovered significant isolates from 119 (0.6%) of 19,463 specimens; isolates were considered significant if the patient had symptoms or pyuria and the *G. vaginalis* cultures were pure or almost pure. Josephson et al. (111) detected $>10^4$ CFU of *G. vaginalis* per ml in 322 (2.3%) of 14,178 urine specimens and recorded pure cultures for 67 women and 5 men. In a study of urine cultures from 12,343 women, Woolfrey et al. (246) reported 163 presumptive *G. vaginalis* isolates, 75% of which were confirmed. It is important to note that incubation was limited to 24 h in two of these studies (111, 246).

An investigation by Lam and Birch (119) demonstrated the importance of certain laboratory procedures for ensuring maximum recovery of *G. vaginalis* from urine specimens. (i) Urine should be refrigerated (4°C) until cultured. Three of 11 urine samples held at 20°C were culture negative after 24 h, and another 6 samples were negative after 48 h; reduction of CFU was greater at pH 5 than pH 6. (ii) Maximum recovery of *G. vaginalis* was obtained on an enriched medium (V agar medium) adjusted to pH 7. (iii) Anaerobic incubation for at least 48 h gave the best recovery. Duplicate cultures on modified colistin-nalidixic acid medium were inoculated with urine specimens from 20 *G. vaginalis*-positive subjects and incubated at 37°C in aerobic and anaerobic atmospheres, both with 5% CO_2. Growth was detected in 5 of the anaerobically incubated cultures after 18 h, in 16 cultures after 48 h, and in all 20 cultures after 72 h. On the other hand, only 1 aerobic culture showed growth after 18 h, 8 cultures were positive after 48 h, and the remaining 12 cultures were still negative after 72 h.

These findings suggest that some surveys have underestimated the incidence of *G. vaginalis* bacteriuria because of suboptimal experimental conditions. A pH of 5 was recorded for urine specimens from 17 of 41 patients (111). The loss of viability that occurs in acidic urine held at room temperature for uncontrolled periods before processing may reduce the CFU below the level considered significant. Furthermore, the use of conventional sheep blood agar and aerobic incubation for only 1 day will reduce the probability of detecting *G. vaginalis*.

Male Urogenital Tract

G. vaginalis infections in men involve the genital tract more often than the urinary tract (120). For example, in two studies of male patients with various urinary tract diseases, bladder urine obtained by suprapubic aspiration yielded *G. vaginalis* in cultures from none of 61 men (67) and 1 of 36 men (68). The presence of *G. vaginalis* in the urethra does not give rise to symptoms in most men, but the bacteria may assume a pathogenic role by extension to the prostate or bladder (225), especially in patients who have undergone a urological procedure (111, 222).

An organism presumed to be *G. vaginalis* was isolated in 1953 by Leopold (126) from cultures of urethral swabs and urine specimens from men suffering from urethritis or prostatitis. More recently, *G. vaginalis* has been recovered from urethral cultures of between 5 and 11.4% of sexually active men (38, 51, 54, 93, 116, 221) and in higher percentages for the sexual partners of women with bacterial vaginosis (1, 72, 170). Since most of these men were asymptomatic, it is necessary when considering patients with urethritis to discriminate between carriage resulting from sexual transmission and actual infection. Furthermore, as Dunkelberg (54) pointed out, a causal role for *G. vaginalis* in urethritis is not established unless *Chlamydia trachomatis* and *U. urealyticum* are excluded.

A urethral discharge and inflammation of the glans penis that may involve the mucous surface of the prepuce is seen in balanoposthitis. Kinghorn et al. (116) recovered *G. vaginalis* from preputial swabs from 21 men with balanoposthitis and isolated concomitant *Bacteroides* spp. from 75% of the cultures. Burdge et al. (29) reported three cases of *G. vaginalis*-associated balanoposthitis with a fishy odor suggestive of anaerobes, observed clue cells in endourethral samples from two men, and pointed out the analogy between this syndrome and bacterial vaginosis. A case of balanoposthitis characterized by erythematous macules, a positive amine test, and *G. vaginalis* involvement was described by Abdennader et al. (1). Signs of balanitis and clue cells were observed by Lefevre et al. (123) in men with *G. vaginalis*-associated urethritis.

The presence of $>10^4$ CFU of *G. vaginalis* per ml in midstream urine specimens is difficult to evaluate because it is found in symptom-free adult males (225) as well as in men suffering from end-stage renal disease, ureteral obstruction, postcatheter cystitis, an infected urinoma, and chronic prostatitis (111, 222). Thus, it was useful to show that the bladder was the site of localization of *G. vaginalis* in three men with urinary symptoms (2, 68). Wilson and Barratt (245) reported the occurrence in a previously healthy young man of a urinary tract infection that led to acute renal failure and septicemia. Finkelhor et al. (69) described a *G. vaginalis*-associated ascending urinary tract infection in a male patient who had received a kidney transplant 5 years earlier.

G. vaginalis was cultured from samples of semen from four asymptomatic sexual consorts of women with bacterial vaginosis (63, 92) and 4 of 12 healthy volunteers (120). Ison and Easmon (99) isolated *G. vaginalis* from 22 (38%) of 58 semen samples and both *G. vaginalis* and obligate anaerobes from 9 (15.5%) others. *G. vaginalis* counts ranged between 10^3 and $>10^7$ CFU/ml, with a geometric mean of 9×10^4 CFU/ml. Clue cells were seen in two semen samples.

Bloodstream

G. vaginalis bacteremia is far more frequent in women than in men (Table 3). Parturition with surgical intervention, postpartum endometritis, and septic abortion are common antecedents of bacteremia. Bacteria gain access to open venous channels in the placental bed during the immediate postpartum period. Since *G. vaginalis* together with other bacteria are often associated with amnionitis or endometritis, it is expected that some bacteremias will have a polymicrobic etiology.

The 30 patients with *G. vaginalis* bacteremia described by Reimer and Reller (184) became afebrile within 1 to 6 days (mean, 3 days) and remained in the hospital for 2 to 15 days (mean, 5 days) after cultures were obtained. Most patients were responsive to limited therapy and recovered without sequelae. Complications were believed to reflect the underlying condition of the patient rather than the virulence of *G. vaginalis*. Meningitis or endocarditis did not develop in any of

TABLE 3. *G. vaginalis* isolated in pure or mixed culture from the bloodstream

Author(s) (reference)	Date[a]	Bacteremic patients			History (no. of patients)	No. of cultures	
		Sex[b]	Age (yr)	No.		Pure[c]	Mixed[d]
Venkataramani and Rathbun (238)	1961–1973	F	14–42	21	Septic abortion (2), ectopic pregnancy (1), cesarean section (10), episiotomy (8)	20	1
	1971	F	19	1	Spontaneous vaginal delivery	1	
	1965	F	60	1	Diabetes mellitus; cellulitis of thigh	1	
	1967	F	32	1	Hysterectomy for carcinoma	1	
	1965–1969	NS[e]	Newborn	4	Prematurity (3), 2 deaths; membranes ruptured at home (1)	4	
	1975	F	23	1	Vaginal bleeding, hydatidiform mole, hysterectomy, bacteremic for 9 days	1[f]	
Rotheram and Schick (194)	1965–1967	F	NS	5	Septic abortion (5)	1	4
Monif and Baer (148)	1970–1973	F	NS	3	Septic abortion, malodorous discharge (3)	3 (2[f])	
		F	NS	8	Post-cesarean section infection (8)	3[f]	5 (3[f])
		F	NS	2	Postpartum endometritis (2)		2[f]
Platt (177)	1971	F	Newborn	1	Necropsy: lungs edematous, pleural effusions	1[f]	
		F	22–35	3	Abortion (2), cesarean section and endometritis (1)	3 (1[f])	
Carney (31)	1971	F	Newborn	1	Maternal chorioamnionitis	1	
		F	18, 30	2	Cesarean section (1), premature delivery (1)	1	1
		F	20	1	Purulent discharge, chorioamnionitis	1[f]	
Regamey and Schoenknecht (183)	1973	F	18	1	Cesarean section, endometritis	1	
Adeniyi-Jones et al. (3)	1975–1977	F	NS	7	Cesarean section (6), episiotomy (1)	6 (3[f])	1[f]
		F	NS	1	Dilatation and curettage, abortion	1[f]	
Reimer and Reller (184)	1977–1981	F	17–50	30	Obstetrical (25), gynecological (5)	14	16
Vontver and Eschenbach (239)	NS–1981	F	NS	14	Febrile postpartum course	14	
Watts et al. (241)	1980–1983	F	Mean, 24	10[g]	Early-onset postpartum endometritis	10	
La Scolea et al. (121)	1982	F	34	1	Chorioamnionitis, cesarean section	1[f]	
Patrick and Garnett (165)	1978	M	57	1	Transurethral prostatectomy, fever, rigors	1[f]	
Wilson and Barratt (245)	1986	M	19	1	Meatal stricture, disseminated intravascular coagulation, acute renal failure	1	
Harper and Jones (85)	1987	M	60	1	Transurethral resection of prostate, rigors	1	
Legrand et al. (124)	1988	M	41	1	Inhalation pneumonia, lung abscess, empyema, fever, chronic alcohol abuse, death	1	
Denoyel et al. (53)	1989	M	65	1	Prostatic adenoma, urinary retention, fever	1[f]	

[a] Year in which patient was hospitalized was given in text or estimated from date of publication.
[b] F, female; M, male.
[c] Many reports of "pure" cultures did not state that anaerobic cultures were made in parallel.
[d] Concomitant isolation of *G. vaginalis* and one or more anaerobic or aerobic bacterial species.
[e] NS, not stated.
[f] Two or more cultures of blood from a given patient yielded the same organism(s). The number in parentheses indicates the number of patients whose blood cultures yielded the same organism(s) two or more times.
[g] Some of the same patients may have been included in the review by Vontver and Eschenbach (239).

the adults mentioned in Table 3. However, cerebrospinal fluid from a 5-day-old baby girl yielded a pure culture of *G. vaginalis*; a culture of her blood was reported as "sterile" (13).

