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METHODS FOR COLLECTING INDIVIDUAL COMPONENTS OF MIXED SALIVA: THE RELEVANCE TO CLINICAL PHARMACOLOGY

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1 Methods are described for collecting constituents of mixed saliva, viz. parotid, submandibular, minor gland and sublingual saliva and gingival fluid.

2 Literature is cited which showed that using these techniques, few antibiotics could be found in mixed saliva or its main components but all were detected in gingival fluid. Rifamicin and clindamycin were found in all components.

Introduction

During the last thirty-five years there have been several publications dealing with the variable presence of sulphonamides and antibiotics in mixed saliva or 'spit' (Fickling, Pincus & Boyd-Cooper, 1939; Bender, Pressman & Tashman, 1953a,b,c; Devine, Knowles, Pierce, Pechinpaugh, Hagerman & Lytle, 1969; Hoeprich, 1971). Measurement of other drugs is more difficult and few have been studied e.g. *p*-aminosalicylic acid (Krakowka, Izdebska-Makosa & Wareska, 1966), aspirin (Graham & Rowland, 1972) and paracetamol (Glynn & Bastain, 1973). Naturally-occurring products can be detected in mixed saliva e.g. immunoglobulins (Brandtzaeg, Fjellanger & Gjeruldsen, 1970), blood group substances (Fiori, Giusti, Panari & Porcelli, 1971) and oestrogens (Heap & Broad, 1974).

However, mixed saliva might not be a satisfactory fluid in which to measure drug secretion as it is not of constant or uniform content, consisting of parotid, submandibular and minor gland saliva, along with gingival fluid, bacteria and desquamated epithelial cells. We have shown that gingival fluid may be a major, if not the only, intra-oral route of secretion of several antibiotics (Stephen & Speirs, 1972).

In this paper we review the development of methods for collecting the components of mixed saliva and discuss the relevance to clinical pharmacology.

Methods

Development of saliva collection techniques

Mixed saliva Several methods have been proposed for the collection of mixed saliva. Wainwright (1934) advocated that the head should be tipped forwards with the mouth pointing vertically downwards. Using this position, Kerr (1961) allowed saliva to drip from the open mouth into a filter funnel or else let saliva accumulate behind closed lips for a fixed time before spitting. He also employed suction through a smooth glass tube so that saliva could be caught in a vacuum trap.

Chewing paraffin wax has been used for at least sixty years to stimulate saliva flow (Bunting & Rickert, 1915) but the taste is unpleasant and the paraffin may contaminate the specimen. Hoeprich & Warshauer (1974) have suggested that paraffin may cause lowering of recorded levels if the drug in question is lipophylic. More recently, Graham & Rowland (1972) recommended chewing a small piece of Teflon. Sucking washed, sterile pebbles has been reported (Hoeprich & Warshauer, 1974) but this could prove dentally hazardous. Shannon (1962) showed that salivation could be stimulated by chewing washed elastic bands and we have previously reported the use of this method (Speirs, Stenhouse, Stephen & Wallace, 1971; Stephen & Speirs, 1972).

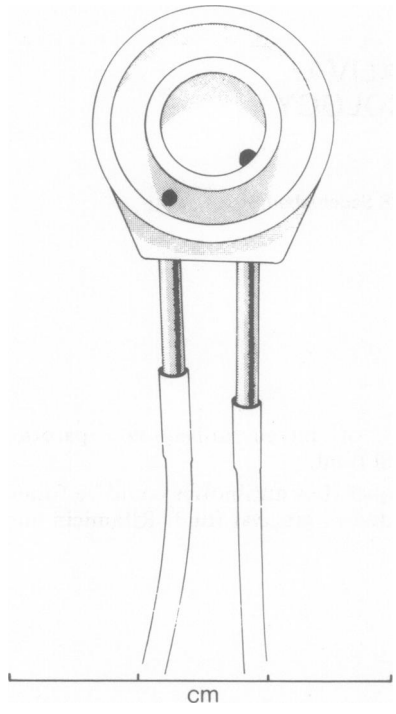

Figure 1 Undersurface of parotid saliva collection cup.

Contamination of mixed saliva specimens with the oral medication can be a problem if uncoated tablets are used, (Speirs *et al.*, 1971) and for this reason we have almost always used capsule formulations. Graham & Rowland (1972) also administered encapsulated aspirin in biopharmaceutical studies as aspirin was retained in the mouth after ingestion of aspirin solutions.

Parotid saliva Prior to 1910, human parotid saliva could be collected only by direct insertion of a cannula into Stenson's duct. Carlson & Crittenden (1910) devised a double-chambered metal cup with two outlet tubes, one of which was connected to a vacuum to hold the cup in place and via the other, the saliva flowed into a collection vehicle. This basic design has since been changed, firstly by Lashley (1916), who is often incorrectly credited with the original design, and then by, Krasnogroski (1931), Jenness & Hackman (1938) and Curby (1953).

