UREA EXCRETION IN THE NORMAL DOG DURING FORCED DIURESIS

JAMES A. SHANNON

From the Department of Physiology, New York University College of Medicine, New York

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In a previous study of the excretion of urea in normal dogs it was shown that at the highest urine flow obtainable by water diuresis forty per cent of the urea filtered through the glomeruli was reabsorbed (Shannon, 1936). The present report is concerned with urea reabsorption at high urine flows obtained by forced diuresis.

The experiments were performed upon two normal dogs which had been used in the previous study. During the period of observation they were maintained on a mixed diet with an adequate salt and vitamin content. Constant creatinine and varying glucose, sulphate and urea concentrations in the plasma were obtained by constant intravenous infusions. All experiments were conducted in a manner similar to the one in table 1. In each experiment an effort was made to include observations over a wide range of urine flows. An observation was not considered valid when the rate of urine flow was changing rapidly, though frequently observations were accepted when the urine flow was slowly increasing. Most experiments were begun with the administration by stomach tube of 40 to 50 ml. of water per kgm., and the rate of the infusion was adjusted so that water and sodium chloride were replaced in amounts approximating their loss in the urine.

Blood and urine samples were collected as described by Shannon and Fisher (1938). In the glucose experiments femoral arterial blood was used for the analyses. Minimal quantities of potassium oxalate were used as an anticoagulant. The bloods were centrifuged and the plasma precipitated immediately upon withdrawal from the animal, using the ferric sulphate-barium carbonate method of Steiner, Urban and West (1932). The urines were diluted to the U/P ratio of creatinine and precipitated by the same method. Creatinine was determined by the Folin and Wu method (1919), urea by the Van Slyke urease method (1927) and glucose by the Folin method (1929). In the glucose experiments the precautions described by Shannon and Fisher (1938) were observed in the analysis of plasma and urine creatinine.
We were fortunate in having available for this investigation two of the dogs (C and G) which had been used in our previous work. Preliminary observations in the normal range of urine flows showed that the relationship between the urea/creatinine clearance ratio and the U/P ratio of creatinine had remained unchanged in the year that had elapsed since previous examination. Our original data on these animals can, therefore, be used as a background for the presentation of our present results. In dog G we have obtained creatinine U/P ratios ranging from 700 during water deprivation to 1.75 during forced diuresis. This range in dog C is from 630 to 2.05.

A single experiment in which glucose was used as a diuretic, and which

<table>
<thead>
<tr>
<th>PERIOD</th>
<th>TIME</th>
<th>URINE FLOW</th>
<th>PLASMA</th>
<th>U/P RATIO</th>
<th>CLEARANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Creatinine</td>
<td>Urea</td>
<td>Glucose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>mgm./100 cc.</td>
<td>mgm.</td>
<td>mgm./100 cc.</td>
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<tr>
<td>0</td>
<td>0:30</td>
<td>420 cc. water by stomach tube</td>
<td>3.40</td>
<td>21.3</td>
<td>15.3</td>
</tr>
<tr>
<td>1</td>
<td>1:40-1:51</td>
<td>420 cc. water by stomach tube, 250 mgm. per kilogram creatinine subcutaneously</td>
<td>3.46</td>
<td>20.8</td>
<td>15.2</td>
</tr>
<tr>
<td>2</td>
<td>2:05</td>
<td>Infusion 6 cc. per minute 5.0 per cent glucose, 0.3 per cent creatinine</td>
<td>3.62</td>
<td>31.8</td>
<td>14.4</td>
</tr>
<tr>
<td>3</td>
<td>2:45-2:58</td>
<td>Infusion 6 cc. per minute 10.0 per cent glucose, 0.4 per cent creatinine</td>
<td>3.42</td>
<td>28.7</td>
<td>12.2</td>
</tr>
<tr>
<td>4</td>
<td>3:10</td>
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<td>3.50</td>
<td>29.4</td>
<td>12.5</td>
</tr>
<tr>
<td>5</td>
<td>3:30-3:45</td>
<td>Infusion 6 cc. per minute 20.0 per cent glucose, 0.4 per cent creatinine</td>
<td>3.50</td>
<td>29.4</td>
<td>12.5</td>
</tr>
<tr>
<td>6</td>
<td>4:00</td>
<td>Infusion 15 cc. per minute 20.0 per cent glucose, 0.16 per cent creatinine</td>
<td>13.55</td>
<td>28.2</td>
<td>10.7</td>
</tr>
<tr>
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<td>4:25-4:36</td>
<td>Infusion 15 cc. per minute 20.0 per cent glucose, 0.16 per cent creatinine</td>
<td>13.55</td>
<td>28.2</td>
<td>10.7</td>
</tr>
<tr>
<td>8</td>
<td>4:45</td>
<td>Infusion 15 cc. per minute 20.0 per cent glucose, 0.16 per cent creatinine</td>
<td>13.55</td>
<td>28.2</td>
<td>10.7</td>
</tr>
</tbody>
</table>

