*Br. J. Pharmac.* (1971), **43**, 242–247.

# Comparison of human serum, parotid and mixed saliva levels of phenoxymethylpenicillin, ampicillin, cloxacillin and cephalexin

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**Summary**

1. A study has been made of serum, mixed and parotid salivary levels attained in normal volunteers following oral dosage of 500 mg phenoxymethylpenicillin tablets, 500 mg crushed phenoxymethylpenicillin tablets in capsules, 500 mg ampicillin, 500 mg cloxacillin and 500 mg cephalexin.

2. High mixed saliva levels were obtained with phenoxymethylpenicillin tablets and it is considered that these were due to rapid intra-oral dissolution of surface powder from friable tablets. No saliva levels were detected when tablets from the same batch were put into capsules.

3. Low or no saliva levels were achieved with ampicillin, cloxacillin and cephalexin.

4. The mode of action of antibiotics in oral infections is discussed.

## Introduction

To date, estimations of benzylpenicillin in saliva have shown that it is present in minute quantities compared with serum levels (Bender, Pressman & Tashman, 1953a; Pellerat, 1963). Similar studies of phenoxymethylpenicillin and cloxacillin do not appear to have been carried out but levels of ampicillin in sputum and saliva have been reported (May & Delves, 1965; Stewart, Fisher, Young & Lutz, 1970). Cephalexin is a recently introduced, orally administered, cephalosporin antibiotic, which is structurally similar to ampicillin. Salivary secretion of cephalexin has not been reported.

Earlier work in the field is of limited value either because the method of obtaining saliva is not stated (Pellerat, 1963; May & Delves, 1965; Stewart *et al.*, 1970) or else mixed saliva ('spit') was studied (Bender *et al.*, 1953a, b, c).

As mixed saliva consists of parotid, submandibular, sublingual and minor gland saliva together with oral bacteria, desquamated epithelial cells and gingival fluid,

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no information is provided regarding levels attained in the individual gland secretions. Because there are physiological differences between salivary glands (Burgen & Emmelin, 1961) it is possible that there may be variations in antibiotic passage through each gland type.

Technically, it is simplest to compare parotid and mixed saliva. In this preliminary investigation we have studied levels of phenoxymethylpenicillin, ampicillin, cloxacillin and cephalexin in human serum, mixed saliva and parotid saliva.

## Methods

Fourteen freely consenting volunteers with no history of drug allergy or salivary gland disease were studied. All were hospital clinicians or students. There were ten males and four females whose ages ranged from 21 to 32 with a mean of 26 years. All began an experiment on one or more occasions following an overnight fast which remained unbroken until one hour after medication. Each subject swallowed 500 mg (2×250 mg) of each antibiotic with 50 ml water as quickly as possible to avoid prolonged contact with the oral mucosa. Those who received phenoxymethylpenicillin tablets B.P. repeated the test with two gelatin capsules each containing one crushed tablet from the same batch. All antibiotics were obtained from a hospital pharmacy.

For the mixed salivary collection, volunteers chewed two clean rubber bands as described by Shannon (1962). The volume accumulated during the first 5 min was discarded, after which 1·5 ml was collected in a sterile bijou bottle. Samples were taken immediately before treatment and subsequently at 0·5, 1, 3 and 6 hours.

Parotid secretion was collected using a sterile Carlson-Crittenden cup, modified as described by Mason, Harden, Rowan & Alexander (1966). Suction to the outer chamber was via a connected dental chip syringe. The cups were sterilized between patients by ethylene oxide gas (Weymes, 1966) and were not used until 24 h after gas exposure. Between sampling sessions, cups and connecting tubing were rinsed with a sterile water-jet, dried by compressed air and returned to their original containers to avoid contamination. Parotid saliva was stimulated using 0·5 ml 10% citric acid solution every 30 seconds. The initial 1·5 ml was discarded and a further 1·5 ml collected in another sterile bijou bottle. Specimens were obtained immediately after the mixed salivary samples.

