# Renal Transport of Glucose by the Aglomerular Fish *Lophius americanus* [1]

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*ABSTRACT* The renal excretion of glucose by the aglomerular fish *Lophius americanus* was studied. It was found that glucose is a normal constituent of *Lophius* urine. U/P ratios were approximately 0.02. However, after elevation of plasma glucose level or injection of phlorizin the U/P ratio for glucose was increased as much as ten fold. This increase in U/P ratio was observed even if urine was collected directly from the ureter rather than from the bladder. These results are consistent with the hypothesis that glucose is able to diffuse across the renal tubular epithelium, which also has the ability to reabsorb glucose which diffuses into the urine.

The renal handling of glucose by the aglomerular fish *Lophius americanus* has been studied by several investigators (Marshall and Grafflin, '28; Marshall, '30). They reported that either no glucose appeared in Lophius' urine or that only trace amounts were detected. Their data indicated that the renal tubule of Lophius is either impermeable to glucose or that all of the sugar diffusing into urine is reabsorbed. Since there is no detectable glucosuria following administration of phlorizin (Marshall and Grafflin, '28), it seemed likely that impermeability of the tubules is the controlling factor.

More recently, Malvin and Fritz ('62) injected L-arabinose, D-arabinose, or fructose into Lophius and found that all of these sugars were excreted into the urine. Since they could not demonstrate the presence of an active transport system for reabsorption of these substances, they concluded that the sugars entered tubular urine by a process of simple diffusion.

These diametrical views on the permeability of the tubular cells to carbohydrates need to be reconciled. It is possible that the difference in the findings results from the fact that the earlier chemical methods used were less specific and less sensitive than those available today. For these reasons we became interested in restudying the problem of possible glucose transport of the aglomerular kidney of *Lophius*.

## METHODS

Fish used in this study were captured in nets dragged along the ocean bottom in the vicinity of Mount Desert Island, Maine. In 11 of the fish urine samples were obtained immediately following capture, from a polyethylene catheter inserted into the bladder through the urinary papilla. The sample was quickly frozen in dry ice and maintained in the frozen state until analysis. All other fish were placed in large metal containers supplied with running sea water until the dragger returned to dock on the same day. Cracked ice was added to the containers which were then covered and transported by truck to the laboratory where they were placed in individual tubs of running sea water. Two types of experiments were performed on a total of 26 fish.

### *Type A — Bladder urine*

A polyethylene catheter was inserted into the urinary papilla of 21 fish and securely tied. This enabled repeated sampling of urine contained in the bladder. (In 9 of these fish only one sample was taken at the time of capture.) The bladder was emptied by gentle pressure on the ventral

Received Oct. 26, '64. Accepted Feb. 4, '65.

[1] This investigation was supported by U. S. Public Health Grant A-3885 and by Nonr-1224(26).

[2] This investigation was supported in part by Public Health Research Career Award Program HE-K3-6375 from the National Heart Institute.

surface of the fish, a small amount of air was blown into the bladder and gentle pressure again applied until the air was expelled. The tip of the catheter was tied closed so that timed collection periods could be started. The urine was obtained at intervals by opening the catheter and allowing the urine to drain freely into a graduated cylinder. When the urine ceased to drain air was blown into the bladder and pressure applied to the ventral surface until the remaining urine and air were expelled. The catheter was then resealed. Blood samples were obtained, using heparinized syringes, from vertebral vessels at the beginning and end of each collection period. The average of these blood samples was taken as the average plasma concentration for the collection period.

In some experiments glucose or phlorizin was injected. Injections of glucose were made in the region of the head either subcutaneously or intramuscularly; injections of phlorizin were made into muscles of the tail.

### *Type B — Ureteral and bladder urine*

Five fish were used to study the possible role of the urinary bladder in glucose transport. In those fish urine was obtained simultaneously from the bladder and from one ureter. The tail of each fish was tied to one end of a board, and the board placed in a tub of running sea water so that only the head of the animal was submerged. After infiltration with 1% procaine, a ventral midline incision was made. One ureter was exposed and catheterized with polyethylene tubing; the ureter being tied off distal to the site of catheterization. The open end of the catheter was placed in a small graduated cylinder or collection bottle about the level of the kidneys. Urine from that kidney drained freely and continously into the container which was replaced at the start of each collection period. In addition bladder urine and blood samples were also collected as described for Type A experiments. Thus in these experiments urine was collected directly from one kidney, while urine from the other kidney was in contact with the bladder during the collection period.

