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COMPARATIVE BIOCHEMICAL STUDIES OF OSMOREGULATION IN SIPUNCULA—I. STEADY-STATE CHARACTERISTICS OF TWO SIPUNCULIDS IN FULL-STRENGTH SEA WATER

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Abstract—1. Chemical analyses are reported for centrifuged coelomic fluid, body wall, retractor muscles and coelomocytes of the Sipunculids *Themiste dyscritum* and *Phascolopsis gouldi* acclimated to full-strength sea water.

2. Both species are isosmotic to sea water. The major coelomic fluid solutes are sodium and chloride; free amino acids (FAA) and glucose do not significantly contribute to the coelomic fluid osmotic pressure.

3. Relative to ambient conditions, *P. gouldi* is slightly hyperionic in Na and iso-ionic in Ca; *T. dyscritum* is iso-ionic in Na and hypo-ionic in Ca. Both species show elevated levels of K and lower levels of Cl, Mg, SO_4 and orthophosphate.

4. All tissues studied appear closely isosmotic to coelomic fluid (*T. dyscritum*). Half to three-fourths of the total measured solute concentration of the tissues is contributed by FAA, phosphate and K.

5. Glycine is the major constituent (*ca.* 80%) of the FAA pool of *T. dyscritum* retractor muscle. Also present in greater than 10 mM concentrations are glutamate, isoleucine, threonine, serine and aspartate.

6. Orthophosphate appears more concentrated in Sipunculid muscle tissue (body wall, retractor muscle) than in coelomocytes. Coelomocytes are possibly distinct in having a higher intracellular glucose level.

INTRODUCTION

THE SIPUNCULA is a small and exclusively marine group of invertebrates (Hyman, 1959; Kohn & Rice, 1972) and has been the subject of numerous studies on osmoregulation (e.g. Botazzi, 1908; Adolph, 1936; Steinbach, 1940; Gross, 1954; Larson, 1964; Virkar, 1966; Oglesby, 1968; Hogue & Oglesby, 1972). In the most recent and extensive review of the osmotic physiology of this group, Oglesby (1969) stresses the dearth of basic information on the chemical composition of the coelomic fluid. Only six species have been examined for more than chloride content, and the data on three are practically worthless since these studies dealt with uncentrifuged coelomic fluid samples, containing variable and unknown numbers of coelomocytes (Hogue & Oglesby, 1972). The remaining studies generally suggest

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weak ionic regulation of the coelomic fluid in high salinities; however, the degree to which specific ions are regulated appears to vary with the species (Oglesby, 1969; Hogue & Oglesby, 1972).

The only tissue analyses of sipunculids acclimated to full strength sea water are those reported by Duchâteau & Florkin (1952) of individual free amino acids from retractor muscles of *Sipunculus nudus*; by Virkar (1966) of the total amino acid pool in *Phascolopsis gouldi* body wall; by Steinbach (1940) of several inorganic ions of *P. gouldi* retractor muscle; and by Towle & Giese (1966) for proteins, lipids and carbohydrates in various tissues of *Phascolosoma agassizi*. For no single species have the tissue osmotic constituents been fully described. Understanding of sipunculid osmotic physiology clearly requires additional information on steady-state levels of intracellular and coelomic fluid solutes.

This paper is the first of three reports on hydromineral balance of the Sipuncula. It is the purpose of the present paper to define for *Themiste dyscritum* and *P. gouldi*, two North American sipunculids commonly used in laboratory studies, the major osmotically important constituents of several tissues and the coelomic fluid of worms acclimated to full-strength sea water. In two future papers (Foster, 1974a, b) the changes in tissue and coelomic fluid solutes will be reported for these species exposed to salinities from 50% to 130% sea water.

