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Dissolution and bioavailability of digoxin tablets

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A dissolution test is described for tablets of digoxin B.P. Dissolution profiles are reported for 11 brands of digoxin tablets available in Gt. Britain in 1972. Good correlations exist between both the area under the serum concentration/time curve from 0–6 h and (1) the amount of digoxin dissolved in 1 h, (2) the reciprocal of the time for 50% dissolution.

Digoxin is a potent drug with a narrow margin between its therapeutic and toxic dose. Many factors influence the cardiac response to a constant dose in an individual, for example age, renal function, serum potassium level and thyroid state.

Following the development of radioimmunoassay procedures for determination of serum digoxin levels, a new variable, that of differences in bioavailability of digoxin tablets, was described by Lindenbaum, Mellow & others in 1971. Similar experiences were reported in 1972 by Shaw, Howard & Hamer. These latter workers, together with Manninen, Melin & Härtel (1971), suggested that differences in bioavailability might be due to differences in rate of disintegration. Fraser, Leach & Poston (1972) pointed out that for a drug so sparingly soluble as digoxin, differences in bioavailability were likely to be dependent on the dissolution rate, rather than the disintegration time. Their preliminary report like that of Beckett & Cowan (1972) supported this suggestion. The purpose of this paper is firstly to describe a method for determination of dissolution rate of single digoxin tablets, secondly to report dissolution profiles of several brands of digoxin tablets available in the United Kingdom and finally to demonstrate the correlation between the *in vitro* test and the *in vivo* findings.

MATERIALS AND METHODS

In vitro *study*

The tablets examined were samples from single batches of 9 brands of digoxin tablets B.P., together with samples from single batches of "Lanoxin" (Burroughs Wellcome & Co.) manufactured before and after May, 1972—referred to as 'Old' and 'New' Lanoxin respectively.

Uniformity of content. Preliminary experiments showed that complete solution of digoxin occurred when single tablets were stirred in 500 ml 0·1M hydrochloric acid at high speed for 4 h. Single tablet assays were performed after such treatment, using the fluorometric procedure described below.

Disintegration time. This was according to the British Pharmacopoeia.

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Dissolution rate. This was based on the beaker method of Levy & Hayes (1960), and the flask method of Poole (1969). A 2 litre round-bottom flask with one side-arm, containing 500 ml of 0·1M hydrochloric acid was immersed in a water-bath at 37° ± 0·5°. The stirring rod was set at a constant height in the dissolution medium and rotated at 70 rev min^{-1} by an electric motor (Citenco Type KQTS/11). The tablet was introduced through the side-arm and was positioned at the bottom of the flask under the stirring rod. Samples were removed from the dissolution medium via the side-arm, using a needle set at a constant height and angle. Five ml samples of the dissolution medium were removed at 5, 15, 30, 45 and 60 min, and then at 30 min intervals to 240 min. After each sample had been removed 5 ml of 0·1M hydrochloric acid was added to the dissolution vessel to maintain constant volume.

The assay method used was modified from that of Wells, Katzung & Meyers (1961). Each 5 ml sample was filtered through a membrane filter. 2·5 ml of each filtrate was then reacted with 0·2 ml of 18·75 mmol $litre^{-1}$ hydrogen peroxide, freshly prepared every 24 h and 7·3 ml of a reagent containing: Analar hydrochloric acid (wt per ml 1·18 g) 7·2 ml; ascorbic acid B.P.C. 4·0 mg; Analar methanol 0·1 ml. This was freshly prepared every 48 h. The reaction mixture was left to stand for 40 min to allow fluorescence to develop. For each batch of samples a blank solution (2·5 ml of 0·1M hydrochloric acid) and a standard solution (Digoxin U.S.P. XVI 0·5 $\mu g\ ml^{-1}$) were treated similarly. The fluorescence obtained after 40 min was shown to be stable for at least 3 h. The intensity of fluorescence was measured on a Perkin Elmer model 204 Fluorimeter at an excitation wavelength of 350 nm and an analyser wavelength of 485 nm and was linearly related to the concentration of digoxin. The coefficient of variation for 17 replicates of 0·5 $\mu g\ ml^{-1}$ was ±1·13%. It appeared that common tablet excipients did not interfere with the assay.

