Fate of Orally Administered $^3$H-Digitoxin in Man with Special Reference to the Absorption

By BJÖRN BEERMANN, M.D., KJELL HELLSTRÖM, M.D., AND ANDERS ROSÉN, M.D.

SUMMARY
$^3$H-digitoxin and polyethylene glycol (nonabsorbable marker) were given orally to five subjects provided with a gastrointestinal tube. Of the radioactivity administered, approximately 15, 30, and 70% had been absorbed when the test solution was passing the stomach, the duodenum, and the upper jejunum, respectively. The absorption was rapid, and the plasma contained detectable amounts of radioactivity after 5 minutes. There was no evidence of a disposition of $^3$H-digitoxin in intestinal aspirates. On a sixth subject labeled digitoxin was instilled in the mid-jejunum. The concentration of label in the plasma and the pattern of elimination of radioactivity in the urine and the feces in this subject indicated that the absorption of digitoxin was effective also in the more distal part of the gut. The combined results from the present study indicate that the absorption of orally administered digitoxin is complete.

The biliary excretion of label 4 to 24 hours after the start of the experiments was calculated from the bilirubin turnover and the mean radioactivity per mg bilirubin in duodenal aspirates obtained after intravenous injections of cholecystokinin. Less than 10% of the administered dose appeared to be excreted with the bile.

The radioactivity recovered in plasma showed less decomposition than that found in duodenal bile and urine.

Additional Indexing Words:
Digitoxin Cardiac glycosides Gastrointestinal absorption Biliary excretion

The gastrointestinal absorption of digitoxin is considered to be very effective since the oral and parenteral dose required for therapeutic effects is about the same. By such calculations the absorption of digoxin has been estimated to approximate 70%. Studies in animals have demonstrated that digitoxin and digoxin participate in an enterohepatic circulation. It is possible that gastric and intestinal diseases may interfere with the absorption of not only the orally administered glycosides but also of the glycosides and their metabolites that are excreted with the bile. Goldfinger et al. recently reported that the plasma concentration of digoxin is significantly lower in patients with malabsorption. This observation underlines the importance of a more extensive knowledge of the gastrointestinal absorption and the metabolic fate of orally administered digitalis glycosides in healthy and diseased subjects.

The main purpose of the present investigation was to study which parts of the gastrointestinal tract are involved in the absorption of orally administered digitoxin in healthy subjects. An attempt was also made to evaluate to what extent digitoxin participates in the enterohepatic circulation.

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Experimental

Table 1

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Subject</th>
<th>Administration</th>
<th>Collection of feces and urine (days)</th>
<th>GI aspiration: distances from nose (cm)</th>
<th>Injection of cholecystokinin: time after administration of label (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E.M.</td>
<td>Oral</td>
<td>21</td>
<td>80</td>
<td>1.2, 5, 4, 7, 10, 24</td>
</tr>
<tr>
<td>2</td>
<td>H.L.</td>
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<td>16</td>
<td>80, 115</td>
<td>4, 10, 24</td>
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<tr>
<td>3</td>
<td>R.W.</td>
<td>Oral</td>
<td>15</td>
<td>50, 80, 115</td>
<td>4, 7, 10, 24, 72</td>
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<tr>
<td>4</td>
<td>K.A.</td>
<td>Oral</td>
<td>21</td>
<td>50, 80, 115</td>
<td>4, 7, 10, 24</td>
</tr>
<tr>
<td>5</td>
<td>S.J.</td>
<td>Oral</td>
<td>21</td>
<td>50, 115</td>
<td>4, 7, 10, 24</td>
</tr>
<tr>
<td>6</td>
<td>S.O.</td>
<td>Intrajejunal</td>
<td>21</td>
<td>—</td>
<td>—</td>
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<tr>
<td>7</td>
<td>S.S.</td>
<td>Intravenous</td>
<td>21</td>
<td>50, 80</td>
<td>1.2, 5, 4, 24</td>
</tr>
</tbody>
</table>

Methods

Experimental Procedures

Healthy male transport workers, 45 to 51 years of age, volunteered for the investigation.