Ten millilitres of blood were taken by venepuncture before medication and subsequently at 1 and 3 hours.

Serum and saliva samples were frozen and transported within 3 h to the laboratory where they were stored at −20° C until assays were carried out during the next 3 days.

To estimate antibacterial activity in small volumes of saliva, a fish-spine diffusion technique was used (Garrod & O'Grady, 1968a). The test plates were 9 cm diameter Petri dishes containing 10 ml nutrient agar at pH 7 (Oxoid peptone 0·5%, Oxoid lablemco 0·3%, sodium citrate 1%, Oxoid agar (No. 3) 1·2%) preseeded with a *B. subtilis* spore suspension. Parotid and mixed saliva specimens were tested undiluted with the exception of mixed saliva specimens taken at 0·5 and 1 h from phenoxymethylpenicillin tablet treated volunteers which were tested also at dilutions

of 1/2, 1/10 in phosphate buffer pH 6. Postmedication sera were tested undiluted and in dilutions of 1/2, 1/10 and 1/20 in human plasma.

Graphs were made from standards in phosphate buffer pH 6 and plasma as follows: phenoxymethylpenicillin and ampicillin 0·03, 0·06, 0·12, 0·25, and 0·5 μg/ml; cloxacillin 0·12, 0·25, 0·5, 1 and 2 μg/ml and cephalexin 2·5, 5, 10 and 20 μg/ml.

## Results

The levels obtained in serum, mixed saliva and parotid saliva after 2×250 mg phenoxymethylpenicillin tablets, 2×250 mg crushed phenoxymethylpenicillin in gelatin capsules, 2×250 mg ampicillin capsules, 2×250 mg cloxacillin capsules and 2 × 250 mg cephalexin monohydrate capsules are shown in Tables 1–5. In all cases, the values shown have been corrected for premedication antibacterial activity. Serum levels which might be therapeutically acceptable were achieved in almost all subjects.

In our initial study of the mixed salivary secretion of phenoxymethylpenicillin following administration of tablets (Table 1), we found a range of <0·03–3·25 μg-ml at 30 minutes. All fell steadily to <0·03 μg/ml at 3 hours. In contrast, no activity >0·03 μg/ml was detectable in any of the parotid specimens.

It might well be assumed from this that the parotid, submandibular and sublingual glands secrete phenoxymethylpenicillin differently. As an uncoated formulation was used, however, the alternative possibility of intra-oral dissolution of powder from the friable tablet surface had to be considered even although each could be swallowed in 5 seconds. We therefore repeated the experiment on the same four subjects using phenoxymethylpenicillin tablets from the same batch, ground and packed into gelatin capsules (2×250) mg). As shown in Table 2, there was no detectable secretion of the drug in either the parotid or mixed saliva, thus confirming our second hypothesis.

TABLE 1. *Concentration of phenoxymethylpenicillin (μg/ml) in serum, mixed and parotid saliva after tablets (2×250 mg)*

| | | Subject | | | |
|---|---|---|---|---|---|
| Specimen | Time (h) | 1 | 2 | 3 | 4 |
| Serum | 1 | 1·75 | 2·26 | 2·88 | 4·13 |
| | 3 | 0·72 | 0·40 | 0·11 | 0·24 |
| | 0·5 | <0·03 | 3·25 | 0·95 | 2·20 |
| Mixed saliva | 1 | <0·03 | 0·24 | 0·10 | 0·75 |
| | 3 and 6 | <0·03 | <0·03 | <0·03 | <0·03 |
| Parotid saliva | 0·5, 1, 3 and 6 | <0·03 | <0·03 | <0·03 | <0·03 |

TABLE 2. *Antibacterial activity (μg/ml) in serum, mixed and parotid saliva after phenoxymethylpenicillin capsules (2×250 mg)*

| | | Subject | | | |
|---|---|---|---|---|---|
| Specimen | Time (h) | 1 | 2 | 3 | 4 |
| Serum | 1 | 2·40 | 2·50 | 3·50 | 3·50 |
| | 3 | 0·17 | 0·33 | 0·07 | 0·30 |
| Mixed saliva | 0·5, 1, 3 and 6 | <0·03 | <0·03 | <0·03 | <0·03 |
| Parotid saliva | 0·5, 1, 3 and 6 | <0·03 | <0·03 | <0·03 | <0·03 |

The serum and saliva levels obtained in four subjects given ampicillin are shown in Table 3. Three subjects had no measurable level of ampicillin at any time but the fourth had a parotid value of 0·12 μg/ml at 3 hours.

