Relationship of Plasma Digitoxin and Digoxin to Cardiac Response Following Intravenous Digitalization in Man

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SUMMARY

The value of plasma digitalis determinations will depend upon the accuracy with which they mirror the myocardial effects of digitalis. Ten volunteers received 0.8 mg of digitoxin intravenously, and after 2 hours the mean plasma digitoxin level was 45.6 ± 6 \(\mu\)g/ml but declined over 4 to 6 hours during plasma-tissue equilibration. The cardiac response, as indicated by decreases in the left ventricular ejection time index (LVETI) and Q-S\(_2\) intervals \((P < 0.01)\), was present at 2 hours and reached maximum at 4 to 6 hours. Subsequently, plasma level, LVETI, and Q-S\(_2\) changed in the same direction during slow excretion.

Six subjects received 1.0 mg of digoxin intravenously. Mean plasma digoxin at 30 min was 6.8 ± 0.3 \(\mu\)g/ml; it fell after 3 to 4 hours to levels usually seen during maintenance digoxin administration \(<3 \mu\)g/ml\) and then declined more slowly. Correlation of individual digoxin levels with ΔLVETI values during the first 4 hours was significant \((P < 0.01)\). The half-life \((T_{1/2})\) for the dominant slope of the plasma curve was 30.5 hours by plasma determinations and 29 hours by LVETI determination.

Plasma levels of digitoxin and digoxin were related to their cardiac effects under the conditions studied. Prior to plasma-tissue equilibration, plasma determinations of digoxin and digitoxin will be higher than levels seen during maintenance administration of these drugs.

Additional Indexing Words:
Left ventricular ejection time index
Plasma digitoxin and digoxin
Heart rate
Systolic interval

METHODS for the measurement of plasma nonradioactive digoxin and digitoxin concentrations are now available.\(^1\)-\(^7\) The value of these determinations will depend on the adequacy with which they reflect the myocardial concentrations or effects of digitalis or both.\(^8\) The plasma-myocardial tissue ratio of concentrations of these glycosides during the steady state after administration is of the order of at least 1:30.\(^9\),\(^10\) Thus far, animal experiments and observations on patients considered to be intoxicated with these drugs suggest that plasma concentration reflects the myocardial concentration or effects, or both.\(^6\),\(^8\) Prior to widespread application of these new methods, the relationships between the plasma levels of these drugs and their myocardial effects in man should be defined more precisely. A recent study has shown that the electrocardiographic effects of digitoxin and digitalis leaf, short of toxic arrhythmias, are too nonspecific for such purposes.\(^7\) Furthermore, the sizable overlap of plasma values between patients considered adequately digitalized and those judged intoxicated\(^6\) suggests

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the need for additional correlative investigations.

The purpose of the present study was to attempt to correlate plasma levels of digitoxin and digoxin during the first 2 to 3 days after acute administration with the myocardial effects of these drugs as reflected in the expected alterations in the systolic portion of the cardiac cycle.

Methods

Subjects

Ten patients and six medical students volunteered for study. Mean age for the 10 patients was 44.6 years (range, 35 to 55); the mean age of the six students was 25 years (range, 24 to 26). Fourteen of the 16 subjects had no evidence of cardiovascular disease. The two cardiac patients had the following conditions: alcoholic cardiomyopathy (subject 1) and mild mitral stenosis (subject 2). None of these patients or students had previously received digitalis; all had normal serum electrolyte determinations at the time of study.

