Infection with minute-colony-forming β-haemolytic streptococci

PAULINE M. POOLE AND G. WILSON

From Chester Public Health Laboratory

SYNOPSIS One hundred and thirty-one strains of minute-colony-forming β-haemolytic streptococci were isolated during the course of routine investigations in clinical bacteriology. Each strain was examined for the presence of polysaccharide antigens of Lancefield's groups A, C, G, and F and characterized in detail as to biochemical and cultural features. On the results of these tests it is concluded that the strains should be placed in the species *Streptococcus milleri*. The clinical details relevant to the various strains are summarized according to the site of isolation, and their pathological significance is discussed.

The cultural characteristics and distribution of minute-colony-forming β-haemolytic streptococci were first described by Long and Bliss (1934) and Long *et al* (1934) using strains isolated from the throat. Bliss (1937) differentiated these streptococci serologically into Lancefield's groups F and G, dividing the F strains further into four antigenic types. One of these types was likewise found in the group G strains. Smith and Sherman (1938) classified 'minute' streptococci of group G as *Streptococcus anginosus* having observed their resemblance to this species as described by Andrewes and Horder (1906). Niven (1957) included 'minute' streptococci of both groups F and G in *Strep. anginosus*. Guthof (1956) isolated a new kind of streptococcus, which he called *Streptococcus milleri*, from dental abscesses and other inflammatory lesions of the mouth. His strains were non-haemolytic, and he was unable to demonstrate the presence of any of Lancefield's antigens. Later, non-haemolytic streptococci biochemically similar to *Strep. milleri* were found in dental root canals (Ottens and Winkler, 1962). These were described as 'indifferent streptococci', and more than half of them were found to contain antigen of Lancefield's groups F, G or C. The group F antigen of the 'indifferent' strains was identical with that of group F haemolytic streptococci. In addition, Ottens and Winkler demonstrated the presence of type-antigens which were shared by both 'minute' haemolytic and 'indifferent' strains. Sometimes type-antigen was present without group-antigen. *Strep. milleri* and the 'indifferent streptococci' were

similar in cell wall composition (Colman and Williams, 1965). *Strep. milleri* and 'indifferent streptococci' were brought together into the species *Strep. milleri* (Colman and Williams, 1972) and shown further to share biochemical similarities as well as common antigens and cell wall components.

These biochemical similarities include hydrolysis of arginine and aesculin, a positive Voges-Proskauer reaction as well as resistance to bacitracin and nitrofurazone. Both grow poorly in air (Winkler and Amerongen, 1959; Colman and Williams, 1973) and are stimulated by carbon dioxide (Liu, 1954; Bateman *et al*, 1975). No protein antigen has been found in either *Strep. milleri* or 'minute' streptococci.

Methods

The minute-colony-forming-β-haemolytic streptococci were initially isolated during the course of routine bacteriological investigations. All anaerobic cultures on blood agar, which might or might not contain neomycin or gentian violet, were inspected for the presence of minute pinpoint colonies surrounded by a zone of β-haemolysis and often giving a sweetish smell. Such colonies were subcultured to blood agar for incubation aerobically, anaerobically, and in 10% carbon dioxide in air at 37°C.

CHARACTERIZATION TESTS
In all these tests the cultures were incubated at 37°C in 10% carbon dioxide (with loose caps where relevant). The incubation period was five days unless otherwise stated.

Acid production from lactose, sucrose, salicin,

raffinose, and trehalose was detected by growth in the serum-free medium of Flynn and Waitkins (1972). Aesculin hydrolysis was tested by growing the streptococci on 0·1 % aesculin agar with the addition of 5 % horse serum, a positive reaction being indicated by black discolouration around the bacterial growth. Arginine hydrolysis was detected by growth in arginine broth (Niven *et al*, 1942) containing 5 % horse serum, Nessler's reagent being added at the end of the incubation period to show the presence of ammonia. The Voges-Proskauer reaction was carried out by growing in a medium composed of 1 % tryptose, 0·5 % yeast extract, 0·5 % di-potassium hydrogen phosphate, 0·5 % glucose, and 5 % horse serum. At the end of incubation the culture was tested for the presence of acetylmethylcarbinol by Barritts' (1936) method. Bile tolerance was shown by inoculation onto 'half' plates containing 10 % or 40 % bile agar on one side and blood agar on the other. The plates were inspected for growth after 24 hours.

