Salicylates in saliva

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The possible excretion of acetylsalicylic acid and salicylic acid into human whole-mouth saliva was studied after the ingestion of 1.0 g of acetylsalicylic acid in gelatine capsules. In addition, the oral clearance of both salicylates was determined after a sham intake of acetylsalicylic acid in solution. No acetylsalicylic acid was excreted in saliva. The maximum concentration of 1.2 μg/ml of the metabolite, salicylic acid, was excreted after 3 hours. Considerable concentrations of both salicylates were retained from 2 to 3 hours in the mouth after the sham intake of the drug in solution. During the retention period, part of the acetylsalicylic acid was hydrolyzed to salicylic acid. *In vitro*, at low concentration levels about 50 % of salicylic acid was bound to salivary proteins. The degree of binding was dependent on the drug concentration. The reason for the absence of excreted acetylsalicylic acid from the saliva was evidently its hydrolysis in the body. Protein binding in the oral cavity may explain the slow clearance of locally applied salicylates. Retention of salicylates in the mouth after the use of drug solutions or effervescent preparations should be considered in, *e.g.* evaluations of local analgesic effects or bleeding disorders.

Key-words: Aspirin; salicylic acid; salicylates; drug therapy; protein binding

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Salicylates, alone or in combinations with other drugs, are widely used in the alleviation of pain in medicine, and inflammatory pain amenable to relief by salicylates is also encountered in the field of dentistry. In addition, these drugs are indiscriminately employed by the laity for many ailments.

The present study of acetylsalicylic and salicylic acid in the saliva of persons taking a therapeutic dose of acetylsalicylic acid had three motivations.

Firstly, evidence has been presented that salicylates have a peripheral analgesic mechanism (*Lim & Guzman*, 1968). As the suggested peripheral blocking of sensory impulse generation and anti-inflammatory effect seem to be important analgesic mechanisms of these drugs, local applications and elevation in saliva salicylate concentration could be of interest in dentistry. Potentially, salicylates block the impulse generation in inflammation by preventing prostaglandin release in tissues in which prostaglandin formation is taking place (*Ferreira & Vane*, 1974). Salicylates seem to have no role in this respect in the dental pulp (*Haegerstam & Edwall*, 1976). However, a salicylate preparation to be used locally in cases of periosteitis after extractions has been tested with good results in several countries (*Menzel*, 1969; *Neuner & Schegg*, 1969; *Kjellman*, 1973).

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Secondly, the ingestion of acetylsalicylic acid by normal people causes a small but definite prolongation of the bleeding time (*Mielke et al.*, 1969) and the hemostasis of people with certain bleeding problems is severely upset (*Quick*, 1970). *In vitro*, even a small amount of acetylsalicylic acid prevents the aggregation of platelets (*Weiss, Aledort & Kochwa*, 1968). However, *in vivo* acetylsalicylic acid is rapidly hydrolyzed to salicylic acid, which is almost inactive in this respect (*O'Brien*, 1968). Thus, the potential bleeding problem mainly concerns gastroenterologists and dentists. The use of acetylsalicylic acid is contraindicated in gastrointestinal ulcers. The question posed in dentistry is to what extent acetylsalicylic acid is excreted in saliva or retained in the oral cavity, *e.g.* after the use of the drug in solutions. In other words, is it present at concentrations that can be significant in the evaluation of bleeding complications in oral surgery?

Thirdly, the excretion and biotransformation of drugs by salivary glands are poorly investigated. As regards salicylates, acetylsalicylic acid hydrolyzes in an aqueous solution and the hydrolysis is accelerated by esterases found in the body (*Harris & Riegelman*, 1967). In spite of this, surprisingly high excretion of short-lived acetylsalicylic acid via the salivary route is reported in some studies (*Sano*, 1959). *Borzelleca & Putney* (1970 a) have postulated that the elimination of salicylic acid across the parotid epithelium involves a passive process dependent upon the relative state of hydration and the pH of the saliva. They also found (1970 b) a limited glucuronide-forming capacity in the salivary glands, but concluded that the conjugation of salicylic acid could not occur to an extent sufficient to alter the excretion of the drug via this route.

