CHARACTERISTICS OF A GRAM-NEGATIVE ANAEROBE ISOLATED FROM MEN WITH NON-GONOCOCCAL URETHRITIS

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SUMMARY. A small, fastidious gram-negative anaerobe was isolated from men with non-gonococcal urethritis (NGU). The isolates are described as NGU-associated anaerobes because they were extremely rare in men with urethritis other than NGU, and in asymptomatic men. They showed twitching motility, had many polar pili and appeared to be a homogenous group culturally, morphologically and biochemically. None of the strains fermented or utilised carbohydrates or organic acids as sole sources of carbon for energy and growth. However, growth of all strains was stimulated by formate and fumarate in liquid and solid media, especially in the former where growth seemed dependent on these growth factors. Unlike most anaerobes they produced cytochrome enzyme(s) that might be involved in oxidation-reduction reactions in the presence of oxygen as some of the strains were capable of growing in 5% oxygen. However, growth and energy generally resulted from anaerobic phosphorylation. Strains of this anaerobe seemed to require a low redox-potential (Eh) for survival during transportation but this was not essential for growth. Comparative studies with the other asaccharolytic anaerobes showed some similarity between the NGU-associated anaerobe, *Bacteroides ureolyticus* and *Wolinella succinogenes*. Like these, some NGU-associated strains pitted agar media and all produced urease. However, unlike these anaerobes, strains of the NGU-associated anaerobe produced enzymes for the hydrolysis of arginine, and the decarboxylation of lysine and ornithine. They also produced oxidase and some strains haemolysed sheep red cells. However, lactic acid was not an end-product of the metabolism of glucose by any of the strains. The NGU-associated anaerobes are strikingly different from anaerobic vibrios, *B. praeacutus* and *B. asaccharolyticus*.

INTRODUCTION

Anaerobes are now thought to be implicated in a wide variety of urogenital-tract

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infections in men (Duerden, 1980). They have also been associated, in women, with the so-called "urethral syndrome", also known as dysuria-frequency syndrome (Maskell, Pead and Allen, 1979; Gargan, Brumfitt and Hamilton-Miller, 1980). Although anaerobes form part of the microflora of the male urethra (Sullivan *et al.*, 1972; Gorbach and Bartlett, 1974; Weinberg, 1974; Smith, 1975) and have been isolated from men with non-gonococcal urethritis (NGU) (Hafiz *et al.*, 1975; Bowie *et al.*, 1977; Hallén *et al.*, 1977), they have not been convincingly implicated as a cause of NGU. Recently, however, in a study of anaerobes in the urethra of men with urethritis we have demonstrated a striking association between a small, gram-negative, non-sporing anaerobe and the occurrence of NGU (Fontaine *et al.*, 1982*a* and *b*). These organisms were isolated from 50% of patients with NGU from whom no other known or putative NGU-causing micro-organisms could be isolated, but rarely from patients without NGU. On first isolation these strains appeared to exhibit some synergism with anaerobic gram-positive cocci, produced very small colonies when subcultured on agar media, and grew poorly in liquid media. They were asaccharolytic, rod-shaped, and some strains pitted or corroded agar media. The organisms were seen to have twitching movements in wet preparations examined by dark-field illumination. Because these anaerobes had some unusual features, we compared them with reference strains of possibly related bacteria, namely *Eikenella corrodens*, *Campylobacter* spp., *Wolinella* spp., anaerobic vibrio-like organisms and two asaccharolytic species of *Bacteroides*—*B. ureolyticus* (*corrodens*) and *B. praeacutus*.

In this paper descriptive, morphological, biochemical and phenotypic characteristics of the anaerobes that we have isolated from NGU are presented. For ease of reference, they are termed "NGU-associated" anaerobes. In order to characterise them more fully some of their features are compared with those of other possibly closely-related species.

MATERIALS AND METHODS

Specimen collection, transport medium and transportation have been described previously (Fontaine *et al.*, 1982*b*).

