Digoxin—Issues and Controversies
Steven J. Soldin

This review deals briefly with the clinical pharmacology of digoxin and reviews in some depth the problems inherent in methods of digoxin measurement. Digoxin-like factors (materials that cross react in digoxin immunoassays) are discussed, and some current evidence suggesting the existence of a new hormone, endoxin (endogenous digoxin), is summarized.

The year 1985 marks the bicentenary of the publication of a classic in medical history, William Withering's An Account of the Foxglove, and Some of its Medical Uses; with Practical Remarks on Dropsey, and Other Diseases. Reportedly (1), Withering first concerned himself with the foxglove in 1775 when his opinion was asked about "a family recipe for the cure of dropsey... kept a secret by an old woman in Shropshire." The medicine is purported to have been composed of 20 or more herbs, but he was quick to recognize that the foxglove was the active component. Withering's (2) detailed description of its toxic effects—"sickness, vomiting, purging, giddiness, confused vision, objects appearing green and yellow; increased secretion of urine, with frequent motions to part with it, and sometimes inability to retain it; slow pulse, even as slow as 35 in a minute, cold sweats, convulsions, syncope and death" (1)—as well as his recording of its uses in the treatment of dropsey (edema) and its diuretic effects are a milestone in medical history. In the early 20th century it became accepted that the primary effect of digitalis was on the heart, and that the digitalis glycosides were useful as anti-arrhythmics for treatment of atrial fibrillation and flutter, and to improve the function of the heart as a pump (i.e., enhance the contractility of myocardial muscular tissue) in the treatment of cardiac failure.

Digitalis may have played a role in the illness of Vincent Van Gogh, and his use of yellow and green color swirls perhaps was generated by his memory of toxic visual symptoms (3). Digitalis toxicity has in fact plagued the medical profession for over 200 years. According to Burchell (4): "The situation qualified as a professional disgrace on the basis of 3 items: the situation persists, physicians are often slow to recognize it and over the decades, writers have been harsh in their denunciation of fellow physicians when toxicity has occurred. The Index Medicus, in the codes Digitalis Toxicity and Digitalis Poisoning lists about 20 papers per year." Withering's account estimates the incidence of toxicity between 1780 and 1784 as 18%, while studies among patients being admitted to hospital in the 1970s quote figures of 20 to 30% (5). Are we doing little better today than Withering was in his time?

"Digitalis" and "digitalis compounds" are terms that encompass the entire group of cardiac glycoside inotropic drugs (those influencing the contractility of myocardial muscular tissue) and chronotropic drugs (those affecting the rate of rhythmic movements such as the heart beat). Cardiac glycosides of medicinal importance are obtained from Digitalis purpurea Linne (Fam. Scrophulariaceae) (digitoxin, digitalis, gitalin), from Digitalis lanata Ehrhart (Fam. Scrophulariaceae) (digoxin, digitoxin, lanatoside C, deslanoside, acetyldigitoxin), Strophantus gratus (ouabain), and Acokanthera schimperi (ouabain) (6-9).

These compounds have a characteristic ring structure (aglycone or genin) to which is coupled one or more sugars. The aglycone portion of the glycoside consists of a steroid nucleus and an α,β-un saturated five- or six-membered lactone ring at the C17 position of the steroid nucleus (6-8). The hydroxy groups at C3 and 14 are in the β-configuration. The sugars are attached usually through the C3 hydroxyl (Figures 1 and 2).

