Studies on the Renal Excretion of Radioactive Digitoxin in Human Subjects with Cardiac Failure

By G. T. Okita, Ph.D., F. E. Kelsey, Ph.D., P. J. Talso, M.D., L. B. Smith, B.S., and E. M. K. Geiling, Ph.D., M.D.

Randomly labeled C14-digitoxin was used in a quantitative study of the renal excretion of unchanged digitoxin and its metabolites in three human subjects with cardiac insufficiency. The elimination of approximately 60 to 80 per cent of an administered dose through the kidney suggests that the major route of elimination of digitoxin in cardiac patients is through the urinary route. There is a marked initial excretion of digitoxin during the first two days after administration of the radioactive drug followed by a gradual leveling off of the excretion gradient thereafter. Minute amounts of unchanged digitoxin have been detected in the urine up to the fortieth day after administration of a single dose of the glycoside, while C14-labeled compounds were detected up to the seventy-fourth day.

UNTIL RECENTLY, the lack of suitable analytic methods has hindered quantitative studies of the renal excretion of digitalis glycosides. Utilizing only bioassay technics, early investigators1-5 concluded that little if any of the various glycosides studied was excreted in the urine of various species of laboratory animals after oral or parenteral administration. Recently, however, Friedman and co-workers,6-9 employing the sensitive embryonic duck heart method, reported that rats, rabbits and dogs excrete negligible amounts of digitoxin in the urine while normal human subjects excrete up to "40 per cent of a digitalizing dose of digitoxin in a physiologically active state" over a period of 12 to 24 days. In 1950 Geiling and co-workers10 using radioactive digitoxin, reported that dogs excrete up to 46 per cent of a single dose of the glycoside in the urine. More recently, Fischer and associates,11 also using radioactive digitoxin, have shown that rats and cats eliminate 30 per cent and 55 per cent, respectively, of unchanged digitoxin and metabolites through the urinary route. Clinically, it has been assumed that cardiac patients either excrete or destroy 0.1 to 0.2 Gm. of digitalis or 0.1 to 0.2 mg. of digitoxin per day.

To gain further information concerning the mode of elimination of digitoxin in human subjects, renal excretion studies have been conducted in cardiac patients using digitoxin uniformly labeled with carbon14. Use of the tracer technic permits not only greater sensitivity for the detection of minute amounts of the labeled drug, but it also enables one to follow the metabolic products of the parent
compound. Furthermore, the method enables one to distinguish between unchanged digitoxin

**LYOPHILIZED RADIOACTIVE URINE**

1. dissolve 2 Gm. in 60 ml. 50 per cent ethanol
2. 2 mg. normal digitoxin added as carrier
3. 120 ml. CHCl₃ added and thoroughly shaken—repeat with two more portions of fresh CHCl₃

<table>
<thead>
<tr>
<th>Chloroform</th>
<th>Ethanol</th>
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<tbody>
<tr>
<td>1. evaporate to small volume</td>
<td>1. evaporate to small volume</td>
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<tr>
<td>2. “plate” 1⁄₁₀ aliquot</td>
<td>2. “plate” aliquot</td>
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<tr>
<td>3. evaporate rest to dryness</td>
<td>on planchet</td>
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<tr>
<td>4. dissolve residue in 50% ethanol</td>
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<td>5. extract with carbon tetrachloride</td>
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<table>
<thead>
<tr>
<th>50% Ethanol</th>
<th>Carbon tetrachloride</th>
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<tr>
<td>1. extract with chloroform</td>
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<tr>
<td>2. “plate”</td>
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<thead>
<tr>
<th>Chloroform</th>
<th>Ethanol</th>
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<tr>
<td>1. evaporate to dryness</td>
<td>1. evaporate to small volume</td>
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<tr>
<td>2. dissolve residue in small amount chloroform and “plate”</td>
<td></td>
</tr>
<tr>
<td>3. filter through alumina column</td>
<td></td>
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<tr>
<td>4. pass following solvents through column</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Chloroform</th>
<th>1% Ethanol in chloroform</th>
<th>10% Ethanol in chloroform</th>
<th>95% Ethanol in chloroform</th>
<th>25% Ethanol in chloroform</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. evaporate each eluate fraction to small volume</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>2. plate each fraction</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>3. determine radioactivity of all “plated” fractions</td>
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</table>

**Fig. 1. Flow-sheet of extraction procedure**

and other possible cardioactive principles. By the tracer method it is possible to assay as little as 0.02 microgram of radioactive digitoxin.