MATERIALS AND METHODS

Source and identification

The two species of Sipuncula used in this study were *Phascolopsis* (= *Golfingia*) *gouldi* (Fisher, 1950) and *Themiste* (= *Dendrostomum*) *dyscritum* (Fisher, 1952). *P. gouldi* was obtained from the Supply Department, Marine Biological Laboratory, Woods Hole, Massachusetts, and was identified according to Smith (1964) following Stephen's (1964) revision of Sipuncula genera. *T. dyscritum* were either personally collected or supplied by Dr. Rimmon Fay, Pacific Bio-marine Supply Company, Venice, California. The commercial supply of *T. dyscritum* (Dr. Rimmon Fay, personal communication) and those sipunculids personally field collected were from the same local population inhabiting the lower intertidal cobble zone at Little Dume Point, Malibu, California. Identification of *T. dyscritum* was based on Fisher's (1952) taxonomic key following the revisions by Stephen (1964). A related and morphologically similar species is *T. zostericolum*. According to Fisher's (1952) taxonomic key, these species are positively identified by the arrangement and site of attachment of three, thread-like fixing muscles running between the body wall and sites on the post-esophageal, intestinal surface.

This method of identification proved impractical in the present study in which several hundred animals were involved. Therefore, from each lot of sipunculids supplied by Pacific Bio-marine all individuals of greater length than 150 mm (relaxed) were eliminated, the rationale being that *T. dyscritum* presumably reaches an upper length of 170 mm while *T. zostericolum* may achieve a length of more than 245 mm (Fisher, 1952). Then, several specimens from the remaining supply were identified according to the arrangement of the three internal fixing muscles. The majority of these worms were identified as *T. dyscritum*, although, on the average, perhaps 20 per cent were *T. zostericolum*. It is important to note that several such identifications were inconclusive due to abnormal or novel arrangements of fixing muscles not discussed by Fisher (1952).

The worms were maintained in 10–15 gallon aquaria of aerated full-strength ("100%") artificial sea water ("Instant Ocean", Aquarium Systems, Inc., Wickliffe, Ohio) kept at

$15 \pm 2°C$, pH 7·8. The sipunculids were allowed to burrow freely through a 3-in. deep substratum of washed and crushed oyster shells. For all *T. dyscritum* studies, 100% sea water (SW) is defined as containing 560 mM Cl^- (580 mM Cl/kg H_2O) and represents approximately 105% Los Angeles sea water. For *P. gouldi* 100% SW is defined as 503 mM Cl^- (522 mM Cl/kg H_2O) and is equivalent to approximately 101% Woods Hole SW.

Following an acclimation period of 1–1½ weeks, the worms were transferred to aquaria containing 2–4 gallons of 100% SW but without crushed oyster shells, until it appeared that all solid material had been voided from their guts (1–2 days). The animals were then collected, gently blotted with paper towelling and dissected.

Sampling procedure

Samples of medio-anterior trunk body wall, retractor muscles of the introvert, coelomocytes and coelomic fluid were isolated by a routine sequence of operations carried out at 15°C and generally completed within 5 min for each individual. *Coelomic fluid* (*ca.* 3 cm^3) was collected in glass vials, covered with Parafilm and centrifuged for 5 min at 1350 ***g***. The supernatant cell-free coelomic fluid was decanted into a similar vial and sealed with Parafilm. The volume of the coelomocyte pellet was determined using micro-hematocrit capillary tubes. This pellet contained primarily hemerythrocytes, although other coelomocyte types and gametes were common but in small proportion (Tétry, 1959). *Body wall* segments (200–500 mg) were wiped free of adhering coelomic fluid, and the ventral nerve cord was removed. Retractor muscles (200–300 mg) were excised with scissors at points approximately ⅛ in. from their attachments on the body wall and on the esophageal–tentacular complex. All tissue and coelomic fluid samples were immediately stored in 1-dram glass vials, securely sealed with Parafilm and frozen.

Analytical methods

Free amino acids. Total free amino acids of coelomic fluid and tissues extracted and deproteinized in 80% (v/v ethanol) were estimated as ninhydrin-positive substances (NPS) according to the colorimetric method of Rosen (1957).

