RENAL TUBULAR TRANSPORT OF INORGANIC DIVALENT IONS BY THE AGLOMERULAR MARINE TELEOST, LOPHIUS AMERICANUS*

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ABSTRACT

A characterization was attempted of the mechanisms involved in the tubular transport of inorganic divalent ions by the aglomerular kidney of *Lophius*, attention being paid particularly to the possible existence of transport maxima (*Tm*) and to competition for transport among related substances undergoing tubular excretion. Excretory rates of divalent ions in non-treated fish during standard laboratory conditions paralleled spontaneous changes in urine flow. *Tm* rates of excretion were reached for magnesium, sulfate, and thiosulfate with corresponding plasma levels of 2 to 5, 5 to 17, and 4 to 12 μM/ml. respectively. Elevation of magnesium chloride levels in plasma markedly depressed calcium excretion; sodium thiosulfate similarly depressed sulfate excretion. Experimental observations suggest the existence of a transport system for divalent cations separate from another for divalent anions. Within each transport system the ion with the higher excretion rate depressed competitively transfer of the other ion. Neither system was influenced by probenecid (benemid) in doses which markedly depressed the simultaneous excretion rate of *p*-aminohippuric acid.

Background information for this study on divalent ion transport in *Lophius* was provided in an earlier paper which presented the complete pattern of electrolyte distribution in plasma and urine (9). Samples were taken both from freshly captured *Lophii* and from fish maintained in the laboratory during the course of the progressive diuresis which is exhibited spontaneously by this and other marine teleosts in captivity. The most important excretory function of aglomerular renal tubules appears to be the active elimination of inorganic divalent ions taken up in plasma consequent to the ingestion of sea water, a

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feature of the water conservation mechanism in marine teleosts (19, 13, 7). Univalent ions, which are also absorbed from the strongly hypertonic sea water in the intestine, are selectively eliminated by secretory activities of the gills. The renal tubule actually acts as a barrier to the free diffusion of the univalent ions, or at most plays only a passive role in their elimination. Residual bladder urine taken from fish at the time of capture is frequently essentially chloride-free, and the concentrations of sodium and potassium in urine are always significantly lower than simultaneous plasma concentrations. During "laboratory diuresis," however, chloride appears in urine in which it effectively balances the principal cation, magnesium, which is transported into urine in increasing amounts as the progressive diuresis runs its course (7, 9).

In the current study attempts are made to characterize those secretory mechanisms involved in the transport of divalent ions by the aglomerular tubule, especially with respect to transfer maxima, competition for transport, and the actions of specific transport inhibitors.

Methods

Lophii were captured by otter trawlnet from waters south of Mount Desert Island, at a depth of 60 to 100 meters. Some specimens were taken by divers in 3 to 6 meters of water at Aldersea, Bar Harbor. In the laboratory, fish were kept individually in tubs supplied with running sea water. Blood samples were taken from the tail vessels by syringe containing heparin as anticoagulant. Injections were given intravenously or intramuscularly. Urine samples were obtained from an indwelling polyethylene catheter inserted through the urinary papilla into the bladder.

Inorganic sulfate was determined according to the method of Power and Wakefield (16), and thiosulfate, when present, was oxidized to tetrathionate with iodine before analysis of the sulfate ion. Thiosulfate in urine was determined by the iodine method, and in plasma by the iodate method according to Gilman *et al.* (11). Calcium plus magnesium in plasma and urine was determined by titration with ethylenediaminetetraacetate (EDTA) using Eriochrome Schwartz as an indicator. Magnesium in urine was determined by the same procedure after precipitation of calcium with ammonium oxalate, and calcium values obtained by the difference. The method is almost identical with that of Friedman and Rubin (10). In plasma, and in urines with less than 10 μM calcium per ml., calcium was determined directly by the method of Rehell (17), and magnesium obtained by difference. Total amino nitrogen was determined by the ninhydrin method of Troll and Cannan (21). *p*-Aminohippurate was determined by the method of Bratton and Marshall as modified by Smith *et al.* (20).

RESULTS

Fig. 1 shows characteristic changes in urine flow and excretion rates for the divalent ions—SO_4, Mg, and Ca—during spontaneous "laboratory diuresis" in one fish during the first 70 hours after capture. Plasma and urine concentrations of these ions are also indicated. In this specimen a progressive increase occurred in the excretion of divalent ions which was associated with parallel

increases in urine volume, so urinary concentrations of these ions remained fairly constant. Frequently, however, both diuresis and excretion of divalent ions levelled off after approximately 24 hours, and the plasma concentrations of magnesium and sulfate then sometimes rose as high as three times the initial values.

