EDITORIAL

Measurement of Serum Digitalis Glycosides
Clinical Implications

IMPROVEMENT in the care of patients with cardiac disease over recent decades has been due in large measure to the development of techniques which allow quantitative evaluation of clinical problems. Thus, objective data obtained at cardiac catheterization are weighed carefully in reaching clinical decisions, and great reliance is placed on the ever increasing array of measurements forthcoming from the clinical chemistry laboratory. With growing evidence of the usefulness of measurement of circulating concentrations of cardiovascular drugs such as quinidine, pro-cainamide, diphenylhydantoin, and lidocaine, the stage is set for exploration of the potential application of quantitation of serum or plasma cardiac glycoside levels.

The practicing cardiologist is all too well aware of the ubiquity of problems related to over- or underdigitalization. Until relatively recently, however, the digitalis glycosides have eluded reliable estimation because usual therapeutic serum concentrations are in the nanogram (10^-9 g) per ml range, well below the sensitivity of conventional physicochemical techniques. The availability of isotopically labeled digitalis derivatives marked the beginning of a new era in the understanding of the pharmacodynamics of cardiac glycosides. Direct measurement of radioactivity in biological fluids and tissues of selected subjects given ^14C or tritium labeled glycosides yielded highly useful information concerning metabolic turnover and rates as well as routes of excretion; also the important observation was made that serum and myocardial digoxin levels have similar half-lives and bear a relatively constant ratio to one another.¹

It remained for Lukas and Peterson,² by a double isotope dilution derivative method, and for Lowenstein and Corrill,³ by cardiac glycoside inhibition of red blood cell ^86Rb uptake, to show that usual therapeutic blood levels of unlabeled digitalis glycosides could be quantitated. The former method uses tritiated digitoxin to monitor procedural losses of digitoxin during extraction and conversion to a ^14C-triaceacetate derivative, which is isolated, purified, and quantitated. This method is technically demanding, requiring several days for completion, and has not been extended to the measurement of digoxin concentrations.

The red cell ^86Rb uptake inhibition approach takes advantage of the well-known capacity of cardiac glycosides to inhibit membrane Na-K activated adenosine triphosphatase (ATPase), an integral part of the pump mechanism which maintains high intracellular potassium and low intracellular sodium concentrations.⁴ Rubidium acts as a potassium analog in this system, and is used because the isotope, ^86Rb, has a more convenient half-life (19 days) than available potassium isotopes. Following extraction from serum or plasma, the amount of cardiac glycoside present in a sample is estimated by comparison of its ability to inhibit active uptake of ^86Rb with the inhibition produced by known amounts of digoxitin or digoxin.

Subsequent progress has been rapid, and today these two general approaches have been joined by Na-K ATPase inhibition⁵ and radioimmunoassay⁶,⁷ in the measurement of serum or plasma digitoxin levels. The ATPase inhibition assay of digitoxin described by Burnett and Conklin⁵ again utilizes the

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inhibitory properties of the drug on Na-K activated ATPase, in this case isolated from brain cortex. Radioimmunoassay methods are based on competition between unlabeled cardiac glycosides present in (or extracted from) the patient's serum or plasma, and isotopically labeled molecules, added in vitro, for specific antibody binding sites. Although not antigenic in the forms used clinically, cardiac glycosides can be covalently coupled as haptons to carrier proteins such as serum albumin.8, 9 These conjugates, when injected into rabbits, will elicit antibodies of high affinity and specificity for the free glycoside.8, 9 Estimation of concentrations in unknown samples is carried out by comparison with a standard curve expressing displacement of the isotopically labeled form of the drug from the antibody binding site by known amounts of unlabeled glycoside. All of these methods, as applied to digitoxin measurements, yield mean values and ranges which are in excellent agreement.

Red cell 86Rb uptake inhibition3, 10 and radioimmunoassay11 methods are in use for determination of digoxin concentrations, and preliminary report has been made of an ATPase enzymatic isotopic displacement technique conceptually similar to radioimmunoassay.12 Greater sensitivity is required of these methods, since serum or plasma digoxin concentrations are some ten times lower than those of digitoxin. Significant discrepancies exist in reported means and ranges of serum digoxin concentrations as measured by the red cell 86Rb uptake inhibition and radioimmunoassay methods, and these remain to be resolved.

