SENSITIVITY OF FOUR SPECIES OF BACTEROIDES TO ANTIBIOTICS

BY

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The genus here referred to as *Bacteroides* (also known as *Fusiformis* and by other names) consists of Gram-negative non-sporing anaerobic bacilli. These are inhabitants of the mouth and lower bowel, and may be found in infections of various parts of both alimentary and respiratory tracts, the uterus, and other organs; such infections are often mixed but sometimes pure, and of every degree of severity from the quiet local necrosis of Vincent's gingivitis up to septicaemia.

There is perhaps no genus of common pathogenic bacteria which has been so little studied except by a few specially interested workers. Their isolation is difficult and their precise identification often much more so. Although they are almost invariably present in any foul-smelling material from the mouth, lung, or a lesion—such, for instance, as a gangrenous appendix—in the lower alimentary tract, it is exceptional for any attempt to be made to identify them.

It is hence not altogether surprising that information about their sensitivity to antibiotics should be fragmentary; few such tests have been made, but the value of some is also diminished by the use of an incomplete range of concentrations or by failure to identify the organisms. Beigelman and Rantz (1949) give an admirable picture of the nature of *Bacteroides* infections in their account of 47 cases, but they did not attempt species identification or use any concentration of penicillin exceeding 0.1 unit/ml. in testing sensitivity. Rantz (1951) also describes a case of bacterial endocarditis due to an unidentified strain that was resistant to 1 unit/ml. of penicillin, and inhibited by 10 and 1 μg./ml. respectively of streptomycin and oxytetracycline: treatment with the latter for 66 days effected a cure.

Bacteroides funduliformis (*necrophorus*) is the subject of six papers in which sensitivity to antibiotics is reported: the results are given in Table I.

Bacteroides fragilis was tested under this name by only two authors: Marson and Meynell (1953) found a single strain from a patient with pylephlebitis to be inhibited by 20 units of penicillin per ml., and 25, 5, 0.1, and 0.4 μg./ml. respectively of streptomycin, chloramphenicol, chlortetracycline, and oxytetracycline: chlortetracycline was successfully used in treatment. King *et al.* (1952) found the inhibitory concentrations for one strain to be streptomycin 250, chloramphenicol 0.47, and chlor- and oxytetracycline 0.11 μg./ml.: a result for penicillin is not given.

According to Foley (1947) four strains of *Bacteroides funduliformis* and eight of *Bacteroides fragilis* were all resistant to 100 units of penicillin per ml. and inhibited by streptomycin only in concentrations of 1,500–4,000 μg./ml. It seems that these depressing results may have been due to the use of a medium containing thioglycollate in which the antibiotics were inactivated, although the author considers this possibility and discounts it.

According to Willich (1952), "*Bact. pyogenes anaerobium* (Buday)" is the most important member of the "funduliformis group," and four strains of it were resistant to 2 units of penicillin per ml., and inhibited by streptomycin, chloramphenicol, and chlor- and oxytetracycline in concentrations of 250, 1–3, 1, and 6 μg./ml. respectively.

A general impression conveyed by these findings is that *Bacteroides* are sensitive to the tetracyclines and chloramphenicol, particularly the former, and relatively much more resistant to streptomycin. On the other hand, the data are highly discordant on the action of penicillin, the antibiotic likely to be used for most infections in which these organisms are concerned. Moreover, they refer to only two identified species. It therefore appeared desirable to test an adequate number of strains of several species by a uniform method in the hope of clarifying this subject.

TABLE I.—*The Sensitivity of Bacteroides necrophorus to Antibiotics*

Authors	No. of Strains	Inhibitory Concentrations of			
		Penicillin	Streptomycin	Chloramphenicol	Chlortetracycline
Cooper and Robson (1947)	1	0·5			
Ruys (1947)	2	0·5			
Ernst and Hartl (1951)[1]	"A great number"	>10	>10		0·31–0·62
McVay and Sprunt (1952)[2]	14	>2–>10	75–>250		2–40
King *et al.* (1952)	1				3·9
Fisher and McCusick (1953)[3]	5	<0·2–50	50–625	1·6–6·3	<0·19–1·6

Penicillin concentrations are in units/ml., the remainder in μg./ml.

