Renal Tubular Transport of $^3$H-Digoxin in Saline Diuresis in Rats
Evaluation by Micropuncture

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SUMMARY We evaluated urinary excretion and tubular transport of $^3$H-digoxin by three different methods in anesthetized rats made diuretic by infusion of 2.5% saline. In one group small volumes of $^3$H-digoxin and $^{14}$C-inulin were injected simultaneously into surface proximal convolutions, and urine was collected serially from both ureters. Digoxin recovery was lower after early (62.1 ± 5.3%) than after late (86.9 ± 7.7%) proximal administration but inulin recovery was complete (99.6 ± 2.7%) after all injections. Most of the digoxin was excreted simultaneously with inulin. Delayed recovery was low. In another group of rats digoxin and inulin were applied directly to the capsule of the left kidney. Two-thirds of the recovered digoxin appeared from the left ureter and one-third from the right. The difference (41.9 ± 7.4%) is an estimate of transtubular digoxin influx. Digoxin excretion preceded inulin only on the left. Digoxin to inulin concentration ratios were 6 times higher from the left than from the right, whereas inulin recoveries from the two sides were similar. In a third group of rats tubular fluid was collected from surface convolutions of proximal and distal tubule. In the accessible segment of the proximal tubule 35.9% of the filtered digoxin was reabsorbed. In the more distal nephron, drug was added into the lumen; this resulted in a net urinary excretion of 80.2 ± 18.2%. These findings are compatible with free filtration of digoxin at the glomerulus followed by passive proximal tubular reabsorption and an influx against a concentration gradient in the distal nephron.

THERAPEUTIC and toxic effects of digoxin depend strongly on its concentration in the blood. Blood levels of digoxin, in turn, are determined by dose and schedule of administration and its physiological disposition. The primary route for elimination of digoxin as the unchanged glycoside is the kidney. Its biotransformation and biliary excretion are limited. It has been shown repeatedly that alterations in renal function cause changes in the rate of elimination of digoxin. The mechanism by which the kidney handles digoxin has been studied extensively in man and experimental animals. Clearance ratios of digoxin to creatinine were found by several investigators to be equal, or close to 1. This led to the generally accepted conclusion that digoxin is quantitatively excreted in the urine after filtration in the glomerulus. More recently, Doherty and associates used stop-flow analysis in dogs and observed digoxin to creatinine concentration ratios of less than 1 in the middle segment of the nephron. This finding suggests that there is tubular reabsorption of the drug. Steiness, on the other hand, found digoxin to inulin clearance ratios which were greater than 1 in man and which suggested an active secretory mechanism that could be inhibited by spironolactone.

In our present experiments we used micropuncture techniques to examine the renal handling of digoxin in rats with saline (2.5%)-induced diuresis, to assess more directly the site and qualitative and quantitative aspects of its renal tubular transport. The drug appears to cross the tubular epithelium in both directions; reabsorption is predominant in the proximal convoluted tubule, and there is a net transtubular influx in the more distal segments of the nephron which leads to an urinary excretion of digoxin that closely approximates its filtered load.

Methods

All experiments were performed on male Wistar rats weighing 200–400 g. The animals were anesthetized by an intraperitoneal (ip) injection of ethyl(1-methylpropyl)-malonylthiourea (Inactin), 100 mg/kg of body wt. The rats were placed on a thermostatically controlled, heated animal board to maintain body temperature at 36°C. After tracheotomy, two polyethylene cannulas (PE 10) were placed in the right external jugular vein for infusions and drug injections. All the rats were made diuretic by intravenous (iv) infusion of 2.5% saline at the rate of 4–5 ml/hr to permit rapid serial collections of urine. An interval of at least 45 minutes was allowed for equilibration before starting the experiment. The left kidney was exposed for micropuncture as described earlier. Ureters were catheterized with polyethylene tubing (PE 50) with a dead space of approximately 50 μl. The surface of the left kidney was bathed in mineral oil and illuminated by a fiber optic source. Areas of all incisions were covered with thin Parafilm to reduce fluid losses. Tubular transit times were measured after injection of 0.05–0.1 ml of a 5% solution of lissamine green through one of the jugular cannulas. Transit time was recorded as the elapsed time between the green flush on the surface of the
TUBULAR MICROINJECTIONS