Culture medium. A previously described defined medium of tryptone soya blood agar (TSBA) (Fontaine and Taylor-Robinson, 1981), was modified by omitting the iron suspension and using 2% agar instead of 3%.

Enriched media. Brain heart infusion (BHI) broth, pH 7.2, was supplemented with the 'growth-stimulating' agents sodium formate, sodium fumarate, and potassium nitrate (BHI-FFN). Aqueous, sterile neutralised solutions of formate, fumarate and nitrate were added to the broth to give final concentrations of 0.2%, 0.3% and 0.1%, respectively.

An agar medium was supplemented similarly with these 'growth-stimulating' agents. Its composition was BHI 3.7%, yeast extract 0.5%, haemin 10 µg/ml, vitamin K₁ (3-phytylmenadione) 10 µg/ml, Tween-80 0.1% and agar 2%. This medium, BHIA-FFN, was pre-reduced with cysteine hydrochloride 0.05% and sodium formaldehyde sulfoxylate 0.03% (pH 7.2), and was sterilised by autoclaving.

Anaerobic bacteria. The fastidious gram-negative anaerobe was isolated from the urethra of 32 of 64 men with NGU, one of seven men with gonorrhoea and 4 of 30 men who were asymptomatic at the time of sampling (Fontaine *et al.*, 1982*b*). From some men two swabs were taken and the anaerobe was isolated from both. In all, 64 isolates, henceforth termed strains, were available for further examination. The following anaerobes and other possibly related bacteria were used for comparative study: *B. asaccharolyticus* NCTC9337, *B. praeacutus* NCTC11158, *B. ureolyticus* (laboratory strain), *W. succinogenes* ATCC29543, *E. corrodens* NCTC10596, *C. coli* NCTC11353, *C. fetus* NCTC5850, and *C. jejuni* I and II NCTC nos. 11168

and 11392, and anaerobic vibrio-like organisms or curved rods supplied by various investigators.

Staining and microscopy. Gram-stained smears of bacteria were made with dilute carbol fuchsin as counterstain and examined by light microscopy.

Organisms grown in tryptone-yeast-glucose medium (Fontaine and Taylor-Robinson, 1981), containing formate-fumarate were negatively stained for electron microscopy. Copper grids (400 mesh) with a formvar-carbon film were floated on a drop of the culture for 2 min, then they were floated on a drop of glutaraldehyde 3% in 0.1 M sodium cacodylate buffer (pH 7.4) for 2 min. The grids were blotted, transferred successively to three separate drops of distilled water, dipped in 1% or 0.5% potassium phosphotungstate (pH 6.5), blotted, air-dried and examined in a Philips EM300 electron microscope.

Characterisation of strains

The NGU-associated strains were characterised mainly by conventional tests (Cowan, 1974), modified as indicated.

Cell and colony morphology. Strains were cultivated on Columbia blood agar (CBA), pre-reduced TSBA and BHIA-FFN. The appearance of colonies was recorded and gram-stained smears prepared after incubation for 5 days.

Pigment production. Colonies on BHIA-FFN were examined for pigmentation after incubation for 1–2 weeks. They were also examined for fluorescence under long and short wave ultraviolet light with an ultraviolet lamp (Mineralight; model UV SL–25).

Motility and H₂S production was assessed with Bacto-SIM (sulphide-indole-motility) semi-solid medium (Difco) supplemented with sodium formate 2 g/L and sodium fumarate 3 g/L. After inoculation, the medium was incubated anaerobically at 37°C for 3 days and examined for the spread of organisms from the site of inoculation. H₂S production was indicated by blackening of the medium and was also tested for by the BBL Minitek Microsystem (see below).

Lipase, lecithinase, oxidase and catalase tests. These were performed after growth for 4 days on Willis and Hobbs' medium (Willis and Hobbs, 1959) modified to contain sodium formaldehyde sulfoxylate 0.03%, cysteine hydrochloride 0.1%, haemin 10 µg/ml, vitamin K₁ 10 µg/ml, sodium formate 0.2% and sodium fumarate 0.3%. Solutions of tetramethyl-p-phenylenediamine dihydrochloride 1% and H₂O₂ 6% were used to test for oxidase and catalase, respectively.