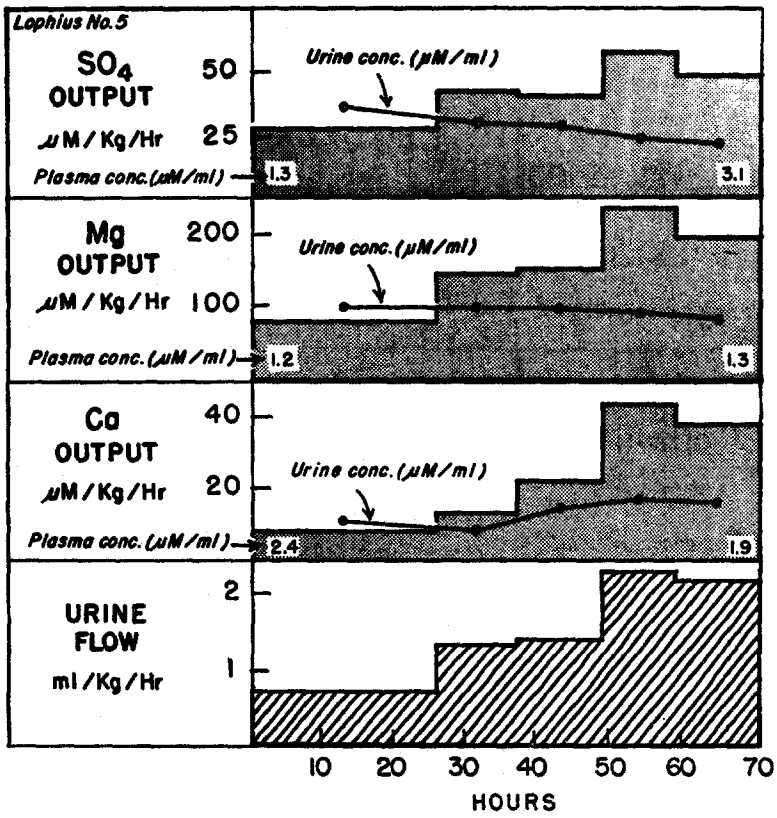

FIG. 1. Direct relationship between urine flow and the excretion of divalent ions during spontaneous "laboratory diuresis." *Lophius* 5. Weight 6.8 kg. Urine flow and excretion of divalent ions are indicated by bar grams, urine concentrations of divalent ions by lines, initial and final plasma concentrations in micromols per milliliter by numbers.

In the following studies, fish after collection were brought to the laboratory before any samples were taken. Zero time in Figs. 2 to 7 therefore corresponds to 2 to 10 hours after capture. Control collections were made for an additional 7 to 20 hours and then various salts of divalent ions were given intravenously. The total amounts administered to each fish varied between 3 and 14 mM per kg. body weight. The qualitative responses were similar over this dose range,

and the doses were non-lethal. Each millimol of injected divalent ion per kilo body weight produced an increase in plasma concentration of approximately 1 μM/ml. for calcium, magnesium, or thiosulfate, and 2 μM/ml. for sulfate.

In two fish calcium chloride was injected intravenously at intervals to elevate calcium plasma concentrations. Marked increases in urinary calcium