Selected tubules were punctured with sharpened volumetric micropipettes with an outer tip diameter of 5–8 μm, and 20–60 nl of a solution containing 3H-digoxin and 14C-inulin were injected. Injection time varied from 60 to 100 seconds. The radioactive mixture was prepared as follows: 250 μCi of 3H-digoxin in 1.0 ml of 9:1 ethanol-benzene was evaporated and then dissolved in 1.2 ml of isotonic saline together with 50 μCi of 14C-inulin. The solution was colored with lissamine green. Specific activities of digoxin and inulin were 9.0 Ci/mmol and 2.29 mCi/g, respectively. Digoxin concentration in the mixture was approximately 18 μg/ml; thus the amount of digoxin delivered in a single microinjection was 0.4–1 ng. The injection rate was adjusted to maintain constant tubular diameter and to prevent retrograde flow. An attempt was made to administer the microinjection in at least one early and one late proximal convolution in each rat. In some, a midproximal convolution also was punctured. After the microinjection, all ureteral urine was collected. Six consecutive 180-second samples were taken from the microinjected (left) kidney. Duplicate reference standards were collected from the nonmicroinjected (right) kidney. Duplicate reference standards were prepared by measuring aliquots of the radioisotope mixture into counting vials with the micropipette which subsequently was used for tubular microinjection. A puncture was considered technically satisfactory if there was no visible leakage at the site of puncture, net inulin recovery was 95–105%, and the total amount of 14C-inulin recovered from the nonmicroinjected kidney was less than 0.5% of the amount administered.

Net digoxin recovery was calculated as follows: Percent digoxin recovery = [(digoxin U/I)/(inulin U/I)] x 100, where U = the amounts of 3H and 14C recovered in the urine and I = the injected quantities. Direct recovery, or the digoxin which was excreted with the same time course as inulin, was calculated as twice the percentage of digoxin recovered with the first 50% of the inulin. Indirect recovery of digoxin was computed as net recovery minus direct recovery. The difference between 100 and net recovery was termed unrecovered fraction. The reasons for these calculations and the interpretation of tracer microinjection data have been previously described.6–10

SURFACE APPLICATION

We evaluated transtubular influx of digoxin by placing a droplet (20–50 μl) of 3H-digoxin and 14C-inulin solution, prepared as described above, on the surface of the left kidney and studying bilateral urinary excretion patterns; 18–22 consecutive 5-minute urine samples were collected from each kidney. The duration of these experiments was taken as the elapsed time between placement of the droplet on the renal capsule and the time when inulin excretion from the left kidney had fallen to 10% of its peak rate. This allowed termination of experiments prior to complete absorption and excretion of the droplet (an extremely slow process). Recovery of digoxin was calculated as the percent of the total amount excreted by both kidneys. Excretion by glomerular filtration from the left (experimental) kidney was equated with the percent recovery from the right after correction for differences in the rate of filtration. The difference between the percent recovery from the left kidney and percent excretion by filtration was taken as an estimate of transtubular digoxin influx. In general, these experiments using surface application were similar to those previously described.6–11

TUBULAR FLUID COLLECTIONS

After administration of the appropriate priming doses the rats in these experiments received a continuous iv infusion of 3H-digoxin and 14C-inulin at rates of 40 μCi/hr and 8 μCi/hr, respectively. In addition to the surgical preparation described above, the right carotid artery also was cannulated to permit blood pressure to be recorded and to provide blood samples. Tubular fluid was collected in silicone-treated, sharpened, glass micropipettes with an outer diameter of 8–12 μm. A large oil block was injected and kept in view by controlled intermittent suction. Urine samples 15–20 minutes in duration were collected with each sample of tubular fluid. A midcollection blood sample also was obtained. The general methods for preparation, micropuncture technique, handling of samples, and calculation of data were similar to those described in previous publications.6–7

Radiochemicals were at least 98% pure as shown by the supplier (New England Nuclear Corp.). In addition, we rechromatographed our solution by paper chromatography (chloroform-formamide). As shown in Figure 1, digoxin appeared in a single peak which indicated the uniformity of the sample. We confirmed this conclusion by counting the eluted peaks for 3H in a liquid scintillation counter. All samples were analyzed in a three-channel, cooled liquid scintillation spectrometer with external standardization. Counting efficiency was 25–30% for 3H and 65–75% for 14C. Appropriate correction was made for 14C overlap into the 3H channel which always was less than 7.5%.

**FIGURE 1** Paper chromatographic separation of 3H-digoxin (chloroform-formamide).
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A. Early Proximal
B. Late Proximal

FIGURE 2. $^3$H-digoxin and $^{14}$C-inulin recovery patterns after microinjection into early (A) and late (B) surface convolutions of proximal tubule during 2.5% saline diuresis.