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**Fig. 1. Structure of digoxin**

**Fig. 2. Structure of key cardiac glycosides**

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Brief Summary of the Clinical Pharmacology of Digoxin

Digoxin is one of the drugs most widely used in current health care delivery and is the fifth most commonly prescribed drug in North America. The main pharmacological property of the cardiac glycosides is their ability to increase the force and velocity of myocardial systolic contraction—positive inotropic action (10). The currently accepted mechanism of action of digoxin is its inhibitory action on the Na⁺/K⁺-transporting ATPase (EC 3.6.1.37) transmembrane pump of the sarcolemma (11, 12). The inhibition results in the accumulation of intracellular sodium ions, which are thought to cause displacement of bound calcium ions. The resulting free calcium ions cause a positive inotropic effect, resulting in a more forceful contraction of the myocardium (13). This is undoubtedly an oversimplification, because the way in which digitalis affects movements of calcium ions in heart muscle is not yet fully understood. Extracellular calcium ions are probably very important in myocardial contraction, as evidenced by the pronounced changes in contractile force brought about by changes in the extracellular concentration of calcium ions. Intracellular calcium ions can increase as a result of either an increased influx from the extracellular environment (14) or enhanced release of bound intracellular calcium ions from sites such as the sarcoplasmic reticulum (15).

In patients with congestive heart failure, increased myocardial contractility and cardiac output reduce sympathetic tone, thus slowing increased heart rate and causing diuresis in edematous patients (6, 7, 16, 17). Glycoside-induced slowing of heart rate in patients without congestive heart failure is negligible and is primarily attributable to vagal (cholinergic) and sympatholytic effects on the sinoatrial node (17, 18), but with toxic doses it is caused by direct depression of sinoatrial node automaticity (19, 20). The major indication for digoxin use is the combination of congestive heart failure and atrial fibrillation. Atelectatic ventricular failure is usually treated with load reduction before therapy with digitalis is instituted. Another indication for use of this drug is chronic congestive failure with sinus rhythm that has failed to respond to diuretics alone. The amount of digoxin available for binding to Na⁺/K⁺-ATPase is influenced by several factors: dose, bioavailability of the preparation used, apparent volume of distribution of the drug, degree of metabolism to pharmacologically active and inactive metabolites, and the rate at which the drug is eliminated.

Factors influencing myocardial sensitivity to digoxin include hypo- and hyperkalemia, hypercalcemia, hypo- and hypermagnesemia, acid–base disorders, myocardial ischemia, hypoxemia, underlying heart disease, and pulmonary disease (13).

Bioavailability: The bioavailability or completeness of absorption of digoxin varies markedly (55 to 85%) from one preparation to the next, and many reports of toxic digoxin concentrations can be attributed to a patient’s changing from one formulation to another (21–23). Intestinal motility affects the absorption of digoxin tablets. For example, malabsorption syndromes that are characterized by intestinal hypermotility and motility-stimulating drugs such as metoclopramide decrease absorption. Drugs that retard gastrointestinal motility, such as the anti-cholinergics, tend to increase bioavailability (24, 25).

Digoxin distribution: Figure 3 shows the time course of digoxin concentration in serum after an intravenous injection or infusion. The distribution of digoxin is aptly described by a two-compartment open pharmacokinetic model (26). The first very rapid decrease in concentration, mainly a result of dilution in blood, takes a few minutes. During the distribution or α phase the drug equilibrates between the central and peripheral compartment (27). The central compartment may be thought to represent plasma and the highly perfused organs such as liver and kidneys; the peripheral compartment represents the deeper tissues, particularly skeletal muscle and myocardium. Skeletal muscle forms the largest storage depot of digoxin. Nevertheless, the digoxin concentration during maintenance therapy is much lower in skeletal muscle than in myocardium. The ratio of the concentrations in plasma and heart reportedly is between 1:30 and 1:200 (28–30). The apparent volume of distribution (Vₐ) of digoxin can be defined as "the volume of body water which would be required to contain the amount of drug in the body if it were uniformly present in the same concentrations in which it is in blood." Vₐ is highly variable and ranges from 3 to 10 L/kg body weight (31). This large Vₐ is a further indication of digoxin’s significant binding to tissues. Vₐ can be markedly altered by factors that alter this digoxin tissue binding. Only 20 to 25% of digoxin is bound to plasma proteins (32, 33).