TABLE 1

An experiment showing the effect of extreme diuresis on the excretion of urea

Experiment 22D, May 26, 1937. Dog G, weight 17.2 kgm., S.A. 0.78 sq. m., maintained on a mixed diet. The concentration of glucose in the urine can be obtained by multiplying the plasma concentration by the U/P ratio.
follows essentially the same procedure as our experiments with sodium sulphate and urea, is given in table 1. The urea/creatinine clearance ratio (or the fraction of filtered urea which is excreted) in all experiments with forced diuresis is plotted against the log of the creatinine U/P ratio in figure 1, which includes our previous observations on these same animals.

The data divide themselves into two rectilinear or nearly rectilinear phases, one which extends from a creatinine U/P ratio of 2.0 to 10 or 20, and a second from a U/P ratio of 20 up to the highest values obtainable. Without implying any mathematical description of the data, these two phases have been emphasized in figure 1 by the two straight lines A and B.

1 It must be noted that rapid intravenous infusions, especially when the fluid is enriched with glucose or sulphate, may considerably increase the filtration rate, presumably because the plasma volume is expanded directly by the administered fluid and by drawing water from the tissues. This increase in filtration rate may be accompanied by a small increase in the urea/creatinine clearance ratio even where there is no significant change in urine flow, as shown in table 1. It is possible that
We have previously pointed out the inadequacy of a uniform process of diffusion, occurring distal to the site of water reabsorption, to explain the reabsorption of urea in the normal dog at creatinine U/P ratios above 20 (i.e., at urine flows of 0.05 to 5 cc. per minute). Our present observations lead us to extend this conclusion to creatinine U/P ratios of 2 to 20 (i.e., at urine flows of 5 to 50 cc. per minute). At a creatinine U/P ratio of 2.0, 20 per cent of the diffusion gradient, as calculated from bladder urine and blood, has already been dissipated, and yet when this ratio is increased to 20, only 46 per cent of the diffusion gradient is dissipated; if a uniform process of diffusion were involved, occurring distal to a single site of water reabsorption, the behavior of the system at a creatinine U/P ratio of 2.0 would lead us to expect that approximately 90 per cent of the diffusion gradient should be dissipated at the higher ratio. We are forced, therefore, to search for some mechanism to account for urea reabsorption which is in better agreement with the quantitative facts.

In considering the data in figure 1 there are four important points to be noted: a, the reabsorptive process is distinctly divisible into two phases, as indicated by lines A and B. b. The fact that line A extrapolates to 1.0 at a creatinine U/P ratio of 1.0 suggests that the reabsorptive process is not an active tubular process and may be due entirely to physical diffusion. c. If increased physical diffusion is held responsible for the increased reabsorption occurring between creatinine U/P ratios of 20 to 700 (phase B), then the portion of the tubules in which this reabsorption occurs must be considered to be relatively impermeable to urea, since a relatively large increase in the time available for diffusion (which is proportional to the creatinine U/P ratio) results in only a small fractional reduction in the diffusion the elevation of the filtration rate from this cause has raised the urea/creatinine clearance ratios to some undetermined but slight extent in phase A. If so, the tendency for the data obtained with urea diuresis to fall below the other data may plausibly be explained by the fact that the urea infusions do not elevate the filtration rate, presumably because urea diffuses into the tissues.

We have not included in the above description the additional and variable lowering of the clearance ratio which is observed in severe dehydration, which may be incident to the lowering of glomerular filtration that accompanies this condition.

These statements are not contradictory to those in the preceding paragraph. This moiety of urea loss, (A), is presumed to take place in the same segment as, and concurrently with water reabsorption, in consequence of the developing diffusion gradient of urea. The increase in time, in terms of the creatinine U/P ratio, available for diffusion under these conditions will be approximately half that calculated when water reabsorption occurs prior to any urea loss. The fractional dissipation of the diffusion gradient of urea in the range of creatinine U/P ratios of 2 to 10 is the expected order of magnitude if simple diffusion, under these conditions, is the mechanism of this urea loss. The data are such as to preclude closer analysis.
gradient. The portion of the tubule responsible for the reabsorption of urea at low U/P ratios (phase A) would appear to be more permeable to urea, since a small increase in the time available for diffusion leads to a relatively large fractional reduction in the diffusion gradient.

