A Method for Measuring Plasma Levels of Digitalis Glycosides

By Jerold M. Lowenstein, M.D.

For both practical and theoretical reasons there has long been a need for measuring blood levels of cardiac glycosides. From the practical standpoint the clinician is constantly confronted with patients in cardiac failure in whom it is difficult to determine whether there is too much or too little digitalis, and the wrong analysis may have serious or even fatal consequences. The problem is made more difficult by the fact that digitalis has one of the lowest toxic-to-therapeutic ratios of any commonly used drugs. The most sensitive nonradioactive assay available has been the duck embryo bioassay of Friedman, and capable of detecting 0.025 μg of digitoxin per milliliter of fluid. This proved inadequate for measuring normal blood levels of the glycoside and it was necessary to do laborious urine extractions. These results are difficult to interpret, because cardiac glycosides are excreted fecally as well as renally, and active metabolic products as well as the originally administered drug appear in the urine. Okita studied blood and tissue levels with C\textsuperscript{14} labeled digitoxin and was able to measure as little as 0.02 μg. He could detect blood levels for about 3 days after an intravenous dose of 0.5 to 1.5 mg. of C\textsuperscript{14} digitoxin. This method also is not sufficiently sensitive to detect ordinary blood levels of orally administered glycosides, and of course not all patients can be given radioactive digitalis.

In 1953 Schatzmann showed that extremely small concentrations of cardiac glycosides inhibit radioactive potassium uptake of human red cells. Glynn further demonstrated that both influx and efflux of both potassium and sodium are partly inhibited by as little as 0.001 μg of digoxin per milliliter of buffer. Glynn and Kahn and Acheson studied a large group of steroids and showed that only the cardiac glycosides inhibit potassium uptake in very small (physiologic) concentrations. Inhibition of potassium transport seems to be intimately connected with the basic inotropic action of cardiac glycosides.

The extreme sensitivity of this effect led the author to look for diminished potassium uptake in the red cells of patients treated with digitalis. Instead of radioactive potassium, radioactive rubidium (Rb\textsuperscript{86}) was used because red cells do not distinguish to any significant extent between potassium and rubidium, and Rb\textsuperscript{86} has a much more convenient half life (19 days) than any available potassium isotope. No significant difference was found between the red cell rubidium uptake of patients taking and not taking digitalis. However, the red cells of digitalized patients were more sensitive to the in vitro blocking effect of digoxin. Though group differences were significant, clinical digitalis levels and individual variation was too great to permit a quantitative measure by this method.

This report describes a new quantitative method for measuring plasma levels of cardiac glycosides. Rather than using the patient's own red cells, we apply the patient's plasma to standard red cells and then measure rubidium uptake from a standard glucose-saline solution. In this way, it is possible to detect as little as 0.05 millimicrograms of digoxin per milliliter of plasma. The variable inhibiting power of different normal plasmas reduces the practical detectability of this method to about one millimicrogram of digoxin per milliliter, which represents a sensitivity 20 times greater than the duck embryo or C\textsuperscript{14} methods.

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Material and Methods

Blood to be tested was drawn in tubes containing ethylenediamine tetraacetic acid (EDTA) as anticoagulant. Plasma was separated from the red cells within 24 hours and red cells were discarded. The plasma was then incubated for 1 hour at 37 C. with type O, Rh-positive red cells, 1 ml. of plasma to 0.5 ml. of red cells. At the end of an hour's incubation, the red cells were washed twice with normal saline and incubated with 1 ml. of saline containing glucose, 100 mg. per cent, and Rb86, about 0.05 µc./ml., specific activity 100 µc./mg. After 1 hour's incubation at 37 C. the cells were washed twice with normal saline, and the radioactivity was measured in a well-type scintillation counter. Per cent uptake was calculated by comparing red cell counts with a standard containing the original Rb86 activity.

The standard red cells were obtained from banked blood, type O, Rh-positive, anticoagulant ACD. Single units (500 ml.) were obtained and preserved by adding 50 mg. of adenine sulfate. At the beginning of each run the needed quantity of whole blood was removed and centrifuged, and the supernatant plasma was discarded. The standard cells were then incubated as described above.

With each run a standard curve was drawn showing the sensitivity of the red cells to varying concentrations of digoxin (Lanoxin). The cells maintained normal sensitivity and rubidium uptake for at least 4 weeks.

