The clinical importance of gram-positive anaerobic cocci isolated at St Bartholomew's Hospital, London, in 1987

D. A. MURDOCH*, I. J. MITCHELMORE and S. TABAQCHALI

Department of Microbiology, St Bartholomew's Hospital, London EC1A 7BE

Summary. The clinical importance of the gram-positive anaerobic cocci (GPAC) isolated in 1987 at St Bartholomew's Hospital, London, is assessed. Of about 800 anaerobic isolates, 209 (27%) were GPAC, of which 67 (32%) were from abscesses and 22 (11%) were in pure growth. Four species comprised 77% of the 168 isolates available for study: *Peptostreptococcus magnus* (55 isolates, 33%), *P. micros* (23, 14%), *P. asaccharolyticus* (24, 14%) and *P. anaerobius* (27, 16%). Different species were associated with different sites, from *P. magnus* (usually skin-associated sites; normally cultured with aerobes, infrequently with other anaerobes), *P. asaccharolyticus* (distributed widely) and *P. anaerobius* (usually genitourinary and gastrointestinal; always below the diaphragm) to *P. micros* (always deep sites with other anaerobes). *P. magnus* was isolated from 15 abscesses and was obtained in pure culture from 11 specimens, six of them abscesses developing from infected sebaceous cysts. *P. micros* was usually isolated from soft tissue abscesses, never from the skin, and with a characteristic mixed flora consisting of "*Streptococcus milleri*" and anaerobic gram-negative rods. *P. heliotrinreducens* was a rare isolate from similar specimens. *P. asaccharolyticus* was cultured from a wide variety of sites, typically mixed with both aerobes and anaerobes, and frequently from abscesses. Most isolates of *P. anaerobius* came from gastrointestinal or female genitourinary specimens, never from above the diaphragm and rarely from the skin; cultures were usually heavily mixed. Isolates of *P. vaginalis* and the "bGAL" group made up 11% of strains and were usually cultured from superficial sites, *P. vaginalis* often from post-operative wound infections with *Staphylococcus aureus*. There were only two isolates of *P. hydrogenalis*, three of *P. tetradius* and none of *P. barnesae*, *P. prevotii*, *P. lacrimalis*, *P. lactolyticus*, *P. productus* or *Peptococcus niger*. GPAC are a heterogeneous group associated with a wide variety of infections, particularly abscesses, and are frequently isolated in pure culture. They deserve further study.

Introduction

Gram-positive anaerobic cocci (GPAC), placed in the genera *Peptostreptococcus* and *Peptococcus*, are neglected but important human pathogens. They are the second commonest group of anaerobes isolated from clinical sources, consistently accounting for 20–40% of all anaerobes.^{1–5} However, little is known about the relative importance of individual species because the classification is unsatisfactory^{6–10} and laboratory identification has been unreliable.^{3, 11–13} Two recent comparative surveys^{3, 4} used identification systems relying heavily on carbohydrate fermentation tests;^{3, 8, 13} unfortunately, most species of GPAC are asaccharolytic,^{8, 10, 14} so that differentiation has depended largely on negative results. Thus, cell size is the method usually recommended^{3, 10, 12, 13} to distinguish between two of the most important species, *Pepto-*

streptococcus magnus and *P. micros*. Five new species have been described recently,^{15–17} but there are few data yet on their identification or clinical importance.

The problems with laboratory identification have been lessened by the introduction of pre-formed enzyme kits,^{10, 18–20} based on tests for the presence of saccharolytic and proteolytic enzymes. These kits were used to characterise a collection of 256 clinical strains, isolated in 1986–1987 at St Bartholomew's Hospital, London.²¹ Of these strains, 246 (96%) were placed in 12 enzyme groups. There was complete agreement between grouping with pre-formed enzyme kits and the results from volatile fatty acid (VFA) analysis by gas-liquid chromatography (GLC). A detailed analysis of the clinical significance of the 209 isolates of GPAC from 1987 described in that report is now presented.

Materials and methods

Isolation of anaerobes from patient samples

Specimens of pus, purulent fluids and antral wash-

Received 24 Aug. 1993; revised version accepted 15 Jan. 1994.

* Present address and address for correspondence: Department of Microbiology, Southmead Hospital, Bristol BS10 5NB.

outs for anaerobic culture were inoculated on to Brucella Agar (Difco), with defibrinated horse blood (Oxoid) 6%, haemin (Sigma) 5 mg/L and vitamin K1 (Roche) 1 mg/L; a second plate with neomycin (Wellcome) 100 mg/L was also inoculated. Part of the specimen was inoculated into Robertson's Cooked Meat Broth (Southern Group Labs, London), which was subcultured at 48 h on to the same media. Cultures were inoculated in air and incubated anaerobically in an atmosphere of N₂ 80%, H₂ 10% and CO₂ 10% for 10 days. The anaerobic chamber used was home-made until August 1987, when a Mark III cabinet (Don Whitley Scientific, Shipley, W Yorks) was brought into use. All anaerobes isolated from these specimens were investigated further. Aerobic cultures were inoculated on to Brucella Agar as above, MacConkey Agar (Oxoid) and, for head and neck specimens, Chocolate Agar (Tissue Culture Services, Botolph Claydon, Bucks). Aerobic cultures were incubated in air + CO₂ 5% at 37°C for 48 h.

Other specimens were inoculated on to pre-poured Blood Agar (Tissue Culture Services) and MacConkey agar, and incubated in air + CO₂ and anaerobically, as above, for 48 h, with neomycin and chocolate plates if appropriate. Anaerobic plates from these specimens were incubated in jars equipped with palladium catalysts and Gaspaks (Becton Dickinson, Oxford). Anaerobes isolated from these specimens were recorded and saved for further study only if they were in heavy growth and reasonably pure.

Identification of GPAC

Strains of GPAC were identified as described previously;²¹ briefly, the techniques used were the gram-stained film and colonial morphology, the detection of VFA production by GLC, the pre-formed enzyme profile obtained with the ATB 32A kit (bioMérieux, Basingstoke, Hants), and the antibiotic sensitivity pattern. A Pye-Unicam series 104 gas chromatograph was used for the detection of VFA. Other anaerobes were named and identified according to Holdeman Moore *et al.*⁸ and the API 20A system (bioMérieux).