Measurement of Plasma Digitoxin and Digoxin

The technic longest used in our laboratory because of its availability was based on that of Lowenstein as modified in 1966. All measurements of plasma digitoxin employed this method. Since certain modifications have been made in our laboratory and since the original description omitted certain key details, a complete description of the technic employed in the present study follows: (1) Fresh blood, 100 ml, is obtained from a normal undigitized donor. (2) The plasma is separated after centrifugation, and the red cells are washed twice with physiologic saline solution and then refrigerated until used. (3) Calibration curves are run for every experiment utilizing serial dilutions of digitoxin or digoxin in plasma containing the following concentrations: 0, 5, 20, 50, and 100 μg/ml. Five empty tubes are set up to obtain the background counts, and each calibration point or sample is run in triplicate or duplicate. (4) One milliliter of dichloromethane is added to 1-ml aliquots of the plasma samples, and the solution is mixed for 15 to 20 sec on a vortex shaker. (5) After a 15-min centrifugation, the plasma layer is discarded, and the remaining solution is evaporated to dryness in a shaker water bath beginning at 50°C and progressively heating to 90°C. (6) After cooling, 1 ml of normal saline is added to each tube to reconstitute the precipitate, and this solution is incubated for 10 min at 37°C in a shaker water bath. (7) One-half milliliter of washed fresh red cells (previously drawn as described above) is added to each tube, and the tubes are incubated at 37°C for 2 hours in a shaker water bath. (8) The tubes are then centrifuged, the supernatant is discarded, and the red cells are washed twice with normal saline. (9) Radioactive rubidium (86Rb) is diluted in a solution of 85 mg% glucose in normal saline to a concentration of approximately 2.5 μC/ml. One milliliter of this solution is added to each tube. (10) The red cells to which the 86Rb solution have been added are then incubated for 1 hour at 37°C in a shaker water bath with occasional shaking in order to keep the cells in suspension. (11) These solutions are centrifuged for 15 min at about 3,500 rpm. The supernatant is discarded, and the cells are washed with normal saline which is also discarded. (12) Red cell uptake of 86Rb is measured in a well-type auto-gamma counter (Packard model no. 402).

Data accumulated in this laboratory have demonstrated that normal red cells from any ABO group are suitable.11 Day to day variations in 86Rb uptake, however, require the running of a fresh calibration curve for each unknown sample or group of samples on any given day.

The background counts are subtracted from the counts of each tube. The calibration curve is constructed by arbitrarily assigning the count of red cells exposed to the blank plasma tube as 100% uptake of 86Rb on the ordinate. Coordinate points are made between known concentrations on the abscissa and actual count on the ordinate for each of the known digitalis glycoside samples. The unknowns are plotted according to the number of counts expressed as per cent of the 100% count value and concentration in μg/ml is read off the abscissa. The value assigned to each known and unknown sample is the average of the values of the triplicate or duplicate determinations.

Recently a major modification of the 86Rb method designed to increase its accuracy for digoxin was reported by Grahame-Smith and Everest.5 It was employed for plasma digoxin determinations with the following alterations: (1) Centrifugations were carried out at room temperature; (2) 2.5 μC of rubidium radioactivity was added to each sample; and (3) every calibration curve point and each unknown or patient sample was carried out in duplicate. The data from a given run were expressed as the mean of these duplicate determinations. Calculations and counting were carried out as described above.

Determinations of Systolic Intervals

Recording and measurement of systolic intervals were carried out as described by Weissler and...
colleagues\textsuperscript{12} utilizing the following equipment. Recordings were made on a research recorder with a direct-writing attachment at a paper speed of 100 mm/sec. Lead II of the electrocardiogram was recorded in standard fashion. Heart sounds at the apex were recorded with the patient recumbent utilizing a model PS-1 pulse sound microphone (Electronics for Medicine, Inc.). The carotid pulse tracings were obtained by utilizing a Hewlett-Packard, model APT-16 coil pickup. Analyses were carried out for heart rate, pre-ejection period (PEP), left ventricular ejection time (LVET), Q-S\textsubscript{p}, Q-1, and left ventricular ejection time index (LVETI). The pre-ejection period and Q-S\textsubscript{p} interval were corrected for heart rate according to the regression curves published by Weissler and associates.\textsuperscript{13} Calculations for ejection fraction (EF) were carried out according to the method of Carrard and co-workers\textsuperscript{14} which has shown a correlation coefficient of 0.88 between angiocardiographically determined ejection fraction (EF) and that determined from the relationship of the PEP and the LVET according to the formula, $EF = 1.25 - 1.25 \frac{PEP}{LVET}$. The LVETI was, where so indicated, corrected for diurnal effects as reported by Weissler and associates.\textsuperscript{12} At least 10 consecutive cardiac cycles were analyzed. Careful checks on consistency and accuracy were made by frequent independent determinations of similar sequences by each of two of the authors (K. N. and W. S.).