Proline and hydroxyproline do not give the characteristic purple color with ninhydrin and are therefore not included in the estimate. Many classes of compounds containing α-amino groups will react with ninhydrin (Rosen, 1957). It is judicious therefore to consider these data as estimates of the "total ninhydrin-positive materials".

In one study the soluble amino acids of *T. dyscritum* retractor muscles were extracted in 3% sulfosalicylic acid at 5°C for 2 days, and the sample heated in a boiling water bath for 5 min to precipitate proteins. The neutral and acidic free amino acids were then assayed by conventional methods on a Phoenix automatic amino acid analyzer equipped with a 150-cm resin bed.

Sodium, potassium, magnesium, calcium. Tissue sodium and potassium were quantitatively extracted with 80% ethanol; magnesium and calcium were extracted with 2% (w/v) trichloroacetic acid (TCA). Aliquots of these samples were suitably diluted with distilled water (Na, Mg) or with a solution of 1% La_2O_3 in 5% HCl containing 100 mM NaCl (Ca, K). La^{2+} in this solution protects Ca^{2+} from interference by phosphate and aluminum, while the high Na level controls for interference from Rb, Li and Ca in the potassium analyses (Instruction Manual to the Perkin–Elmer 290B). All samples were measured with a Perkin–Elmer 290B atomic absorption spectrophotometer using an air–acetylene combustion mixture and a three-slot burner head (No. 303–0202). Working standards of each ion were prepared from 1000 μg/l. stock solutions using distilled water (Na, Mg) or the lanthanum solution (Ca, K).

Chloride. Chloride was measured by electrometric titration on an Aminco-Cotlove Automatic Chloride Titrator equipped with the direct millequivalent Readout accessory. Standard assay procedures were followed for coelomic fluid, sea water and 80% EtOH tissue extracts (Cotlove *et al.*, 1958; Cotlove & Nishi, 1961).

Sulfate. The soluble, protein-free sulfate level of 80% EtOH tissue and coelomic fluid extracts and sea water was determined turbidmetrically as the barium sulfate precipitate at 420 mμ on a Coleman spectrophotometer. Except for a reduction in volumes and use of a saturated $BaCl_2$ solution rather than crystals, the analytic details followed the parent method (*Standard Methods for the Examination of Water and Waste Water* (*S.M.E.W.W.W.*), 1965).

Phosphate. Orthophosphate was assayed colorimetrically at 625 mμ as the molybdenum blue complex by the standard stannous chloride method (Section B, *S.M.E.W.W.W.*, 1965). Ethanol extracts of body wall or retractor muscle were dried at 80°C and the residue taken up with distilled water. Coelomocytes were first lysed in distilled water before analysis, but coelomic fluid and sea water were measured directly.

Ammonia . The Permutit method of Folin & Bell (1917) and the microdiffusion method of Seligson & Seligson (1951) were used for the determination of tissue and fluid ammonia. Both methods determine ammonia spectrophotometrically at 500 mμ as the dimercuric ammonium iodide complex resulting from nesslerization.

pH. The pH of uncentrifuged coelomic fluid was determined immediately following collection using a micro-electrode assembly on a Leeds and Northrup pH meter with scale expansion.

Glucose. A glucose oxidase assay (Sigma) was used to estimate glucose in 1 N $Ba(OH)_2$ extracts of coelomic fluid and tissues after protein precipitation with $ZnSO_4$.

Water. Tissues and coelomic fluid water contents were determined from the wet weight–dry weight difference after drying a sample to constant weight on tared aluminum foil pans at 100 ± 5°C. Weights were recorded on a Mettler balance, Model P-1200, or a Roller–Smith Precision Balance to the nearest 0·001 g.

Osmotic pressure. The determinations of sea water and coelomic fluid osmotic pressure (osmolarity) were performed on a Mechrolab vapor pressure osmometer, Model 301A, using standard methods and NaCl reference standards. Only freshly collected, centrifuged samples of coelomic fluid were assayed.

Statistics

Variability for all data is expressed as the mean ± standard deviation.

