Determination of Bioavailability of Digitoxin Using the Radioimmunoassay Procedure

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Abstract □ A commercial digitoxin radioimmunoassay procedure, normally used to evaluate equilibrium-state plasma levels, was modified such that the sensitivity was increased 20-fold. Due to the extensive metabolism of digitoxin in man, the specificity of the assay procedure for digitoxin was determined against several known metabolites. These studies indicated that the assay procedure was more sensitive to digoxin and the monodigitoxoside and bisdigitoxoside of digitoxigenin than to digitoxin itself. It also had a lesser degree of sensitivity to the aglycone digitoxigenin. For the bioavailability study, single 0.1-mg. doses of digitoxin were administered orally, as one of two commercial brands of tablets, to a panel of eight subjects in a crossover study with 56 days separating the two doses. Plasma levels were measured for 15 days following dosing. Significant differences in the plasma levels between brands, as determined by analysis of variance, were found at 0.5, 1, 72, and 240 hr. The peak plasma levels were found to differ significantly. No statistical difference in the relative bioavailability, as measured by the area under the plasma level curve, was found between the two tablets tested.

Keyphrases □ Digitoxin plasma levels—radioimmunoassay determination, two commercial tablets, man □ Bioavailability, two commercial digitoxin tablets—determination of plasma levels, man □ Radioimmunoassay technique—determination, digitoxin plasma levels, two commercial tablets, man

During the past few years, this laboratory has been involved in a continuing effort to study the generic equivalence or inequivalence of marketed drug products. Past studies have included ephedrine (1), warfarin (2), and propoxyphene (3).

The importance of uniform dosage and bioavailability is perhaps epitomized by the cardiac glycosides as with no other series of therapeutic agents, because these agents produce such a strong inotropic effect on the heart and frequently represent the difference between life and death for many patients suffering from congestive heart failure. Since digitoxin is known to have a long half-life, it is particularly important that uniform dosage and bioavailability limits are maintained. These difficulties are further complicated by the fact that a fairly low dose of the cardiac glycoside is used to achieve the desired therapeutic results, and manufacturing tablets with uniform drug content apparently is difficult when such low levels of medication are required. The manufacturing difficulties are evidenced by the number of tablets of various cardiac glycosides that have been recalled during a 1-year period. As determined from the "Drug Recall" section of the *Journal of the American Pharmaceutical Association* during the period of June 18, 1970, to June 16, 1971, approximately 25 million tablets containing cardiac glycosides were recalled by the Food and Drug Administration (FDA).

Assay methodology to perform adequate bioavailability studies on digitoxin tablets was lacking, since assay sensitivity was usually not sufficient to follow plasma levels long enough to measure bioavailability. With the advent of the red blood cell ^{86}Rb-uptake assay (4) and the Na,K-ATPase inhibition procedure (5), new levels of assay sensitivity for digitoxin were achieved. Nonetheless, both procedures are too impractical for routine assays as are needed for a bioavailability study. The development of the radioimmunoassay procedure for digitoxin (6) unquestionably provided the researcher with an assay technique that could be made sufficiently sensitive to measure the levels of drug for an adequate length of time to determine the bioavailability of a single dose of digitoxin.

This report is the first in a series on the bioavailability of digitoxin and digoxin commercial tablets. The commercial radioimmunoassay[1] procedure, normally used to determine equilibrium-state plasma levels for patients receiving the medication chronically, was modified and the sensitivity was increased at least 20-fold to achieve the necessary assay sensitivity for a single-dose bioavailability study. Thus, a reproducible sensitivity of 0.2 ng./ml. was achieved. In the study reported here, plasma levels of digitoxin and its metabolites were measured in eight normal volunteers in a crossover study for 15 days following the administration of a 0.1-mg. dose of the drug. The innovators' product was compared to one other commercial digitoxin tablet USP.

EXPERIMENTAL

The radiotracer, antiserum, standard digitoxin solution, and buffer components used for this radioimmunoassay procedure were all obtained from the radioimmunoassay kit[1]. The dextran-coated charcoal[2] suspension was prepared from laboratory materials. The assay samples were counted in a liquid scintillation spectrometer[3], using a liquid scintillation cocktail[4]. The reference standard used for preparation of a quench curve was standard tritiated water[5], and the most suitable quenching agent for the scintillation cocktail was found to be chloroform. All other chemicals used were reagent grade and were used as received.

Study Conditions and Subjects—Both brands of tablets used conformed to the USP XVIII requirements with respect to identity, disintegration time, content, content uniformity, and other digitoxosides. Individual tablets of Treatment A[6] were assayed by FDA and were found to contain an average of 101.3% of label, with a range of 98.6–104.9%. Treatment B[7] tablets were also assayed at FDA laboratories and were found to contain an average of 96.5% of label, with a range of 89.7–103.5%.

