Endogenous Digoxin-Like Immunoreactive Factors: Impact on Digoxin Measurements and Potential Physiological Implications

Roland Valdes, Jr.

Various laboratories have reported endogenous digoxin-like immunoreactive factor(s) (DLIF) in blood from patients in renal failure or liver failure, from newborn infants, and from third-trimester pregnant women. Similar immunoreactivity has been detected in amniotic fluid, in cord blood, and in urine and serum from normal subjects. The factor(s) giving rise to this immunoreactivity cross react with antibodies used in many currently available immunoassays for digoxin, sometimes causing apparent digoxin concentrations exceeding the therapeutic range obtained for exogenous digoxin, with consequent errors in measurement and in subsequent clinical interpretation of digoxin results. Here, I summarize findings in our laboratory and those of others. DLIF evidently exist in three states in serum: tightly protein-bound, weakly protein-bound, and unbound (free). In normal subjects, >90% of the total DLIF in serum is tightly but reversibly bound to serum proteins and is not readily detectable by direct measurement of digoxin in serum with conventional immunoassays. However, there seems to be a redistribution of the more weakly bound and unbound components in patients with renal failure, pregnant women, and newborns. The increased values detected in these groups are attributable to increased amounts of weakly bound and unbound DLIF rather than to increased total DLIF. Carrier proteins may play a prominent role in the transport of these factors in blood. I discuss the potential physiological and pharmacological implications of detecting endogenous immunoreactive factors that cross react with antibodies to drugs.

Additional Keyphrases: liver function • kidney function • newborns • pregnancy • interference with assays for cardia
tive drugs • urine

Digoxin is the cardiac glycoside most widely prescribed and used in the United States. The therapeutic use of this digitalis derivative is constrained by its narrow therapeutic range, 0.8 to 2.0 µg per liter of plasma. Its toxic effects are generally seen at concentrations in plasma (or serum) above 2.5 µg/L (1). Because of this narrow therapeutic range and the large population on this therapy, numerous assays have been developed and marketed for measuring digoxin in serum or plasma. As early as 1965, using an assay based on the inhibition of rubidium uptake by human erythrocytes, Lowenstein and Corrill (2,3) observed false-positive digoxin measurements in several patients who had no pathological condition in common. Interestingly, some of those measurements were as high as 10 µg/L (in terms of digoxin) when in fact the subjects had taken neither digitalis compounds nor any drug known to interfere with the assay and had no evidence of digitalis toxicity. Extraction of digoxin from the samples with organic solvents before assay eliminated the false-positive interference. Similar false-positive digoxin results were later observed by Burnett and Conklin (4), who were using a sodium-potassium ATPase (from hog brain) inhibition assay for digoxin. In 1969, Smith et al. (5) described a radioimmunoassay for digoxin and they, as well as others, have applied this technique to determine digoxin in serum. Since then, several studies in which radioimmunoassays were used have defined the narrow therapeutic range mentioned above (1). Those studies have revealed substantial overlap between effective therapeutic and toxic concentrations of digoxin in the blood. In three specific clinical conditions, attempts have been made to further define therapeutic ranges and appropriate digoxin dosages: (a) renal failure, digoxin elimination being primarily controlled by the kidney (6), (b) infancy (7), and (c) mother and fetus during pregnancy (8).

Several reports have presented evidence for substantial variation among digoxin results obtained by radioimmunoassays of serum or plasma (9,10). In most cases the variations (as large as 3.0 µg/L) between assays could not be accounted for by digoxin metabolites (11), the presence of known interfering drugs (12), or assay dependence on the protein concentration of the specimen (9,12). In recent years there have been reports of endogenous factors detected in some fluids and tissues from animal (13,14) and human (15) sources that cross react with antibodies raised against digoxin. The discovery of endogenous digoxin-like immunoreactive factors (DLIF)1 has offered an explanation for the above-noted discrepancies in digoxin measurements. It may also explain why there is substantial overlap in the currently defined therapeutic and toxic reference ranges in serum for patients taking digoxin (1). Because these endogenous factors interfere with accurate measurement of digoxin, it will ultimately be important to identify these compounds. Moreover, as with many other endogenous substances that mimic the therapeutic effects of administered drugs, under-

1 Throughout this article the abbreviation DLIF (digoxin-like immunoreactive factor) will be used for endogenous substance(s) that cross react with antidigoxin antibodies (i.e., competitively displace labeled digoxin). Names used by other investigators for substances with this property include cardioglin, endocardin, endodigin, endoxin, DLS (digoxin-like substance), and DLIS (digoxin-like immunoreactive substance). I use the term "factor" in that it, or they as a group, possess the noted immunoreactivity.