Source of clinical information

The St Bartholomew's Hospital computerised data base was used to list all anaerobic isolates from 1986–1987. Limited information was available from patient request forms; this was supplemented where appropriate by consulting the patients' notes.

Results

In 1986–1987, 440 strains of GPAC were isolated at St Bartholomew's Hospital; there were 598 strains of gram-negative anaerobic rods, 344 strains of *Clostridium* spp. (67% of which were faecal isolates of *C. difficile*) and 18 strains of *Veillonella* spp. (table I). Of

the GPAC isolated in 1986, only 88 (38%) were still available for study, compared to 168 (80%) of 209 in 1987. Because of the large difference, this report is based on the 1987 strains, although 1986 strains are discussed briefly for rare groups such as *P. hydrogenalis*. Five isolates from 1987 were no longer viable and 36 had not been stored. Data on the other micro-organisms with which 1987 isolates were cultured were available in all but four cases (2%), but there was no additional clinical information available for another 12 (6%) isolates. Of the 168 available strains, 88 came from pus and other fluids, and 80 from other specimens; 55 (33%) were identified as *P. magnus*, 23 (14%) as *P. micros*, 24 (14%) as *P. asaccharolyticus* and 27 (16%) as *P. anaerobius*. These four species represented 77% of all available strains (table II).

Analysis by site of specimen

Superficial sites (table III) yielded 71 isolates (33% of total): 34 from the upper body (face, neck, breast, trunk, axilla and umbilicus), 24 from superficial pelvic sites and 13 from the leg. Most were identified as *P. magnus* (28 of the 59 isolates available for study, 48%). Of the 34 isolates from superficial sites on the upper body, 20 (59%) were from abscesses (including 10 *P. magnus* and five *P. asaccharolyticus* strains; table IV). The pelvic strains were isolated from the perineum, superficial groin or thigh wounds and pressure sores, and were frequently accompanied by a heavy mixed culture suggestive of faecal contamination; only six (25%) were from abscesses. The superficial sites on the leg were mostly ulcers or infected post-surgical wounds; none was an abscess. Thirteen isolates from superficial sites were cultured pure, of which eight (including six *P. magnus*) were from infected sebaceous cysts on the upper body and five (including three *P. magnus*) were from post-operative wounds. *Staphylococcus aureus* was cultured from nine (69%) of 13 specimens from the leg but from only six (18%) of the 34 specimens from the upper body. There were only

Table I. Comparison of groups of anaerobic bacteria isolated at St Bartholomew's Hospital 1986–1987

Anaerobic groups	Number of isolates in		
	1986	1987	Total
Gram-negative rods			
<i>Bacteroides</i> *	197	283	480
<i>Fusobacterium</i>	22	46	68
Unidentified	46	4	50
Total	265	333	598
<i>Veillonella</i> spp.	6	12	18
<i>Clostridium</i> spp.			
faecal	69	164	233
other sites	47	64	111
Total	116	228	344
GPAC	231	209	440

* The genus *Bacteroides* is defined according to the 2nd edition of Bergey's Manual of Systematic Bacteriology, in use when these isolates were identified.

Table II. Number of isolates of GPAC and organisms with which they were cultured

Species	Number (%) of isolates of GPAC		Number (%) of specimens from which associated organisms were isolated			
	Total	Isolated pure	Aerobic/facultative†	<i>S. aureus</i>	" <i>Str. milleri</i> "	Obligate anaerobes
VFA-negative group						
<i>P. magnus</i>	55	11 (20)	42 (76)	12 (22)	2 (4)	19 (35)
<i>P. micros</i>	23	0	12 (55)	2 (9)	8 (36)	22 (96)
<i>P. heliotrinreducens</i>	5	0	2 (40)	0	2 (40)	5 (100)
Unidentified	1	0	1	0	0	1
Butyric group						
<i>P. asaccharolyticus</i>	24	3 (12)	21 (87)	3 (12)	2 (8)	19 (79)
<i>P. indolicus</i>	1	1	0	0	0	0
<i>P. tetradius</i>	3	0	3	0	0	3
<i>P. vaginalis</i>	10	1 (10)	7 (70)	5 (50)	0	1 (10)
"bGAL" group	8	0	5 (63)	1 (12)	0	6 (75)
<i>P. hydrogenalis</i>	2	0	1	0	0	1
Other	6	0	5	2	0	5
Isovaleric group						
"Ivoricus" group	1	0	1	0	0	1
Isocaproic group						
<i>P. anaerobius</i>	27	1 (4)	19 (73)	4 (15)	3 (11)	20 (77)
Caproic group						
Hare group VIII	2	0	2	0	0	1
Total	168					

* The numbers in brackets refer to the percentage of all isolates of that species.

† The isolates of "*Str. milleri*" were not included in "aerobic/facultative organisms".**Table III.** Isolation of GPAC from selected superficial sites

Species	Number of isolates from							Total (%)
	face and neck	axilla	breast	skin of trunk	umbilical	superficial pelvic	superficial leg	
<i>P. magnus</i>	4	3	1	3	4	8	5	28 (51)*
<i>P. micros</i>								0
<i>P. heliotrinreducens</i>				1				1 (20)
<i>P. asaccharolyticus</i>	2		2	1		4		9 (38)
<i>P. vaginalis</i>				1		2	3	6 (60)
"bGAL" group			2	1		3		6 (75)
<i>P. hydrogenalis</i>				2				2 (100)
Other butyrate-producing					1	1	1	3
<i>P. anaerobius</i>					1	2	1	4 (15)
Unsaved strains	1	1	1	2		4	3	12
Total	7	4	6	11	6	24	13	71

* Number in brackets refers to the percentage of all isolates of that species.

four isolates of *P. anaerobius* and none of *P. micros* from skin-associated sites.

