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Use of whole saliva for bioavailability studies with reference to phenytoin

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The most popular method of determining the relative bioavailability of a drug formulation is to undertake single dose crossover studies in healthy volunteers and compare the area under the serum drug concentration-time curve (AUC) extrapolated to infinity (Koch-Weser 1974). The method entails the collection of enough blood samples to define the absorption and elimination portion of the concentration-time curve. We wondered whether bioavailability testing could be undertaken using whole saliva samples instead of blood in single dose crossover studies. As differences in the bioavailability of phenytoin formulations (Neuvonen 1979) have been reported to produce relapses in seizure control (Lund 1974) or signs of intoxication (Tyrer et al 1970) when one formulation is substituted for another, we have examined two oral phenytoin formulations available in New Zealand. These were 100 mg phenytoin sodium capsules (Parke-Davis) and 100 mg phenytoin sodium tablets (Kempthorne Prosser).

Methods. Five healthy adult volunteers (3 males and 2 females) gave their informed consent for the study which was conducted under clinical supervision. All declared that they were in good health with no known intolerance to any drug. On general physical examination they were normal with normal hepatic and renal function. No other medication was taken for at least one week before and during the study.

Subjects were fasted overnight until 1200 h on day 1. A single oral dose of 300 mg phenytoin (3 capsules or tablets) was given at 0800 h with 200 ml cold water. This dose is small enough to avoid the complication of non-exponential decline in serum concentrations in most subjects (Paxton et al 1977c). The first drug was assigned at random and alternated by crossover after at least 2 weeks. Venous blood samples were withdrawn immediately before drug administration and twice daily until 80 h after in four subjects. For subject 1, blood sampling was at 2 hourly intervals over days 1 and 2 and thereafter twice daily to 80 h. Blood samples were allowed to clot and the serum separated by centrifugation and stored frozen at − 20 °C for not more than 2 weeks. Whole saliva samples were obtained at 2 hourly intervals during the 4 days after drug administration. The saliva specimens were collected (after stimulation with one crystal of citric acid if necessary) by expectoration into universal containers and stored frozen. Before assaying, the whole saliva samples were

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Table 1. Serum and salivary bioavailability parameters for two formulations of phenytoin administered orally.

Subject	AUC^{∞} (μmol litre^{-1} h)		Peak concn (μmol litre^{-1})		Time to peak concn. (h)		$t_{\frac{1}{2}\beta}$ (h)	
	C	T	C	T	C	T	C	T
1. Serum	738	706	13·0	18·1	3·0	7·3	15·6	13·1
Saliva	62	44	1·49	1·22	3·0	3·0	14·3	15·2
2. Serum	483	502	14·7	12·8	8·3	7·0	13·9	14·0
Saliva	46	40	1·82	1·07	6·0	5·0	14·7	15·4
3. Serum	780	827	18·9	24·7	8·4	7·0	16·6	16·7
Saliva	57	78	1·48	2·23	10·0	5·0	16·4	14·8
4. Serum	774	745	16·7	15·1	7·0	8·7	15·7	16·0
Saliva	72	70	1·73	1·78	7·0	13·0	14·9	16·4
5. Serum	840	823	20·7	19·6	9·0	9·0	17·6	19·6
Saliva	61	62	1·62	1·52	7·0	8·7	17·5	19·4
* Serum	723 (140)	721 (133)	16·8 (3·1)	18·1 (4·5)	7·1 (2·4)	7·7 (0·9)	15·9 (1·4)	15·9 (2·5)
Saliva	60 (9)	59 (16)	1·63 (0·15)	1·56 (0·46)	6·6 (2·5)	7·0 (4·0)	15·6 (1·3)	16·2 (1·9)

C, phenytoin capsules; T, phenytoin tablets.
* Mean (with s.d.). None of the differences between tablets and capsules were statistically significant.

thawed, centrifuged to remove any precipitated matter and the clear supernatant used. After the appropriate dilutions of serum and saliva with assay buffer, concentrations of phenytoin were measured by radioimmunoassay (Paxton et al 1977a).