MATERIAL AND METHODS

Ten healthy volunteers (5 women and 5 men), laboratory workers aged from 24 to 35 years and weighing 65 $\pm$ 10 kg, participated in the study. They were instructed to take no drugs (other than oral contraceptives if these were used regularly) for 1 week before the experiments and to abstain from coffee, tea, cola and alcoholic beverages for 24 hours before, and during the study.

Acetylsalicylic acid was taken orally in the morning, 1 hour after a light breakfast. Two different ways of drug administration were used. Excretion of salicylates through the salivary glands was studied after ingestion of a single dose of 1.0 g acetylsalicylic acid (Ph. Nord.) in two gelatine capsules with 50 ml of water. Secondly, clearance of salicylates from the oral cavity was studied after a sham intake of 1.0 g of acetylsalicylic acid in 50 ml of water; the suspension was kept in the mouth for 10 sec., but not ingested. The mouth was briefly washed afterwards, so that no particles were retained.

Samples of stimulated whole-mouth saliva were collected immediately before and at 0.5, 1, 2, 3, 4 and 5 hours after intake of the drug. The subjects spat into small wide-mouthed vials while chewing a piece of paraffin. The samples were immediately frozen and stored in a freezer pending assay.

Salicylates were extracted into chloroform. The method was slightly modified from that of *Schanker et al.* (1957). For the determination of total salicylate (acetylsalicylic acid and salicylic acid after hydrolysis), 0.1 ml of concentrated HCl and 20 ml of chloroform were shaken for 15 min with 6 ml of saliva. After the centrifugation a 15-ml aliquot was shaken 10 min with 3 ml of 0.1 N NaOH to

obtain hydrolysis. Two millilitres of the aqueous phase were added to 1 ml of 0.5 N HCl. The optical density was read at 300 nm (UV absorbance in Hitachi 101 spectrophotometer). The recovery from sodium salicylate was 95 ± 4 %.

For the determination of acetylsalicylic acid, the extraction from saliva was done as described above, but 0.3 ml of concentrated HCl was used. The extraction from the chloroform phase was done with 5 ml of phosphate buffer (pH 6.7) to retard the hydrolysis. Three millilitres of the buffer phase were mixed with 0.1 ml of concentrated HCl and the optical density was read at 277 nm and 300 nm. The values were read immediately, since acetylsalicylic acid hydrolyzes in phosphate buffer ($t_{1/2}$ at 25°C about 2.5 days), liberating salicylic acid (*Rowland & Riegelman*, 1967).

The concentration of salicylic acid was read from a standard curve. The absorbance at 300 nm is due to salicylic acid only. Another standard curve for salicylic acid was made at 277 nm and the absorbance corresponding to the concentration was read. The value for acetylsalicylic acid alone was obtained from a third standard curve, after the subtraction of salicylic absorbance at 277 nm from the acetylsalicylic acid absorbance at 277 nm. The recoveries of acetylsalicylic acid and salicylic acid from the mixtures were satisfactory, 93 ± 2 % and 95 ± 3 % respectively.

The *in vitro* binding of salicylic acid to saliva proteins was studied with dextran gel filtration. The preparation of columns from Sephadex G 25 and the elution process were performed as described by *Potter & Guy* (1964). Saliva samples of 0.5 ml from resting secretion were incubated 30 min with salicylic acid (1.2—12.0 μgml) at 39°C.

RESULTS

Acetylsalicylic acid was not excreted into saliva with the dose used in this study. The values fell to the zero level, when they were corrected with the absorbance of the metabolite at 277 nm, which was found to be the peak of the emission spectrum of acetylsalicylic acid. The metabolite, salicylic acid, was excreted in saliva. The peak concentration after 3 hours was 1.2 ± 0.3 μg/ml (Fig. 1).

