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An effective antibiotic cover for the prevention of endocarditis following dental and other post-operative bacteraemias[1]

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SYNOPSIS Pre- and post-extraction blood cultures were taken from 242 patients. The post-extraction ones were taken from 100 unpremedicated patients, from 42 with an erythromycin estolate cover, and from 100 patients after protection with pyrrolidino methyl tetracycline. The 100 post-extraction blood cultures from unpremedicated patients gave 64 positive results which yielded 155 strains, 88 of which were not aerobes.

One hundred and fifteen representative strains were tested for sensitivity to 22 antibiotics. Of the 42 patients who received the erythromycin orally, 16 yielded positive blood cultures of mixtures of aerobes and anaerobes and of the 100 given one intravenous injection of the tetracycline three only developed a bacteraemia of a single type of aerobe. The serum concentrations obtained with the tetracycline given intravenously were 15 to 20 times higher than the serum levels obtained with the erythromycin given orally.

There is a strong indication for using this kind of efficient antibiotic cover for dental extractions and other operative procedures known to be followed by a bacteraemia.

Bacterial endocarditis is in many cases the result of bacteraemia produced by operative procedures, among which is tooth extraction. It is stated that 25 to 50% of endocarditis patients had one or more teeth extracted some two months before the onset of the valvular ailment (Cates and Christie, 1951). Northrop and Crowley (1943) reported 23 such cases, and 94 other cases are on record by various other authors.

The bacteraemia of tooth extraction is transient, lasts about 10 minutes (Northrop and Crowley, 1943; Okell and Elliott, 1935), and the bacteria are most numerous in the blood stream within the first two minutes of their introduction into the circulation (Reichel, 1939). In persons with heart valves damaged by rheumatic fever, syphilis, or a congenital defect, the platelet-and-fibrin thrombi on the surface of the diseased valves trap some of the bacteraemic organisms, which then multiply and start an endocardial vegetation. The circulation time from the site of a tooth socket to an elbow vein is less than 18 seconds (Koch, 1922).

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For an antibiotic cover during dental extraction various investigators tried sulphonamide compounds, penicillin, and other antibiotics, but all except one failed to isolate one single anaerobe in their unpremedicated post-extraction control blood cultures. Using sulphanilamide, Pressman and Bender (1944) obtained 7% reduction (from 83% to 76%) and Northrop and Crowley (1943) 5·4% reduction (from 15% to 9·6%). With penicillin the reductions varied from 9% (Hirsch, Viveno, Merril, and Dowling, 1948) to 42% (Schirger, Martin, Royer, and Needham, 1960). With antibiotics other than penicillin the reductions were 61% (Khairat: the present work, the highest reduction ever obtained, in a series of 100 patients with 88 anaerobes isolated), 44% (Roth, Cavallaro, Parrott, and Celentano, 1950) with aureomycin orally, 28% (Bender, Pressman, and Tashman, 1958) using streptomycin sulphate intramuscularly, and 25% (Cooley and Haberman, 1957) with terramycin orally. The failure of some antibiotics completely to prevent the bacterial invasion of the blood stream after exodontia explains the occasional occurrence of endocarditis despite a penicillin cover.

In most of the published work no anaerobic culture was attempted. The present work completes the

picture of the bacteraemic risks by determining the incidence of anaerobes as well as aerobes and by finding a safe antibiotic effective against both.

METHODS

THE BLOOD CULTURES This paper deals with a study of the incidence of positive blood cultures after tooth extraction in otherwise healthy patients; of the types of aerobic and anaerobic bacteria concerned; and the sensitivity of the freshly isolated bacteraemic strains to all the currently used antibiotics, and of the effect of premedication with two antibiotics on the incidence of positive blood cultures.

The incidence and nature of the bacteraemia was established in a series of pre- and post-extraction blood cultures from 100 unmedicated patients taken at random, regardless of the presence of pyorrhoea or of the number of teeth extracted. By sensitivity tests *in vitro*, two antibiotics were likely to be most active against the wide variety of bacterial species encountered in this condition. A second series of 142 patients, also taken at random, was tested: 42 after premedication with erythromycin estolate (Ilosone Lilly) orally, and 100 after premedication with pyrrolidino methyl tetracycline (Reverin Hoechst) intravenously.

For each of the pre- and the post-extraction blood cultures 30 ml. blood samples were taken by a modification of the technique described by Khairat (1939): three 7 ml. volumes of the blood were each added to 14 ml. of 0·05% Liquoid broth, 3 ml. into 6 ml. Liquoid broth for making three pour plates and 5 ml. for a sample of the patients' serum for antibiotic assay. The serum was removed from the clot after one hour and stored at −20°C. A blood broth and one pour plate were incubated aerobically, another set in air containing 5% CO_2 (Khairat, 1964), and the third in an anaerobic jar fitted with a room temperature Deoxo catalyst (Khairat, 1965). The pour plates were made within three minutes of taking the blood sample.