Procedures

Intravenous Digitoxin

Digitoxin, 0.8 mg, was given intravenously to 10 patient volunteers after control samples of blood had been obtained, and systolic intervals determined. These procedures were done at approximately 7:30 a.m. Subsequent blood sampling and systolic interval determinations were performed in 2, 4, 6, and 24 hours, in six patients in 48 hours, and in three patients in 72 hours. The environmental circumstances during these studies deserve note. The patients were allowed to partake of their usual meals. Between determinations they were allowed to walk about or sit in the hospital corridors and in their rooms. They were brought to the laboratory 30 min prior to each determination in order to rest in the supine position before each measurement; blood was drawn after systolic interval determinations. The digitoxin series of experiments was performed during the course of a heat wave in an unairconditioned environment. The temperature in the laboratory in which the study was done ranged between 80 and 85 F, in the morning and rose to approximately 98 F at the end of each work day. These conditions contrast with those under which the base-line data of Weissler and associates\textsuperscript{12} were obtained, that is, postabsorptive fasting state, recumbent during entire digitalis assay study, and an air-conditioned environment. The conditions of this portion of the present study reflect those that may often be present in clinical settings.

Intravenous Digoxin

These studies more closely simulated the conditions of the study described by Weissler and co-workers\textsuperscript{12} on digitalis assay. The six medical student volunteers arrived in the laboratory at 7:00 a.m. in the postabsorptive fasting state on separate occasions. They remained recumbent and fasting throughout the 4-hour study period on the first day and were in the postabsorptive condition and resting for 60 min prior to the morning determinations at the 24 and 48-hour intervals. Following control observations, 1 mg of digoxin was administered intravenously. Repeat samples of plasma and determinations of systolic intervals were obtained at 3, 1, 1/2, 2, 3, and 4 hours, and then at 24 and 48 hours in all six subjects. Room temperature throughout this series was approximately 72 F. These sampling intervals allowed determination of the $T_{1/2}$ of the slow decline according to the method of Doherty and associates.\textsuperscript{15}

Paired $t$-test analyses were used to check significance of deviations from control of each test employed. All statistical tests were carried out on an Olivetti desktop computer, model 101.

Results

Intravenous Digitoxin

Selected data obtained before and after the intravenous administration of 0.8 mg of digitoxin are presented in figure 1 and table 1. Control plasma digitoxin level was 0.44 ± 0.12 $\mu g/ml$; range, 0 to 0.9 $\mu g/ml$. The mean 2-hour level of 45.5 ± 5.9 $\mu g/ml$ declined toward the slow excretion slope within 4 hours. The 48 and 72-hour values reveal the gradual continued decline of plasma digitoxin concentration. The $T_{1/2}$ of this slow decline was estimated to be 58 hours. Significant declines were seen in the LVETI and Q-S\textsubscript{p} at 2 hours ($P < 0.01$), and these persisted at 24 hours ($P < 0.05$). The maximal inotropic effect appeared to occur at 4 hours, but the exact time of its occurrence could not necessarily be gleaned from the data because of the time increments chosen for the observations. Heart rate was essentially unchanged. The pre-
Mean values for plasma digitoxin and simultaneous alterations in LVETI for 10 subjects during the 24 hours following intravenous administration of 0.8 mg of digitoxin.

ejection period and the derived ejection fraction were not significantly altered.