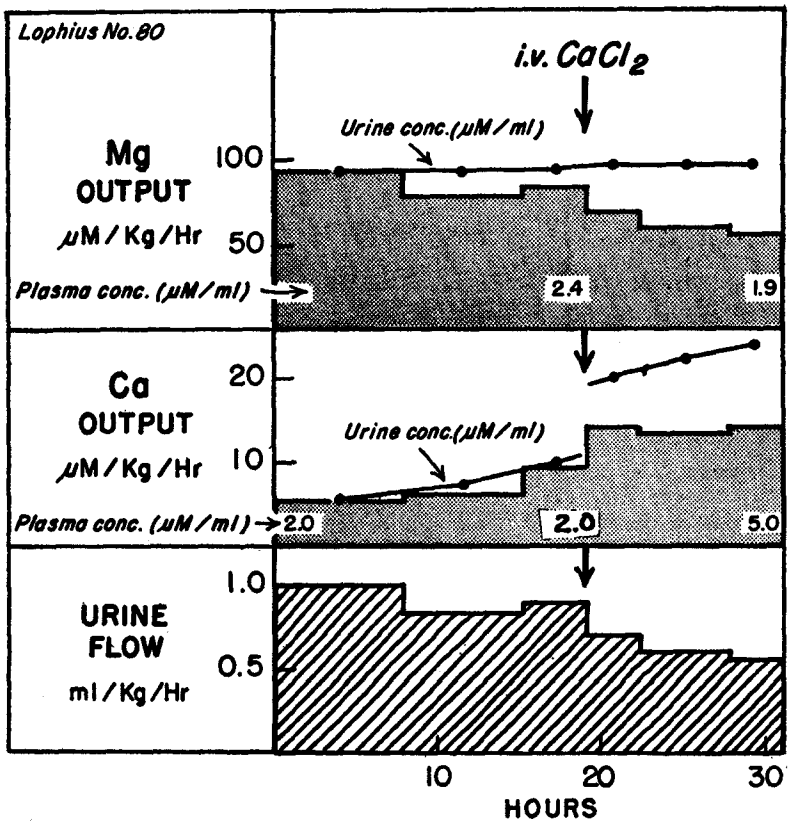

FIG. 2. Effect of elevated plasma calcium levels on urine flow, calcium and magnesium excretion. *Lophius* 80. Weight 7 kg. 27 mM $CaCl_2$ intravenously at arrow. Symbols as in Fig. 1.

concentration as well as total excretion occurred in spite of diminished urine flows. In *Lophius* 80 (Fig. 2) a maximum rate of secretion apparently was reached at a plasma level of 5 μM/ml., but in the other fish such a maximum rate was not demonstrated. Plasma concentrations and urinary excretion of magnesium were depressed in both fish, but the concentration of magnesium in urine remained practically unchanged.

Intravenous injections of magnesium chloride in three *Lophii* raised urine

flow and increased the excretion of magnesium in spite of a slight lowering of its urinary concentration (Fig. 3). With plasma concentration falling from 5.2 to 1.9 μM/ml., magnesium excretion remained relatively constant; evidently a maximum rate of secretion (*Tm*) prevailed over this range of plasma magnesium

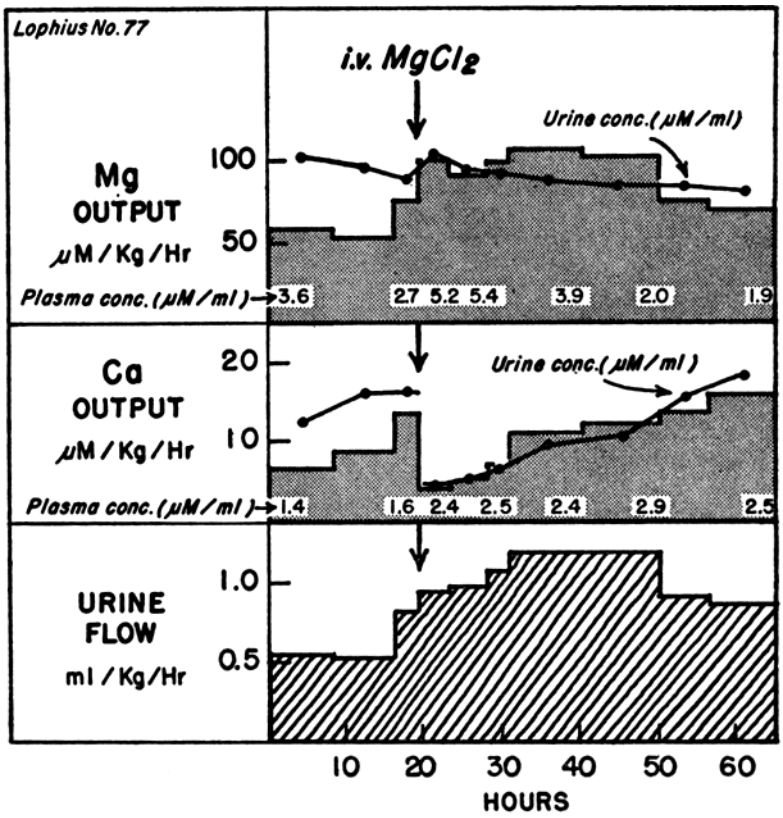

FIG. 3. Effect of elevated plasma magnesium levels on urine flow, calcium and magnesium excretion. *Lophius* 77. Weight 7.3 kg. 20 mM $MgCl_2$ intravenously at arrow. Symbols as in Fig. 1.

concentrations. Magnesium chloride administration also produced a marked depression of calcium excretion. In the two fish in which plasma magnesium concentrations were measured, calcium excretion varied inversely with plasma magnesium concentrations.