The three blood broths were not subcultured before the sixth day even when macroscopic indications of growth appeared earlier, to allow slow-growing anaerobes to multiply. No colony was picked from the subcultured blood agar plates except under a stereo microscope, and no blood culture was discarded as sterile unless plate subcultures of the three blood broths showed no growth after five days' incubation in the three atmospheres.

TESTS *in vitro* OF ANTIBIOTIC SENSITIVITY These were made for each of 115 of the freshly isolated bacteraemic strains, by flooding three blood agar plates with a young broth culture of the strain, 18 hours' culture for aerobic and CO_2-dependent organisms, and 48 hours' culture for anaerobes, diluted 1:5. Excess culture fluid was removed and 15 minutes later commercially obtained sensitivity discs containing the lowest available concentration of antibiotic or other chemotherapeutic agent were applied, eight to each plate. The plates were incubated in the atmosphere most suitable for the organism. The 22 antibacterial substances used are listed in Table II. The 115 representative organisms tested were : 37 viridans streptococci including CO_2-dependent ones, 18 anaerobic streptococci, a *Str. faecalis*, 37 corynebacteria including non-aerobic corynebacteria, eight *Bacteroides corrodens* strains never before isolated from the circulating blood (Wilson and Miles, 1964, p. 623), two *Fusiformis* strains, two *Bacteroides melaninogenicus*, two other *Bacteroides* strains, three *Neisseria pharyngis*, one *N. catarrhalis*, two *Veillonella orbiculus*, one *Klebsiella aerogenes*, and one *Micrococcus*.

ASSAY OF ANTIBIOTIC IN SERUM For the assay of erythromycin estolate and pyrrolidino methyl tetracycline's blood concentration, the patient's serum was serially diluted twofold in broth. A 16 hours' broth culture of *Staph. aureus* (A.T.C.C. 6538 P) was diluted 1/20 and 0·5 ml. added to each 0·5 ml. of serum-broth mixture. The end-point of inhibition was recorded as the serum dilution totally inhibiting growth after 16 hours' incubation. This strain of *Staph. aureus* proved the most suitable test organism for both erythromycin estolate and pyrrolidino methyl tetracycline, as judged by previous tests with it in parallel with *Sarcina lutea* (*Micrococcus luteus*) (A.T.C.C. 9341), *B. subtilis* (A.T.C.C. 6633), *B. cereus* (A.T.C.C. 11778), and *Str. pyogenes* (A.T.C.C. 8668).

RESULTS

BACTERAEMIA IN UNMEDICATED PATIENTS All but one of the 242 pre-extraction blood cultures were sterile. The one exception yielded an albus type of staphylococcus, which, since the pour-plates indicated an average of 40 colonies per millilitre of circulating blood, was clearly not a contaminant. Indeed *Staph. albus* (nine colonies per ml.) was isolated from the same patient 11 days later from a pre-extraction blood culture.

From the 100 unpremedicated patients, the post-extraction blood cultures were positive in 64, yielding a total of 155 strains (Table I). Eighty-eight of the strains were non-aerobes and 67 were aerobes (Khairat, 1966). The non-aerobes included five strictly CO_2-dependent organisms, some of them CO_2-dependent viridans streptococci, 46 obligate anerobes, and 37 which grew both in hydrogen and in air containing 5% CO_2. One third of the 64 positive blood cultures grew one type of organism, and the maximum number of different types of bacteria growing in one blood culture was seven. Half the aerobes were viridans streptococci; some of the anerobic streptococci isolated produced black colonies. The degree of bacteraemia was such that in 75% of the positive cases the pour plates indicated that the circulating blood contained one colony per millilitre.

Neither 'rocking' (wrenching) during teeth extraction, *i.e.*, excessive trauma, nor epinephrine in the local anaesthetic had any effect on the number of positive blood cultures obtained.