Biochemical tests were performed with the BBL Minitek Microsystem (Stargel *et al.*, 1976) for indole, H₂S and urease production, aesculin and arginine hydrolysis, nitrate reduction, lysine and ornithine decarboxylation and fermentation of arabinose, cellobiose, fructose, glucose, galactose, glycerol, glycogen, inositol, lactose, maltose, mannitol, mannose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, starch, sucrose, trehalose and xylose. Organisms from agar cultures were suspended in Minitek medium (10⁷ cfu/ml) supplemented with sodium formate 0.2% and sodium fumarate 0.3%. Three drops (0.06 ml) of this suspension were added to appropriate disks and the results of tests were read after anaerobic incubation at 37°C for 48 h.

Other tests for aesculin hydrolysis and urease production. Organisms were grown at 37°C anaerobically for 3 days in a medium of tryptone 1%, ferric chloride 0.05%, aesculin 0.1%, sodium chloride 0.5%, agar 0.7%, sodium formate 0.2% and sodium fumarate 0.3% (pH 7.2). Hydrolysis was indicated by a blackening of the medium. Agar slopes containing urea (Oxoid) were supplemented with sodium formate 0.2% and sodium fumarate 0.3%, inoculated and incubated anaerobically at 37°C for 3 days. A pink colour indicated urease production.

Dextran hydrolysis. Strains were grown for 4 days in BHI supplemented with Hartley digest broth 2%, yeast extract 0.2%, sodium formate 0.2%, sodium fumarate 0.3%, haemin 10 µg/ml, vitamin K₁ 10 µg/ml, sucrose 5% and dextran (Pharmacia) 0.5%. The hydrolysis of dextran in the medium was indicated by the formation of a slimy mucopolysaccharide.

Gelatin liquefaction was observed in a medium of peptone 0.5%, yeast extract 1%, glucose 0.1%, sodium formate 0.2%, sodium fumarate 0.3%, sodium carbonate 0.05%, cysteine hydrochloride 0.05% and gelatin 12% (pH 7.0). After inoculation and anaerobic incubation at 37°C for 3 days, both inoculated and uninoculated media were placed at 4°C and observed for liquefaction.

Tests for other enzymes. API-ZYM strips (API Laboratory Products Ltd, Grafton Way, Basingstoke, Hants RG22 6HY) were used to test for various constitutive enzymes.

Gas liquid chromatography. Both volatile and non-volatile short chain fatty acid metabolic end-products produced after growth in chopped meat medium (Southern Group Laboratory, Hither Green Hospital, Hither Green Lane, London SE13 6RU) were analysed by gas-liquid chromatography (Holdeman and Moore, 1977). Additionally, isolates were screened for the production of succinic acid from the metabolism of fumaric acid after growth in BHI-FFN. Chromatography was performed with a Pye-Unicam 104 chromatograph with a dual flame ionisation detector fitted with a 5 ft x 4 mm diameter glass column packed with "Gas Chrom Q" treated with phosphoric acid 2% and Carbowax 20 M 10% (Phase Separations Ltd, Deeside Industrial Estate, Queensferry, Clwyd CH5 2LR). Analyses were performed isothermally at an oven temperature of 135°C.