In vivo *study*

Six healthy male volunteers took single doses of 0·5 mg each (two tablets) of 'New' and 'Old' Lanoxin and 2 other brands. At least seven days separated each test. Volunteers fasted from midnight, took the tablets about 09.00 h and fasted for a further 4 h. Blood samples were taken at zero time, at quarter hourly intervals until 1 h, at half hourly intervals until 2 h, hourly until 4 h, and after 6, 8 and 24 h. Serum digoxin levels were determined by radioimmunoassay using the Lanoxitest β kit method (Wellcome Reagents Ltd.); the only modification was the use of pooled normal digoxin-free human serum, rather than bovine albumin for addition to digoxin standards. Serum was stored at −20° until analysed. All samples were assayed at least twice in different batches. The standard deviations for the determination of digoxin in commercial control sera were 0·24 and 0·20 ng ml^{-1} for mean digoxin concentrations of 3·62 and 1·65 ng ml^{-1}. These were assayed 12 and 15 times respectively in parallel with the batches of experimental sera throughout the study.

Serum concentrations were plotted against time on linear graph paper. The area under the concentration/time curve was taken as the estimate of bioavailability, and this was quantitated by cutting out and weighing. The coefficient of variation of the weight of 20 samples of the graph paper used for the above plots was ±3·1%. Serum digoxin concentrations in the time period 6 to 24 h were of the same order as the precision of the assay. For this reason estimates of bioavailability were made for the period 0–6 h. The bioavailability of 'Old' Lanoxin was arbitrarily selected as the standard so that the bioavailability of the other brands could be compared.

Table 1. *Individual tablet assay.*

	Manufacturer	Mean digoxin per tablet μg (% of labelled content)	Range % of labelled content
1	'New' Lanoxin	261 (104)	101–106
2		292 (117)	104–125
3	(6 samples)	303 (121)	120–125
4		297 (119)	104–135
5	(3 samples)	264 (105)	105–106
6		253 (101)	82·8–120
7		276 (110)	101–120
8	'Old' Lanoxin	246 (98·4)	93·6–103
9		265 (106)	97·2–110
10		286 (114)	100–121
11		227 (90·8)	87·2–96·0

RESULTS AND DISCUSSION

In vitro *study*

Uniformity of content. Single tablet assays were performed on five samples of each brand since variation in digoxin content from tablet to tablet would be expected in a formulation with such a small amount of active ingredient (about 0·25%) no matter how satisfactory the trituration, granulation and tableting processes. Such variation might influence dissolution and serum level experiments and also, if significant, might directly affect therapeutic response. The results for the uniformity of content are shown in Table 1. It is apparent that standards of processing vary from manufacturer to manufacturer, and such results underline the importance of the recently introduced uniformity of content standard in the British Pharmacopoeia.

Disintegration test B.P. All samples disintegrated within 15 min and therefore passed the test. As expected for a sparingly soluble drug like digoxin the disintegration rates in Table 2 bear little relation to the dissolution rate.

Dissolution studies. Tablets from single batches of the 11 samples were examined for their dissolution characteristics. The dissolution pattern for single tablets was consistent, results usually agreeing within ±5% except for the first two readings which might have a range of ±10%. For this reason only two samples of each

Table 2. *Disintegration test and t50 dissolution results*

Manufacturer	Time in minutes taken for 50% digoxin to dissolve	Disintegration time—B.P.
1	4	2 min 8 s
2	8	47 s
3	11	4 min 47 s
4	35	29 s
5	53	1 min 27 s
6	105	9 min 0 s
7	110	3 min 23 s
8	110	3 min 52 s
9	160	9 min 6 s
10	210	2 min 18 s
11	227	3 min 40 s

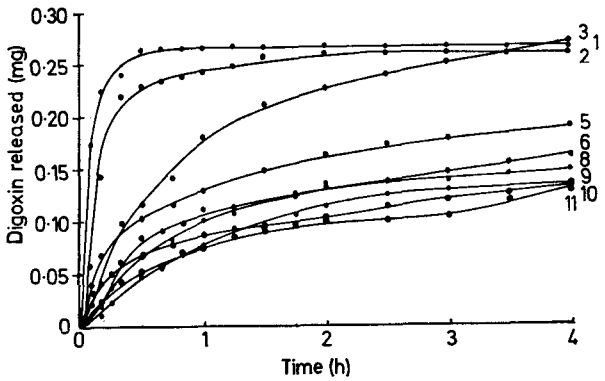

FIG. 1. Dissolution of 9 samples of digoxin tablets B.P. (1 = "New", 8 = "Old" Lanoxin).

brand were examined and their means were plotted against time. Nine representative dissolution profiles are shown in Fig. 1. The wide differences are striking, and that some tablets are less than 60% dissolved even after 4 h might well account for poor absorption.

