Contribution of Quantitative Assay Techniques to the Understanding of the Clinical Pharmacology of Digitalis

By THOMAS W. SMITH, M.D.

SUMMARY

Despite recent advances in understanding of the pharmacokinetics and electrophysiological effects of cardiac glycosides, digitalis toxicity remains distressingly common in clinical practice. Another substantial group of patients is markedly underdigitalized, failing to gain the full therapeutic benefits of optimal use of these drugs. Since cardiac digitalis toxicity is a dose-related phenomenon, and serum or plasma digoxin and digitoxin concentrations rise with increasing doses, at least a statistical correlation between circulating levels and clinical state might be expected. Increasing availability of serum or plasma digitalis concentration measurements thus offers the clinician a potential means of improving the patient's chances of benefiting from treatment with cardiac glycosides. Assay methods in current use include a double-isotope dilution derivative method (digitoxin), red cell 86Rb-uptake inhibition (digitoxin and digoxin), Na+-K+ ATPase inhibition (digitoxin), ATPase enzymatic displacement (digitoxin and digoxin), gas chromatography (digitoxin), and radioimmunoassay (digitoxin, digoxin, and ouabain).

The rapidly expanding literature reporting clinical experience with these technics reflects general agreement that mean serum or plasma digoxin and digitoxin levels are significantly higher in patients with clinical evidence of toxicity compared with nontoxic patients. Nevertheless, multiple factors influence individual responses, and blood level data must be interpreted in the overall clinical context. Hypokalemia, hypercalcemia, hypomagnesemia, acid-base disturbances, hypoxemia, and hypothyroidism all tend to decrease tolerance to any given digitalis dose or blood level. Autonomic nervous system tone and other drugs concurrently received must also be considered. Advanced heart disease in general, and coronary artery disease in particular, appear to predispose patients to apparent digitalis toxicity at relatively lower serum or plasma levels.

Cardiac glycoside assay technics have also proven useful in various studies of the clinical pharmacology of digoxin, digitoxin, and ouabain. Handling of digoxin by patients on cardiopulmonary bypass has been assessed, and gastrointestinal absorption has been evaluated in normal subjects; poor and erratic absorption of the drug has been documented in patients with malabsorption syndromes. Potentially important drug-drug interactions of agents such as phenobarbital and phenylbutazone with digitoxin have been studied, as well as the effects of steroid-binding resins on digoxin and digitoxin metabolism. Studies of ouabain pharmacokinetics by radioimmunoassay have demonstrated a plasma half-life of 21 hours, indicating that, as in the case of digoxin and digitoxin, half-life of serum or plasma concentration after establishment of blood-tissue equilibrium bears a close relationship to duration of clinical effect.

Additional Indexing Words:
Digoxin Digitoxin Ouabain Radioimmunoassay
Red cell 86Rb-uptake inhibition Na+-K+ ATPase inhibition
Enzymatic isotopic displacement Digitalis intoxication

From the Cardiac Unit, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts.
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THE NEED FOR more objective means of judging appropriate digitalis dosage has long been apparent to the clinician. The narrow margin between therapeutic and toxic effects of digitalis was all too apparent even to Withering, who published his classic monograph in 1785 in part lest "a medicine of so much efficacy... be condemned and rejected as dangerous and unmanageable." A recent prospective study of 931 consecutive admissions to a single medical service has documented a 23% incidence of toxicity in patients on maintenance digitalis glycosides, with an additional 6% judged possibly toxic. At the other extreme, 11% of patients were markedly underdigitalized. The experience of Henderson and his colleagues in an outpatient clinic suggested that as many as 36% of patients presumed to be on adequate maintenance doses of digitalis were underdigitalized, while 10% showed electrocardiographic signs of digitalis excess. Variability of absorption and excretion, as well as variable myocardial sensitivity, complicate the problem faced by the clinician. Moreover, the Food and Drug Administration has recently issued warnings that marked tablet-to-tablet variations in digoxin content were found in some preparations tested.