The successful detection of *G. vaginalis* bacteremia depends partly on the culture methods, including the specimen volume, culture medium, and conditions and time of incubation. Direct plating (121) or poured plates (183) were found useful. Reimer and Reller (184) attributed their success in recovering *G. vaginalis* to the omission of sodium polyanetholesulfonate, an anticoagulant commonly used since the 1960s, or to the inclusion of gelatin in the blood culture medium to detoxify this anticoagulant, which otherwise could inhibit *G. vaginalis* growth. A heightened index of suspicion may have contributed to the recognition of bacteremia in four men during the period 1986 to 1989 (Table 3), whereas only one case was recorded earlier.

IMMUNOLOGICAL RESPONSES TO *G. VAGINALIS* INFECTION

An immune response to intrauterine infection was demonstrated by Møller et al. (147) in a study of women with premature rupture of fetal membranes. Immunofluorescence

microscopy using fluorescein-conjugated anti-human globulin revealed that amniotic fluid contained antibody-coated bacteria, and *G. vaginalis* was recovered in cultures of amniotic fluid, placenta, and fetal membranes from one patient. Immunoglobulin A and secretory piece could be detected prior to the appearance of signs of intrauterine infection.

Secretory immunoglobulin A, which may be present in vaginal secretions (43), could affect the progress of bacterial vaginosis by coating *G. vaginalis* and blocking adherence to epithelial cells. Levison et al. (127) suggested that this could account for the absence of clue cells in some vaginal fluids.

In a study of postabortal pelvic inflammatory disease, Heisterberg et al. (87) determined the titers of agglutinating antibodies against *G. vaginalis* in sera from patients who had undergone first-trimester abortion and who harbored *G. vaginalis* in the cervix. A titer of 2,560 was found in serum from one woman who experienced pelvic inflammatory disease, whereas the titers were not higher than 320 in 29 women with uncomplicated postabortal courses.

Kristiansen et al. (118) studied three patients with clinical signs of endometritis who underwent hysterectomy; in each case, *G. vaginalis* was isolated in pure culture from the fundus of the uterine cavity. Titers of serum antibodies that reacted with *G. vaginalis* were 64, 256, and 512 as determined by an immunofluorescence assay method in which a titer of $\geq$16 was regarded as positive.

Sera from 28 women with clinical signs of "vaginitis" but with no evidence of extravaginal infection were studied by Ghione et al. (76). They used various *G. vaginalis* isolates as antigens in immunofluorescence and enzyme-linked immunosorbent assays. Both methods detected immunoglobulins that reacted with each patient's own strain but not with *G. vaginalis* vaginal isolates from other subjects. Significantly, tests of sera from 15 women with asymptomatic *G. vaginalis*-associated vaginosis showed either negative reactions (12 subjects) or only low levels of reactivity.

Isolates from blood and urine specimens from a male patient with *G. vaginalis* septicemia were used by Wilson and Barratt (245) as antigens in indirect immunofluorescence tests. Serum taken during the acute and convalescent phases showed an increase of antibody titer from <8 to >128 against both the blood and urinary isolates.

When considering the design of serological tests, it is important to recognize that *G. vaginalis* strains isolated from different patients exhibit antigenic differences (20, 57, 60, 76, 100). Elevated serum antibody titers can be detected in some infections if tests are performed with *G. vaginalis* antigens prepared from each patient's infecting strain. Reports of the lack of humoral response to *G. vaginalis* in patients with extravaginal infections (77) are difficult to evaluate when results were obtained with test antigens from *G. vaginalis* strains different from, and possibly unrepresentative of, the patients' strains.

Impaired immunity has been suggested as a factor in the association between *G. vaginalis* and urinary tract infections of kidney transplant patients (69, 169) and between *G. vaginalis* and vaginitis emphysematosa (112).

G. vaginalis is opsonized for phagocytosis and killing by human neutrophils in the presence of normal human serum in a process primarily mediated by complement activated by the alternative pathway (57). However, the organism is resistant to killing by human serum plus complement or by mouse antiserum (19, 57).

CONCLUSIONS AND PROSPECTS

G. vaginalis has emerged from obscurity to become an object of active investigation. Controversies concerning its role in bacterial vaginosis and its potential pathogenicity in extravaginal sites have been largely resolved by the use of improved media and methods for the isolation and identification of the organism; standardization of criteria for the clinical and laboratory diagnosis of bacterial vaginosis; and recognition that certain anaerobic bacteria, as well as *G. vaginalis*, are participants in the disease process. Detection of *G. vaginalis*, *Mobiluncus* spp., and *Mycoplasma hominis* in anorectal specimens suggests that there may be an endogenous source of bacterial vaginosis in addition to sexual transmission.

One of the challenging areas for future investigation is the development of a suitable animal model of bacterial vaginosis. This would permit (i) determination of the relative pathogenicity of various bacteria incriminated in bacterial vaginosis and extravaginal infections; (ii) testing of the pathogenic potential of *G. vaginalis* strains that exhibit differences in piliation, adherence to epithelial cells, and production of cytolytic toxin and enzymes of interest, such as lipase and phospholipases; and (iii) comparison of the therapeutic efficacies of antibacterial drugs. Further, new systems for typing *G. vaginalis* merit development and application in future studies of strains cultured from vaginal fluid samples from women with healthy and diseased vaginas, isolates from anorectal specimens, and isolates from extravaginal sites of infection. The finding of antigenic heterogeneity of *G. vaginalis* strains calls for methods that make use of antigens prepared from each patient's infecting strain in studies to evaluate immune responses to *G. vaginalis*-associated infections.

The metabolism, biochemical features, and ultrastructure of *G. vaginalis* and other possible *Gardnerella* spp. require further study. Also, strains isolated from patients, human carriers, and mares should be compared. Certain differential tests need to be performed quantitatively; these include lysis of horse, human, rabbit, and sheep erythrocytes and susceptibility to inhibition by bile salts and fecal lipids. Investigators should include well-defined standard strains that will facilitate comparisons between the results of different studies. The detection of *Gardnerella*-like bacteria in mares may stimulate interest in the genital flora of other domestic animals. Genetic studies of collections of *Gardnerella*-like bacteria are required in order to determine the possible existence of distinct, additional species of *Gardnerella*. Utilization of DNA-rRNA hybridization and nucleic acid sequencing ultimately will reveal the phylogenetic relationships of this enigmatic genus.

ACKNOWLEDGMENT

I thank Vicki J. Schnadig for generously providing the original unpublished photographs for Fig. 1 and 3.

REFERENCES

1. **Abdennader, S., I. Casin, N. Brunat, M. Janier, Y. Perol, and P. Morel.** 1990. Sexual transmission of *Gardnerella vaginalis*. Genitourin. Med. **66**:45. (Letter.)
2. **Abercrombie, G. F., J. Allen, and R. Maskell.** 1978. *Corynebacterium vaginale* urinary-tract infection in a man. Lancet **i**:766. (Letter.)
3. **Adeniyi-Jones, C., D. J. Groves, A. Mannethu, and J. Righter.**