RESULTS AND DISCUSSION

Coelomic fluid

The analyses of *T. dyscritum* and *P. gouldi* coelomic fluid (CF) presented in Table 1 are the most detailed reports concerning steady-state solute levels of centrifuged body fluids of any sipunculid species. In general, both species show weak inorganic ion regulation; the individual electrolyte levels closely approximate those in the ambient sea water (CF/SW ratio *ca.* 1·00). These coelomic fluids are not strikingly different from sea water in their general salt composition, a feature suggested as early as 1852 by Williams and in several more recent reports (Bethe & Berger, 1931; Bialascewicz, 1933; Oglesby, 1968, 1969; Hogue & Oglesby, 1972).

Hypo-ionic regulation of Mg, Cl, PO_4 and possibly SO_4 and hyper-ionic K balance are characteristic of both worms. Coelomic fluid Na is elevated above sea-water level in *P. gouldi* but is iso-ionic for *T. dyscritum.* Conversely, *T. dyscritum* appears to maintain Ca below ambient level but *P. gouldi* is iso-ionic. In a study dealing with the time course of coelomic fluid ion adjustments to sudden osmotic stress (50–130% SW), *T. dyscritum* maintained in 100% SW for 7 days, as in the present work, was also iso-ionic in Ca balance. In this study, however,

TABLE 1—STEADY STATE COMPOSITION OF COELOMIC FLUID COMPONENTS OF *T. dyscritum* AND *P. gouldi* IN 100% SW*

Solutes	*T. dyscritum*		*P. gouldi*	
	Conc.	CF/SW	Conc.	CF/SW
Na^+	486·2 ± 9·9 (N = 3)	0·998	456·8	1·063
Cl^-	558·5 ± 6·5 (N = 8)	0·963	508·0	0·973
K^+	11·67 ± 0·63 (N = 3)	1·053	12·50	1·179
Mg^{2+}	44·00 ± 3·28 (N = 4)	0·799	33·02	0·651
Ca^{2+}	10·13 ± 0·89 (N = 4)	0·911	11·67	0·997
SO_4^-	26·2 ± 2·2 (N = 8)	0·989	22·3	0·937
PO_4^{2-}†	33·3 ± 2·6 (N = 3)	0·411	39·6	0·541
NH_4^+	0·31 ± 0·25 (N = 5)	—	—	—
NPS‡	3·74 ± 1·81 (N = 18)	—	1·42	—
Glucose	2·2 ± 1·6 (N = 4)	—	—	—
Protein§	*ca.* 0·07	—	—	—
pH	7·53 ± 0·31 (N = 11)	0·964	7·84	1·004
Density (24°C)	1·024 ± 0·005 (N = 9)	—	1·024 ± 0·003 (N = 4)	—
H_2O‖	96·28 ± 0·50 (N = 9)	—	96·01 ± 0·96 (N = 4)	—
Osmotic pressure (mOsm)	1043 ± 32 (N = 13)	0·993	—	—
Total solutes	1142·6	0·999	1045·3	1·020

Results are expressed as m-moles/kg H_2O and represent the average ± SD (*T. dyscritum*) and the pooled mean (N = 5) (*P. gouldi*).

* 100% SW is defined as containing 580 mM Cl^-/kg H_2O for *T. dyscritum* and 522 m-moles Cl^-/kg H_2O for *P. gouldi*.

† Total orthophosphate, μmoles/kg H_2O.

‡ NPS = ninhydrin-positive substances.

§ Sulfosalicylic acid estimation, g BSA equivalents/100 ml.

‖ g H_2O/100 ml.

during the next 11 days the condition became progressively hypo-ionic (CF/SW = 0·858, N = 10) (Foster, 1974a). Possibly *T. dyscritum* in the field maintains iso-ionic Ca balance but slowly becomes hypo-ionic after prolonged maintenance in the laboratory, as already reported for Cl in this species (Hogue and Oglesby, 1972).