Deep sites from above the diaphragm (table V) yielded 35 strains of GPAC (17%); the dominant species at most sites was *P. micros*, which accounted for 15 (47%) of the 32 available strains. Both brain abscesses yielded a mixed growth of *P. micros*, "*Streptococcus milleri*", a *Fusobacterium* sp. and a *Bacteroides* sp. Strains of *P. micros* were also isolated from four peritonsillar abscesses and one abscess in the roof of the mouth, with streptococci and other anaerobes (*Fusobacterium* spp., four strains; *Bacteroides* spp., five strains) in all cases. Apart from one strain of *P. heliotrinreducens* from a tooth abscess, *P. micros* was the only GPAC isolated from the mouth. Antral washouts from 23 cases of sinusitis, which were

usually chronic and purulent, yielded GPAC, notably *P. magnus* (10 isolates), *P. micros* (six isolates) and the only two strains of Hare group VIII; a wide range of organisms other than GPAC was also cultured. Other anaerobes were always present if *P. micros* was isolated but they were cultured from only two of 10 specimens with *P. magnus*. Four pleural empyemas yielded two strains of *P. micros* (both with fusobacteria and other anaerobes), one strain of *P. heliotrinreducens* (with "*Str. milleri*" and two *Bacteroides* spp.) and one of *P. vaginalis* (with *Pseudomonas aeruginosa*). There were no pure cultures of GPAC from deep sites above the diaphragm.

Abdominal and anorectal specimens yielded 38 strains (18%, table V), 23 of them from abscesses. Most groups of GPAC were represented. *P. anaerobius* was

Table IV. Isolation of GPAC from abscesses

Species	Number of isolates from abscesses of											Total (%)
	brain	intra-oral	skin of upper body	pleural empyema	breast	abdomino-pelvic	anorectal	thigh/groin/pilonidal	scrotum	Bartholin's gland	unknown	
<i>P. magnus</i>			9		1		3	1	1			15 (27)*
<i>P. micros</i>	2	5		2		2	2					13 (57)
<i>P. heliotrinreducens</i>		1	1	1		1	1					5 (100)
<i>P. asaccharolyticus</i>			3		2	3	1	1				10 (42)
<i>P. indolicus</i>											1	1 (100)
<i>P. vaginalis</i>				1								1 (10)
"bGAL" group					1			1				2 (25)
Other butyrate-producing								1				1
<i>P. tetradius</i> / <i>P. prevotii</i>												0
<i>P. anaerobius</i>						3	6		1	1		11 (37)
Unsaved strains			2		1		1	2	1	1		8
Total	2	6	15	4	5	9	14	6	3	2	1	67 (32)

* Number in brackets refers to the percentage of all isolates of that species.

Table V. Isolation of GPAC from selected deep sites

Species	Number of isolates from							Total (%)
	brain abscess	intra-nasal	intra-oral	pleural empyema	abdomino-pelvic abscess	anorectal abscess	female genitourinary infection	
<i>P. magnus</i>		10			5	3	3	21 (38)*
<i>P. micros</i>	2	6	5	2	2	2	3	22 (96)
<i>P. heliotrinreducens</i>			1	1	1	1		4 (80)
<i>P. asaccharolyticus</i>		2			3	1	5	11 (46)
<i>P. tetradius</i>							3	3 (100)
<i>P. vaginalis</i>				1				1 (10)
"bGAL" group					1		1	2 (25)
Other butyrate-producing					1	1	1	3
<i>P. anaerobius</i>					5	6	9	20 (74)
Hare group VIII		2						2 (100)
Unsaved		3			3	3	6	15
Total	2	23	6	4	21	17	31	104

* Number in brackets refers to the percentage of all isolates of that species.

the commonest species, accounting for 11 (34%) of the 32 available strains, but, like other GPAC from these sites, it was almost always associated with a heavy mixed growth of other organisms. Post-operative wound infections of the abdominal wall yielded four of the eight isolates of *P. magnus* (two in pure growth), but twice *P. magnus* was cultured with faecal flora from anorectal abscesses. A primary ischiorectal abscess in a previously well 27-year-old woman yielded an isolate of *P. asaccharolyticus* in pure growth.

Genitourinary tract specimens yielded 40 (19%) isolates. Of the nine specimens from males, one strain of *P. magnus* (in pure culture) and one of *P. anaerobius* (with a *Bacteroides* sp.) were from scrotal abscesses. Five strains from semen probably reflect the normal flora of the distal urethra. Of the 31 strains from women (table V), *P. anaerobius* (nine of the 25 available strains, 36%) was again the commonest species but was not associated with any specific clinical condition. The placenta of a case of intra-uterine death

yielded an isolate of *P. magnus* in pure culture. Two Bartholin's abscesses yielded a pure growth of *P. anaerobius* and a pure growth of an organism that died on subculture. The remaining strains included most groups of GPAC in association with vaginal flora. The variety of organisms isolated from IUCDs suggests that the specimen was often contaminated with vaginal flora, before or during removal.

Other sites. A strain of *P. magnus* was isolated with a coagulase-negative staphylococcus from an infected aortic graft; an infected femorofemoral graft yielded an unidentified organism similar to *P. micros* in association with *Porphyromonas asaccharolytica* and *S. epidermidis*. In 1987, there were no isolates of GPAC from blood and only two from musculoskeletal sites (one *P. anaerobius*, one unsaved), both mixed growths from cases of chronic osteomyelitis. However, in 1986, specimens from joints yielded three isolates of *P. magnus* (septic bursitis of the knee, with *S. aureus*; septic effusion of the elbow, with a coagulase-negative

staphylococcus; and an indolent tibial sinus, with a coliform and an *Enterococcus* sp.) and three isolates which were not available for study.

Analysis by species of GPAC

P. magnus was much the commonest species of GPAC, accounting for 55 isolates—33% of those available for identification and *c.* 7% of all anaerobes isolated at St Bartholomew's Hospital in 1987. It was the commonest GPAC in superficial sites (48% of the isolates available for identification) and antral wash-outs (50%) but was relatively scarce in specimens from the abdomen and gastrointestinal tract (25%) and the female genitourinary tract (12%). It was the most frequent organism in abscesses (table IV) but it was recorded proportionally less often than several other species. Most of the abscesses, and nine of the 11 specimens from which it was cultured pure, were from superficial sites; 76% of isolates were cultured with aerobic or facultative organisms but only 35% with other anaerobes (table II).