1980. *Hemophilus vaginalis* bacteremia. Can. Med. Assoc. J. **122**:424–426.
4. **Åkerlund, M., and P.-A. Mårdh.** 1974. Isolation and identification of *Corynebacterium vaginale* (*Haemophilus vaginalis*) in women with infections of the lower genital tract. Acta Obstet. Gynecol. Scand. **53**:85–90.
5. **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 epidemiologic associations. Am. J. Med. **74**:14–22.
6. **Bailey, R. K., J. L. Voss, and R. F. Smith.** 1979. Factors affecting isolation and identification of *Haemophilus vaginalis* (*Corynebacterium vaginale*). J. Clin. Microbiol. **9**:65–71.
7. **Balsdon, M. J., and D. Jackson.** 1981. Metronidazole metabolite and *Gardnerella vaginalis* (*Corynebacterium vaginale*). Lancet **i**:1112. (Letter.)
8. **Balsdon, M. J., G. E. Taylor, L. Pead, and R. Maskell.** 1980. *Corynebacterium vaginale* and vaginitis: a controlled trial of treatment. Lancet **i**:501–504.
9. **Bannatyne, R. M., J. Jackowski, R. Cheung, and K. Biers.** 1987. Susceptibility of *Gardnerella vaginalis* to metronidazole, its bioactive metabolites, and tinidazole. Am. J. Clin. Pathol. **87**:640–641.
10. **Bartley, D. L., L. Morgan, and M. E. Rimsza.** 1987. *Gardnerella vaginalis* in prepubertal girls. Am. J. Dis. Child. **141**:1014–1017.
11. **Bejar, R., V. Curbelo, C. Davis, and L. Gluck.** 1981. Premature labor. II. Bacterial sources of phospholipase. Obstet. Gynecol. **57**:479–482.
12. **Benito, R., J. A. Vazquez, S. Berron, A. Fenoll, and J. A. Saez-Nieto.** 1986. A modified scheme for biotyping *Gardnerella vaginalis*. J. Med. Microbiol. **21**:357–359.
13. **Berardi-Grassias, L., O. Roy, J. C. Berardi, and J. Furioli.** 1988. Neonatal meningitis due to *Gardnerella vaginalis*. Eur. J. Clin. Microbiol. Infect. Dis. **7**:406–407.
14. **Beveridge, T. J.** 1988. Wall ultrastructure: how little we know, p. 3–20. *In* P. Actor, L. Daneo-Moore, M. L. Higgins, M. R. J. Salton, and G. D. Shockman (ed.), Antibiotic inhibition of bacterial cell surface assembly and function. American Society for Microbiology, Washington, D.C.
15. **Bhalla, P., N. Rewari, and P. Chadha.** 1989. *Gardnerella vaginalis* vaginitis in Cu T 200 users. Indian J. Med. Res. **89**:80–86.
16. **Blackwell, A. L., and D. Barlow.** 1984. Anaerobic vaginosis: clinical and diagnostic aspects. Scand. J. Urol. Nephrol. **86**(Suppl.):129–133.
17. **Blackwell, A. L., A. R. Fox, I. Phillips, and D. Barlow.** 1983. Anaerobic vaginosis (non-specific vaginitis): clinical, microbiological, and therapeutic findings. Lancet **ii**:1379–1382.
18. **Borchardt, K. A., B. S. Adly, R. F. Smith, J. Eapen, and C. B. Beal.** 1989. Importance of *Gardnerella vaginalis* as an etiological agent in bacterial vaginosis. Genitourin. Med. **65**:285. (Letter.)
19. **Boustouller, Y. L., and A. P. Johnson.** 1986. Resistance of *Gardnerella vaginalis* to bactericidal activity of human serum. Genitourin. Med. **62**:380–383.
20. **Boustouller, Y. L., A. P. Johnson, and D. Taylor-Robinson.** 1986. Detection of species-specific antigen of *Gardnerella vaginalis* by Western blot analysis. J. Gen. Microbiol. **132**: 1969–1973.
21. **Boustouller, Y. L., A. P. Johnson, and D. Taylor-Robinson.** 1987. Pili on *Gardnerella vaginalis* studied by electronmicroscopy. J. Med. Microbiol. **23**:327–329.
22. **Bowie, W. R., C. E. Shaw, D. G. W. Chan, J. Boyd, and W. A. Black.** 1986. In vitro activity of difloxacin hydrochloride (A-56619), A-56620, and cefixime (CL 284,635; FK 027) against selected genital pathogens. Antimicrob. Agents Chemother. **30**:590–593.
23. **Bratos, M. A., A. Orduña, J. Medina, R. O. de Lejarazu, and A. Rodriguez-Torres.** 1986. Diagnosis of *Gardnerella vaginalis* infection by an enzyme-linked immunosorbent assay. Eur. J. Clin. Microbiol. **5**:46–48.
24. **Briselden, A. M., and S. L. Hillier.** 1990. Longitudinal study of the biotypes of *Gardnerella vaginalis*. J. Clin. Microbiol. **28**:2761–2764.
25. **Bro, F.** 1989. Vaginal microbial flora in women with and without vaginal discharge registered in general practice. Dan. Med. Bull. **36**:483–485.
26. **Brown, M. I.** 1984. Anaerobic vaginosis. Lancet **i**:337. (Letter.)
27. **Bump, R. C., and W. J. Buesching III.** 1988. Bacterial vaginosis in virginal and sexually active adolescent females: evidence against exclusive sexual transmission. Am. J. Obstet. Gynecol. **158**:935–939.
28. **Bump, R. C., F. P. Zuspan, W. J. Buesching III, L. W. Ayers, and T. J. Stephens.** 1984. The prevalence, six-month persistence, and predictive values of laboratory indicators of bacterial vaginosis (nonspecific vaginitis) in asymptomatic women. Am. J. Obstet. Gynecol. **150**:917–924.
29. **Burdge, D. R., W. R. Bowie, and A. W. Chow.** 1986. *Gardnerella vaginalis*-associated balanoposthitis. Sex. Transm. Dis. **13**:159–162.
30. **Cano, R. J., M. A. Beck, and D. V. Grady.** 1983. Detection of *Gardnerella vaginalis* on vaginal smears by immunofluorescence. Can. J. Microbiol. **29**:27–32.
31. **Carney, F. E.** 1973. Hemophilus vaginalis septicemia. Obstet. Gynecol. **41**:78–79.
32. **Catlin, B. W.** 1970. *Neisseria meningitidis* (meningococcus), p. 76–81. *In* J. E. Blair, E. H. Lennette, and J. P. Truant (ed.), Manual of clinical microbiology, 1st ed. American Society for Microbiology, Washington, D.C.
33. **Catlin, B. W.** 1975. Cellular elongation under the influence of antibacterial agents: way to differentiate coccobacilli from cocci. J. Clin. Microbiol. **1**:102–105.
34. **Catlin, B. W.** 1978. Characteristics and auxotyping of *Neisseria gonorrhoeae*, p. 345–380. *In* T. Bergan and J. R. Norris (ed.), Methods in microbiology, vol. 10. Academic Press Ltd., London.
35. **Catlin, B. W.** 1990. *Branhamella catarrhalis*: an organism gaining respect as a pathogen. Clin. Microbiol. Rev. **3**:293–320.
36. **Chen, K. C. S., R. Amsel, D. A. Eschenbach, and K. K. Holmes.** 1982. Biochemical diagnosis of vaginitis: determination of diamines in vaginal fluid. J. Infect. Dis. **145**:337–345.
37. **Ching, L. Q., K. A. Borchardt, R. F. Smith, and C. B. Beal.** 1988. A 24 hour plastic envelope method for isolating and identifying *Gardnerella vaginalis* (PEM-GVA). Genitourin. Med. **64**:180–184.
38. **Chowdhury, M. N. H.** 1986. *Gardnerella vaginalis* carriage in male patients. Trop. Geogr. Med. **38**:137–140.
39. **Chowdhury, M. N. H., and S. K. Desilva.** 1986. Episiotomy wound infection due to *Gardnerella vaginalis*. Eur. J. Clin. Microbiol. **5**:164–165.
40. **Chowdhury, M. N. H., F. A. Jabbar, and A. M. Kambal.** 1985. Isolation of *Gardnerella vaginalis* from high vaginal swabs. Trop. Geogr. Med. **37**:328–333.
41. **Chowdhury, M. N. H., and A. M. Kambal.** 1985. A case of conjunctivitis in a neonate due to *Gardnerella vaginalis*. Trop. Geogr. Med. **37**:365–366.
42. **Clarke, R. W., L. E. Collins, and R. Maskell.** 1989. *Gardnerella vaginalis* as a urinary pathogen. J. Infect. **19**:191–193. (Letter.)
43. **Cohen, M. S., J. R. Black, R. A. Proctor, and P. F. Sparling.** 1984. Host defences and the vaginal mucosa. A re-evaluation. Scand. J. Urol. Nephrol. **86**(Suppl.):13–22.
44. **Cook, R. L., G. Reid, D. G. Pond, C. A. Schmitt, and J. D. Sobel.** 1989. Clue cells in bacterial vaginosis: immunofluorescent identification of the adherent gram-negative bacteria as *Gardnerella vaginalis*. J. Infect. Dis. **160**:490–496.
45. **Coyle, M. B., and B. A. Lipsky.** 1990. Coryneform bacteria in infectious diseases: clinical and laboratory aspects. Clin. Microbiol. Rev. **3**:227–246.
46. **Cristiano, L., N. Coffetti, G. Dalvai, L. Lorusso, and M. Lorenzi.** 1989. Bacterial vaginosis: prevalence in outpatients, association with some micro-organisms and laboratory indices. Genitourin. Med. **65**:382–387.
47. **Criswell, B. S., C. L. Ladwig, H. L. Gardner, and C. D. Dukes.** 1969. *Haemophilus vaginalis*: vaginitis by inoculation from