The screening method used for this study was previously reported by Wagner *et al.* (2). The subjects selected were eight normal males from 22 to 30 years of age. The subjects were arranged into two groups of four as follows: Group I, Subjects 1–4, weight 54.4–78 kg., age 22–29 years; and Group II, Subjects 5–8, weight 81.2–93 kg., age 24–30 years.

[1] Tritiated-digitoxin Radioimmunoassay Kit, Schwarz/Mann, Orangeburg, NY 10962

[2] Dextran T70, Pharmacia, Uppsala, Sweden; and Norit A charcoal, Sigma Chemical Co., St. Louis, Mo.

[3] Packard Tri-Carb, model 3320.

[4] Unogel, Schwarz/Mann, Orangeburg, N. Y.

[5] New England Nuclear, Boston, Mass.

[6] Treatment A was one tablet of Purodigin, 0.1 mg., Wyeth, Lot 1693870.

[7] Treatment B was one tablet of digitoxin USP, 0.1 mg., Squibb, Lot 9L695.

The protocol was as previously reported by Wagner *et al.* (2) with some modifications. The changes in protocol were related to the changes in medication, sampling times, and period of treatments. First, samples of whole blood were taken from a forearm vein at time zero, 0.5, 1, 2, 4, 6, 12, 24, 72, 120, 240, and 360 hr. after dosing. The two doses administered in the crossover study were separated by 56 days or approximately 9–10 half-lives of elimination.

During Phase I of the study, Subjects 1–4 received a dosage form designated as Treatment A[6], while Subjects 5–8 received Treatment B[7]. Fifty-six days later, Subjects 1–4 received Treatment B and Subjects 5–8 received Treatment A.

***In Vitro* Dissolution Studies**—The dissolution of individual tablets was conducted in a 1-l. three-necked flask containing 500 ml. of distilled water maintained at 37°. A single-bladed, Teflon stirrer was rotated at 50 r.p.m., and 10-ml. samples were removed at predetermined time periods with immediate replacement with fresh solvent. At least four dissolution trials were conducted for each brand of tablets.

The samples were analyzed by the fluorometric method of Wells *et al.* (7) in a spectrophotofluorometer[8], using an activation wavelength of 395 nm. and an emission wavelength of 570 nm. The procedure was used to analyze quantitatively aqueous solutions containing 0.01–0.25 mcg./ml. of digitoxin. With different sets of reagents, the slope varied slightly; therefore, a separate calibration curve (0.2–8.0 ng./ml.) was run with each individual dissolution trial. All assays were carried out at least in duplicate.

Radioimmunoassay—The procedure used for the analysis of total digitoxin derivatives in plasma was a modification of the one described in the commercially available digitoxin radioimmunoassay kit[1]. The commercial procedure essentially was that reported earlier by Smith (6).

Whole blood, obtained by venipuncture, was collected from patients in vacutainers containing sodium citrate as an anticoagulant. The plasma samples were stored in capped polystyrene test tubes at −15° for up to 2 weeks prior to assay. It had been previously determined that these storage conditions did not adversely affect the assay of digitoxin.

Two significant changes were made in the recommended procedure. The modifications involved changing the ratio of the tracer to antiserum to obtain the necessary assay sensitivity in the concentration range of 0.2–8.0 ng./ml. Yalow and Berson (8) discussed the factors involved in the antigen–antibody binding process and gave an excellent background discussion for the radioimmunoassay procedure. Also, since it was necessary to change the quantity of plasma used in the assay procedure to 1 ml., the concentration of charcoal in the dextran-coated charcoal suspension had to be changed. The factors that determine the amount and type of adsorbent used in radioimmunoassay procedures were discussed by Herbert *et al.* (9). The charcoal suspension used in the experiment described in this paper consisted of 250 mg. dextran and 10 g. charcoal in 100 ml. of pH 7.4 phosphate-buffered saline. The phosphate-buffered saline was prepared from the kit material, and the pH was adjusted as described in the commercial kit.

The assay procedure involved the following steps. To 1 ml. of patient plasma or plasma spiked with digitoxin standard solution, 10 μl. of antiserum and 5 μl. of tracer were added. To the blank plasma sample, only 5 μl. of tracer was added. Calibrated Lang Levy constriction pipets[9] were used to measure all microliter quantities, because automatic pipeting devices were found to be too inaccurate. The material was then gently mixed on a vortexer and allowed to incubate at ambient temperature for 30 min. Following the incubation, 0.5 ml. of the dextran-coated charcoal suspension was rapidly added to each sample, the material was vortexed gently, and after 5 min. the material was centrifuged at 1500×*g* for 20 min. The clear supernate was then decanted into 15 ml. of liquid scintillation-solubilizer fluid and counted in a liquid scintillation counter.