The present communication is in the nature of a preliminary report. Results presented herein indicate that the major route of elimination of digitoxin in human subjects with cardiac failure is through the renal system.

**METHOD**

Three patients with heart disease were selected for this study on the basis of their willingness and ability to cooperate. All three subjects had arteriosclerotic heart disease with congestive failure of varying degrees of severity. Subjects E. W., a 58 year old male weighing 67.6 Kg., and E. S., a 71 year old male weighing 61.5 Kg., had auricular fibrillation. Subject O. L., a 65 year old woman weighing 54.2 Kg., had a sinus rhythm. Throughout the period of study all patients were hospitalized in the metabolic ward of Billings Hospital where a constant diet and a constant intake of distilled water was administered. Records of the basal pulse rate, both apical and radial, and body weight were made each morning with the patient in the post-absorptive state. Prior to the administration of the labeled digitoxin, digitalis was withheld for varying lengths of time. In the case of O. L. this period was six days, for E. W. 14 days, and for E. S. 34 days.

Subjects O. L. and E. W. were then given a single intravenous dose of 0.5 mg. of the labeled digitoxin and subject E. S. a dose of 1.5 mg. Twenty-four hour urine samples were collected during the period of observation which varied for each subject. For O. L. this period was 12 days, for E. W. 23 days and for E. S. 84 days. Urine samples were stored under refrigeration during the 24 hour collection period and then lyophilized by the freeze-dry method. During the period of observation in E. W. no cardiac glycosides or diuretic agents were given. In the case of O. L., 0.5 mg. of digitoxin (Eli Lilly) was administered four days after giving the labeled digitoxin, and 15 days later a mercurial diuretic was administered as symptoms and signs of congestive failure supervened. Subject E. S., who received the largest dose of radioactive digitoxin, was given 1.5 mg. of digitoxin (Eli Lilly) 29 days after receiving the labeled dose. Electrocardiograms were taken at one to three day intervals.

The radioactive drug administered to the patients was prepared by biosynthesis with *digitaria purpurea* plants exposed to an atmosphere of carbon¹⁴ dioxide. Extraction and purification of the glycoside was performed by initial solvent extractions followed by the use of chromatographic methods for the ultimate isolation of digitoxin as a single entity.¹² Specific activity of two batches of the labeled drug used in this study was 525,000 counts per minute per milligram or 0.364 microcurie per milligram and 620,000 counts per minute per milligram or 0.430 microcurie per milligram.

The extraction procedure employed for the isola-
tion of unchanged digoxin and fractionation of its metabolic products from lyophilized urine of cardiac patients receiving uniformly labeled carbon$^{14}$-digoxin is shown on the flow-sheet in figure 1. Radioactivity determinations of the various extracted fractions were made by an internal gas-flow Geiger counter. $^3$ Self-absorption, dilution and background corrections, was made on the counting data. Using a known amount of radioactive digoxin as a control and subjecting it to the extraction procedure described in figure 1, recoveries of 97 ± 2 per cent have been obtained.

The various radioactive fractions extracted from the urine of cardiac patients were divided into three categories: "unchanged" digoxin, chloroform soluble metabolites, and water soluble metabolites. "Unchanged" digoxin was found in the 10 per cent ethanol in chloroform eluate. The word "unchanged" is placed in quotation marks since conventional identification and characterization methods could not be employed for the compound due to the minute amount of drug recovered from the urine. The chloroform soluble metabolites occurred in all the various fractions obtained from the original chloroform soluble residue other than the 10 per cent ethanol in chloroform eluate. The water soluble metabolites occurred in the original 50 per cent aqueous ethanol solution. The significance of the chloroform soluble metabolites lies in the fact that they may be closely related in structure to the parent compound, while the water soluble metabolites are all probability conversion products of digoxin. It should be noted that the metabolic compounds were not isolated and characterized as single entities but only fractionated according to their solubility properties and chromatographic behavior on an alumina column.