TABLE I

THE SPECIES DISTRIBUTION OF 115 POST-EXTRACTION STRAINS FROM 100 UNPREMEDICATED PATIENTS AND THEIR GASEOUS GROWTH REQUIREMENTS

Strain	*Growth*				*Total*
	Aerobically and in Other Atmospheres	*Only in Air containing 5% CO_2*	*Only Anaerobically*	*Only Anaerobically and in Air Containing 5% CO_2*	
Viridans streptococci	34	1	—	9	44
Anaerobic streptococci	—	—	13	7	20
Black colonied anaerobic streptococci	—	—	4	—	4
Str. pyogenes	2	—	—	—	2
Str. faecalis	1	—	—	—	1
Corynebacteria	21	3	2	16	42
Bacteroides corrodens	—	—	16	—	16
Fusiformis organisms	—	1	3	5	9
Bacteroides melaninogenicus	—	—	2	—	2
Other *Bacteroides* species	—	—	4	—	4
Neisseria pharyngis	3	—	—	—	3
N. catarrhalis	1	—	—	—	1
Veillonella orbiculus	—	—	2	—	2
Staphylococcus aureus	2	—	—	—	2
Staph. albus	1	—	—	—	1
A Gram-positive *Micrococcus*	1	—	—	—	1
Klebsiella aerogenes	1	—	—	—	1
Totals	67	5	46	37	155

The significant number of CO_2-dependent and anaerobic bacteria isolated emphasizes the importance of making blood cultures (and indeed all cultures) in triplicate and incubating not only in air, but also in the other two atmospheres used (Wilson and Miles, 1964).

ANTIBIOTIC SENSITIVITY TESTS The test *in vitro* of 115 freshly isolated post-extraction bacteraemic strains with 22 antibiotics and antibacterial chemotherapeutic substances indicated four antibiotics that inhibited over 85% of the strains (Table II). Excluding the two furadantoins, the four were erythromycin estolate, demethyl chlor-tetracycline (Declomycin or Ledermycin, Lederle), tetracycline and ampicillin (Penbritin Beecham) as shown in Table II. Of these, erythromycin estolate and tetracycline in the form of the pyrrolidino methyl compound were selected for clinical trial.

The broad-spectrum penicillin ampicillin was not considered further because of the frequency of anaphylactic reactions associated with the use of penicillins. Erythromycin estolate was chosen for clinical testing not only because it gave good results *in vitro* (Table II) but because blood concentrations (in the region of 1 to 2 μg./ml.) are attainable one hour or even 30 minutes after administration, a feature of some importance for the controlled premedication of dental out-patients with little attendant risk of the development of resistant strains

TABLE II

SENSITIVITY OF 115 FRESHLY-ISOLATED POST-EXTRACTION BACTERAEMIA STRAINS TO 22 ANTIBIOTICS AND ANTIBACTERIAL COMPOUNDS

Antibacterial Agent	*Number of Strains Tested*	*Number of Resistant Strains*	*Percentage of Sensitive Strains*
Furadantin, 100 units	114	3	96·5
Erythromycin estolate, 2 μg.	86	6	93·0
Demethyl chlor-tetracycline, 5 μg.	115	10	91·3
Tetracycline, 5 μg.	115	13	88·7
Altafur, 50 units	79	9	88·6
Ampicillin, 2 μg.	115	16	86·1
Erythromycin (stearate), 2 μg.	110	21	80·9
Penicillin, 2 units	115	27	76·6
Chloramphenicol, 5 μg.	113	28	75·2
Vancomycin, 5 μg.	113	36	68·1
Novobiocin, 5 μg.	114	38	66·7
Ristocetin, 5 μg.	110	38	65·5
Bacitracin, 2 units	112	39	65·2
Oleandomycin, 2 μg.	81	36	55·6
Soframycin, 100 units	93	47	49·5
Gantrisin[1], 1 mg.	114	86	24·6
Kanamycin, 5 μg.	114	96	15·8
Polymyxin B, 50 units	113	97	14·2
Streptomycin, 2 μg.	114	103	9·6
Colymycin, 2 μg.	114	105	7·9
Neomycin, 5 μg.	115	109	5·2
Nystatin, 100 units	40	40	0·0

[1]Representing all the sulphonamides.

(Garrod and Waterworth, 1962). The furadantoins and Altafur were included only for their bacteriological interest, being compounds unsuitable for use for the treatment of bacteraemia. Pyrrolidino methyl

tetracycline was chosen because it had been in use since 1957 without any recorded toxic sequelae, including thrombophlebitis.

ANTIBIOTIC PROPHYLAXIS AGAINST BACTERAEMIA The results of premedication with erythromycin estolate and pyrrolidino methyl tetracycline are summarized in Table III.