FIGURE 3. Cumulative $^3$H-digoxin and $^{14}$C-inulin recovery patterns following intratubular microinjection into early (A) and late (B) proximal segments of rat nephron during 2.5% saline diuresis.

Data are presented as the mean ± 1 sd. To evaluate statistical significance we used Student's $t$-test.

Results

TUBULAR MICROINJECTIONS

We performed a total of 25 microinjections of tracer in 13 rats. Urine flow in these animals averaged 24.7 ± 9.1 $\mu$l/min per 100 g of body wt, and was similar during all injections. Inulin recovery was 99.6 ± 2.7% and inulin leak was 0.8 ± 0.7%; this substantiates the fact that the renal tubule is impermeable to inulin. Recovery patterns of $^3$H-digoxin and $^{14}$C-inulin after microinjection into an early and a late proximal tubule are illustrated in Figure 2A and B; the percentage of the injected isotope recovered in each urine collection is plotted against time. Inulin recovery showed a

| Table 1: Urinary $^3$H-Digoxin Recoveries after Proximal Microinjections in Rats with Saline Diuresis |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Early proximal  | Middle proximal | Late proximal   |                 |
| Transit time (sec)  | 4.5 ± 1.0       | 8.8 ± 3.3       | 11.6 ± 3.1      |                 |
| Net digoxin recovery (%) | 62.1 ± 5.3 NS | 69.7 ± 8.6      | 86.9 ± 7.7      | <0.001          |
| Direct digoxin recovery (%) | 52.7 ± 5.8 NS | 55.4 ± 12.0     | 76.4 ± 8.4      | <0.001          |
| Indirect digoxin recovery (%) | 9.4 ± 5.4 NS | 14.3 ± 8.8      | 10.9 ± 7.2      | NS              |
| Unrecovered digoxin (%) | 37.9 ± 5.2 NS | 30.2 ± 6.6      | 13.2 ± 7.7      | <0.001          |

Values are mean ± sd; NS = not significant; $n$ = number of observations.

* Early proximal vs. middle proximal.
† Middle proximal vs. late proximal.
‡ Early proximal vs. late proximal.
longer delay, had a lower peak, and was more prolonged after early proximal injection than after late proximal injection. The digoxin recovery peak was similar to the inulin peak after late proximal injection, but was lower after early proximal administration. There was no evidence for precession of digoxin over inulin, and delayed digoxin recovery was minimal in both cases as indicated by the slight crossing over of the lines on the downslope of the curves.

The time course of cumulative digoxin and inulin recoveries from a single experiment are illustrated in Figure 3. After early proximal injection, inulin recovery was nearly complete in 5 minutes, whereas digoxin recovery reached a plateau of around 60% in 7 minutes, an indication that in this experiment about 40% of the injected digoxin left the nephron. After late proximal microinjection, both inulin and digoxin recoveries approximated 100% in about 4 minutes. These observations suggest a marked efflux of digoxin from the lumen of the proximal convoluted tubule but only small losses in the more distal segments of the nephron.

Results describing digoxin recovery from all tracer microinjection experiments are summarized in Table 1. Mean digoxin recovery was significantly lower after early (62.1 ± 5.3%) than after late (86.9 ± 7.7%) proximal injections; this finding indicates removal of a significant fraction of the luminal digoxin in the proximal nephron. Indirect recoveries were low and comparable after all injections. Most of the digoxin recovered in the urine appeared with a time course similar to that of inulin (direct recovery). As indicated in Table 1, the fraction of the digoxin that was not recovered in the urine was significantly higher after early than after late proximal microinjections.

SURFACE APPLICATIONS

To examine transtubular influx of digoxin, we placed a droplet of inulin-digoxin mixture on the capsule of the left kidney in six rats under the experimental conditions described above. The duration of these experiments averaged 55 ± 17 minutes. The time course of recovery of inulin and digoxin from the left and right kidney under these conditions is illustrated in Figure 4. Excretion patterns for inulin were similar from the left and right kidney, indicating that inulin appeared in the urine exclusively by glomerular filtration after absorption into the blood and recirculation to both kidneys. Digoxin recovery from the right (control) kidney (Fig. 4A) was similar to inulin recovery, but the urinary digoxin to inulin ratio (0.33 ± 0.08) at peak excretion was less than that of the droplet (1.2). Digoxin excretion from the left kidney (Fig. 4B) initially was significantly higher than either ipsilateral inulin or contralateral digoxin excretion. From all six experiments, urinary digoxin to inulin ratios from the left kidney averaged 1.87 ± 0.46 during peak urinary digoxin excretions. This value was significantly higher (P < 0.001) than either the ratio of 0.33 for the right kidney or 1.2 in the applied mixture.