$^3$H-digoxin was given orally to five subjects, by intrajejunal instillation to a sixth, and intravenously to a seventh (Table 1). The oral dose ($50 \mu$Ci) was dissolved in 50 ml water together with 0.1 mg nonlabeled digitoxin and 5 g polyethylene glycol (PEG, nonabsorbable marker). The intake of the test solution was immediately followed by 100 ml water. When instilled intrajejunally, the same amount of labeled and nonlabeled digitoxin was dissolved in 10 ml 5.5% glucose and injected as a bolus. The intravenous dose (3.5 $\mu$Ci mixed with 0.1 mg nonlabeled digitoxin) was dissolved in 3.5 ml 11% ethanol. The experiments were started in the morning before breakfast, with the subjects having fasted overnight.

Before the experiments the subjects were provided with an intestinal double-lumen tube that was inserted through the nose and allowed to pass to the desired level. The position of the tube was controlled by X-ray. Gastric aspirates were obtained through a separate tube.

Gastrointestinal aspirates were drawn at various time intervals during 24 hours and analyzed for total radioactivity, PEG, and bilirubin. In some instances the label in chloroform extracts of the aspirates was fractionated by paper chromatography. To obtain concentrated bile, 37.5 Ivy units of cholecystokinin were injected intravenously on several occasions (Table 1).

Blood samples were drawn intermittently, and the urine and feces collected continuously for 15, 16, or 21 days (Table 1). Total radioactivity was determined in all samples. The label in some specimens of urine and blood was fractionated in a manner similar to that of the gastrointestinal aspirates.

Material*

$^3$H-Digoxin (specific activity 4.5 Ci/m mole according to the manufacturer), the radioactivity randomly distributed, was purified on the day before each experiment by thin-layer chromatography on glass plates (20 x 20 cm) covered with a layer (250 $\mu$) of silica gel. Ethyl-acetate-butanol (90:10, v/v) was used as the solvent system. The label with the same RF-value as that of unlabeled digitoxin was recovered by subsequent extraction with ethanol of the gel that had been scraped off the plate. On refractionation by descending paper chromatography (see below), more than 99% of the label accumulated within the same spot as unlabeled digitoxin.

Determination of Radioactivity

All determinations of radioactivity were performed with a liquid scintillator (model 3003†). Plasma samples (1 ml) were dissolved in 15 ml of an emulsifier (Insta-Gel†). Aliquots of gastrointestinal aspirates (0.1 ml) and urine (1 ml) were pipetted into 15 ml of the scintillation liquid, as

*The $^3$H-Digoxin was obtained from the New England Nuclear Corp., Boston, Massachusetts. Unlabeled digitoxin and digoxin were supplied by ACO Läkemedel, Stockholm, Sweden. Digitoxigenin and digoxigenin were gifts from Dr. Wartburg, Sandoz A.G., Basel, Switzerland. Cholecystokinin was obtained from the GIH research group, Chemistry Department, Karolinska Institutet, Stockholm. Polyethylene glycol (PEG, mol wt 4,000) was purchased from Kebo A.B., Stockholm. Bilirubin (used for the determination of the bilirubin turnover) was obtained from ACO Läkemedel, Stockholm. $\beta$-glucuronidase (Helix pomatia) and bacterial $\beta$-glucuronidase were supplied by Sigma Company, St. Louis, Missouri, and Mylase P by Nutritional Biochemicals, Cleveland, Ohio. Silicic acid (Silicar, 100-200 mesh) was manufactured by Mallinckrodt Chemical Works, St. Louis, Missouri.

†Packard Instrument Co., Downers Grove, Illinois.
described by Bray. To study the possible presence of volatile label, aliquots of gastrointestinal aspirates were analyzed in the same way after being evaporated to dryness at 45°C and redissolved in water. Feces were homogenized and lyophilized. Aliquots of the dry powder were analyzed for radioactivity by means of a modified Schöninger combustion technic. Quenching was corrected for by internal standardization.