Digoxin elimination and metabolism: The elimination half-life of digoxin in healthy test subjects reportedly varies between 26 and 45 h (32), although a significantly longer t½ has been reported for patients with reduced renal function (34). Renal clearance of digoxin occurs by glomerular filtration, tubular reabsorption, and tubular secretion, with the last accounting for half the digoxin in the urine (35). Digoxin is distributed across the placenta, and some is secreted into breast milk (36, 37). Digoxin and its metabolites are also present in bile. The small intestine is a major site of absorption of digoxin, so enterohepatic recycling may be a significant factor in the pharmacokinetics. An excellent discussion of digoxin pharmacokinetics can be found in several reviews (13, 32, 38, 39), and Table 1 summarizes some of the pharmacokinetic variables for digoxin in man.

Some years ago it was believed that relatively polar glycosides such as digoxin, deslanoside, and ouabain were not metabolized appreciably (40, 41, 42). Today it is appreciated that metabolism can be extensive and can involve reduction of the lactone ring to form dihydrotigoxin and the stepwise removal of sugar molecules, followed by epimerization of the 3β-hydroxyl to the 3α-(epi) position and conjugation to give the polar metabolites 3-epi-glucuronide and 3-epi-sulfate (43–46). Figure 4 shows the pathways involved. Gault et al. (46) studied digoxin biotransformation in patients with normal renal function or with advanced renal failure. Some patients were found to have extensive biotransformation, with polar metabolites predominating (47).
Table 1. Some Pharmacokinetic Variables for Digoxin

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral dose absorbed</td>
<td>55–85%</td>
</tr>
<tr>
<td>Protein bound</td>
<td>20–25%</td>
</tr>
<tr>
<td>Half-life</td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td>26–45 h</td>
</tr>
<tr>
<td>Children</td>
<td>11–50 h</td>
</tr>
<tr>
<td>Time to peak plasma concn.</td>
<td>1.5–5.0 h</td>
</tr>
<tr>
<td>Time to steady state</td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td>7–11 days</td>
</tr>
<tr>
<td>Children</td>
<td>2–10 days</td>
</tr>
<tr>
<td>Volume of distribution</td>
<td>3–10 L/kg</td>
</tr>
<tr>
<td>Effective blood concns.</td>
<td>1.0–2.5 nmol/L</td>
</tr>
<tr>
<td>Toxic blood concns.</td>
<td>&gt; 2.5 nmol/L</td>
</tr>
</tbody>
</table>

![Pathways of digoxin metabolism](image)

These authors developed a liquid-chromatographic procedure for separating all of the digoxigenin bis-digitoxiside, the monodigitoxiside, digoxigenin, the 3-epidigoxigenin, the 3-keto-digoxigenin, and the polar metabolites of digoxin. [45]. [3H]Digoxin-12α was administered to individuals with normal and poor renal function. Six hours after the dose, only 58% and 64% of the radioactivity present in the serum samples was associated (co-chromatographed) with digoxin in those individuals with impaired and normal renal function, respectively. And 28% and 22% of the radioactivity in these respective two groups was associated with the so-called polar metabolites. The dihydro derivatives co-chromatographed with the unreviewed precursor compounds in the study described above. The percentage of the label present as digoxin 6 h after the dose will therefore be even smaller than the figures given by these authors. In this regard, Clark and Kalman [48] reported urinary dihydrodigoxin to account for 7 to 47% of the labeled digoxin products present, while Greenwood et al. [49] found that an average of 16.4% of the oral digoxin dose was excreted as dihydrodigoxin. In a study in which digoxin was administered orally, Gault et al. [50] reported that <2% of the byproducts in the urine were dihydro derivatives. It is postulated that the dihydro derivatives are formed in the intestine by the bacterial flora [51–53]; consequently, the route of administration will govern the extent of conversion to these compounds. In a recent study [54] 19 patients' sera were studied, 6 h after they had ingested 12α-[3H]digoxin, by both a liquid chromatography/RIA procedure and a regular RIA method. Metabolites accounted for 1 to 99% of the total plasma radioactivity, averaging 40%. It is therefore imperative that any reliable procedure for the measurement of digoxin be specific. Studies assessing the degree of cross reactivity with digoxin metabolites and digoxin-like factors are also essential. Unfortunately, few if any of the commercially available antibodies now meet these requirements for specificity.