Sensitivity tests were carried out by growth on blood agar for 24 hours in the presence of discs (Mast) impregnated with penicillin, 1 unit, tetracycline, 25 μg, erythromycin, 5 μg, bacitracin, 0·1 unit, and nitrofurazone, 10 μg, and on Sensitivity Test Agar (Oxoid) containing 5 % lysed blood with discs containing sulphamethoxazole, 50 μg, and trimethoprim, 2·5 μg. Strains were recorded as either sensitive or resistant. Sensitivity was indicated by a zone of inhibition, the diameter of which was at least 20 mm, and resistance by growth up to the edge of the disc. Partial sensitivity was not observed.

Polysaccharide group antigens of groups A, C, F, and G were identified by both extraction with formamide (Fuller, 1938) and treatment with Maxted's (1948) enzyme. Representative strains were grown on a digest agar containing 5 % sucrose and 5 % horse serum, and the cultures were examined for

the presence of colonies characteristic of dextran and levan production.

Results

'Minute' streptococci were isolated from 131 sites, sometimes on more than one occasion. For the purpose of this account multiple isolates from the same site are treated as single strains. Eight contained polysaccharide of Lancefield's group A, 29 of group C, 59 of group F, and 15 of group G. Twenty strains failed to react with grouping sera and are referred to as ungroupable. The original isolates were made anaerobically. On subculture to blood agar incubated aerobically, 104 strains grew more poorly than anaerobically, and 27 representative of all the groups failed to grow. All strains, including those of group G, grew better in 10 % carbon dioxide than in air, frequently producing comparatively luxuriant colonies. This finding resembles that of Liu (1954) but differs from that of Colman and Williams (1972) whose minute strains of group G were not so stimulated. Beta-haemolysis was most striking in anaerobic culture. Enhanced zones of haemolysis were shown by all 15 strains of group G, by one of group C, and by two of group F.

The biochemical reactions varied (table I). Sucrose and salicin were fermented, arginine was hydrolysed, and the Voges-Proskauer test was positive in the majority of strains in all groups. Strains of group C and G generally fermented lactose and hydrolysed aesculin, whereas in isolates of the other groups these were less common features. Strains of group C differed from the majority in only occasionally fermenting trehalose. Growth of 'minute' streptococci in the presence of bile salts was infrequent. Group G strains were the most active in this feature, nine (60 %) of the isolates growing in the presence of 40 % bile. Group G strains were

| | Group | | | | | |
	A	C	F	G	Ungroupable	Total
Total strains	8	29	59	15	20	131
Acid from lactose	2 25·0	23 79·0	8 13·5	14 93·3	6 30·0	53 40·4
sucrose	8 100·0	18 62·0	59 100·0	15 100·0	20 100·0	120 91·6
salicin	7 87·5	18 62·0	54 91·5	14 93·3	14 70·0	107 81·6
raffinose	—	—	—	7 46·0	—	—
trehalose	7 87·5	4 13·7	48 81·3	12 80·0	15 75·0	86 65·0
Hydrolysis of aesculin	1 12·5	26 89·6	22 35·5	15 100·0	5 25·0	69 52·6
Hydrolysis of arginine	8 100·0	29 100·0	56 94·9	15 100·0	20 100·0	128 97·6
Acetoin from glucose	7 87·5	23 79·0	55 93·2	14 93·3	20 100·0	119 90·8
Growth in 10 % bile	0 0·0	0 0·0	4 6·7	9 60·0	2 10·0	15 11·4
40 % bile	0 0·0	0 0·0	2 3·4	9 60·0	2 10·0	13 10·0

Table I   *Biochemical reactions of minute-colony-forming β-haemolytic streptococci: number and percentage of strains by Lancefield group that give the stated reaction*

additionally inoculated into raffinose, seven fermenting this sugar.