**3H-Digitoxin and Labeled Metabolites**

Aliquots of plasma and gastrointestinal aspirates were extracted for 30 min with 30 volumes chloroform-methanol (2:1, v/v) at reflux temperature. The insoluble material was removed by filtration, and the water-methanol phase was re-extracted with 15 volumes chloroform. The combined chloroform extract was assessed for radioactivity, evaporated to dryness under vacuum, redissolved in 1 ml 20% benzene in chloroform, and applied on a silicic acid column (10 g silicic acid/g organic extract). The column (inner diameter 10 mm, height 32 mm per g silicic acid) was eluted (30 ml of each solvent/g silicic acid) with (a) 20% benzene in chloroform, (b) 20% ethanol in chloroform, and (c) 75% ethanol. In control experiments a mean of 98% of 3H-digitoxin added to nonradioactive serum samples was recovered in fraction b. The label in this fraction was submitted to descending paper chromatography with methylisobutyl ketone-diisopropyl ether-methanol (80:20:10, v/v) as the solvent system. The paper (30 × 400 mm Whatman no. 1) had been soaked in 25% formamide in acetone and saturated over the mobile phase for 12 hours before the start of the chromatography. Carrier digitoxin (40 μg) was added to each sample to be spotted on the paper. Digitoxin, digoxin, digitoxigenin, and digoxigenin were run on a parallel strip, the Rf-values being 0.65, 0.34, 0.76, and 0.56, respectively. The spots of the reference substances were made visible by spraying the paper with Kedde's reagents (2 g 2,5-dinitrobenzoic acid, 100 ml 2M KOH, and 100 ml methanol). The paper strip was then cut so that the label in a segment with an Rf-value identical with that of a reference substance could be determined separately. These segments, as well as the intermediate ones and other parts of the paper strip, were then analyzed for radioactivity with the combustion technic mentioned above. Aliquots of urine were extracted three times with 1.5 volumes of chloroform. Ninety-nine percent of the 3H-digitoxin added to nonradioactive urinary specimens was recovered in the chloroform phase. The combined chloroform extract was analyzed for radioactivity and submitted to the same type of descending paper chromatography as described above. Other aliquots of urine were mixed with 0.2M acetate buffer pH 4.2 (urine: buffer: 1:3 v/v) and β-glucuronidase (Helix pomatia, 500 Fischer units per ml urine) and incubated for 16 hours in air. Incubation of Mylase P (10 mg/ml urine, 0.125M acetate buffer, pH 6.0) and bacterial β-glucuronidase (1.5 mg/ml urine, 0.125M acetate buffer, pH 6.8) was performed in some experiments in which the ratio of urine to buffer was 1:4, v/v. The label in the incubated specimens was fractionated by extraction with chloroform.

**Absorption of 3H-Digitoxin in Stomach and Upper Part of Small Intestine**

The uptake of radioactivity was calculated by estimating the following ratio (A/T ratio):

\[
\text{radioactivity per mg PEG in the aspirates} / \text{radioactivity per mg PEG in the test solution}
\]

The A/T ratios were calculated only for aspirates in which the concentration of PEG exceeded

<table>
<thead>
<tr>
<th>Table 2</th>
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<tbody>
<tr>
<td><strong>GI aspiration: distance from nose (cm)</strong></td>
</tr>
<tr>
<td>50</td>
</tr>
<tr>
<td>50</td>
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<tr>
<td>50</td>
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<tr>
<td>80</td>
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<tr>
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<tr>
<td>115</td>
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<td>115</td>
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<tr>
<td>115</td>
</tr>
</tbody>
</table>
ABSORPTION OF ³H-DIGITOXIN

A:

\[ \frac{A}{T} \]

0.6

0.2

PGE

PEG; 50.000 ± 20.000

Radioactivity (cpm/ml) and PEG (mg/ml) in aspirates from the stomach (50 cm), the duodenum (80 cm), and the proximal jejunum (115 cm from the nose) after oral administration of ³H-digitoxin and PEG (lower section). The ratios between radioactivity per mg PEG of the aspirates and that of the test solution = \( \frac{A}{T} \) ratios (upper section). Experiment 3.