In the six subjects followed 48 hours, LVETI and Q-S₂ continued to be significantly reduced. The three subjects followed longer had a mean plasma digitoxin value at 72 hours of 18.9 ± 5.6 μg/ml, a decline of approximately 8 μg/ml from the value at 48 hours of 27 ± 7.5 μg/ml. Their mean LVETI and Q-S₂ were below the control value at 72 hours, but statistical significance was not present for these few observations.

**Intravenous Digoxin**

The mean plasma digoxin values following intravenous administration of 1.0 mg of digoxin are presented in figure 2 and table 2. The mean control value was 0.07 ± 0.02 μg/ml with a range of 0 to 0.15. The plasma levels at 30 min averaged 6.84 ± 0.28 and these values rapidly declined to a mean value at 4 hours of 1.85 ± 0.09 μg/ml. Progressive decreases were measured at 24 hours and 48 hours. The T½ of the rapid

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**Table 1**

*Mean Values for Plasma Digitoxin and Selected Systolic Intervals Before and Following Intravenous Administration of 0.8 mg of Digitoxin*

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>No.</th>
<th>Plasma digitoxin (μg/ml)</th>
<th>Heart rate (b/min)</th>
<th>LVETI (msec)</th>
<th>Q-S₂,*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>0.44 ± 0.12†</td>
<td>77 ± 4.9</td>
<td>401 ± 6.0</td>
<td>537 ± 5.8</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>45.5 ± 5.9</td>
<td>78 ± 4.3</td>
<td>392 ± 5.2‡</td>
<td>529 ± 8.0‡</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>30.0 ± 5.1</td>
<td>81 ± 4.5</td>
<td>385 ± 4.6‡</td>
<td>518 ± 5.7‡</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>28.1 ± 4.9</td>
<td>79 ± 4.6</td>
<td>387 ± 5.3‡</td>
<td>520 ± 6.0‡</td>
</tr>
<tr>
<td>24</td>
<td>10</td>
<td>28.9 ± 4.8</td>
<td>76 ± 4.5</td>
<td>393 ± 5.2§</td>
<td>524 ± 6.6**</td>
</tr>
<tr>
<td>48</td>
<td>6</td>
<td>26.9 ± 4.7</td>
<td>75 ± 4.7</td>
<td>393 ± 7.5**</td>
<td>523 ± 6.7§</td>
</tr>
<tr>
<td>72</td>
<td>3</td>
<td>18.9 ± 5.6</td>
<td>80 ± 4.9</td>
<td>391 ± 8.4</td>
<td>517 ± 11.0</td>
</tr>
</tbody>
</table>

*Corrected for rate.
†Values represent mean ± standard error of mean.
‡, §, **P values compared to control: †P < 0.01; §P < 0.05; **P < 0.02.
PLASMA DIGITOXIN AND DIGOXIN

Table 2

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Plasma digoxin (mg/ml)</th>
<th>Heart rate (b/min)</th>
<th>LVETI (msec)</th>
<th>Q-S2,§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.067 ± 0.02†</td>
<td>69 ± 3.0</td>
<td>419 ± 3.6</td>
<td>547 ± 7.0</td>
</tr>
<tr>
<td>0.5</td>
<td>6.84 ± 0.28</td>
<td>62 ± 2.6</td>
<td>404 ± 4.1†</td>
<td>524 ± 6.0†</td>
</tr>
<tr>
<td>1</td>
<td>5.92 ± 0.49</td>
<td>63 ± 2.5</td>
<td>404 ± 3.5†</td>
<td>524 ± 7.1†</td>
</tr>
<tr>
<td>1.5</td>
<td>4.77 ± 0.58</td>
<td>60 ± 3.3</td>
<td>400 ± 2.9†</td>
<td>517 ± 6.5†</td>
</tr>
<tr>
<td>2</td>
<td>3.41 ± 0.41</td>
<td>60 ± 1.7</td>
<td>395 ± 2.9†</td>
<td>516 ± 6.4†</td>
</tr>
<tr>
<td>3</td>
<td>2.58 ± 0.37</td>
<td>63 ± 2.9</td>
<td>390 ± 3.7‡</td>
<td>516 ± 6.8‡</td>
</tr>
<tr>
<td>4</td>
<td>1.85 ± 0.09</td>
<td>64 ± 2.3</td>
<td>389 ± 2.5‡</td>
<td>515 ± 4.8‡</td>
</tr>
<tr>
<td>24</td>
<td>1.11 ± 0.15</td>
<td>64 ± 3.6</td>
<td>406 ± 3.6‡</td>
<td>532 ± 5.5§</td>
</tr>
<tr>
<td>48</td>
<td>0.63 ± 0.10</td>
<td>68 ± 3.5</td>
<td>409 ± 4.1§</td>
<td>536 ± 6.1§</td>
</tr>
</tbody>
</table>