Approximately a quarter of the isolates from all groups were tested for the ability to produce extracellular polysaccharides from glucose. All gave a negative result.

The 'minute' streptococci were uniformly sensitive to penicillin, erythromycin, and trimethoprim, the inhibition zones being generally far greater in diameter than minimum (20 mm), taken as evidence of sensitivity. All strains were resistant to sulphamethoxazole and nitrofurazone. One hundred and twenty-six strains were resistant to bacitracin, including all of those forming antigen of group A. Eight strains were resistant to tetracycline (table II).

CLINICAL FEATURES

Respiratory tract
Forty-nine isolates were from the respiratory tract, 41 of these from the throat. Of 25 patients complaining of sore throat or tonsillitis, 21 (84%) yielded the organism in heavy growth compared with five (33%) of 16 symptomless carriers. Six of the remaining eight isolates occurred in moderate to heavy growth, and five of six where the information was recorded were associated with the presence of pus. Two strains, mixed with coliforms and Proteus, were from tracheostomy wounds. Four were from patients with bronchopneumonia or chest infections. Of these, three were found in sputum, associated with incidental flora only. The fourth, mixed with *Staphylococcus aureus*, was isolated from the lung at necropsy. Another strain, also mixed with *Staph. aureus*, was from an abscess in a thoracotomy wound. The last was isolated repeatedly in pure growth.

A man aged 42 had undergone pneumonectomy for carcinoma of the bronchus six years previously. Immediately postoperatively the pleural space was sterile. In May 1974, he had a respiratory illness and the space became infected. A large amount of foul-smelling pus was drained through the site of an earlier drainage tract. 'Minute' haemolytic strepto-cocci of group A were isolated. After treatment with local irrigations and instillations of antibiotics the space became apparently sterile. Within a few days of removal of the drainage tube, however, discomfort recurred and drainage was re-established. 'Minute' streptococci were again found and remained present until one month later when a stoma was fashioned. Swabs from the stoma became sterile and all appeared well until six months later when it began to discharge again. 'Minute' streptococci were once more isolated and were found on several further occasions.

Appendix
Eighteen isolates were made from patients with appendicitis. Thirteen patients were less than 15 years old. Five strains were grown from the appendix itself, two from the peritoneal cavity, and the remainder from discharge, either through drainage tubing or from the wound. Sixteen of the isolates, 14 in heavy growth, were associated with a mixed faecal type flora. Two, one from a pelvic abscess and one from an appendix, were in heavy pure growth. In six patients the appendix was recorded as being gangrenous, and pus was observed in the peritoneal cavity of a further seven. In five of these seven, there was an obvious perforation of the appendix. The four patients remaining were described as having mildly or acutely inflamed appendices. Despite drainage, the wounds became infected and broke down in 13 cases, with the formation of large amounts of malodorous pus and delay in healing.

Other abdominal
Seventeen infections were found in patients who had undergone other abdominal surgery. The colon was sectioned in eight cases, five were gastrectomies, one a resection for a perforated duodenal ulcer, and three isolates were from miscellaneous sites. Pus was a constant feature. The organisms were commonly present in heavy growth and mixed with a faecal flora. In two cases, however, the isolates were in pure culture, one from a hemicolectomy wound, the other from a patient who had had a gastrectomy.