Twenty-three strains of *P. micros* were isolated, 15 (65%) of them from deep sites above the diaphragm; it was never cultured from superficial sites. *P. micros* was strongly associated with soft tissue abscesses (13 isolates, 57%, including both brain abscesses, all four peritonsillar abscesses, two of the four pleural empyemas and two anorectal abscesses) but it was also cultured from three IUCDs and six purulent specimens from cases of chronic sinusitis. Unlike *P. magnus*, it was isolated with aerobic or facultative organisms in only 55% of specimens but it was invariably cultured with other anaerobes; it was frequently associated with a distinctive abscess-type flora, typically streptococci (11 isolates, eight of them "*Str. milleri*"), *Bacteroides* spp. (18 isolates) and *Fusobacterium* spp. (15 isolates). It was cultured less often with *S. aureus* (two isolates) than it was with *Veillonella* spp. (three isolates). The five isolates of *P. heliotrinreducens* were all from polymicrobial abscesses but from a wide variety of sites: a superficial skin abscess in a diabetic, a pleural empyema, and tooth, abdominal wall and perianal abscesses. The abscess flora was remarkably similar to that found with *P. micros*: "*Str. milleri*" (two isolates), *Bacteroides* spp. (nine isolates; all five specimens), *Fusobacterium* spp. (two isolates) and coliforms (two isolates).

Butyrate-producing GPAC were often very difficult to characterise satisfactorily.²¹ However, 24 isolates were identified as *P. asaccharolyticus*. This species was not associated with any particular disease process or anatomical site; nine isolates were from superficial sites, four from abdominal specimens and five from the female genitourinary tract. It was cultured from 10 abscesses, two in pure growth—one abscess developing from a submandibular sebaceous cyst and the ischio-rectal abscess mentioned above. It was also the only isolate from a urethral swab in a case of urethritis, although its significance is questionable. It was usually

isolated with aerobic or facultative organisms (87% of specimens) and other anaerobes (79%).

Of the other recognised butyrate-producing species, *P. indolicus*, a species of veterinary importance,^{22, 23} was cultured on only one occasion, in pure growth from a superficial abscess. Regrettably, full clinical information was not available. The sole isolate from 1986 was cultured from a perineal abscess with faecal flora. There were only three isolates of *P. tetradius*, all from vaginal specimens, and none of *P. prevotii*. Of the five newly described species,^{16, 17} two correspond to previously characterised groups, Hare group III with *P. hydrogenalis*^{21, 24} and the "ADH" group with *P. vaginalis*.^{19, 21} The two isolates of *P. hydrogenalis* came from a purulent sternotomy wound (with *Escherichia coli*) and an infected pacemaker site (with a "bGAL" group strain of GPAC). The five isolates of *P. hydrogenalis* in 1986 were also cultured from superficial sites (long-standing foot ulcer; preputial sac; labial cyst; mastoidectomy wound; umbilical swab). Ten isolates (6% of those available for study) were identified as *P. vaginalis*. Five were from post-surgical wound infections (two from the leg, two inguinal and one from the trunk), of which three were cultured with *S. aureus* and one was pure (from an infected skin graft on the leg). One was isolated from a pleural empyema with *Ps. aeruginosa*. The clinical information on the other four strains was, unfortunately, very incomplete, although two were cultured with *S. aureus* from the foot. Of the eight isolates placed in the "bGAL" group, two came from skin-related abscesses (of the breast and inguinal region) and four others from other superficial sites—a fungating carcinoma of the breast, an infected post-cardiac thigh wound, the pacemaker site mentioned above and tissue from a bed sore. Isolates of *P. vaginalis* were usually cultured with aerobes rather than with other anaerobes but "bGAL" group strains were associated with both aerobes and anaerobes (table II). The other six butyrate-producing isolates were heterogeneous. One, with a *Bacteroides* sp., came from a primary thigh abscess in a diabetic.

The distribution of *P. anaerobius* (27 isolates) was unique: it was not cultured from above the diaphragm but it was the commonest GPAC in gastrointestinal specimens (11 of the 32 available isolates, 35%) and the female genitourinary tract (nine of 25, 36%). Only four isolates (15%) were from superficial sites. Most isolates were cultured with aerobic or facultative organisms and anaerobes in a heavily mixed faecal-type flora. Eleven isolates were from abscesses, nine of them intra-abdominal or anorectal, which were also usually heavily mixed; the isolate from a Bartholin's abscess was the only pure culture. The one isolate of the "ivoricus" group in 1987 was from normal skin flora. The isolates from 1986 were from an IUCD, an infected leg wound (in heavily mixed culture) and the preputial sac of a case of balanitis. Both isolates of Hare group VIII²⁴ in 1987 were in mixed culture from antral washouts from cases of chronic sinusitis; there

was none in 1986. There were no isolates of *P. barnesae*, *P. productus*, *P. lacrimalis*, *P. lactolyticus* or *Peptococcus niger* in 1986 or 1987, nor of *Str. parvulus*,²⁵ which has recently been placed in the genus *Atopobium*.²⁶

Discussion

A standard text²⁷ recently stated that "the need for determining [the identity of] species [of GPAC] is often questioned since differences in pathogenic potential for the various anaerobic gram-positive cocci are not well defined". This report shows clearly that there are major differences, in the frequency with which they are isolated, the sites from which they are cultured and the organisms with which they are associated. However, this survey has major limitations: firstly, it is a retrospective analysis—some of the clinical information is incomplete and 20% of the GPAC isolated in 1987 were not available for study; secondly, the patient population at St Bartholomew's Hospital is not typical, as there were few orthopaedic and no obstetric specimens. This is unfortunate as *P. magnus* is recognised as a significant cause of septic arthritis, usually in the presence of joint prostheses,^{28,29} and because the original descriptions of GPAC as human pathogens (in the guise of "anaerobic streptococci") came from cases of puerperal sepsis.^{30,31} Fortunately, this condition is now very rare in developed countries, although it may not be in the developing world.³² Regrettably, the placental specimen from which *P. magnus* was obtained in pure culture was sent from another hospital and we could not obtain more complete information on the cause of intra-uterine death. The collection and transport of specimens were not as rigorous as in some surveys, such as that of Brook;⁴ nevertheless, some extremely fastidious species, e.g., *P. heliotrinreducens*, were cultured successfully.