[1] Concentrations for streptomycin and chlortetracyline stated as mg.%, but are presumably meant to be μg./ml.

[2] It is not clear in this paper whether all the strains examined were considered to be of the same species.

[3] Only 5 of the 14 strains were identified as *Bacteroides necrophorus*: the values stated are those for these five, with a possible omission because it is not clear which of two strains from one patient was so identified.

Materials and Methods

Most of the organisms studied were recovered from cultures made for diagnostic purposes on blood agar and incubated in McIntosh and Fildes jars anaerobically with 5% added CO_2 for periods up to one week.

Bacteroides fragilis.—This species was recognized by its regular morphology and translucent circular colony with entire edge, and its identity was confirmed by showing that it produced H_2S in broth, gas from serum peptone water, and no indole. Of the 23 strains examined, one was No. 8560, N.C.T.C., the remainder were all isolated from abdominal lesions, either in direct association with the lower bowel or in areas liable to contamination from it (appendicitis, pelvic abscesses, stitch abscesses following abdominal operations, etc.). An appearance in a penicillin-sensitivity test-plate suggested that one strain of this organism formed penicillinase: this was confirmed by adding a liquid culture of it to penicillin solution, which was found to be destroyed. Of eight other strains available at that time, one also behaved in this way: the remainder did not. Penicillinase formation by this species has not been recorded before.

Bacteroides fusiformis (*F. fusiformis,* Vincent's fusiform bacillus).—This organism was recognized by its morphology, which was perfectly retained in culture, and by its characteristic umbonate colony with an arborescent periphery and irregular edge. This species was found more difficult to isolate and maintain than any other. Despite the kindness of my colleagues in the dental department, which I gratefully acknowledge, in sending me cases of Vincent's gingivitis, only four strains from this source survived long enough to be tested. Of two others, one was obtained from the sputum of a patient with lung abscess, and one from pus from cervical actinomycosis with accompanying bacterial infection.

Bacteroides necrophorus (funduliformis).—Of the four strains examined, one was No. 7155, N.C.T.C., for one I am indebted to Dr. J. M. Alston, and for two to Dr. Per Holm, of the State Serum Institute, Copenhagen.

Bacteroides melaninogenicum.—This organism was first described by Oliver and Wherry (1921), and later more fully by Burdon (1928). Schwabacher *et al.* (1947), showed that the pigment produced in its colonies on blood agar is not melanin but haematin, and proposed that the name *nigrescens* replace the misconceived *melaninogenicum,* a change which awaits but obviously merits official adoption. The organism is extremely common, and would be more often recognized if anaerobic cultures on blood agar were incubated longer. The pigment, which is derived from haemoglobin, cannot be formed until haemolysis has occurred, and is not often seen well in less than five days. The colonies are then of a very deep sepia or actually black colour, with glistening surface, and the medium underlying an area of profuse growth is decolorized. These remarkable colonies, usually mixed with those of other bacteria, on a clear and almost colourless medium shading off into red where growth is less profuse or absent, afford one of the most brilliant pictures in all bacteriology, but its aesthetic merits are much diminished by an abominable odour. The 22 strains examined were isolated from cases with gingivitis and other oral lesions, from sputum, and from several cases of uterine sepsis.

Sensitivity Tests

Antibiotic solution and horse blood freshly lysed with saponin, each in a volume of 0.5 ml., were pipetted into Petri dishes, and 14 ml. of meat extract peptone agar *p*H 7.4, at 47° C., was added and well mixed before setting. Uniform distribution of the blood, which could be seen, gave an assurance that the antibiotic was similarly distributed. Lysed blood was used for two reasons: lysis liberates V factor and, whether or not for this reason, has been found to improve growth; a clear medium is also helpful for inspecting growth and for seeing markings on the under-surface of the dish. This was divided into 12 compartments, and each area so enclosed was inoculated with a different strain of *Bacteroides,* the inoculum being one 1-mm. loopful (loop made of very fine wire) of an undiluted blood broth culture spread over an area about 2 cm. in diameter. The cultures were inspected for presence or absence of growth after two to five days' incubation anaerobically with 5% CO_2 (two days gave adequate growth of *Bacteroides fragilis*; others required longer).