Sensitivity tests. (i) *Antibiotics.* Strains were tested by a disk-diffusion method (Sutter and Finegold, 1971; Leigh and Simmons, 1977) with 'An-ident' multodisks (Oxoid) and with individual sensitivity disks (Oxoid). The 'An-ident' multodisks contained: colistin 10 µg, erythromycin 60 µg, kanamycin 1000 µg, penicillin 2 units, rifampicin 15 µg, and vancomycin 5 µg. The single sensitivity disks contained: cefoxitin 30 µg, clindamycin 10 µg, co-trimoxazole 25 µg, erythromycin 30 µg, gentamicin 30 µg, metronidazole 5 µg, minocycline 30 µg, nalidixic acid 30 µg, neomycin 10 µg, streptomycin 10 µg, sulphafurazole 100 µg, sulphamethoxazole 25 µg and tetracycline 10 µg. The organisms were grown on BHIA-FFN, and the colonies dispersed in phosphate-buffered saline (PBS). This agar medium was seeded by spreading the suspension of organisms uniformly over the medium with an impregnated swab. Zones of inhibition were recorded after anaerobic incubation at 37°C for 3 days. Organisms were considered resistant if colonies developed up to the edge of the disc.

(ii) *Bile salts.* Suspensions of organisms prepared from pure cultures were seeded on BHIA-FFN and tested with Whatman A/A filter-paper disks (Baird and Tatlock) impregnated with sodium taurocholate 2.5%, sodium deoxycholate 0.5%, and a mixture of both (Duerden *et al.*, 1980). Zones of inhibition were recorded after anaerobic incubation at 37°C for 3 days.

(iii) *Dyes.* Suspensions of organisms prepared from pure cultures were seeded on BHIA-FFN and tested with disks impregnated with brilliant green 0.001%, gentian violet 0.001%, and victoria blue 0.001%. The cultures were incubated anaerobically at 37°C for 3 days and examined for zones of inhibition.

Reduction of dyes. Each strain was grown in BHI-FFN containing basic fuchsin 0.005%, methyl violet 0.005% and neutral red 0.01%. These cultures and media containing dyes but no organisms were incubated anaerobically at 37°C and observed for dye reduction.

Carbon utilisation. Twenty-six strains were tested for ability to grow in a carbon-free medium composed of yeast extract 0.02%, sodium chloride 0.2%, calcium chloride 0.02%, magnesium chloride 0.02%, K₂HPO₄ 0.1%, and KH₂PO₄ 0.1% to which were added the following organic compounds as single carbon sources: (i) carbohydrates—arabinose, cellobiose, fructose, glucose, glycogen, inositol, maltose, mannose, ribose, sorbitol and trehalose, all at 0.5%; (ii) sodium salts of organic acids—acetate 0.2%, aspartate 0.002%, butyrate 0.2%, caproate 0.2%, formate 0.2%, fumarate 0.005%, heptanoate 0.2%, lactate 0.2%, malonate 0.005%, oxaloacetate 0.01%, propionate 0.2%, pyruvate 0.2%, and succinate 0.2%; (iii) amino acids—cysteine hydrochloride 0.5%, arginine 0.002%, lysine 0.002%, ornithine 0.005% and tryptophan 0.1%; (iv) alcohols—glycerol 0.2, butanol 0.2% and phenol 0.2%.

RESULTS

Cultural features

Colony morphology. In primary cultures, after incubation at 37°C for 7 days on TSBA, colonies of the gram-negative NGU-associated anaerobe protruded from adjacent colonies of the mixed population. They were translucent, shiny, brownish, pin-point colonies with a flattened periphery and almost imperceptible until viewed

under oblique lighting and at various angles. Prolonged incubation resulted in extension of the peripheral zone. On subculture, colonies varied from 0.4 to 0.8 mm in diameter.

Additional growth factors and morphology. Various growth factors, other than vitamin K₁ and haemin, were incorporated in the medium because of the small size of the colonies. The addition of sodium formate 0.2% and sodium fumarate 0.3% to the medium (BHIA-FFN) changed the morphology of the colonies and increased their size (fig. 1). In primary cultures, colonies appeared light-yellow, umbonate and usually 1–2 mm in diameter. However, on subculture on BHIA-FFN, the colonies appeared grey. They did not fluoresce under UV light.

Growth in liquid media. Growth, as indicated by turbidity, was poor or absent after incubation at 37°C for 2 days. However, it was enhanced by sodium formate 0.2% and sodium fumarate 0.3%, usually proceeding from the bottom of the culture as filamentous tufts, which disrupted on agitation to give appreciable turbidity.