culture. Obstet. Gynecol. **33**:195–199.
48. **Criswell, B. S., J. H. Marston, W. A. Stenback, S. H. Black, and H. L. Gardner.** 1971. *Haemophilus vaginalis* 594, a gram-negative organism? Can. J. Microbiol. **17**:865–869.
49. **Criswell, B. S., W. A. Stenback, S. H. Black, and H. L. Gardner.** 1972. Fine structure of *Haemophilus vaginalis*. J. Bacteriol. **109**:930–932.
50. **Csángó, P. A., N. Hagen, and G. Jagars.** 1983. Characterization of *Gardnerella vaginalis* by gas chromatography. Scand. J. Infect. Dis. **40**(Suppl.):19–22.
51. **Dawson, S. G., C. A. Ison, G. Csonka, and C. S. F. Easmon.** 1982. Male carriage of *Gardnerella vaginalis*. Br. J. Vener. Dis. **58**:243–245.
52. **Defalco, J., J. Cannillo, and K. Nath.** 1990. Isolation and restriction endonuclease analysis of *Gardnerella vaginalis* chromosomal DNA. J. Microbiol. Methods **12**:207–216.
53. **Denoyel, G.-A., E. B. Drouet, H. P. De Montclos, A. Schanen, and S. Michel.** 1990. *Gardnerella vaginalis* bacteremia in a man with prostatic adenoma. J. Infect. Dis. **161**:367–368. (Letter.)
54. **Dunkelberg, W. E.** 1977. *Corynebacterium vaginale*. Sex. Transm. Dis. **4**:69–75. (Review.)
55. **Dunkelberg, W. E., Jr., and I. McVeigh.** 1969. Growth requirements of *Haemophilus vaginalis*. Antonie van Leeuwenhoek J. Microbiol. Serol. **35**:129–145.
56. **Dunkelberg, W. E., Jr., S. Skaggs, and D. S. Kellogg, Jr.** 1970. A study and new description of *Corynebacterium vaginale* (*Haemophilus vaginalis*). Am. J. Clin. Pathol. **53**:370–377.
57. **Easmon, C. S. F., L. Clark, J. P. Crane, and R. Green.** 1985. Phagocytosis and killing of *Gardnerella vaginalis* by human neutrophils. J. Clin. Pathol. **38**:747–749.
58. **Easmon, C. S. F., C. A. Ison, C. M. Kaye, R. M. Timewell, and S. G. Dawson.** 1982. Pharmacokinetics of metronidazole and its principal metabolites and their activity against *Gardnerella vaginalis*. Br. J. Vener. Dis. **58**:246–249.
59. **Edmunds, P. N.** 1960. *Haemophilus vaginalis*: morphology, cultural characteristics and viability. J. Pathol. Bacteriol. **79**: 273–283.
60. **Edmunds, P. N.** 1962. The biochemical, serological and haemagglutinating reactions of "*Haemophilus vaginalis*." J. Pathol. Bacteriol. **83**:411–422.
61. **Edwards, D. I.** 1979. Mechanism of antimicrobial action of metronidazole. J. Antimicrob. Chemother. **5**:499–502.
62. **Eley, A., and T. M. Clarry.** 1990. Tetracycline resistance in *Gardnerella vaginalis*. J Antimicrob. Chemother. **26**:452–453. (Letter.)
63. **Elsner, P., and A. A. Hartmann.** 1987. *Gardnerella vaginalis* in the male upper genital tract: a possible source of reinfection of the female partner. Sex. Transm. Dis. **14**:122–123.
64. **Eschenbach, D. A., P. R. Davick, B. L. Williams, S. J. Klebanoff, K. Young-Smith, C. M. Critchlow, and K. K. Holmes.** 1989. Prevalence of hydrogen peroxide-producing *Lactobacillus* species in normal women and women with bacterial vaginosis. J. Clin. Microbiol. **27**:251–256.
65. **Eschenbach, D. A., S. Hillier, C. Critchlow, C. Stevens, T. DeRouen, and K. K. Holmes.** 1988. Diagnosis and clinical manifestations of bacterial vaginosis. Am. J. Obstet. Gynecol. **158**:819–828.
66. **Ezzell, J. H., Jr., and W. J. Many, Jr.** 1988. *Gardnerella vaginalis*: an unusual case of pyogenic liver abscess. Am. J. Gastroenterol. **83**:1409–1411.
67. **Fairley, K. F., and D. F. Birch.** 1983. Unconventional bacteria in urinary tract disease: *Gardnerella vaginalis*. Kidney Int. **23**:862–865.
68. **Fairley, K. F., and D. F. Birch.** 1989. Detection of bladder bacteriuria in patients with acute urinary symptoms. J. Infect. Dis. **159**:226–231.
69. **Finkelhor, R. S., E. Wolinsky, C. H. Kim, P. Tchou, and J. D. Frengley.** 1981. *Gardnerella vaginalis* perinephric abscess in a transplanted kidney. N. Engl. J. Med. **304**:846. (Letter.)
70. **Fredricsson, B., K. Englund, L. Weintraub, A. Ölund, and C.-E. Nord.** 1989. Bacterial vaginosis is not a simple ecological disorder. Gynecol. Obstet. Invest. **28**:156–160.
71. **Furman, L. M.** 1988. Neonatal *Gardnerella vaginalis* infection. Pediatr. Infect. Dis. J. **7**:890. (Letter.)
72. **Gardner, H. L.** 1980. *Haemophilus vaginalis* vaginitis after twenty-five years. Am. J. Obstet. Gynecol. **137**:385–390.
73. **Gardner, H. L.** 1983. "Non-specific" vaginitis: a non-entity. Scand. J. Infect. Dis. **40**(Suppl.):7–10.
74. **Gardner, H. L.** 1983. Pathogenicity of *Gardnerella vaginalis* (*Haemophilus vaginalis*). Scand. J. Infect. Dis. **40**(Suppl.):37–40.
75. **Gardner, H. L., and C. H. Dukes.** 1955. *Haemophilus vaginalis* vaginitis: a newly defined specific infection previously classified as "nonspecific" vaginitis. Am. J. Obstet. Gynecol. **69**: 962–976.
76. **Ghione, M., P. A. Clerici, G. Piragine, and E. Magliano.** 1989. Humoral circulatory immune response to *Gardnerella vaginalis*. J. Clin. Microbiol. **27**:2138–2139.
77. **Gibbs, R. S., M. H. Weiner, K. Walmer, and P. J. St. Clair.** 1987. Microbiologic and serologic studies of *Gardnerella vaginalis* in intra-amniotic infection. Obstet. Gynecol. **70**:187–190.
78. **Goldberg, R. L., and J. A. Washington II.** 1976. Comparison of isolation of *Haemophilus vaginalis* (*Corynebacterium vaginale*) from peptone-starch-dextrose agar and Columbia colistin-nalidixic acid agar. J. Clin. Microbiol. **4**:245–247.
79. **Greaves, W. L., J. Chungafung, B. Morris, A. Haile, and J. L. Townsend.** 1988. Clindamycin versus metronidazole in the treatment of bacterial vaginosis. Obstet. Gynecol. **72**:799–802.
80. **Greenwood, J. R.** 1983. Current taxonomic status of *Gardnerella vaginalis*. Scand. J. Infect. Dis. **40**(Suppl.):11–14.
81. **Greenwood, J. R., and M. J. Pickett.** 1979. Salient features of *Haemophilus vaginalis*. J. Clin. Microbiol. **9**:200–204.
82. **Greenwood, J. R., and M. J. Pickett.** 1980. Transfer of *Haemophilus vaginalis* Gardner and Dukes to a new genus, *Gardnerella*: *G. vaginalis* (Gardner and Dukes) comb. nov. Int. J. Syst. Bacteriol. **30**:170–178.
83. **Hammerschlag, M. R., S. Alpert, I. Rosner, P. Thurston, D. Semine, D. McComb, and W. M. McCormack.** 1978. Microbiology of the vagina in children: normal and potentially pathogenic organisms. Pediatrics **62**:57–62.
84. **Hansen, W., B. Vray, K. Miller, F. Crokaert, and E. Yourassowsky.** 1987. Detection of *Gardnerella vaginalis* in vaginal specimens by direct immunofluorescence. J. Clin. Microbiol. **25**:1934–1937.
85. **Harper, E. A., and A. S. Jones.** 1987. Comparison of radiometric and gas capture systems for blood cultures. J. Clin. Pathol. **40**:243. (Letter.)
86. **Harper, J. J., and G. H. G. Davis.** 1982. Cell wall analysis of *Gardnerella vaginalis* (*Haemophilus vaginalis*). Int. J. Syst. Bacteriol. **32**:48–50.
87. **Heisterberg, L., P. E. Branebjerg, A. Bremmelgaard, J. Scheibel, and L. Høj.** 1987. The role of vaginal secretory immunoglobulin A, *Gardnerella vaginalis*, anaerobes, and *Chlamydia trachomatis* in postabortal pelvic inflammatory disease. Acta Obstet. Gynecol. Scand. **66**:99–102.
88. **Hill, G. B., D. A. Eschenbach, and K. K. Holmes.** 1984. Bacteriology of the vagina. Scand. J. Urol. Nephrol. **86** (Suppl.):23–39.
89. **Hillier, S., M. A. Krohn, D. H. Watts, P. Wolner-Hanssen, and D. A. Eschenbach.** 1990. Microbiologic efficacy of intravaginal clindamycin cream for the treatment of bacterial vaginosis. Obstet. Gynecol. **76**:407–413.
90. **Hillier, S. L., J. Martius, M. Krohn, N, Kiviat, K. K. Holmes, and D. A. Eschenbach.** 1988. A case-control study of chorioamnionic infection and histologic chorioamnionitis in prematurity. N. Engl. J. Med. **319**:972–978.
91. **Holmes, K. K., C. Spiegel, R. Amsel, D. A. Eschenbach, K. C. S. Chen, and P. Totten.** 1981. Nonspecific vaginosis. Scand. J. Infect. Dis. **26**(Suppl.):110–114.
92. **Holst, E.** 1990. Reservoir of four organisms associated with bacterial vaginosis suggests lack of sexual transmission. J. Clin. Microbiol. **28**:2035–2039.
93. **Holst, E., P.-A. Mårdh, and I. Thelin.** 1984. Recovery of anaerobic curved rods and *Gardnerella vaginalis* from the urethra of men, including male heterosexual consorts of female carriers. Scand. J. Urol. Nephrol. **86**(Suppl.):173–177.

