Renal tubular handling of zinc in the dog

WINONA VICTERY, JACQUELYN M. SMITH, AND ARTHUR J. VANDER
Department of Physiology, University of Michigan, Ann Arbor, Michigan 48109

VICTERY, WINONA, JACQUELYN M. SMITH, AND ARTHUR J. VANDER. *Renal tubular handling of zinc in the dog.* Am. J. Physiol. 241 (Renal Fluid Electrolyte Physiol. 10): F532–F539, 1981.—Clearance and stop-flow techniques, performed on anesthetized dogs, were used to obtain evidence for renal tubular reabsorption or secretion of ^{65}Zn (administered intravenously with varying amounts of carrier $ZnCl_2$). Ultrafilterability of plasma ^{65}Zn was determined in vitro with Amicon CF-50A filter cones. Clearances were obtained under six conditions: antidiuresis, mannitol diuresis alone and following the infusion of chlorothiazide, citrate, cysteine, or histidine. The percentage of ultrafilterability averaged, respectively, 1.15, 1.38, 0.91, 1.53, 19.90, and 11.61, while fractional zinc excretion averaged 0.046, 0.10, 0.31, 0.43, 0.78, and 0.12, respectively. Control stop-flow patterns invariably showed net Zn reabsorption in the distal nephron. Stop-flow patterns after chlorothiazide revealed significant net secretion in the far distal area in all animals. Patterns after either citrate or histidine infusion revealed significant net reabsorption in the proximal nephron. Those after cysteine were similar to the control patterns. Thus, both clearance and stop-flow experiments revealed net reabsorption as the major tubular mechanism of zinc handling, tubular secretion occurring only during chlorothiazide administration.

chlorothiazide; hyperzincuria; stop-flow studies; clearance techniques

ALTHOUGH URINARY EXCRETION OF ZINC is normally not the major route for the homeostatic control of zinc balance (5), large clinically significant urinary losses can occur in a variety of diseases (8) and experimental situations including amino acid infusions (16) and administration of the diuretic chlorothiazide (4, 22). Little is known about the basic mechanisms for the renal tubular handling of zinc or the alterations that occur in the situations characterized by hyperzincuria. The primary reason for this lack of information is that clearance procedures require the measurement of ultrafilterable zinc concentrations in plasma, a procedure fraught with difficulties because of the very small percentage that is ultrafilterable and the potential zinc contamination of filtration or dialysis membranes. To data there has been only one previously published study in which clearances of ultrafilterable zinc have been reported. Using an in vitro ultrafiltration technique, Yunice et al. (23) found that zinc normally underwent net tubular reabsorption, but that secretion occurred during infusion of cysteine. However, as detailed in the DISCUSSION, we believe that their values for ultrafilterable zinc concentrations are invalid. In addition, a paper being published concurrently by Abu-Hamdan et al. (1) reports changes in fractional excretion of zinc after administration of large amounts of zinc and during amino acid infusion.

The study reported here presents data from renal clearance experiments for zinc in anesthetized dogs using a different ultrafiltration method. In addition, the stop-flow technique was used to evaluate mechanisms for renal zinc handling. The stop-flow technique has three advantages in this regard: *1*) its interpretation does not depend on the validity of in vitro ultrafiltration measurements; *2*) it permits coarse localization of sites of tubular transport of an ion; and *3*) it may reveal transport in different directions in the same nephron site (under different experimental conditions) or in different nephron sites at the same time.

In all experiments, we have used radioactive ^{65}Zn in order to avoid potential problems of contamination of ultrafiltration or dialysis materials and to permit detection of the very small concentrations of zinc in the urine during stop-flow analysis performed without zinc loading, i.e., at normal plasma concentrations of zinc. After clearance and stop-flow collections under control conditions were performed, several agents previously reported to produce hyperzincuria were also studied in an attempt to determine whether they altered ultrafilterability, reabsorption, or secretion. Histidine, cysteine, and citrate are thought to be the major normal low molecular weight ligands for zinc in plasma (10), and administration of the first two has been reported to cause significant hyperzincuria (16). Only chlorothiazide, of the commonly used natriuretic drugs, has been reported to be effective in causing hyperzincuria (4, 22).

METHODS

Studies were performed on 20 nonfasted male dogs weighing 12.7–20 kg. After pentobarbital anesthesia (30 mg/kg i.v. with supplements given as required), the animals were prepared surgically for renal clearance and stop-flow techniques; both of the cephalic and femoral veins and one femoral artery were catheterized with polyethylene tubing for intravenous infusion and blood sampling. The right ureter was catheterized via a small flank incision. Creatinine and inulin (prime, 600 mg of each dissolved in 20 ml isotonic saline, followed by an infusion of 6 mg/min in 0.21 ml/min of isotonic saline) were administered to determine, respectively, creatinine clearance in the clearance protocols and water reabsorption in the stop-flow experiments. At the same time, the dogs were given an infusion (0.2–0.3 ml/min) containing ^{65}Zn (New England Nuclear, NEZ-111) with varying amounts of carrier Zn (as $ZnCl_2$) added: 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$ in 16 animals, 500 $\mu g \cdot kg^{-1} \cdot h^{-1}$ in one animal, and 2,000 $\mu g \cdot kg^{-1} \cdot h^{-1}$ in three animals. The lowest dose of carrier zinc was estimated (using the specific activity of

the infusate) to raise plasma zinc concentration an average of 2.83 ± 0.73 (SE) μg/ml, approximately a 2–4% increase. The infusion vehicle for the Zn was either isotonic saline or a mixture of 1:1 autologous serum and isotonic saline; there were no differences in the results observed for these two infusions and the data have, therefore, been pooled. The dose of ^{65}Zn varied between 50 and 500 μCi/h, the larger doses being required to give sufficient radioactivity in mannitol-diuretic urine for stop-flow analysis.