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standing the biochemistry and pathophysiological role of these substances could lead to the development of better therapeutic modalities in human disease. In this review I summarize the findings of our laboratory and those of other investigators with respect to the detection and characterization of endogenous digoxin-like immunoreactivity in humans.

Detection of Endogenous DLIF in Human Extracellular Fluid

Endogenous DLIF in blood from adults in renal or hepatic failure. Recently our laboratory reported anomalous values for digoxin measured in the serum of one patient in acute renal failure who was treated with digoxin (10). Measured digoxin continuously increased (see Figure 1) for 10 days after his last dose. Not only was the increasing digoxin of concern, but discrepant values for "digoxin" were found when different radioimmunoasays were used (Clinical Assays, 3.9; Corning, 3.6; Beckman, 2.2; and Syva, 1.9 µg/L) for the same serum specimen. In contrast, serum from a nonuremic control showed no significant between-assay difference. Our subsequent studies (16) comparing a series of patients with normal renal function and those with renal impairment, both taking digoxin, demonstrated that results were consistently discrepant among the immunoasays in the renal-failure group as compared with controls with normal renal function (Table 1). The observed discrepancies were not correlated with the assay’s mode of hapten separation, tracer-label used, or type of immunoassay (radioimmunoassay vs homogeneous enzymoimmunoassay). In addition, studies suggested that the discrepancy was not due to cross reactivity of known digoxin metabolites (17). In another recent report (17) a patient in acute renal failure was also noted to have a serum digoxin concentration increasing from 2.9 to 4.2 µg/L (by Kallestad Laboratories’ Digoxin RIA) within 11 days after his last dose of digoxin. The authors concluded that, based on total digoxin dosage and injections of trifluorinated digoxin, the increase in serum digoxin was most likely ascribable to a gradual decrease in the patient’s apparent volume of distribution, of unknown cause. Decreased numbers of digitalis receptors, altered structures of receptors, or inhibition of digoxin binding (perhaps by DLIF?) have been proposed as possible explanations (17).

To learn more about endogenous DLIF, we subsequently studied a group of patients who were in renal failure but not taking digoxin. Figure 2 summarizes our results. Evidently many of the immunoasays currently in use will detect DLIF in patients with renal impairment. In that particular group of subjects some of the false-positive values measured were as high as 1.0 µg/L (digoxin-equivalent). Our data also showed that measured values of DLIF in any one specimen were not always concordant between assays, nor were they correlated with the degree of renal impairment or with the hemodialysis status of the patients studied (16).

Similar false-positive digoxin measurements have been

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Table 1. Summary of Differences between Digoxin Assays for Patients with and without Renal Impairment

<table>
<thead>
<tr>
<th>No. (and %) of sera with difference of 0.3 µg/L or more</th>
<th>Maximum difference, µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method C vs D</td>
<td></td>
</tr>
<tr>
<td>Renal-impaired</td>
<td>48/102 (47)</td>
</tr>
<tr>
<td>Normal</td>
<td>1/32 (3)</td>
</tr>
<tr>
<td>Method A vs D</td>
<td></td>
</tr>
<tr>
<td>Renal-impaired</td>
<td>38/102 (37)</td>
</tr>
<tr>
<td>Normal</td>
<td>3/32 (9)</td>
</tr>
<tr>
<td>Method A vs C</td>
<td></td>
</tr>
<tr>
<td>Renal-impaired</td>
<td>39/102 (38)</td>
</tr>
<tr>
<td>Normal</td>
<td>4/32 (12)</td>
</tr>
</tbody>
</table>

From reference 16. Methods A, C, and D are as in Figure 2.