This last was a man of 28 years who one month

Lancefield group	No of strains tested	Antibacterial agent						
		Penicillin	Erythromycin	Tetracycline	Bacitracin	Trimethoprim	Sulphamethoxazole	Nitrofurazone
A	8	100·0	100·0	100·0	0	100·0	0	0
C	29	100·0	100·0	96·5	10·3	100·0	0	0
F	59	100·0	100·0	94·9	1·7	100·0	0	0
G	15	100·0	100·0	86·2	0	100·0	0	0
Ungroupable	20	100·0	100·0	90·0	5·0	100·0	0	0
Total	131	100·0	100·0	93·9	3·8	100·0	0	0

Table II *Sensitivity of minute-colony-forming β-haemolytic streptococci to antibacterial agents: percentage sensitive by Lancefield group*

after gastrectomy developed an abscess in a midline scar. The abscess was opened and drained. The infection appeared subcutaneous, the deeper layers of the wound being intact. He subsequently developed a series of abscesses, both in his recent midline scar and in an earlier paramedian vagotomy scar. Ungroupable 'minute' haemolytic streptococci were grown from all these lesions. A large volume of pus was present and eventually a chronic sinus developed. A sinugram revealed the tract of a fistula from the duodenal loop.

Vagina
Thirty-one isolates were made from the vagina. Six patients had offensive lochia, 24 complained of discharge, and one was suffering from vaginitis. Growth was heavy in 19 patients. Pus cells were found in 18 cases, but there was no correlation between the amount of growth and the presence of pus. Three patients had simultaneous infections with *Candida albicans*, two with *Trichomonas vaginalis*, and in four the presence of anaerobic streptococci was noted. Otherwise the flora was incidental and not recorded specifically.

Perineum and adjacent sites
Ten isolates were from patients with lesions either in or adjacent to the perineum. Five, four of which were puerperal, were from perineal wounds. One was from a perianal ulcer and one from the vulva. Two were from abscesses, one of the scrotum and one in a pilonidal sinus. The tenth was from a sacral pressure sore. Growth was heavy in nine patients, and in six the presence of pus was recorded. All were in mixed culture, anaerobic streptococci, Bacteroides, and coliforms predominating.

Other sites
Two strains were isolated from infected fingers and one from a lesion on the thumb. The cultures were in heavy mixed growth, but no other specific pathogen was present. 'Minute' streptococci were also found mixed with anaerobic streptococci in a breast abscess, and in pure growth from an abscess of the

chin and from the purulent urine of one of the patients who yielded a similar strain from sputum.

ANATOMICAL DISTRIBUTION BY LANCEFIELD GROUP
Although 'minute' haemolytic streptococci were found in all parts of the body, the different groups showed individual preferences for certain sites (table III). This may be a reflection of their normal occurrence at these sites in the absence of active infection. Group A strains occurred equally in the respiratory tract and in the vaginoperineal area, none being found in the abdomen. Group C strains were predominantly respiratory and abdominal. In the abdomen, group C strains were associated equally with appendicitis and other surgical conditions. They were, however, found only infrequently in the vagina and perineum. Group F strains formed just under half of the total isolates and occurred most frequently in the vagina, although they were also represented in the respiratory tract and abdomen. In the abdomen, group F strains differed from those of group C, in that they were found twice as often associated with appendicitis as with other abdominal conditions. Group G strains were largely respiratory. The ungroupable strains were distributed throughout all sites. A quarter were found in the perineum or adjacent areas, equalling the total number of isolates of all other groups found at this site.

Discussion

A detailed identification of the 131 strains of 'minute'-colony haemolytic streptococci was made. They were distinguished from large-colony strains of haemolytic streptococci not only by size but by several other features. Growth in air was poor but was enhanced by the presence of carbon dioxide. Generally the Voges-Proskauer test was positive, and all strains were resistant to nitrofurazone and sulphonamides. All the 'minute' strains having group A antigen were resistant to bacitracin. Only four 'minute' strains of group C fermented trehalose unlike large colony group C strains, which usually

	Group							
	A		C		F		G	
Respiratory	4	50·0	14	48·2	15	25·4	10	66·0
Surgery for appendicitis	0	0	5	17·2	11	18·6	0	0
Other abdominal surgery	0	0	6	20·7	5	8·5	1	6·6
Vagina	3	37·5	3	10·3	21	35·6	3	20·0
Perineum and adjacent sites	1	12·5	1	3·4	3	5·1	0	
Other	0	0	0	0	4	6·7	1	6·6
Total	8	100·0	29	100·0	59	100·0	15	100·0