In 12 of the animals, clearances (see description below) were performed 60–90 min after administration of the ^{65}Zn infusion; these clearances are termed antidiuretic clearances. Following their completion, an infusion of 15% mannitol in isotonic saline was begun in all but one of these animals at the rate of 0.75 $ml \cdot kg^{-1} \cdot min^{-1}$; in seven other animals, no antidiuretic clearances were performed and the mannitol infusion was begun simultaneously with Zn infusion. In both groups (18 dogs) approximately 40 min after the mannitol infusion was begun and after stabilization of urine flow at 6–13 ml/min, clearances and/or stop-flow procedures (see description below) were performed. A second set of postmannitol clearances and/or stop-flow collections was then obtained in 15 dogs 20–45 min after one of several experimental manipulations: five animals received sodium citrate (a prime of 0.1 g/kg administered as a 1% solution over 10 min, followed by addition of 0.36% sodium citrate to the mannitol infusion); four animals were given cysteine (3.5–10 g/h in isotonic saline infused at 1.1 ml/min); two animals received histidine infusion (3 g/h infused in 1.1 ml of isotonic saline per minute); five animals received chlorothiazide (5 mg/kg as a prime administered over 15 min, followed by a 5 $mg \cdot kg^{-1} \cdot h^{-1}$ infusion).

Clearance protocol. The methodology for determining clearance of ultrafilterable ^{65}Zn was the same as that described previously for ^{203}Pb (15). Each clearance datum is the average of two successive 15-min (during antidiuresis) or 5-min (during mannitol diuresis) urine collections with an arterial blood sample obtained at the end of the first 5- or 15-min urine collection. This blood sample consisted of two 15-ml aliquots collected anaerobically in Vacutainer tubes containing no anticoagulant as well as a 5-ml aliquot collected aerobically for measurement of whole blood ^{65}Zn concentration. The 35-ml volume of blood was replaced with an equal volume of isotonic saline. The samples in the Vacutainer tubes were centrifuged immediately for 10 min, and the plasma was then ultrafiltered by low-speed centrifugation (250 *g*) through Amicon CF-50A cones (50,000 mol wt cutoff). Ultrafiltrates were routinely checked for protein (dipstick method) and values discarded if protein exceeded 1+ (20 mg/dl).

Clearance of ultrafilterable ^{65}Zn was calculated by the equation

$$C_{ult\text{-}Zn} = \frac{[Zn]_{urine} \times \text{urine flow rate}}{[Zn]_{ult}}$$

where $[Zn]_{urine}$ and $[Zn]_{ult}$ are the ^{65}Zn concentrations in urine and plasma ultrafiltrate, respectively. The fractional excretion of filtered ^{65}Zn is given by the ratio $C_{ult\text{-}Zn}/C_{Cr}$, where C_{Cr} is the creatinine clearance, which was used to estimate glomerular filtration rate (GFR). A ratio less than unity signifies Zn reabsorption, whereas a ratio greater than unity indicates net secretion. In several of the animals with high doses of ^{65}Zn, we collected only urinary excretion data to avoid handling of blood and plasma samples. For these animals, no data for ultrafilterability or fractional excretion are available.

It must be emphasized that these clearance data are for exogenous zinc only. No firm conclusion can be drawn from them concerning the renal handling of endogenous zinc, since we have no knowledge of the rates of equilibration of the exogenous zinc with endogenous pools. In previous studies (17, 18), however, we have documented that the administration of either glucagon (17) or inorganic lead (18) to dogs in protocols very similar to the present ones induced virtually identical percentage increases in the excretion rates of endogenous zinc and acutely infused ^{65}Zn. This suggests that ^{65}Zn, in association with the carrier doses used, does, in fact, equilibrate rapidly with the endogenous pool of zinc contributing to renal excretion.

Stop-flow experiments. A standard stop-flow procedure (3) was performed in 13 dogs. After completion of relevant clearances, two or three urine samples ("free-flow" samples) were collected over 30 s, and immediately thereafter the ureteral catheter was completely occluded for 5 min. Following release of the occlusion, urine was collected in 30–50 sequential aliquots, each approximately 0.5 ml ("stop-flow" samples). The concentration of ^{65}Zn in each stop-flow (SF) sample was divided by the [Zn] in the free-flow (FF) sample preceding the occlusion, and this ratio, $[Zn]_{SF}/[Zn]_{FF}$, was divided by the analogous ratio for inulin, $[In]_{SF}/[In]_{FF}$. The values obtained by this double ratio calculation reflect sequential changes in $[Zn]_{SF}$ relative to free flow after elimination of any concentration changes due solely to water reabsorption. A value of this double ratio greater than unity signifies net zinc secretion into that sample during ureteral occlusion; a value less than unity signifies the occurrence of net Zn reabsorption from that sample during ureteral occlusion.

Analytical methods. Samples were prepared for radioisotopic counting by aliquoting approximately 1-g samples of whole blood, plasma ultrafiltrate, or urine (stop-flow samples were transferred quantitatively) into stoppered tared tubes. The tubes were then reweighed and counted for ^{65}Zn in a Searle Analytic model 1195 AutoGamma counter peaked for the 1.12 MeV gamma ray. No correction for decay was necessary because of the 243-day half-life of this isotope; activity is expressed as counts per minute per milliliter of sample. No attempt was made to measure endogenous Zn. Inulin was determined in each stop-flow sample by the method of Shreiner (9) in 50-μl aliquots of urine. Creatinine was measured in urine and ultrafiltrate samples by the method of Bonsnes and Taussky (2). Urinary [Na] was measured using an Instrumentation Laboratory flame photometer and urinary [Ca] by atomic absorption flame spectrophotometry.

Grouped data are expressed as means ± SE. The method used for evaluation of statistical significance was either the standard or paired-sample *t* test.

