Role of the Liver in Excretion and Destruction of Digitoxin

By SHIRLEY ST. GEORGE, PH.D., RENÉ BINE, JR., M.D., AND MEYER FRIEDMAN, M.D.

The role of the liver in the excretion and destruction of digitoxin was studied in the rat, rabbit, dog and man (in part). It was found that no more than 10 per cent of a given dose of digitoxin is excreted in the bile of the animals tested and none could be detected in the bile of two human subjects. Evidence was obtained which suggested that in the rat and rabbit, the liver was able to destroy a large amount of injected digitoxin.

It has generally been assumed that because the liver excretes the major portion of administered ouabain or strophanthin it probably excretes digitoxin in the same manner. However, Hatcher and Eggleston have been the only investigators who have attempted to study the hepatic handling of digitoxin and their results were inconclusive because of the relative inadequacy of their assay method. Thus, exact quantitative knowledge of the role of the liver in the excretion or destruction of digitoxin in the animal body is completely lacking.

The embryonic duck heart preparation, however, has allowed the microassay of digitoxin in various biologic tissue and fluids including the liver. We have been able in the present study to assay digitoxin, when present, in the bile of various animals. It has been possible therefore by (1) analyses of bile as well as by (2) analyses of blood and tissues, before and after (a) bile ligation, and (b) partial hepatectomy, to determine the probable role of the liver in the elimination or destruction of digitoxin in various animals including man. The present report contains these results.

Methods

A. Physiologic Procedures

Bile was collected from the unanesthetized rat and rabbit by a method previously described. Care was taken to collect bile from the common duct of the rabbit proximal to the cystic duct. Bile was collected from the unanesthetized dog by means of a T-tube type of cannula previously inserted into the common duct.

Partial hepatectomy was performed in all species by surgical removal of 55 to 76 per cent of the liver. The excision was performed by application of a ligature to the base of each lobe to be removed, followed by excision of the lobe. Care was taken to insure the continued biliary excretion of the hepatic remnant left behind. Digitoxin was injected intravenously immediately after hepatectomy had been performed.

B. Assay of Digitoxin in Various Biologic Media

The bile content of digitoxin was assayed in the following manner. One cc. quantities of bile to which 1, 2, 4, 6, 8, 10, and 12 microgram quantities of digitoxin respectively had been added were extracted with 20 cc. of absolute alcohol by shaking for one hour. They were filtered and the filtrate evaporated to dryness at 70 C. in vacuo. The residue was taken up in 5 cc. chloroform, concentrated to one-third volume, stored at 20 C. for one-half hour, and then the supernatant fluid was decanted. It was evaporated to dryness and taken up in 20 cc. of Tyrode's solution by shaking for one hour. The average time taken for the occurrence of the "digitalis effect" was observed for each sample. In this manner, standards were established by which a bile sample containing as little as 1.0 microgram of digitoxin per cubic centimeter of bile could be assayed.

The digitoxin content of tissues and serum were assayed according to methods previously described.

Results

A. The Hepatic Excretion of Digitoxin

1) In the Rat. A small but significant fraction of digitoxin is excreted in the bile of the rat. After an average intravenous injection of 312 micrograms of digitoxin into a rat, the bile collected during the first 24 hours (see table 1)
contains 32 micrograms or about 10 per cent of the administered dose. None could be detected in the bile excreted the second day.

The above discovery of the relatively small amount of digitoxin excreted by the liver of the rat was confirmed in several different experimental studies. First, the digitoxin content of the serum of 24 rats that had been subjected to biliary obstruction was compared with that of a similar number of normal rats, 6 and 24 hours after the intravenous injection of 1.0 microgram of digitoxin per gram of body weight. After six hours the average content of digitoxin was 0.6 and 0.4 microgram per cubic centimeter of serum in the ligated and normal rats respectively. At the end of 24 hours, no digitoxin was found in the serum of either group. These observations, therefore, indicated that biliary obstruction, per se, resulted at best in only a slight retardation in the rate of disappearance of digitoxin from the blood, and probably the body also, of the rat.