Ungroupable		Total	
6	30·0	49	37·4
2	10·0	18	13·7
5	25·0	17	12·9
1	5·0	31	23·6
5	25·0	10	7·6
1	5·0	6	4·6
20	100·0	131	100·0

Table III *Frequency of isolation of minute-colony-forming β-haemolytic streptococci from various body sites; number and percentage of strains by Lancefield group isolated from stated site*

ferment this sugar. The 'minute' strains of group G differed from their large colony counterparts in that seven fermented raffinose and nine grew in the presence of 40% bile.

The behaviour of the minute-colony-forming β-haemolytic streptococci, however, confirms the validity of their inclusion in the species *Strep. milleri* as first postulated by Colman and Williams (1972). It may be that eventually the haemolytic strains will prove to have other distinctive characteristics which will establish their position within a separate variety of the species. For the present, however, haemolysis as a single feature is insufficient to merit such a distinction. Both haemolytic and non-haemolytic strains grow better anaerobically or in the presence of carbon dioxide than in air. Both ferment sucrose and salicin, give a positive Voges-Proskauer reaction, hydrolyse arginine, and are resistant to nitrofurazone and bacitracin. Haemolytic strains are resistant to sulphamethoxazole, and there is some evidence that non-haemolytic strains behave likewise (personal observation). Neither produce extracellular polysaccharide from sucrose. Haemolytic strains tend to be less active than non-haemolytic in fermenting lactose or trehalose and in growth in the presence of bile salts. The degree of activity in these tests varies to some extent among the haemolytic strains according to their Lancefield group (table I).

The normal habitat of both haemolytic and non-haemolytic strains included here in *Strep. milleri* has been investigated by several workers. Although Long *et al* (1934) isolated 'minute' strains of group F and G from the throat in 6-8% of normal subjects and Hare (1935) stated that strains of group F occurred mainly in the nasopharynx, more recent work does not support their observations on the importance of this site. Haemolytic strains occurred in only 41 (0·6%) of an estimated 7000 throat swabs examined in the present study, and Wort (1975) reported an isolation rate of only 0·04% of group F strains from similar material. Guthof (1956) was unable to demonstrate non-haemolytic *Strep. milleri* in the nasopharynx, and Mejarè and Edwardsson (1975) likewise found that this organism, although abundantly present on teeth, was isolated from the oral cavity in less than 1% of individuals. The occurrence of *Strep. milleri* on teeth was, however, consistent with the presence of both 'indifferent' and 'minute' strains in dental root canals (Winkler and Van Amerongen, 1959). Haemolytic strains of group F have been found in the vagina of puerperal women (Lancefield and Hare 1935; Wort, 1975), although experience in our study suggests that haemolytic strains occur infrequently at this site, 31 (0·6%) strains only being isolated from an estimated 4900

vaginal swabs. It seems likely, however, that they occur more commonly in the gastrointestinal tract. Haemolytic strains of group F were grown from the faeces of 5% of normal puerperal women (Hare and Maxted, 1935) and from 11% of appendices (Rogers, 1957). It would be valuable to elucidate more accurately the distribution in the normal subject of both haemolytic and non-haemolytic strains, thus identifying possible sources of infection.

