Fractional reabsorption is substantial and is seen to be independent of urine flow rate. Fractional reabsorption in the collecting ducts can also be estimated from individual experiments by measuring the change in velocity of the leading edge of the urinary bolus as it proceeds through the papillary ducts. Such measurements give similar values for the fractional reabsorption.

The results show that in spite of the fast flow of urine through the collecting duct, fractional fluid reabsorption is substantial. The mechanism for this reabsorption must now be re-examined in light of the new information. The authors wish to thank Dr. Clifford Patlak for helpful discussions. This work was supported by NIH grant 5 RO1 AM15972-09.

FREE AMINO ACID POOLS IN INTERTIDAL NEMERTINA AND OLIGOCHAETA

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Two species of worms, Procephalothrix spiralis (Nemertina; Anopla) and Clitellio arenarius (Annelida; Oligochaeta) were analyzed for free amino acids in order to focus on the amino acids potentially available for cell volume regulation. Worms were collected simultaneously from under rocks at Salsbury Cove, Maine, and maintained at 7°C in recirculating seawater aquaria without food for at least one week prior to analysis. Intact specimens of each species were pooled due to small size, gently drained of adhering seawater, weighted (C. arenarius 77.58 mg and P. spiralis 33.47 mg wet weight) and homogenized in 95% EtOH. Homogenates were washed (95% EtOH) into glass vials, placed in a boiling water bath, allowed to stand 30 minutes and subsequently freeze dried. For free amino acid analysis (Table 1), freeze-dried samples were dissolved in 0.2 N lithium citrate buffer pH 2.83 and then developed on a column of Beckman AA-10 resin (2.8 x 300 mm) using three buffer systems (I - 0.2 N lithium citrate, pH 2.83, II - 0.2 N lithium citrate, pH 3.7 and III - 1.0 N lithium citrate, pH 3.75) on a Beckman amino acid analyzer model 119 CL AA.

Table 1. Pool sizes of free amino acids in clitellio arenarius and procephalothrix

Major Differences in Pool Sizes Amino Acid	mmoles/mg dry wt CA	PS
Taurine	< 0.2	308.6
Aspartic Acid	3.6	41.2
Asparagine	114.0	3.6
Proline	24.2	0.2
Glycine	7.6	134.9
Arginine	2.3	72.1

Other Amino Acids Amino Acid	mmoles/mg dry wt CA	PS
Serine	14.1	8.2
Glutamic Acid	9.1	9.7
Glutamine	4.0	< 0.2
Alanine	64.0	26.5
Valine	3.8	< 0.2
Ethanolamine	< 0.2	6.7
Ammonia	22.2	7.8
Ornithine	< 0.2	3.8
Lysine	< 0.2	12.8
Histidine	< 0.2	3.6

Species differences were apparent in pool sizes of total free amino acids and particularly in the amino acids comprising that in each species. C. arenarius (CA) was found to have a total free amino acid pool size of 247.7 n moles/mg

dry wt compared to 632.2 n moles/mg dry wt in P. spiralis (PS) (sum of columns, Table 1). Such a species difference in the pool size of free amino acids may reflect a greater dependence on free amino acids for cell volume regulation in P. spiralis. Of particular significance were the major differences in the pool sizes of the individual free amino acids between species (Table 1). The major free amino acids in C. arenarius were asparagine, proline and alanine which made up 61.6% of the total free amino acid pool. However, in P. spiralis, taurine, aspartic acid, glycine and arginine comprised 88.1% of the free amino acis. Of these, it is interesting that the levels of proline, alanine, taurine and glycine are most commonly reported to be affected during regulatory volume decrease in marine invertebrates. Hence, it may be expected that C. arenarius utilizes asparagine, proline and/or alanine during regulatory volume decrease, whereas in P. spiralis taurine, glycine and/or arginine would be most available. This work was supported by NIH grant 5 RO1 AM15973-09.

INHIBITION OF CYCLIC AMP-STIMULATED CHLORIDE TRANSPORT IN THE RECTAL GLAND OF SQUALUS ACANTHIAS BY A RELATED SERIES OF "LOOP" DIURETICS

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Coupled NaCl cotransport systems have been suggested to occur in a number of epithelial tissues (for review, see Frizzell, Am. J. Physiol. 236:F1, 1978) including dogfish rectal gland, where they may contribute to active Cl transport across the tissue layer. In many of these cases active Cl movements are susceptible to inhibition by the diuretic furosemide. However, high concentrations (~1 mM) of this drug are frequently needed to block transport, and this has raised questions as to the specificity of furosemide's action. Recently, a series of benzoic acid derivatives related to furosemide has been synthesized (Neilsen and Feit, Am. Chem. Soc. Symp., 83:12, 1978), several of which have been shown to be potent diuretics in vivo in dogs. It has also been shown that representatives of this group of agents are highly active as specific inhibitors of a cyclic AMP activated Cl-dependent NA^+ plus K^+ contransport system in avian erythrocytes (Palfrey, Feit and Greengard, Am. J. Physiol., in press, 1979). The relative potency of these compounds in erythrocytes correlated well with their diuretic efficacy, measured as Na^+ excretion, in dogs; some analogs being totally ineffective in both systems. We tested various members of this series on total fluid and Cl secretion by the perfused dogfish rectal gland with the purpose of: (a) comparing the relative potency of the compounds in this tissue with that already established in erythrocytes, and (b) finding inhibitors that would be more potent, and possibly more specific, than furosemide.

Dogfish rectal glands were perfused and volume and Cl analyses performed as previously described (Soloman et al., Bull. MDIBL., 17:59, 1977). Dibutyryl cyclic AMP (0.05 mM) and theophylline (0.1 mM) were routinely added to the perfusate to obtain maximal secretory rates. The diuretic compounds used in this study were obtained from Dr. P.W. Feit, Leo Pharmaceutical Products, Ballerup, Denmark and their structures are indicated in Table 1. Stock solutions were made up in DMSO at 1000X the final concentration in the perfusion medium; 0.1% DMSO alone was found to have no effect on fluid or electrolyte secretion by the rectal gland. Ten minute collection periods were employed, the usual experimental protocol being 30 min control (no drug), 30 min drug, 30 min control. Occasionally more samples were collected when incomplete effects of a drug or reversal were suspected. The results for total fluid and Cl secretion by the rectal gland are compiled in Table 1.

The most potent analog tested was 3-benzylamino-4-phenyl-5-sulfamoylbenzoic acid (Compound I), greater than half-maximal inhibition (IC_50) with this compound being obtained at $< 10^{-6}$ M. This agent was thus more than two hundred times as potent as furosemide ($IC_{50} > 5 \times 10^{-4}$ M). The relative efficacy of the series of compounds closely matched that found previously in the avian erythrocyte (Table 1), although the sensitivity of the rectal gland trans-