Figure 2 Correlation between dissolution at 30 (closed symbols) and 60 min (open symbols) and the urinary 24 h digoxin excretion.

Göttingen). With that method correlation obtained between dissolution rates and bioavailability was poor.

Serum digoxin concentration peaks occur at about 30 to 60 min after digoxin intake. Therefore dissolution achieved later than at 30 min would not be used in predicting digoxin bioavailability.

Among the tablets tested by us one product (B) with good bioavailability dissolved very slowly. In earlier experiments (Reissell, Manninen, Ojala & Karjalainen, 1974) tablets from a different batch of the manufacturer B dissolved very rapidly and had a good bioavailability. Deviation of product B in the present work from the others indicates that dissolution tests in their present form cannot always be used to predict digoxin bioavailability. However, if the tablets are manufactured in a

suitable way, this simple test can be used in the control of the stability of digoxin tablets between different batches of tablets and to predict the bioavailability, thus diminishing the need for cumbersome testing of bioavailability in man.

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EFFECT OF VITAMIN C SUPPLEMENT ON ANTIPYRINE DISPOSITION IN MAN

Previous studies have shown that concomitant administration of vitamin C with certain drugs can inhibit the rate at which the drug undergoes

biotransformation (Houston & Levy, 1975; Houston & Levy, 1976a; Houston & Levy, 1976b). This may result in a potentiation of the pharma-

cological effect of the drug (Houston, Wilkens & Levy, 1976). The present study investigates further vitamin C-drug interactions in man; specifically the effect of chronic vitamin C administration on the oxidative route of hepatic drug biotransformation.

Antipyrine was used as a model drug in this investigation for the following reasons. In man, 95% of the drug undergoes oxidative biotransformation (Brodie & Axelrod, 1950; Eichelbaum, Schomerus, Spannbrucker & Zietz, 1976). The total body clearance of antipyrine is low, consequently it is not influenced by changes in hepatic blood flow and it will directly reflect changes in the activity of the hepatic drug-metabolising enzymes (Branch, Shand, Wilkinson & Nies, 1974). Investigations from a number of laboratories (Welch, de Angelis, Wingfield & Farmer, 1975; Vesell, Passananti, Glenwright & Dvorchik, 1975; Fraser, Mucklow, Murray & Davies, 1976; Chang, Wood, Dixon, Conney, Anderson, Eiseman & Alvares, 1976) have demonstrated a 1:1 relationship between the concentration of antipyrine in saliva and plasma over the range 0.5-30 µg/ml. This enables pharmacokinetic parameters for antipyrine disposition to be derived solely from saliva data. This approach was used in the following study.

Five healthy male subjects, aged 24-36 years, weighing 65-80 kg, took part in this study. No drugs or vitamin preparations were allowed 1 week prior to the study. Normal diets were maintained throughout the study. All but one subject (WM) were non-smokers. Following an overnight fast, the drug (Antipyrine, B.P. 10 mg/kg) was taken orally and mixed saliva samples collected at -0.5, 3, 6, 9, 12, 24, 30, 36 and 48 h. Food was allowed after the second sample was given. No coffee or tea was consumed within the hour prior to samp-

ling. On the third day of the study, after the 48 h sample was collected, each subject received 1g of vitamin C (Boots, high dose effervescent). On each of the following 9 days (i.e. days 4-11 of the study) a further 1g of vitamin C was consumed. On day 10 (after 7 days of the vitamin supplement) each subject received a second antipyrine dose (taken 2 h prior to vitamin intake) and the above saliva sampling procedure repeated. Saliva samples were extracted and assayed for antipyrine by gas chromatography (with a nitrogen specific detector) as described by Van Boxtel, Wilson, Lindgren & Sjoqvist (1976). The half-life ($T_{1/2}$) of antipyrine was determined from the slope of the least-squares regression line of the logarithm of the saliva concentration versus time plot. The apparent volume of distribution (V_d) of antipyrine was calculated from the relationship

$$V_d = \frac{FD}{(AUC).K}$$

where F was the fraction of the antipyrine dose (D) absorbed and was assumed to be 1 (Andreassen & Vesell, 1975), AUC was the area under the saliva concentration-time curve between zero and infinity calculated by the trapezoidal rule and K was the disposition rate constant ($0.693/T_{1/2}$). Total body clearance (TBC) was calculated from the equation

$$TBC = \frac{FD}{AUC}$$

The results of this study are summarised in Table 1. Chronic vitamin C administration resulted in a shortening of the saliva half-life of antipyrine in each of the five subjects ($P < 0.05$ by paired *t*-test). A saliva concentration-time course for antipyrine before and after the vitamin supplement for a typical subject is shown in Figure 1.

