THE CHEMOPROPHYLAXIS OF GAS GANGRENE

BY

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IT is generally agreed that penicillin contributed substantially to the prevention of gas gangrene in World War II casualties from D Day, 1944, onwards, when systemic administration for this purpose was first regularly practised. At that time no other antibiotic was available. There are now many, including several others with an action on clostridia comparable to that of penicillin. Is any of these now to be preferred to penicillin for this purpose?

Gas gangrene is rare in civilian practice, and an effective comparison of different methods of preventing it would occupy many years, if indeed it could be made at all. Only war provides material for such a study, and it is therefore natural to turn to the war in Korea for possible enlightenment. In fact, experience there, although highly successful in the prevention of this infection, affords little new information about any part which antibiotics can play in this. It is evident from the papers of many authors in Vol. III of the American Army publication *Battle Casualties in Korea* that the evacuation of the wounded on this front, often by helicopter, was exceptionally rapid, and that definitive surgery was carried out after an interval considerably shorter than has been possible in any previous campaign. Adequate and early surgery is much the most important factor in the prevention of gas gangrene, and it is therefore not surprising that this infection was rarely seen. Howard & Inui (1954, 1955) saw only four cases among 4,900 battle casualties during a fifteen-month period; four others were diagnosed at an evacuation hospital among a total of 12,000 casualties. These eight cases are described, and some of them appear to have had ischæmic gangrene rather than true clostridial myositis, if the latter be defined as the invasion of normal muscle from the primary focus. None of them died, six had wounds involving large arteries, (femoral, popliteal, or both anterior and posterior tibial), and in one case "gas gangrene" developed "distal to the tourniquets" on both legs. All casualties were given a moderate dose of penicillin at the battalion aid station. Treatment was continued with penicillin, often with the addition of streptomycin, and sometimes with chlortetracycline. No attempt was made to compare the efficacy of penicillin and chlortetracycline in either prevention or treatment.

PREVIOUS LABORATORY STUDIES

The expectation that antibiotics other than penicillin, and in particular the tetracyclines, may be of value in gas gangrene is based in the first instance on laboratory tests.

In vitro activity. Almost all authors who have studied the action of tetracyclines on clostridia *in vitro* have found them highly active, usually more so

than penicillin, and in most experiments chlortetracycline has been found the most active of the three. Bliss, Warth & Chandler (1950) tested chlortetracycline and oxytetracycline against five species: the former inhibited growth in concentrations five times lower. English, P'an, McBride, Gardocki, van Halsema & Wright (1953-54) in tests with all three tetracyclines against three species also found substantial differences in favour of chlortetracycline. Willich (1952) in tests with three antibiotics against 13 species of clostridia found the order of activity to be oxytetracycline > chlortetracycline > chloramphenicol. Eveland, Newton, Pohutsky, Purdy & Frick (1955) made tests with 103 strains of fourteen species and four antibiotics and found their order of activity to be chlortetracycline > penicillin > oxytetracycline > chloramphenicol. Several papers report tests of strains isolated in Korea. Newton, Strawitz, Lindberg, Howard & Artz (1955) tested 56 strains comprising nine species and found considerable variations in sensitivity to the four antibiotics studied. The range of minimum inhibitory concentrations in μg./ml. was for chlortetracycline 0.0125-0.8, for oxytetracycline 0.05-3.2, for penicillin 1.2-3.8, and for chloramphenicol 1.0-32. They relate these findings to the maximum concentrations of the antibiotics produced in the blood by ordinary doses. On this basis the only antibiotic inhibiting all the strains was chlortetracycline. (In fairness to penicillin it should be observed that the blood levels assumed for this purpose are the comparatively low ones produced by procaine penicillin). The most extensive tests of this kind have been those of Lindberg & Newton (1954-55), who examined 507 strains embracing eighteen species and fifty-nine unidentified organisms, 457 being from wounds in Korea and 50 from soil or clothing. The wound strains were rather less sensitive than the latter group, possibly as the result of antibiotic treatment before material for culture was obtained. Streptomycin was not tested, because of its known inactivity. The order of activity of the four antibiotics tested was chlortetracycline > oxytetracycline > penicillin > chloramphenicol. In a few tests by a simpler method polymyxin was found to be without effect and bacitracin highly active. The findings for 126 of these strains are also reported elsewhere by Lindberg, Wetzler, Marshall, Newton, Strawitz & Howard (1955), whose paper contains the significant sentences: "During the Korean conflict, penicillin was used almost exclusively for the prophylaxis of wound infections. Perhaps the re-evaluation of antibiotic therapy after wounding would lead to the more extensive use of the newer antibiotics."