The values obtained by Marcus and co-workers13 by direct measurement of radioactivity in the blood of volunteers given maintenance doses of tritiated digoxin appear to serve as the most unequivocal source of data against which other methods may be judged. Levels in comparable subjects determined by radioimmunoassay are in close agreement with these direct measurements.11, 14

The advantages and disadvantages of these various approaches have been reviewed in detail recently.15 From the standpoint of the clinician faced with a decision regarding use of digitalis glycosides, radioimmunoassay methods requiring no serum extraction step have the potential advantage of being able to be completed in 1 hour or less.

What, then, are the clinical implications of the rapidly growing availability of such methods? Clearly, these techniques will be of use in pursuing further studies of general patterns of the clinical pharmacology of digitalis glycosides, and several such investigations have been carried out.16-19

It is much more difficult to define the usefulness of knowledge of serum levels in the management of individual problem patients. Certain situations recur where such information is unquestionably useful: for example, in the case of the patient from whom an accurate history of type of digitalis medication or dosage cannot be obtained, the patient with overt or suspected gastrointestinal disease in whom absorption of digoxin is uncertain,18 and the hemodynamically unstable patient with fluctuating renal function and digoxin excretion.

Whether methods for measurement of serum glycoside levels will be useful for diagnostic purposes in cases of questionable digitalis intoxication, however, is less easy to answer. Several studies7, 10, 11, 14, 16, 20-22 have demonstrated significantly higher serum or plasma digoxin or digitoxin concentrations in patients with digitalis intoxication compared with nontoxic controls. In a relatively large series of patients studied by radioimmunoassay, 90% of 131 patients without evidence of digoxin toxicity had serum levels of 2.0 ng/ml or below, while 87% of patients meeting strict criteria for digoxin intoxication had levels above 2.0 ng/ml.14 It must be noted, however, that studies published to date generally compare patients with obvious, unequivocal signs of digitalis intoxication with those in whom there are no signs or symptoms of toxicity whatever. Thus the groups compared lie at opposite ends of the clinical spectrum, where the decision to give or withhold cardiac glycosides is usually straightforward. The only
group of patients with equivocal electrocardiographic signs of digoxin toxicity thus far reported proved to have a mean serum digoxin concentration between those observed for obviously toxic and nontoxic patients, with substantial overlap in both directions.\textsuperscript{14}

The available evidence, then, both in the case of digoxin and of digitoxin,\textsuperscript{7, 20} indicates that no arbitrary level can be chosen that clearly differentiates toxic from nontoxic serum or plasma glycoside concentrations. Multiple factors in the clinical setting, including (but undoubtedly not limited to) serum potassium, sodium, calcium, and magnesium concentrations, acid-base balance, presence or absence of hypoxia, thyroid status, autonomic nervous system influences, and other drugs concurrently received, all have an important bearing on the tolerance of a given patient to cardiac glycosides.\textsuperscript{21} Some of these factors, including potassium,\textsuperscript{24} sodium,\textsuperscript{25} and thyroid function,\textsuperscript{26} appear to affect the ratio of serum or plasma to myocardial digoxin concentration.

Perhaps most importantly, the nature and severity of underlying heart disease will be determinants of digitalis sensitivity. Thus, serum digoxin concentration is rather poorly correlated with ventricular response to atrial fibrillation in unselected patients, many of whom have intrinsic disease of the conduction system resulting in slow ventricular rates on or off the drug.\textsuperscript{16} The correlation becomes much better among patients who are able to respond with a rapid ventricular rate when not receiving digitalis. Another dramatic example of difference in sensitivity is afforded by patients who ingest massive doses of digitalis with suicidal intent. Recent experience\textsuperscript{27} supports the clinical impression that young subjects with normal hearts tend to respond to these toxic doses with atrioventricular conduction disturbances alone, while older patients with established coronary artery disease develop life-threatening ventricular tachyarrhythmias.

In general, our experience with over 6,000 serum digoxin and digitoxin assays supports the concept that knowledge of serum or plasma digitalis glycoside concentrations can be of substantial clinical usefulness, provided this information is interpreted in the context of the various factors discussed above.

Pendulum swings from initial uncritical enthusiasm to disillusionment and unwarranted pessimism all too often accompany the introduction of new clinical tools. One hopes that early critical appraisal of measurement of circulating levels of cardiac glycosides will result in the establishment of an appropriate place in the diagnostic armamentarium of the clinician. Whatever that place may be, it will neither replace the need for frequent, careful observation of the patient, nor the advisability of using small increments of the more rapidly excreted forms of digitalis when problems related to dosage arise.

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