A quench correction for all samples was made through use of an automatic external standard, and a standard curve was prepared from reference standard tritiated water[5]. The data obtained were then corrected to disintegrations per minute, and the percent of tritiated digitoxin bound to the antiserum could be calculated. The percent bound was then plotted against the concentration of unlabeled digitoxin in solution to obtain the standard curve shown in Fig. 1. In addition to the patient samples and the standards determined with each assay group, it was also necessary to determine the appropriate blanks (consisting of all components except antiserum), total counts for the tracer added (consisting of adding 5 μl. of the tracer directly to the liquid scintillation flask), and the total amount of tracer bound by the antiserum in the absence of unlabeled drug, which in this case was 30–35%. The total count value was used as a control for the number of counts added to each sample, and the blanks were used as a background value for correction of all counts obtained for the assayed samples.

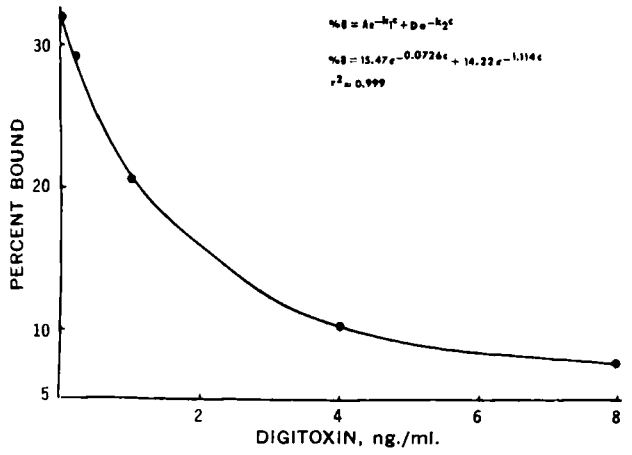


Figure 1 – *Example of typical calibration curve determined for each set of samples analyzed.*

An example of a typical calibration curve is shown in Fig. 1. Each curve was fitted to a biexponential equation using the program NONLIN[10] on a computer[11]. In all cases, the fits were essentially perfect, giving a coefficient of determination[12] of at least 0.999. An example of the equation used for the calibration curves and the parameter estimates for the sample calibration curve shown are found inset in Fig. 1. Since all samples were assayed in duplicate, it was possible to calculate a mean normalized difference between duplicates in terms of percentages. In all, 352 samples (not including calibration curves) were analyzed. The mean normalized difference between duplicates was 12.3% with a standard deviation of 14.1%. This value represents the actual differences found from day to day for all patient samples and at all concentrations levels.