Several observations have suggested that serum digitalis concentration measurements might be of use in evaluating patients receiving these drugs. At the most fundamental level, there is the overwhelming weight of evidence that rhythm disturbances due to digitalis are dose-related phenomena. Since studies from a number of laboratories have shown that serum or plasma digitalis levels rise with increasing dosage, at least a statistical correlation between clinical state and blood level would be expected. In addition, Doherty and his co-workers have demonstrated that the ratio of serum to myocardial digoxin concentrations is relatively constant after the completion of uptake and distribution of the drug.

Finally, there is growing evidence implicating Na\(^+\)–K\(^+\) activated "transport" ATPase as a mediator of digitalis action on the heart. Experiments with the squid giant axon and with red blood cells indicate that this cell membrane-bound enzyme is accessible only to digitalis present at the external cell surface. Thus it may be that the digitalis receptor is in relatively close proximity to the extracellular compartment, which would tend to enhance the translation of plasma concentration to myocardial effect.

Animal experimental studies have demonstrated a significant correlation between serum digoxin concentration and electrophysiologic effects as reflected by both acetylcholine and repetitive ventricular responses to low-energy endocardial stimuli.

Digitalis Assay Technics

Recent advances in the ability to quantitate accurately and conveniently the minute serum or plasma concentrations of digitalis glycosides in patients on usual doses of these drugs have opened new avenues of investigation. The various approaches which have proven useful in quantitating circulating concentrations are listed in table 1. The limited sensitivity of the duck-embryo bioassay led investigators to obtain \(^{14}\)C- or \(^{3}\)H-labeled compounds, allowing the extremely useful studies of Okita, Doherty, Marcus.

Table 1

<table>
<thead>
<tr>
<th>Approaches to Digitalis Assay</th>
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<tbody>
<tr>
<td>Duck-embryo bioassay</td>
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<tr>
<td>Radioisotope labeling</td>
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<tr>
<td>Physicochemical methods:</td>
</tr>
<tr>
<td>Double-isotope dilution derivative</td>
</tr>
<tr>
<td>Gas chromatography</td>
</tr>
<tr>
<td>ATPase inhibition:</td>
</tr>
<tr>
<td>Red cell (^{86})Rb-uptake inhibition</td>
</tr>
<tr>
<td>Micromosomal Na(^+)–K(^+) ATPase inhibition</td>
</tr>
<tr>
<td>Competitive binding:</td>
</tr>
<tr>
<td>Radioimmunoassay</td>
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<tr>
<td>ATPase enzymatic isotope displacement</td>
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</table>
and their colleagues. The many important studies of these and other workers by direct use of isotopically labeled digitalis glycosides were recently summarized by Doherty,36 and important contributions continue to derive from this approach.38-44

Classical physicochemical approaches have been difficult to devise because of the stringent sensitivity requirement of nanogram per milliliter concentrations. Nevertheless, a double-isotope dilution derivative assay for digoxin was developed by Lukas and Peterson,12 and Watson and Kalman have recently developed a useful gas chromatographic approach to digoxin measurement.45

Realization that cardiac glycosides are potent inhibitors of Na+-K+ activated "transport" ATPase46, 47 led to Lowenstein's development of the red cell 86Rb-uptake inhibition assay,48 since modified by several workers,16, 49-53 and to the ATPase-inhibition method used for digitoxin assay in a large clinical experience by Burnett and Conklin and their co-workers.13, 14 The principle of competitive binding of radioactively labeled and unlabeled cardiac glycosides to a specific binding site provided by an antibody or Na+-K+ activated ATPase underlies, respectively, radioimmunoassay methods14, 17, 55-59 and the enzymatic displacement technic developed by Brooker and Jelliffe.60

In addition to these various approaches to measurement of serum or plasma digitalis levels, salivary electrolyte concentration determinations have recently been found to be a clinically applicable tool in the assessment of digitalized patients.61

Radioimmunoassay Technics

The pioneering work of Yalow and Berson62 demonstrated the feasibility of using specific antibodies to quantitate trace amounts of circulating polypeptide hormones by radioimmunoassay. An analogous approach to measurement of cardiac glycosides was first employed by Oliver et al. in the measurement of serum concentrations of digitoxin.55 Methods for obtaining digoxin-specific antibodies of high affinity and specificity63, 64 soon allowed the development of a radioimmunoassay technic for serum digoxin concentrations in the range encountered clinically.56 Because of the marked cross reactivity between digoxin and deslanoside,64 the latter glycoside can also be measured with high sensitivity. A radioimmunoassay method for measurement of sub-nanogram amounts of ouabain in plasma and urine has also been developed recently.59

