Stop-flow analysis of renal reabsorption and excretion of sulfate in the dog

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Absorption of sulfate, has been discussed by Drenckhahn and Meissner (4), who found cellular uptake of labeled sulfate limited to the epithelium of collecting ducts.

In the present study we have investigated more directly the site of reabsorption of sulfate, utilizing the stop-flow technique of Malvin, Sullivan and Wilde (5), which permits a gross localization of renal transport mechanisms along the nephron. The data to be presented provide evidence that inorganic sulfate is partially reabsorbed in 'proximal parts' of the nephron. The data also demonstrate how distal tubular mechanisms (acidification, secretion of NH₃, and potassium) are affected by the presence of a load of inorganic sulfate. These experiments have been reported previously in abstract form (6).

METHODS

The stop-flow method has been described in detail in previous communications from this laboratory (7). The results to be reported were obtained in experiments performed on 10 female mongrel dogs, weighing between 13 and 18 kg and anaesthetized with 30 mg/kg of pentobarbital given intravenously. The right ureter was catheterized in each instance. A solution containing creatinine, p-aminohippurate (PAH) and sodium sulfate was infused to elevate and maintain plasma levels of these substances. A second infusion of mannitol dissolved in saline served to establish osmotic diuresis. When urine flow was stable at 8-10 ml/min., the stop-flow experiment was performed. Clamping times varied from 4 to 8 minutes; multiple urine samples were collected for the 3 minutes immediately following release of the clamp. Inulin, to signal the appearance of newly formed glomerular filtrate, was injected 1 minute prior to the release of the clamp.

In some of our experiments a control stop-flow run was performed at endogenous plasma levels of sulfate. Following the control run, sulfate was infused and after a waiting period of 10-25 minutes, the experiment was repeated at elevated plasma levels. Colorimetric analyses of creatinine, PAH, and inulin were performed on di-

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luted urine aliquots and plasma filtrates (7). The pH of urine samples was determined after equilibration with air, using a Beckman pH meter (model GS) with the one-drop attachment. Ammonia was determined by the microdiffusion method of Conway (8), and potassium and sodium were analyzed with a Baird flame photometer (internal standard method). Sulfate was determined in 0.5-ml aliquots of urine or plasma by precipitation of sulfate with barium chloride and back titration of the excess barium with versene, by the volumetric method of Manns, Reschovsky and Certa (9). Erichrome black T was used as indicator. Amino nitrogen was determined in urine and tungstic acid filtrates of plasma by the ninhydrin method of Troll and Cannan (10).

**RESULTS**

Figure 1 presents data obtained in a single stop-flow experiment in which an isotonic solution containing sodium sulfate, PAH and creatinine in saline was infused at 5 ml/min. A second infusion containing 20% mannitol was administered at a rate of 7 ml/min. The experiment consists of a series of stop-flow collection periods, which are interposed between six conventional clearance periods. The ureter was clamped for 8 minutes; multiple urine samples were collected for 3 minutes. Cumulative volumes of stop-flow urine samples are plotted along the abscissa as percentage of the total volume of urine trapped within the kidney during the clamping period. This volume has been arbitrarily assumed to be that represented by all samples collected up to the one which contains 50% of the maximum concentration of inulin. Points on the right side of the chart represent fractions of urine which have been in contact with more distal parts of the nephron; points on the left side represent fractions of urine which have been in contact with more proximal parts of the nephron. Data from clearance periods are shown at the extreme right and left sides.
Creatinine U/P ratios, plotted at the bottom of the chart are indicative of water reabsorption along the nephron. PAH, inorganic sulfate, sodium and potassium U/P ratios have been divided by the simultaneously determined creatinine U/P ratio. These expressions have the connotation of clearance of the substance in question, divided by the simultaneously determined filtration rate. Clearance ratios greater than one indicate active tubular secretion, whereas ratios less than one indicate tubular reabsorption. As is shown by the third curve from the bottom, U/PeSO4/U/PCr ratios are significantly lower in proximal urine samples than in distal and in control samples. The lowest value, 0.18 is at 81% volume. This indicates tubular reabsorption at the same proximal site at which PAH is maximally secreted. The two upper curves show distal reabsorption of sodium and distal secretion of potassium as described previously (7). Note that peak potassium secretion occurs distal to the site of maximal sodium reabsorption.