TABLE III

CLINICAL TRIAL OF PREMEDICATION WITH TWO ANTIBIOTIC COVERS

Antibiotic Cover	*Dose (mg.)*	*Timing (min.) of Premedication before Extraction*	*Number of Patients*	*Number of Positive Blood Cultures*
Nil. (controls)	—	—	100	64
Erythromycin estolate, orally	250	90	16	6
	500	90	9	3
	500	120	2	2
	500	240	6	2
	1000	240	9	3
Pyrrolidino-methyl tetracycline, intravenously	275	3	100	3

Erythromycin estolate With one 250 mg. erythromycin estolate capsule orally, six cultures were positive, yielding different combinations of anaerobic and viridans streptococci, corynebacteria, *N. pharyngis* and *Bacteroides melaninogenicus*. Neither a twofold nor a fourfold increase of the dose, nor an increase up to four hours in the period for absorption of the drug, improved on this result. The dose was changed as soon as positive blood cultures were obtained. The bacteria in the 10 remaining positive blood cultures included those named above and also *Fusiformis* and *Veillonella* spp. The dose could not be further increased for fear of toxic effects. Thus erythromycin estolate failed as a certain antibiotic cover, protecting only 26 of the 42 patients: 38% of the cultures were positive, a confidence limit of 23·5 to 54·3% positive.

The strains isolated from the 16 failures were all sensitive *in vitro* to 2 μg. erythromycin estolate discs, *i.e.*, they were very sensitive to it. But even after the full 1 g. dose given four hours before extraction, the *Staph. aureus* inhibition titre of the serum varied from 1/8 to 1/32, an average of 1/20. A serum titre of 1/32 is equivalent to 1·0 μg./ml. of Ilosone four hours after a dose of 250 mg. (Perry, Hall, and Kirby, 1958).

It was perhaps too much to expect that a concentration of drug sufficient to destroy a bacteraemic infection could be obtained in the blood by giving a single oral dose. The same applies to intramuscular injection and to slow intravenous infusion drip.

Pyrrolidino methyl tetracycline Effective concentrations would clearly be more readily obtained by intravenous administration of an antibiotic in a concentrated form given immediately before tooth extraction. Accordingly pre-operative injections of 275 mg. pyrrolidino methyl tetracycline dissolved in 10 ml. sterile distilled pyrogen-free water were given to 100 patients. The injections were made very slowly, in two to three minutes, and as far as possible, the tooth or teeth were extracted immediately afterwards. The serum titre of pyrrolidino methyl tetracycline in samples taken at the time of blood culture ranged between 1/256 and 1/512, corresponding to a blood concentration of 18 to 25 μg./ml. (Wagner, 1964) which was 15 to 20 times the concentration achieved after the oral erythromycin treatment. The bacteriological results were striking: only three of the 100 patients gave positive blood cultures, each due to a single type of bacterium, viridans streptococci in two cases, and a corynebacterium in one case. It is noteworthy that these were all aerobic species. This was a reduction in the number of the bacteraemia strains isolated, from 57 aerobes plus 88 non-aerobes (a total of 155) to three aerobes, and a reduction in the positive blood cultures of 61% (from 64% to 3%). The two viridans strains were resistant to 5 μg. but not to 30 μg. tetracycline discs (there are no Reverin discs). The corynebacterium was sensitive to both concentrations.

Of the 100 patients receiving a pyrrolidino methyl tetracycline cover, none manifested any side effects, except that about half of them experienced a taste of soap or ether during the injection.

DISCUSSION

The corynebacteria isolated from the unpremedicated patients were not contaminants, for none of the pre-extraction blood samples, taken from the same patients two or three minutes before the post-extraction ones, yielded any organisms. All samples were taken by the author, using exactly the same technique.

Though no antibiotic inactivator was incorporated in the blood culture medium, since none is known for the erythromycins or for the tetracyclines, one of the two antibiotics used failed in its series of blood cultures whereas the other did not.

In testing 115 representative strains against 22 antibiotics to determine their sensitivity precisely, the results with the disc method only give a rough guide to the sort of antibiotic that might be used, and despite the large number of strains tested (Table II), the same applies to the percentage differences between the closely allied drugs, especially since no accurately

assayed control for each disc (obtainable from each manufacturer) was put.

As to the large number of anaerobes isolated in the present work, this might explain why many of the endocarditis patients with certain clinical signs repeatedly give negative (aerobic) blood cultures. The cause is undoubtedly lack of proper anaerobic culturing technique.

Although a few of the 22 antibacterial drugs tested are intended for topical use only, *e.g.*, soframycin, or are mainly active against fungi, *e.g.*, nystatin, they were nevertheless tested since there is little recorded information about sensitivity of the various aerobic and anaerobic dental and oral flora to the antibiotics in common use. It should be stressed that most of the dental bacteraemia organisms isolated were Gram positive, and, as was to be expected, were not susceptible to antibiotics active mainly on Gram-negative bacteria.