Freshly collected, uncentrifuged coelomic fluid of both species is slightly alkaline but no large hydrogen-ion gradient appears to occur across the body wall. The concentrations of organic solutes such as amino acids, glucose and proteins are low and presumably of minor osmotic importance. The low protein level also suggests little Gibbs–Donnan effect on the general ion distribution arising from charged proteins in the coelomic fluid.

An expected, consequence of weak ionic regulation of the body fluids is osmotic equilibrium between the coelomic fluid and sea water. Only centrifuged coelomic fluid of *T. dyscritum* was measured for osmotic pressure. This fluid is nearly

isosmotic to the medium (CF/SW = 0·993, $N = 13$), in close agreement to CF/SW values of 1·004 and 1·015 reported by Oglesby (1969) and by Hogue & Oglesby (1972), respectively. Likewise, the total coelomic fluid solute concentration of both sipunculids nearly matches that of sea water. This total solute concentration equivalence strongly suggests that *P. gouldi* coelomic fluid is also isosmotic to 100% SW.

Overall, the coelomic fluids of *T. dyscritum* and *P. gouldi* appear isosmotic in the steady state in 100% SW, with the total solute concentration almost entirely made up by inorganic electrolytes—in particular, by sodium and chloride. Both worms show weak electrolyte regulation, yet quantitative differences (relative to ambient salt levels) do occur. Ion balance of *P. gouldi* coelomic fluid is unique and although "100% SW" salinity for this species is approximately 90 per cent that defined as 100% SW for *T. dyscritum*, the coelomic fluid of *P. gouldi* is clearly not equivalent to a diluted form of *T. dyscritum* coelomic fluid.

Tissues

Chemical analyses of body wall, retractor muscle and coelomocytes of *T. dyscritum* and *P. gouldi* are presented in Tables 2,3,4 and 5.

TABLE 2—STEADY STATE COMPOSITION OF BODY WALL FROM *T. dyscritum* AND *P. gouldi* IN 100% SW*

	T. dyscritum		*P. gouldi*	
Solutes	Conc.	BW/CF	Conc.	BW/CF
Na^+	235·3 ± 56·1 ($N = 9$)	0·484	—	—
Cl^-	284·1 ± 30·2 ($N = 5$)	0·524	184·2	0·363
K^+	88·62†	7·884	—	—
Mg^{2+}	42·80 ± 5·65 ($N = 4$)	0·770	—	—
Ca^{2+}	15·31 ± 3·81 ($N = 3$)	1·511	—	—
SO_4^{2-}	23·1 ± 6·9 ($N = 5$)	0·882	37·2	1·668
NH_4^+	44·1 ± 7·0 ($N = 8$)	142·3	—	—
PO_4^{3-}‡	14·66 ± 2·83 ($N = 9$)	*ca.* 440	4·64	*ca.* 117
NPS	302·6 ± 13·5 ($N = 5$)	80·91	389·9	389·9
Glucose	4·18 ± 1·89 ($N = 6$)	1·900	—	—
H_2O§	68·60 ± 0·70 ($N = 5$)	—	71·59 ± 2·10 ($N = 8$)	—
Total solutes	1010·7	0·885	615·9	0·589

Results are expressed as m-moles/kg tissue H_2O and represent the average ± S.D. (*T. dyscritum*) or the pooled mean, $N = 5$ (*P. gouldi*).

* 100% SW is defined as containing 580 mM Cl^-/kg H_2O for *T. dyscritum* and 522 m-moles Cl^-/kg H_2O for *P. gouldi*.

† Pooled mean, $N = 5$.

‡ Total orthophosphate, μmoles/kg H_2O.

§ g H_2O/100 g tissue.