### *Chemical methods*

All samples of urine and plasma were frozen and maintained in the frozen state until the time of analysis. Plasma and urinary levels of glucose were estimated using the Glucostat reagent supplied by Worthington Biochemical Corporation, Freehold, New Jersey. The Glucostat method is specific for D-glucose. Total reducing substance of urine and plasma were determined by the method of Nelson and Norton ('44).

## RESULTS

All urine samples collected from the 11 fish catheterized immediately upon capture were analyzed for glucose using the Glucostat reagent. Glucose was found to be present in all urines. The mean concentration was 0.66 mg %, and the range was 0.29–1.39 mg %. Plasma levels ranged from 10.6 mg % to 107 mg %, with a mean of 48.7 mg %.

### *Total reducing substance*

Fifteen samples of urine and 15 plasma samples from four fish were analyzed for both total reducing substance and for glucose concentration. In both plasma and urine the total reducing substance was considerably higher than the glucose concentration. For plasma the average ratio, total reducing substance / glucose, was 3.5, S.D. 0.35. The mean ratio for urine was much greater and more variable, mean 16.0, S.D. 5.6. The range of this ratio in urine was 8.6–27.3.

### *Type A experiments*

Tables 1, 2 and 3 show the results for three experiments in which the glucose concentration of urine was measured. Table 1 shows the results of an experiment from a series of four in which the plasma concentration of glucose was increased by injection. In this experiment, the concentration of glucose in plasma was tripled by the injection. However, urinary glucose increased 11 fold, with the result that the U/P ratio for glucose increased approximately four fold.

Administration of phlorizin to five fish also resulted in a large increase in excretion of glucose. Results of one representa-

TABLE 1

*Effect of injection of glucose on the U/P ratio for glucose*

| Time in minutes | Urine volume | Glucose mg % | | U/P ratio |
|---|---|---|---|---|
| | | Urine | Plasma | |
| | *ml/hr* | | | |
| 0–6 | 2.0 | 2.50 | 86.5 | 0.029 |
| 60–92 | 2.7 | 1.74 | 88.8 | 0.020 |
| 92 | 10 gms glucose injected in head | | | |
| 92–175 | 3.0 | 13.0 | 162 | 0.081 |
| 175–246 | 2.9 | 20.2 | 250 | 0.081 |
| 246–327 | 3.7 | 19.1 | 273 | 0.070 |
| 327–411 | 4.0 | 22.9 | 283 | 0.081 |
| 411–641 | 3.9 | 26.4 | 278 | 0.095 |
| 641–716 | 3.9 | 22.7 | 260 | 0.087 |

TABLE 2

*Effect of injection of phlorizan on the U/P ratio for glucose*

| Time in minutes | Urine volume | Glucose mg % | | U/P ratio |
|---|---|---|---|---|
| | | Urine | Plasma | |
| | *ml/hr* | | | |
| 0–80 | 4.5 | 1.17 | 85.6 | 0.014 |
| 80–168 | 4.4 | 1.31 | 91.5 | 0.014 |
| 168 | 82 mg/kg Phlorizan 1 M in tail | | | |
| 168–225 | 4.1 | 7.98 | 99.2 | 0.081 |
| 255–320 | 5.0 | 11.1 | 110 | 0.101 |
| 320–389 | 4.9 | 11.5 | 123 | 0.094 |
| 389–485 | 4.7 | 16.7 | 137 | 0.122 |