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Fig. 1. Concentrations of digoxin and creatinine in serum, and calculated creatinine clearances vs time in a digoxin-treated patient who developed acute renal failure

Method used: GammaCoat Digoxin RIA (Clinical Assays). Reproduced, with permission, from ref. 10

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Fig. 2. Apparent digoxin concentrations in serum as measured with six immunoasays for digoxin in patients not taking digoxin

The group with renal impairment (Renal) included as many as 54 patients known to be free of digoxin. The group with normal renal function (Non-renal) included as many as 14 healthy adult laboratory technicians who had never had digoxin. Each point represents duplicate determinations. Digoxin methods used were: RIA-Phase, Beckman Instruments, Inc. (A); Digoxin RIA Kit, Beckon Dickinson Immunodiagnostics (B); GammaCoat, Clinical Assays (C); Immophase, Corning Medical and Scientific (D); RAINEN Digoxin RIA, New England Nuclear (E); extx, Syva (F). Reproduced, with permission, from ref. 16
reported by DiPiro et al. (12) in a series of patients with hepatic failure. These authors concluded that in patients with active alcoholic cirrhosis the mean "apparent serum digoxin" concentration was 0.74 μg/L (SD 0.4, maximum value 1.3 μg/L, n = 10). There were no significant correlations between apparent digoxin and serum albumin or bilirubin concentrations. Interestingly, of their control group of 10 patients (noncirrhotic, and receiving no cardiac glycosides), six had detectable apparent digoxin (mean 0.4, SD 0.3 μg/L, maximum 0.9 μg/L). In their digoxin RIA they used rabbit antiserum to digoxin (Biology, Inc.) and 125I-labeled digoxin (Wellcome Reagents, Ltd.).

Endogenous DLIF in blood from newborns. Apparent digoxin has been detected in plasma from cord blood of neonates (18) and in plasma from premature, low-birthweight infants (19, 20). We examined for DLIF sera from a series of normal newborns two to four days postpartum, using several commonly used digoxin immunoassays (Figure 3). DLIF values ranged from 0.1 to 1.4 μg/L digoxin-equivalent (21). As measured by one of these commercially available assays (Clinical Assays), 16% of the results exceeded 0.8 μg/L even though none of these infants was receiving digoxin. By these (Figure 3) RIA methods, over 90% of the infants had detectable apparent digoxin (>0.1 μg/L) in their serum. In that same study we demonstrated that values for DLIF were additive to those for digoxin added in vitro. In addition, preliminary time-course studies of a limited series of infants suggested the measured endogenous DLIF in many cases increased during the first few postnatal days, then decreased (21), suggesting that the DLIF may not originate from either the mother or the placenta. Subsequently, a more detailed study by Pudek et al. (22), comparing seven digoxin immunoassays, corroborated our initial findings. Their data also suggest that the DLIF is dehydroepiandrosterone, a steroid commonly present in abnormally high concentrations in neonatal blood. In a more recent report (23) these investigators measured DLIF concentrations in serum during the first 14 postnatal days in 24 premature neonates (Figure 4). All infants had values exceeding 0.6 μg/L by Nuclear Medical Laboratory's digoxin RIA assay (range 0.6–5.3 μg/L) and showed a distinct peak at about the fourth (SD 1.6) postnatal day. In addition, the peak values for DLIF obtained during the first eight postnatal days correlated inversely with gestational age and birth weight (23). That same study also showed that the interference by DLIF in digoxin measurements in serum after digoxin administration was additive in vivo.

Therefore, several reports involving more than 10 different digoxin immunoassay kits with presumably different anti-digoxin antibodies have firmly established the presence of endogenous digoxin immunoreactivity in the serum and plasma of two- to eight-day-old infants. Unlike values for the maternal serum, which rapidly decrease after parturition (see below), DLIF values for newborns can remain high for as long as two weeks (21, 23). The magnitude of the DLIF is striking, in many patients being within or above the generally accepted therapeutic range for digoxin.