TABLE 3—STEADY STATE COMPOSITION OF RETRACTOR MUSCLE FROM *T. dyscritum* AND *P. gouldi* IN 100% SW*

Solutes	*T. dyscritum* Conc.	*T. dyscritum* RM/CF	*P. gouldi* Con.	*P. gouldi* RM/CF
Na^+	124·5 ± 25·4 (N = 8)	0·256	—	—
Cl^-	127·3 ± 22·9 (N = 5)	0·235	155·4	0·306
K^+	112·9†	10·05	—	—
Mg^{2+}	23·55 ± 1·25 (N = 4)	0·544	—	—
Ca^{2+}	4·125 ± 0·120 (N = 4)	0·407	—	—
SO_4^{2-}	33·9 ± 3·8 (N = 3)	1·294	23·9	1·071
PO_4^{3-}‡	26·98 ± 4·57 (N = 6)	*ca.* 810	5·22	*ca.* 132
NH_4^+	18·2 ± 6·1 (N = 6)	58·71	—	—
NPS	547·2 ± 24·1 (N = 5)	146·3	543·3	543·3
Glucose	3·50 ± 2·47 (N = 3)	1·591	—	—
H_2O§	75·87 ± 0·98 (N = 13)	—	76·17 ± 7·47 (N = 8)	—
Total solutes	1004·0‖	0·879	727·8	0·697

Results are expressed as m-moles/kg tissue H_2O and represent the average ± SD (*T. dyscritum*) or the pooled mean, N = 5 (*P. gouldi*).

* 100% SW is defined as containing 580 mM Cl^-/kg H_2O for *T. dyscritum* and 522 m-moles Cl^-/kg H_2O for *P. gouldi*.

† Pooled mean, N = 5.

‡ Total orthophosphage, μmoles/kg H_2O.

§ g H_2O/100 g tissue.

‖ NH_4 excluded since included in NPS estimate.

TABLE 4—LEVELS OF MAJOR FREE AMINO ACIDS IN RETRACTOR MUSCLE FROM *T. dyscritum* IN 100% SW

Amino acid	100% SW
Alanine	Present*
Aspartic acid	12·3
Asparagine and glutamine	11·7‡
Glutamic acid	32·7‡
Glycine	517·6
Isoleucine (?)	26·4
Methionine	2·7‡
Proline	Trace
Serine	14·3‡
Taurine (?)	Present†
Threonine	26·2‡
Urea (?)	Present†
Total	643·9

Results expressed as m-moles/kg tissue H_2O and are the pooled means of four retractor muscles (N = 2 worms).

* Too close to glycine to estimate.

† Probably present but no standards run to confirm this.

‡ "Net Total Estimate" (NTE) of amino acid level, accuracy probably not better than ± 10%. Other estimates are accurate within 2%.

TABLE 5—STEADY-STATE COMPOSITION OF COELOMOCYTES FROM *T. dyscritum* AND *P. gouldi* IN 100% SW*

Solutes	*T. dyscritum*		*P. gouldi*	
	Conc.	CC/CF	Conc.	CC/CF
Na^+	98·4 ± 27·7 ($N = 7$)	0·202	—	—
Cl^-	192 ± 27 ($N = 3$)†	0·354	153	0·301
K^+	138·0 ± 14·8 ($N = 3$)†	10·08	—	—
Mg^{2+}	13·51 ± 0·97 ($N = 4$)	0·307	—	—
Ca^{2+}	2·595 ± 0·465 ($N = 7$)	0·256	—	—
SO_4^{2-}	23·6 ± 6·8 ($N = 5$)	0·901	—	—
PO_4^{3-}‡	0·221 ± 0·065 ($N = 3$)	6·634	—	—
NH_4^+	28·9 ± 4·6 ($N = 6$)	93·23	—	—
NPS	397·3 ± 27·4 ($N = 11$)	106·2	—	—
Glucose	9·0 ± 4·2 ($N = 3$)	4·09	—	—
H_2O§	*ca.* 74·6 ($N = 2$)	—	—	—
Total solutes				
(a) mM/l. cells	874·6	0·765	—	—
(b) mM/kg H_2O	1172·4	1·026	—	—

Results are expressed as m-moles/l. cells ± SD.

* 100% SW is defined as containing 580 mM Cl^-/kg H_2O for *T. dyscritum* and 522 m-moles Cl^-/kg H_2O for *P. gouldi*.