Two fish were injected intravenously with sodium sulfate at various intervals (Fig. 4). Only in one, No. 66, did this produce a significant rise in urinary concentration and excretion of sulfate. This fish had a low plasma concentration of sulfate during the control periods. In both fish sulfate excretion remained

fairly constant when plasma concentrations exceeded 5 μM/ml., thereby suggesting a secretory transfer maximum for sulfate reached at some plasma level below this value.

In three *Lophii*, sodium thiosulfate was injected intravenously (*e.g.* Fig. 5). It is evident that thiosulfate is actively secreted by the aglomerular kidney.

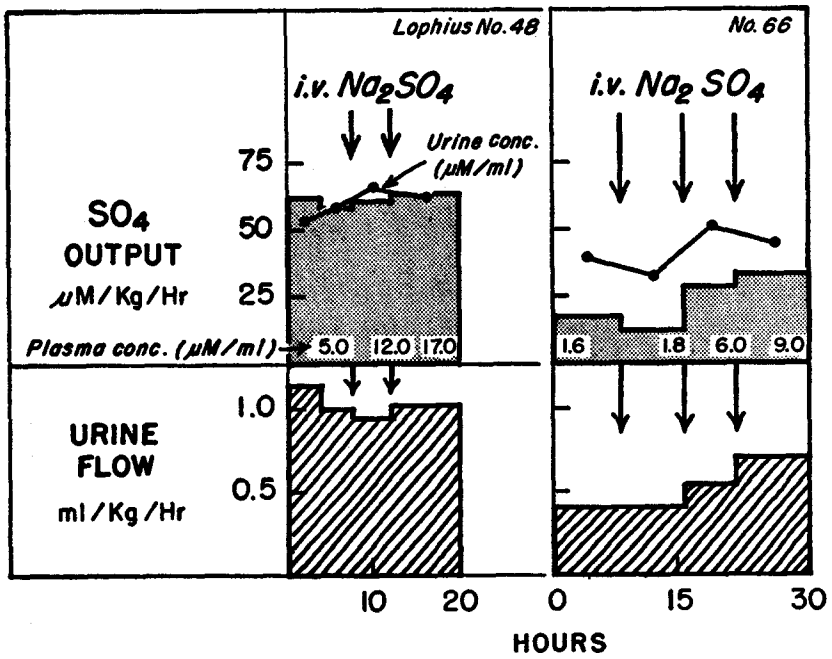

FIG. 4. Effect of elevated plasma sulfate levels on urine flow and sulfate excretion. Left, *Lophius* 48. Weight 3.5 kg. 7 mM (first arrow) and 14 mM (second arrow) Na_2SO_4 intravenously. Right, *Lophius* 66. Weight 5.8 kg. 6 mM Na_2SO_4 intravenously at each arrow. Symbols as in Fig. 1.

Thiosulfate excretion remained constant, evidently at maximal levels, when plasma concentrations varied from 4 to 12 μM/ml. The simultaneous urine: plasma concentration ratios for thiosulfate were 2 to 5 times as high as for sulfate, indicating that thiosulfate is secreted more actively than sulfate.

The simultaneous presence of thiosulfate inhibited sulfate excretion. The depression was so marked that sometimes with thiosulfate present the concentration of sulfate in urine was lower than in plasma. These observations, together with earlier studies on active resorption of these anions in the dog (1), indicate the existence of a common transfer mechanism subject to mutual inhibition.

After injection of sodium sulfate or thiosulfate, a diuresis was produced only

in one fish. In this specimen the plasma sulfate concentration was below 5 μM/ml. before injection, but was above this value in all others. Evidently maximum excretion of divalent anions was already present in these fish, and injections of sulfate or thiosulfate did not further raise the excreted osmotic load or urine flow.