Seven antibiotics were used, doubled dilutions being made from freshly prepared solutions. Oxytetracycline was chosen as representative of the tetracyclines, being preferred to chlortetracycline for its greater stability.

Results

The results are stated in Table II. End-points were almost invariably sharp. In some tests higher concentrations of streptomycin were used than those included in this Table: apart from two strains of *Bacteroides melaninogenicum* resistant to 512 μg./ml., all strains of all species were inhibited by this concentration or one between it and 16 μg./ml. These degrees of sensitivity seem to have no practical consequence.

TABLE II.—*Numbers of Strains of Four Species of* Bacteroides *Inhibited by Different Concentrations of Seven Antibiotics*

Species of *Bacteroides* and Antibiotics	Minimum Inhibitory Concentration (μg. per ml.)												Resistant*	
	0.007	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16		
Fragilis:														
Penicillin												10	13	>16
Streptomycin ...												1	23	>16
Chloramphenicol ...										3	19			
Oxytetracycline ...							12	2	9					
Erythromycin ...									3	5			15	> 4
Polymyxin ...													23	>16
Bacitracin													23	>16
Fusiformis:														
Penicillin				2	2	1	1							
Streptomycin ...										2	2		2	>16
Chloramphenicol								3	2	1				
Oxytetracycline ...					4		2							
Erythromycin† ...									1		1		3	> 4
Polymyxin ...				2		1	1	2						
Bacitracin													6	>16
Melaninogenicum:														
Penicillin	2	5	8	7										
Streptomycin ...											2	6	14	>16
Chloramphenicol								5	8	6	3			
Oxytetracycline ...					6	8	6	2						
Erythromycin ...		1	5	6	6	3	1							
Polymyxin ...				2	2	5	2						11	> 0·5
Bacitracin									4	6	8	3	1	>16
Necrophorus:														
Penicillin				3	1									
Streptomycin ...												1	3	>16
Chloramphenicol								2	1	1				
Oxytetracycline ...					1	1	2							
Erythromycin ...										4				
Polymyxin ...				1	1	1	1							
Bacitracin ...													4	>16

* Numbers given are, first, the number of strains, then the concentration to which they were resistant.
† One strain not tested.

Discussion

Although there is a good deal of strain variation in sensitivity, the results afford a fairly clear picture. Three of the four species are fully sensitive to penicillin: this accords with the majority of published results for *Bacteroides necrophorus*, and is in accordance with expectation for *Bacteroides fusiformis* in view of the therapeutic action of penicillin in Vincent's infection. *Bacteroides melaninogenicum* is very highly sensitive: to know this may be of importance in connexion with a form of subcutaneous gangrene spreading from an abdominal operation wound and accompanied by a foul-smelling dark brown discharge, in two cases of which I have found this organism. On the other hand, *Bacteroides fragilis* is much more resistant, and authors who have reported such resistance in unidentified *Bacteroides* organisms may have been dealing with this species. Since this species is so closely associated with the lower bowel, it might be a sound policy to assume that most *Bacteroides* infections arising in this area will be penicillin-resistant.

Streptomycin clearly has no place in treating any of these infections. Oxytetracycline is consistently more active than chloramphenicol, and it is likely that other tetracyclines will behave similarly : chlortetracycline has been used successfully by several authors already quoted. The superiority of oxytetracycline over any other antibiotic in activity against *Bacteroides fragilis* is interesting in view of the remarkable efficacy of this antibiotic in treating peritonitis due to a perforated viscus, although in this connexion action on other bacteria must also be important. Veillon and Zuber (1898) found this organism constantly present in the pus of appendicitis, and although they are rarely identified, such organisms are frequently numerous in the peritoneal exudate due to perforation of any part of the lower bowel.