The relatively small role of the biliary tract in the excretion of digitoxin was suggested also by the relatively small increase in the urinary excretion of digitoxin observed in rats, after biliary ligation. The average urine of eight ligated rats contained 11 micrograms (range: 8 to 14 micrograms) and that of normal rats, 6 micrograms (range: 4 to 10 micrograms) during the first 24 hours after injection of digitoxin. These results also show that the relatively unchanged rate of disappearance of digitoxin from the blood after biliary ligation, noted above, was not due to the substitution of renal for hepatic excretion of digitoxin.

2) In the Rabbit and Dog. Digitoxin was found in the bile of both the rabbit and dog. The bile from four rabbits during the first hour after intravenous injection of digitoxin (0.5 microgram per gram of body weight) was found to contain approximately 3 micrograms per cubic centimeter or an average total of 25 micrograms (see table 1).

The biliary excretion of digitoxin in the rabbit was studied in a second manner. A previous study had demonstrated that injected digitoxin rapidly disappeared from the heart, lung, and liver of the rabbit after administration of the drug. Therefore, these organs in both normal rabbits and those subjected to biliary ligation were analyzed for their digitoxin content one hour after its intravenous injection (0.5 microgram per gram of body weight). If biliary excretion were responsible for the observed disappearance of digitoxin from tissue, then the tissue content of digitoxin in the ligated rabbits would have been higher than that of the controls. Such was not found to be the case. The average digitoxin content of the heart, lung, and liver was 0.3, 0.5, and 0.6 microgram per gram of wet tissue respectively in the control group of six rabbits, and 0.40, 0.5, and 0.5 microgram per gram of tissue in the group of five ligated rabbits. It appeared then that the rabbit was similar to the rat in its biliary handling of digitoxin.

Biliary excretion in the dog appeared to be similar to that observed in the other two species. The average biliary excretion of digitoxin by two dogs for the first 24 hours after the intravenous injection of approximately 1375 micrograms (0.1 microgram per gram) was 163 micrograms or about 12 per cent of the administered dose.

3) In Man. Bile was obtained from two different individuals who had had biliary fistulas following gall bladder surgery. Liver function of both patients was normal. Preliminary assay indicated that if 0.5 microgram of digitoxin was added to 1.0 cc. of this bile, it could be detected. Each of the patients was given 1.2 mg. of digitoxin by vein, and then the bile was collected daily for three days and assayed. The first patient, a man of 68 years, drained 160 and 375 cc. of bile respectively the first and second days. The second patient, a man of 50 years, drained 89 and 114 cc. respectively the first and second days. No digitoxin could be detected in any of the four samples of the two individuals.

These negative findings, of course, do not indicate that the human liver excretes no digitoxin. They do suggest, however, that bile contains less than 0.5 microgram of digitoxin per cubic centimeter after injection of 1200 micrograms of the drug. Since the average person excretes approximately 500 cc. of bile per day, this suggests that an amount of digitoxin less than 250 micrograms of the 1200 micrograms
given is the maximal quantity capable of being excreted in bile.

Table 1.—Digitoxin Content of Bile after Injection of Digitoxin

<table>
<thead>
<tr>
<th>Rat</th>
<th>Amount Digitoxin Injected (pg.)</th>
<th>Bile (0-24 Hours)</th>
<th>Bile (24-48 Hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Volume (cc.)</td>
<td>Digitoxin Concentration (pg./cc.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>290</td>
<td>7.5</td>
<td>4</td>
</tr>
<tr>
<td>41</td>
<td>312</td>
<td>11.0</td>
<td>2</td>
</tr>
<tr>
<td>78</td>
<td>224</td>
<td>9.5</td>
<td>4</td>
</tr>
<tr>
<td>79</td>
<td>360</td>
<td>15.5</td>
<td>2</td>
</tr>
<tr>
<td>90a</td>
<td>365</td>
<td>17.0</td>
<td>2</td>
</tr>
<tr>
<td>91</td>
<td>333</td>
<td>17.5</td>
<td>2</td>
</tr>
<tr>
<td>99</td>
<td>300</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>312</td>
<td>13.0</td>
</tr>
</tbody>
</table>

