Correlation of Serum Concentrations with Heart Concentrations of Digoxin in Human Subjects

By H.-G. Güllner, M.D., E. B. Stinson, M.D., D. C. Harrison, M.D., and S. M. Kalman, M.D.

SUMMARY
Biopsies of cardiac tissue were taken from patients undergoing surgery for coronary artery or valvular disease. All subjects were on maintenance doses of digoxin, which were stopped 48 hours before surgery. The ratios, heart:serum, for digoxin content were remarkably similar and the variance was small.

Additional Indexing Words:
Atrial biopsies  Digoxin in human heart  Tissue:serum ratios of digoxin

The utility of the assay of serum levels of a drug implies a systematic relationship between this measurement and concentration of the drug at the site of action during "steady state" conditions. Data to support this relationship with respect to digoxin have been sparse, particularly with respect to man. One noteworthy finding is the observation both in animals and in man that digoxin concentrations in heart as well as in certain other tissues are about one order of magnitude greater than serum levels of digoxin. A wide variation in this ratio, tissue:serum concentration, has been reported in man. To investigate the distribution of digoxin in serum and in myocardial tissue we have measured this drug in samples of atrial tissue taken from patients undergoing cardiovascular surgery.

Methods
Tissue samples from the tip of the right atrial appendage were obtained from 12 patients receiving maintenance doses of digoxin (0.25 mg daily orally) 5 min before institution of cardiopulmonary bypass. For all of these patients the last dose of digoxin was given 48 hours before surgery. The patients suffered from either valvular or coronary artery disease and were taking digoxin because of a history of congestive heart failure. All samples were taken by the same surgeon (E.B.S.). Venous blood samples were drawn 10–15 min before the initiation of cardiopulmonary bypass, immediately centrifuged and the serum saved for digoxin determination. All patients had normal serum electrolyte and blood urea concentrations at the time of surgery.

Biopsy specimens weighing 80–790 mg (wet weight) were stored in closed vials under refrigeration. At the time of determination adherent fat was removed from the muscle tissue which was then weighed and minced with a scissors. Then it was transferred into a Kontes Duall tissue grinder and homogenized in 3 ml of 0.04 M phosphate buffer, pH 6.6. The homogenate was transferred into a separatory funnel, 15 ml of dichloromethane were added, and the mixture shaken vigorously by hand for two minutes. This extraction step was repeated once. The dichloromethane phases were pooled, placed in a round bottom flask, and evaporated to dryness in a Büchi Rotavapor-R evaporator. The residue was dissolved in 2 ml of tris-buffered human serum albumin (BHSA) containing 0.14 M NaCl and 0.1 M tris(hydroxymethyl) aminomethane adjusted to pH 7.5 with 2 N HCl. One ml of BHSA solution containing tissue extract was assayed for digoxin by radioimmunoassay as described by Smith et al. Digoxin in blood serum was assayed by the same method.

To check the recovery of 3H-digoxin from heart tissue three female Sprague-Dawley rats weighing 200–250 grams were injected intraperitoneally with 10 μCi 3H-digoxin each. They were sacrificed 8 hours later. Ventricles were divided longitudinally into two sections of 150–200 mg. One section was analyzed by the dichloromethane extraction method, the second was analyzed by combustion in a Packard Tri Carb Sample Oxidizer. The results are shown in table 1. The recovery was slightly higher using the oxidation method than the dichloromethane extraction method. The recovery of the extraction method appears to be between 95 and 100%. The variability among animals was small.

No digoxin was detected by radioimmunoassay in control atrial myocardium from a patient not receiving digoxin.

Results
The ratios heart:serum for digoxin were based on heart concentration (ng/g):serum level (ng/ml). It is
Table 1

Comparison of Extraction vs. Oxidation

<table>
<thead>
<tr>
<th>Rat #</th>
<th>Extraction (dpm/g)</th>
<th>Oxidation (dpm/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6066</td>
<td>7070</td>
</tr>
<tr>
<td>2</td>
<td>6019</td>
<td>6207</td>
</tr>
<tr>
<td>3</td>
<td>4740</td>
<td>4886</td>
</tr>
</tbody>
</table>

*Corrected for loss of counts on oxidizer based on introduction of pure 3H-digoxin.

apparent that there is a constant ratio, with a relatively small standard deviation between these ratios for 12 patients (table 2).

Discussion

Monitoring of digoxin serum levels to evaluate the state of digitalization of the heart has found widespread application since the radioimmunoassay for digoxin was introduced by Smith et al. In order to be meaningful digoxin concentrations in serum should reflect directly concentrations of the drug in the myocardial tissue. Doherty and coworkers showed that there were high concentrations of digoxin in human kidney, liver, heart, and skeletal muscle. Using 3H-digoxin Doherty and Perkins demonstrated heart to serum ratios from 35:1 to 58:1 in dogs with a mean ratio of 43:1. They also were able to show that the release of digoxin from cardiac muscle paralleled the fall in digoxin serum concentration of the glucoside.