Tests in vivo. Experimental study of the treatment of gas gangrene is hampered by the fact that the most suitable animal, the guinea-pig, is also the only species to which the administration of penicillin or a tetracycline may itself be fatal. Altemeier, McMurrin & Alt (1950) found both chlortetracycline and chloramphenicol in moderate doses to be as effective as massive doses of penicillin in the prophylaxis of *Clostridium welchii* infection in guinea-pigs. Sandusky, Keeble, Wharton & Taylor (1950) also found these antibiotics effective in *Cl. welchii* infection in guinea-pigs, if administered within a short time of inoculation. Some of the chlortetracycline-treated animals died later, evidently from the toxic action of the drug, and the results given by chloramphenicol were

irregular. Most other workers have used mice. Bliss *et al.* (1950) found penicillin as good as or better than other antibiotics, including chlortetracycline and oxytetracycline, in infection by *Cl. septicum* and *Cl. tetani*. Kiser, Mello, Reichard & Williams (1952) found that the order of merit of penicillin and chlor- and oxytetracycline differed for the five species of clostridia with which they inoculated mice, but chlortetracycline came first against four of them and oxytetracycline against the other (*Cl. novyi*), penicillin always occupying second or third place. The therapeutic tests in mice of Anwar & Turner (1956) were concerned exclusively with infection by *Cl. tetani*. Under the conditions of their experiments the PD_{50} (in mg.) were oxytetracycline 1.4, penicillin 8.1, chlortetracycline 10.6, erythromycin 11.6, and tetracycline 14.1. (Only one dose of antibiotic was given daily, and since potassium penicillin was used, which the mouse excretes very rapidly, the results afford little indication of what is to be expected from dosage ensuring continuous action. The difference in effect between oxytetracycline and the other tetracyclines is remarkable and not vitiated by any such consideration.) Lennert-Petersen (1954) also found oxytetracycline inferior to chlortetracycline in preventing the development of experimental *Cl. tetani* infection in mice. He compared these with no other antibiotic.

None of these laboratory studies covers the entire field of this rather complex subject. Not all pathogenic species of clostridia are represented in any author's tests (although some of these embrace various species of doubtful importance), and about some of the newer antibiotics there is little or no information. It therefore seemed worth while to perform a fresh series of *in vitro* tests to fill these gaps in knowledge and to reassess the activity of the major antibiotics in a comparison made by a uniform method.

EXPERIMENTAL

Strains of clostridia. For the great majority of these I am much indebted to Dr. S. T. Cowan of the National Collection of Type Cultures. Strains from this source are denoted in the table by their N.C.T.C. number. Two named strains of *Cl. welchii* were originally isolated in this department from cases of gas gangrene. "R220" is a strain of *Cl. tetani* received from Professor W. J. Tulloch in 1927, and "Birmingham" a strain recently received from Dr. M. J. Meynell. "R.F.T." is a stock culture of *Cl. sporogenes* maintained in this department.

Method of Test. Solutions of pure antibiotics in sterile distilled water in a series of twofold dilutions were added in 0.5 ml. volumes to 14 ml. volumes of nutrient agar, pH 7.4, together with 0.5 ml. of horse blood previously lysed with saponin (to give a transparent medium), and thoroughly mixed. Plates so prepared were divided into compartments by cutting narrow ditches (since most of the organisms studied give spreading growth) and each area was inoculated with a 1 mm. loopful of undiluted twenty-four-hour Robertson's meat broth culture. The plates were incubated anaerobically with approximately 5 per cent added CO_2 in McIntosh and Fildes jars and read for the presence or absence of growth after twenty-four hours.

Table 1. *Sensitivity of* 10 *species of clostridia to* 16 *antibiotics*
Minimum Inhibitory Concentrations (μg/ml.)