Endogenous DLIF in normal and hypertensive pregnant women. Our laboratory also examined serum from 51 pregnant women in their third trimester for DLIF (24). Values of 0.1 to 0.6 μg/L digoxin-equivalent were found (see Figure 5)—values lower than those reported for neonates or for adults in renal failure but still potentially clinically relevant. The rapid elimination of DLIF from the serum of pregnant women after parturition (Figure 6) suggests an elimination half-life of 6 h or less (24). Six endogenous sex steroid hormones known to be increased in serum during pregnancy—estradiol, estriol, progesterone, pregnanediol, pregnanetriol, and pregnenolone—were examined for possible cross reactivity with the antibody used to detect DLIF in pregnancy. The most cross reactive of these, progesterone, cross reacted by 0.04% at a concentration of 3.7 × 10^6 μg/L.
Endogenous DLIF increases in serum throughout gestation (Figure 7), and values in third-trimester hypertensive women are higher than in normal pregnant controls (25). Other laboratories have confirmed this finding, both for serum (26, 27) and in amniotic fluid (28). Seccombe et al. (23) showed that DLIF concentrations in amniotic fluid seem to remain constant from the 16th to 33rd week of gestation, then increase from the 33rd week to term.

By modifying our assay for DLIF (29), we have been able to measure digoxin-like immunoreactivity without interference from the specimen's ionic strength, protein concentration, or heat-denatured serum proteins. Table 2 summarizes results of measurements obtained after heating sera from nonpregnant controls, normal pregnant controls, and hypertensive pregnant women. There is a significant increase in DLIF in the hypertensive group as compared with controls, especially in the immunoreactive factors measured directly in serum before the serum sample is heated, which, as discussed below, represents the weakly protein-bound and

Table 2. Enhancement of DLIF by Heating Serum of Normal and Hypertensive Pregnant Women

<table>
<thead>
<tr>
<th></th>
<th>Before heating</th>
<th>After heating</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLIF, ng/L (digoxin equivalent)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 4)</td>
<td>55 ± 3</td>
<td>578 ± 44</td>
<td>523</td>
</tr>
<tr>
<td>Preg. normal, 3rd trimester (n = 8)</td>
<td>141 ± 12</td>
<td>686 ± 53</td>
<td>545</td>
</tr>
<tr>
<td>Preg. hypertensive, 3rd trimester (n = 13)</td>
<td>196 ± 16</td>
<td>759 ± 69</td>
<td>563</td>
</tr>
<tr>
<td>Hyper- vs normotensives, % increase</td>
<td>39</td>
<td>10</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Reproduced, with permission, from reference 25. Values are means ± SE.
unbound components of DLIF combined. The finding of abnormally high values for DLIF in hypertensive pregnancy is of particular interest in view of a report of similar activity in the plasma of hypertensive monkeys (30).

**DLIF in normal subjects during cardiovascular stress.** It has been proposed that DLIF may represent endogenous digitalis-like compounds (31). At present little information is available to relate DLIF and cardiovascular function. A recent report demonstrated increased DLIF in the serum of rats with induced cardiac overload (32). After induced cardiac hypertrophy, a positive correlation was noted between the weight of the heart and concentrations of DLIF in serum, as determined by using the EMIT-cad (Syva) assay for digoxin. We have found increased serum DLIF in healthy individuals exercised by walking on a treadmill (33). In these experiments the combined unbound and weakly protein-bound components of DLIF in serum rose from a baseline of 52 ± 6 to 91 ± 8 ng/L digoxin-equivalent (mean ± SE, n = 6) after 3 h of continuous exercise. These values are below the detection sensitivities (100 to 200 ng/L) of conventional immunoassays for digoxin. Baseline total DLIF measured after heating the specimen was 580 ± 54 ng/L and increased to 945 ± 104 ng/L during these experiments. These latter concentrations of total DLIF in serum are the largest we have observed in adult humans who were not taking digoxin (29). The reason for these increases is under investigation.

**DLIF in normal subjects after water and salt ingestion.** The possibility has been raised that DLIF may represent endogenous natriuretic compounds and therefore be associated with salt and water homeostasis (13). However, as in the case with the issue of cardiovascular function, there are few data to support this proposition. Gault et al. (34) suggested that both acute and chronic salt loading of healthy individuals increases the measured DLIF in their plasma by 130% from a plasma baseline value of 46 ng/L. The increases in measured digoxin immunoactivity did not correlate with the decreases in plasma renin or aldosterone. In experiments on humans in which renal excretion of water (diuresis) or salt (natriuresis) was selectively stimulated, urinary excretion of DLIF was correlated to rate of urine flow but not with natriuresis (35). Klingmuller et al. (15) reported the isolation of a digoxin-like immunoreactive substance from the urine of humans after chronic salt-loading. However, urinary excretion of this substance by control groups not loaded with salt was not reported. Nevertheless, our experiments, as well as theirs (15, 34, 35), do establish that these studies are feasible in human subjects and that DLIF can be measured in urine as well as in serum. Physicochemical studies have suggested that metabolites of serum DLIF are excreted in the urine (29).