Considerable evidence has accumulated, indicating that on occasion these streptococci are of pathogenic significance. Group F strains showing varying degrees of haemolysis have been described most frequently. Although only haemolytic strains were studied in the present investigation the results support their potential pathogenic role. Long *et al* (1934) claimed that 'minute' streptococci of group F and G occurred much more frequently in the throats of patients suffering from rheumatic fever and glomerulonephritis than in those of healthy persons or those suffering from tonsillitis. In the results presented here, however, a trend was discernible towards heavier growths from the throats of patients with symptoms than from carriers. Elsewhere in the respiratory tract group F streptococci, sometimes in pure culture, have been associated with lesions by several workers. Isolations have been made from paranasal sinuses and a chest abscess (Long and Bliss, 1934), from tonsils and chest fluid (Lancefield and Hare, 1935), from an infected antrum (Plummer, 1941), from empyemas (Foley, 1947), and, more recently, from a maxillary sinus and an ear (Wort, 1975). In our series four strains of various groups were found in lung infections. In the mouth non-haemolytic strains have been described in dental abscesses (Guthof, 1956), and both haemolytic and non-haemolytic strains in infected root canals (Winkler and van Amerongen, 1959). Group F streptococci have been grown in pure culture from a patient with peritonitis and abscess (Foley, 1947) and in mixed culture from the peritoneal cavities of eight patients, seven of whom had acute appendicitis (Wort, 1975). Bateman *et al* (1975) have isolated non-haemolytic strains, two of group F and one ungroupable, in pure culture from three patients with multiple liver abscesses. They also quote data from the Streptococcus Reference Laboratory, London, showing that 21% of 75 streptococci isolated from pus in viscera or the central nervous system were *Strep. milleri*. The isolation of group F streptococci from cerebrospinal fluid of patients with meningitis and from brain abscesses has also been reported by other workers (Thomas, 1939; Rantz, 1942; Wheeler and Foley, 1943; Koepke, 1965). Wort (1975) noted two isolates from urine. In this investigation strains of groups C and F as well as ungroupable strains

were found in a variety of abdominal lesions, although no isolates were made from the central nervous system.

Our results reaffirm the findings of previous workers. Generally the isolates were in mixed culture, in gangrenous lesions of the appendix and elsewhere, in pelvic and other abscesses, in offensive lochia, in perineal and other wounds. The lesions were purulent, often foul-smelling and of long duration with delay in healing. On seven occasions, however, the isolates representative of all groups were found in pure culture from infections in the abdomen, the chest, the urine, and an abscess. Such pure cultures may indicate that at these sites the organisms were pathogens and may confirm their contributory pathogenic role when mixed with other species of bacteria.

References

Andrewes, F. W. and Horder, T. J. (1906). A study of the streptococci pathogenic for man. *Lancet*, **2**, 708-713.

Barritt, M. M. (1936). The intensification of the Voges-Proskauer reaction by the addition of α-naphthol. *J. Path. Bact.*, **42**, 441-454.

Bateman, N. T., Eykyn, S., and Phillips I. (1975). Pyogenic liver abscess caused by *Streptococcus milleri*. *Lancet*, **1**, 657-659.

Bliss, E. A. (1937). Studies upon minute hemolytic streptococci. 3. Serological differentiation. *J. Bact.*, **33**, 625-642.

Colman, G. and Williams, R. E. O. (1965). The cell walls of streptococci. *J. gen. Microbiol.*, **41**, 375-387.

Colman, G. and Williams, R. E. O. (1972). Taxonomy of some human viridans streptococci. In *Streptococci and Streptococcal Diseases*, edited by W. Wannamaker and J. M. Matsen, pp. 281-299. Academic Press, London and New York.

Colman, G. and Williams, R. E. O. (1973). Identification of human streptococci. In *Recent Advances in Clinical Pathology*, Series 6, edited by S. C. Dyke, pp. 293-303. Churchill Livingstone, Edinburgh and London.

Flynn, J. and Waitkins, S. A. (1972). A serum free medium for testing fermentation reactions in *Neisseria gonorrhoeae*. *J. clin. Path.*, **25**, 525-527.

Foley, G. E. (1947). Further observations on the occurrence of streptococci of groups other than A in human infection. *New Engl. J. Med.*, **237**, 809-811.

Fuller, A. T. (1938). The formamide method for the extraction of polysaccharides from haemolytic streptococci. *Brit. J. exp. Path.*, **19**, 130-139.