In three experiments we examined the time course of digoxin excretion more closely by collecting urine at intervals of 0.5–1.0 minutes rather than at intervals of 5 minutes. Data from these experiments are presented in Table 2. Digoxin appeared earlier than inulin in the urine collected from the left kidney, whether expressed as time of first appearance, time of 50% recovery, or time of peak recovery. Peak digoxin recovery rate was nearly 4 times higher from the ipsilateral kidney than from the contralateral side, whereas inulin recovery rates from the two sides were similar. The results of one of these experiments are illustrated in Figure 5; counts per minute of digoxin and inulin in each sample are plotted on the ordinate and elapsed time on the abscissa. The left kidney excreted more digoxin than the right kidney. Also, digoxin excretion from the left clearly preceded inulin excretion, indicating that digoxin penetrated the renal tubule from the peritubular side.

Time courses of cumulative recovery of digoxin and its components from a single experiment are illustrated in Figure 6. Nearly 70% of the recovered digoxin appeared in the urine of the left kidney. The difference between recovery of digoxin from left and right kidneys is an estimate of transtubular digoxin influx, because net recovery from the right, corrected for differences in glomerular filtration rate.

![Figure 4](image-url)  
**Figure 4** Urinary recovery patterns of digoxin and inulin after placement of a droplet containing ¹H-digoxin and ¹⁴C-inulin on the capsule of the left kidney.
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Figure 5. Time course of digoxin and inulin recovery after surface application with short urine collection periods.

Figure 6. Patterns of cumulative $^3$H-digoxin recovery and its components from the left kidney following surface application.

(GFR), approximates filtered digoxin. In this experiment, estimated transtubular influx (40%) exceeded digoxin excretion by filtration (30%).

Data for recovery of urinary digoxin and inulin from all six experiments on surface application are summarized in Table 3. Mean values for inulin recovery from the left and right kidney were similar, whereas digoxin recovery was significantly higher from the left (68.9 ± 4.3%) than from the right (31.1 ± 4.3%; $P < 0.001$).

TUBULAR FLUID COLLECTIONS

A total of 34 collections of free-flowing tubular fluid were made in six rats with saline-induced diuresis. Simultaneous clearance data were obtained for all rats. The results are summarized in Table 4. Mean urine flow, GFR, and ratios of tubular fluid to plasma inulin concentration were similar to published observations for rats undergoing hypertonic saline diuresis.

Digoxin clearance was usually lower than inulin clearance, indicating net tubular reabsorption of digoxin. Urinary excretion of digoxin averaged 80.2% (Table 4) of the filtered load. Plasma concentration averaged 1.0 ng/ml, a value which compares favorably with usual therapeutic concentrations of this drug.

The percentages of filtered water and digoxin remaining at different sites along the nephron are shown in Figure 7. Water was reabsorbed along the entire length of the renal tubule as expected. Digoxin also was reabsorbed in the proximal convoluted tubule. At the early proximal collection site 107.1 ± 23.0% of the filtered digoxin remained, whereas at the late proximal puncture site only 71.2 ± 10.6% remained. The difference, 35.9%, represents digoxin that was reabsorbed along the proximal tubule. This value agrees well with the reabsorption of 30-40% found in the microinjection studies. Some digoxin was lost between late proximal and distal puncture sites and probably from the pars recta of the proximal tubule. In the distal nephron there was no evidence for continued digoxin reabsorption; instead, the percent of digoxin remaining increased, suggesting an addition of digoxin to the tubular fluid. However, the

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**Table 3** Urinary $^3$H-Digoxin and $^{14}C$-Inulin Recovery after Placement of the Mixture on the Surface of the Left Kidney in Six Rats with Saline Diuresis