Pooled plasma was obtained from the Presbyterian Hospital Clinical Laboratory by pooling plasma of blood drawn in EDTA tubes for routine laboratory tests. In addition, the plasma of two individuals working in the laboratory was run most days as an absolute basis of comparison.

Results

The sensitivity of red cell rubidium uptake to inhibiting effect of digoxin is shown in figure 1. Five hundredth millimicrogram per milliliter regularly produced slight inhibition of uptake, whereas 0.02 mµg./ml. produced none. In figure 2 the same data are plotted on a linear rather than a logarithmic scale. In figure 3 is shown the comparative Rb86 uptake of three groups of patients: those receiving no digitalis, whose average for each run is arbitrarily taken as 100 per cent (actual 1-hour Rb86 uptakes were 35 to 50 per cent); patients on maintenance digoxin 0.25 to 0.5 mg./day; patients on maintenance digitalis.
Figure 4

Plasma glycoside levels (as digoxin equivalent) based on the data in figures 3 and 1, with standard deviations.

leaf, 0.1 Gm./day. In figure 4 these data are converted into the equivalent level of plasma-digoxin based on the curve of figure 1. With very few exceptions patients not on digitalis had no substances in their plasma that inhibited red cell rubidium uptake. The digoxin group averaged 0.4 μg./ml. with a range corresponding to one standard deviation of 0.8 μg./ml. The group on digitalis leaf averaged 12 μg./ml. (digoxin equivalent) with a range of 3.5 to 18.5.

In the series described above no absolute standard was employed, but each day’s average Rb⁸⁶ uptake of the “no digitalis” group was taken as 100 per cent (= 0 plasma digoxin). A second series of patients was studied, with the plasma of two laboratory workers (which consistently gave identical Rb⁸⁶ uptakes) taken as a daily absolute standard of 100 per cent, thus giving consistent standardization from day to day. By the previous method, the actual average, taken as 100 per cent, might vary from day to day. The results, however, shown in figures 5 and 6, are very similar to those of the first series. Two additional groups were included here: pooled plasma, which averaged a slightly higher Rb⁸⁶ uptake than patients not on digitalis (97.6 per cent compared to 96.2); and five patients with digoxin toxicity, with an average plasma level of 6 μg./ml. compared with 0.05 μg./ml. for the nontoxic group. The average Rb⁸⁶ uptake of patients not on digitalis (96.2 per cent) was taken as the 0 plasma level (fig. 6). The probability distributions in these various groups are given in table 1. In table 2 are listed data on patients not taking digitalis who had low Rb⁸⁶ uptake (i.e., high apparent plasma digitalis levels).

One patient was given a single intravenous injection of 0.5 mg. of digoxin and blood samples were drawn at 1, 4, 10, 15, 40, 60, 90, and 120 minutes. The plasma digoxin levels are shown in figure 7 and the curve, replotted on semilog paper in figure 8, seems to have two exponential phases; the first, with a halftime of 1.5 minutes, extrapolates to a distribution volume of 2.5 L., which is just the estimated plasma volume of this patient. The second slope, with a half-time of 16 minutes, has a distribution volume of 47.6 L. (greater than the estimated total body water for a 56-
Table 1

**Distribution of Apparent Plasma Digitalis Levels in the Groups Studied**

<table>
<thead>
<tr>
<th>Digoxin level, μg./ml.</th>
<th>Pooled plasma</th>
<th>Probability of finding a value higher than that in left-hand column</th>
<th>Digoxin</th>
<th>Digoxin (toxic)</th>
<th>Digitalis leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.7%</td>
<td>47%</td>
<td>76%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>10</td>
<td>38</td>
<td>80</td>
<td>76</td>
</tr>
<tr>
<td>1.0</td>
<td>0</td>
<td>7</td>
<td>27</td>
<td>80</td>
<td>67</td>
</tr>
<tr>
<td>5.0</td>
<td>0</td>
<td>6</td>
<td>2</td>
<td>80</td>
<td>57</td>
</tr>
<tr>
<td>10.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>38</td>
</tr>
<tr>
<td>15.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>20.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Kg. individual). At 90 and 120 minutes the plasma level of digoxin was no longer detectable (i.e., less than 0.05 μg./ml).

**Discussion**

The method described here appears to be capable of measuring plasma levels of digitalis at clinical dosage levels. If 1 μg./ml. be taken as the minimum practically detectable dose, 67 per cent of patients on digitalis leaf and 27 per cent of patients on digoxin were found to have higher values, while four of five patients with digoxin toxicity had levels greater than 6 μg./ml. There was a remarkable difference in the average levels of digoxin and digitalis leaf—0.05 compared with 2.5 μg./ml. (in Rb86-uptake equivalents). This would seem to indicate a marked difference in the relative affinities of heart, red cells, and plasma for the two drugs; these data imply indirectly that the heart-to-plasma ratio is higher for digoxin, since there is an equivalent action on the heart at much lower plasma levels.