RESULTS

Clearance data from all animals receiving 10 μg Zn·kg^{-1}·h^{-1} are summarized in Fig. 1. *Panel A* depicts mean values (± SE, except where sample size is only two) for the percentage of plasma ^{65}Zn that was ultrafilterable under each experimental condition, and *panel B* presents fractional excretion of ^{65}Zn in the same animals. At the end of 1–2 h of Zn infusion at this dose, the percentage of ultrafilterable ^{65}Zn in plasma was 1.15 ± 0.13%. Fractional excretion of ^{65}Zn during antidiuresis was 0.046 ± 0.010, indicating marked net reabsorption (95.4%) of Zn. Mannitol infusion did not alter the percentage of plasma ^{65}Zn that was filterable (1.38 ± 0.19%); however, fractional excretion was significantly elevated ($P < 0.05$) to 0.10 ± 0.02. Chlorothiazide treatment tended to lower ultrafilterable ^{65}Zn but increased fractional excretion approximately threefold. In four other animals given chlorothiazide, zinc excretion was elevated an average of 2.9-fold; fractional excretion could not be calculated because no data were obtained from ultrafilterability. Citrate did not significantly elevate the percentage of ultrafilterable zinc (1.53 ± 0.22%) but did significantly increase fractional excretion to 0.43 ± 0.17 ($P < 0.05$). In one dog fractional excretion exceeded unity (1.1). Cysteine and histidine infusion elevated the percentage of ultrafilterable ^{65}Zn approximately 20-fold and 10-fold, respectively (statistical significance was not achieved because of the small sample size and large variation). Fractional Zn excretion increased to a much larger degree with cysteine infusion than with histidine; the value exceeded unity (1.25) in only one of the three dogs receiving cysteine.

Examples of the control ^{65}Zn stop-flow pattern are illustrated in Figs. 2–4. The sodium concentration pattern provides coarse localization of nephron segments in that the minimal sodium concentration, i.e., the point of maximal reabsorption, corresponds to fluid trapped during ureteral occlusion in distal portions of the nephron (probably including collecting duct, distal tubule, and the ascending loop of Henle) (3). The beginning of the plateau in sodium concentration (approximately 8 ml in Fig. 2) at its preocclusive values is presumed to represent fluid trapped only in the proximal portions of nephrons during occlusion, since these are samples in which *p*-aminohippurate concentration peaks and glucose concentration dips (3). The most striking feature of the control stop-flow pattern for ^{65}Zn is the net reabsorption (double ratio less than unity) occurring in the segment corresponding to the distal reabsorptive minimum for sodium. This was seen in all 13 dogs. As shown in Table 1, the mean (±SE) double ratio in this distal region for all dogs was 0.20 ± 0.05, a value significantly different from unity ($P < 0.001$). There was more variability in the double-ratio pattern for the first few samples collected after