Species	Number or Name	Penicillin	Streptomycin	Chloramphenicol	Chlortetracycline	Oxytetracycline	Tetracycline	Erythromycin	Spiramycin	Oleandomycin	Carbomycin	Novobiocin	Vancomycin	Bacitracin	Polymyxin	Neomycin
Cl. welchii	1265	0.06	>128	4	0.03	0.12	0.25	2*	64	16*	1*	32	1	4	>128	>128
	8237	0.06	>128	4	0.03	0.12	0.12	2*	64	16*	1*	32	1	4	>128	>128
	8246	0.12	>128	4	0.06	0.12	0.12	2*	64	16*	1*	32	2	2	>128	>128
	"Leyton"	0.12	>128	4	0.06	0.25	0.12	2*	32	16*	1*	32	1	4	>128	>128
	"Neighbour"	0.25	>128	4	0.12	0.12	0.12	2*	32	16*	1*	32	1	4	>128	128
Cl. septicum	285	0.03	>128	2	0.06	0.12	0.12	0.5	16	4	1	16	2	8	>128	>128
	501	0.03	>128	4	0.06	0.12	0.12	0.5	16	4	1	16	2	8	>128	>128
	504	0.03	>128	2	0.06	0.12	0.12	0.5	16	4	1	16	1	8	>128	>128
Cl. œdematiens	278	0.015	>128	2	0.015	0.12	0.12	0.25	8	2	0.12	32	1	0.5	>128	>128
	538	0.007	>128	2	0.06	0.12	0.12	0.25	4	2	0.12	32	0.5	0.25	>128	>128
	2908	0.007	>128	2	0.03	0.06	0.06	0.12	8	4	0.12	64	0.5	0.25	>128	>128
Cl. tetani	279	0.007	>128	2	0.12	0.25	0.25	0.5	8	4	0.12	0.06	16	1	128	>128	>128
	9567	0.03	>128	2	0.12	0.25	0.12	0.5	8	4	0.12	0.12	16	1	>128	>128	>128
	9572	0.015	>128	4	0.06	0.25	0.06	0.5	8	4	0.25	0.12	16	2	>128	>128	>128
	"R220"	0.015	>128	2	0.06	0.25	0.03	0.5	8	4	0.12	0.12	16	2	>128	>128	>128
	"Birmingham"	0.3	>128	2	0.03	0.06	0.03	0.25	8	4	0.25	0.12	16	2	>128	>128	>128
Cl. histolyticum	503	0.03	64	4	0.03	0.03	0.06	2	4	8	0.25	0.25	8	2	128	>128	>128
	2915	0.03	128	2	0.06	0.25	0.06	1	4	4	0.25	0.25	8	1	128	>128	128
	7123	0.03	64	4	0.12	0.25	0.25	2	8	4	0.25	0.25	8	2	>128	>128	128
Cl. sporogenes	532	0.12	>128	2	0.03	0.06	0.06	1	8	16	0.25	0.25	64	8	>128	>128	>128
	534	0.06	>128	2	0.06	0.25	0.06	1	16	16	0.5	0.25	64	8	>128	>128	>128
	"R.F.T."	0.12	>128	2	0.06	0.25	0.06	1	8	16	0.5	0.25	64	8	>128	>128	>128
Cl. bifermentans	1341	0.12	>128	8	0.06	0.25	0.25	0.12	8	2	0.25	0.25	64	0.5	8	>128	>128
	6798	0.25	128	8	0.03	0.06	0.06	0.12*	16	16*	1	0.12	16	2	128	>128	>128
Cl. sphenoides	507	2	64	8	0.06	0.12	0.12	0.5	8	8	1	0.25	16	1	>128	>128	128
Cl. tetanomorphum	288	0.12	>128	2	0.03	0.12	0.06	0.5	8	1	0.5	0.06	16	4	>128	>128	>128
	500	0.25	>128	2	0.03	0.06	0.06	1	8	4	0.25	0.12	8	1	>128	>128	>128
Cl. tertium	541	1	128	1	0.03	0.06	0.06	1	16	16	0.5	0.12	8	2	2	>128	>128
	2917	1	>128	4	0.03	0.06	0.06	1	32	16	0.5	0.25	16	4	8	>128	>128

* Concentration inhibiting normal growth : See text.

RESULTS

The minimum inhibitory concentrations so determined of 16 antibiotics for the 29 strains of 10 species of clostridia studied are stated in Table 1. All the results were quite clear-cut except for the action of erythromycin, oleandomycin and carbomycin on all strains of *Cl. welchii*. Normal growth was inhibited by the concentration stated, but on medium containing two, or even four, times this amount there was a faint haze of growth consisting microscopically of exceedingly long Gram-positive filaments.

It is at once apparent that streptomycin, neomycin and polymyxin can be excluded from further consideration. The activity of novobiocin is also too low to afford any promise of clinical usefulness. The same may be said of bacitracin, which affords the only example of wide inter-species differences, only one, *Cl. œdematiens*, being highly sensitive to it. In what may be called the erythromycin group, spiramycin and oleandomycin are much less active than erythromycin or even than carbomycin, which is actually more inhibitory to some species than erythromycin itself, a finding in marked contrast to their relative activity against most bacteria. Antibiotic E129 (Garrod & Waterworth, 1956) is uniformly and fairly highly active. The activity of chloramphenicol is strikingly uniform, but at an unpromisingly high concentration level. That of vancomycin is similar, and in view of the present difficulty of administering this antibiotic, its use for this purpose is not in any case likely to be considered. These observations therefore serve to show that none of the newer antibiotics can rival penicillin and the tetracyclines for the prevention of clostridial infection.