These differences in dissolution rate may be largely due to variation in particle size as suggested by Jounela & Sothman (1973). The times for 50% dissolution were estimated from these profile data and are reported in Table 2.

In vivo *study*

For tablets which dissolve rapidly the *in vivo* results showed high peaks occurring soon after ingestion; these peaks declined steeply as redistribution and excretion occurred. This effect is shown in Fig. 2 where the mean serum concentrations for each brand in six subjects are plotted against time. One volunteer experienced severe nausea about 1½ h after administration of 'New' Lanoxin, when serum concentrations were at a peak of about 3 ng ml.$^{-1}$ Similar results were reported by Falch, Teien & Bjerkelund (1973). Thus tablets with a rapid dissolution rate may be more likely to produce this side-effect unless given in divided doses during the day.

The bioavailability results are shown in Table 3. Application of the *t*-test to these estimates of bioavailability showed that the areas under the concentration/time curves

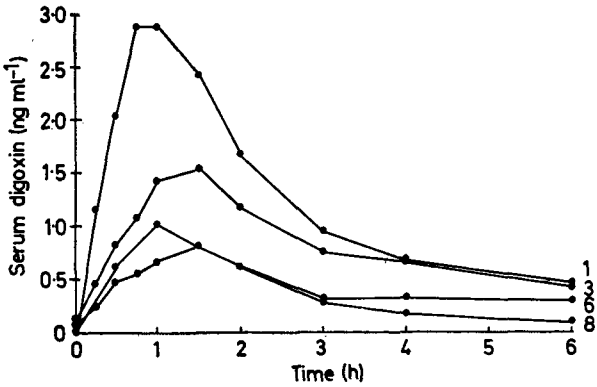

FIG. 2. Mean serum concentration *vs* time curves in 6 subjects (1 = "New", 8 = "Old" Lanoxin).

Table 3. *Bioavailability* results in 6 subjects.*

Subject	Old Lanoxin	New Lanoxin	Manufacturer 3	Manufacturer 6
1	0·66	3·02	2·83	0·61
2	1·78	2·34	2·33	0·73
3	0·65	4·02	1·26	1·35
4	0·53	3·02	1·03	0·66
5	1·45	2·45	1·94	0·78
6	0·93	2·71	2·26	1·27
Mean	1·00	2·93	1·94	0·90
s.d.	0·51	0·60	0·68	0·32

* $\frac{\text{Mean area under serum concn/time curve 0–6 h for other brands}}{\text{Mean area under serum concn/time curve 0–6 h for 'Old' Lanoxin}}$

Table 4. *Correlation between* in vitro *parameters and bioavailability for 4 brands of digoxin tablets studied.*

Parameter	Correlation Coefficient	
Wt digoxin dissolved after ½ h	0·944	0·05 $< P <$ 0·1
" " " " 1 h	0·995	$P <$ 0·01
" " " " 2 h	0·981	$P <$ 0·05
" " " " 3 h	0·926	0·05 $< P <$ 0·1
" " " " 4 h	0·889	$P >$ 0·1
Reciprocal of dissolution half time (1/t50)	0·984	$P <$ 0·05

in Fig. 2 were significantly different ($P <$0·05) from each other except the comparison of 'Old' Lanoxin with tablets from manufacturer 6.

Bioavailability was estimated from the serum profiles in the period of 0–6 h. This is a short period in relation to the half life of the drug, but was dictated by the relative insensitivity of the radioimmunoassay.

In vitro—in vivo *correlation*

Multiple correlations were calculated to investigate how far the dissolution data for the four brands studied related to the estimates of bioavailability. Correlation coefficients are shown in Table 4 between bioavailability and (1) the amount of digoxin dissolved at various times and (2) the reciprocal of the half time for dissolution (1/t50).

Good correlations were obtained at the 1 h period and also for the 1/t50. This strongly suggests that determination of the weight of digoxin dissolved at 1 h or estimation of the reciprocal of the dissolution half time would be useful quality control standards to which each batch of digoxin tablets could be submitted.

These studies confirm the differing bioavailabilities of tablets of digoxin and, for the first time, describe an *in vitro* test that should prove useful in controlling this variable.

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