TABLE 3

*Control experiment showing stability of U/P ratio for glucose*

| Time in minutes | Urine volume | Glucose mg % | | U/P ratio |
|---|---|---|---|---|
| | | Urine | Plasma | |
| | *ml/hr* | | | |
| 0–43 | 8.1 | 0.37 | 11.2 | 0.033 |
| 43–134 | 3.6 | 0.31 | 11.0 | 0.028 |
| 134–220 | 3.8 | 0.25 | 11.7 | 0.021 |
| 220–280 | 3.8 | 0.26 | 12.8 | 0.021 |
| 280–360 | 3.8 | 0.35 | 14.5 | 0.024 |
| 360–444 | 4.4 | 0.42 | 16.1 | 0.026 |
| 444–1394 | 4.4 | 0.52 | 16.3 | 0.028 |
| 1394–1488 | 3.1 | 0.95 | 15.9 | 0.060 |

tive experiment are shown in table 2. During the two control periods the U/P ratio for glucose remained constant. After administration of phlorizin the U/P ratio for glucose reached a level almost nine times that of the control. During this time there was no significant change in the rate of urine production; and only a small increase in the concentration of glucose in plasma. A consistent elevation of plasma concentration of glucose was not observed following injection of phlorizin, but a large increase in U/P ratio was recorded in each case.

Data presented in table 3 are the results of a single experiment in which repeated collections of urine and plasma of an untreated fish were made. No significant change in the U/P ratio for glucose was found even though the experiment lasted twice as long as any in the previous series.

*Type B experiments*

In three fish, urine samples were collected from the ureter and bladder during the control period only. In two fish a similar control period was obtained and then glucose was injected into the mus-

cles of the head and additional periods were obtained. In all samples derived from the ureter glucose was present. In addition no significant difference was found in the concentration of glucose in ureteral urine as opposed to bladder urine. Figure 1 presents all the data derived from this series. Along the abscissa is plotted the U/P ratio for glucose in urine collected from the ureter. Along the ordinate is plotted the same ratio for urine collected simultaneously from the bladder of the same fish. If the glucose concentrations in both urines were the same all points would fall along the line drawn which has a slope of 1. Although there is some scatter it is clear that no significant difference exists in the U/P ratios for glucose between ureteral and bladder urine.

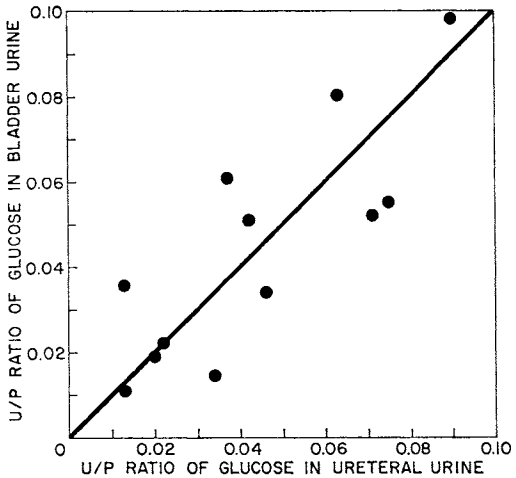


Fig. 1 Comparison of U/P ratios for glucose in urine obtained simultaneously from the bladder and ureter.

## DISCUSSION

The presence of glucose in all samples of urine, and the fact that glucose concentration is the same in samples taken simultaneously from the bladder and the ureter indicate that this sugar is a normal constituent of the urine of *Lophius americanus*. In addition it is clear that the bladder is not the site of entry. The amount of glucose present is not related to time of sampling relative to time of capture; nor does the U/P ratio increase in prolonged experiments. These findings vitiate the premise that glucose diffuses into urine only of fish whose renal function is deteriorating. Moreover, this premise cannot account for the action of phlorizin on the U/P ratio of glucose. The marked increase in excretion of glucose following intramuscular injection of phlorizin was prompt and was maintained for a long period of time. It was not influenced by plasma level of glucose nor rate of urine flow.

In view of the finding that concentration of total reducing substance greatly exceeds urinary concentration of glucose (ave. = 16:1), it is clear why earlier workers could not be certain that glucose was present in urine of *Lophius*.

Since functional glomeruli do not exist, glucose must be able to cross the renal tubular epithelium of *Lophius* by a process of passive or facilitated diffusion. This idea receives support in the present experiments, for both glucose loading and injection of phlorizin lead to increased renal excretion of glucose. The nephron of *Lophius*, which is considered in its entirety to be homologous to the proximal tubule of mammals (Marshall and Grifflin, '28), has thus retained an active transport system for glucose, even though it lost its glomeruli.

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