94. **Holst, E., L. Svensson, A. Skarin, L. Weström, and P.-A. Mårdh.** 1984. Vaginal colonization with *Gardnerella vaginalis* and anaerobic curved rods. Scand. J. Urol. Nephrol. **86**(Suppl.):147–152.
95. **Holst, E., B. Wathne, B. Hovelius, and P.-A. Mårdh.** 1987. Bacterial vaginosis: microbiological and clinical findings. Eur. J. Clin. Microbiol. **6**:536–541.
96. **Huth, E. J.** 1989. Style notes: bacterial vaginosis or vaginal bacteriosis? Ann. Intern. Med. **111**:553–554.
97. **Ison, C. A., S. G. Dawson, J. Hilton, G. W. Csonka, and C. S. F. Easmon.** 1982. Comparison of culture and microscopy in the diagnosis of *Gardnerella vaginalis* infection. J. Clin. Pathol. **35**:550–554.
98. **Ison, C. A., and C. S. F. Easmon.** 1984. Studies on the mechanism of adhesion of *Gardnerella vaginalis* to human erythrocytes. Scand. J. Urol. Nephrol. **86**(Suppl.):191–193.
99. **Ison, C. A., and C. S. F. Easmon.** 1985. Carriage of *Gardnerella vaginalis* and anaerobes in semen. Genitourin. Med. **61**:120–122.
100. **Ison, C. A., D. G. Harvey, A. Tanna, and C. S. F. Easmon.** 1987. Development and evaluation of scheme for serotyping *Gardnerella vaginalis*. Genitourin. Med. **63**:196–201.
101. **Janda, W. M., J. J. Bradna, and P. Ruther.** 1989. Identification of *Neisseria* spp., *Haemophilus* spp., and other fastidious gram-negative bacteria with the MicroScan *Haemophilus-Neisseria* identification panel. J. Clin. Microbiol. **27**:869–873.
102. **Jantzen, E., B. P. Berdal, and T. Omland.** 1980. Cellular fatty acid composition of *Haemophilus* species, *Pasteurella multocida*, *Actinobacillus actinomycetemcomitans* and *Haemophilus vaginalis* (*Corynebacterium vaginale*). Acta Pathol. Microbiol. Scand. Sect. B **88**:89–93.
103. **Joesoef, M. R., S. L. Hillier, S. Josodiwondo, and M. Linnan.** 1991. Reproducibility of a scoring system for Gram stain diagnosis of bacterial vaginosis. J. Clin. Microbiol. **29**:1730–1731.
104. **Johnson, A. P., and Y. L. Boustouller.** 1987. Extra-vaginal infection caused by *Gardnerella vaginalis*. Epidemiol. Infect. **98**:131–137.
105. **Johnson, A. P., and H. A. Davies.** 1984. Demonstration by electron microscopy of pili on *Gardnerella vaginalis*. Br. J. Vener. Dis. **60**:396–397.
106. **Johnson, A. P., C. A. Ison, C. M. Hetherington, M. F. Osborn, G. Southerton, W. T. London, C. S. F. Easmon, and D. Taylor-Robinson.** 1984. Vaginal colonization of pig-tailed macaques by *Gardnerella vaginalis*. Scand. J. Urol. Nephrol. **86**(Suppl.):207–210.
107. **Jolly, J. L. S.** 1983. Minimal criteria for the identification of *Gardnerella vaginalis* isolated from the vagina. J. Clin. Pathol. **36**:476–478.
108. **Jones, B. M., I. Geary, A. B. Alawattegama, G. R. Kinghorn, and B. I. Duerden.** 1985. *In-vitro* and *in-vivo* activity of metronidazole against *Gardnerella vaginalis*, *Bacteroides* spp. and *Mobiluncus* spp. in bacterial vaginosis. J. Antimicrob. Chemother. **16**:189–197.
109. **Jones, B. M., I. Geary, M. E. Lee, and B. I. Duerden.** 1989. Comparison of the in vitro activities of fenticonazole, other imidazoles, metronidazole, and tetracycline against organisms associated with bacterial vaginosis and skin infections. Antimicrob. Agents Chemother. **33**:970–972.
110. **Jones, B. M., G. R. Kinghorn, and B. I. Duerden.** 1988. In vitro activity of azithromycin and erythromycin against organisms associated with bacterial vaginosis and chancroid. Eur. J. Clin. Microbiol. Infect. Dis. **7**:551–553.
111. **Josephson, S., J. Thomason, K. Sturino, R. Zabransky, and J. Williams.** 1988. *Gardnerella vaginalis* in the urinary tract: incidence and significance in a hospital population. Obstet. Gynecol. **71**:245–250.
112. **Josey, W. E., and W. G. Campbell, Jr.** 1990. Vaginitis emphysematosa: a report of four cases. J. Reprod. Med. **35**:974–977.
113. **King, A., C. Boothman, and I. Phillips.** 1989. Comparative in-vitro activity of meropenem on clinical isolates from the United Kingdom. J. Antimicrob. Chemother. **24**(Suppl. A):31–45.
114. **King, A., C. Boothman, and I. Phillips.** 1990. Comparative in vitro activity of cefpirome and cefepime, two new cephalosporins, Eur. J. Clin. Microbiol. Infect. Dis. **9**:677–685.
115. **King, A., and I. Phillips.** 1986. The comparative *in-vitro* activity of pefloxacin. J. Antimicrob. Chemother. **17**(Suppl. B):1–10.
116. **Kinghorn, G. R., B. M. Jones, F. H. Chowdhury, and I. Geary.** 1982. Balanoposthitis associated with *Gardnerella vaginalis* infection in men. Br. J. Vener. Dis. **58**:127–129.
117. **Klebanoff, S. J., S. L. Hillier, D. A. Eschenbach, and A. M. Waltersdorph.** 1991. Control of the microbial flora of the vagina by H_2O_2-generating lactobacilli. J. Infect. Dis. **164**:94–100.
118. **Kristiansen, F. V., S. Øster, L. Frost, Y. Boustouller, B. Korsager, and B. R. Møller.** 1987. Isolation of *Gardnerella vaginalis* in pure culture from the uterine cavity of patients with irregular bleedings. Br. J. Obstet. Gynaecol. **94**:979–984.
119. **Lam, M. H., and D. F. Birch.** 1991. Survival of *Gardnerella vaginalis* in human urine. Am. J. Clin. Pathol. **95**:234–239.
120. **Lam, M. H., D. F. Birch, and K. F. Fairley.** 1988. Prevalance of *Gardnerella vaginalis* in the urinary tract. J. Clin. Microbiol. **26**:1130–1133.
121. **La Scolea, L. J., Jr., D. M. Dryja, and W. P. Dillon.** 1984. Recovery of *Gardnerella vaginalis* from blood by the quantitative direct plating method. J. Clin. Microbiol. **20**:568–569.
122. **Lee, W., L. E. Phillips, R. J. Carpenter, M. G. Martens, and S. Faro.** 1987. *Gardnerella vaginalis* chorioamnionitis: a report of two cases and a review of the pathogenic role of *G. vaginalis* in obstetrics. Diagn. Microbiol. Infect. Dis. **8**:107–111.
123. **Lefevre, J.-C., J.-P. Lepargneur, R. Bauriaud, M.-A. Bertrand, and C. Blanc.** 1991. Clinical and microbiologic features of urethritis in men in Toulouse, France, Sex. Transm. Dis. **18**:76–79.
124. **Legrand, J. C., A. Alewaeters, L. Leenaerts, P. Gilbert, M. Labbe, and Y. Glupczynski.** 1989. *Gardnerella vaginalis* bacteremia from pulmonary abscess in a male alcohol abuser. J. Clin. Microbiol. **27**:1132–1134.
125. **Leighton, P. M., B. Bulleid, and R. Taylor.** 1982. Neonatal cellulitis due to *Gardnerella vaginalis*. Pediatr. Infect. Dis. **1**:339–340.
126. **Leopold, S.** 1953. Heretofore undescribed organism isolated from the genitourinary system. U. S. Armed Forces Med. J. **4**:263–266.
127. **Levison, M. E., I. Trestman, R. Quach, C. Sladowski, and C. N. Floro.** 1979. Quantitative bacteriology of the vaginal flora in vaginitis. Am. J. Obstet. Gynecol. **133**:139–144.
128. **Liebowitz, L. D., J. Saunders, G. Fehler, R. C. Ballard, and H. J. Koornhof.** 1986. In vitro activity of A-56619 (difloxacin), A-56620, and other new quinolone antimicrobial agents against genital pathogens. Antimicrob. Agents Chemother. **30**:948–950.
129. **Lien, E. A., and S. L. Hillier.** 1989. Evaluation of the enhanced rapid identification method for *Gardnerella vaginalis*. J. Clin. Microbiol. **27**:566–567.
130. **Lin, J. Y., K. C. S. Chen, J. Hale, P. A. Totten, and K. K. Holmes.** 1986. Two-dimensional thin-layer chromatography for the specific detection of hippurate hydrolysis by microorganisms. J. Clin. Microbiol. **23**:118–123.
131. **Lossick, J. G.** 1982. *Gardnerella vaginalis*-associated leukorrhea: the disease and its treatment. Rev. Infect. Dis. **4**(Suppl.):S793–S800.
132. **Lossick, J. G.** 1990. Treatment of sexually transmitted vaginosis/vaginitis. Rev. Infect. Dis. **12**(Suppl. 6):S665–S681.
133. **Malone, B. H., M. Schreiber, N. J. Schneider, and L. V. Holdeman.** 1975. Obligately anaerobic strains of *Corynebacterium vaginale* (*Haemophilus vaginalis*). J. Clin. Microbiol. **2**:272–275.
134. **Mårdh, P.-A., E. Holst, and B. R. Møller.** 1984. The grivet monkey as a model for study of vaginitis: challenge with anaerobic curved rods and *Gardnerella vaginalis*. Scand. J. Urol. Nephrol. **86**(Suppl.):201–205.
135. **Mårdh, P.-A., and L. Weström.** 1976. Adherence of bacteria to vaginal epithelial cells. Infect. Immun. **13**:661–666.