Gault et al. [45] found that readily extractable metabolites, such as the mono- and bis-digitoxisides of digoxigenin, digoxigenin itself, and 3-keto-digoxigenin cross react, on the average, by 90% or more, confirming earlier reports of lack of specificity of digoxin antibodies [55, 56].

Table 2 gives the percentage cardioactivity of digoxin metabolites. Stepwise removal of the sugar residues leads to a stepwise loss of cardioactivity, while reduction of the lactone ring results in almost total loss of pharmacological activity [57–59].

Treatment of digoxin toxicity with Fab antibody fragments: The concept of using hapten-specific antibodies to reverse the toxic effects of a drug has been advanced [60–63]. More recently, digoxin-specific Fab antibody fragments have been purified and used to treat patients with advanced, life-threatening digoxin toxicity [64–66]. Although in such circumstances such an immunological approach is feasible, practical, can be life saving, and has been used experimentally for over 10 years, the antibody fragments are not freely available to institutions in North America, an issue which should be of some concern to us all.

Immunoaassays for Digoxin

Digoxin is routinely measured in serum by immunoassay, an antibody to digoxin being raised against a conjugate of bovine serum albumin and digoxin, which is prepared by periodate oxidation of the vicinal hydroxyl groups of the terminal sugar and then coupling of the resulting aldehyde groups to the amino groups of the bovine serum albumin [67]. Thus the conjugate linkage is through the carbohydrate moiety of digoxin. It is not surprising, therefore, that the antibodies generated against this conjugate are directed for the most part against the steroid moiety of digoxin. As a consequence, digoxigenin bis- and monodigitoxiside and digoxigenin itself all react with the antibodies, whereas dihydrodigoxigenin and dihydrodigoxin metabolites—in which C22 is reduced—show little or no cross reactivity. The cross reactivity of the metabolites of digoxin with the anti-digoxin antibodies is well known and is usually stated for most commercial antisera. What has not been appreciated previously is that in many patients (approximately two out of three) digoxin undergoes considerable to extensive metabolism, and measurement of "digoxin" concentrations in serum with nonspecific antibodies 6 to 24 h after a dose clearly can lead to problems in interpreting results. This is probably responsible for the lack of a consensus as to the correlation of serum digoxin concentration with either its therapeutic or its toxic effects. Some investigators have found a good relationship between the serum digoxin concentration and indices of improved contractility (68–71); others have not (72–75).

The relationship between serum digoxin concentration as measured by use of currently available nonspecific antibodies and the reduction of ventricular rate in patients with atrial fibrillation is also poor [76–78]. The rationale for the therapeutic monitoring of a particular drug requires that the drug meet certain established criteria:

Table 2. Percentage Cardioactivity of Digoxin Metabolites

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Activity relative to digoxin, %</th>
<th>Reference no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dihydrodigoxin</td>
<td>2–6</td>
<td>57, 58</td>
</tr>
<tr>
<td>Dihydrodigoxigenin</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>Digoxigenin</td>
<td>4–21</td>
<td>57, 58</td>
</tr>
<tr>
<td>Digoxigenin mono-digitoxiside</td>
<td>66</td>
<td>59</td>
</tr>
<tr>
<td>Digoxigenin bis-digitoxiside</td>
<td>77</td>
<td>59</td>
</tr>
</tbody>
</table>
interactions between able in reported lites chromatography sugars.