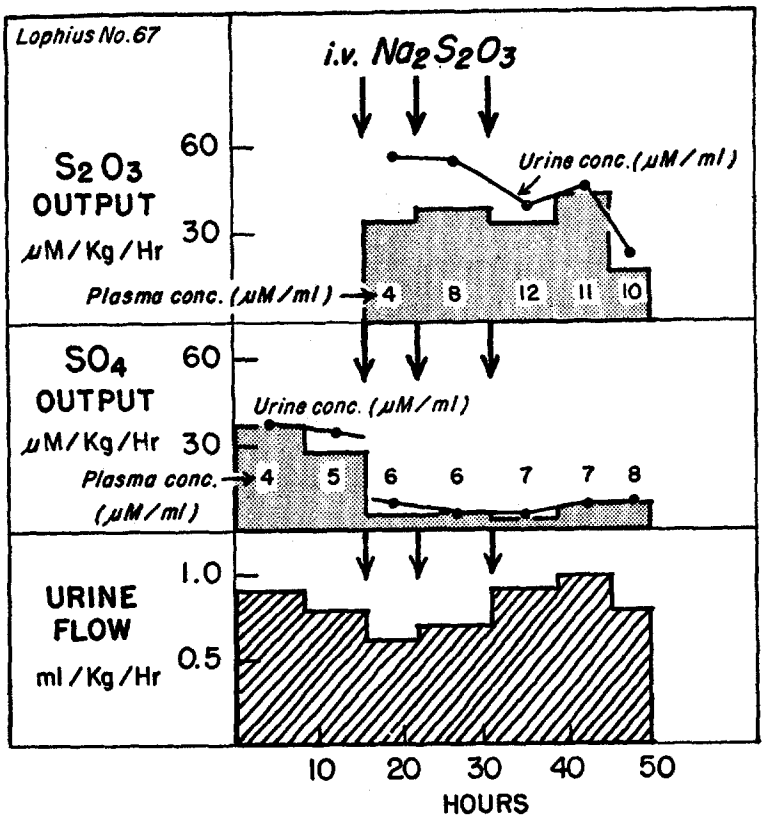

FIG. 5. Excretion of thiosulfate, marked depression of sulfate excretion after injection of sodium thiosulfate. *Lophius* 67. Weight 5 kg. 16 mM $Na_2S_2O_3$ intravenously at each arrow. Symbols as in Fig. 1.

In two fish glycine was injected intravenously to test whether amino acids depress the renal tubular transport of sulfate in *Lophius* as they do the reabsorption of sulfate in the dog (2). There was no consistent change in urinary sulfate concentration or excretion coincident with elevated glycine values in plasma (Fig. 6). Subsequent to glycine injections, amino-N concentrations in the urines were slightly higher than in corresponding plasma samples. This, however, hardly signifies active secretion of amino acids, as the blood samples

were obtained at the end of each urine collection, and the plasma concentration was continuously falling in both fish.

Independence between the divalent cation and anion transport mechanisms was demonstrated in experiments on three fish injected with magnesium chloride, which showed that when plasma magnesium concentrations were raised