Figure 2 illustrates the results of a two-run stop-flow experiment, performed on the same dog at a constant, elevated plasma sulfate level. Prior to the second run glycine was infused in order to achieve high urinary levels of this amino acid. In this manner it was possible to compare the sites of reabsorption of sulfate and glycine in the same experiment. It is seen that glycine and inorganic sulfate share a common reabsorptive site. Note that maximal acidification was observed in distal samples where the lowest pH was 5.48 in the first run and 6.38 in the second run. Corresponding concentrations

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of ammonia were 6.35 μEq/ml before and 15.4 μEq/ml after the infusion of glycine.

Figure 3A illustrates two consecutive experiments performed on the same dog, the first serving as a control run, the second after elevation of the plasma sulfate level to 7.0 μEq/ml, serving to demonstrate the action of sodium sulfate on distal tubular functions. The data for potassium exhibit the only striking difference between the two experiments. In the stop-flow run before sulfate infusion, potassium data are characterized by a biphasic curve with a minimum at 42% and a slight, but possibly significant maximum at 30% volume. Clearance ratios in the control experiment did not exceed 0.6. Infusion of sulfate resulted in an upward shift of the curve. Secretion of potassium is indicated at 30% volume by $\frac{U}{P_\text{K}}/\frac{U}{P_\text{Cr}} = 1.9$.

The next double experiment (fig. 3B) differs from the experiment just described in two respects: a) following the control run the infusion of a more concentrated solution of sodium sulfate resulted in elevation of plasma sulfate to 19 μEq/ml. b) Neither the sulfate nor the mannitol infusion contained sodium chloride. The time interval between onset of the infusion of sulfate and second stop-flow run was essentially the same as in experiment 3.

A comparison of the three upper pairs of curves demonstrates activation of acidification, secretion of ammonia and secretion of potassium in distal segments following the infusion of a more concentrated sulfate solution. The concentration of ammonia in distal samples increased from 3.75 μEq/ml before infusion of sulfate to 9.41 μEq/ml afterwards and pH decreased from 6.4 to 5.3. Clearance ratios for potassium exceeding 2 in the second experiment demonstrate a distinct secretion of potassium by the same segment where urine is acidified. Many other experiments from our laboratory indicate that these effects are produced by the infusion of large quantities of any poorly absorbed anion, e.g. phosphate, sulfate or ferrocyanide.

**DISCUSSION**

**Site of reabsorption of sulfate.** It is known from clearance studies on dogs that inorganic sulfate is filtered and partially reabsorbed by an active transport system with a limiting maximum rate (about 130 μmole/100 ml GFR (1, 11)). According to Cohen et al. (1) the tubular maximal transport capacity is affected by glucose and phlorrhizin. Increased rates of reabsorption of glucose depress $\text{Tm}_{\text{SO}_4}$ while blockade of glucose reabsorption by phlorrhizin results in a reversal of the depressant effect and elevation of the rate of sulfate reabsorption above control levels. It was concluded from these experiments, that glucose and sulfate share some common reaction during the reabsorption. This suggests that reabsorption of sulfate occurs in proximal tubules, the site of glucose reabsorption, if we assume that interrelated reabsorptive mechanisms are placed at the same anatomical site within the kidney. Autoradiographic studies, however, pointed to the collecting ducts as the site of sulfate reabsorption (4).

Since only indirect evidence was available, we performed experiments with the purpose of determining the site of sulfate reabsorption more directly by using stop-flow analysis. Our data demonstrate that inorganic sulfate is reabsorbed in 'proximal parts' of the nephron at the site of maximal secretion of PAH. The site of PAH secretion, however, corresponds with the site of reabsorption of glucose as reported by Malvin et al. (12). It is therefore assumed that reabsorption of sulfate and secretion of PAH are functions of the proximal convoluted tubule, the site of reabsorption of glucose in both the amphibian (13) and the mammalian kidney (14).