Several tests were used in the identification of the "unchanged" digoxin recovered from the urine of cardiac patients. Comparison of $R_f$ values of the radioactive drug with nonradioactive crystalline digoxin (Lilly) by paper partition chromatography $^{32}$ indicated that the two compounds were identical. Also polarographic analysis of the reisolated digoxin in 50 per cent ethanol gave a half-wave potential of −1.95 volts which is within the published range of −1.934 to −1.988 volts. $^{14}$ Color reaction tests with a p-dimethylaminobenzaldehyde reagent gave a specific blue color for both the radioactive drug and normal digoxin. The use of the isotope dilution method for determining a constant specific activity of a compound also confirmed the presence of digoxin in the 10 per cent ethanol in chloroform eluate.

**RESULTS**

**Clinical Effects**

In subjects E. W. and E. S., who were fibrillating, the basal pulse rate responded to the administration of the radioactive digoxin in a manner very similar to that effected by the nonradioactive digoxin. Figure 2 shows the basal pulse rate record of E. S. Subject O. L. had no significant effect on the basal pulse rate. The serial electrocardiograms in subjects E. W. and E. S. reflected the effect of the administered doses of digitoxin by a slowing of the ventricular rate but with only insignificant changes in the S-T segments and T waves. O. L.'s serial electrocardiograms responded to the radioactive preparation and to the nonradioactive drug by exhibiting a temporary partial heart block and numerous ventricular premature systoles.

![Figure 2: Basal pulse rate of subject E.S. (1) Digitalis discontinued; (2) 1.5 mg. C$^{14}$ digitoxin I.V.; (3) 1.5 mg. nonradioactive digitoxin (Eli Lilly) I.V.](image)

**Experimental Effects**

The daily renal excretion rates of "unchanged" digoxin and its metabolic products for the three cardiac subjects who received a single intravenous dose of radioactive digitoxin are shown in figures 3 and 4.

The term "microgram equivalent" noted in the charts is used to express the amount of original drug converted into the metabolic products, that is, if 1 microgram of radioactive digitoxin has a certain number of disintegrations per minute and if a metabolic product of the drug contains the same number of disintegrations per minute, then the metabolite is considered to amount to 1 "microgram equivalent." It should be stressed that the term "microgram equivalent" does not express the actual amount of the metabolic product but only the equivalent amount of C$^{14}$ converted from the parent compound.
1. Persistence

It can be seen from results shown in figures 3 and 4 that the injected radioactive digitoxin subjects O. L. and E. W. were not of sufficient duration, extrapolation of the excretion curves indicates that the drug persists in the body

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\text{Data showing the persistence of the drug in the three subjects are summarized in table 1. Although the experimental periods for both for about 31 to 42 days. Subject E. S., who was studied the longest and received the largest dose, was still excreting "unchanged" digitoxin in the urine up to the fortieth day}
\]
after administration of the drug, while it’s radioactive metabolic products were detected up to the seventy-fourth day.

2. Excretion Gradient

The relatively rapid elimination of the cardiac glycoside during the first three days after its administration is noteworthy. As illustrated by the curves in figures 3 and 4, there is a very rapid initial loss during the first three days followed by a gradual leveling off after the seventh and eighth days. During the initial three-day period, 20 per cent of the administered dose is eliminated with about half of this amount being excreted during the first 24 hours. In the case of the "unchanged" digitoxin there is approximately five times as much drug eliminated during the first 24 hour period as there is during the second 24 hour period. Analyses of the first 24 hour urine samples at six hour intervals show that most of the "unchanged" digitoxin eliminated during the first day is excreted during the initial six hours.

3. Comparison between "Unchanged" Digitoxin and its Metabolites

Another significant finding was the relatively small amount of "unchanged" digitoxin eliminated through the renal route in comparison with the larger amount of metabolic products. (See table 2.) Although experimental periods for both O. L. and E. W. were not of sufficient duration, extrapolation of their excretion curves indicate that less than 9 per cent of the digitoxin would have been excreted unchanged.

**Table 1.—Length of Time Required for Excretion of Various Radioactive Compounds after Single Administration of C14-Digitoxin**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dose (mg.)</th>
<th>No. of Experimental Days</th>
<th>Excretion Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. L.</td>
<td>0.5</td>
<td>12</td>
<td>&quot;Unchanged&quot; Digitoxin</td>
</tr>
<tr>
<td>E. W.</td>
<td>0.5</td>
<td>23</td>
<td>*27 ± 2 days</td>
</tr>
<tr>
<td>E. S.</td>
<td>1.5</td>
<td>85</td>
<td>†40–50 days</td>
</tr>
</tbody>
</table>

* Figures estimated by extrapolation of excretion curves.
† Urine of intermediate days not collected.