The arithmetic means in μg./ml. of the minimum inhibitory concentrations of these four major antibiotics for all 29 organisms, in descending order of activity, are chlortetracycline 0.055, tetracycline 0.106, oxytetracycline 0.147, penicillin 0.215. There is not much strain variation in sensitivity to these antibiotics, nor are most of the interspecies differences wide, although it is noteworthy that penicillin is actually more active than the tetracyclines against some of the more important species (*Cl. septicum*, *Cl. œdematiens* and most strains of *Cl. tetani*), and owes its position in the order of activity to the lesser sensitiveness of relatively unimportant organisms lower in the list. Excluding the aberrant results with two of these species, the arithmetic mean of its inhibitory concentrations becomes 0.088, ranking it second in order of activity.

Tests of combined action. In view of the possibility that penicillin and a tetracycline may be administered together, it is necessary to know how they act in combination. Determinations of minimum inhibitory concentrations were made by the same method, on medium containing a suitable range of concentrations of penicillin and chlortetracycline alone, and containing varying concentrations of each and fixed concentrations of the other (the latter were penicillin, 0.015 and 0.003 μg./ml. and chlortetracycline, 0.03 μg./ml.). The organisms so tested were four strains of *Cl. welchii* and three each of *Cl. œdematiens*, *Cl. septicum* and *Cl. tetani*. The results need not be given in full, because they are covered by the statement that when both antibiotics were present in in-

dependently effective concentrations, the result was an additive effect. No suggestion of antagonism was seen.

An attempt was also made to study independent and combined bactericidal action by adding antibiotics singly and in various combinations, in the fixed concentration of 10 μg./ml., to blood broth, inoculating fairly heavily and subcultivating on blood agar after overnight anaerobic incubation. The results of this proceeding were irregular and difficult to interpret, probably for three reasons: the difficulty of ensuring that the culture used contained no spores, which would in any case probably not be killed, the impossibility of judging the proportion of survivors of organisms with a spreading habit of growth, and the possibility that a sterile subculture might only exemplify the reluctance of clostridia to grow from a small inoculum.

DISCUSSION

It appears from these results that none of the more recent antibiotics is likely to have any outstanding value for the prevention of clostridial infection. The findings for penicillin and the tetracyclines agree generally with those of previous workers, and it is clear that reliance must be placed on one of these. The question is: does the *in vitro* superiority of chlortetracycline indicate that it should replace penicillin for this purpose?

Any answer to this question can only be an opinion, since there are no adequate therapeutic comparisons, either experimental or clinical, to provide the necessary confirmation. The differences in activity of these four antibiotics are small, and it is likely that any of them given in adequate doses, and in the favourable circumstances of civilian practice, would achieve its object. Which of them is to be preferred for a battle casualty whose evacuation and surgical treatment may be delayed? There is a serious objection to the use of tetracyclines in these circumstances, namely, that absorption of an orally administered drug may be unsatisfactory in a man suffering from shock. Intravenous administration is obviously impracticable in the field. For this reason, and because its worth has been proved, penicillin may still be considered the antibiotic of choice. Casualties in Korea were given 300,000 units (sometimes 600,000) of procaine penicillin. Newton *et al.* (1955) found that not all their Korean strains of clostridia were inhibited by the concentration of penicillin known to be attained in the blood after these doses of this form of penicillin, and it is difficult to understand why the preparation used did not contain some sodium or potassium penicillin to give higher blood levels, or why the dose was not larger. A greater margin of safety would be afforded by an initial dose of say 400,000 units each of potassium and procaine penicillin, repeated at intervals of 6 hours.

SUMMARY

The action of 16 antibiotics on 29 strains of 10 species of clostridia has been studied *in vitro*. None of the newer antibiotics, about which there is little or no previous information, has any outstanding activity.

The arithmetic means of the minimum inhibitory concentrations for all these organisms of the four most active antibiotics were (in μg./ml.) chlortetracycline 0.055, tetracycline 0.106, oxytetracycline 0.147, penicillin 0.215; excluding the exceptional results with two species of lesser importance, the mean for penicillin was 0.088.

Reasons are given for the belief that penicillin should be preferred to the tetracyclines for the immediate prophylaxis of gas gangrene in battle casualties.

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