In view of the above facts, we are led by inference to relate the reabsorption of urea to the reabsorption of water. Smith (1937) has recently suggested that the reabsorption of water in the mammalian nephron is divisible into two processes; first, an obligatory process which is essentially isotonic in nature, and which accompanies or is made possible by the active reabsorption of chloride and other osmotically active constituents from the tubular urine. This process presumably occurs in the proximal segment of the tubule. Second, the facultative or variable reabsorption of water which is potentially hypertonic in nature, and which is controlled by the antidiuretic hormone of the pituitary gland. This process presumably takes place in the distal segments of the tubule. No means is available at the present time for differentiating these processes in the mammal, but the hypothesis lends itself admirably to the explanation of our observations on urea reabsorption.

We may identify phase A in urea reabsorption with reabsorption in the proximal tubule; in this process a major portion of the water filtered is reabsorbed, and it is conceivable that in this process of concentrating glomerular filtrate, about 40 per cent of the filtered urea diffuses back into the blood. If the obligatory reabsorption of water (which is isotonic) is blocked by the introduction into the glomerular filtrate of osmotically significant quantities of glucose, sulphate or urea, the reabsorption of urea at this site fails to occur both because of a decrease in the extent to which urea is concentrated and because of a decrease in the time available for diffusion. We may identify phase B in urea reabsorption with the facultative reabsorption of water in the distal portions of the tubules, which may be presumed to be less permeable to urea. This is indicated by the smaller slope of line B as compared to line A. Since the obligatory reabsorption of water is completed in the normal animal at the highest urine flow obtainable by water diuresis, phase B in urea reabsorption invariably begins with a urine from which about 40 per cent of the filtered urea has already been reabsorbed. It is recognized that the two phases of urea reabsorption, as set forth above, are not subject to sharp separation or independent examination. It is probable that some urea reabsorption occurs in the distal portions of the tubule even when the creatinine U/P ratio is 10 or below. Since phase A extrapolates to a urea/creatinine clearance ratio of 1.0 at a creatinine U/P ratio of 1.0, physical diffusion may be tentatively accepted as the cause of reabsorption until evidence to the contrary is adduced.

The present observations on forced diuresis lend some support to Smith’s
hypothesis. It is known that the dog's kidney can concentrate the urine above a $\Delta 3.0^\circ C.$, the normal range varying from 1.0 to 2.0°C. (unpublished observations). During glucose diuresis of the type reported here, the concentration of glucose in the urine is invariably below 5.0 per cent $(-\Delta 0.517^\circ C.)$ and may be lower at the higher urine flows than at the low urine flows. Since at these high urine flows the concentration of urea and chloride and other constituents is negligible, it is difficult to understand how the diuresis can be due to the osmotic pressure of the urine as it traverses the distal segments. But our results are explicable in the view that the presence of an unabsorbed, osmotically active constituent in the glomerular filtrate blocks the isotonic (obligatory) reabsorption of water in the proximal tubule, and that fluid is therefore delivered to the distal segments too rapidly for the reabsorptive system in this portion of the nephron to effect significant further concentration.

SUMMARY

The excretion of urea has been studied in the normal dog by the simultaneous determination of creatinine and urea clearances during diuresis induced by the intravenous administration of glucose, sodium sulphate and urea. As the creatinine U/P ratio is reduced from 10 (the minimal value during water diuresis), progressively less urea is reabsorbed. The fact that the urea/creatinine clearance ratio extrapolates to 1.0 at a creatinine U/P ratio of 1.0 indicates that there is no active reabsorption of urea.

The reabsorption of urea is interpreted under the hypothesis that water reabsorption takes place at two sites in the nephron. The diffusion gradient created by the reabsorption of water in the proximal tubule accounts for the deficit in the urea clearance at the highest urine flow during water diuresis. The further deficit associated with low urine flows is attributed to diffusion in the distal portions of the nephron.

I wish to express my thanks for technical assistance to Dr. Elmer Alpert.

REFERENCES

Folin, O. J. Biol. Chem. 82: 83, 1929.
Shannon, J. A. This Journal 117: 206, 1936.