Details of radioimmunoassay methods in current use in our laboratory have been published elsewhere.14, 56, 57, 64, 65 Briefly, 0.1-0.5 ml of serum from the patient to be studied is brought to a convenient volume with phosphate-buffered saline. A suitable amount of a tritiated tracer glycoside is then added, followed by antibody with high affinity and specificity for the substance to be measured. After a brief period of equilibration, during which labeled and unlabeled glycosides compete for a limited number of antibody binding sites, dextran-coated charcoal is added. Free glycoside binds to the charcoal and is centrifuged down, leaving antibody-bound tracer in the supernatant phase which is then decanted into a toluene-detergent base scintillation fluid and counted in a liquid scintillation spectrometer. After correction for background and quenching, results are plotted on a semilogarithmic scale of percent antibody-bound tracer vs concentration of unlabeled substance to be measured, or as reciprocal bound tracer against cardiac glycoside concentration. The latter method yields a rectilinear relationship which lends itself to computer usage. Sensitivity, even using a 0.1-ml serum sample, extends well below the concentration ranges encountered in clinical practice. With minor modifications, the procedure can be completed in an hour without compromising accuracy significantly.

Since all assay methods in current use depend upon comparison of data from unknown samples with "known" standards, accuracy of results is entirely dependent on the accuracy with which the standards are prepared and used. Meticulous technic is particularly important in this phase of all assay procedures.
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Falsely low cardiac glycoside concentration measurements can result from presence of exogenous sources of radioactivity in the patient's serum, as from various radioisotope scanning procedures in clinical use. It is therefore useful to check an auxiliary channel on the scintillation spectrometer for presence of gamma-emitting isotopes such as $^{131}$I. When present, such potentially interfering substances may be removed by preliminary extraction techniques, or appropriate correction may be made.

Using this general approach, we have carried out over 10,000 assays for serum glycoside levels in a broad spectrum of clinical settings. Digoxin and digitoxin determinations are done on a routine clinical basis, and a single technician performs an average of 30–40 determinations per day. The commercial availability of radioimmunoassay kits with a choice of tritium or radioiodine tracers has made digoxin and digitoxin assays widely available to laboratories with the necessary counting equipment.

Clinical Experience

Although absolute values have varied somewhat from method to method and from laboratory to laboratory, recent publications reflect general agreement concerning digoxin levels encountered in clinical practice. Table 2 summarizes data from published series. Ritzmann et al. have noted generally similar values. Other factors being equal, larger doses of digoxin result in higher serum digoxin concentrations. As demonstrated by earlier studies, impaired renal function is also associated with higher serum concentrations of digoxin. A tendency to relatively higher serum digoxin concentration values as measured by red cell $^{86}$Rb-uptake inhibition in some initial studies is less evident in subsequent experience.

In the case of digitoxin, comparable serum or plasma concentrations have also been observed in several laboratories using various methods, as summarized in table 3. Serum or plasma digitoxin concentrations are approximately tenfold higher than those of digoxin, probably due in large part to the substantially greater degree of albumin binding of digitoxin compared with digoxin.

Patients receiving digitals leaf in usual maintenance doses have been shown to have serum digitoxin concentrations comparable to those of patients on crystalline digitoxin when measured by Na+-K+-ATPase inhibition or by radioimmunoassay.

The relationship between blood levels of digitals glycosides in patients with and without clinical evidence of toxicity is at once the most difficult and most interesting.