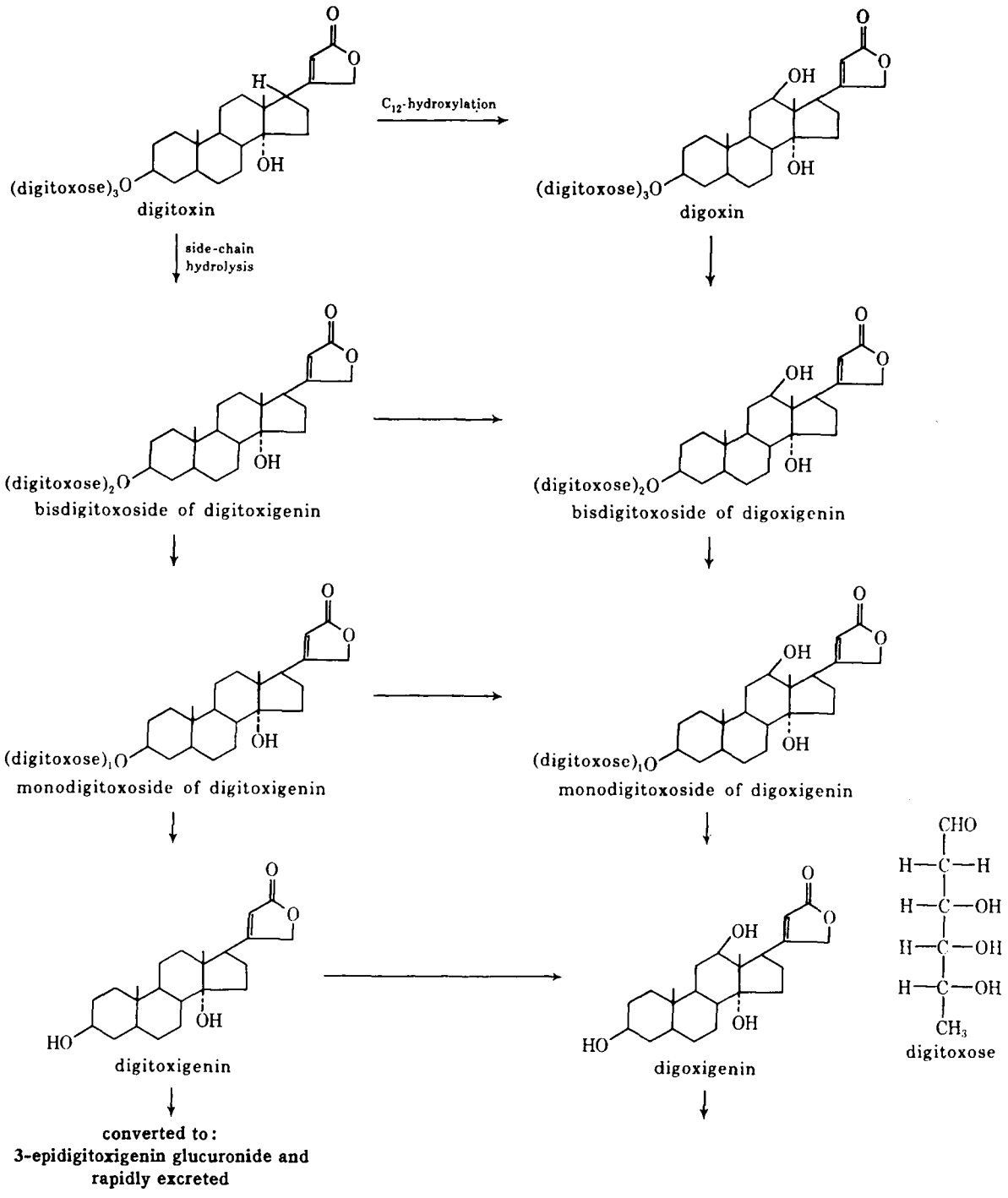
Cross-Reactivity Studies Since it has been noted that digitoxin is extensively metabolized in man and other species (10–13), it was deemed necessary to evaluate the binding of the major digitoxin metabolites with the antiserum under the assay conditions. Thus, the degree of binding of digoxin[1], the bisdigitoxoside[13] and the monodigitoxoside[13] of digitoxigenin, and digitoxigenin[13] with the antiserum was studied. Since these compounds interact with the antiserum on a molecular basis, the comparison of their reactivity with the antiserum was made on a molar basis. Previous studies (6, 14) showed that most endogenous steroid compounds produce virtually no interference with the digitoxin antiserum. Usually, levels of 1000 10,000 ng./ml. of progesterone or testosterone were necessary to inhibit antibody binding of tritiated digitoxin. In the present studies, approximately 20% of the blank serum samples produced small false-positive responses. The "apparent" concentration of drug in the blanks was usually calculated to be less than 0.1–0.2 ng./ml. For those subjects where such apparent positive blank values occurred, the percent tritiated digitoxin bound was corrected or normalized for all samples.

RESULTS

The scheme for the metabolism of digitoxin (Scheme I) was proposed by Lauterbach and Repke (15) following *in vitro* and *in vivo* studies with rats. A similar pattern for the metabolism of digitoxin

[8] Aminco-Bowman, model 4-8166, American Instrument Co., Silver Spring, Md.
[9] A. H. Thomas Co., Philadelphia, PA 19105

[10] The program NONLIN was supplied by Dr. C. M. Metzler, The Upjohn Co., Kalamazoo, Mich.
[11] IBM 360/67.
[12] Coefficient of determination = $r^2 = (\Sigma obs^2 - \Sigma dev^2)/\Sigma obs^2$.
[13] Boehringer Mannheim Corp., New York, N. Y.



Scheme I—Proposed metabolic scheme for digitoxin by Lauterbach and Repke (15)

in man has evolved primarily through the works of Okita and coworkers (12, 13), Ashley *et al.* (11), Hermann and Repke (16), and Repke (17). Therefore, the effects of these various metabolites on the analysis of digitoxin in plasma were determined.

Figure 2 shows the results of the studies comparing the effect of nonradiolabeled digitoxin and digoxin on the binding of ^{3}H-digitoxin with the antiserum. It is apparent that, on an equimolar basis, digoxin displaces the tracer from the antiserum to a larger extent than digitoxin itself. This was not too surprising since the antiserum used was prepared by challenging rabbits with a digoxin-albumin conjugate; thus, such cross-reactivity was anticipated. Although the relative proportions of digitoxin to digoxin necessary to produce the same percent of tracer bound to antiserum are variable, for most of the curve it takes approximately four times as much digitoxin as digoxin to produce the same decrease in the percent tracer bound.

A separate study was undertaken to determine the sensitivity of the radioimmunoassay to the various digitoxigenin derivatives of digitoxin, and all of the metabolites interacted to a varying degree with the antiserum (Fig. 3).