Table 1 Effect of chronic vitamin C administration on the pharmacokinetics of antipyrine

Subject	$T_{1/2}$ (h)		Apparent volume of distribution (l kg ⁻¹)		Total body clearance (ml kg ⁻¹ h ⁻¹)	
	Control	After vitamin C	Control	After vitamin C	Control	After vitamin C
MC	13.3	9.3	0.450	0.408	23.4	36.3
MD	12.0	9.0	0.606	0.603	35.0	46.4
BH	10.0	8.0	0.493	0.535	34.1	46.3
JH	8.3	7.0	0.505	0.513	42.2	50.8
WM	6.2	6.0	0.576	0.590	63.9	68.4
Mean	10.0	7.9	0.526	0.530	39.7	49.6
s.d.	2.8	1.4	0.064	0.078	15.1	11.7
Statistical difference from control		$P < 0.05$		NS		$P < 0.005$

Figure 1 Time course for antipyrine saliva concentrations in a typical subject (MD) before (○) and after (●) the vitamin supplement.

It should be noted that although this investigation, and that of others where plasma samples were obtained (Pagliaro & Benet, 1975), have shown large intersubject variations in antipyrine half-life, it has been documented that individual antipyrine half-lives show good reproducibility (Lindgren, Collste, Norlander & Sjoqvist, 1974; Kellermann, Luyten-Kellermann, Horning & Stafford, 1976).

The mean total body clearance increased from $39.7 \text{ ml kg}^{-1} \text{ h}^{-1}$ to $49.6 \text{ ml kg}^{-1} \text{ h}^{-1}$ ($P < 0.005$ by paired *t*-test) after the vitamin course. There was a significant correlation ($r = 0.913$, $P < 0.02$) between the total body clearance in the control study and the percentage increase in total body clearance after vitamin administration for the subjects studied (Figure 2).

There was no evidence to indicate that the partitioning of antipyrine between saliva and blood was altered by the vitamin supplement. The apparent volume of distribution of antipyrine was not altered by administration of the vitamin (Table 1). These values were very similar to those reported from plasma data (Lindgren *et al.*, 1974; Vestal, Norris, Tobin, Cohen, Shock & Andres, 1975; O'Malley, Stevenson & West, 1975). Also

Figure 2 Relationship between total body clearance of antipyrine in control study and the increase in total body clearance of antipyrine following the vitamin supplement in five subjects.

there was no significant difference between the antipyrine concentration in the first saliva samples (obtained 3 hours after administration) from the control study ($15.7 \pm 2.2 \text{ μg/ml}$, mean $\pm$ s.d.) and from the study after vitamin administration ($15.5 \pm 3.4 \text{ μg/ml}$).

Vitamin C and/or its metabolites had no apparent effect on the antipyrine assay. Several saliva samples obtained after administration of antipyrine alone were diluted with an equal volume of saliva either from unmedicated subjects or from subjects taking vitamin C only. The analytical results were not significantly different.

The results of this study show that a 1 g per day supplement of vitamin C for 7 days can cause a statistically significant increase in the total body clearance of antipyrine. This is in agreement with the study by Feetam, Leach & Meynell (1975) who demonstrated that in man steady state plasma warfarin concentrations (but not prothrombin times) were decreased significantly ($P < 0.05$ by paired *t*-test) by vitamin C supplements. Investigations using guinea pigs have shown that high intake of vitamin C can influence the components of the hepatic, oxidative drug-metabolism complex: increases in the concentrations of cytochromes P-450 and b_5 (Degkwitz & Kim, 1973) and enhancement of cytochrome P-450 reductase and o-demethylase activity (Sato & Zannoni, 1974). The biochemical basis for this enhancement of oxidative biotransformation remains unclear (Sato & Zannoni, 1976).

While the present study was taking place, the results of another investigation into the influence of vitamin C on drug oxidation was published (Wilson, Van Boxtel, Alvan & Sjoqvist, 1976). Although this study involved thirteen subjects it is

difficult to draw conclusions from their data since five different vitamin regimens were used. Subjects receiving vitamin doses $< 1.2\text{g}$ daily ($n = 7$) showed a significant increase ($P < 0.05$ by paired t -test) in total body clearance of antipyrine whereas higher daily doses ($n = 6$) tended to decrease total body clearance. The net effect when all data was considered was no significant difference. An unusual dose-response relationship may exist for vitamin C and hepatic oxidative function and clarification of this phenomenon must await further investigations.

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