With these reservations, over 200 strains of GPAC were isolated in this laboratory in 1987—almost one per working day. They were cultured in pure growth from 22 specimens (11% of total), almost always in situations where they were of clinical significance; 67 (32%) isolates were from abscesses. These findings agree closely with other surveys.¹⁻⁴ *P. magnus* stands out as the most pathogenic species of GPAC. It was much the commonest species overall and by far the commonest species isolated in pure culture; it was associated particularly with abscesses arising from sebaceous cysts and superficial wound infections. It was the second commonest of all anaerobes in four major surveys, those of Wren *et al.*,¹ Holland *et al.*,² Rosenblatt³ and Brook,⁴ in which it accounted for 6%, 12%, 13% and 5% of all anaerobic isolates respectively, compared to 7% in this survey. Sanderson,³³ using cases from Wren's survey, described three diabetics with infections of the foot in which *P. magnus* was cultured pure. It was isolated from 15–20% of diabetic foot infections (abscesses, osteomyelitis and

necrosis) by Wheat *et al.*³⁴ The retrospective study of Bourgault *et al.*²⁸ documented 222 patients from whom *P. magnus* was isolated, in pure culture in 32 cases (12%); it was the commonest species of GPAC isolated over a 3-year period and was particularly associated with soft tissue and bone and joint infections. It was more usually isolated with aerobes or facultative organisms than with other anaerobes, but, in contrast to this report, only one pure culture from a sebaceous abscess was documented. Some of these cases were analysed further in a series by Fitzgerald *et al.*,²⁹ in which *P. magnus* was the commonest anaerobe in cases of post-traumatic and post-operative anaerobic septic arthritis; there are similar reports, usually associated with the presence of prosthetic implants.^{35,36} Brook⁴ associated it with bone and chest infections. There are also case reports of fatal endocarditis,³⁷ paravalvular abscess round a bio-prosthetic aortic valve,³⁸ purulent pericarditis and mediastinitis,³⁹ and anaerobic necrotising pneumonia complicated by pyopneumothorax.⁴⁰ These reports illustrate the range of serious and potentially fatal infections which *P. magnus* can cause; most can be linked to the skin as the source of the organism. However, the identification methods used in some of these cases are unclear or open to criticism. Recently, Edmiston *et al.*⁴¹ reported that *P. magnus* was the commonest organism cultured from a series of 52 non-puerperal breast infections; GPAC constituted 49% of all isolates and predominated in both acute and chronic cases. *P. magnus* is undoubtedly one of the commonest anaerobic pathogens; its importance, particularly in the development of post-operative wound infections and superficial soft tissue infections, deserves much fuller study.

The pathogenic potential of other species of GPAC is very poorly understood, mainly because of problems with identification, but also because most isolates come from mixed cultures, often from superficial sites where they could be part of the normal commensal flora. Therefore, it is very often difficult to know whether they are part of the disease process or are just "fellow travellers". *P. micros* is a good example. This species accounted for only 4% of all GPAC in Brook's survey⁴ but 9% in Rosenblatt's;³ it has also been implicated in a case of vertebral osteomyelitis,⁴² four cases of post-operative septic arthritis,²⁹ nine of non-puerperal breast infection,⁴¹ five of bacteraemia⁴³ and four diabetic foot infections.³⁴ In this survey, it was the only GPAC isolated from peritonsillar abscesses and it may indeed be the predominant GPAC in oral pathology: it was the commonest organism (17% of total) in a study of endodontic abscesses⁴⁴ and has been associated with progressive periodontitis.⁴⁵ However, these figures would presumably include *P. heliotrinreducens*, which can best be differentiated by its pre-formed enzyme profile.^{21,46} *P. micros* is a slow-growing organism that is unlikely to be cultured successfully unless grown under strictly anaerobic conditions. We found that familiarity with the highly

distinctive colonial morphology^{21, 47} led to a steadily increasing isolation rate, from 4.5% of all GPAC isolated in 1986 to 10% in the first half of 1987 and 19% in the second half. We believe that isolates of *P. micros* are often missed. This is even more true of *P. heliotrinreducens*, of which the colonies are less distinctive and take even longer to grow.⁴⁶ In this survey, the natural history of these organisms was remarkably similar—both were isolated typically from polymicrobial abscesses in association with a distinctive group of other anaerobes. Are they just “passengers” that manage to colonise the nutritive environment of an abscess after, for instance, isolates of “*Str. milleri*” have done the initial damage? Or do they contribute greatly to abscess formation? In a polymicrobial specimen, it is very difficult to know. Certainly, in pre-formed enzyme kits, both species are highly proteolytic—more so than any other species of GPAC. In a subcutaneous abscess model in the mouse,⁴⁸ abscesses were formed more easily by combinations of GPAC and facultative organisms than by GPAC alone. We suggest that with both of these species, we are seeing only “the tip of the iceberg”; it is natural to perform more thorough microbiology on important specimens such as abscesses, particularly from sites such as the brain or pleural cavity. These organisms may be present quite regularly in a wide variety of specimens, but die during transport, or be overgrown by more rapidly growing bacteria such as coliforms; they may not be visible before cultures are discarded, or they are just not recognised.

The position of *P. asaccharolyticus* is less clear-cut, both taxonomically and clinically. It accounted for 14% of all available isolates and 17% of those from abscesses, and was cultured pure in three instances. However, unlike the other three major species, it was not associated with any particular site or infection. Organisms identified as *P. asaccharolyticus* accounted for 21% of GPAC in Rosenblatt's series³ and 18% of Brook's,⁴ and have been isolated from two paediatric intracranial abscesses,⁴⁹ five cases of bacteraemia,⁴³ 12 diabetic foot infections,³⁴ 12 breast infections⁴² and one peritonsillar abscess.⁵⁰ Standard identification manuals^{3, 8, 12} have characterised all indole-positive, butyrate-producing GPAC as *P. asaccharolyticus* or *P. indolicus*, but this group is more heterogeneous.^{6, 10, 19–21} Another indole-positive species, *P. hydrogenalis*, has recently been described¹⁶ and a few isolates of *P. vaginalis* produce indole.²¹ There is strong genetic and phenetic evidence that *P. asaccharolyticus* as currently defined⁸ is not a homogeneous species^{6, 10, 21} and may include indole-negative strains.^{21, 51} Seven (29%) isolates in this survey were provisionally placed in a different group B,²¹ of which three were isolated from superficial abscesses (of the face, neck and breast), two from sacral sores and two from antral washouts. Before the clinical importance of *P. asaccharolyticus* can be defined accurately, the classification needs to be improved.