Cell morphology. The shape and size of the gram-negative NGU-associated anaerobic organisms are shown in fig. 2. They were straight to slightly curved rods, 1–6 μ m long and 0.2–0.6 μ m wide with rounded ends.

In negatively stained preparations, the organisms were seen mainly as straight rods with up to 35 polar pili, each measuring 5–10 nm in width (fig. 3).

FIG. 1.—Colonies of NGU-associated anaerobe on pre-reduced selective blood agar medium supplemented with the sodium salts of formate, fumarate and nitrate.

FIG. 2.—Gram-stained preparation ($\times 950$) of the anaerobic bacilli $(0.2-0.6) \times (1-6) \mu\text{m}$ isolated from the urethra of men with non-gonococcal urethritis.

Biochemical and phenotypic features

Biochemical tests. None of the NGU-associated strains utilised any of the carbohydrates tested and they were unable to use organic acids, amino acids, or alcohols as sole carbon sources.

Phenotypic characteristics. Some other characteristics of the NGU-associated strains are shown in table I. Of the 64 strains tested, none produced catalase,

FIG. 3.—Electronmicrograph of negatively stained organism. Note the large number of polar pili. Bar represents 500 nm.

TABLE I
Some phenotypic characteristics of NGU-associated strains

Test	Reaction	Number of strains	
		that gave the indicated reaction	that gave an atypical reaction
Catalase production	—	64	0
Lecithinase production	—	64	0
Lipase production	—	64	0
Urease production	+	64	0
Pigment production	—	64	0
Indole production	—	64	0
Aesculin hydrolysis	—	64	0
Dextran hydrolysis	—	64	0
Milk digestion	—	64	0
Gelatin liquefaction	+	53	2
H ₂ S production	—	36	21
Nitrate reduction	+	57	5

lecithinase, lipase, pigment or indole, hydrolysed aesculin or dextran, or digested milk. Most strains liquefied gelatin and reduced nitrate to nitrite with very little further reduction of the nitrite. They did not produce H₂S but all produced urease.

Characteristics that are helpful in differentiating between NGU-associated strains and the other asaccharolytic anaerobes are shown in table II. Of particular note are multiple polar pili, haemolysis, H₂S production, and decarboxylation of lysine and ornithine. The strains were anaerobic; none grew in air or air enriched with CO₂ 10%, but many were able to grow in the presence of 5% oxygen, or anaerobically at 43°C. In broth and plate cultures, growth was stimulated by formate and fumarate. The strains were quite dissimilar from *B. asaccharolyticus*, *E. corrodens*, anaerobic vibrio-like organisms and the *Campylobacter* spp. studied.

Some of the NGU-associated strains produced a poorly-resolved beta-haemolysis on blood agar containing 5% sheep erythrocytes. Pitting or corroding of the agar was a variable characteristic not seen on primary isolation; only 25% of the NGU-associated strains examined exhibited this feature after several subcultures.

Analysis of broth cultures containing sodium formate and sodium fumarate by gas liquid chromatography showed that large amounts of succinic acid were produced, with little or no acetic acid, and no other short chain fatty acids.

Production of constitutive enzymes. Of the 64 NGU-associated strains examined, all produced esterase and esterase lipase, 62 produced acid and alkaline phosphatases and 54 produced phosphoamidase. About half of the strains produced an arylamidase for cystine, leucine and valine but other enzymes were not produced by most of the strains.

Sensitivity tests. The susceptibility of NGU-associated strains to antibiotics, dyes and bile-salts is shown in table III. All the strains were sensitive to penicillin, neomycin, tetracycline, rifampicin, metronidazole and erythromycin, about half of the strains were sensitive to sulphafurazole and septrin, but most were resistant to vancomycin. Generally, the organisms were intolerant of dyes and were inhibited to some extent by bile salts.