Rabbit Bile

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Amount Digitoxin Injected (pg.)</th>
<th>Bile (0-60 Minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Volume (cc.)</td>
</tr>
<tr>
<td>1</td>
<td>1596</td>
<td>11.0</td>
</tr>
<tr>
<td>2</td>
<td>1822</td>
<td>6.5</td>
</tr>
<tr>
<td>3</td>
<td>1471</td>
<td>11.0</td>
</tr>
<tr>
<td>4</td>
<td>1053</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>1371</td>
</tr>
</tbody>
</table>

Dog Bile

<table>
<thead>
<tr>
<th>Dog</th>
<th>Amount Digitoxin Injected (pg.)</th>
<th>Bile (0-24 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Volume (cc.)</td>
</tr>
<tr>
<td>1</td>
<td>1250</td>
<td>128</td>
</tr>
<tr>
<td>2</td>
<td>1500</td>
<td>198</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>1375</td>
</tr>
</tbody>
</table>

Human Bile

<table>
<thead>
<tr>
<th>Case</th>
<th>Amount Digitoxin Injected (pg.)</th>
<th>Bile (0-24 Hours)</th>
<th>Bile (24-48 Hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Volume (cc.)</td>
<td>Digitoxin Concentration (pg./cc.)</td>
</tr>
<tr>
<td>1 A. S.</td>
<td>1200</td>
<td>160</td>
<td>N.D.</td>
</tr>
<tr>
<td>2 A. A.</td>
<td>1200</td>
<td>89</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>375</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>114</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

* None detectable.

B. The Hepatic Destruction of Digitoxin

1) In the Rat. Removal of 66 per cent of the liver of a rat leads to a marked reduction in the rate of disappearance of digitoxin from both its tissues and blood. Thus (see table 2),
(0.4 microgram per cubic centimeter) 24 hours after injection of the drug. Moreover, although there was no detectable digoxin in the heart, lung, or liver of the normal rat at this time, the heart, lung, and liver of the partially hepatectomized rat contained 0.30, 0.10, and 1.30 micrograms of digoxin per gram of tissue respectively. These results indicated that the liver either absorbed or destroyed a considerable fraction of the digoxin given to the rat.

2) In the Rabbit and Dog. Drastic subtotal hepatectomy interfered moderately but not profoundly (see table 2) with the rate of disappearance of digoxin from the tissue of the rabbit. The heart, lung, and liver tissues of six normal rabbits contained an average of 0.3, 0.5, and 0.6 microgram of digoxin per gram respectively, one hour after intravenous injection of the drug (0.5 microgram per gram of body weight). Similar tissues of five rabbits subjected to partial hepatectomy contained an average of 0.6, 1.10, and 0.9 microgram of digoxin per gram respectively. In other words, hepatectomy of this degree led to a retention of about twice as much digoxin in the tissues, one hour after injection of the drug. Here again, the results are in contrast with those following biliary obstruction alone.

Partial hepatectomy of three dogs (see table 2) did not appear to influence the rate of disappearance of digoxin from the representative tissues studied.

**DISCUSSION**

The present studies indicate that the liver of various laboratory animals is able to excrete about 10 per cent of a given dose of digoxin during the first 24 hours after its administration. No biliary excretion of digoxin, however, was observed in any animal 24 hours after injection. These observations indicate that the major pathway for the disappearance of digoxin in the animal is not by hepatic excretion of the intact, unchanged glycoside, or aglycone. Our inability to detect digoxin in the bile of either of two patients only allows us to postulate that the human subject cannot excrete more than 20 per cent of a given dose. He possibly excretes much less or none.