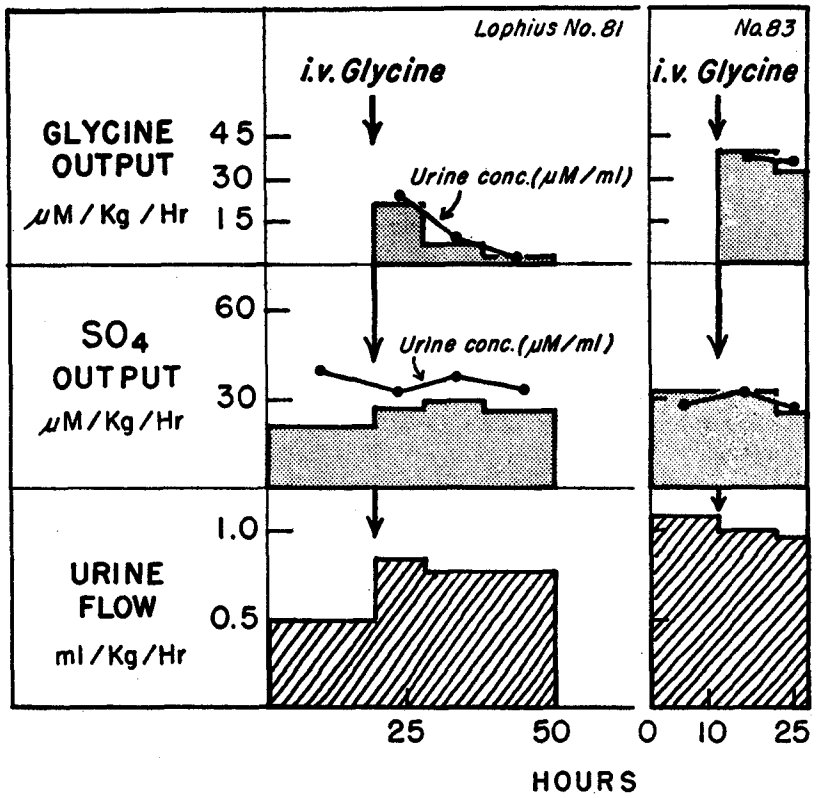

FIG. 6. Lack of effect of glycine on sulfate excretion. Left, *Lophius* 81. Weight 7 kg. Right, *Lophius* 83. Weight 2.7 kg. 80 mM glycine intramuscularly + 27 mM glycine intravenously at arrows. Symbols as in Fig. 1.

as high as twice control levels there was no evidence of competition with sulfate excretion. Although the urinary concentrations of sulfate dropped between 8 and 26 per cent, the total excretion rate at the same time increased between 30 and 57 per cent. The changes were ascribed to increased urine flow produced by the magnesium chloride, rather than to any direct interrelationship between the magnesium and the sulfate transfer mechanisms. Furthermore, a threefold increase in the plasma level of sulfate produced no consistent changes in either the urine concentration or excretion of magnesium.

Probenecid (benemid) previously has been shown to inhibit the tubular

secretion of penicillin, *p*-aminohippuric acid, phenol red, and other organic acids (3). It also inhibits the tubular secretion of urea by the frog (8). As seen from Fig. 7, a dose of probenecid, sufficient to depress PAH secretion by 75 per cent, had no significant effect on the excretion of magnesium and sulfate in *Lophius*.

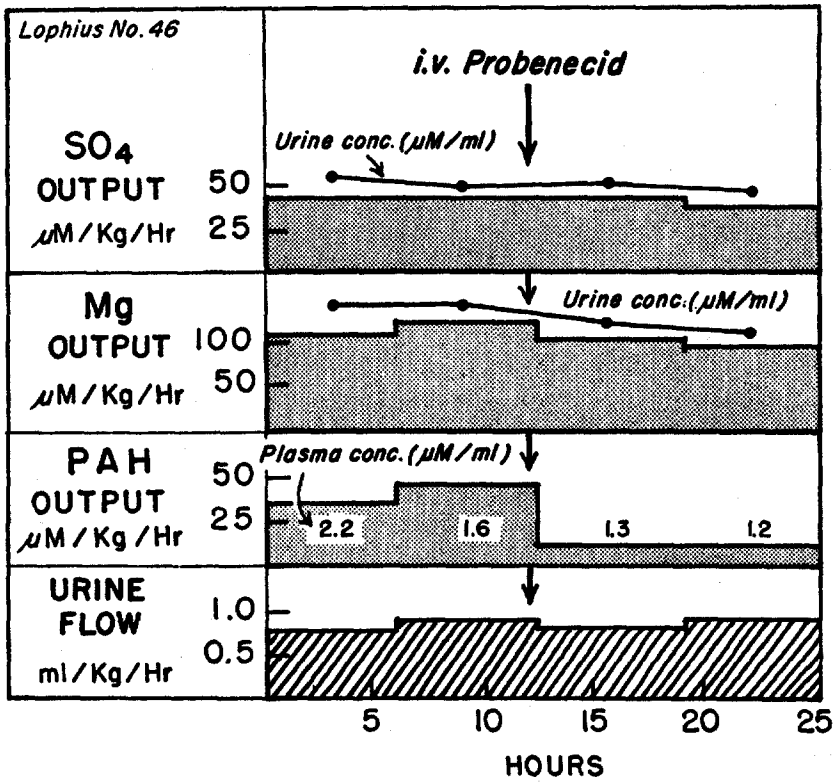

FIG. 7. Lack of effect of probenecid on excretion of inorganic ions, in dose suppressing PAH transport. *Lophius* 46. Weight 3.6 kg. Pretreatment, 9 mM PAH intramuscularly 15 hours before experiment. Probenecid 0.1 gm. intravenously + 0.1 gm. intramuscularly at arrow. Symbols as in Fig. 1.

