bolite, are advantages over microbiological assay procedures.

It may be applied with some modification to the determination of a series of penicillins in serum samples.

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan. The authors acknowledge the excellent technical assistance of Miss S. Nishikawa and Miss M. Hayakawa.

June 26, 1978

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# The pH dependent absorption of propranolol and indomethacin by Parafilm, a stimulant of salivary secretion

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The possible use of drug concentration in saliva as a means of predicting plasma concentrations of the drug has attracted attention (Spiers, 1977). To obtain a sample of suitable volume for analysis it is sometimes necessary to stimulate salivary flow, the type of stimulus depending on the investigator's preference. One particular stimulus is the chewing of a waxy strip (Parafilm sealing tissue, Gallenkamp), by the subject, this is then discarded when sufficient saliva has been produced. Because the concentrations of drugs in biological fluids may be affected by the materials they come into contact with on collection and storage (Cotham & Shand, 1975; Rosseel & Bogaert, 1976) the possibility of uptake of drugs by Parafilm has been examined. For this purpose two lipid-soluble drugs, propranolol (a weak base) and indomethacin (a weak acid) were used (Study 1). The pH of saliva increases as salivary flow increases, independently of the stimulus (Dawes & Jenkins, 1964). We have therefore measured the changes in pH associated with increased salivary volume as a result of chewing Parafilm in 10 subjects (Study 2). We also wished to discover if Parafilm absorbed propranolol from the mouth and if the predicted reduction in saliva/plasma drug ratio occurred when salivary flow was stimulated by chewing the material (Study 3).

*Study* 1. Strips of Parafilm, 10 × 5 cm (each cut into 32 equal pieces about 1 $cm^2$) were added to 5 ml aliquots of Sörensen's phosphate buffer at pH 5·0, 6·0, 7·0 and 8·0 containing propranolol (200 ng $ml^{-1}$) or indomethacin (50 $\mu g\ ml^{-1}$). These were mechanically shaken at room temperature (25°) in 30 ml stoppered tubes for 3, 6, 9 or 12 min. Immediately after shaking the film was removed and the solution frozen to −20°. Propranolol was assayed fluorimetrically (Shand, Nuckolls & Oates, 1970) and indomethacin spectrophotometrically (Hvidberg, Lausen & Jansen, 1972). The procedure was repeated at each pH with buffer and film without drugs and the same results were obtained as with buffer alone. During the first 3 min of shaking there was a pH-dependent absorption of both drugs into the film; shaking for a further 9 min produced little change. At higher pH the absorption of propranolol was greater and that of indomethacin smaller (Table 1).

*Study* 2. The saliva produced by each subject over 2 min was collected by continually drawing the expelled samples into a pre-weighed 5 ml syringe. Air was excluded from the sample since the pH of saliva rises rapidly as carbon dioxide is lost into air (Dawes & Jenkins, 1964). The weight and volume of saliva were noted and its pH measured. After a further 5 min, saliva production was stimulated by the chewing of a

Table 1. *Percent (mean ± s.e., n = 4) of propranolol and indomethacin remaining in solution after shaking with Parafilm for 3 and 12 min at pH 5·0, 6·0, 7·0 and 8·0.*

| | Propranolol | | Indomethacin | |
|---|---|---|---|---|
| Time (min) pH | 3 | 12 | 3 | 12 |
| 5·0 | 100 | 100 | 78 ± 4 | 71 ± 3 |
| 6·0 | 91 ± 3 | 86 ± 3 | 95 ± 1 | 90 ± 1 |
| 7·0 | 80 ± 2 | 71 ± 2 | 94 ± 4 | 97 ± 2 |
| 8·0 | 63 ± 3 | 61 ± 1 | 97 ± 3 | 96 ± 3 |

* Correspondence.

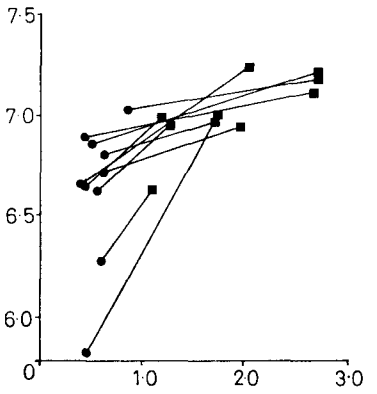


FIG. 1. Relation between salivary flow rate (g $min^{-1}$) (abscissa) and salivary pH (ordinate) in 10 subjects. ● unstimulated salivary flow; ■ stimulated salivary flow by chewing Parafilm.