This is even more true of the indole-negative,

butyrate-producing species, of which there are now five—*P. prevotii* and *P. tetradius*, and the newly described *P. lacrimalis*, *P. lactolyticus* and *P. vaginalis*.¹⁰ Many studies^{6–8, 19, 20} have shown that the present classification has major deficiencies which makes analysis of clinical importance very tentative. DNA-DNA hybridisation studies,⁶ HPLC studies⁵¹ and pre-formed enzyme analysis^{20, 21} agree that the type strain of *P. prevotii* is a very unusual strain of GPAC, closely related to the type strain of *P. tetradius* but not to other butyrate-producing GPAC. None of the isolates in this survey was identified as *P. prevotii sensu stricto*, and only three (1.5%) as *P. tetradius*, all from the female genitourinary tract; this site has yielded other isolates since. Strains identified as *P. prevotii* accounted for 9% of all GPAC in Rosenblatt's series³ and 15% in that of Brook,⁴ and have been cultured by other workers from a similar range of clinical specimens to *P. asaccharolyticus*.^{4, 29, 34, 42} However, many of these isolates may have been indole-negative strains of *P. asaccharolyticus*,^{6, 21} *P. vaginalis* or the “bGAL” group. The latter group is still not adequately defined (or named!) but, in this survey, *P. vaginalis* and “bGAL” group strains accounted for 18 isolates (11% of those available for study) and were often cultured from important specimens, particularly post-surgical wound infections. Both groups were usually isolated from skin-related sites, but whereas *P. vaginalis* was most frequently cultured with *S. aureus*, “bGAL” isolates were more likely to be cultured with other anaerobes. Isolates of *P. hydrogenalis* also came from superficial sites, but their limited number suggests that this species may be less significant. We conclude that the classification and laboratory identification of all butyrate-producing cocci need much more thorough study; in particular, the value of the indole test, on which great emphasis has been placed,^{8, 10, 12, 13} is very much open to doubt.^{6, 21, 51, 52}

As with *P. micros*, *P. anaerobius* was associated with polymicrobial, soft-tissue abscesses; however, most isolates were from intra-abdominal sepsis or the female genitourinary tract, in association with faecal flora—often coliforms and enterococci—unlike the organisms associated with *P. micros*. It was isolated occasionally from superficial sites, but usually where faecal contamination was likely. *P. anaerobius* is the easiest species of GPAC to identify^{12, 21}—colonies are usually visible after overnight incubation and have a characteristic “plasticine” smell—so, unlike other species, it is likely that the majority of significant isolates in this survey were cultured successfully. Its pathogenic importance in this study was much less clear-cut than that of the three other major species. Yet it has been reported from three cases of septic arthritis,²⁹ 13 diabetic foot infections,³⁴ an endodontic abscess,⁴⁴ three peritonsillar abscesses,⁵⁰ three breast infections⁴² and a post-thoracotomy sternal wound infection⁵³—a much wider variety of sites than recorded in this study. It accounted for 10% of GPAC in Rosenblatt's survey³ and 18% in Brook's.⁴ The possibility that it is an

important component of mixed anaerobic infections, particularly gynaecological, deserves further study. The clinical contexts of the few isolates of "ivoricus" and Hare group VIII do not suggest that these groups are of much clinical importance.

Therefore, there appears to be a spectrum from mainly skin-related species (*P. magnus* and *P. vaginalis*), through "bGAL" group strains, *P. asaccharolyticus* and *P. anaerobius* to the species associated with deep infections, *P. micros* and *P. heliotrinreducens*. The sites from which the different species were isolated correlated with the associated organisms and the proportion of aerobes to anaerobes cultured. Most anaerobic infections are believed to develop from the endogenous flora,⁵ and GPAC are known to be present on most body surfaces^{8,13,27,54} but commensal isolates of GPAC have rarely been identified to the species level. Recent studies have led to the recognition of four new species,^{16,17} including *P. vaginalis*, but there are undoubtedly several more species which have not yet been properly described—some of which (for instance, "bGAL", "ivoricus" and Hare group VIII strains) were isolated from clinical specimens in this survey. Therefore, further and more detailed studies of the commensal flora are needed, with the aims of identifying isolates to the species level and the description of new species. Until a more satisfactory classification for GPAC is available, a significant proportion of clinical isolates will be unidentifiable—or misidentified.

Other areas need further study, particularly culture methods. At present, primary isolation usually requires prolonged incubation—often for 5 days—by which time many isolates have been overgrown by more rapidly growing organisms, or the results are no longer of much clinical relevance; hence there is an urgent need to develop selective media that enrich for GPAC, to permit more rapid isolation. Other research should include prospective studies of the range of infections caused by GPAC, supported by careful specimen collection as detailed by Brook.⁴ Very little is known of the pathogenesis of infections caused by GPAC. This report cannot do more than document the association of certain species, for instance *P. vaginalis* with *S. aureus*. However, Brook and Walker⁴⁸ have developed a mouse abscess model to study the importance of individual isolates in mixed cultures,

and have shown that only heavily capsulate isolates of anaerobes were able to induce abscesses. Recently, Krepel *et al.*^{55,56} have extended the work of Edmiston *et al.*,⁴² by comparing isolates from non-puerperal breast abscesses, diabetic foot ulcers and intra-abdominal sites. Finding that abscess and ulcer isolates were much more likely to produce collagenase than intra-abdominal strains, they suggested that production by *P. magnus* of proteolytic enzymes could have an adjunctive effect on the development of soft-tissue infections. Studies of virulence factors might clarify the pathogenic potential of different species of GPAC.

This survey and others^{28,29} have shown that GPAC, particularly *P. magnus* and *P. asaccharolyticus*, can be isolated in pure culture from significant infections. We recommend that specimens to be cultured for anaerobes should include pus from superficial infections and all joint fluids, particularly when foreign material is present. Agents with known activity against GPAC, such as penicillin or metronidazole, should be considered in empirical therapy for these infections. GPAC should be fully identified by routine laboratories when they are isolated in pure growth or from important specimens such as blood and deep organ abscesses. Greater awareness of the laboratory characteristics of the different species of GPAC would increase isolation rates; colonies of *P. micros* and *P. anaerobius*, in particular, show a highly distinctive morphology.²¹

GPAC have received much less attention than some other groups of anaerobes, particularly clostridia and *Bacteroides* spp., a neglect disproportionate to their clinical importance. This report has demonstrated that different species are isolated from different infections and are associated with distinct groups of other micro-organisms. The recent advances in classification and identification should stimulate more thorough study of an interesting and important group of human pathogens.