TABLE II
Characteristics of the NGU-associated anaerobe and related organisms

Character	Presence or absence of character in				
	NGU-associated strains	<i>W. succinogenes</i>	<i>B. ureolyticus</i>	<i>B. praeacutus</i>	Anaerobic vibrios
Cell shape	straight to curved (0.2-0.6) × (1-6)	straight to curved (0.5-1) × (2-6)*	straight to curved 0.5 × (2-4)†	straight (0.9-1) × 4-16‡	curved (0.5-1) × (1-4)§
Cell size (µm)	t	+	t	+	+
Motility	-	polar (1)	-	?	sub-polar (2-8)
Flagella	+	-	+ - †	- †	-
Pili	+	-	-	-	-
Growth in air + CO ₂	-	+	-	+	-
Growth in O ₂ 5%	+	+	-	-	-
Growth in air	-	-	-	-	-
Growth stimulated by formate-fumarate	+	+	+	-	-
Haemolysis	β (some)	-	-	-	-
Pitting of agar on primary culture	-	-	+	-	-
Oxidase production	+	-	+	-	-
Urease production	+	-	+	-	-
H ₂ S production	-	+	+	+	-
Arginine dihydrolase	+	-	+	-	-
Lysine decarboxylase	+	-	-	-	-
Ornithine decarboxylase	+	-	-	-	-
GLC: acetic acid	- ^a	- ⁺	- ^a	+	+
lactic acid	-	- ^a	- ^a	+	+
succinic acid	+	+	+	+	+

+ = all strains gave positive result; - = all strains gave negative result; + - = most strains positive; - - = most strains negative; -^a = little or none; t = twitching.

* Tanner *et al.* (1981); † Jackson and Goodman (1978); ‡ Holdeman *et al.* (1977); § Sprott *et al.* (1983).

TABLE III

Susceptibility of NGU-associated strains to antibiotics, dyes and bile-salts

Test	Result	Percentage of strains that gave the indicated result
<i>Antibiotic sensitivity</i>		
Penicillin 2 units	S	100
Neomycin 10 µg	S	100
Tetracycline 10 µg	S	100
Rifampicin 15 µg	S	100
Metronidazole 5 µg	S	100
Erythromycin 30 µg	S	100
Co-trimoxazole 25 µg	S	53
Sulphafurazole 100 µg	S	56
Vancomycin 15 µg	R	94
<i>Resistance to dyes</i>		
Victoria blue	R	100
Gentian violet	R	97
Brilliant green	R	84
<i>Bile salts inhibition</i>		
Taurocholate	S	50
Deoxycholate	R	69

S = sensitive; R = resistant.

DISCUSSION

The small, gram-negative, non-sporing, anaerobic bacilli that we have studied were isolated from the urethra of men with NGU (Fontaine *et al.*, 1982*a* and *b*). They were the predominant organisms among a number of other urethral anaerobes and were isolated more frequently from men with NGU than from symptomless men and are, therefore, called "NGU-associated" anaerobes. In the previous study it was difficult to isolate the anaerobic bacilli from urethral swabs transported in anaerobic liquid transport medium with a redox-potential (Eh) of about -150 mV (pH 7.0). However, recovery was enhanced by reducing the Eh to between -300 and -400 mV with titanium trichloride-citrate complex. Although the organisms seemed to require a low Eh for survival in transport medium, it was not a prerequisite for growth because it was later found that some strains could grow in O₂ 5%. First attempts at isolation on pre-reduced blood agar produced colonies that were almost imperceptible and apparently in symbiosis with those of anaerobic gram-positive cocci, identified as *Gaffkya anaerobia*. Even so, it was possible to recover the NGU-associated anaerobe in pure culture by subculture on to solid media, although growth was poor or non-existent in liquid media. In view of this and because the organisms were asaccharolytic with straight to curved rod morphology, it was thought that they might be related to *Vibrio succinogenes*, now renamed *Wolinella succinogenes* (Wolin, Wolin and Jacobs, 1961; Niederman and Wolin, 1972; Smibert and Holdeman, 1976; Tanner *et al.*, 1981). Therefore, we investigated whether the NGU-associated organisms had a formate-fumarate or nitrate energy metabolism and included these substrates in both liquid and agar media. Growth was greatly enhanced, particularly in liquid medium. Moreover, the symbiosis exhibited on original isolation on agar medium was less

apparent. The addition of formate and fumarate either separately or as a mixture resulted in larger, umbonate, light-grey colonies instead of pinpoint brownish colonies.