1 mg/ml. PEG was analyzed as described by Hydén.\(^1\)

**Biliary Excretion of Label**

An attempt was made to determine the amount of label excreted with the bile. The mean radioactivity/mg bilirubin during the time of observation was calculated planimetrically for specimens of duodenal bile collected after injection of cholecystokinin. The biliary excretion of label was estimated as the mean radioactivity per mg bilirubin \( \times \) the bilirubin turnover. Bilirubin was measured according to the method of Nosslin.\(^1\)

The bilirubin turnover was determined as described by Engstedt et al.\(^1\)

**Results**

**Oral Administration**

**Absorption of Label from Stomach and Upper Part of Small Intestine**

In all series of gastric and small intestinal aspirates, the amount of radioactivity per mg PEG was less than that of the test solution (table 2). In gastric aspirates collected for up to 30 min from three subjects, the mean A/T ratios ranged between 0.78 and 0.86. In duodenal aspirates the mean A/T ratios recorded for four subjects amounted to 0.62 to 0.77. The lowest A/T ratios (0.16 to 0.42) were found in the aspirates that were drawn from the proximal jejunum of four subjects. The A/T ratios, as well as the concentration of label and PEG in aspirates at various levels from subject R.W., are shown in figure 1.

Some duodenal aspirates, obtained when the major part of the radioactivity and PEG was passing, were analyzed with regard to content of "³H-digitoxin." All radioactivity was extractable with chloroform; 97 to 98% of the label from the paper chromatograms had the same Rf-value as that of unlabeled digitoxin.

**Plasma Level of Label**

The radioactivity in plasma reached a maximal level 20 to 45 min after the

\[ \text{figure 2} \]

Radioactivity in the total plasma volume after oral (means and range limits), intrajejunal, and intravenous administration of ³H-digitoxin.
administration of the test solution (fig. 2). At that time the plasma volume contained 16 to 20% of the administered dose. The concentration of radioactivity decreased rapidly during the next few hours. When calculated from the fourth hour to the twenty-first day, the half-life of the label was approximately 13 days.

All radioactivity in serum samples collected 30 min to 15 days after the administration of the test solution could be extracted with chloroform. In one sample, collected on the twenty-first day, 15% of the label remained in the methanol-water phase. Paper chromatography of the chloroform extracts from specimens obtained within 2 days showed that more than 80% of the radioactivity was accumulated within the digoxin area. From 6 to 21 days the proportion of label with an Rf-value below digoxin became more prominent, and "3H-digitoxin" constituted approximately 55% of the label (fig. 3).

**Biliary Excretion of Label**

The major part of the test solution passed the duodenum within 30 min. In one subject who received an injection of cholecystokinin at 1 hour, the concentration of label was higher in the aspirate obtained before rather than after the stimulation of the flow of bile. After 2.5 hours, however, the same procedure resulted in more than a tenfold increase of the label, which had a concentration of 11,700 cpm/mg bilirubin. The mean concentration of radioactivity from 2.5 to 24 hours in this subject was 5,455 cpm/mg bilirubin. The bilirubin turnover was 273 mg/24 hr, and the calculated biliary excretion of label corresponded to 10.1% of the given dose. The mean radioactivity in duodenal bile collected from 4 to 24 hours in three other subjects was 7,670 (6,840 to 8,500) cpm/mg bilirubin, and the calculated biliary excretion averaged 6.9 (6.2 to 7.5)% of the label administered. For practical reasons the small intestinal tubes had to be withdrawn after 24 hours in all but one subject. On the third day specimens of this subject's duodenal bile contained 3,700 cpm/mg bilirubin.

Specimens of duodenal bile collected at 4, 7, and 24 hours from two subjects were taken for further analyses. Of the total radioactivity in the 4-hour samples, 63 and 60%, respectively, were recovered in the chloroform phase. The corresponding values at 7 hours were 41

**Figure 3**

Paper chromatographic separation of the radioactivity in plasma.