Figure 4 is a plot of the *in vitro* dissolution data for the two brands of tablets used in the *in vivo* studies. The curves for each brand were obtained from the average data of four dissolution trials. The vertical bars represent 1 standard deviation on either side of the mean. The results of the analysis of variance for the dissolution of the two dosage forms are shown in Table I.

Table II presents the average plasma levels of digitoxin and its metabolites obtained at each indicated time period. The values reported are the mean plasma levels of the eight subjects. Also shown are the average peak plasma concentrations and the average elimination half-lives. The area under the plasma level–time curve, shown

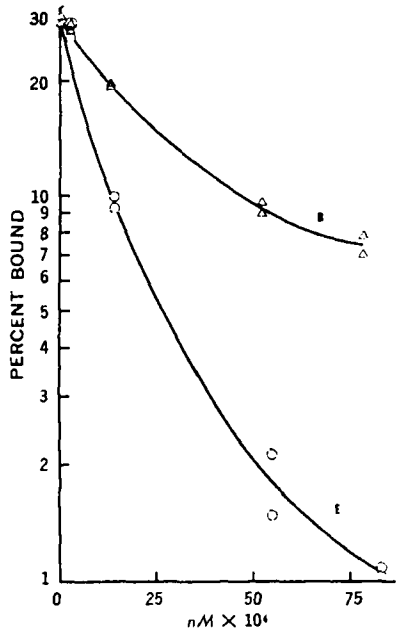


Figure 2—*Effect of digoxin on digitoxin radioimmunoassay. Key: B, △, digitoxin; and E, ○, digoxin.*

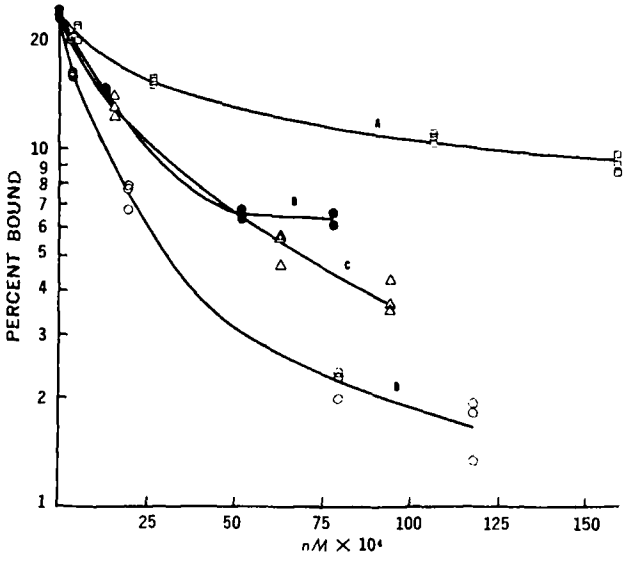


Figure 3—*Affinity of various digitoxigenin metabolites of digitoxin for antiserum in commercial radioimmunoassay procedure. Key: A, □, digitoxigenin; B, ●, digitoxin; C, △, bisdigitoxoside of digitoxigenin; and D, ○, monodigitoxoside of digitoxigenin.*

as average area in this table, is an indication of the relative availability of each dosage form. Data for the 0–360-hr. period and the estimated area from zero to time infinity are also reported.

DISCUSSION

The metabolic pathway shown in Scheme I was developed primarily from urinary excretion data. Very little information is available on the blood levels of these various metabolites, primarily because of the extremely low levels of drug and metabolites obtained following normal doses of digitoxin. This problem has not been satisfactorily resolved to date. However, urinary excretion studies have indicated that digitoxin is extensively metabolized in man and, as reported by Okita (12), only approximately 8% of an administered dose of ^{14}C-digitoxin was recovered as unchanged drug in the urine over a 3-week period; approximately 70% of the dose was recovered as metabolic products over the same time interval (12). A major component of the metabolic products was determined to be digoxin (12).

Since the kit[1] used in this study had an antiserum that was prepared by challenging rabbits with a digoxin albumin conjugate, it was apparent that some cross-reactivity would exist between digitoxin and its metabolite digoxin. Since all of the other metabolites shown in Scheme I have also been detected in urine, the possibility of their existence in the blood should be considered. As shown in Fig. 2, the antiserum exhibited a much greater affinity for digoxin than for digitoxin.