136. **Martens, M. G., S. Faro, M. Maccato, G. Riddle, and H. A. Hammill.** 1991. Susceptibility of female pelvic pathogens to oral antibiotic agents in patients who develop postpartum endometritis. Am. J. Obstet. Gynecol. **164:**1383–1386.
137. **Masfari, A. N., B. I. Duerden, and G. R. Kinghorn.** 1986. Quantitative studies of vaginal bacteria. Genitourin. Med. **62:**256–263.
138. **Mazzulli, T., A. E. Simor, and D. E. Low.** 1990. Reproducibility of interpretation of gram-stained vaginal smears for the diagnosis of bacterial vaginosis. J. Clin. Microbiol. **28:**1506–1508.
139. **McCarthy, L. R., P. A. Mickelsen, and E. G. Smith.** 1979. Antibiotic susceptibilities of *Haemophilus vaginalis* (*Corynebacterium vaginale*) to 21 antibiotics. Antimicrob. Agents Chemother. **16:**186–189.
140. **McCormack, W. M., C. H. Hayes, B. Rosner, J. R. Evrard, V. A. Crockett, S. Alpert, and S. H. Zinner.** 1977. Vaginal colonization with *Corynebacterium vaginale* (*Haemophilus vaginalis*). J. Infect. Dis. **136:**740–745.
141. **McDowall, D. R. M., J. D. Buchanan, K. F. Fairley, and G. L. Gilbert.** 1981. Anaerobic and other fastidious microorganisms in asymptomatic bacteriuria in pregnant women. J. Infect. Dis. **144:**114–122.
142. **McFadyen, I. R., and S. J. Eykyn.** 1968. Suprapubic aspiration of urine in pregnancy. Lancet **i:**1112–1114.
143. **McGregor, J. A., D. Lawellin, A. Franco-Buff, and J. K. Todd.** 1991. Phospholipase C activity in microorganisms associated with reproductive tract infection. Am. J. Obstet. Gynecol. **164:**682–686.
144. **Mickelsen, P. A., L. R. McCarthy, and M. E. Mangum.** 1977. New differential medium for the isolation of *Corynebacterium vaginale*. J. Clin. Microbiol. **5:**488–489.
145. **Mohanty, K. C., and R. Deighton.** 1987. Comparison of 2 g single dose of metronidazole, nimorazole and tinidazole in the treatment of vaginitis associated with *Gardnerella vaginalis*. J. Antimicrob. Chemother. **19:**393–399.
146. **Moi, H., R. Erkkola, F. Jerve, G. Nelleman, B. Bymose, K. Alaksen, and E. Tornqvist.** 1989. Should male consorts of women with bacterial vaginosis be treated? Genitourin. Med. **65:**263–268.
147. **Møller, M., J. Sørensen, F. L. Jepsen, and A. C. Thomsen.** 1986. Diagnosis of intrauterine infection by demonstration of antibody-coated bacteria in the amniotic fluid. Br. J. Obstet. Gynaecol. **93:**240–244.
148. **Monif, G. R. G., and H. Baer.** 1974. *Haemophilus* (*Corynebacterium*) *vaginalis* septicemia. Am. J. Obstet. Gynecol. **120:**1041–1045.
149. **Moran, D. J., and A. Payne.** 1989. Subclinical intra-amniotic infection with *Gardnerella vaginalis* associated with preterm delivery. Case report. Br. J. Obstet. Gynaecol. **96:**489–490.
150. **Moran, O., O. Zegarra-Moran, C. Virginio, and G. Rottini.** 1991. Voltage-dependent cationic channels formed by a cytolytic toxin produced by *Gardnerella vaginalis*. FEBS Lett. **283:**317–320.
151. **Moss, C. W., and W. E. Dunkelberg, Jr.** 1969. Volatile and cellular fatty acids of *Haemophilus vaginalis*. J. Bacteriol. **100:**544–546.
152. **Müller, M.** 1981. Action of clinically utilized 5-nitroimidazoles on microorganisms. Scand. J. Infect. Dis. **26**(Suppl.)**:**31–41.
153. **Nagy, E., M. Petterson, and P.-A. Mårdh.** 1991. Antibiosis between bacteria isolated from the vagina of women with and without signs of bacterial vaginosis. APMIS **99:**739–744.
154. **Nath, K., D.-J. Choi, and D. Devlin.** 1991. The characterization of *Gardnerella vaginalis* DNA using non-radioactive DNA probes. Res. Microbiol. **142:**573–583.
155. **Nightingale, L. M., C. B. Eaton, A. E. Fruehan, J. B. Waldman, W. B. Clark, and M. L. Lepow.** 1986. Cephalhematoma complicated by osteomyelitis presumed due to *Gardnerella vaginalis*. JAMA **256:**1936–1937.
156. **Nugent, R. P., M. A. Krohn, and S. L. Hillier.** 1991. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of Gram stain interpretation. J. Clin. Microbiol. **29:**297–301.
157. **O'Donnell, A. G., D. E. Minnikin, M. Goodfellow, and P. Piot.** 1984. Fatty acid, polar lipid and wall amino acid composition of *Gardnerella vaginalis*. Arch. Microbiol. **138:**68–71.
158. **O'Hare, M. D., G. Ghosh, D. Felmingham, and R. N. Gruneberg.** 1990. In-vitro studies with ramoplanin (MDL 62,198): a novel lipoglycopeptide antimicrobial. J. Antimicrob. Chemother. **25:**217–220.
159. **Paavonen, J.** 1983. Physiology and ecology of the vagina. Scand. J. Infect. Dis. **40**(Suppl.)**:**31–35.
160. **Pandit, D. V., S. M. Barve, and L. P. Deodhar.** 1989. Biotypes of *Gardnerella vaginalis* isolated from non-specific vaginitis patients in Bombay. Indian J. Med. Res. **89:**435–438.
161. **Pao, C. C., S. S. Lin, and T. T. Hsieh.** 1990. The detection of *Gardnerella vaginalis* DNA sequences in uncultured clinical specimens with cloned *G. vaginalis* DNA as probes. Mol. Cell. Probes **4:**367–373.
162. **Papapetropoulou, M., N. J. Legakis, J. Detorakis, E. Kalambokas, and T. Lymberopoulou.** 1986. Frequency and epidemiologic associations of different types of vaginitis in symptomatic women in Greece. Eur. J. Clin. Microbiol. **5:**447–449.
163. **Papapetropoulou, M., and A. Pappas.** 1987. The acute urethral syndrome in routine practice. J. Infect. **14:**113–118.
164. **Park, C. H., M. Fauber, and C. B. Cook.** 1968. Identification of *Haemophilus vaginalis*. Am. J. Clin. Pathol. **49:**590–593.
165. **Patrick, S., and P. A. Garnett.** 1978. *Corynebacterium vaginale* bacteraemia in a man. Lancet **i:**987–988. (Letter.)
166. **Peeters, M., and P. Piot.** 1985. Adhesion of *Gardnerella vaginalis* to vaginal epithelial cells: variables affecting adhesion and inhibition by metronidazole. Genitourin. Med. **61:**391–395.
167. **Petersen, E. E., and K. Pelz.** 1983. Diagnosis and therapy of nonspecific vaginitis: correlation between KOH-test, clue cells and microbiology. Scand. J. Infect. Dis. **40**(Suppl.)**:**97–99.
168. **Petersen, E. E., and K. Pelz.** 1984. Anaerobic vaginosis. Lancet **i:**337–338.
169. **Petit, P. L. C., and R. P. Mouton.** 1985. *Gardnerella vaginalis* in urinary tract infections of immunocompromised patients. Eur. J. Clin. Microbiol. **4:**357. (Letter.)
170. **Pheifer, T. A., P. S. Forsyth, M. A. Durfee, H. M. Pollock, and K. K. Holmes.** 1978. Nonspecific vaginitis: role of *Haemophilus vaginalis* and treatment with metronidazole. N. Engl. J. Med. **298:**1429–1434.
171. **Piot, P.** 1984. Bacterial vaginosis: an evaluation of treatment. Scand. J. Urol. Nephrol. **86**(Suppl.)**:**229–235.
172. **Piot, P.** 1991. *Gardnerella*, *Streptobacillus*, *Spirillum*, and *Calymmatobacterium*, p. 483–487. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
173. **Piot, P., and E. Van Dyck.** 1983. Isolation and identification of *Gardnerella vaginalis*. Scand. J. Infect. Dis. **40**(Suppl.)**:**15–18.
174. **Piot, P., E. Van Dyck, M. Goodfellow, and S. Falkow.** 1980. A taxonomic study of *Gardnerella vaginalis* (*Haemophilus vaginalis*) Gardner and Dukes 1955. J. Gen. Microbiol. **119:**373–396.
175. **Piot, P., E. Van Dyck, M. Peeters, J. Hale, P. A. Totten, and K. K. Holmes.** 1984. Biotypes of *Gardnerella vaginalis*. J. Clin. Microbiol. **20:**677–679.
176. **Piot, P., E. Van Dyck, P. A. Totten, and K. K. Holmes.** 1982. Identification of *Gardnerella* (*Haemophilus*) *vaginalis*. J. Clin. Microbiol. **15:**19–24.
177. **Platt, M. S.** 1971. Neonatal *Haemophilus vaginalis* (*Corynebacterium vaginalis*) infection. Clin. Pediatr. **10:**513–516.
178. **Ralph, E. D.** 1983. Comparative antimicrobial activity of metronidazole and the hydroxy metabolite against *Gardnerella vaginalis*. Scand. J. Infect. Dis. **40**(Suppl.)**:**115–120.
179. **Ralph, E. D., and Y. E. Amatnieks.** 1980. Relative susceptibilities of *Gardnerella vaginalis* (*Haemophilus vaginalis*), *Neisseria gonorrhoeae*, and *Bacteroides fragilis* to metronidazole and its two major metabolites. Sex. Transm. Dis. **7:**157–160.
180. **Ralph, E. D., T. W. Austin, F. L. M. Pattison, and B. C. Schieven.** 1979. Inhibition of *Haemophilus vaginalis* (*Corynebacterium vaginale*) by metronidazole, tetracycline, and ampi-