Guthof, O. (1956). Ueber pathogene „vergrünende Streptokokken": Streptokokken Befunde bei dentogenen Abszessen und Infiltraten im Bereich der Mundhöhle. *Zbl. Bakt.*, 1 *Abt. Orig.*, **166**, 553-564.

Hare, R. (1935). The classification of haemolytic streptococci from the nose and throat of normal human beings by means of precipitin and biochemical tests. *J. Path. Bact.*, **41**, 499-511.

Hare, R. and Maxted, W. R. (1935). The classification of haemolytic streptococci from the stools of normal pregnant women and of cases of scarlet fever by means of precipitin and biochemical tests. *J. Path. Bact.*, **41**, 513-520.

Koepke, J. A. (1965). Meningitis due to *Streptococcus anginosus* (Lancefield group F). *J. Amer. med. Ass.*, **193**, 739-740.

Lancefield, R. C. and Hare, R. (1935). The serological differentiation of pathogenic and non-pathogenic strains of haemolytic streptococci from parturient women. *J. exp. Med.*, **61**, 335-349.

Liu, P. G. (1954). Carbon dioxide requirement of Group F and minute colony G hemolytic streptococci. *J. Bact.*, **68**, 282-288.

Long, P. H. and Bliss, E. A. (1934). Studies upon minute hemolytic streptococci. 1. The isolation and cultural characteristics of minute β-hemolytic streptococci. *J.exp. Med.*, **60**, 619-631.

Long, P. H., Bliss, E. A. and Walcott, C. F. (1934). Studies upon minute hemolytic streptococci. 2. The distribution of minute hemolytic streptococci in normal and diseased human beings. *J. exp. Med.*, **60**, 633-641.

Maxted, W. R. (1948). Preparation of streptococcal extracts for Lancefield grouping. *Lancet*, **2**, 255-256.

Mejarè, B. and Edwardsson, S. (1975). Observations on the characteristics of *Streptococcus milleri* and its occurrence as a regular inhabitant of the human mouth. *Caries Res.*, **9**, 308.

Niven, C. F., Jr. (1957). *Streptococcus anginosus*. In *Bergey's Manual of Determinative Bacteriology*, 7th Edition, edited by R. S. Breed *et al.*, pp. 516-517. Baillière, Tindall and Cox, London. Williams and Wilkins, Baltimore.

Niven, C. F. Jr., Smiley, K. L., and Sherman, J. M. (1942). The hydrolysis of arginine by streptococci. *J. Bact.*, **43**, 651-660.

Ottens, H. and Winkler, K. C. (1962). Indifferent and haemolytic streptococci possessing group-antigen F. *J. gen. Microbiol.*, **28**, 181-191.

Plummer, H. (1941). A serological and biochemical study of hemolytic streptococci. *J. Immunol.*, **42**, 91-107.

Rantz, L. A. (1942). Streptococcal meningitis. Four cases treated with sulfonamides in which the etiological agent was an unusual streptococcus. *Ann. intern. Med.*, **16**, 716-726.

Rogers, K. B. (1957). The association of acute appendicitis with infective diarrhoea. *Proc. R. Soc. Med.*, **50**, 1025-1026.

Smith, F. R. and Sherman, J. M. (1938). The hemolytic streptococci of human feces. *J. infect. Dis.*, **62**, 186-189.

Thomas, A. (1939). Purulent meningitis produced by the minute hemolytic streptococcus (Long and Bliss) and *B. coli*. *J. Mt. Sinai Hosp.*, **5**, 702-704.

Wheeler, S. M. and Foley, G. E. (1943). A note on non-group A streptococci associated with human infection. *J. Bact.*, **46**, 391-392.

Winkler, K. C. and van Amerongen J. (1959). Bacteriologic results from 4,000 root canal cultures. *Oral Surg.*, **12**, 857-875.

Wort, A. J. (1975). Observations on group F streptococci from human sources. *J. med. Microbiol.*, **8**, 455-457.