Table 2

<table>
<thead>
<tr>
<th>Authors</th>
<th>Method</th>
<th>Mean conc (ng/ml)</th>
<th>Stat signif</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nontoxic</td>
<td>Toxic</td>
</tr>
<tr>
<td>Beller et al.</td>
<td>Radioimmunoassay</td>
<td>1.0</td>
<td>2.3</td>
</tr>
<tr>
<td>Bertler, Redfors</td>
<td>$^{86}$Rb uptake</td>
<td>0.8–1.3*</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Brooker, Jelliffe</td>
<td>Enzymatic displacement</td>
<td>1.4</td>
<td>3.1</td>
</tr>
<tr>
<td>Chamberlain et al.</td>
<td>Radioimmunoassay</td>
<td>1.4</td>
<td>3.1</td>
</tr>
<tr>
<td>Evered, Chapman</td>
<td>Radioimmunoassay</td>
<td>1.38</td>
<td>3.36</td>
</tr>
<tr>
<td>Fogelman et al.</td>
<td>Radioimmunoassay</td>
<td>1.4</td>
<td>1.7</td>
</tr>
<tr>
<td>Grahame-Smith, Everest</td>
<td>$^{86}$Rb uptake</td>
<td>2.4</td>
<td>5.7</td>
</tr>
<tr>
<td>Hoeschen, Proveda</td>
<td>Radioimmunoassay</td>
<td>0.8–1.3*</td>
<td>2.8</td>
</tr>
<tr>
<td>Morrison, et al.</td>
<td>Radioimmunoassay</td>
<td>0.76–1.25*</td>
<td>3.35</td>
</tr>
<tr>
<td>Oliver, et al.</td>
<td>Radioimmunoassay</td>
<td>1.6</td>
<td>3.0</td>
</tr>
<tr>
<td>Smith, et al.</td>
<td>Radioimmunoassay</td>
<td>1.1–1.4*</td>
<td>3.3</td>
</tr>
<tr>
<td>Smith, Haber</td>
<td>Radioimmunoassay</td>
<td>1.4</td>
<td>3.7</td>
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*Means for patients receiving 0.25 or 0.50 mg/day oral maintenance digoxin, stated in that order.

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areas under current investigation. It is apparent that a myriad of factors influences the response of the heart to any given blood or myocardial level of digitalis, including serum potassium, sodium, calcium, and magnesium concentrations, acid-base balance, presence or absence of hypoxia, thyroid status, autonomic nervous system tone, and other drugs concurrently received, in addition to the type and severity of underlying heart disease. In comparing the work of different investigators, time after last digitalis dose must also be considered, as well as criteria for patient selection, criteria for toxicity, and details of measurement methods.

Despite all of these variables, a fairly consistent picture has emerged in most studies published to date. Table 2 summarizes the digitalin findings from a number of laboratories. These series comprise a total of about 1000 patients studied. Mean levels in toxic patients tend to be about twofold higher than those in patients without evidence of toxicity, and the difference was statistically significant in all studies except that of Fogelman and his co-workers.69 Significant overlap between toxic and nontoxic levels has been the general experience, probably related in large part to various factors affecting myocardial sensitivity just mentioned. In addition, since there are no absolute clinical criteria which unequivocally define digitalis toxicity, at least part of the overlap is probably due to the uncertainties of assignment of patients to "toxic" or "nontoxic" groups. In any case, this overlap demands that serum or plasma levels of digitalis glycosides be evaluated in the clinical context, taking into account all variables which bear on the emergence of toxicity. Intermediate serum digoxin concentrations around 2 ng/ml are particularly likely to be encountered in patients with equivocal signs of digoxin excess such as first-degree atrioventricular (A-V) block or occasional ventricular premature beats.14

Table 3 summarizes studies of the relationship between digoxin levels and presence or absence of toxicity. Again, significant differences in mean concentrations were generally observed in patients with and without electrocardiographic evidence of toxicity. If anything, the degree of overlap in levels of nontoxic and toxic patients tends to be greater than in the case of digoxin, perhaps related at least in part to the greater extent of plasma protein binding of digitoxin71 and hence greater opportunity for this variable to affect the distribution of the drug in the body.