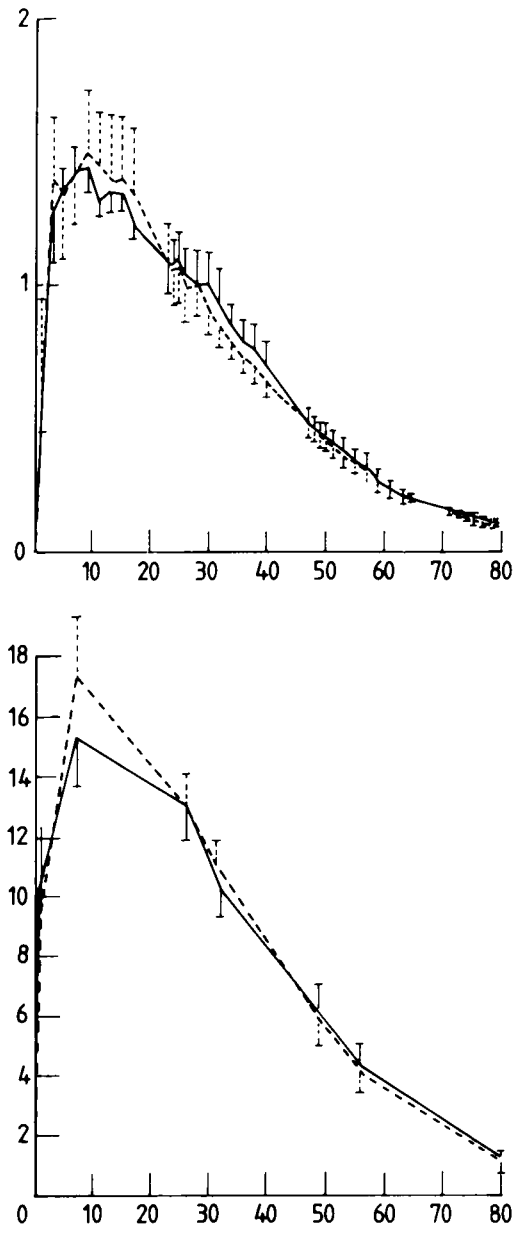

FIG. 1. Mean serum and salivary phenytoin concentration-time curves in 5 healthy volunteers after a single 300 mg oral dose of phenytoin sodium as capsules (——) and tablets (– – – –). Mean and s.e.m. are given. Ordinates: upper graph, saliva concentration (μmol litre^{-1}) and lower graph, serum concentration (μmol litre^{-1}). Abscissa: time (min).

Serum and salivary concentrations were plotted against time and AUC^{80} from zero time to 80 h calculated by the trapezoidal rule. Extrapolation of the AUC to infinity was achieved by the equation $AUC^{\infty} = AUC^{80} + C_{80}/\beta$ where β is the slope of the terminal linear segment of the log concentration-time plot fitted by the method of least squares regression analysis and C_{80} is the theoretical phenytoin concentration at 80 h calculated from this. For comparison of the results, Student's *t*-test for paired observations was used with differences being regarded as significant when $P < 0{\cdot}05$.

Results. The mean plots of the salivary and serum phenytoin concentrations (plus standard errors) for the formulations are in Fig. 1. The bioavailability parameters estimated from the data and the statistical comparisons between the two formulations are summarized in Table 1. There was no significant difference in serum or salivary AUC^{∞} values, peak concentrations, time to peak concentration and elimination half-lives after tablets or capsules, indicating equivalent bioavailability for the two formulations. However, because of the paucity of serum data on the first day (except for subject 1), the inaccuracy of serum peak concentrations and time to peak concentration must be accepted.

Comparison of salivary and corresponding serum concentrations gave a linear relationship with a gradient 0·11 and a highly significant correlation coefficient ($r = 0{\cdot}989$, $P < 0{\cdot}001$). The time to peak concentration and the elimination half-life ($t_{\frac{1}{2}}\beta$) did not differ significantly in serum or saliva. The mean peak concentration in saliva was approximately 9% of that in serum while the AUC^{∞} in saliva was approximately 8% of that in serum.