Of the remaining antibiotics tested, erythromycin is highly active only against *Bacteroides melaninogenicum*, and this is the only species against which bacitracin has any action at all in the concentrations used. Polymyxin is uniformly active against all species except *Bacteroides fragilis*. It seems unlikely that the use of any of these three antibiotics could be specially indicated for these infections.

The mere existence of a *Bacteroides* infection can often be demonstrated or confidently deduced within a short time, but to isolate and identify the organism and test its sensitivity to antibiotics will take at least a week, although some useful information may be obtained by a sensitivity test (cup or disk method) in primary culture. It is thus desirable to have some general policy for the immediate treatment of these infections. The policy suggested by these results is that one of the tetracyclines should be used for infections originating in the lower bowel, and penicillin for all others, unless they are mixed, when the choice will depend partly on the sensitivities of the other bacteria.

Summary

Existing information about the sensitivity of different species of *Bacteroides* to antibiotics is incomplete and contradictory.

Bacteroides fusiformis (6 strains), *necrophorus* (4), *fragilis* (23), and *melaninogenicum* (22) have been tested for sensitivity to seven antibiotics by a falling dilution method over a full range of therapeutically attainable concentrations.

Penicillin equals or exceeds other antibiotics in activity against three of these species: *Bacteroides fragilis* is resistant to penicillin, and most sensitive to oxytetracycline. All species are relatively resistant to streptomycin and moderately sensitive to chloramphenicol.

A therapeutic policy for these infections is proposed, to be applied in the absence or in advance of laboratory data.

Almost all the difficult and laborious work of isolating and maintaining these strains, as well as the sensitivity testing, was carried out by Miss Pamela M. Waterworth, to whom I express my grateful thanks.

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ASPIRIN AND GASTRIC HAEMORRHAGE

BY

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Since the observations of Douthwaite and Lintott (1938) there has been no doubt that aspirin can cause gastric haemorrhage, but most of the information on this topic has been in the form of reports of one case or of a small number of cases—for example, Hurst and Lintott (1939), Crismer (1947), Dumont-Ruyters (1948), Richmond (1950). There had been little published on its general frequency as a cause of haematemesis or melaena serious enough to cause admission to hospital until Muir and Cossar's (1955) observations that about one-third of patients with peptic ulcer, and about one in twenty of a series of control patients, complained of dyspepsia after taking this drug. They suggested that in one in eight cases of haematemesis aspirin had been "at least a contributory cause" of the bleeding.

In every series of cases of haematemesis and melaena there are some which defy diagnosis in spite of every investigation, including exploratory laparotomy and even post-mortem examination. Probably, as Tidy (1950) says, Hurst (1943) overstated the case when he alleged that about half the "undiagnosed" cases might be found to be due to aspirin. Avery Jones (1952) confessed to "a strong clinical impression" that an occasional case is due to aspirin idiosyncrasy. In the course of a recent survey of a series of cases of gastric and duodenal haemorrhage patients were asked about their "aspirin habits."

The object of this inquiry was threefold : (1) To find out the "aspirin habits" of the patients concerned, especially their reasons for taking the drug, their method of taking it, and whether it usually caused any symptoms. (2) To discover whether any cases might reasonably be attributed to the drug alone—that is, in patients with no evidence of any other possible cause of the bleeding. (3) To investigate the circumstances of any such cases, and, by comparison with reported cases, to find any common features which led to the administration of the aspirin, with a view to preventing a recurrence.

The patients were asked about their habits, and whether they had observed any untoward effects. A few have been omitted, mainly those who were too ill to be interrogated or those who were mentally incapable of giving a coherent answer or even of understanding the question. Of the former, it is almost certain that none were cases of aspirin haemorrhage, the lesion