**DLIF in Organs from Animals and Humans**

Several studies have been aimed at measuring DLIF in extracts of various animal organs. We recently compared digoxin-like immunoreactivity in serum (normalized to protein content) with activity extracted from tissues of male Sprague–Dawley rats (36). The organs were removed and homogenized. The centrifuged supernatant fluids were analyzed for DLIF and for total protein. The digoxin immunoreactivities measured in the organs, in nanograms of digoxin-equivalent per gram of protein, were: adrenal 39.5, liver 5.2, kidney 1.1, and brain, heart, or lung 0.0. For comparison, the immunoreactivity measured in normal human or rat serum averaged 7.3 ng of digoxin-equivalent per gram of protein as measured with the most sensitive assay for DLIF (29). DLIF isolated from human tissues followed the same pattern of distribution in tissue as in rats and has the same biochemical characteristics as DLIF isolated from human serum and urine. A study by Schreiber et al. (14) supports these findings. They found that, in extracts of rabbit adrenals fractionated by thin-layer chromatography and assayed for endogenous DLIF (Syva, EMIT) and for digitalis glycoside-like biological activity (inhibition of 86Rb uptake by erythrocytes), both DLIF and biological activity were present in two distinct chromatographic fractions migrating between corticosterone and deoxycorticosterone, with a mobility different from that of aldosterone. No studies to date have firmly established the adrenal gland as a definitive source of DLIF; however, our preliminary data based on catheterization of adrenal veins of dogs (manuscript in preparation) supports the hypothesis that the adrenal organ is a source of the DLIF measured in serum.

Other tissues have also been reported to possess digoxin-like immunoreactivity. Beyers et al. (37) and Diamandis et al. (38) have demonstrated the presence of endogenous DLIF in human placental extracts. Our own work has shown that supernates from homogenates of human placental tissue at term (prepared in our laboratory by the same method as the rat organs above) contain almost threefold as much DLIF as normal human serum, per gram of protein (our unpublished results).

**Characterization of DLIF, and Its Binding to Serum Proteins**

**Binding of DLIF to serum proteins.** Characterization of DLIF has demonstrated common physical properties for the factor(s) isolated from the sera of all human subjects we have studied. Of interest is that most of the total DLIF is tightly but noncovalently bound to serum proteins (29). Chromatography on Sephadex G-25 (Figure 8) and membrane ultrafiltration have been used to isolate DLIF from its binding proteins in serum. Thermal protein denaturation—by boiling diluted serum—significantly increases the quantity of DLIF measured by immunoassay. The magnitude of the increase depends on the clinical groups studied (see Table 3). A substantial portion (90% for normal controls and 80% for the clinical groups studied) of the total DLIF present in human serum remains tightly but reversibly associated with the protein, even after denaturation by boiling. One present hypothesis is that total DLIF is present in serum as three distinct components defined as: (a) tightly protein-bound, not released by boiling; (b) weakly protein-bound,
released by boiling, i.e., rendered ultrafilterable; and (c) unbound (free). Whether these components are chemically identical or whether they exist in dynamic equilibrium with each other is not yet known. However, equilibrium ultrafiltration studies do suggest that the immunoactivity measured directly in serum (before boiling) represents both the weakly protein-bound and the unbound components of DLIF (Figure 9), and not that which is tightly bound to proteins. The distribution of DLIF components changes (i.e., the unbound and weakly bound fractions increase; see Figure 10 post-salt-I chromatography fraction) in the clinical groups we studied. Interestingly, the unbound and weakly protein-bound components of DLIF are the components detected as "false-positive interference" by conventional immunoassays for digoxin (29).