Discussion. In the comparison of bioavailabilities of different drug formulations using serum parameters, the area under the serum concentration-time curve has been considered to be the most reliable parameter after single dose studies (Koch-Weser 1974). Our serum data indicated that the capsules and tablets were bioequivalent with similar absorption characteristics and serum concentration-time profiles in the body. The same conclusion was reached using the salivary data. This non-invasive technique provides a method for obtaining a large number of specimens, and determining the bioavailability of a drug without loss of blood or exposure of the subject to any discomfort or infection. In addition it would appear that the greater frequency of sampling of whole saliva would allow a more accurate determination of the bioavailability parameters. Previous studies (Paxton et al 1977b; 1977c) have shown the salivary concentration of phenytoin to be independent of degree of stimulation and pH of saliva, and representative of the free pharmacologically active fraction of the drug in serum. Although we found a good linear correlation between serum and salivary concentrations, it would seem worthwhile to consider the salivary concentrations as representing the free

fraction rather than extrapolating the results to represent total serum drug concentrations. With drugs such as phenytoin, whose measurement in saliva has been validated, salivary rather than serum bioavailability studies may be clinically more relevant, as comparisons of free pharmacologically active drug fractions would appear to give a better measurement of a drug's concentration at its receptor site and better quantitation of clinical response.

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Freeze-drying of haemoglobin in the presence of carbohydrates

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Compounds that make it possible to freeze-dry haemoglobin without oxidation have been studied in our laboratory for several years (Labrude et al 1976; Labrude & Vigneron 1980). Of such compounds, glucose is known to be very effective (Amberson et al 1942; Farr et al 1947; Smith & Pennell 1952; Fateeva & Gruzova 1979). In 1976 we demonstrated the effectiveness of several other carbohydrates. More recently Pristoupil et al (1978) and De Venuto et al (1979) have confirmed these results.

To our knowledge however, no systematic study has been reported of the protective actions of hexoses, of substituted derivatives of glucose or levulose, or of other carbohydrates and related C4, C5, C7, etc. compounds. Therefore we have tried to discern possible relationships between the properties of these compounds and their protective activities.

All the compounds were of analytical quality and were from Sigma, Calbiochem, Boehringer, Merck, Fluka, and Prolabo. Only water-soluble compounds were studied, since the absence of this characteristic is incompatible with effective protection. The compounds studied, in order of increasing complexity, are as follows:

Trioses: DL-glyceraldehyde, dioxyacetone, glycerol, propane-1,3-diol.

Tetroses: D-erythrose, erythritol.

Pentoses: D-ribose, D-arabinose, D-xylose, D-lyxose and substituted derivatives of ribose (Table 1).

α-D-glucose (Sigma G 5000 with 5% β anomer) β-D-glucose (Sigma G 5250), L-glucose (Sigma G 5500, mixed anomers);

D-series of aldohexoses: allose, altrose, glucose, mannose, idose, galactose, talose. Substituted derivatives of glucose (Table 2).

Ketohexoses: fructose, L-sorbose, D-tagatose, sodium fructose-1-phosphate, trisodium fructose-1,6-disphosphate . $7H_2O$, and barium fructose-6-phosphate.

Heptoses: D-mannoheptulose, D-sedoheptulose hydrate, α-D-heptagluco-γ-lactone, calcium heptagluconate.

Disaccharides: D-sucrose, D-trehalose hydrate, D-cellobiose, lactulose, palatinose, maltose hydrate, gentobiose.

The haemoglobin solution, at a concentration of 45 ± 5 g litre^{-1}, was prepared as described before (Labrude & Vigneron 1980). The carbohydrates were dissolved in it, without adjustment of the pH, to a concentration of 0·2 M. All other operations were as described by Labrude & Vigneron (1980). Of the parameters studied (colour, dissolution, saturation, p 50, etc.), we report only the methaemoglobin concentration, measured according to Evelyn & Malloy's method (1938). Each carbohydrate was studied twice. A compound was considered to be effective if it led to a methaemoglobin concentration less than or equal to 10% (Tables 1, 2); in the absence of protector the mean value was 49% (n = 30).

The trioses and tetroses were ineffective. The four aldopentoses were very effective, as were almost all the ribose derivatives studied. The α, β, L, or D character of the glucose did not affect its protective activity. The six aldehexoses were as effective as glucose. In the substituted aldohexoses series the most unfavourable modifications for the protective activity were those on carbon atoms 1 and 2. Changes at position 6 seemed to be less important, although most of the borderline cases (leading to 10% methaemoglobin) occurred there and the double modification on positions 1 and 6 seems best avoided. Of the six ketohexoses, only the 6-phosphate and 1,6-diphosphate derivatives of fructose were

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