Of the four volunteers taking cloxacillin, detection of the drug in saliva was possible in only one mixed saliva specimen at 6 h (Table 4). In view of the serum levels obtained in this subject, the possibility of another source of antibacterial activity cannot be ruled out.

The serum and saliva levels of six subjects given cephalexin are shown in Table 5. No antibiotic activity >2·5 μg/ml was detectable in parotid or mixed saliva at any time in any subject.

## Discussion

We have been unable to demonstrate secretion of phenoxymethylpenicillin in mixed or parotid saliva of four adult volunteers after administration of 2×250 mg capsules although serum levels were satisfactory. When these four subjects were given 2×250 mg tablets phenoxymethylpenicillin B.P. however, high levels were detected in mixed saliva of three for more than 1 h yet none was noted in parotid saliva at any time. Although the subjects were able to swallow each tablet within 5 s it is considered that intra-oral dissolution of surface powder from the friable tablet was responsible for the prolonged antibacterial activity. The *British Pharmacopoeia* (1968) regulation regarding the disintegration of uncoated tablets is that under standard conditions, they should disintegrate within 15 minutes. There

TABLE 3. *Concentration of ampicillin (μg/ml) in serum, mixed and parotid saliva after capsules (2×250 mg)*

| | | Subject | | | |
|---|---|---|---|---|---|
| Specimen | Time (h) | 1 | 2 | 3 | 4 |
| Serum | 1 | 0·96 | 1·20 | 1·20 | 13·00 |
| | 3 | 13·00 | 1·80 | 3·00 | 10·50 |
| Mixed saliva | 0·5, 1, 3 and 6 | <0·03 | <0·03 | <0·03 | <0·03 |
| Parotid saliva | 0·5, 1, 3 and 6 | <0·03 | <0·03 | <0·03 | <0·03* |

*Level of 0·12 μg/ml at 3 h.

TABLE 4. *Concentration of cloxacillin (μg/ml) in serum, mixed and parotid saliva after capsules (2×250 mg)*

| | | Subject | | | |
|---|---|---|---|---|---|
| Specimen | Time (h) | 1 | 2 | 3 | 4 |
| Serum | 1 | 0·36 | 3·50 | 4·00 | 4·40 |
| | 3 | <0·12 | 0·25 | 0·80 | <0·12 |
| Mixed saliva | 0·5, 1, 3 and 6 | <0·12* | <0·12 | <0·12 | <0·12 |
| Parotid saliva | 0·5, 1, 3 and 6 | <0·12 | <0·12 | <0·12 | <0·12 |

*Level of 0·85 μg/ml at 6 h.

TABLE 5. *Concentration of cephalexin (μg/ml) in serum, mixed and parotid saliva after capsules (2×250 mg)*

| | | Subject | | | | | |
|---|---|---|---|---|---|---|---|
| Specimen | Time (h) | 1 | 2 | 3 | 4 | 5 | 6 |
| Serum | 1 | 7·22 | 10·00 | 13·00 | 13·00 | 19·00 | 29·50 |
| | 3 | 2·50 | 3·50 | <2·50 | 2·75 | 4·25 | <2·5 |
| Mixed saliva | 0·5, 1, 3 and 6 | <2·5 | <2·5 | <2·5 | <2·5 | <2·5 | <2·5 |
| Parotid saliva | 0·5, 1, 3 and 6 | <2·5 | <2·5 | <2·5 | <2·5 | <2·5 | <2·5 |

is no minimum time within which they should not disintegrate. Tablets may be film-coated for a variety of reasons such as disguising an unpleasant taste but phenoxymethylpenicillin tablets are rarely coated. It would seem likely that many batches of these tablets are acting as lozenges. Penicillin lozenges were condemned by the American Dental Association in 1956 because of the risk of hypersensitivity reactions (Speirs & Stephen, 1968), and Pellerat (1963) has also drawn attention to the potential hazards of the use of benzylpenicillin lozenges. Dangers of topical oral antibiotics might, however, have been overstated (Garrod & O'Grady, 1968b).