Physicochemical properties of DLIF. Most of the few studies dealing with the physical and chemical characterization of DLIF agree that it is water soluble, heat stable, not digestible by proteolytic enzymes, and has a molecular mass of less than 1000 Da (13, 14, 29). One study suggests that DLIF isolated from plasma is sensitive to hydrolysis by HCl at boiling temperature (39). Our studies show that the apparent molecular mass of DLIF isolated from human serum (200 Da as estimated by membrane ultrafiltration) is less than that of DLIF isolated from human urine (400 Da). Additionally, exposure to acid and heat causes the apparent molecular size of the DLIF isolated from urine to more closely match that isolated from serum. Hence, the factor in urine may represent a conjugated metabolite of the factor in serum (29).

Immunoreactive Factors and Endogenous Drug-like Compounds

Theory of common antibody-receptor sites and the detection of endogenous drug-like compounds. A rationale for the existence of endogenous substances that cross react with antibodies directed against physiologically active drugs is based on the principle of structural complementarity between the endogenous substance and the drug. Gintzler et al. (40) propose that if both the endogenous substance and the drug elicit the same or similar biological responses, they

**Table 3. Enhancement of Measured DLIF in Serum in Various Physiological Conditions**

<table>
<thead>
<tr>
<th>Source of serum</th>
<th>Before heating*</th>
<th>After heating*</th>
<th>Ratio after/before</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy adults (n = 7)</td>
<td>61 ± 3</td>
<td>596 ± 33</td>
<td>9.8 ± 0.06</td>
</tr>
<tr>
<td>Renal-failure patients (n = 13)</td>
<td>128 ± 38</td>
<td>636 ± 61</td>
<td>5.0 ± 0.50</td>
</tr>
<tr>
<td>Third-trimester pregnant women (n = 8)</td>
<td>141 ± 12</td>
<td>686 ± 53</td>
<td>4.9 ± 0.04</td>
</tr>
<tr>
<td>Newborns (n = 10)</td>
<td>230 ± 7</td>
<td>1290 ± 49</td>
<td>5.6 ± 0.04</td>
</tr>
</tbody>
</table>

* Heating to 100°C for 5 min after threefold dilution with water. All measurements are corrected for dilution and reported as mean ± SE. Reproduced, with permission, from reference 29.

![ELUTION FRACTIONS](image)

Fig. 10. Comparison of the percent of total DLIF (activity) in Sephadex eluate fractions for pooled normal human serum (A); pooled serum from patients with renal failure, pregnant women, and neonates (B); amniotic fluid (C); and urine (D).

The column fractions were designated according to the procedure used in Fig. 8. Reproduced, with permission, from ref. 29.

![Figure 9](image)

Fig. 9. Comparison of endogenous DLIF (digoxin immunoactivity) measured before heating of serum samples and measurements of the ultrafiltrates of the same heated serum.

The points (left to upper right) are for serum pools from: normal adults, pregnant women, adults in renal failure, and newborns. DLIF measured before heating is evidently rendered ultrafilterable by heating. Reproduced, with permission, from ref. 29.
may interact at the same or similar receptor sites. Consequently, their structural similarity at the active site (epitope) would then lead to antibody cross-reaction (Figure 11). This principle has been successfully used in identifying endogenous nonpeptide opiate-like (40) and benzodiazepine-like (41) compounds. By analogy, other endogenous drug-like substances (in particular, those resembling the cardiac glycosides such as digoxin) have been proposed by several investigators (31, 42, 43). A digoxin-like substance has been postulated since 1942 to be the endogenous mediator of enhanced cardiac contractility by its assumed interaction with membrane bound ouabain-sensitive sodium—potassium ATPase (43). Therefore, the possibility that DLIF may possess digitalis-like biological activity is intriguing.

However, care should be taken when interpreting reports suggesting identity between "digoxin-like" and "ouabain-like" substances, especially if the basis of the measurements are made by cross reactivity with anti-digoxin antibodies. Ouabain itself is not a potent cross reagent in digoxin immunoassays (13); therefore, the implication of "ouabain-like" does not readily extend to "digoxin-like," as measured by immunoreactivity. Also, the recognition of topographical features on molecules by receptors is not limited to compounds of chemically identical classes (42)—for example, morphine, an alkaloid, has biological activity similar to the endorphins, which are polypeptides. Thus DLIF, even if they were biologically active in the same way as digoxin, would not have to be chemically homologous to it; i.e., they need not have a steroid-lactone ring structure (11).