Retention of acetylsalicylic acid was observed in saliva, when the drug suspension was allowed to be in contact with the oral cavity (Fig. 3). Partial hydrolysis of retained acetylsalicylic acid occurred in the mouth, since after 30 min the concentration of total salicylate (36.4 ± 5.8 μg/ml) far exceeded that of acetylsalicylic acid (10.8 ± 1.5 μg/ml) (Figs. 2 & 3). The clearance of salicylic acid from the saliva was also slow. The salicylic acid retained after 3 hours (2.9 ± 1.3 μg/ml) exceeded the amount excreted at that time point through the salivary glands (Figs. 1 & 2).

Salicylic acid was extensively bound (63—44 %) to salivary proteins at low drug levels (Fig. 4). The relative extent of binding decreased with increasing salicylic acid concentration.

DISCUSSION

Concentrations of salicylic acid in saliva are dose-related and linearly proportional to concentrations in plasma, at least up to a concentration of 50 μg/ml (*Borzelleca & Doyle*, 1966; *Graham & Rowland*, 1972). This study was focused on the proportions of acetylsalicylic acid and salicylic acid excreted through the salivary glands and the clearance of salicylates from the oral cavity.

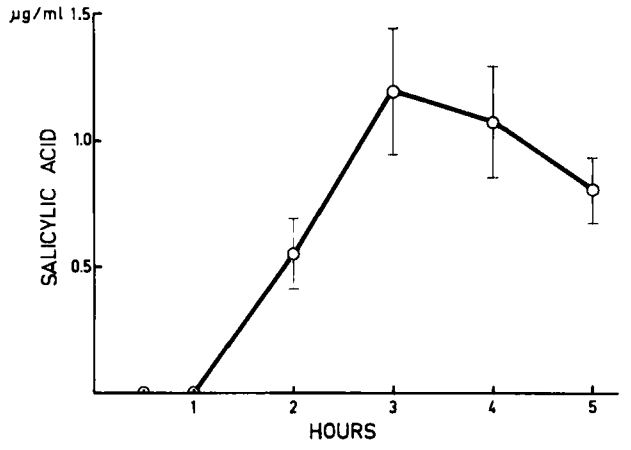

Fig. 1. The excretion of salicylic acid into whole-mouth saliva after ingestion of 1.0 g of acetylsalicylic acid in two gelatine capsules. The means (± S.D.) are from 10 subjects.

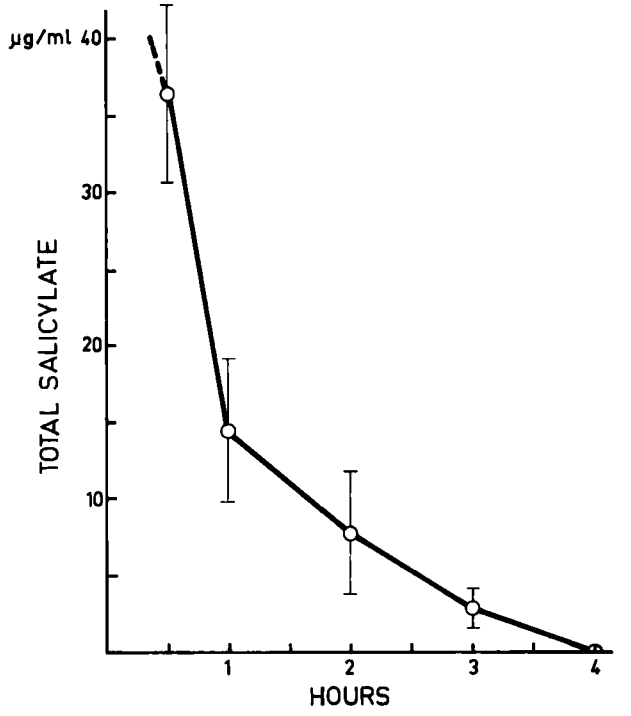

Fig. 2. The oral clearance of total salicylate after the sham intake of acetylsalicylic acid in solution. The means (± S.D.) represent the concentrations in saliva after hydrolysis to salicylic acid.