† Average of three pooled means of $N = 10$.

‡ Total orthophosphate, μmoles/kg H_2O.

§ g H_2O/100 g centrifuged cell pellet.

It is generally accepted that animal cells are normally in an isotonic, steady state in relation to the extracellular (= pericellular) body fluids amd maintain their intracellular osmotic pressure equal to the ambient osmotic pressure (Conway & McCormack, 1953; Robinson, 1960; Robertson, 1965, 1970; Dick, 1966). The ratio of total measured tissue solutes to total measured coelomic fluid solutes is close to unity for *T. dyscritum* body wall, retractor muscle and coelomocytes (Tables 2, 3 and 5). If it is assumed that the tissue osmolality can be reasonably estimated from the sum of the extractable tissue constituents, then these tissues are within 100 m-moles/kg H_2O of being isosmotic to the coelomic fluid and hence to sea water. The apparent solute deficit of body wall and retractor muscle is likely to be made up by several ninhydrin-insensitive organic compounds such as trimethylamine oxide, betaine, lactate, glycerol and various organic phosphates. The combined solute concentration of these substances in mantle muscle of several cephalopod molluscs, for example, exceeds 200 mM/kg tissue (Robertson, 1965).

Analyses of constituents of *P. gouldi* tissues are less complete. However, the levels of tissue solutes measured in this species, i.e. Cl, SO_4, PO_4, NPS, are comparable to the levels of the same solutes in homologous tissues of *T. dyscritum*.

If this proportionality exists for the other, unassayed tissue solutes, then *P. gouldi* tissues are probably also nearly isosmotic to their coelomic fluid.

It must be noted that these assays are liable to certain errors. The tissue "solute deficit" just mentioned may be greater (unlikely) or less than suggested due to the practice of adding up individual solute estimates based on small sample size and from samples derived from different specimens. For one thing, considerable individual variation is seen in each species, e.g. *T. dyscritum* body wall Na = 235·3 ± 56·1 (S.D.) mM/kg H_2O (Table 2). Additionally, some estimates such as ninhydrin-positive substances (NPS) may be less sensitive than desirable. If, for example, the value for the total free amino acid pool of *T. dyscritum* retractor muscle derived from summation of the individual amino acid estimates, 644 mM (Table 4), were used in Table 2 instead of the estimate based on total ninhydrin-positive substances, 547 mM, the total measured solute pool for this tissue would essentially match the total measured solute level of the coelomic fluid (Table 1).

Homologous tissues of each worm are remarkably similar in the content and relative abundance of specific solutes. This parallelism holds for each tissue studied, i.e. body wall (BW), retractor muscle (RM) and coelomocytes (CC), and may be summarized for *T. dyscritum* and similarly (where data exists) for *P. gouldi* (BW, CC) by the following hierarchy of relative solute concentrations:

$$\text{NPS} > \text{Cl} \geqslant \text{Na} \geqslant \text{K} > \text{Mg} > \text{Ca (BW, RM, CC)},$$
$$\text{Mg} > \text{SO}_4 \text{ (RM)}, \quad \text{SO}_4 \geqslant \text{Mg (BW, CC)}.$$

Such generalizations should not obscure the fact that solute levels of similar tissues of each species do in fact exhibit distinct quantitative differences, as is also true for the coelomic fluid electrolyte profiles (Table 1). It is impressive, however, that these species, of separate genera, display much similarity in their overall pattern of ion regulation. This view is also consonant with the opinion of P.L. ILLG (see Kohn & Rice, 1971) who has characterized the Sipuncula as a phylum behaving as a genus with respect to its evolution of character sets.