We usually use a modification of the device described by Mason, Harden, Rowan & Alexander (1966). It has an overall diameter of 16 mm, a depth of 3 mm and an inner chamber of 7 mm diameter (Figure 1). It is obtainable from N. Harvey Esq., 186 Auchinairn Road, Bishopbriggs, Glasgow. This two-chambered structure is machined from a block of Teflon. A stainless steel orthodontic tube of 1 mm internal diameter passes outside from the inner chamber and to this, a 10 cm length of Esco 'Silescol' translucent silicone rubber tube of 1 mm bore is attached. A similar steel tube passes out from the outer chamber and to this a 30 cm length of 'Silescol' tubing is fitted.

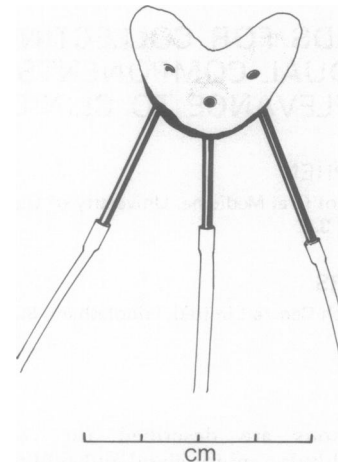

Figure 2 Undersurface of submandibular saliva collection device.

The inner chamber can be positioned over the parotid duct orifice for collection of saliva whilst suction applied to the outer chamber, holds the cup in place. Suction is achieved simply with a dental chip syringe or by a water pump (e.g. 'Speedivac'), although recently we have employed a more sophisticated suction apparatus (Mason, Chisholm, Ferguson, Hunter, Lyell & Stephen, 1974).

Parotid saliva is stimulated by applying 1 ml of 10% citric acid to the dorsum of the tongue every 30 s, then it is collected in sterile bijou bottles. To allow for gland wash-out the first 1.5 ml of saliva should be discarded (Stephen, Harden & Mason, 1971). Depending on the age and state of health of the subject, flow rate is 1-3 ml/min, and in normal volunteers we have found the pH range to be 7.85-8.30.

Between subjects, cups and tubes should be rinsed in running water, blown through with compressed air, dried, then sterilized by ethylene oxide gas (Weymes, 1966), and stored in double-packaged polyethylene bags until required. It is recommended that cups sould not be used for at least five days after gas sterilization (Weymes & Robertson, 1972).

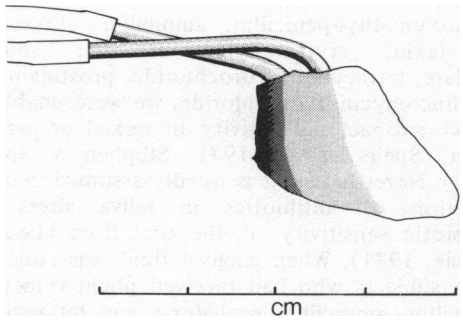

Figure 3 Lateral view of submandibular saliva collection device.

Submandibular saliva collection The appliance we use is a modification of the 'universal' design described by Truelove, Bixler & Merrit (1967). As shown in Figure 2, this is a V-shaped collector. It is fashioned in a pliable polyvinyl chloride ('Vinatex Natural' – NP/60/7801; Vinatex Ltd.) by an injection moulding process. The stabilizing side-arms are approximately 15 mm long and 8 mm wide. Viewed from the side, the device is wege-shaped, the higher anterior portion being 5 mm deep (Figure 3). Stainless steel orthodontic tubes of 1.2 mm internal diameter pass from both outer suction chambers, and from the inner collection chamber. They are bent at an angle of 110° to allow clearance over the incisal edges of the lower anterior teeth. 'Silescol' tubing is fitted to all three steel tubes; 13 cm to the central collecting tube and 30 cm to the outer suction tubes.

Submandibular salivation is also stimulated by 10% citric acid but here, a 2 ml wash-out volume must be discarded. The usual stimulated flow-rate is 1-4 ml/min and the pH range is 6.85-7.60. To test the peripheral seal, a few drops of 10% methylene blue solution can be placed in the floor of mouth, any leakage being indicated by colouration of the collected saliva. Alternatively, a few drops of fluid placed on a blood agar plate for over-night incubation will readily indicate whether or not there was any contamination. Ethylene oxide gas can be used to sterilize these collectors.

Labial minor gland saliva Kutscher, Mandel, Zegarelli, Denning, Eriv, Ruiz, Ellgood & Phalen (1967) employed either 1 μl, 5 μl or 10 μl capillary tubes to collect minor gland saliva from the dried, everted surface of the lower lip. By noting the time taken to fill any particular tube, they could calculate flow rates. We routinely use 10 μl capillary tubes ('Microcaps', Drummond Scientific Company, U.S.A.), and the collected saliva is expressed into pre-weighed bottles by means of a rubber bulb attachment. Bottles can be reweighed and, by noting the collection time, flow rate data may be obtained if necessary as the specific gravity is approximately 1. Otherwise, the contents can be expressed directly on to sterile 2 mm² Whatman No. 1 filter paper squares for bioassay. Sterile 2″ x 2″ surgical swabs are suitable for drying the lip surface prior to salivary stimulation with 10% citric acid. The saliva flow rate is only 0.1-0.5 μl/min and the pH range 7.5-8.0.