After ingestion, acetylsalicylic acid is rapidly hydrolyzed to salicylic acid. Hydrolysis occurs in the gastrointestinal wall, body fluids and liver. Only about 70 % of an oral dose of 650 mg in solution reaches the peripheral circulation intact (*Rowland et al.*, 1972). Unhydrolyzed acetylsalicylic acid is present at demonstrable levels in the blood up to 1 hour after an oral dose, but if appreciable levels are to be attained, the drug must be administered in rapidly absorbed form or in large doses (*Levy & Leonards*, 1966).

We did not find any excretion of acetylsalicylic acid into saliva after the

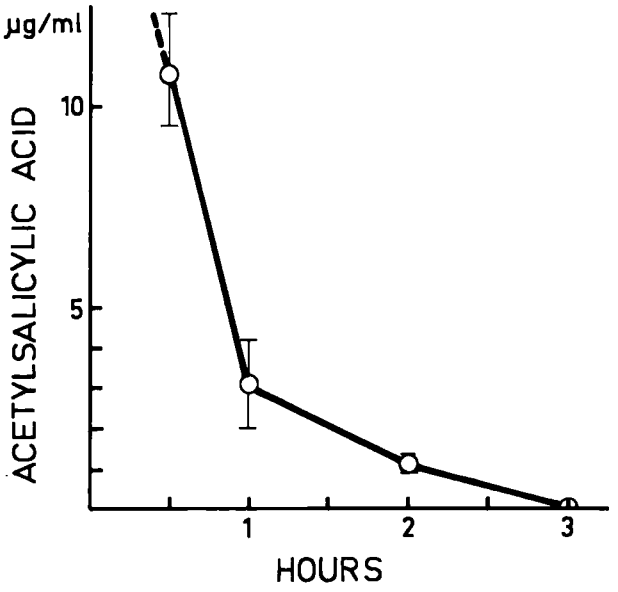

Fig. 3. The oral clearance of acetylsalicylic acid after the sham intake of acetylsalicylic acid in solution. The means (± S.D.) of the concentrations in saliva are shown.

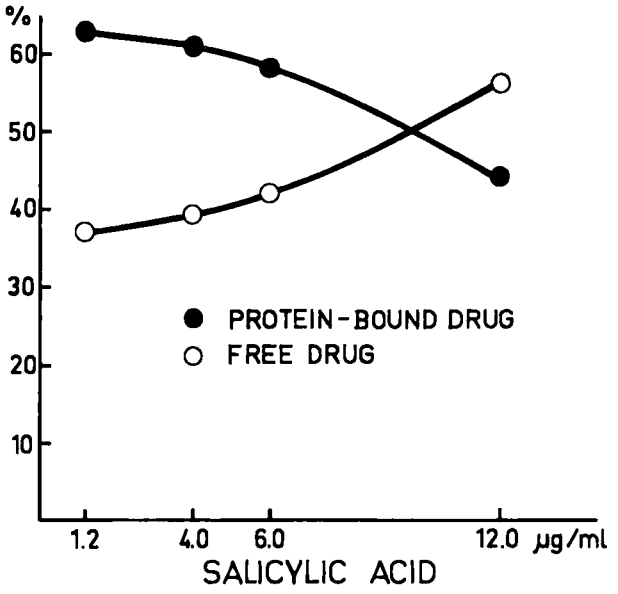

Fig. 4. The *in vitro* binding of salicylic acid to proteins of whole saliva.

ingestion of 1 g in gelatine capsules. This is in accordance with a short notice of *Graham & Rowland* (1972), as they state that »no significant quantities of hydrolyzable salicylate (aspirin) were detected in saliva» after the ingestion of 650 mg in gelatine capsules. These results are in sharp contrast to the massive excretion of acetylsalicylic acid reported by *Sano* (1959).