Physiological implications of endogenous DLIF in humans. Several investigators have suggested correlations between the presence of endogenous DLIS, inhibition of transmembrane sodium—potassium exchange (a proposed mechanism for natriuresis and (or) peripheral vasoconstriction), and hypertension (30, 44). Because patients with essential hypertension and (or) renal failure, newborn infants, and pregnant women are volume-expanded, a hypothesis linking volume expansion, natriuresis, and increased DLIF is plausible (13). Enriched extracts of DLIF from human urine injected into rats produced natriuresis (15, 45). Precipitation with antibody specific for digoxin further enhanced the natriuretic activity by almost 10-fold (15). Interestingly, one report has documented that injection of anti-digoxin antibodies into rats that have been made hypertensive by treatment with desoxycorticosterone acetate (DOCA) and salt lowers their blood pressure (46). Another report has suggested an increase of DLIF in plasma of DOCA-treated hypertensive rats relative to controls (47). These combined results are consistent with the idea of an endogenous substance that cross reacts with digoxin and plays a role in some forms of hypertension. Kojima (47) also presents evidence of increased ouabain-like binding activity of DLIF to rat brain sodium—potassium ATPase in vitro. Along those same lines, the work of Graves and Williams (28) suggests a correlation between DLIF in amniotic fluids and ouabain-like sodium—potassium ATPase inhibitory activity in vitro. Finally, Schreiber et al. (32) demonstrated increases of digoxin-like immunoreactivity in the serum of rats after experimentally induced cardiac overload. This finding may be related to the increases in serum DLIF measured in human subjects during prolonged cardiovascular stress (33). However, it remains an open question as to if or how the presence of DLIF in either blood or organs is related to cardiac or renal physiology.

In conclusion, above-normal concentrations of endogenous digoxin-like immunoreactive factors have been detected in the serum and plasma of patients in renal failure and hepatic failure, neonates, and pregnant women, as measured with more than 10 commercially available digoxin immunoassays. Normal subjects also have detectable concentrations. The presence of these factors raises two important issues: the problem of erroneous digoxin measurements in these groups of patients and the potential physiological relevance of these factors. Several studies reviewed in this article clearly establish that these factors cause falsely positive digoxin measurements in serum, the magnitude of which can range from barely detectable (0.1-0.2 μg/L) to values in excess of the therapeutic reference range for digoxin (>2.0 μg/L). At present, the antibody heterogeneity in antisera used for digoxin assays and the scatter in DLIF values obtained for the various clinical groups with any one immunoassay make it necessary for each individual laboratory to evaluate the particular digoxin assay kit it is using for the extent of this interference. Changes in DLIF concentrations with time after birth in neonates and with time of gestation in pregnant women make it difficult to establish useful therapeutic and toxic ranges for digoxin or for DLIF baseline values for serum before such patients are given digoxin. Changes of serum DLIF with time have not been studied for patients in renal or hepatic failure. In these patients it is not yet possible to say whether stable baseline DLIF values could be determined before doing with digoxin. Nor has it been established whether measurements of DLIF itself are useful, because the bioactivity of DLIF is unknown. Eliminating the detection of this endogenous immunoreactivity by currently available digoxin immunoassays may be possible by manipulating assay conditions (especially because protein binding plays a role in its detection), by using more-specific antibodies, or by using solvent-extraction techniques. No studies dealing with these improvements have yet been reported.

On the other hand, the detection and accurate quantification of these endogenous immunoreactive factors will play an important role in their isolation and identification, and in establishing their physiological role, if any. From this point of view, more-specific methods suitable for accurate measurement of DLIF still need to be established. An important question remaining is whether substances detected by various laboratories as "digoxin-like immunoreactive" are in fact the same or related substances—a question that can be answered only when these substances have been isolated and characterized. In any event, the presence of endogenous DLIF noted in the sera of patients in renal failure, liver failure, pregnant women, and newborn infants compromises the accurate measurement and clinical interpretation of results for digoxin obtained with current immunoassays. The physiological relevance of DLIF and its detection in other clinical conditions is the subject of ongoing research. Discovery of other endogenous drug-like substances by using antibodies raised against compounds in our present pharmacopeia is an intriguing possibility.
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References