Table 4 presents the concentrations of neutral and acidic amino acids extracted from retractor muscles of *T. dyscritum* acclimated to 100% SW. The neutral amino acid glycine is impressively concentrated by retractor muscle and constitutes over 80 per cent of the tissue free amino acid pool. The five most common free amino acids of this tissue may be ranked, in order of decreasing abundance:

$$\text{glycine} \gg \text{glutamic acid} \geqslant \text{isoleucine} \geqslant \text{threonine} \geqslant \text{serine}.$$

The only other quantitative analysis of free amino acid profiles in sipunculid tissue is that of Duchâteau *et al.* (1952), who report similarly high glycine levels in *Sipunculus nudus* muscle, constituting approximately 78 per cent of the total free amino acid concentration. As in *T. dyscritum* (Table 4), the other free amino acids are considerably less concentrated. In *S. nudus*, however, the relative concentrations of the five major amino acids do not closely follow the pattern in *T. dyscritum*:

$$\text{glycine} \gg \text{arginine} > \text{alanine} \geqslant \text{glutamic acid} \geqslant \text{aspartic acid}.$$

Not surprisingly, the tissue-free amino acid profile appears to show greater species specificity than the tissue inorganic electrolyte profile.

The question has been raised (Hogue & Oglesby, 1972) whether the species, *T. dyscritum*, collected from southern California and used in the present study, is not actually *T. zostericolum*. Using two-dimensional paper chromatography, Kittredge *et al.* (1962) have qualitatively studied the pattern of free amino acids extractable with 80% EtOH from tissue (unspecified) of *T. zostericolum* collected on the coast of San Diego county. It is relevant to the question of species that they found high concentrations of aspartic acid, whereas it is clear that in *T. dyscritum* tissue (Table 4) aspartic acid represents less than 2 per cent of the free amino acid pool.

High orthophosphate levels are commonly reported for invertebrate muscle tissue, e.g. cephalopod molluscs (Robertson, 1965), echiurans (DeJorge & Ditadi, 1969), horseshoe crab (Robertson, 1970), lamellibranch molluscs (Potts, 1958). The body wall musculature and retractor muscles of *T. dyscritum* and *P. gouldi* follow this general pattern and strongly concentrate phosphate to over one hundred times the ambient (coelomic fluid) level. Non-muscle tissue, such as coelomocyte (Table 5), is relatively low in phosphate. Both histological examination and wet weight measurements of the separated tissue layers of *T. dyscritum* body wall suggest that nearly 60 per cent of the body wall is constructed of circular and longitudinal muscle, the remainder being primarily cuticle. Interestingly, the orthophosphate level is nearly 60 per cent that of "pure" (retractor) muscle in this species, suggesting that all *T. dyscritum* muscles generally possess equivalent intracellular phosphate levels. Sufficient information is lacking, but the situation in *P. gouldi* may be similar, since body wall phosphate content is less than that in retractor muscle.

Glucose contents of body wall and retractor muscle of *T. dyscritum* are less than half that of coelomocytes. However, there appears to be no difference in the total carbohydrate level of these tissues in *Phascolosoma agassizi* (Towle & Giese, 1966). All tissues of *T. dyscritum* and *P. agassizi* nevertheless maintain greater carbohydrate levels than the extracellular fluid.

Overall, sipunculid tissues like most other marine invertebrate tissues (Lange, 1968; Florkin & Schoffeniels, 1969) appear characteristically to possess a sizeable free amino acid pool. Combined with potassium and orthophosphate, this solute fraction constitutes the major osmotic pool in *T. dyscritum* and *P. gouldi* tissues. The solutes most common to the coelomic fluid, namely Na and Cl, are considerably less concentrated and probably largely represent coelomic fluid trapped in the extracellular tissue spaces. It is apparent from published data on *Sipunculus nudus* (Duchâteau *et al.*, 1952) and on *T. dyscritum* and *P. gouldi* reported here that specific solutes in tissues differ in concentration between species somewhat more than is true of solute pools in their coelomic fluids. However, interspecies differences are less remarkable than the similarities. Coelomic fluid isosmotic balance in the Sipuncula appears to be primarily handled through regulation of Na and Cl, whereas the tissues of animals in full-strength sea water appear to establish osmotic

equilibrium with the coelomic fluid largely through control of the intracellular free amino acid level.

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