It is important to note that nearly all of the data cited in these studies are from patients with cardiac disease. In our own experience, advanced cardiac disease has been especially

Table 3

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<thead>
<tr>
<th>Authors</th>
<th>Method</th>
<th>Mean concentration (ng/ml)</th>
<th>Stat signif</th>
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<tr>
<td>Beller et al.3</td>
<td>Radioimmunoassay</td>
<td>20</td>
<td>Yes</td>
</tr>
<tr>
<td>Bentley et al.13</td>
<td>ATPase inhibition</td>
<td>13 (0.05 mg/day)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>22 (0.1 mg/day)</td>
<td></td>
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<td></td>
<td></td>
<td>47 (0.2 mg/day)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>23 (mean for all patients)</td>
<td></td>
</tr>
<tr>
<td>Lowenstein, Corrill49</td>
<td>86Rb uptake</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Lukas, Peterson13</td>
<td>Double-isotope dilution derivative</td>
<td>20 (range)</td>
<td></td>
</tr>
<tr>
<td>Morrison, Killip30</td>
<td>Radioimmunoassay</td>
<td>25 (0.1 mg/day)</td>
<td></td>
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<td></td>
<td></td>
<td>44 (0.2 mg/day)</td>
<td></td>
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<tr>
<td>Oliver et al.25</td>
<td>Radioimmunoassay</td>
<td>about 20</td>
<td></td>
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<tr>
<td>Ritzmann et al.70</td>
<td>86Rb uptake</td>
<td>19</td>
<td></td>
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<tr>
<td>Smith57</td>
<td>Radioimmunoassay</td>
<td>17</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34</td>
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prevalent in patients with clinical evidence of toxicity. In the prospective study of patients admitted to Boston City Hospital, 74% of toxic patients were in functional class III or IV as opposed to 54% of nontoxic patients.²

It is generally agreed, then, that no arbitrary set of levels can be selected which clearly separate toxic from nontoxic patients. Although uncommon, patients with digoxin levels below 1.0 ng/ml occasionally meet usual criteria for toxicity. In the presence of cardiac and/or pulmonary disease, a number of factors tend to increase the slope of phase 4 spontaneous diastolic depolarization in specialized conduction tissues,⁷⁹ ⁷⁴ increase disparity of recovery times (enhancing probability of reentrant rhythm disturbances),⁷³ ⁷⁵ or to depress A-V conduction.⁷³ Since cardiac glycosides tend to produce all of these same electrophysiologic effects,⁷⁶ ⁷⁹ it is not surprising that these drugs may precipitate clinical toxicity in hearts precariously balanced by cardiopulmonary disease.

An apparent example of this sort of phenomenon is the recent experience of Morrison and Killip,⁷⁹ who found that hypoxemia associated with chronic lung disease tended to predispose patients to digitalis toxic rhythm disturbances at serum glycoside concentrations lower than those associated with toxicity in a population without advanced hypoxemia.

For reasons which remain unclear, occasional patients with supraventricular tachyarrhythmias such as atrial flutter or atrial fibrillation require serum digoxin concentrations of 3 ng/ml or more to maintain a therapeutically adequate degree of A-V block and sustain these relatively high levels without overt evidence of toxicity.

A further example of the interaction between intrinsic cardiac disease and response to digitalis is found in the British experience at St. Bartholomew’s and the National Heart Hospitals. Chamberlain et al. studied the relationship between serum digoxin concentration and ventricular response to atrial fibrillation in an unselected series of 116 patients.¹⁵ Since many of these patients had intrinsic disease of the A-V conduction system with slow ventricular rates on or off digitalis, the overall correlation between serum digoxin concentration and ventricular rate was poor. However, when the evaluation was restricted to patients who were capable of responding with a rapid rate (and hence had reasonably normal A-V conduction systems), the much better correlation shown in figure 1 resulted.

Acute coronary artery disease has long been suspected to predispose to digitalis toxicity.⁸⁰ Based on their experience in the measurement of serum digoxin and digitoxin concentrations in the setting of acute myocardial infarction, Morrison and Killip have suggested that alterations in the myocardial response may lower the toxic threshold to digitalis during the first 24 hours.⁸¹ It is, of course, particularly difficult to assign a definite etiology to rhythm disturbances occurring in digitalized patients at this time because of the high incidence of arrhythmias caused by the underlying disease process. Experimental studies in the dog indicate marked inhomogeneity of early digoxin uptake by the infarcted ventricle,⁸² which
may play a role in the alteration of normal sensitivity to the drug.