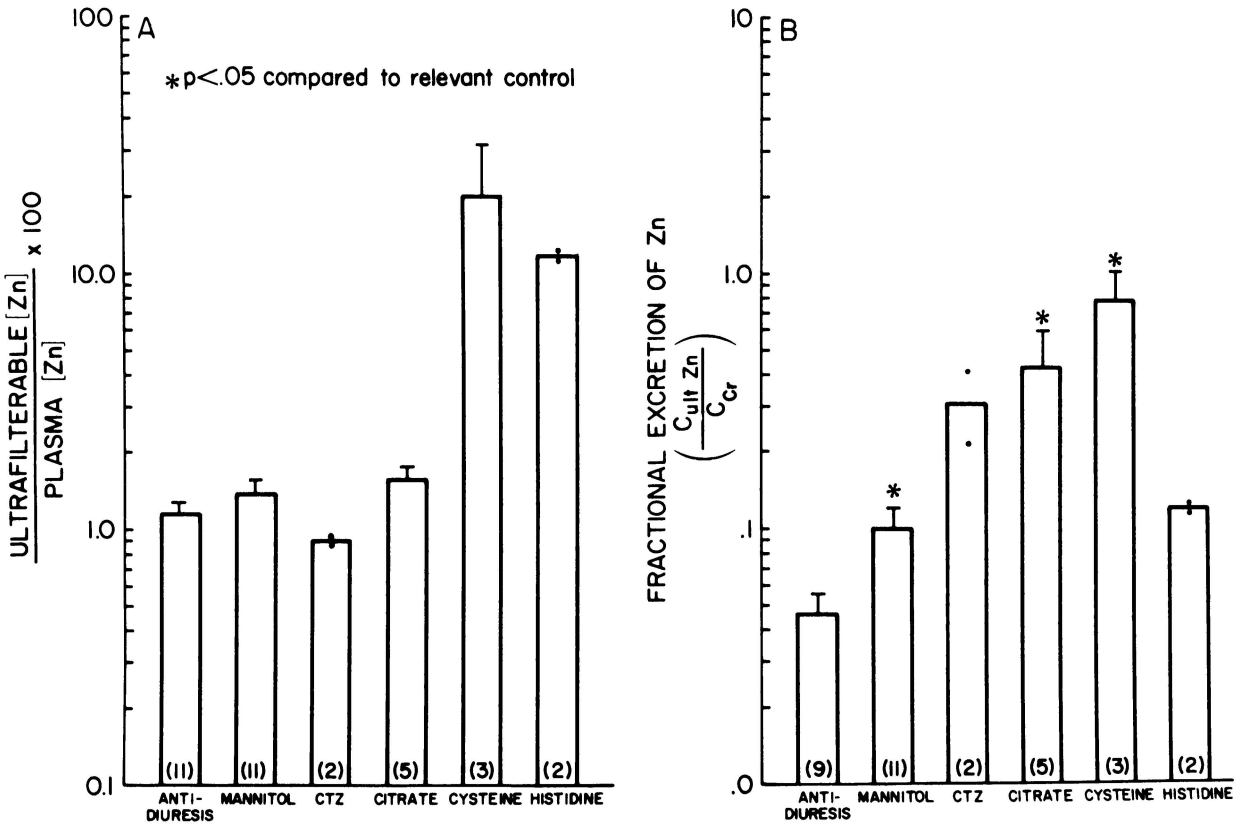

FIG. 1. *A*: percent of ultrafilterable plasma ^{65}Zn in each experimental protocol (designated on *x*-axis). Each bar represents mean value ± SE for treatment group when the number of animals was greater than 2. Individual data points are shown when there were only 2 animals per treatment group. The number of animals is given in parentheses. Statistical comparison is with appropriate control group; antidiuresis is the control for the mannitol group, and mannitol is the control for all other treatments. No statistical comparisons were made for the chlorothiazide (CTZ) and histidine groups. *B*: fractional excretion of ^{65}Zn ($C_{ult\text{-}Zn}/C_{Cr}$) for each treatment group. Symbols and statistical comparisons as in *A*. Note that there are 2 fewer animals for this variable than for *A* because in 2 animals the urinary counts were too low to be dependable.

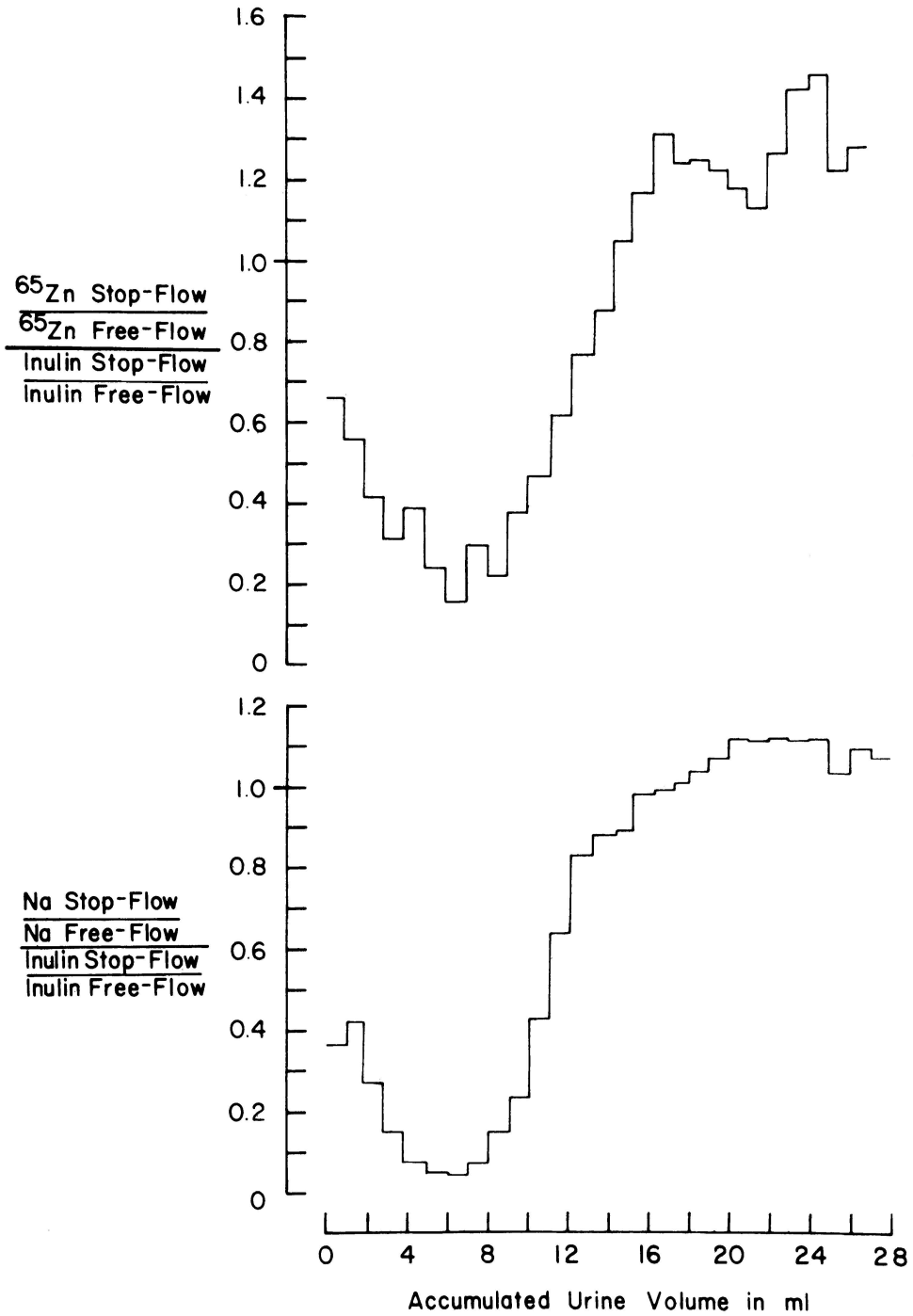

FIG. 2. Control stop-flow pattern for ^{65}Zn (*upper panel*) and for Na (*lower panel*). ^{65}Zn and Na concentrations in each stop-flow sample are corrected for water reabsorption as described in text. That the double ratio exceeds unity in proximal samples in this figure does not indicate secretion but rather a new plateau reflecting a change in function during period of occlusion. There must be a clear-cut peak for evidence of secretion (3).

release of occlusion (far distal nephron) and for the proximal nephron. However, as shown in Table 1, for the entire control group there was no evidence for either zinc reabsorption or secretion in these areas; far distal double ratio = 1.18 ± 0.15 and proximal double ratio = 1.14 ± 0.16. Finally, the dose of carrier zinc administered had no effect on the stop-flow patterns.