**Figure 4**

Cumulative excretion of radioactivity in urine after oral (means and range limits), intrajejunal, and intravenous administration of 3H-digitoxin.

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and 60% and at 24 hours 45 and 65%. The chloroform extracts from one of these subjects were submitted to column and paper chromatography. The amount of radioactivity (per cent of total radioactivity in the duodenal bile specimen) with the same Rf-value as that of unlabeled digitoxin amounted to 45, 35, and 34% at 4, 7, and 24 hours, respectively. Of the radioactivity on the paper chromatograms, 5 to 16% was distributed within areas which had Rf-values below that of digitoxin.

**Excretion of Radioactivity in Urine and Feces**

All urinary specimens collected during 21 days contained radioactivity. The cumulative excretion of label ranged between 22 and 36% and averaged 30%. The highest recovery was encountered within 5 days (fig. 4).

The radioactivity in urinary specimens obtained at various time intervals was fractionated by the addition of chloroform. The label extractable with chloroform ranged between 80.6 and 45.8%, the highest recovery being encountered in a single specimen collected at 6 hours (table 3). Incubation with bacterial β-glucuronidase or enzymes from *Helix pomatia* (β-glucuronidase + sulphatase) resulted in an increased recovery of chloroform-extractable radioactivity in all five specimens analyzed that were collected on the first to the fifth day (table 3). Such changes were not observed in two specimens obtained at 6 hours. Incubation with sulphatase (Mylase P, two specimens) did not consistently influence the distribution of label between the chloroform and the methanol-water phases (table 3).

The radioactivity with the same Rf-value as digitoxin constituted 53% of the total label from a urinary specimen obtained at 6 hours. The corresponding figure for urinary specimens collected from 2 to 21 days was 20 to 35%.

The cumulative excretion of label in the feces for 21 days was 23 (19 to 25)% of the given dose. The water that condensed during lyophilization of the fecal homogenates contained 0.1% of the label recovered in feces.

**Intrajejunal Instillation**

The concentration of label in plasma was lower after intrajejunal instillation than after oral administration (fig. 2); otherwise, the plasma curves were very similar in the two types of experiments. The maximal content of label was recorded at 20 min and corresponded to 12% of the 3H-digitoxin in the test solution. In specimens taken at 30 min and 21 days, 87 and 58% of the total radioactivity, respectively, accumulated within the same chromatographic segment as digitoxin.

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**Table 3**

*Chloroform Extractable Radioactivity in Urinary Specimens*

<table>
<thead>
<tr>
<th>Subject</th>
<th>Time of collection (days)</th>
<th>Urine (untreated)</th>
<th>Per cent of label extractable with CHCl₃:</th>
<th>Bacterial β-glucuronidase</th>
<th>Helix pomatia enzymes</th>
<th>Mylase P</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.W.</td>
<td>½</td>
<td>80.6</td>
<td></td>
<td>76.8</td>
<td>63.3</td>
<td></td>
</tr>
<tr>
<td>K.A.</td>
<td>½</td>
<td>65.8</td>
<td>65.3</td>
<td>76.4</td>
<td>63.3</td>
<td></td>
</tr>
<tr>
<td>R.L.</td>
<td>1</td>
<td>61.6</td>
<td></td>
<td>81.7</td>
<td>85.8</td>
<td></td>
</tr>
<tr>
<td>R.W.</td>
<td>2</td>
<td>65.4</td>
<td></td>
<td>88.3</td>
<td>56.2</td>
<td></td>
</tr>
<tr>
<td>H.L.</td>
<td>3</td>
<td>64.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.J.</td>
<td>3</td>
<td>63.1</td>
<td>87.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K.A.</td>
<td>5</td>
<td>61.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.J.</td>
<td>8</td>
<td>57.0</td>
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<tr>
<td>R.W.</td>
<td>10</td>
<td>66.0</td>
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<td></td>
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<tr>
<td>S.J.</td>
<td>12</td>
<td>45.8</td>
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<tr>
<td>R.W.</td>
<td>15</td>
<td>66.4</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*Notes:* All urinary specimens were collected during 21 days. The cumulative excretion of label ranged between 22 and 36% and averaged 30%. The highest recovery was encountered within 5 days (fig. 4).
The cumulative excretion of label in urine (fig. 4) and feces for 21 days was 32 and 27%, respectively.