Gingival fluid collection Gingival fluid appears in the crevice between the gingival margin and tooth surface. It was first described by Brill & Krasse in 1958, and is thought to be physiological or an inflammatory exudate (Brandtzaeg, 1965), or a serum transudate modified by crevicular cells (Weinstein & Mandel, 1964). Mann & Stoffer (1964) used disposable 2 μl micropipettes for collection of this fluid from patients with moderate to severe gingivitis. For subjects with healthy gingivae however, we developed an alternative technique (Stephen & Speirs, 1972). The gingival margins of at least two upper anterior teeth, neither of which has a Russell's periodontal score >1 (Russell, 1956), are brushed for 2 min by Stillman's method (Stillman, 1932), then dried for 30 s with a warm air jet. Sterile 2 mm squares of filter paper are gently slipped into the labial gingival crevices by sterile needles, left *in situ* for 2 min, then transferred to preseeded test plates for microbiological assay. The gingival fluid collection is unlikely to exceed 0.1 μl/min when the gingivae are healthy. The usual pH range is 7.5-8.0.

Sublingual saliva collection Schneyer (1955) described a 'Segregator' device which enables secretions from the right and left sublingual glands to be collected separately from the combined secretions of both submandibular glands. We have increased the stability of this appliance by the incorporation of orthodontic cribs or clasps (Figure 4). The original design has also been modified by inserting 1 mm internal diameter orthodontic tubes into the acrylic base-plate rather than have polythene tubes leading directly into the body of the appliance. With this device, secretions of right and left sublingual glands can be collected from the two outer tubes while the combined secretions of right and left submandibular gland are collected from the single central tube. No suction is required to hold the appliances in place as they are individually made to fit each subject. They can be sterilized by ethylene oxide gas provided it is cycled at 30° C and not the usual 55° C, as the impression material used in part of

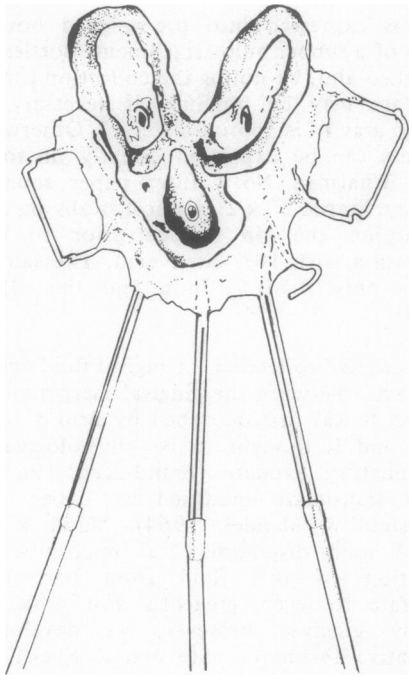

Figure 4 Undersurface of combined sublingual and submandibular collector, showing outer sublingual tubes and inner submandibular tube.

the construction is heat-labile. Pilot studies at present underway with this appliance show that citric acid stimulation produces sublingual saliva at a rate of 0.01-0.1 ml/min and a range of pH 7.5-8.0.

Discussion

It has been shown that the measurement of some drugs in mixed saliva alone might be a reliable guide to blood levels (Graham & Rowland, 1972; Glynn & Bastain, 1973), but we have found this to be rarely the case. Studying normal, over-night fasted, volunteers who had received single doses of phenoxymethyl-penicillin, ampicillin, cloxacillin, cephalexin, erythromycin stearate, sodium fusidate, tetracyline hydrochloride, prostinamycin and lincomycin hydrochloride, we were unable to detect antibacterial activity in mixed or parotid saliva (Speirs *et al.*, 1971; Stephen & Speirs, 1972). Nevertheless, it is usually assumed that the secretion of antibiotics in saliva alters the antibiotic sensitivity of the oral flora (Leading Article, 1971). When gingival fluid was collected from subjects who had received phenoxymethyl-penicillin, ampicillin, cephalexin and tetracycline hydrochloride, a variable amount of antibacterial activity was detected for at least 3 h after medication (Stephen & Speirs, 1972; Macfarlane, McCrossan, Stephen & Speirs, 1974), and this could well affect the organisms of the gingival crevice.

All subjects who received erythromycin estolate secreted erythromycin in gingival fluid and some also had levels in mixed and parotid saliva (Stephen & Speirs, 1972), while rifampicin, and to a lesser estent clindamycin, were present in mixed saliva, gingival fluid and also parotid, submandibular and minor gland saliva (Stephen & Speirs, 1972; Macfarlane *et al.*, 1974). It was also noted that drugs not present in parotid, submandibular and minor gland saliva had low partition coefficients between *n*-octanol and aqueous buffer solutions over the range of pH 6.6-8.6 (Macfarlane *et al.*, 1974).

As analytical methods are now being developed for many drugs other than antibiotics, we suggest that the saliva collection methods which we have described could be used to study their secretion in saliva constituents. Such work might add to our understanding of the factors which influence the transfer of drugs across membranes and secretion by glands of different cell types.

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