With digitoxiside, antigenic molecule. raising the exercise ic measurement. reliable

Bisdigitoxisido Digoxigenin, ~1Fluorescence rarelly all these compounds, we wondered whether some commonly used drugs, injected into laboratory animals, would give false-positive readings for digoxin. These drugs themselves do not interfere in the immunoassay systems studied. However, they might be converted to compounds or else elicit release of substances that do interfere in the immunoassay systems studied. Hydrocortisone and epinephrine were injected into a sheep or dog in doses commonly used for humans. Considerable digoxin immunoreactivity was generated in samples withdrawn from the injection site 2 and 5 min later (Table 6).

During the past 15 years, digoxin in body fluids has been routinely measured almost exclusively through the use of various immunoassay procedures (90–96). While liquid-chromatographic (97–100) and gas chromatographic–mass spectrometric (101) procedures have been described, they have not been widely applied in the routine clinical chemis-

<table>
<thead>
<tr>
<th>Compound</th>
<th>Measured digoxin, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concn in buffer or serum tested, mg/L</td>
</tr>
<tr>
<td>Psycocain</td>
<td>500</td>
</tr>
<tr>
<td>α-Hydroxyymristic acid</td>
<td>500</td>
</tr>
<tr>
<td>α-Hydroxyauric acid</td>
<td>1000</td>
</tr>
<tr>
<td>Cerebrosides Type II</td>
<td>500</td>
</tr>
<tr>
<td>Cerebrosides Type I</td>
<td>500</td>
</tr>
<tr>
<td>L-α-Monopalmitoyl-lecithin</td>
<td>500</td>
</tr>
<tr>
<td>L-α-Monomystoyl-lecithin</td>
<td>500</td>
</tr>
<tr>
<td>1-Allyl-2-hydroxyphosphadiehcoline</td>
<td>1000</td>
</tr>
<tr>
<td>Monosoycardioilin</td>
<td>1000</td>
</tr>
<tr>
<td>Cerebrosides Type I</td>
<td>1000</td>
</tr>
<tr>
<td>Sulfatides</td>
<td>1000</td>
</tr>
</tbody>
</table>

* Fluorescence polarization immunoassay.
try laboratory, owing to their marked lack of sensitivity, which would necessitate lengthy sample preparation and large sample size. The lack of specificity of present immunoassays for digoxin is a serious shortcoming to the rational application of the principles of therapeutic drug monitoring to the improvement of patient care in this area.

Digoxin-Like Immunoreactive Substances and the Concept of Endogenous Digoxin

For several years digoxin immunoreactivity has been reported in dog and human plasma and urine from individuals known never to have received the drug (87, 102–116). Considerable interest has been generated by these findings, with many investigators believing that we are on the brink of discovering a natriuretic hormone. The term "endoxin" has been coined by Gruber et al. (114) because the factor of interest binds to digoxin antibodies. The postulated natriuretic hormone (endoxin) should not be confused with atrial natriuretic factor. In 1959, Bompiani et al. (117) reported histological studies of granular structures between the myocytes of rat-heart atria. In 1979, de Bold (118) reported that the number of these granules changed during water deprivation. He and others later reported (119) that intravenous injection of rat atrial extracts caused a striking natriuresis and diuresis in rats. This discovery resulted in atrial natriuretic factor becoming the focus of attention in many laboratories, and a number of peptides—variously named "atriopeptins," "auriculins," "cardionatrias," and "atrial natriuretic factors" have recently been purified and sequenced (120–123). It is beyond the scope of this article to review these atrial natriuretic factor(s); good reviews are available (124–126). Atrial natriuretic factors cause a natriuresis, diuresis, and drop in blood pressure, but they neither cross react with digoxin antibodies nor inhibit Na+/K+-ATPase. In contrast, the postulated natriuretic hormone has been isolated from plasma (106, 114, 127), placenta (128), and brain (129); it cross reacts with digoxin antibodies, inhibits Na+/K+-ATPase, and also causes a natriuresis and diuresis. Studies by Gault et al. (127), Hamlyn et al. (130), and Devynck et al. (106) lend credence to the probable existence of an endogenous natriuretic hormone (endoxin), a concept previously postulated by LaBella (131) and others. Gault's group showed in a preliminary study that the concentration of digitalis-like factor(s) in plasma more than doubled in response to both an intravenous salt load and a high-salt diet, in patients with mild hypertension. Hamlyn et al. (130), using a kinetic Na+/K+-ATPase assay, demonstrated a significant correlation between concentrations of an inhibitor of Na+/K+-ATPase activity in plasma and the mean arterial blood pressure of normotensive and hypertensive individuals. Devynck et al. (106) found that in two-thirds of untreated hypertensives and several of the normotensive subjects with a family history of hypertension, the potency of the digitalis-like compound, as measured by its interference with ouabain binding to the erythrocyte, was significantly greater than in the controls.