Chlorothiazide was administered prior to the second stop-flow in five animals. Fig. 3 presents typical results from one such experiment. In every animal, compared with the previous control pattern for Zn, there was an increase in the far distal double ratio for Zn (exceeding unity in every case), and the distal Zn minimum after chlorothiazide was not as low as in the control collection: far distal double ratio before and after chlorothiazide, respectively, 1.11 ± 0.21 and 1.46 ± 0.16 ($P < 0.01$, paired-sample analysis); distal double ratio, 0.12 ± 0.074 and 0.36 ± 0.069 ($P < 0.001$, paired-sample analysis). The far distal double ratio after chlorothiazide was significantly different from unity ($P < 0.05$), constituting evidence for net secretion in this area.

As mentioned above, proximal reabsorption of zinc was not manifest in control stop-flows. To bring out a possible proximal reabsorptive pathway (see DISCUSSION and Refs. 20 and 21), we added citrate to the mannitol infusion. The results of one such experiment are shown in Fig. 4, which presents the stop-flow patterns before and after citrate for zinc, calcium, and sodium; results were

identical in the other two experiments. After administration of citrate, there was no change in the reabsorptive pattern for Na, but there was obvious proximal (as well as distal) reabsorption for Ca and Zn. For the three dogs, the proximal double ratio for ^{65}Zn was 0.50 ± 0.094, significantly different from unity ($P < 0.05$). A stop-flow pattern during histidine infusion is shown in Fig. 5. This pattern is identical to the pattern seen with citrate in that it shows clearcut proximal (as well as distal) reabsorption of Zn. The proximal double ratios for the two dogs receiving histidine were 0.36 and 0.37.

In contrast to citrate and histidine, cysteine produced no change from the control stop-flow pattern despite the marked increase in ^{65}Zn in all the urine samples. None of the animals exhibited net Zn secretion during stop-flow anywhere along the nephron.

TABLE 1. *Summary of stop-flow double ratio data in 13 control dogs*

	$\frac{^{65}Zn_{stop\text{-}flow}}{^{65}Zn_{free\text{-}flow}} / \frac{Inulin_{stop\text{-}flow}}{Inulin_{free\text{-}flow}}$
Far distal nephron	1.18 ± 0.15
Distal nephron	0.20 ± 0.05
Proximal nephron	1.21 ± 0.21

Far distal denotes the highest double ratio found within the first 3 ml collected after release of occlusion. Distal refers to the sample containing the lowest double ratio (usually identical to or within one sample of the sodium minimum). Proximal refers to an average of three consecutive samples at the beginning of the sodium plateau.

DISCUSSION

The clearance data obtained in this study strongly support the hypothesis that net reabsorption, averaging approximately 95%, is the dominant tubular mechanism for the renal handling of acutely administered zinc under basal conditions. Yunice et al. (23), in experiments with endogenous Zn rather than ^{65}Zn, came to a similar conclusion, but their data differ from ours in that their estimate of ultrafilterability under control conditions was much higher—approximately 10% of total plasma zinc

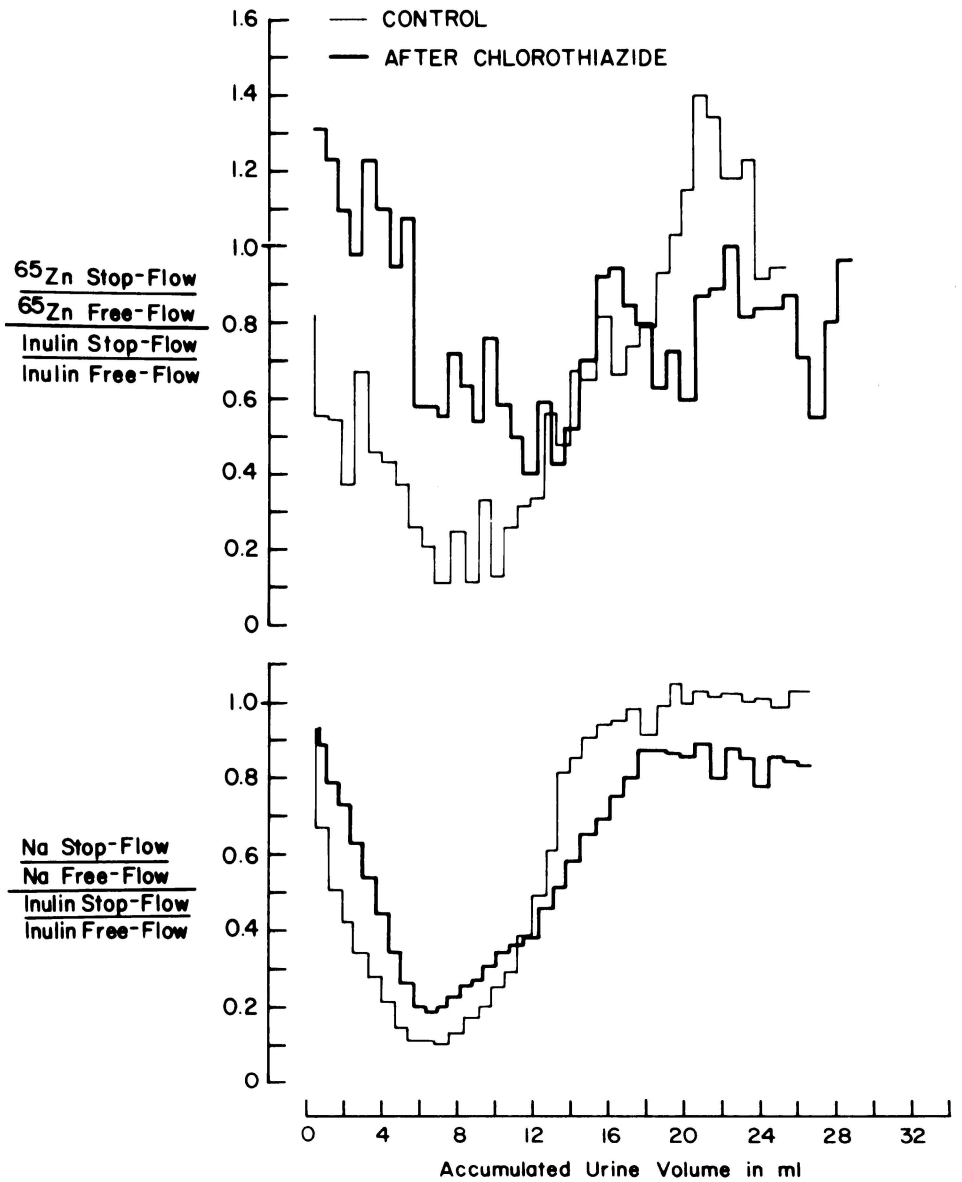

FIG. 3. Stop-flow patterns for ^{65}Zn and Na prior to and approximately 20 min subsequent to chlorothiazide treatment (see text for description).