Above 1.0 μg./ml. there remained 7 per cent of false positives in the group not taking digitalis. There do not seem to be any consistent diagnoses or medications in this group (table 2), but it is noteworthy that two had metastatic carcinoma, and Scott12 found decreased red cell Rb86 uptakes in patients and rodents with metastatic cancer. One patient, in renal shutdown, had severely deranged plasma electrolytes and pH and another was

Table 2

**Patients not on Digitalis with High Apparent Plasma Digitalis Levels**

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Clinical data</th>
<th>Medication</th>
<th>Rb86 uptake, %</th>
<th>Digoxin equivalent, μg./ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>Metastatic breast ca.</td>
<td>Phenobarbital</td>
<td>89.8</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5-Fluorouracil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>Diabetic, alcoholic, fasting glucose 260</td>
<td>Tolbutamide</td>
<td>89.9</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Darvon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>Status asthmaticus</td>
<td>Aminophyllin</td>
<td>91.4</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Amytal</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chlorotrimeton</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nembutal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>Recurrent prostatic ca., creatinine 1.7</td>
<td>Hydrocortisone</td>
<td>91.3</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Meperidine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>Crush injury, renal shutdown, Na 123 K 7.3 CO2 13.3</td>
<td>Dramamine</td>
<td>92.6</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Librium</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Empirin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chlorothiazide</td>
<td>89.3</td>
<td>10.0</td>
</tr>
</tbody>
</table>

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in diabetic acidosis; an acid pH is known to decrease red cell potassium uptake. Finally, with regard to the patient in heart failure, there have been many reports of digitalis-like substances in the plasma, particularly in heart failure, and such a substance—palmitoyl lysolecithin—has been isolated from mammalian tissue. While this material has similar effects to digitalis on the isolated amphibian heart, the author is not aware of any reports of its effect on potassium and rubidium uptake in red cells. Work is in progress in this laboratory to discover the plasma fraction with the greatest digitalis-like activity.

Investigations during the past few years make it appear very probable that cardiac glycosides affect cellular cation transport by inhibiting membrane adenosine triphosphatase. The resulting loss of intracellular potassium and decrease in intracellular pH may greatly increase the force of heart muscle contraction and in part account for the beneficial action of digitalis in heart failure. Clinical studies of cardiac glycosides have been severely hampered in the past for want of a method for measuring blood and tissue levels. Therefore a practical technic requiring no special equipment (except the well-type scintillation counter now present in almost all isotope laboratories) should find wide application.

**Summary**

A method is described for measuring plasma levels of cardiac glycosides, utilizing their inhibitory effect on the rubidium-86 uptake of human red cells. As little as 0.05 mµg. of digoxin/ml. of plasma produces detectable inhibition, but due to the variable effect of other substances in human plasma the practical limit of the method is 1.0 mµg./ml., about 20 times as sensitive as the duck embryo heart or the C¹⁴-labeled techniques.

Patients on digitalis leaf were found to have much higher circulating levels than patients on digoxin (average value 7.5 mµg./ml. of digoxin equivalent for digitalis leaf, 0.05 mµg./ml. for digoxin). Patients toxic on digoxin averaged 6 mµg./ml.

Of patients not taking digitalis, 7 per cent had significant levels of digitalis-like substances in their plasma; these were mostly individuals with metastatic carcinoma, severe fluid and electrolyte disturbances, or heart failure. No drug (other than cardiac glycosides) gave consistent high levels by this test.
References


The Endless Journey

A great discovery is not a terminus, but an avenue leading to regions hitherto unknown. We climb to the top of the peak, and find that it reveals to us another higher than any we have yet seen and so it goes on. The additions to our knowledge of physics made in a generation do not get smaller or less fundamental or less revolutionary as one generation succeeds another. The sum of our knowledge is not like what mathematicians call a convergent series . . . where the study of a few terms may give the general properties of the whole. Physics corresponds rather to the other type of series called divergent, where the terms which are added one after another do not get smaller and smaller, and where the conclusions we draw from the few terms we know cannot be trusted to be those we should draw if further knowledge were at our disposal.—J. J. Thomson.
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