Recent fast-atom bombardment mass-spectrometric and nuclear magnetic resonance data obtained in our laboratory on digoxin-like factors purified from placenta (unpublished) indicated the possible presence of mono- and diglycerides. We already knew that similar compounds could cross react in digoxin immunoassays (55), but we had not evaluated their ability to inhibit 86Rb uptake by the erythrocyte. This experiment revealed that some of these compounds possess considerable ability, in micromolar concentrations, to inhibit 86Rb uptake. Data recently published by Tamura et al. (132) indicate that linoleic and oleic acids act as endogenous Na+/K+-ATPase inhibitors. These compounds were isolated and identified from porcine plasma after the pigs had undergone volume-expansion experiments. Is endogenous digoxin a family of monocacyl and diacyl glycerols, and do these compounds play some role as endogenous regulators of the ubiquitous enzyme Na+/K+-ATPase?

The etiology of essential hypertension, a disease prevalent in cultured societies, is unknown. The discovery of two new hormones, one of which has already been structurally identified and which affect the body's handling of salt and water, is encouraging and gives some hope that the pathogenesis of essential hypertension may begin to unravel in the years ahead.

### Table 5. Cross Reactivity in Digoxin Immunoassays

<table>
<thead>
<tr>
<th>Compounds dissolved in buffer</th>
<th>Measured digoxin, nmol/L</th>
<th>Standards in buffer</th>
<th>Standards in serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concen, mg/L</td>
<td>FPIA</td>
<td>RIA</td>
</tr>
<tr>
<td>11α-Hydroxy-progesterone</td>
<td>25</td>
<td>6.4</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>&gt;6.4</td>
<td>&gt;6.4</td>
</tr>
<tr>
<td>Cortisone</td>
<td>25</td>
<td>1.8</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>3.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Progesterone</td>
<td>25</td>
<td>2.6</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>3.8</td>
<td>2.2</td>
</tr>
<tr>
<td>Sulfatides</td>
<td>500</td>
<td>0.4</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0</td>
<td>6.1</td>
</tr>
</tbody>
</table>

### Table 6. False-Positive Digoxin Measurements after Injection of Epinephrine and Hydrocortisone into Sheep and Dogs

<table>
<thead>
<tr>
<th>Body weight, kg</th>
<th>Drug &amp; dose</th>
<th>Assay</th>
<th>Time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>Hydrocortisone, 100 mg</td>
<td>FPIA &lt;0.4*</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RIA</td>
<td>&lt;0.4</td>
</tr>
<tr>
<td>Sheep</td>
<td>Hydrocortisone, 500 mg</td>
<td>FPIA &lt;0.4</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RIA</td>
<td>&lt;0.4</td>
</tr>
<tr>
<td></td>
<td>Epinephrine, 0.5 mg</td>
<td>FPIA</td>
<td>&lt;0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RIA</td>
<td>&lt;0.4</td>
</tr>
</tbody>
</table>

*Digoxin equivalents, nmol/L.

### References


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