On an equimolar basis, the ability of each of the digitoxigenin derivatives to interfere with the ^{3}H-digitoxin antiserum binding process was found to be the monodigitoxoside of digitoxigenin > the bisdigitoxoside of digitoxigenin ≃ digitoxin > digitoxigenin (Fig. 3). Thus, all metabolites of digitoxin tested so far would in a very real sense interfere with the analysis of digitoxin, depending on the relative proportions of each in the blood. It would appear that the level of digitoxigenin would be extremely small since it has been reported that this material is rapidly conjugated and excreted. Also, since this compound has the least affinity for the antiserum, it would contribute very little in the analysis of digitoxin in plasma. However, both the monodigitoxoside and bisdigitoxoside of digitoxigenin would markedly affect the radioimmunoassay of digitoxin in plasma. The digoxigenin derivatives (Scheme I) were not studied in this experiment. However, recent studies in these laboratories with the digoxin radioimmunoassay procedure indicated that these derivatives have essentially the same affinity for the antiserum as digoxin itself. Thus, again depending upon their plasma levels in man, these components could potentially interfere with the analysis of digitoxin. Having verified that many of these substances have at least the same or greater affinity for the antiserum as digitoxin itself, it can be concluded that the digitoxin radioimmunoassay procedure used in this study was not specific for just digitoxin and most likely was actually a measure of total drug and metabolites in the plasma.

It is felt that this information in no way detracts from the clinical usefulness of the radioimmunoassay procedure. It is still the most sensitive, convenient, and economical procedure available at present for measuring cardiac glycosides in the blood. It has been shown (18) that all of the metabolites are cardioactive to varying degrees in both the guinea pig and the cat. Digitoxin and the monodigitoxoside and bisdigitoxoside of digoxigenin were approximately equipotent. Digitoxigenin itself was only about one-half as potent as digitoxin. The same is true for digoxin and the monodigitoxoside and bisdigitoxoside of digoxigenin, which have about the same potency, with digoxigenin having only approximately one-tenth the activity of digoxin. Nonetheless, all important metabolites of digitoxin ap-

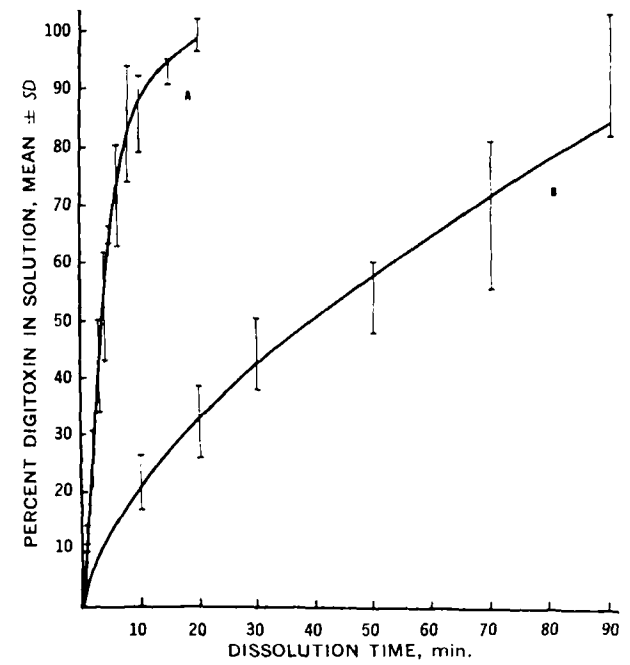


Figure 4—In vitro *dissolution of two commercial digitoxin tablets in distilled water at 37°, Treatments A and B.*

Table I—Summary of Analyses of Variance of $t_{25\%}$, $t_{50\%}$, and $t_{75\%}$ Values for Two Brands of Digitoxin Tablets Supplied by FDA

Brand	Brand[b] Average $t_{25\%}$[a], min.	$t_{50\%}$[a], min.	$t_{75\%}$[a], min.
Treatment A	1.9	3.9	6.7
Treatment B	14.6	46.5	74.1
F-value from ANOVA	26.17[c]	119.96[c]	141.50[c]
Significance level	$0.025 > p > 0.01$	$0.055 > p > 0.001$	$0.005 > p > 0.001$

[a] $t_{25\%}$, $t_{50\%}$, and $t_{75\%}$ are the times in minutes necessary for dissolution of 25, 50, and 75% of the total tablet contents, respectively. [b] Each average is based on four tablets drawn randomly from the same bottle. [c] Significant.