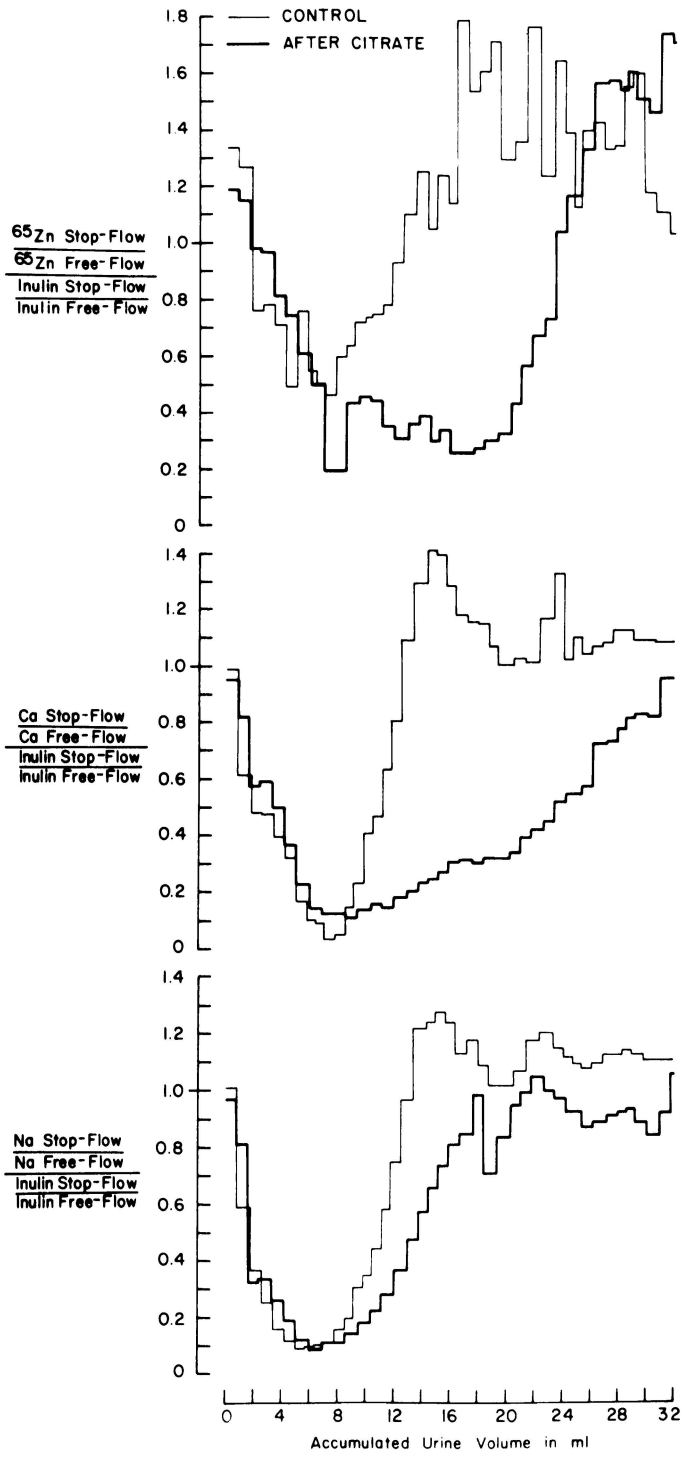

FIG. 4. Example of stop-flow patterns for ^{65}Zn, Ca, and Na before and 20 min after citrate treatment (see text for dose).

compared with our 1.5%. Because their value was obtained by using the Amicon UM-2 membrane (mol wt cutoff 1,000 daltons), we prepared, using the UM-2 filter, ^{65}Zn ultrafiltrates from plasma obtained from dogs undergoing antidiuresis. We found that same value, 9–10%, as Yunice et al. (23). However, all of these samples tested heavily positive for protein (100–300 mg/dl), which could easily explain their high value for ultrafilterable [Zn]. This leakage of protein may be due to the fact that UM-2 filtrates are prepared under 70 psi pressure for several hours. Our ability to reproduce their result also argues strongly that the difference in ultrafilterability between the two studies does not reflect a difference between the ultrafilterability of endogenous Zn and that of acutely administered ^{65}Zn. Finally, our value of 1.5%, achieved using the Amicon CF-50A filter, is virtually identical to that reported by Prasad and Oberleas (6) who used a totally different dialysis technique.

It must be emphasized that the present experiments were performed while plasma ^{65}Zn was stabilized by a continuous infusion. Rubini et al. (7) reported clearance values for a single intravenous ^{65}Zn injection that indicated that the clearance of plasma zinc was very low immediately after injection and then rose to extremely high values after plasma concentration had fallen to barely detectable levels. No measurement was made of ultrafilterable [Zn], and the very high clearance values were almost certainly artifacts resulting from non-steady-state conditions (15).

The stop-flow patterns for control dogs further substantiate the view that net reabsorption predominates under basal conditions, since distal reabsorption was the only consistently observed event.