Intravenous Administration

The plasma radioactivity curve is shown in figure 2. From 4 hours to 21 days the half-life of the label was 9 days. Duodenal aspirates were drawn at various times from 1 to 24 hours. The concentration of radioactivity varied between 127 and 462, the mean value being 290 cpm/mg bilirubin. The biliary excretion of label during the first 24 hours was estimated to be 4.9%. In this subject the cumulative excretion of label in urine (fig. 4) and feces amount to 43 and 26%, respectively.

Discussion

The present technic of calculating the uptake of digitoxin in the upper small intestine is based on the assumption that 3H-digitoxin does not undergo decomposition before being absorbed and that the label remains attached to the molecule. The possibility of such changes was tested by analyses of the gastrointestinal aspirates when the test solution passed the stomach and the upper small intestine. Since 97 to 98% of the label in these aspirates had the same paper chromatographic properties as digitoxin, it is most likely that the absorption of label was reflecting the uptake of the glycoside.

The absorption of digitoxin, when administered orally in a water solution, was very rapid, as indicated by the appearance of radioactivity in plasma after only 5 min, and was effective, as demonstrated by the A/T ratios at various levels in the gastrointestinal tract. Fifteen per cent of the given dose had been absorbed when the test solution was passing the stomach. The absorption continued in the duodenum and the very proximal part of the jejunum, at which level approximately 70% of the label had been taken up (fig. 5). The low excretion of radioactivity in feces during the first days of these experiments indicates that digitoxin is effectively taken up, even in the jejunum and/or the ileum. This view is further supported by the observation of the similarity in plasma concentration of label and in the fecal and urinary excretion of radioactivity after oral and intrajejunal administration. From the combined results of these studies, it appears that the absorption of orally administered digitoxin should be complete.

In the interpretations of the data concerning the radioactivity in blood, bile, urine, and feces, it is essential to consider that the original compound was labeled randomly. As much as 67% of the tritium in the molecule can be associated with the digitoxose residues and only 33% with the genin. Since tritium in a molecule may be replaced by hydrogen in the body, the total radioactivity in any tissue or excreta may represent a mixture of the label in digitoxin, its metabolites, and other substances not derived from the parent compound. The magnitude of this latter fraction was not established. The radioactivity in digitoxin was measured on paper chromatography of a chloroform extract that had passed a silicic acid column procedure which should have removed most of the nonrelevant radioactivity.

After a 4-hour period of equilibration of the label in the body, the half-life of the plasma radioactivity was about 13 days. Within 2 days about 80% of the plasma label...
showed the same chromatographic property as that of digitoxin. This fraction then decreased and was in part replaced by label with an Rf value lower than that of the glycoside. Consequently, the half-life of the “nonmetabolized” glycoside appears to be lower than that of the total label. Without considering the possibility that the specific activity may have changed with time because of an exchange of tritium for hydrogen, the half-life of digitoxin in serum was calculated to be approximately 8 days. Shorter periods of half-life have been observed by Lukas and Peterson (4 to 6 days) and Okita et al. (2 to 2.5 days). They studied patients with cardiac failure under experimental conditions that differed from the present ones. Lukas and Peterson used a double-isotope derivative assay to determine nonradioactive digitoxin and measured the half-life after cessation of treatment in two patients who had been given 0.1 mg digitoxin orally for a long time. Okita et al. administered intravenously up to 1.5 mg 14C-labeled digitoxin of very low specific activity. In the latter study the short turnover time of the label in the plasma contrasted to the half-life of 9 days observed for the labeled compounds in the urine. The total recovery of label in urine and feces collected for 21 days in the present study ranged between 53 and 69%, which is compatible with a half-life of 13 days for the label in plasma.