In humans, postmortem analyses of heart tissue after a single intravenous dose of tritiated digoxin was shown by Doherty and coworkers to display average concentrations 30 times higher than in serum. The subjects studied by Doherty’s group died at various times, from 3.5 hours to several days after the administration of the tracer dose of 3H-digoxin. In addition, they died of a variety of diseases, some of which may have altered the capacity of the heart for binding the drug. The data obtained provide a rough estimate of the relation between heart and serum concentrations, but the wide variation obtained in tissue:serum ratios cannot be used to demonstrate a systematic relationship.

The study by Binnion et al. did not help our understanding because of its bizarre design. They used blood from one set of patients and heart tissue from another set, and concluded that there was “no apparent relation between plasma and myocardial digoxin concentrations.” Coltart et al. using papillary muscle from eight patients undergoing surgery for valve replacement, found ratios ranging from 39:1 to 155:1. Although no information about the diseases of these patients was given, it can be assumed that variable but significant amounts of connective tissue were present in the heart samples. This may explain the failure of previous work to demonstrate a constant relationship between heart and blood concentrations of digoxin. Carroll et al. reported ratios ranging from 25:1 to 128:1 with a standard deviation of 28:1. In this study washing of the myocardial samples in isotonic saline prior to digoxin determination may have resulted in variability in wet weight and residual digoxin. The wide range of intervals, 24-48 hours, between discontinuation of the drug and time of surgery could also have influenced the results.

Thus, most published results have not demonstrated a fixed ratio between myocardial levels and serum digoxin. The lack of such a relationship would make interpretation of serum levels clinically difficult. Our data confirm the estimates made by

Table 2

Digoxin Concentrations and Ratios

<table>
<thead>
<tr>
<th>Subject</th>
<th>Serum (ng/ml)</th>
<th>Myocardium (ng/g)</th>
<th>Myocardium/Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (R.A.) C.A.D.</td>
<td>1.2</td>
<td>29.8</td>
<td>24.8</td>
</tr>
<tr>
<td>2 (H.B.) C.A.D.</td>
<td>1.9</td>
<td>33.6</td>
<td>17.7</td>
</tr>
<tr>
<td>3 (M.B.) C.A.D.</td>
<td>0.5</td>
<td>14.0</td>
<td>28.0</td>
</tr>
<tr>
<td>4 (J.D.) Aortic stenosis</td>
<td>1.0</td>
<td>29.1</td>
<td>29.1</td>
</tr>
<tr>
<td>5 (E.H.) Aortic insufficiency</td>
<td>1.0</td>
<td>23.9</td>
<td>23.9</td>
</tr>
<tr>
<td>6 (K.P.) Aortic stenosis</td>
<td>1.1</td>
<td>21.1</td>
<td>19.2</td>
</tr>
<tr>
<td>7 (S.R.) Aortic + mitral stenosis</td>
<td>3.5</td>
<td>86.6</td>
<td>24.7</td>
</tr>
<tr>
<td>8 (E.S.) Mitral stenosis</td>
<td>1.7</td>
<td>39.2</td>
<td>23.0</td>
</tr>
<tr>
<td>9 (W.S.) Aortic stenosis</td>
<td>1.0</td>
<td>21.6</td>
<td>21.6</td>
</tr>
<tr>
<td>10 (L.S.) C.A.D.</td>
<td>1.0</td>
<td>23.7</td>
<td>23.7</td>
</tr>
<tr>
<td>11 (J.W.) Aortic + mitral stenosis</td>
<td>1.0</td>
<td>24.9</td>
<td>24.9</td>
</tr>
<tr>
<td>12 (R.W.) Aortic stenosis</td>
<td>2.4</td>
<td>63.7</td>
<td>26.5</td>
</tr>
</tbody>
</table>

Mean ± SEM | 1.4 ± 0.8 | 34.3 ± 3.7 | 23.9 ± 3.2 |

Abbreviation: C.A.D. = coronary artery disease.
Doherty and his colleagues in showing a high concentration in heart, but show a uniformity not apparent in the other studies. A constant ratio between heart tissue and serum concentrations of digoxin in humans was demonstrated in table 2. The remarkable correspondence between 12 of these cases is a finding not heretofore approached. The standard deviation is sufficiently small to lend support to the ratio obtained. Several factors may explain the reasons for our findings. First, in our experiment all 12 tissue samples were taken by the same surgeon and always from the same site of the heart, the tip of the right atrial appendage. The use of a single site for biopsy is important since different amounts of fibrotic and fatty tissue are present at different sites of the heart. Digoxin appears to be taken up predominantly by myocardial cells and only to a small extent by connective tissue and fat. We believe that the hearts involved in this study were in sufficiently good condition that there was no great variability in the fat and fibrous tissue of biopsy samples (unpublished experiments, Coltart, Gyllner, Kalman and Harrison). Secondly, the patients involved in this study all had normal electrolytes; this is important in the transport of digoxin into and out of myocardial cells. Thirdly, all had been on maintenance doses for relatively long periods of time before the drug was stopped. Finally, all 12 of these patients had had their digoxin maintenance doses stopped 48 hours before surgery, a conventional procedure in cardiac surgery.

References
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Circulation. 1974;50:653-655
doi: 10.1161/01.CIR.50.4.653

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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