The second aim of these experiments was to evaluate the mechanisms underlying the hyperzincuria produced by various agents. The induction of mannitol diuresis significantly elevated fractional excretion of zinc with no change in ultrafilterable [Zn]. This effect was probably the result of enhanced tubular flow rates rather than of specific changes in tubular pathways for Zn transport. Steele (11) has reported a small increase in Zn excretion in human subjects during volume expansion with saline, and this increase correlated with the associated change in urine flow.

Chlorothiazide was studied in the present experiments because, in contrast to other antidiuretic drugs, it has been found to increase Zn excretion (4, 22). In the clearance experiments reported here, chlorothiazide's hyperzincuric effect was clearly on the renal tubular handling of Zn, since the drug did not affect ultrafilterable [Zn]. Stop-flow analysis revealed that chlorothiazide induced far distal tubular secretion of Zn and that, in addition, the distal reabsorptive minimum for Zn was elevated.

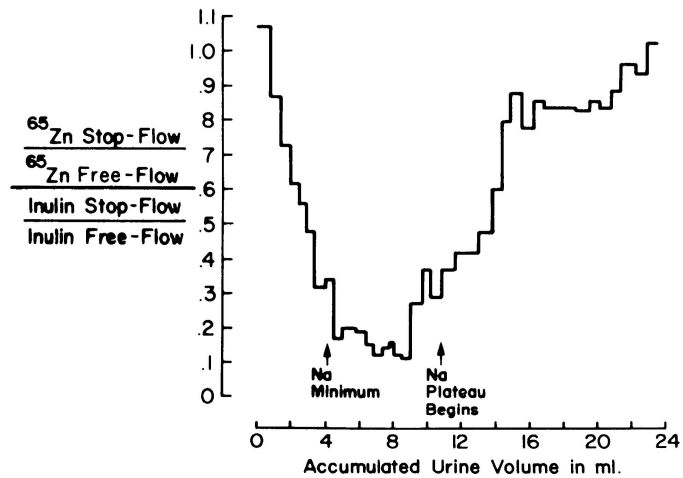

FIG. 5. Stop-flow pattern for ^{65}Zn obtained during histidine infusion.

The latter might possibly be due to a decrease in distal reabsorption of Zn, but it is also possible that this elevated minimum is the result of the induction of far distal secretion. In other words, the far distal secretory area for Zn sensitive to chlorothiazide may also extend into the distal reabsorptive area so that the distal minimum would be the resultant of simultaneously occurring reabsorptive and secretory movements (as is the case for potassium, the stop-flow pattern for which is altered in a similar manner by chlorothiazide [14]).

Cysteine and histidine were infused because it has been postulated that these anions are the major low molecular weight ligands for Zn in plasma (10) and that alternations in their plasma concentrations are major determinants (acting via changes in the filtered load of Zn) of Zn excretion (16). Citrate might also be a potential ligand for Zn in plasma. All three anions increased both absolute zinc excretion and fractional excretion, but the clearance and stop-flow data taken together suggest that the mechanisms underlying these changes vary for the three anions.

Citrate, for example, did not alter ultrafilterable [Zn], and its effect (at least at the doses used) must, therefore, be exerted mainly on tubular transport. The stop-flow pattern revealed no Zn secretion after citrate infusion, so it is probable that the key effect is inhibition of tubular reabsorption, most likely resulting from the binding of Zn by citrate within the tubular lumen. (That such binding would occur at this site but not in plasma is not surprising given the changes that occur in anion concentrations and pH as fluid flows through the tubule.) It might seem paradoxical that a substance that retards reabsorption would be associated with a clear-cut stop-flow proximal reabsorption pattern not seen in the absence of the anion. However, this is precisely what one would predict since, as analyzed elsewhere for lead (20), anions might unmask a proximal reabsorptive site for an action during stop-flow by preventing achievement of a limiting concentration for that cation during free-flow.

Cysteine and histidine, unlike citrate, produced a marked increase in ultrafilterable [Zn], and this effect can account for a large fraction, perhaps all, of the hyperzincuria produced by these two anions, as has been suggested by others (16, 23). In addition, it is clear that for any given increment in the filtered load of Zn, cysteine induces a much greater increase in fractional excretion of Zn than does histidine. A likely possibility is that cysteine retards Zn reabsorption to a greater degree than does histidine, a view consistent with the stop-flow data. Another theoretical possibility is that cysteine might induce tubular secretion of Zn. One dog did, in fact, manifest a fractional Zn excretion of 1.25 during cysteine infusion, but give the uncertainties inherent in quantifying ultrafilterable Zn, a single value of this magnitude cannot be construed as conclusive or even strong evidence. Against the existence of tubular secretion is the fact that the stop-flow patterns for Zn during cysteine infusion yielded no evidence for a secretory pathway, even in the one animal whose fractional excretion had exceeded unity.

Our findings for cysteine constitute another difference from those of Yunice et al. (23), who reported that infusions of similar quantities of cysteine produced fractional excretion values for zinc consistently well above unity, i.e., net secretion. We believe, however, that these values, like the values obtained in the basal state, may be invalidated by problems in the UM-2 ultrafiltration technique. Consistent with this view is the fact that these authors observed no increase in ultrafilterable [Zn] during cysteine infusion despite the fact that cysteine is known to be a strong ligand for zinc. In contrast, at the same dose our ultrafiltration technique showed a 20-fold increase in ultrafilterability. As described for plasma obtained under basal situations, we verified that this discrepancy was due to the difference in filtration technique (not the use of ^{65}Zn) by finding that use of the UM-2 filter yielded a value for ultrafilterability of ^{65}Zn of 10%, unchanged from basal, in plasma obtained from cysteine-infused dogs. The question of why the UM-2 filter should underestimate ultrafilterability of Zn in cysteine-rich plasma was not studied.

In summary, this combination of clearance and stop-flow techniques has revealed that the amount of Zn excreted is normally the resultant of filtration and reabsorption. At least one drug—chlorothiazide—induces far distal secretion, whereas citrate and amino acids influence mainly filtration and/or reabsorption. Which pathways are altered in response to physiological inputs or in pathological states remains to be determined.