It has been described by Berglund and Lortspeich (2) that similar interaction occurs between the reabsorptive mechanisms for sulfate and glycine. Glycine among other amino acids depresses maximal sulfate reabsorption when filtered in significant amounts. The reabsorption of glycine has been shown by the stop-flow method to occur in the proximal segment in a region coextensive with that which secretes PAH (15). We have, therefore, compared the sites of reabsorption of sulfate and glycine in the same experiment. As demonstrated by figure 2, the assumption that closely linked transfer systems are localized within the same segment, is confirmed. Glycine, measured as $\alpha$-amino nitrogen, and inorganic sulfate are both maximally reabsorbed from proximal stop-flow samples.

There was no evidence of secretion of sulfate in our experiments, not even when dogs were loaded with large quantities of sulfate (clearance ratios approaching 1.0 in control periods). Neither could we confirm distal reabsorption of sulfate, as postulated by Drenckhahn and Meissner (4) from autoradiographic studies. These authors observed radioactivity stored in the walls of collecting ducts of guinea pigs 5–24 hours after a single injection of tracer amounts of labeled sulfate. Our experiments, performed during acute loading with Na$_2$SO$_4$, are certainly concerned with tubular transport of inorganic sulfate. The experiments of Drenckhahn and Meissner, in contrast, are probably concerned with the incorporation of S$^{35}$ into ethereal sulfates or proteins of tubular cells, not with transport of inorganic sulfate as such. If what they observe is in reality indicative of the site of tubular transport of organic conjugates of sulfate, it is evident that they are handled differently from inorganic sulfate and at different sites within the kidney. However, the autoradiographic studies of Norhagen and Odeblad (16), performed on the mouse indicate that the epithelium of collecting ducts contains less S$^{35}$ than proximal parts of the nephron 6–48 hours after the injection of S$^{35}$O$_4$.

Radioautographic studies of distribution of an element known to be incorporated into tissue constituents are not likely to shed light on site of renal tubular reabsorption, especially if the study is performed long after administration of that element.
Effects of sodium sulfate on acidification, secretion of ammonia and secretion of potassium. In control experiments acidification and secretion of ammonia occur in the more distal parts of the nephron. The pH and ammonia concentration of proximal stop-flow urine samples do not differ significantly from free flow clearance values. Control curves for potassium are characterized by a minimum in distal parts of the nephron. In some dogs a less marked maximum (fig. 3) was observed more distally corresponding with the site of maximal acidification. Proximal clearance ratios for potassium increase progressively towards free flow ratios. We interpret the control curve for potassium as evidence for reabsorption of this ion proximal to the site of acidification. A slight secretion of potassium distal to the site of potassium reabsorption may contribute to the distal maximum.

The effects of sulfate infusion on distal tubular functions are clearly demonstrated by experiment 4. In response to a load of sodium sulfate, acidification and secretion of ammonia are enhanced in the terminal nephron. Furthermore, a distinct secretion of potassium is observed at the same site.

Enhancement of potassium secretion, although less in magnitude, is also apparent in experiment 3. The response seems to be proportional to the load of sulfate infused and to the plasma sulfate concentration achieved (7.0 μEq/ml in experiment 3; 19 μEq/ml in exp. 4).

REFERENCES


The higher the plasma concentration of sulfate, the greater the filtered load of sodium sulfate presented to distal segments where exchange of potassium for sodium occurs.

One might argue similarly that acidification of the urine and ammonia secretion should be proportional to sulfate load. Although this may well be true, the two experiments do not demonstrate this conclusively. In experiment 4 a distinct activation of acidification and ammonia secretion was observed in response to a large load of sulfate. In experiment 3 no increment in acidification and ammonia secretion was observed when less sulfate was infused. However, in the control series of experiment 3, the urine was extensively acidified. More intense acidification might not have occurred even if a large load of sulfate had been infused.

It may be noted that in experiment 2, unlike the other experiments, lower pH of distal urine samples corresponds to a relatively low concentration of ammonia. After the infusion of glycine the distal urine is less acid but contains higher concentrations of ammonia. The more glycine available, the greater the filtered load of sodium sulfate presented to distal segments where exchange of potassium for sodium occurs. This reciprocity between urinary pH and ammonia concentration is explained by the fact that glycine serves as precursor of NH3 (17). The more glycine available, the greater is the secretion of ammonia and the more completely are the urinary hydrogen ions neutralized.