We have reviewed our clinical experience for further clues to the role of coronary artery disease in predisposing to digitalis intoxication. A total of 367 digitalized patients in whom detailed clinical data and serum digoxin or digitoxin concentrations were available were reviewed. Coronary artery disease was present in 61% of the 279 patients without digitalis toxicity. However, among 88 toxic patients 75% had coronary artery disease. This difference was of borderline statistical significance (P = 0.05).

A somewhat more striking result was obtained when patients at the extremes of the overlapping range of serum levels were evaluated as a group. A total of 40 patients had rhythm disturbances consistent with digitalis toxicity at relatively low serum concentrations, or had no signs or symptoms of toxicity at relatively high levels. Of the 16 "toxic" patients with digoxin levels ≤ 1.0 or digitoxin levels ≤ 10 ng/ml, 15 (91%) had coronary artery disease. On the other hand, of 24 nontoxic patients with serum levels of digoxin ≥ 2.0 or digitoxin ≥ 26 ng/ml, only 11 (46%) had coronary disease. This difference is statistically significant, with a P value less than 0.01. Thus, coronary artery disease in particular appears to predispose occasional patients to rhythm disturbances due to (or closely mimicking) digitalis excess at relatively low serum levels.

Two other types of patients bear mention who appear to differ from the bulk of digitalized adult patients with cardiac disease. One of these groups is encountered in pediatric practice, where relatively large doses of digoxin on a milligram per kilogram basis are routinely given to infants with congenital heart disease. These doses result in relatively high blood levels, which are generally quite well tolerated in this younger age group.83, 84 Young adults with normal hearts also tolerate remarkably large doses or serum concentrations of digoxin, as we have observed in our experience with accidental and suicidal ingestions.85

An objective approach to the evaluation of the problem of sensitivity of the heart to increments of digitalis is the acetylstrophanthidin tolerance test.86 Barr et al. have correlated serum digoxin concentration with acetylstrophanthidin tolerance in a series of hospitalized patients receiving maintenance digoxin.87 Patients with evidence of diminished cardiac tolerance to increments of acetylstrophanthidin had a significantly higher mean serum digoxin concentration than did patients without evidence of increased cardiac sensitivity. Despite the evident statistical correlation, varying acetylstrophanthidin sensitivity was seen among individual patients with similar serum digoxin concentration, presumably as a result of the various myocardial sensitivity factors mentioned previously.

Clinical settings in which serum or plasma cardiac glycoside concentration measurements have proven particularly useful include the evaluation of patients who are unable to give an accurate history of type and/or dosage of digitalis preparation taken. Radioimmunoassay procedures for both digoxin and digitoxin indicate which drug is being taken, as well as giving an estimate of the patient's body stores. Serum level measurements are also quite useful in the complex clinical situation following cardiac surgery, where cardiac and renal function frequently undergo considerable swings, and digitalis dosage schedules tend to be more irregular than usual. Abnormalities of gastrointestinal4 or renal function6-8 frequently result in situations in which serum concentration measurements are very helpful.

Special Problems in the Clinical Pharmacology of Digitalis Glycosides

In addition to providing information regarding individual problem patients, cardiac glycoside assay technics have proven useful in a number of studies of special problems in the clinical pharmacology of digoxin, digitoxin, and ouabain. The question of digoxin handling by patients undergoing cardiopulmonary bypass has recently been investigated by two groups using radioimmunoassay technics. The studies of Coltart et al. have demonstrated negligible losses of digoxin from the body.
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during bypass. An initial fall in serum concentration due to hemodilution from oxygenator prime returned to or above baseline levels early in the postoperative period as reequilibration of the large tissue digoxin stores occurred. Morrison and his co-workers have reported a similar experience.21

Debate over the completeness of absorption of digoxin from the gastrointestinal tract has been ongoing since the introduction of the drug into clinical use. Studies of absorption of tritiated digoxin by Doherty and his colleagues38, 39 have been informative, but this approach does not allow the drug to be given in the tablet form actually used clinically. White et al. determined the time course of absorption of digoxin tablets in normal healthy subjects.90 As shown in figure 2, in fasting subjects, peak plasma levels were reached at about 1 hour, falling to plateau after 6 hours. The same subjects after a meal had slightly lower and later peaks, but plateau values were not significantly different. A 4-week crossover study of 21 patients on maintenance therapy confirmed that digoxin taken in the fasting state resulted in plasma concentrations similar to those obtained when the drug was taken after meals. It should be noted that blood levels drawn soon after doses, before blood-tissue equilibrium has been reached, are difficult to interpret and have little meaning in the usual clinical context.