*Corrected for rate.
† Values represent mean ± standard error of mean.
‡, §P values compared to control: †P < 0.01; §P < 0.05.

decay observed from the 30-min to 4-hour period was 48 min. The T₉₀ of the slow decline extrapolated from the values at 3, 4, 24, and 48 hours was 30.5 hours.

The mean values for selected systolic time intervals and the associated mean plasma digoxin levels are seen in table 2 and figure 3. Significant decreases in LVETI and Q-S₂ were seen 30 min after injection. The LVETI shortening progressed to its maximum at 3 and 4 hours. While the 24 and 48-hour values were significantly less than control, they were returning toward the initial value. A similar pattern of change was noted in the Q-S₂ interval. The mean PEP decreased within the first 2 hours, but was not materially different from control afterward. Heart rate, ejection fraction, S₁-S₂, isovolumic contraction time, and Q-1 were not significantly altered. The T₉₀ of the ΔLVETI derived from the slope of these values was approximately 29 hours.

The maximal inotropic effects seen at 3 and 4 hours, as determined from the maximum ΔLVETI, occurred as plasma digoxin declined into a slow excretion phase. During the rapid early fall of the plasma level, inotropic effects began. During the subsequent slow decline, the inotropic effects, as reflected in shortened LVETI and Q-S₂, paralleled the plasma digoxin values.

Correlation of Falling Plasma Concentration with ΔLVETI

Figure 4 illustrates the relationship between

![Figure 3](image_url)

**Figure 3**

Mean values for plasma digoxin and simultaneous alterations in LVETI in six patients followed 48 hours after 1 mg of digoxin was given intravenously.

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individual plasma digoxin levels and individual ΔLVETI values during the initial 4 hours following administration of digoxin. The r value for this correlation was −0.77 (P < 0.01).

A similar analysis was attempted with the digitoxin data, but the correlation was not significant. A correlation of the mean plasma digitoxin values and the mean ΔLVETI values during the first 6 hours did show a similar slope yielding an r value of 0.9.

Discussion

This investigation indicated that the modified 86rubidium-uptake inhibition bioassay method as described and modified can be used for detecting digitoxin and digoxin in plasma. Furthermore, it shows a relationship between the plasma level of these two drugs and the onset and the time course of inotropic effect as measured by systolic time intervals following acute digitalization in man.

The plasma digitoxin values were similar to those obtained in other laboratories using similar methods.2, 7, 11 The observed approximate T1/2 of the slow decline for digitoxin in the present study was 58 hours as compared with the whole blood T1/2 reported by Okita for C-14 digitoxin of 44 to 52 hours16 and the physiologic half time of 102 to 112 hours reported by Weissler and colleagues.12 Confidence in the absolute reliability of this T1/2 value must be tempered by the short observation period and the small number of subjects carried beyond 48 hours. While the presently employed method for digitoxin appeared to be adequate, its resolution seems to have been improved by an adaptation11 of the Grahame-Smith and Everest method.5 The major reason for this increased resolution was that the determinations of concentration in plasma may be performed on the steep portion of the calibration curve rather than the flattened, insensitive portion obtained with the Lowenstein and Corrill method.2, 11