We are grateful to the staff of the Department of Medical Microbiology, St Bartholomew's Hospital, London, for their interest and co-operation, in particular I. Harrison for providing essential computer back-up. Dr A. Lovering, Southmead Hospital, Bristol, assisted with preparation of the tables. Professor D. C. E. Speller made constructive criticisms of an early draft. A generous grant from the Sir Jules Thorn Trust supported a year's research and made this study possible.

References

1. Wren MWD, Baldwin AWF, Eldon CP, Sanderson PJ. The anaerobic culture of clinical specimens: a 14-month study. *J Med Microbiol* 1977; **10**: 49–61.
2. Holland JW, Hill EO, Altemeier WA. Numbers and types of anaerobic bacteria isolated from clinical specimens since 1960. *J Clin Microbiol* 1977; **5**: 20–25.
3. Rosenblatt JE. Anaerobic cocci. In: Lennette EH, Balows A, Hausler WJ, Shadomy HJ (ed) *Manual of clinical microbiology*, 4th edn. Washington, USA, American Society for Microbiology. 1985: 445–449.
4. Brook I. Recovery of anaerobic bacteria from clinical specimens in 12 years at two military hospitals. *J Clin Microbiol* 1988; **26**: 1181–1188.
5. Finegold SM, George WL. *Anaerobic infections in humans*. New York, Academic Press. 1989.
6. Ezaki T, Yamamoto N, Ninomiya K, Suzuki S, Yabuuchi E. Transfer of *Peptococcus indolicus*, *Peptococcus asaccharolyticus*, *Peptococcus prevotii* and *Peptococcus magnus* to the genus *Peptostreptococcus* and proposal of *Peptostreptococcus tetradius* sp. nov. *Int J Syst Bacteriol* 1983; **33**: 683–698.
7. Huss VAR, Festl H, Schleifer KH. Nucleic acid hybridization studies and deoxyribonucleic acid base compositions of anaerobic gram-positive cocci. *Int J Syst Bacteriol* 1984; **34**: 95–101.
8. Holdeman Moore LV, Johnson JL, Moore WEC. Genus *Peptococcus* Kluyver and van Niel 1936, 400^{AL}. In: Sneath PHA (ed) *Bergey's manual of systematic bacteriology*, vol. 2. Baltimore, Williams and Wilkins. 1986: 1082–1092.
9. Taylor EA, Jackman PJH, Phillips I. The differentiation of asaccharolytic anaerobic gram-positive cocci by protein electrophoresis. *J Med Microbiol* 1991; **34**: 339–348.