**Table 2.—Amount of Various Radioactive Compounds Excreted during Experimental Period after Single Administration of C14-Digitoxin**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dose (mg.)</th>
<th>No. of Experimental Days</th>
<th>Excretion Products</th>
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<tbody>
<tr>
<td>O. L.</td>
<td>0.5</td>
<td>12</td>
<td>&quot;Unchanged&quot; Digitoxin</td>
</tr>
<tr>
<td>E. W.</td>
<td>0.5</td>
<td>23</td>
<td>5.6 ± 0.10 28.3 ± 0.53.9 ± 0.1419.6 ± 0.733.6 ± 1.5</td>
</tr>
<tr>
<td>E. S.</td>
<td>1.5</td>
<td>85</td>
<td>6.8 ± 0.11 34.4 ± 0.53.9 ± 0.1419.5 ± 0.768.1 ± 3</td>
</tr>
</tbody>
</table>

* µg. equivalent of C14-digitoxin.
† Figure indicates amount excreted up to fortieth day, urine not collected daily thereafter.
the main excretory route for the elimination of digitoxin and its metabolic end products.

4. Excretion Ratio between Metabolites and Digitoxin

In figure 5 is illustrated the metabolite-digitoxin ratio curve as calculated from the cumulative excretion rates at various intervals after the single administration of digitoxin. It will be noted that the metabolite-digitoxin ratio of the two subjects who received 0.5 mg. of digitoxin increases much more rapidly on successive days than it does for the subject who received a dose three times as large. This seems to indicate that over a given period there is a greater percentage conversion of digitoxin to its metabolic products with a small dose than with a larger one.

It is also interesting to note that during the first 24 hours the metabolite-digitoxin ratio is relatively low, whereas with an increase in the time interval there is an increase in the ratio.

**Discussion**

The renal excretion rate of uniformly labeled carbon\(^{14}\)-digitoxin and its metabolic products in human subjects with cardiac insufficiency has been studied using the extremely sensitive isotope tracer technic.

Contrary to the concept held by many of the earlier investigators\(^3, 4, 15, 16\) the major route of excretion of digitoxin in human beings seems to be through the kidneys and not by way of the liver and gastrointestinal tract. This is supported by the fact that approximately 60 to 80 per cent of the administered dose is eliminated through this route either in the form of “unchanged” digitoxin or its metabolic products.

However, in some animals, the major route of digitoxin excretion seems to be through the gastrointestinal tract. Recently, Fischer and co-workers\(^1\) have reported that rats (digitoxin-resistant animals) excrete most of the drug through the gastrointestinal tract, while cats (digitoxin sensitive animals) excrete it about equally between the renal and gastrointestinal system. In light of these facts and those of the present investigation, there is the possibility that the more sensitive an animal species is to digitoxin the more likely it will be to excrete a larger portion of the drug through the kidneys.

Probably the main reason why many of the earlier investigators placed so little importance on the kidneys as the major route of excretion is attributable to the fact that they had no way of measuring the large amount of metabolic products excreted in the urine. Our data indicate that 52 to 72 per cent of the original drug was excreted as either chloroform or water soluble metabolites. Only 6 to 10 per cent of the drug was excreted as “unchanged” digitoxin.

In 1919, Pardee\(^17, 18\) reported that the body was able to excrete a uniform amount of the drug daily which did not depend upon the quantity in the body. Gold in 1923\(^15\) and more recently Friedman and his associates\(^8\) presented evidence that this is not the case and reported that the amount excreted daily was dependent upon the amount in the body. Our results confirm the findings of Gold and of Friedman.