These data showing the changes in nonradioactive plasma digoxin following intravenous administration appear to be in agreement with the data on tritiated digoxin published by Doherty and his colleagues.15, 17 The actual plasma values in the slow excretion phase agree with those reported by the radioimmunoassay technic of Smith and associates.6 The T1/2 of the slow decline was almost in perfect agreement with the tritiated digitoxin studies in man.15, 17

When digitoxin or digoxin was administered intravenously, the early high plasma values rapidly diminished as plasma-tissue equilibration took place. The onset of the subsequent slow excretion was at approximately 3 to 4 hours with digitoxin and between 4 and 6 hours after administration of digitoxin.

The control data and pattern of change in the systolic time intervals following digitalization were similar to those previously reported by Weissler and his colleagues.12

The availability of plasma glycoside determinations, a long sought-after achievement, may provide important clinical information if the relationships of these measurements to onset and offset of myocardial effects of the drugs can be defined. The large plasma-to-myocardial tissue ratio of digoxin (at least 1:30)6, 10 and the reportedly wide range of myocardial tissue concentrations have led to the suggestion that little of importance concerning myocardial effects might be expected from plasma determination.10 The present data suggest great value from properly timed plasma samplings.

A comparison of the early plasma level-physiologic response relationship (fig. 4) with gross myocardial tissue uptake may provide insight into the time required for digitalis glycosides to gain access to presumed active sites. The progressive slowing of the rapid, early decline in plasma digoxin may be related to a limited rate of uptake by such sites (figs. 2 and 3).

The patterns of change observed for plasma glycoside levels and the systolic intervals provide guide lines for optimal sampling time when these technics are employed clinically. During the plasma-tissue equilibration period, the values for digitoxin and digoxin rapidly fall from their early peak levels while the systolic intervals are altered from control

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toward their maximum values. Samples taken during this period will provide spuriously high glycoside and systolic interval values if they are used to gauge steady-state conditions. Following equilibration, plasma glycoside levels and systolic intervals paralleled each other, indicating that each would be a useful guide to the myocardial effects of the digitalis glycosides tested.

Both humid\textsuperscript{18} and dry heat\textsuperscript{19, 20} have been associated with circulatory adaptations considered to stimulate, and, therefore, stress the cardiovascular system.\textsuperscript{18} Since these effects would be mediated by mechanisms increasing the inotropic status of the myocardium, this environmental condition may be considered to have blunted the effects of the modest dose of digitoxin administered during that series of studies. Indeed, if the control data in table 1 (digitoxin study in hot environment) are compared with those in table 2 (digitoxin study in normal thermal environment), the former shows faster heart rate and shorter LVET and Q-S\textsubscript{2} values. Despite this environmental disadvantage, the pattern and significance of the data appear to warrant the conclusions drawn.

These data do not provide a precise explanation for the large overlap in values found by the radioimmunoassay technic\textsuperscript{2, 6} where large numbers of intoxicated patients had plasma levels in the range of patients clinically considered to be adequately digitalized and not intoxicated. From present knowledge, it is apparent that serum potassium may well modify the glycoside plasma level associated with intoxication just as low serum potassium appears to enhance tissue uptake of digitalis in the experimental animal.\textsuperscript{21, 22} Time of sampling following administration of the drug is another factor that could contribute toward obtaining falsely high or low levels.

References

2. Lowenstein JM, Corbull EM: An improved method for measuring plasma and tissue levels of digitalis glycosides. J Lab Clin Med 67: 1048, 1966
Thermal regulation during acclimatization in a hot, dry (desert type) environment. Amer J Physiol 163: 585, 1950


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It should be the chief aim of a university professor to exhibit himself in his own true character—that is as an ignorant man thinking.—WHITEHEAD AN: Aims of Education. New York, Mentor Books, 1949, p. 26.
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