10. Ezaki T, Oyaizu H, Yabuuchi E. The anaerobic gram-positive cocci. In: Balows A *et al.* (eds) *The prokaryotes*, 2nd edn. New York, Springer-Verlag. 1992: 1879–1892.
11. Holdeman LV, Cato EP, Moore WEC. *Anaerobe laboratory manual*, 4th edn. Blacksburg, Anaerobe Laboratory, Virginia Polytechnic Institute and State University. 1977.
12. Sutter VL, Citron DM, Edelstein MAC *et al.* *Wadsworth anaerobic bacteriology manual*, 4th edn. Los Angeles, Star Publishing Co. 1985.
13. Hillier S, Moncla BJ. Anaerobic gram-positive nonspore-forming bacilli and cocci. In: Balows A, Hausler WJ, Herrmann KL, Isenberg HD, Shadomy EP (eds) *Manual of clinical microbiology*, 5th edn. Washington, American Society for Microbiology. 1991: 532–535.
14. Ezaki T, Yabuuchi E. Oligopeptidase activity of gram-positive anaerobic cocci used for rapid identification. *J Gen Appl Microbiol* 1985; **31**: 255–265.
15. Schiefer-Ullrich H, Andreesen JR. *Peptostreptococcus barnesae* sp. nov., a gram-positive, anaerobic, obligately purine-utilising coccus from chicken faeces. *Arch Microbiol* 1985; **143**: 26–31.
16. Ezaki T, Liu S-L, Hashimoto Y, Yabuuchi E. *Peptostreptococcus hydrogenalis* sp. nov. from human faecal and vaginal flora. *Int J Syst Bacteriol* 1990; **40**: 305–306.
17. Li N, Hashimoto Y, Adnan S, Miura H, Yamamoto H, Ezaki T. Three new species of the genus *Peptostreptococcus* isolated from humans: *Peptostreptococcus vaginalis* sp. nov., *Peptostreptococcus lacrimalis* sp. nov., and *Peptostreptococcus lactolyticus* sp. nov. *Int J Syst Bacteriol* 1992; **42**: 602–605.
18. Stenson MJ, Lee DT, Rosenblatt JE, Contezac JM. Evaluation of the AnIdent system for the identification of anaerobic bacteria. *Diagn Microbiol Infect Dis* 1986; **5**: 9–15.
19. Murdoch DA, Mitchelmore IJ, Tabaqchali S. Identification of gram-positive anaerobic cocci by use of systems for detecting pre-formed enzymes. *J Med Microbiol* 1988; **25**: 289–293.
20. Murdoch DA, Mitchelmore IJ, Nash RA, Hardie JM, Tabaqchali S. Preformed enzyme profiles of reference strains of gram-positive anaerobic cocci. *J Med Microbiol* 1988; **27**: 65–70.
21. Murdoch DA, Mitchelmore IJ. The laboratory identification of gram-positive anaerobic cocci. *J Med Microbiol* 1991; **34**: 295–308.
22. Madsen M, Hoi-Sorensen G, Aalbaek B, Hansen JW, Bjorn H. Summer mastitis in heifers: studies of the seasonal occurrence of *Actinomyces pyogenes*, *Peptostreptococcus indolicus* and Bacteroidaceae in clinically healthy cattle in Denmark. *Vet Microbiol* 1992; **30**: 243–255.
23. Madsen M, Aalbaek B, Hansen JW. Comparative bacteriological studies on summer mastitis in grazing cattle and pyogenes mastitis in stabled cattle in Denmark. *Vet Microbiol* 1992; **32**: 81–88.
24. Hare R. The anaerobic cocci. In: Waterson AP (ed) *Recent advances in medical microbiology*. London, Churchill. 1967: 284–317.
25. Hardie JM. Anaerobic streptococci. In: Sneath PHA (ed) *Bergey's manual of systematic bacteriology*, vol. 2. Baltimore, Williams and Wilkins. 1986: 1066–1068.
26. Collins MD, Wallbanks S. Comparative sequence analyses of the 16S rRNA genes of *Lactobacillus minutus*, *Lactobacillus rimae* and *Streptococcus parvulus*: proposal for the creation of a new genus *Atopobium*. *FEMS Microbiol Lett* 1992; **74**: 235–240.
27. Bartlett JG. Anaerobic cocci. In: Mandell GL, Douglas RG, Bennett JE (eds) *Principles and practice of infectious diseases*, 3rd edn. New York, Churchill Livingstone. 1990: 1867–1869.
28. Bourgault A-M, Rosenblatt JE, Fitzgerald RH. *Peptococcus magnus*: a significant human pathogen. *Ann Int Med* 1980; **93**: 244–248.
29. Fitzgerald RH, Rosenblatt JE, Tenney JH, Bourgault AM. Anaerobic septic arthritis. *Clin Orthop* 1982; **164**: 141–148.
30. Schottmueller H. Zur bedeutung einiger anaeroben in der pathologie, insbesondere bei puerperalen erkrankungen. *Mitt Grenzgeb Med Chir* 1910; **21**: 450–490.
31. Schwarz D, Dieckman WJ. Anaerobic streptococci: their role in puerperal infection. *South Med J* 1926; **19**: 470–479.
32. Hare R, Polunin I. Anaerobic cocci in the vagina of native women in British North Borneo. *J Obs Gyn Br Emp* 1960; **67**: 985–989.
33. Sanderson PJ. Infection of the foot with *Peptococcus magnus*. *J Clin Pathol* 1977; **30**: 266–268.
34. Wheat LJ, Allen SD, Henry M *et al.* Diabetic foot infections. Bacteriologic analysis. *Arch Int Med* 1986; **146**: 1935–1940.
35. Hunter T, Chow AW. *Peptostreptococcus magnus* septic arthritis—a report and review of the English literature. *J Rheumatol* 1988; **15**: 1583–1584.
36. Davies UM, Leak AM, Dave J. Infection of a prosthetic knee joint with *Peptostreptococcus magnus*: *Ann Rheum Dis* 1988; **47**: 866–868.
37. Cofsky RD, Seligman SJ. *Peptococcus magnus* endocarditis. *South Med J* 1985; **78**: 361–362.
38. Pouedras P, Donnio PY, Sire JM, Avril JL. Prosthetic valve endocarditis and paravalvular abscess caused by *Peptostreptococcus magnus*. *Clin Infect Dis* 1992; **15**: 185.
39. Phelps R, Jacobs RA. Purulent pericarditis and mediastinitis due to *Peptococcus magnus*. *JAMA* 1985; **254**: 947–948.
40. Panagou P, Papandreou L, Bouros D. Severe anaerobic necrotizing pneumonia complicated by pyopneumothorax and anaerobic monoarthritis due to *Peptostreptococcus magnus*. *Respiration* 1991; **58**: 223–225.
41. Edmiston CE, Walker AP, Krepel CJ, Gohr C. The non-puerperal breast infection: aerobic and anaerobic microbial recovery from acute and chronic disease. *J Infect Dis* 1990; **162**: 695–699.
42. Papasian CJ, McGregor DH, Hodges GR, Kennedy J. *Peptostreptococcus* vertebral osteomyelitis. *J Clin Microbiol* 1986; **24**: 633–635.
43. Topiel MS, Simon GL. *Peptococcaceae* bacteraemia. *Diagn Microbiol Infect Dis* 1986; **4**: 109–117.
44. Williams BL, McCann GF, Schoenkecht FD. Bacteriology of dental abscesses of endodontic origin. *J Clin Microbiol* 1983; **18**: 770–774.
45. Rams TE, Feik D, Listgarten MA, Slots J. *Peptostreptococcus micros* in human periodontitis. *Oral Microbiol Immunol* 1992; **7**: 1–6.
46. Murdoch DA, Mitchelmore IJ. Isolation of *Peptostreptococcus heliotrinireducens* from human polymicrobial abscesses. *Lett Appl Microbiol* 1989; **9**: 223–225.
47. Murdoch DA, Mitchelmore IJ, Tabaqchali S. *Peptostreptococcus micros* in polymicrobial abscesses. *Lancet* 1988; **1**: 594.
48. Brook I, Walker RI. The role of encapsulation in the pathogenesis of anaerobic gram-positive cocci. *Can J Microbiol* 1985; **31**: 176–180.
49. Brook I. Bacteriology of intracranial abscess in children. *J Neurosurg* 1981; **54**: 484–488.
50. Jokipii AMM, Jokipii L, Sipilä P, Jokinen K. Semiquantitative culture results and pathogenic significance of obligate anaerobes in peritonsillar abscesses. *J Clin Microbiol* 1988; **26**: 957–961.
51. Harpold DJ, Wasilauskas BL. Rapid identification of obligately anaerobic gram-positive cocci using high-pressure liquid chromatography. *J Clin Microbiol* 1987; **25**: 996–1001.
52. Murdoch DA, Wilks M, Mitchelmore IJ, Tabaqchali S. Identification of gram-positive anaerobic cocci by gas-liquid chromatography and preformed enzyme profile using a commercial kit, ATB 32A. In: Borriello SP *et al.* (eds) *Clinical and molecular aspects of anaerobes*. Petersfield, Wrightson Biomedical. 1990: 285–291.
53. Brook I. Microbiology of postthoracotomy sternal wound infection. *J Clin Microbiol* 1989; **27**: 806–807.
54. Neut C, Lesieur V, Romond C, Beerens H. Analysis of gram-positive anaerobic cocci in oral, faecal and vaginal flora. *Eur J Clin Microbiol* 1985; **4**: 435–437.
55. Krepel CJ, Gohr CM, Edmiston CE, Farmer SG. Anaerobic pathogenesis: collagenase production by *Peptostreptococcus magnus* and its relationship to site of infection. *J Infect Dis* 1991; **163**: 1148–1150.
56. Krepel CJ, Gohr CM, Walker AP, Farmer SG, Edmiston CE. Enzymatically active *Peptostreptococcus magnus*: association with site of infection. *J Clin Microbiol* 1992; **30**: 2330–2334.