<table>
<thead>
<tr>
<th></th>
<th>% (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inulin recovery, left kidney</td>
<td>48.1 ± 2.1</td>
</tr>
<tr>
<td>Net digoxin recovery, left kidney</td>
<td>68.9 ± 4.3</td>
</tr>
<tr>
<td>Excretion of digoxin by filtration, left kidney</td>
<td>27.4 ± 3.7</td>
</tr>
<tr>
<td>Transtubular digoxin influx, left kidney</td>
<td>41.9 ± 7.4</td>
</tr>
<tr>
<td>Inulin recovery, right kidney</td>
<td>51.9 ± 2.1</td>
</tr>
<tr>
<td>Net digoxin recovery, right kidney</td>
<td>31.1 ± 4.3</td>
</tr>
</tbody>
</table>
### TABLE 4
Summary of Clearance and Tubular Fluid Collection Data on the Renal Handling of *H-Digoxin in Rats during 2.5% Saline Diuresis

<table>
<thead>
<tr>
<th></th>
<th>No. of observations</th>
<th>Results (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine flow (µl/min per 100 g body wt)</td>
<td>34</td>
<td>30.1 ± 7.1</td>
</tr>
<tr>
<td>GFR (µl/min per 100 g body wt)</td>
<td>34</td>
<td>828 ± 148</td>
</tr>
<tr>
<td>Digoxin clearance (µl/min per 100 g body wt)</td>
<td>34</td>
<td>644 ± 110</td>
</tr>
<tr>
<td>Early proximal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inulin F/P</td>
<td>8</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Digoxin F/P</td>
<td>8</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>Late proximal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inulin F/P</td>
<td>20</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>Digoxin F/P</td>
<td>20</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Distal tubular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inulin F/P</td>
<td>6</td>
<td>7.2 ± 0.8</td>
</tr>
<tr>
<td>Digoxin F/P</td>
<td>6</td>
<td>4.5 ± 2.0</td>
</tr>
<tr>
<td>Ureteral urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inulin U/P</td>
<td>34</td>
<td>28.5 ± 7.5</td>
</tr>
<tr>
<td>Digoxin U/P</td>
<td>34</td>
<td>22.6 ± 6.5</td>
</tr>
<tr>
<td>Water excretion (%)</td>
<td>34</td>
<td>3.8 ± 1.0</td>
</tr>
<tr>
<td>Digoxin excretion (%)</td>
<td>34</td>
<td>80.2 ± 18.2</td>
</tr>
</tbody>
</table>

GFR = glomerular filtration rate; F/P = tubular fluid to plasma concentration ratio; U/P = urine to plasma concentration ratio.

increase in percent of digoxin remaining between late proximal site and the ureter was statistically significant only at the 98.5% confidence limit (P < 0.025). The increase from distal tubule to ureter reached only the 90% confidence limit, probably because of the small number of distal samples. Therefore, these observations are suggestive rather than conclusive evidence for distal tubular digoxin secretion.

### Discussion

Data obtained in the present study by tracer microinjection and collections of tubular fluid clearly show net transtubular efflux of digoxin in the proximal tubule of the rat. After early proximal injections, 37.9 ± 5.2% of the administered digoxin was not recovered in the urine. This value represents digoxin efflux along the entire length of the nephron; that is, absorption distal to early proximal puncture site. Part of this loss (13.2 ± 7.7%) occurred beyond the last accessible convolution of the proximal tubule and most likely in the thick descending segment of the loop of Henle (pars recta), since it is a continuation of the proximal tubule and is structurally and functionally similar to the pars convoluta. However, data presented here do not allow exact localization of this efflux. The difference of 24.7% represents digoxin that left the tubular lumen in the convoluted portion of the proximal tubule. Indirect recovery, that is, digoxin excreted with a different time course than that of inulin, was small (about 10%) and similar after all injections, indicating a lack of significant transient cellular uptake and surface adsorption, or a greater volume of distribution of digoxin than of inulin within the kidney. Microinjections do not mimic the conditions that exist in digitalized patients. Here, peritubular capillary digoxin concentration is negligible and provides a maximum concentration gradient favoring removal of digoxin from the tubule. Therefore, the magnitude of absorption observed in these experiments probably overestimates net proximal tubular transport of digoxin.

Experiments using tubular fluid collection more closely approximate conditions which exist after digitalization. In these experiments, 35.9% of the filtered *H-digoxin was reabsorbed in the accessible portion of the proximal tubule. The values of proximal digoxin reabsorption obtained by the two different methods are similar, and indicate that under the present experimental condition the rat proximal tubule reabsorbed about one-third of the luminal digoxin. These observations are in general agreement with the findings of Doherty et al. that digoxin can be reabsorbed by the tubes, although the methods they used did not allow an accurate localization of the site of reabsorption.