These results, of course, suggest that biliary

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**Table 2.—The Effect of Partial Hepatectomy on Rate of Disappearance of Digitoxin from Animal Tissues**

<table>
<thead>
<tr>
<th>No. of Animals</th>
<th>Intact Animals (Control)</th>
<th>Partially Hepatectomized Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Digoxin in Tissues (μg./Gm.)</td>
<td>No. of Animals</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>Lung</td>
</tr>
<tr>
<td>(A. Rat)†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>N.D.*</td>
<td>N.D.</td>
</tr>
<tr>
<td>Range</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>S. E. Mean</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(B. Rabbit)‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Range</td>
<td>(0.2-0.5)</td>
<td>(0.1-1.0)</td>
</tr>
<tr>
<td>(C. Dog)§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Range</td>
<td>(0.2-0.4)</td>
<td>(0.1-0.2)</td>
</tr>
</tbody>
</table>

* No Digitoxin detectable (less than 0.05 micrograms per gram).
† Each rat was given 1 microgram of digitoxin per gram of body weight and the tissues were obtained and analyzed 24 hours later.
‡ Each rabbit was given 0.5 microgram of digitoxin per gram of body weight and the tissues were obtained and analyzed one hour later.
§ Each dog was given 0.2 microgram of digitoxin per gram of body weight and the tissues were obtained and analyzed one hour later.
excretion is not the major pathway for the 
elimination of digitoxin from the blood and 
tissues of either animal or man. On the other 
hand, the liver of the rat, and probably of the 
rabbit too, appears to have some method of 
ridding the body of this drug. This was shown 
by the marked change in the rate of disapp 
pearance of digitoxin effected by partial hepa 
tectomy. It would appear that this hepatic 
process is one of destruction or alteration of 
the drug. Thus, it was found in a previous study 
that although rat liver rapidly took up, and 
stored digitoxin after its intravenous admin 
istration, the drug nevertheless could not be 
found in this organ 24 hours after its injection. 
Since this disappearance could not be accounted 
for by escape to some other tissue, by renal 
excretion, by intestinal excretion, or as shown in 
the present study, by biliary excretion, the 
conclusion appeared inescapable that a destruc 
tion or drastic alteration of the digitoxin had 
ocurred in the liver itself.

These conclusions are contrary to those 
drawn by Farah and Smusko 
wicz concerning the role of the rat’s liver in respect to stro 
phathin. These authors found that the rat’s liver 
adsorbed but apparently did not destroy stro 
phathin. The differences between their find 
ings and ours, of course, may be due not only to 
the fact that different glycosides have been 
studied, but also to the fact that theirs was an 
in vivo, and ours an in vivo study of hepatic 
glycoside relationships. Certainly to date, we 
also have not been able to detect any destruc 
tion of digitoxin by the isolated liver slice. This 
is not surprising when it is remembered that it 
is extremely doubtful if the metabolism of any 
cyclopentanophenanthrene compound proceeds 
efficiently in isolated liver slices after several 
hours.

If there appears to be a potent extrabiliary 
process in the rat liver (and to a lesser extent 
in the rabbit liver) for the destruction of digi 
toxin there seems little evidence suggesting a 
similar capacity possessed by the liver of the 
dog or of man. The latter, for example, con 
tinues to excrete via his kidneys’ remnants of 
a single small dose of digitoxin for well over 14 
days until about 40 per cent of the original 
amount is thus eliminated. It seems highly 
improbable that this very slow renal process 
would be utilized if a potent mode of hepatic 
excretion or destruction were at hand.

Summary

1) Quantitative studies were made of the 
hepatic excretion of digitoxin by the rat, rab 
bbit, dog, and man. A small but significant 
fraction of the administered drug was detected 
in the bile of each species except man.

2) Intrahepatic destruction of digitoxin was 
found to occur in the rat and rabbit but not in 
the dog.

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