We thank Allan Klaiman, Ulana Bereza, and Peter Schoeps for their technical assistance, and Judy Schoonmaker for preparing the illustrations.

The investigation was supported by Grants 5R01-ES01141 from the National Institute of Environmental Health Sciences and 5R01-OH00913 from the National Institute of Occupational Safety and Health, and by a grant from the Kidney Foundation of Michigan.

W. Victery is a postdoctoral scholar supported by grants from the Kidney Foundation of Michigan and the National Institute of Environmental Health Sciences.

An abstract of this study was previously published in *Federation Proc.* (38: 1392, 1979).

Received 30 October 1980; accepted in final form 27 May 1981.

REFERENCES

1. ABU-HAMDAN, D. K., S. D. MIGDAL, R. WHITEHOUSE, P. RABBANI, A. S. PRASAD, AND F. D. MCDONALD. Renal handling of zinc: effect of cysteine infusion. *Am. J. Physiol.* 241 (*Renal Fluid Electrolyte Physiol.* 10): F487–F494, 1981.
2. BONSNES, R. W., AND H. H. TAUSSKY. On the colorimetric determination of creatinine by the Jaffee reaction. *J. Biol. Chem.* 158: 581–591, 1945.
3. MALVIN, R. L., AND W. S. WILDE. Stop-flow technique. In: *Handbook of Physiology. Renal Physiology*, edited by J. Orloff and R. W. Berliner. Washington, DC: Am. Physiol. Soc., 1973, p. 119–128.
4. PAK, C. Y. C., B. RUSKIN, AND E. DILLER. Enhancement of renal excretion of zinc by hydrochlorothiazide. *Clin. Chim. Acta* 39: 511–517, 1979.
5. PRASAD, A. S. Clinical, biochemical and pharmacological role of zinc. *Annu. Rev. Pharmacol. Toxicol.* 20: 393–426, 1979.
6. PRASAD, A. S., AND D. OBERLEAS. Binding of zinc to amino acids and serum proteins in vitro. *J. Lab. Clin. Med.* 76: 416–425, 1970.
7. RUBINI, M. E., G. MONTALVO, C. P. LOCKHART, AND C. R. JOHNSON. Metabolism of zinc^{65}. *Am. J. Physiol.* 200: 1345–1348, 1961.
8. SANDSTEAD, H. H., K. P. VO-KHACTU, AND N. SOLOMONS. Condi-

tioned zinc deficiencies. In: *Trace Elements in Human Health and Disease*, edited by A. S. Prasad and D. Oberleas. New York: Academic, 1976, p. 33–49.
9. SCHREINER, G. E. Determination of inulin by means of resorcinol. *Proc. Soc. Exp. Biol. Med.* 74:117–120, 1950.
10. SIGEL, H. *Mixed-Ligand Complexes.* New York: Dekker, 1973, vol. 2, p. 2–31, 168–202.
11. STEELE, T. H. Dissociation of zinc excretion from other cations in man. *J. Lab. Clin. Med.* 81: 205–213, 1973.
12. SULLIVAN, L. P., W. S. WILDE, AND R. L. MALVIN. Renal transport sites for K, H, and NH_3. Effect of impermeant anions on their transport. *Am. J. Physiol.* 198: 244–254, 1960.
13. VANDER, A. J. Potassium secretion and reabsorption in distal nephron. *Am. J. Physiol.* 201: 505–510, 1961.
14. VANDER, A. J., R. L. MALVIN, W. S. WILDE, AND L. P. SULLIVAN. Localization of the site of action of chlorothiazide by stop-flow analysis. *J. Pharmacol. Exp. Ther.* 125: 19–22, 1959.
15. VANDER, A. J., D. L. TAYLOR, K. KALITIS, D. R. MOUW, AND W. VICTERY. Renal handling of lead in dogs: clearance studies. *Am. J. Physiol.* 233 (*Renal Fluid Electrolyte Physiol.* 2): F532–F538, 1977.
16. VAN RIJ, A. M., P. J. GODFREY, AND J. M. MCKENZIE. Amino-acid infusion and urinary zinc excretion. *J. Surg. Res.* 26: 293–299, 1979.
17. VICTERY, W., R. LEVINSEN, AND A. J. VANDER. Effect of glucagon on zinc excretion in anesthetized dogs. *Am. J. Physiol.* 240 (*Renal Fluid Electrolyte Physiol.* 9): F299–F305, 1981.
18. VICTERY, W., N. E. SOIFER, J. S. WEISS, AND A. J. VANDER. Acute effects of lead on the renal handling of zinc in dogs. *Toxicol. Appl. Pharmacol.* In press.
19. VICTERY, W., A. J. VANDER, AND D. R. MOUW. Effect of acid-base status on renal excretion and accumulation of lead in dogs and rats. *Am. J. Physiol.* 237 (*Renal Fluid Electrolyte Physiol.* 6): F398–F407, 1979.
20. VICTERY, W., A. J. VANDER, AND D. R. MOUW. Renal handling of lead in dogs: stop-flow analysis. *Am. J. Physiol.* 237 (*Renal Fluid Electrolyte Physiol.* 6): F408–F414, 1979.
21. VISHWAKARMA, P., AND T. MILLER. Renal tubular transport of citrate: relations with calcium. *Am. J. Physiol.* 205: 281–285, 1963.
22. WESTER, P. O. Trace elements and urine from hypertensive patients before and during treatment with chlorithalidone. *Acta Med. Scand.* 194: 505–512, 1973.
23. YUNICE, A. A., R. W. KING, JR., S. KRAIKITPANITCH, C. C. HAYGOOD, AND R. D. LINDEMANN. Urinary zinc excretion following infusions of zinc sulfate, cysteine, histidine, or glycine. *Am. J. Physiol.* 235 (*Renal Fluid Electrolyte Physiol.* 4): F40–45, 1978.