The maximum level of salicylic acid excretion was reached at about 3 hours after the intake of the intact drug. This indicates retardation of absorption by the capsules. A coat-dependent delay in the appearance of salicylic acid in saliva has

been demonstrated (*Graham & Rowland*, 1972).

Preliminary observations indicated that salicylates were retained in saliva after ingestion as solutions or tablets. This led us to the use of gelatine capsules and to the more detailed study of clearance of salicylates from the mouth. Interestingly, the retention lasted for several hours, which is appreciably more, for example, than the 30 min recorded for glucose and the 20 min for citrate (*Ericsson*, 1967). The reason for this retarded clearance is unknown, but it is possible that protein binding plays a role, especially if binding to other than mobile salivary proteins occurs.

When the possible local effects of salivary salicylates are considered, attention must be paid to their respective concentrations. *Lester, Lolli & Greenberg* (1946) were the first to suggest that acetylsalicylic acid acts as an analgesic by itself, and not through salicylic acid after hydrolysis. This view has been supported later by several authors; in any case acetylsalicylic acid has a clear clinical advantage over sodium salicylate (salicylic acid) (*Lim*, 1966). The superiority also extends to acute inflammatory pain (*Lasagna*, 1961). Thus hardly any analgesic effect can be expected from the excreted drugs, because no acetylsalicylic acid is present and the concentration of salicylic acid is small, compared with the usual plasma levels. In our study the peak of excreted salicylic acid (1.2 μg/ml) was about the same as that found by *Graham & Rowland* (1972) with a dose of 650 mg in a capsule.

On the other hand, the levels of salicylates retained in saliva after the use of the acetylsalicylic acid solution are equal to the plasma levels observed in many studies (*Rowland et al.*, 1972). Whether these concentrations are pharmacologically active in the oral cavity depends on protein binding and access to active sites. In addition, the ratio between the concentrations of the two salicylates is time-dependent, owing to the hydrolysis of acetylsalicylic acid observed in the mouth. The enzymatic hydrolysis of acetylsalicylic acid in whole blood is rapid: $t_{1/2}$ at 37°C is 30 min (*Harris & Riegelman*, 1967). The corresponding nonenzymatic hydrolysis in Krebs-Ringer solution is slow: $t_{1/2}$ at 37°C is 15.5 hours (*Rowland et al.*, 1972). This suggests that hydrolysis in the mouth is also enzymatic. Nonspecific esterases have been found along the entire human gastrointestinal tract, including saliva and dental plaque (*Chauncey et al.*, 1954; *Dawson & Pryse-Davis*, 1963; *Larmas*, 1972).

Salicylic acid is strongly bound to plasma proteins, in contrast to acetylsalicylic acid, which is weakly bound (*Lester et al.*, 1946). The protein concentration of oral fluid is only about 3 mg/ml (*Rauch*, 1959), which is considerably less than in plasma. Accordingly, the binding of salicylic acid to saliva proteins was most pronounced at low drug levels, the percentage of drug bound depending on salicylic acid concentration. The same phenomenon is found in plasma at much higher salicylate levels (*Levy & Leonards*, 1966). The binding of acetylsalicylic acid in saliva was not studied, because of methodological difficulties involved in studying this compound on a micro scale.

The absorption of salicylates across the biological membranes occurs by passive diffusion of non-ionized molecules (*Schanker et al.*, 1957). Since acetylsalicylic acid (pKa 3.5) is a weaker acid than salicylic acid (pKa 3.0), it will be less ionized than salicylic acid at low pH. It is possible that the lipid solubility of unionized acetyl-

salicylic acid is a factor permitting more rapid access to receptor sites, *e.g.* under the acidic tissue conditions of an inflammatory process.

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