The explanation of failure in the three cases is not surprising because even at bactericidal concentrations a few organisms commonly persist and on incubation the drug will degenerate to less than the bacteriostatic level which will allow growth. From a practical point of view this does not matter because in the patient natural antibacterial mechanisms would be at work to demolish the few persisting organisms, whereas in the culture such mechanisms are inactive.

The pour plates of these patients showed very low counts: one colony per millilitre in two of the cases and no colonies in the third. Low pour plate-counts were also met with in the 16 erythromycin estolate failures. However, the argument that because there were few colonies in the pour plates the original number released into the blood was low is not valid, because as the blood contained 18 to 25 μg./ml. of the tetracycline, the concentration in the plates (assuming 15 ml. medium per plate) was between 1·2 and 1·6 μg./ml. which may have been high enough to inhibit primary growth, allowing just a few comparatively resistant colonies to appear. However, without the use of any antibiotic cover the degree of bacteraemia in 64 positive post-extraction blood cultures was very low: one colony per millilitre of the circulating blood in 75% of these positive cases (Khairat, 1966). It is thus obvious that there cannot have been a large number of highly resistant organisms in the blood in these three cases.

The sensitivity *in vitro* of the 115 freshly isolated bacteraemia strains to both antibiotics was almost similar, but the 20 times higher blood levels of the intravenous pyrrolidino methyl tetracycline over the oral erythromycin estolate explains why the first succeeded and the second failed in combatting the bacteraemia. During the 10 minutes following an injection of 275 mg. pyrrolidino methyl tetracycline (which is the usual duration of the transient bacteraemia) the average blood level starts at 27 to 30 μg./ml. and ends with 14 to 19 μg./ml. (Wagner, 1964). This concentration at the end of 10 minutes is still 10 to 15 times the concentrations attained with oral antibiotics. A concentrated intravenous antibiotic, such as pyrrolidino methyl tetracycline, is certainly more likely to kill the bacteria as soon as they are released into the circulation and before they establish a foothold on the heart valves, or at least more likely to damage the bacteria within the 10-minute period of bacteraemia. It must be realized however, that pyrrolidino methyl tetracycline sterilized blood cultures but that it might not necessarily sterilize a bacteraemia. However, it can be properly concluded that pyrrolidino methyl tetracycline was active against anaerobes as well as aerobes and that, in so far as one can argue from the results obtained in the present work, a substance of this class would be a good choice in any attempt to give cover before operative procedures, using the intravenous route, for a rapidly attained high concentration of circulating antibiotic.

Bender *et al.* (1958) administered 1 g. chloramphenicol in a single intravenous dose to 32 patients half an hour before extraction and obtained a reduction from 85% to 22% positive blood cultures though they isolated no anaerobes in their work. They did not incorporate an inactivator in their blood culture medium as there is no inactivator known for chloramphenicol.

Although Roth *et al.* (1950), who used aureomycin orally in a small group of 25 patients, claimed a reduction from 64% to 4% (the 4% figure ought to be corrected to 20% because they dropped four positive blood cultures which yielded albus staphylococci and 'diphtheroids'), counting these as contaminants even though the organisms grew in both the blood broths and the pour plates, and in addition the pre-extraction samples from all 25 unpremedicated controls were free from contaminants.

With the exception of the work of Francis, De Vries, Soomsawasdi, and Platonow (1962), who isolated 30 anaerobic strains though they attained a reduction of only 28%, from 36% to 8%, in a series of 25 patients given penicillin, none of the other workers who tested an antibacterial cover record any cultivation of anaerobes. Their recorded reductions should therefore be further drastically lowered, for it would be grossly unfair to compare their reductions, which varied from 5·4% to 44%, with the 61% reduction in the present work where 88 non-aerobes were isolated in addition to 76 aerobes.

As to whether an effective antibiotic cover such as pyrrolidino methyl tetracycline should be used for all dental extractions, opinions differ. All agree that

there is a good case for its use in patients known to have had rheumatic fever, and certainly in those with signs of valvular disease. There is a strong case for using an effective antibiotic cover as a precautionary measure before every extraction and also before other operative procedures known to be followed by a bacteraemia, such as tonsillectomy, since not every patient remembers if he or she has had an attack of rheumatic fever in childhood, and we should not forget that some affected valves are murmurless; the preparation and injection of the antibiotic do not take more than four minutes; that this intravenous antibiotic gave negative blood cultures in 97% of the cases immediately following the extractions when the bacteraemia was at its height; and because in addition to its efficacy the blood concentration of pyrrolidino methyl tetracycline remains high long enough to deal with bacteria that might settle on the valves (Wagner, 1964). McGregor (1962) favours this course, at least 'to protect murmurless valves'.

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