cillin. Sex. Transm. Dis. **6**:199–202.
181. **Ralph, E. D., and D. A. Clarke.** 1978. Inactivation of metronidazole by anaerobic and aerobic bacteria. Antimicrob. Agents Chemother. **14**:377–383.
182. **Ratnam, S., and B. L. Fitzgerald.** 1983. Semiquantitative culture of *Gardnerella vaginalis* in laboratory determination of nonspecific vaginitis. J. Clin. Microbiol. **18**:344–347.
183. **Regamey, C., and F. D. Schoenknecht.** 1973. Puerperal fever with *Haemophilus vaginalis* septicemia. JAMA **225**:1621–1623.
184. **Reimer, L. G., and L. B. Reller.** 1984. *Gardnerella vaginalis* bacteremia: a review of thirty cases. Obstet. Gynecol. **64**:170–172.
185. **Reimer, L. G., and L. B. Reller.** 1985. Use of a sodium polyanetholesulfonate disk for the identification of *Gardnerella vaginalis*. J. Clin. Microbiol. **21**:146–149.
186. **Reimer, L. G., and L. B. Reller.** 1985. Effect of sodium polyanetholesulfonate and gelatin on the recovery of *Gardnerella vaginalis* from blood culture media. J. Clin. Microbiol. **21**:686–688.
187. **Rempen, A., J. Martius, A. A. Hartmann, and I. Wecker.** 1987. Transmission rate of *Ureaplasma urealyticum*, *Mycoplasma* spp., *Gardnerella vaginalis*, B-streptococci, *Candida* spp. and *Chlamydia trachomatis* from the mother to the newborn. Arch. Gynecol. Obstet. **241**:165–170.
188. **Reyn, A., A. Birch-Andersen, and S. P. Lapage.** 1966. An electron microscopic study of thin sections of *Haemophilus vaginalis* (Gardner and Dukes) and some possibly related species. Can. J. Microbiol. **12**:1125–1136.
189. **Roberts, M. C.** 1990. Characterization of the Tet M determinants in urogenital and respiratory bacteria. Antimicrob. Agents Chemother. **34**:476–478.
190. **Roberts, M. C., and S. L. Hillier.** 1990. Genetic basis of tetracycline resistance in urogenital bacteria. Antimicrob. Agents Chemother. **34**:261–264.
191. **Roberts, M. C., S. L. Hillier, J. Hale, K. K. Holmes, and G. E. Kenny.** 1986. Tetracycline resistance and *tetM* in pathogenic urogenital bacteria. Antimicrob. Agents Chemother. **30**:810–812.
192. **Roberts, M. C., S. L. Hillier, F. D. Schoenknecht, and K. K. Holmes.** 1985. Comparison of Gram stain, DNA probe, and culture for the identification of species of *Mobiluncus* in female genital specimens. J. Infect. Dis. **152**:74–77.
193. **Rosene, K., D. A. Eschenbach, L. S. Tompkins, G. E. Kenny, and H. Watkins.** 1986. Polymicrobial early postpartum endometritis with facultative and anaerobic bacteria, genital mycoplasmas, and *Chlamydia trachomatis*: treatment with piperacillin or cefoxitin. J. Infect. Dis. **153**:1028–1037.
194. **Rotheram, E. B., Jr., and S. F. Schick.** 1969. Nonclostridial anaerobic bacteria in septic abortion. Am. J. Med. **46**:80–89.
195. **Rotimi, V. O., Z. Yakubu, O. O. Abudu, and T. O. Banjo.** 1991. Direct Gram's stain of vaginal discharge as a means of diagnosing bacterial vaginosis. J. Med. Microbiol. **35**:103–106.
196. **Rottini, G., A. Dobrina, O. Forgiarini, E. Nardon, G. A. Amirante, and P. Patriarca.** 1990. Identification and partial characterization of a cytolytic toxin produced by *Gardnerella vaginalis*. Infect. Immun. **58**:3751–3758.
197. **Sadhu, K., P. A. G. Domingue, A. W. Chow, J. Nelligan, N. Cheng, and J. W. Costerton.** 1989. *Gardnerella vaginalis* has a gram-positive cell-wall ultrastructure and lacks classical cell-wall lipopolysaccharide. J. Med. Microbiol. **29**:229–235.
198. **Salmon, S. A., R. D. Walker, C. L. Carleton, S. Shah, and B. E. Robinson.** 1991. Characterization of *Gardnerella vaginalis* and *G. vaginalis*-like organisms from the reproductive tract of the mare. J. Clin. Microbiol. **29**:1157–1161.
199. **Sautter, R. L., and W. J. Brown.** 1980. Sequential vaginal cultures from normal young women. J. Clin. Microbiol. **11**: 479–484.
200. **Scandinavian Journal of Infectious Diseases.** 1983. Panel discussion. First International Conference on Vaginosis, Kristiansand, Norway, April 16–17, 1982. Scand. J. Infect. Dis. **40**(Suppl.):121–126.
201. **Scandinavian Journal of Urology and Nephrology.** 1984. Reports from working groups. The symposium on bacterial vaginosis (Stockholm, January 1984). Scand. J. Urol. Nephrol. **86**(Suppl.):259–266.
202. **Schnadig, V. J., K. D. Davie, S. K. Shafer, R. B. Yandell, M. Z. Islam, and E. V. Hannigan.** 1989. The cytologist and bacteriosis of the vaginal-ectocervical area. Clues, commas and confusion. Acta Cytol. **33**:287–297.
203. **Scott, T. G., B. Curran, and C. J. Smyth.** 1989. Electron microscopy of adhesive interactions between *Gardnerella vaginalis* and vaginal epithelial cells, McCoy cells and human red blood cells. J. Gen. Microbiol. **135**:475–480.
204. **Scott, T. G., and C. J. Smyth.** 1987. Haemagglutination and tissue culture adhesion of *Gardnerella vaginalis*. J. Gen. Microbiol. **133**:1999–2005.
205. **Scott, T. G., C. J. Smyth, and C. T. Keane.** 1987. In vitro adhesiveness and biotype of *Gardnerella vaginalis* strains in relation to the occurrence of clue cells in vaginal discharges. Genitourin. Med. **63**:47–53.
206. **Sehgal, S. C., and V. Nalini.** 1990. The role and prevalence of *Gardnerella vaginalis* in anaerobic vaginosis. Infection **18**:83–85.
207. **Shanker, S., and R. Munro.** 1982. Sensitivity of *Gardnerella vaginalis* to metabolites of metronidazole and tinidazole. Lancet **i**:167. (Letter.)
208. **Shaw, C. E., M. E. Forsyth, W. R. Bowie, and W. A. Black.** 1981. Rapid presumptive identification of *Gardnerella vaginalis* (*Haemophilus vaginalis*) from human blood agar media. J. Clin. Microbiol. **14**:108–110.
209. **Skarin, A., and P.-A. Mårdh.** 1981. Scanning electron microscopic examination of *Bacteroides fragilis* and *Gardnerella vaginalis* after exposure to concentration gradients of metronidazole and tinidazole. Scand. J. Infect. Dis. **26**(Suppl.):54–59.
210. **Skarin, A., and J. Sylwan.** 1986. Vaginal lactobacilli inhibiting growth of *Gardnerella vaginalis*, *Mobiluncus* and other bacterial species cultured from vaginal content of women with bacterial vaginosis. Acta Pathol. Microbiol. Immunol. Scand. Sect. B **94**:399–403.
211. **Smith, R. F.** 1979. Comparison of two media for isolation of *Haemophilus vaginalis*. J. Clin. Microbiol. **9**:729–730.
212. **Smith, R. F., and W. E. Dunkelberg, Jr.** 1977. Inhibition of *Corynebacterium vaginale* by metronidazole. Sex. Transm. Dis. **4**:20–21.
213. **Sobel, J. D.** 1989. Bacterial vaginosis—an ecologic mystery. Ann. Intern. Med. **111**:551–553.
214. **Sobel, J. D., P. Myers, M. E. Levison, and D. Kaye.** 1982. Comparison of bacterial and fungal adherence to vaginal exfoliated epithelial cells and human vaginal epithelial cell tissue culture cells. Infect. Immun. **35**:697–701.
215. **Sobel, J. D., J. Schneider, D. Kaye, and M. E. Levison.** 1981. Adherence of bacteria to vaginal epithelial cells at various times in the menstrual cycle. Infect. Immun. **32**:194–197.
216. **Spiegel, C. A.** 1991. Bacterial vaginosis. Clin. Microbiol. Rev. **4**:485–502.
217. **Spiegel, C. A., R. Amsel, D. Eschenbach, F. Schoenknecht, and K. K. Holmes.** 1980. Anaerobic bacteria in nonspecific vaginitis. N. Engl. J. Med. **303**:601–607.
218. **Spiegel, C. A., R. Amsel, and K. K. Holmes.** 1983. Diagnosis of bacterial vaginosis by direct Gram stain of vaginal fluid. J. Clin. Microbiol. **18**:170–177.
219. **Spiegel, C. A., P. Davick, P. A. Totten, K. C. S. Chen, D. A. Eschenbach, R. Amsel, and K. K. Holmes.** 1983. *Gardnerella vaginalis* and anaerobic bacteria in the etiology of bacterial (nonspecific) vaginosis. Scand. J. Infect. Dis. **40**(Suppl.):41–45.
220. **Spiegel, C. A., and M. Roberts.** 1984. *Mobiluncus* gen. nov., *Mobiluncus curtisii* subsp. *curtisii* sp. nov., *Mobiluncus curtisii* subsp. *holmesii* subsp. nov., and *Mobiluncus mulieris* sp. nov., curved rods from the human vagina. Int. J. Syst. Bacteriol. **34**:177–184.
221. **Staerfelt, F., T. J. Gundersen, A. M. Halsos, C. Barlinn, A. G. Johansen, K. M. Nørregaard, and J. Eng.** 1983. A survey of genital infections in patients attending a clinic for sexually transmitted diseases. Scand. J. Infect. Dis. **40**(Suppl.):53–57.
222. **Sturm, A. W.** 1989. *Gardnerella vaginalis* in infections of the