In contrast with normal subjects, poor and erratic gastrointestinal absorption of digoxin has been documented in patients with malabsorption syndromes and steatorrhea.4 Patients with sprue, short bowel syndromes, radiation enteritis, and marked hypermotility showed low serum digoxin concentrations on usual oral maintenance digoxin doses. Patients with maldigestion due to pancreatic insufficiency, on the other hand, had values close to the usual range.

A particularly enlightening study has been carried out by Lindenbaum et al.15 Because relatively large oral doses of digoxin had been required to manage certain patients adequately, they used a radioimmunoassay method to study the absorption of digoxin produced by different manufacturers. The brand studied by White et al.90 gave a very similar curve of serum levels after an oral dose, but the other preparations studied gave lower values despite comparable amounts of total digoxin present in all tablets. One preparation gave a peak

![Figure 2](https://example.com/figure2.png)

**Figure 2**

*Plasma digoxin concentrations (mean ± standard error) in seven healthy fasting subjects after an oral dose of 0.5 mg digoxin given as two 0.25-mg tablets. (Reprinted with permission from Brit Med J.90)*
serum concentration only one seventh that of the highest peak observed. The clinical implications of this variability in biologic availability are apparent.

An interesting variation in the usual time course of the decline of serum digoxin concentration has been observed by radioimmunoassay in patients following large accidental or suicidal ingestions of digoxin. Apparent serum half-times between 5 and 48 hours after ingestion were substantially shorter than those observed with ordinary doses of digoxin, increasing to the usual range only when the serum level had fallen to 2 ng/ml or less.

Serum digitoxin concentration measurements by the red cell ⁸⁶Rb-uptake inhibition technic have been used to evaluate drug interactions between digitoxin and other agents such as phenobarbital and phenylbutazone which appear to accelerate hepatic digitoxin metabolism. Effects of steroid-binding resins on digitoxin and digitoxin metabolism have also been studied by this technic as well as by radioimmunoassay and by direct measurement of tritiated digitoxin.

We have recently been interested in learning more about some of the shorter acting cardiac glycosides such as ouabain. The plasma pharmacokinetics of ouabain have been defined by radioimmunoassay in dogs and in normal human subjects. After 6-7 hours, an exponential decline in plasma ouabain concentration with a half-life of 21 hours is reached in human subjects. This value is in good agreement with prior estimates of pharmacologic half-life based on ventricular rate responses of patients in atrial fibrillation and estimates of half-life of positive inotropic effect studied by systolic time intervals. Thus, the duration of effect of ouabain, like digoxin and digitoxin, bears a close relationship to the half-life of serum or plasma concentration after blood-tissue equilibrium has been reached.

Concluding Remarks

Recent methodologic advances have placed quantitation of clinically relevant cardiac glycoside concentrations within reach of the average hospital clinical laboratory. Knowledge of serum or plasma digitalis concentrations has proven useful in the management of individual patients. Since overlap between toxic and nontoxic levels occurs in the intermediate range, however, such information must be evaluated in the overall clinical context, taking into account the various factors which influence cardiac response to these drugs. It is hoped that more detailed analysis of many of these factors will be facilitated by the ability to measure serum or plasma digitalis concentrations accurately and conveniently. Clearly, quantitative technics in current use in no way lessen the necessity for frequent, detailed observation of the digitalized patient.

Apart from clarifying some of the complexities of responses of individual patients, the methods discussed above have been and should continue to be useful in elucidating a broad range of problems in the clinical pharmacology of digitalis glycosides.

Acknowledgment

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THOMAS W. SMITH

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