The present experiments do not provide conclusive evidence concerning the nature of the proximal tubular transport of digoxin. Tubular fluid to plasma concentration ratios of digoxin did not fall below unity; therefore, it is not necessary to postulate an active transport system. The fluid to plasma ratios tended to remain fairly low (1.3 ± 0.2) and relatively constant at all proximal puncture sites. These
observations suggest that digoxin is reabsorbed passively, perhaps by diffusion, along a concentration gradient established by the sodium transport-dependent water loss. The proximal epithelium appears to be quite permeable to digoxin, since its luminal concentrations did not rise more than 1½ times above the peritubular plasma levels.

Digoxin is a polar compound and its degree of ionization could be influenced by hydrogen ion concentration of its environment. However, the change in pH of tubular fluid along the proximal nephron is relatively small, therefore “ion trapping” is probably not a major factor in determining proximal efflux of this drug.

In the more distal segments of the nephron, digoxin apparently was added back into the tubular lumen. It may be estimated from the tubular fluid collection experiments that an amount of digoxin equal to 20% of its filtered load entered the distal nephron. Digoxin concentration was 4.5 ± 2.0 times higher in the distal tubular fluid samples than in the peritubular blood, indicating low permeability of distal epithelium to digoxin. In the surface application experiments, in which the concentration gradient was optimal for digoxin influx, the drug readily entered the tubule from the peritubular side. The mean digoxin to inulin concentration ratio in these experiments was 6 times higher in the urine of the left kidney than the right, providing strong evidence for transtubular digoxin influx. Detection of transtubular influx of substances was first reported by Chinard and Enns 11 after intravascular injection. Influx can be determined even more readily after placement of a substance on the renal surface, as described by Gottschalk and associates. 8, 11 because the circulation time from the peritubular capillaries to the glomerulus is longer than from the renal artery to the glomerulus. Accordingly, transtubular influx of a substance is disclosed by its appearance in the urine earlier than the appearance of simultaneously applied inulin which enters the urine only by filtration. The time separation, or precension, is greater if the secretion occurs in the distal rather than in the proximal tubule. In all of the surface application experiments digoxin appeared earlier and in greater quantities from the left kidney, where it was applied, than did inulin from either kidney or digoxin from the contralateral side. Precension of digoxin varied from 1.5 to 2.0 minutes, suggesting a distal site of digoxin entry. Influx accounted for one-half or more of the excreted digoxin in these experiments. Again, it should be noted that the conditions in these rats were ideal for digoxin entry, and tubular influx probably was overestimated.

The present study does not reveal the exact mechanism of transtubular digoxin influx. It is unlikely that passive diffusion could account for the high distal concentration gradient and the magnitude of influx, unless the lowered pH of the distal tubular fluid markedly increases digoxin dissociation and the ionized form becomes trapped in the lumen because permeability of the membrane to the charged moiety is low. The uncharged form would remain in equilibrium across the tubular wall. Information available for the physicochemical properties of digoxin does not allow an estimation of its dissociation at usual distal tubular fluid pH values and the contribution of “ion trapping” to its transtubular influx. The data show that digoxin can be added to the tubular fluid beyond late proximal puncture sites, perhaps in the distal nephron. An active distal secretion which can be inhibited in man by spironolactone has been suggested by Steiness. 4 Although the present investigations do not provide supportive evidence for active distal secretion of digoxin, the results are compatible with such a transport mechanism.

Results of our present study are compatible with previous observations that filtration at the glomerulus plays a primary role in the renal handling of digoxin. In these rats, the drug was freely filtrable, as indicated by the presence of the entire filtered load at early proximal puncture site. Others 1-4 have estimated that 0-30% of the digoxin may be protein-bound and this would retard its filtration. Hyperosmolar saline loading could have reduced protein binding in the present experiments.

Digoxin clearance averaged 20% less than inulin clearance and suggests the existence of experimental conditions more favorable for increased reabsorption or reduced influx than in the digitalized man, where the clearance ratio tends to be closer to unity.

In conclusion, our present investigations show that there are three renal mechanisms, filtration, reabsorption, and transtubular influx, all may play a role in the renal handling of digoxin. Its net urinary excretion depends on the magnitude of the contribution of each function and there is a general tendency to excrete an amount equal to the filtered load. Reabsorption occurs in the proximal tubule but net transtubular influx appears to be localized to the more distal nephron. If the tubular handling of the drug is similar in rat and man, our results suggest ways of improving maintenance therapy and developing methods for accelerated detoxification.

Acknowledgments

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References

Renal tubular transport of 3H-digoxin in saline diuresis in rats.
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