urinary tract. J. Infect. **18**:45–49.
223. **Sturm, A. W.** 1989. Chemotaxis inhibition by *Gardnerella vaginalis* and succinate producing vaginal anaerobes: composition of vaginal discharge associated with *G. vaginalis*. Genitourin. Med. **65**:109–112.
224. **Sturm, A. W., J. H. A. de Leeuw, and N. T. C. M. de Pree.** 1983. Post-operative wound infection with *Gardnerella vaginalis*. J. Infect. **7**:264–266.
225. **Sugarman, I. D., L. J. Pead, S. R. Payne, and R. M. Maskell.** 1990. Bacteriological state of the urine in symptom-free adult males. Br. J. Urol. **66**:148–151.
226. **Symonds, J., and A. K. Biswas.** 1986. Amoxycillin, augmentin, and metronidazole in bacterial vaginosis associated with *Gardnerella vaginalis*. Genitourin. Med. **62**:136. (Letter.)
227. **Taylor, E., and I. Phillips.** 1983. The identification of *Gardnerella vaginalis*. J. Med. Microbiol. **16**:83–92.
228. **Taylor-Robinson, D.** 1984. The bacteriology of *Gardnerella vaginalis*. Scand. J. Urol. Nephrol. **86**(Suppl.):41–55.
229. **Thakur, A., P. Bhalla, and D. S. Aggarwal.** 1986. Incidence of *Gardnerella vaginalis* in non-specific vaginitis. Indian J. Med. Res. **83**:567–574.
230. **Thomason, J. L., S. M. Gelbart, R. J. Anderson, A. K. Walt, P. J. Osypowski, and F. F. Broekhuizen.** 1990. Statistical evaluation of diagnostic criteria for bacterial vaginosis. Am. J. Obstet. Gynecol. **162**:155–160.
231. **Tjiam, K. H., J. H. T. Wagenvoort, B. van Klingeren, P. Piot, E. Stolz, and M. F. Michel.** 1986. In vitro activity of the two new 4-quinolones A56619 and A56620 against *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Gardnerella vaginalis*. Eur. J. Clin. Microbiol. **5**:498–501.
232. **Tønjum, T., G. Bukholm, and K. Bøvre.** 1989. Differentiation of some species of *Neisseriaceae* and other bacterial groups by DNA-DNA hybridization. APMIS **97**:395–405.
233. **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.
234. **Vandamme, P., E. Falsen, R. Rossau, B. Hoste, P. Segers, R. Tytgat, and J. De Ley.** 1991. Revision of *Campylobacter*, *Helicobacter*, and *Wolinella* taxonomy: emendation of generic descriptions and proposal of *Arcobacter* gen. nov. Int. J. Syst. Bacteriol. **41**:88–103.
235. **van der Meijden, W. I.** 1984. Clinical aspects of *Gardnerella vaginalis*-assosciated vaginitis. Scand. J. Urol. Nephrol. **86**(Suppl.):135–141.
236. **van der Meijden, W. I., H. Koerten, W. van Mourik, and W. C. de Bruijn.** 1988. Descriptive light and electron microscopy of normal and clue-cell-positive discharge. Gynecol. Obstet. Invest. **25**:47–57.
237. **Vejtorp, M., A. C. Bollerup, L. Vejtorp, E. Fanøe, E. Nathan, A. Reiter, M. E. Andersen, B. Strømsholt, and S. S. Schrøder.** 1988. Bacterial vaginosis: a double-blind randomized trial of the effect of treatment of the sexual partner. Br. J. Obstet. Gynaecol. **95**:920–926.
238. **Venkataramani, T. K., and H. K. Rathbun.** 1976. *Corynebacterium vaginale* (*Hemophilus vaginalis*) bacteremia: clinical study of 29 cases. Johns Hopkins Med. J. **139**:93–97.
239. **Vontver, L. A., and D. A. Eschenbach.** 1981. The role of *Gardnerella vaginalis* in nonspecific vaginitis. Clin. Obstet. Gynecol. **24**:439–460.
240. **Watson, R. A.** 1985. *Gardnerella vaginalis*: genitourinary pathogen in men. Urology **25**:217–222.
241. **Watts, D. H., D. A. Eschenbach, and G. E. Kenny.** 1989. Early postpartum endometritis: the role of bacteria, genital mycoplasmas, and *Chlamydia trachomatis*. Obstet. Gynecol. **73**:52–59.
242. **Weber, P., S. Dubois, and Y. Boussougant.** 1989. In-vitro activity of pristinamycin and its components against Gram-negative anaerobic bacilli and *Gardnerella vaginalis*. J. Antimicrob. Chemother. **23**:825–830.
243. **Wells, J. I., and S. H. Goei.** 1981. Rapid identification of *Corynebacterium vaginale* in non-purulent vaginitis. J. Clin. Pathol. **34**:917–920.
244. **Wilkins, E. G. L., S. R. Payne, P. J. Pead, S. T. Moss, and R. M. Maskell.** 1989. Interstitial cystitis and the urethral syndrome: a possible answer. Br. J. Urol. **64**:39–44.
245. **Wilson, J. A., and A. J. Barratt.** 1986. An unusual case of *Gardnerella vaginalis* septicaemia. Br. Med. J. **293**:309.
246. **Woolfrey, B. F., G. K. Ireland, and R. T. Lally.** 1986. Significance of *Gardnerella vaginalis* in urine cultures. Am. J. Clin. Pathol. **86**:324–329.
247. **Yong, D. C. T., and J. S. Thompson.** 1982. Rapid microbiochemical method for identification of *Gardnerella* (*Haemophilus*) *vaginalis*. J. Clin. Microbiol. **16**:30–33.
248. **Younes, M. S.** 1970. In vitro effects of furazolidone on the fine structure of *Haemophilus vaginalis*. Am. J. Obstet. Gynecol. **108**:210–214.
249. **Zinnemann, K., and G. C. Turner.** 1963. The taxonomic position of "*Haemophilus vaginalis*" [*Corynebacterium vaginale*]. J. Pathol. Bacteriol. **85**:213–219.