Schmiedeberg in 1883\(^19\) theorized that the prolonged effect of digitoxin was due to the storage of the drug in the tissues of the body and to its slow excretion. Since the work of Hatcher in 1912\(^20\) this has been generally accepted, although there has been no incontrovertible evidence of the persistence of the drug in the body until the recent work of Friedman and his co-workers.\(^8\) This group reported that a “digitalizing” dose of digitoxin...
toxin persisted in the body from 12 to 24 days after a single administration of the glycoside. Our studies indicate that unchanged drug is still excreted in the urine up to 40 days after an intravenous administration of 1.5 mg. of the radioactive drug. Degradation products of digitoxin were detected up to the seventy fourth day. However, the significance of the excretion of metabolites after the fortieth day is difficult to evaluate since it is possible that the radioactivity in the metabolites may come from compounds resynthesized from the one- and two-carbon fragment pool.

To elucidate this point further, urea was isolated from urine of patients receiving digitoxin and recrystallized a minimum of eight times. With an ionization chamber as the method of assay, urea samples from all three patients showed radioactivity. Specific activity of the urea ranged from 7 dps to 10 dps per gram of sample. Recently Hellman and Eidinoff cited evidence that the carbon of urea is derived from the carbon dioxide pool, which is chiefly in the form of carbonic acid. This would therefore suggest that at least part of the digitoxin molecule is broken down into one-carbon fragments, permitting the resynthesis of various biochemical compounds. However, due to the extremely low specific activity of the urea, its radioactivity could not be detected with a windowless gas flow geiger counter. It will be recalled that all the metabolic products mentioned previously were detected with this type of counting device. Therefore, it would seem reasonable to believe that since urea could not be detected by the latter method, compounds resynthesized from one- and two-carbon fragments would also be undetected due to their extremely low specific activity. For this reason it is believed that since the metabolic products cited in our data are of sufficient specific activity, they may be conversion products of carbon 14 digitoxin and not necessarily compounds resynthesized from the body carbon dioxide pool.

The nature of the prolonged retention of the glycoside in the body is not apparent from the data. Such investigators as H. Fischer, Lendle and others have cited the ability of tissue proteins to bind cardiac glycosides. It is highly possible that there may be various types of tissue proteins to which the drug can bind reversibly, with some proteins binding the glycoside more tenaciously than others. This persistence also adds support to the concept of cumulative action of digitoxin as observed by clinicians.

Little is known as yet concerning the nature of the metabolic end products. Due to the minute amount of radioactive metabolites excreted in the urine it is not possible at the present time to chemically identify and characterize the metabolites. However, biologic tests will be made on the metabolites to test them for their cardiotonic activity.

Presently further investigation on the renal excretion of digitoxin is being conducted on a larger sampling of cardiac patients.

**SUMMARY**

1. Using the isotope tracer technic, a quantitative study was made of the renal excretion of intravenously administered radioactive digitoxin in three human subjects suffering from cardiac insufficiency.

2. The elimination of approximately 60 to 80 per cent of an administered dose through the kidneys suggests that the major route of elimination of digitoxin in cardiac patients is through this route.

3. There is a very marked initial excretion of digitoxin during the first two days after administration of the glycoside followed by a gradual leveling off of the excretion gradient after about the fifth day.

4. A minute amount of “unchanged” digitoxin is detected in the urine for as long as 40 days after administration of a single dose of radioactive digitoxin, while carbon14-labeled compounds can be detected up to the seventy-fourth day.

5. Most of the carbon14 from the labeled drug is eliminated as metabolic products while only 6 to 10 per cent of the original drug is excreted as “unchanged” digitoxin.

6. The cumulative effect of digitoxin appears to be related to its persistence in the body.

**ACKNOWLEDGMENTS**

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SUMARIO ESPAÑOL

Digitoxina rotulada con C$^{14}$ fue usada en un estudio cuantitativo de excreción renal de digitoxina no alterada y sus productos metabólicos en tres sujetos humanos con insuficiencia cardíaca. La eliminación de aproximadamente 60 a 80 por ciento de la dosis administrada por medio del riñón sugiere que la ruta mayor de eliminación de la digitoxina en pacientes cardíacos es por el vía urinaria. Hay una marcada excreción de digitoxina durante los dos primeros días después de administración de la droga radioactiva seguido de una nivelación gradual de la pendiente de excreción más largo. Cantidades minutas de digitoxina no alterada han sido descubiertas en la orina hasta el cuadragésimo día después de la administración de una sola dosis del glicósido, mientras que substancias rotuladas con C$^{14}$ fueron percibidas hasta el día septuagésimo cuarto.

REFERENCES

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