Table II—Summary of Average Plasma Concentrations of Digitoxin

Hours	Average Plasma Concentration, ng./ml. Treatment A	Treatment B	—From Analysis of Variance for Crossover Design— Coefficient of Variation[a]	Significant Level of Differences between Averages[b]
0.5	5.4	2.3	52.5	Sig. ($p < 0.001$)
1.0	5.4	4.1	24.8	Sig. ($0.01 > p > 0.005$)
2.0	3.3	3.0	19.0	N.S. ($p > 0.25$)
4.0	2.2	2.4	16.7	N.S. ($p > 0.25$)
6.0	1.9	1.9	20.5	N.S. ($p > 0.25$)
12.0	1.4	1.4	17.4	N.S. ($p > 0.25$)
74.0	1.5	1.3	22.2	N.S. ($0.25 > p > 0.10$)
72.0	1.3	1.0	22.1	Sig. ($0.05 > p > 0.025$)
120.0	1.0	0.9	27.4	N.S. ($0.25 > p > 0.10$)
240.0	0.7	0.5	31.2	Sig. ($0.025 > p > 0.01$)
360.0	0.5	0.5	42.2	N.S. ($p > 0.25$)
Average peak plasma concentration	6.6	4.6	30.1	Sig. ($0.005 > p > 0.001$)
Average area, 0–360 hr.	338.0	291.0	27.9	N.S. ($p > 0.25$)
Average estimated area, zero to infinity	467.0	446.0	41.5	N.S. ($p > 0.25$)
Average elimination $t_{1/2}$, days	8.03	9.85	32.9	N.S. ($p > 0.25$)

[a] Coefficient of variation (%) = $\frac{\sqrt{\text{residual mean square}}}{\text{grand average}} \times 100$. [b] Sig. = significant, and N.S. = not significant.

pear to be cardioactive and thus the assay procedure is a measure of the active drug in the blood following any given dose of digitoxin. However, pharmacokinetic analysis of digitoxin data cannot be carried out using this assay procedure unless the metabolites are separated from the parent compounds prior to analysis.

Tablets of two different manufacturers were subjected to *in vitro* dissolution tests to determine which tablets would be used for the *in vivo* bioavailability studies. The dissolution rate curves for the two tablets used in the studies are shown in Fig. 4. The dissolution of the Treatment B tablet was significantly slower than that of the Treatment A tablet, but in all cases dissolution was found to be complete within 2 hr. Table I shows the data for the average time required to dissolve 25, 50, and 75% of the digitoxin from the two tablet forms studied. As determined by analysis of variance, the differences between the average dissolution time at 25, 50, and 75% dissolved were highly significant.

Table II presents the average plasma levels of digitoxin and its metabolites obtained at each indicated time period. The differences between the average plasma levels of digitoxin during the first two sampling times of 0.5 and 1.0 hr. were highly significant. This finding correlates with the significant differences in the *in vitro* dissolution rate of the two tablets. Also, the average peak plasma concentration for the two tablets was found to be significantly different by analysis of variance for crossover design. However, the difference between the average areas under the plasma level curve following the two tablets was not statistically significant for either the 0–360-hr. period or the estimated area from zero to infinite time.

The data indicate that the differences in *in vivo* dissolution rates for the two tablets were not of sufficient magnitude to depress the relative bioavailability of the tablet that dissolves more slowly *in vitro*. The slower absorption rate and the consequent lower peak level, as noted in these studies, correlated well with the *in vitro* results.

As indicated in Table II, the average half-life, estimated from terminal plasma concentrations, was found to be 8.03 days for Treatment A. The range in half-lives for this dosage form for all eight patients varied between 6.5 and 9.9 days. In the case of Treatment B, the mean half-life was 9.84 days with a range of 5.0–13.7 days. Again, since the assay is nonspecific, this value is a measure of the total drug in the plasma. These values should, therefore, be comparable to the half-life of digitoxin determined with radiolabeled digitoxin, a procedure that also is nonspecific. Okita and coworkers (13, 19), measuring the urinary excretion of digitoxin and its metabolites, found the half-life to be 9.0 days for both a 1.2- and a 0.5-mg. dose of ^{14}C-digitoxin. Caldwell *et al.* (20) measured the levels of radioactivity in the blood following a 1.2-mg. oral dose of ^{3}H-digitoxin in hydroalcoholic solution. In their study, the mean half-life was 11.5 ± 2.3 days. However, in subjects who received a 4-g. dose of cholestyramine 4 hr. after dosing with digitoxin, there was a significant change in the mean plasma half-life to 6.6 ± 1.9 days. The apparent drop in the plasma half-life of the drug following treatment with cholestyramine, a compound that binds digitoxin, was due to the interruption of the enterohepatic circulation of the drug. Okita (21) showed that, in man and several other species, digitoxin and its metabolites are excreted in bile into the small intestine and subsequently reabsorbed. Okita also was able to relate the species differences in the half-life of digitoxin to differences in the enterohepatic circulation. There is little doubt that enterohepatic circulation plays an important role in regulating the time course of digitoxin and its metabolites in the body. It is also quite apparent that this effect can cause considerable variability in the apparent half-life of digitoxin from one subject to another or conceivably within the same subject. This would be expected since the compounds excreted in the bile are concentrated in the gallbladder and gallbladder emptying is dependent upon the time at which food or fatty materials are consumed. Nevertheless, the half-life values obtained in this study generally agree well with the reported literature values.