10 × 5 cm strip of Parafilm for 5 min; the saliva was collected and treated as before.

The densities of the unstimulated and stimulated saliva samples were equal. Fig. 1 shows the increase in pH with increase in flow rate. The mean ± s.e.m. flow rate increased from 0·54 ± 0·04 to 1·91 ± 0·20 g $min^{-1}$ ($P<0.001$, paired $t$-test) and the mean ± s.e.m. pH of saliva increased from 6·63 ± 0·11 to 7·02 ± 0·06 ($P<0.001$, paired $t$-test) when Parafilm was used to stimulate salivary flow.

*Study 3.* The concentration ratio of a drug in saliva to that in plasma may be calculated by the formula used by Matin, Wan & Karam (1974) as follows:

for weak bases:

$$\frac{C_s}{C_p} = \frac{1 + 10^{(pK_a - pH_s)}}{1 + 10^{(pK_a - pH_p)}} \times \frac{f_p}{f_s}$$

for weak acids:

$$\frac{C_s}{C_p} = \frac{1 + 10^{(pH_s - pK_a)}}{1 + 10^{(pH_p - pK_a)}} \times \frac{f_p}{f_s}$$

where $C_s$ = concentration of drug in saliva; $C_p$ = concentration of drug in plasma; $pK_a$ = $pK_a$ of drug (for propranolol $pK_a$ = 9·45); $pH_s$ = pH of saliva; $pH_p$ = pH of plasma (assumed to be 7·40); $f_s$ = fraction of drug in saliva unbound to salivary proteins (assumed to be zero for propranolol); $f_p$ = fraction of drug in plasma unbound to plasma proteins (assumed to be 0·16 for propranolol).

The protein binding values for propranolol were taken from Mucklow, Bending & others (1978). This equation predicts that an increase in salivary pH from 6·63 to 7·02 would reduce the saliva/plasma ratio of propranolol at equilibrium from 0·94 to 0·38. To test this three patients receiving regular therapy with propranolol for the treatment of hypertension were asked to produce 4 ml of saliva without salivary stimulation. A 10 ml sample of venous blood was taken at the same time and the plasma separated, frozen and stored at −20°. The patients then produced a further 4 ml saliva sample by chewing a 10 × 5 cm strip of Parafilm. All saliva samples were collected and treated as described in Study 2 and were stored at −20°. Propranolol was assayed as in Study 2.

The used pieces of Parafilm were washed in water, blotted dry, and each then mechanically shaken with 3·5 ml of 0·01 M hydrochloric acid in 30 ml stoppered tubes. The film was removed and the fluorescence of the solution measured immediately. A blank of unused Parafilm shaken with acid yielded a zero reading. The mean ± s.e.m. amount of propranolol extracted from each piece of film was 56 ± 4 ng.

For the unstimulated saliva samples the mean ± s.e.m. pH was 6·78 ± 0·04 and the mean of saliva/plasma ratios was 1·26 ± 0·49 while for the stimulated samples the mean ± s.e.m. pH was 7·21 ± 0·02 and that of the saliva/plasma ratios was 0·56 ± 0·16.

These studies have shown that, over a range of pH which covers that of saliva in man (Dawes & Jenkins, 1964), the *in vitro* absorption of both drugs by Parafilm is according to the pH-partition hypothesis. Propranolol, a weak base with a $pK_a$ of 9·45, is less dissociated at high pH and it is the lipid soluble, unionized form of the drug which passes into the film. Indomethacin, a weak acid with a $pK_a$ of 4·5, is less ionized and hence absorbed to a greater extent at lower pH. *In vivo*, Parafilm absorbed propranolol and some absorption of weak acids may also occur although this will be less pronounced. We have also shown that the increase in pH due to increase in salivary volume when chewing Parafilm further modifies drug concentrations and suggest that Parafilm or materials with similar physicochemical properties should not be used to stimulate salivary flow for drug measurements.

July 26, 1978

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