Three of our subjects who received 2×250 mg capsules ampicillin had no measurable levels in parotid or mixed saliva. The fourth at 3 h had 0·12 μg/ml in parotid saliva. Marked individual variation of ampicillin secretion in saliva has been reported by Stewart *et al.* (1970) who found that 75% patients receiving 500 mg Q.I.D. of ampicillin had a "saliva" level of less than 0·06 μg/ml 2 h after the last dose, although the mean level for all patients was 0·11 μg/ml. The antibiotic was in fact measured in spit (Stewart personal communication).

Cloxacillin is usually considered to be of value in treating infections due to penicillin resistant *S. aureus*. Since the late 1950's, most causes of acute septic parotitis have been caused by this organism. (Speirs & Mason, 1971). In this study, however, we detected antibacterial activity >0·12 μg/ml only in 6 h mixed saliva specimen of one of four healthy volunteers.

Cephalexin is a new orally administered cephalosporin antibiotic which is structurally rather similar to ampicillin. By using *B. subtilis* as the test organism, standards of cephalexin were approximately 100 times greater than for ampicillin. Measurement of cephalexin antibacterial activity <2·5 μg/ml was not possible without extrapolation of the standardization graph below the lowest standard. As a result, saliva levels <10−30% serum levels could not accurately be detected. None of six subjects had parotid or mixed saliva levels >2·5 μg/ml.

We have shown that few normal subjects secrete significant quantities of the above antibiotics in either mixed or parotid saliva in spite of satisfactory blood levels. This might suggest that these antibiotics would be of limited value in treating salivary gland infections. In fact it has been shown that antibiotic therapy often fails to cure acute septic parotitis, especially if caused by *S. aureus* and that surgical intervention might be essential. (Krippaehne, Hunt & Dunphy, 1962). Even in the treatment of subacute or chronic septic parotitis it has been suggested that antibiotics should be instilled into the parotid duct (Blatt, 1966).

On the other hand, however, it has been suggested that even minute quantities of antibiotic secreted in saliva will inhibit oral organisms and enable the body's defence mechanisms to dispose of such bacteria more easily (Bender *et al.*, 1953a). It has been claimed that although a 15 mg/g troche of chlortetracycline will, after 3 h, give a mixed saliva level 100 times in excess of that which could be achieved by salivary secretion after high oral dosage, both are comparable in reducing oral flora (Kraus, 1951).

Although it is generally believed that an antibiotic is effective because of its tissue penetration, it is rarely possible to estimate tissue levels. A clinician usually depends on a knowledge of blood levels and in certain circumstances the level achieved in excreted or secreted fluid. May & Delves (1965) have shown that ampicillin concentration is highest in purulent bronchial secretion but that this level is rarely

proportional to blood levels. Nonetheless they postulate the possibility of a " blood-bronchus barrier " comparable with the generally accepted " blood-brain barrier " for drugs, which is influenced by inflammation. A similar " barrier " might exist for salivary tissue as secretion of drugs in saliva is dependent on molecular size (or membrane porosity), lipid solubility and degree of ionization (Schanker, 1964).

In view of the uncertainty regarding the mode of action of antibiotics in the management of oral infections further studies are being carried out with other groups of antibiotics.

We thank Professor D. K. Mason, Department of Oral Medicine and Pathology, University of Glasgow Dental School, for his encouragement and advice throughout this study.

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(*Received October* 12, 1970)