In summary, one main problem associated with the determination of digitoxin plasma levels in a single-dose study is sensitivity. The only procedures available with the required sensitivity are the radioimmunoassay, the ^{86}Rb-uptake assay, and the Na,K-ATPase

inhibition procedures. All of these procedures are plagued with the same lack of specificity and, as stated earlier, in our opinion the radioimmunoassay is the most convenient and readily available procedure of these three.

For bioavailability studies, when unlabeled drug must by necessity be used, a separation of unchanged digitoxin and its metabolites by a chromatographic procedure is virtually impossible. This is because of: (*a*) the extremely low plasma levels of drug following a single oral dose, and (*b*) the number of samples that must be processed for a bioavailability study. Furthermore, the problem of assay specificity is in reality more involved than just separating the drug from its metabolites. Studies have shown that the metabolites of digitoxin also are cardioactive in animals. Since this is the case, metabolism in the intestinal wall and in the GI tract should be considered. Furthermore, studies should be undertaken to determine the cardioactivity of these compounds in man. Only if these factors are known would it be possible to determine the availability of active drug from a given dosage form of digitoxin. It is presently thought that all of the metabolites except the aglycone of digitoxigenin and digoxigenin are cardioactive in man, but this has not been quantitatively nor qualitatively reported in the literature. If the various metabolites are all cardioactive, as is believed, then to some measure the radioimmunoassay would be a measure of total active drug. To this extent in carefully controlled crossover studies, the radioimmunoassay is presently the best method for bioavailability studies.

The results of the human bioavailability study reported herein should not be construed as suggesting that all digitoxin tablets made by different manufacturers are equally bioavailable. Only two manufacturers' tablets were studied, and the results should not be extrapolated to any other manufacturer's products.

REFERENCES

(1) P. G. Welling, K. P. Lee, J. A. Patel, J. E. Walker, and J. G. Wagner, *J. Pharm. Sci.*, **60**, 1629(1971).

(2) J. G. Wagner, P. G. Welling, K. P. Lee, and J. E. Walker, *ibid.*, **60**, 666(1971).

(3) J. G. Wagner, P. G. Welling, S. B. Roth, E. Sakmar, K. P. Lee, and J. E. Walker, *Int. J. Clin. Pharmacol.*, **5**, 371(1972).

(4) J. M. Lowenstein and E. M. Corrill, *J. Lab. Clin. Med.*, **67**, 1048(1966).

(5) G. H. Burnett and R. L. Conklin, *ibid.*, **71**, 1040(1968).

(6) T. W. Smith, *J. Pharmacol. Exp. Ther.*, **175**, 352(1970).

(7) D. Wells, B. Katzung, and F. H. Meyers, *J. Pharm. Pharmacol.*, **13**, 389(1961).

(8) R. S. Yalow and S. A. Berson, in "Protein and Polypeptide Hormones; Part I Radioimmunoassay of Protein and Polypeptide Hormones," M. Margonlies, Ed., Excerpta Medica Foundation, Amsterdam, The Netherlands, 1969, pp. 71–76.

(9) V. Herbert, K. Lau, C. W. Gottlieb, and S. J. Bleicher, *J. Clin. Endocrinol*, **25**, 1375(1965).

(10) J. E. Doherty, *Amer. J. Med. Sci.*, **255**, 382(1968).

(11) J. J. Ashley, B. T. Brown, G. T. Okita, and S. E. Wright, *J. Biol. Chem.*, **232**, 315(1958).

(12) G. T. Okita, *Pharmacologist*, **6**, 45(1964).

(13) G. T. Okita, P. J. Talso, J. H. Carry, Jr., F. D. Smith, Jr., and E. M. Geiling, *J. Pharmacol. Exp. Ther.*, **115**, 371(1955).

(14) T. W. Smith, V. P. Butler, Jr., and E. Haber, *Biochemistry*, **9**, 331(1970).

(15) F. Lauterbach and K. Repke, *Arch. Exp. Pathol. Pharmakol.*, **239**, 196(1960).

(16) I. Hermann and K. Repke, *ibid.*, **248**, 370(1964).

(17) K. Repke, in "Proceedings of First International Pharmacological Meeting," vol. 3, W. Welbrandt, Ed., Macmillan, New York, N. Y., 1963, pp. 47–70.

(18) A. Kroneberg, *Arch. Exp. Pathol. Pharmakol.*, **237**, 222 (1959).

(19) G. T. Okita, *J. Amer. Geriat. Soc.*, **5**, 163(1957).

(20) J. H. Caldwell, C. A. Bush, and N. J. Greenberger, *J. Clin. Invest.*, **50**, 2683(1971).

(21) G. T. Okita, *Fed. Proc.*, **26**, 1125(1967).

(22) B. G. Katzung and F. H. Meyers, *J. Pharmacol. Exp. Ther.*, **154**, 575(1966).

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