The high concentration of radioactivity in a series of duodenal aspirates obtained after administration of cholecystokinin, even 3 days after the start of the experiment, demonstrates an elimination of label in the bile. The present technic of calculating the biliary excretion of label quantitatively is based on some assumptions. First, the bilirubin glucuronides excreted in the bile should not enter an enterohepatic circulation. Although this may happen in the rat, the reabsorption in man appears to be small. Lester and Schmid infused conjugated 14C-labeled bilirubin into the gastrointestinal tract of two patients. Less than 5% of the administered isotope was recovered with external biliary drainage. Second, the formation of bilirubin should be approximately the same per time unit. No direct experimental data relating to this matter are available. However, this technic was used in a previous study on the gastrointestinal absorption of labeled butylscopolamine. The 24-hour biliary excretion of label was calculated to be 2%, which was in good agreement with the 0.6 to 5.7% recovered during the same period of time in the bile of patients with a bile duct drainage.

A biliary excretion of radioactivity after the administration of various labeled glycosides of digitalis to animals provided with a bile duct fistula has been reported by several authors. Okita suggests that the long half-life of digitoxin compared with that of digoxin is due to an extensive enterohepatic circulation. The importance of such a circulation was also stressed by Katzung and Meyers, who found that 39% of the label of 3H-digitoxin intravenously administered to dogs was eliminated through a bile duct fistula within 8 hours. The half-life of the plasma label in these dogs was 6 hours, whereas it amounted to 14 hours in control sham-operated animals. Katzung and Meyers suggested that aspiration of duodenal bile might be an effective way to eliminate digitoxin in an acutely intoxicated patient. However, the enterohepatic circulation of digitoxin shows large species variation and appears to be smaller in man than in dogs.

The calculated biliary excretion of label in the present subjects was in all instances less than 10%, and it seems less likely that the enterohepatic circulation is a good explanation for the long retention of digitoxin in man.

The metabolism of digitoxin has been studied by several authors. The lactone may be saturated. There is a stepwise hydrolysis of the trisaccharide portion to bidigitoxoside, monodigitoxoside, and the genin. Any of these compounds may be hydroxylated at the 12 β-position. This results in the formation of digoxin out of digitoxin. The inactive 3 α-hydroxy epimer of the genins is formed in the liver. The α-hydroxylated compounds may be conjugated with glucuronic or sulfuric acid.

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With the technic used in the present study, it was not possible to get detailed information about the metabolic pathways of the glycoside. However, the pattern of distribution of the label on solvent partition column and paper chromatography may give some hints. Digitoxin, digitoxigenin, and the nonconjugated derivatives of these compounds should accumulate in the chloroform phase during the extraction procedures employed. The radioactivity in all but one of the plasma samples and in all duodenal aspirates obtained within 1 hour was extractable with chloroform (fig. 6). The chloroform fraction of duodenal aspirates obtained on later occasions contained proportionally less radioactivity. In specimens of duodenal bile collected after injection of cholecystokinin at 7 and 24 hours, only 60% of the label was extractable with chloroform (fig. 6). Part of the labeled compound(s) in urine appeared to be conjugated with glucuronic acid. The nature of these compounds was not established. It is interesting to note that Lukas and Peterson were unable to find digitoxin glucuronide in the urine of patients treated with digitoxin.\(^{14}\)

The chloroform extract was further fractionated by paper chromatography by which digitoxin, digoxin, digitoxigenin, and digoxigenin could be separated. Because of the possible contamination of other radioactive compounds, the label within each segment represents a maximal concentration of the corresponding compound. By using this definition for identification, the amount of "nonmetabolized" digitoxin was proportionally higher in the blood than in the duodenal bile and the urine (fig. 6). The label that accumulated within the segments of digoxin, digitoxigenin, and digoxigenin in all three types of specimens varied among the subjects, although not consistently. The combined amount of label with the same Rf-value as these three metabolites ranged between 10 and 15%.

**Acknowledgment**

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