No outstanding differences in morphology were found among the 64 strains examined. Slight differences in cell length or colony size were probably due to the presence or absence of the growth factors. We believe that the NGU-associated strains probably form a homogenous group of anaerobes which display twitching motility by virtue of their multiple polar pili.

In an attempt to establish the identity of the NGU-associated strains, their characteristics were compared with those of four other asaccharolytic anaerobes—*B. ureolyticus*, *B. praeacutus*, *W. succinogenes* and anaerobic vibrios—taking into account our observations and some published ones. All except *B. ureolyticus* exhibited classical motility. Some strains of the latter had twitching motility and polar pili, features similar to those of the NGU-associated strains. Morphologically, the cells of *B. ureolyticus* and *W. succinogenes* were similar to those of the NGU-associated strains. All the microorganisms were anaerobic although most strains of the NGU-associated anaerobe, like those of *W. succinogenes*, were tolerant of O₂ 5%. In contrast, *B. ureolyticus* tolerated O₂ 1% only. Formate and fumarate stimulated the growth of all the anaerobes except *B. praeacutus* and the anaerobic vibrios on solid medium, although some workers have reported enhancement of growth of the anaerobic vibrios in broth (Holst, Skarin and Mårdh, 1982).

Strains of the asaccharolytic anaerobes, apart from the NGU-associated strains, did not produce haemolysis in blood agar. The poorly-resolved beta-haemolysis of the latter may be due to lipolytic enzymes rather than a haemolysin. This is suggested also by the production of esterases by the NGU-associated strains. Pitting or corroding of the agar occurred with some of the NGU-associated strains but was not a distinguishing feature. However, the gas liquid chromatographic profiles were helpful because lactic acid was produced by all anaerobes except the NGU-associated strains. An even more striking and distinguishing characteristic of the NGU-associated strains, but not of the other anaerobes, was the production of enzymes for arginine hydrolysis and the decarboxylation of lysine and ornithine.

Several workers have recently reported the isolation of comma-shaped anaerobic bacteria from women with non-specific vaginitis (NSV) (Hjelm *et al.*, 1981; Holst *et al.*, 1982; Phillips and Taylor, 1982; Sprott *et al.*, 1983). These organisms are apparently similar to those described much earlier by Curtis (1913). We obtained strains from each of the aforementioned workers for comparative purposes and the NGU-associated anaerobes are clearly different morphologically, culturally and biochemically. Interestingly, we have not been able to isolate anaerobic curved rods from men with NGU. However, curved and semi-circular gram-variable facultative rods have been observed. These may or may not be one of the variants isolated from NSV but were probably corynebacteria.

In view of the possible transfer of organisms between sexual partners, we cannot as yet explain the absence of 'vaginitis' organisms from the male urethra. However, we have isolated organisms morphologically similar to the NGU-associated anaerobe from women with lower genital-tract infections. Preliminary results obtained from testing vaginal specimens of six women with NSV on a selective medium, have demonstrated that five harboured this anaerobe (unpublished data). This observation is interesting in view of recent reports on the putative or likely causes of NSV. To our

knowledge no one has reported an association between this disease and the organism we have just described. However, it was apparent from these results that the isolation of the NGU-associated anaerobe in NSV may be even higher than in NGU. Further studies are continuing to determine the taxonomic status of the NGU-associated anaerobe, the prevalence of these and anaerobic vibrios in both men and women with lower genital-tract infections and whether the NGU-associated anaerobes have a significant role in the pathogenesis of NGU.

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