Carinamide, which inhibits tubular secretion of thiosulfate in mammalian kidneys (Bucht, H., *Scand. J. Clin. Lab. Inv.*, 1949, **1**, 270), depressed sulfate excretion 60 and 67 per cent respectively in two *Lophii*, and depressed thiosulfate excretion 29 per cent in another *Lophius*. There was no effect on the excretion of magnesium + calcium.

DISCUSSION

Maximal tubular secretory rates have been demonstrated previously for phenol red in *Lophius* (18) and for many additional actively transported com-

pounds among other vertebrates (19). In our experiments, maximal secretory rates were exhibited for sulfate, with plasma concentrations varying between 5 and 17 μM/ml., for thiosulfate, with plasma concentrations between 4 and 12 μM,ml., and for magnesium, with plasma concentrations between 2 and 5 μM/ml. A secretory *Tm* for calcium could, however, not be consistently demonstrated. The pharmacodynamic effects of calcium ions, reflected in depression of urine flow after injection of calcium chloride, and the dominance of the simultaneous secretion of magnesium, are two factors which might explain our failure to demonstrate a *Tm* for calcium.

The competition studies yield some information concerning specificity of the tubular transfer systems. Magnesium and calcium appear to share one transport mechanism, while sulfate and thiosulfate share another. Magnesium shows a higher *U/P* concentration ratio than calcium, and elevated plasma magnesium levels depress calcium secretion. Thiosulfate similarly shows a higher *U/P* concentration ratio than sulfate,[1] and its presence in plasma markedly depresses sulfate excretion. An inhibitory action of thiosulfate on sulfate transport also occurs in the dog (1). In this respect the sulfate transport is identical in the two animals, although in the dog sulfate is reabsorbed subsequent to glomerular filtration instead of being secreted.

In the dog, amino acids are actively reabsorbed, and during this process elevated levels depress sulfate reabsorption (2). In *Lophius*, on the other hand, glycine is not actively transported by the tubules and has no effect on the excretion of sulfate.

As with divalent anions, there are also reports indicating competition in the tubular reabsorption of divalent cations in the mammalian kidney. Magnesium salts, given parenterally to dogs, cats, or rabbits, increase urinary excretion of calcium (14), and intravenous injections of magnesium salts in man yield similar results (22) without altering glomerular filtration rate or total serum calcium. Strontium chloride, given intravenously, also enhances calcium excretion in dogs (6). Magnesium excretion is similarly increased in rabbits and dogs after intravenous administration of calcium chloride (15). These findings indicate mutual interference between calcium, magnesium, and strontium in their tubular reabsorption.

The failure of probenecid to depress tubular excretion of divalent ions suggests that the excretory mechanisms involved here are not identical with the system which transports PAH, penicillin, phenosulfonphthalein, and other organic acids in *Lophius* and other vertebrates.

In the aglomerular toadfish (*Opsanus tau*), urine flow is increased by the ad-

[1] Although secretion of thiosulfate by aglomerular kidneys has been mentioned earlier in the literature (12), we have been unable to trace this note back to any report of performed experiments.

ministration of Mg^{++}, Ca^{++}, Sr^{++}, and Hg^{++} as chloride salts, and the relative effectiveness of these salts bears an inverse relation to the molecular weight (4). Bieter also showed that sodium sulfate and thiosulfate caused a diuresis in toadfish (4). These toadfish were kept in brackish water, and the concentrations of calcium, magnesium, and sulfate in the plasma were probably lower in these fish than in the *Lophii* used in our experiments. This might explain the apparent discrepancy between Bieter's and our results. Although magnesium chloride consistently produced a diuresis in *Lophius*, calcium chloride did the opposite. The lowering of the magnesium concentration in the plasma might be held responsible for this antidiuretic effect, or the high plasma calcium in our experiments may have had some toxic effect similar to that associated with the decreased glomerular filtration rate noted in dogs after the intravenous injection of calcium chloride (5). In only one *Lophius* did sodium sulfate or thiosulfate have any diuretic effect; the plasma sulfate level prior to injection in this fish was below 5 μM/ml. For the other three *Lophii* in this series the *Tm* for sulfate, and consequently maximum diuretic effect, was presumably reached before the injections. These studies, as well as those of Bieter (4), support the concept that the urine flow from aglomerular kidneys is determined mainly by the osmotic load of actively secreted divalent ions.

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