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THE ACCUMULATION AND DISTRIBUTION OF VANADIUM, IRON, AND MANGANESE IN SOME SOLITARY ASCIDIANS

H. MICHIBATA[1], T. TERADA[2], N. ANADA[1], K. YAMAKAWA[1], AND T. NUMAKUNAI[3]

[1]*Biological Institute, Faculty of Science, Toyama University, Toyama 930;* [2]*Department of Chemistry, Toyama College of Technology, Toyama 930-11; and* [3]*Asamushi Marine Biological Station, Tohoku University, Aomori 039-34, Japan*

ABSTRACT

The vanadium, iron, and manganese contents of 15 species of solitary ascidians belonging to the suborders Phlebobranchia and Stolidobranchia were determined by thermal neutron activation analysis. Vanadium was detectable in all species examined. In general, the vanadium content in various tissues of the Phlebobranchia was considerably higher than the iron and manganese contents. The blood cells especially contained a large amount of vanadium. The highest value (21 μg vanadium/mg dry weight) was obtained from blood corpuscles of *Ascidia ahodori*. Species in the suborder Stolidobranchia, on the other hand, had smaller quantities of vanadium in comparison with those in the suborder Phlebobranchia. The iron and manganese contents did not differ greatly between the two suborders. The data are considered in the light of physiological roles of these transition metals in ascidians.

INTRODUCTION

The ability of many species of ascidians to concentrate vanadium and other metals from seawater is one of the physiological peculiarities which distinguishes these organisms from other classes of animals. Recent data give concentrations of dissolved vanadium in seawater as about 35 n*M* (Cole *et al.,* 1983; Collier, 1984), while the concentration of vanadium in ascidian blood cells can be as high as 0.15 *M*, a value about seven orders of magnitude higher (Macara *et al.,* 1979a).

In studies with *Phallusia mammillata,* Henze (1911) was the first to find that ascidian blood contained unexpectedly high amounts of vanadium. Many investigators subsequently analyzed metal content in species belonging to all three suborders (the Aplousobranchia, Phlebobranchia, and Stolidobranchia). The resulting data have shown generally that several species in the suborder Aplousobranchia have a high vanadium content, and that significant vanadium is likewise found in representatives of the Phlebobranchia, whereas species in the suborder Stolidobranchia contain relatively smaller amounts of vanadium, but a high iron content (Goodbody, 1974; Swinehart *et al.,* 1974; Biggs and Swinehart, 1976; Hawkins *et al.,* 1983). Little more information has previously been available on the vanadium accumulated in the species of the Stolidobranchia, however, because of the detection limits of analytical methods used in former experiments (Swinehart *et al.,* 1974; Danskin, 1978; Macara *et al.,* 1979a). The present studies were designed to fill this void in our knowledge of metal accumulation by stolidobranchial species and simultaneously to identify particular vanadium-rich tissues of the species of Phlebobranchia using neutron acti-

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vation analysis. The data are transformed to molar concentrations per unit of tissue for consideration of the roles of these metals in ascidian physiology.

Materials and Methods

Species were collected at the following locations. *Ciona savignyi**, *Ascidia ahodori, A. sydneiensis samea, Chelyosoma siboja, Styela plicata,* and *Halocynthia roretzi:* the Asamushi Marine Biological Station, Tohoku University on the Bay of Mutsu, Japan; *Polycarpa cryptocarpa* and *Pyula sacciformis:* the Noto Marine Laboratory, University of Kanazawa on the Inlet of Tsukumo, Japan; *H. aurantium:* Hakodate Aquarium on the Bay of Hakodate, Japan; *Molgula manhattensis:* the Nakajima Marine Biological Station, Ehime University on the Strait of Sekido, Japan; *C. intestinalis**, *A. malaca, Phallusia mammillata, H. papillosa,* and *Microcosmus sulcatus:* the Stazione Zoologica di Napoli on the Gulf of Naples, Italy.

At least five specimens of each species were analyzed for metals with the exception of *M. manhattensis,* of which three specimens were examined. Each specimen was cleaned of extraneous materials. Blood was obtained via heart puncture, whereupon plasma and corpuscles were separated by centrifuging the blood for 10 min at 2000 $\times g$. The test, mantle, branchial basket, stomach, liver, and gonads were rinsed three times with filtered seawater. All tissues were weighed wet before they were dried in porcelain crucibles in a drying oven at 110°C to a constant dry weight per sample. Samples (about 20 mg dry weight) sufficient for analysis of metals by means of neutron activation analysis, were mineralized in a muffle furnace at 500°C for 24 h. The ash was dissolved in 5.0 ml of 0.1 N HNO_3 (super special grade, Wako Pure Chemical Indust. Ltd., Japan) and put into a polyethylene capsule. For applying the neutron activation analysis to measuring the amount of vanadium and manganese, each sample was irradiated for 2 min in the TRIGA MARK II nuclear reactor at Rikkyo University with thermal neutrons having a flux of 5×10^{11} n/cm$^2 \cdot$s^{-1}. The radioactivity of ^{52}V produced in the irradiated sample was measured with a 50-cm^3 Ge(Li) γ-ray spectrometer (Canberra Inc.) 2 min after irradiation. Sixty min after irradiation, the radioactivity of ^{56}Mn was measured in the same manner as described above to avoid the interference of ^{27}Mg produced in the irradiated sample. The photon energies of ^{52}V and ^{56}Mn used for measurement were 1432 KeV and 846 KeV, respectively. The amounts of vanadium and manganese were determined by comparing the γ-ray spectrograms with those of standard samples. Smoothing of spectral data and calculation of the peak area were done by the method proposed by Adams and Dams (1970) employing an automatic computer system. This analytical method has already been verified to be a most sensitive method of detection of vanadium in the ascidian (Papadopoulou and Kanias, 1977; Michibata, 1984).

After the radionuclides were allowed to decay to negligible concentrations, aliquots of each sample were submitted for analysis of iron by atomic absorption spectrometry (Equipment: Hitachi GA-2 flameless atomic absorption spectrometer). The absorption line used was 3719.9 nm.

Results

The vanadium, iron, and manganese concentrations for different tissues of the chosen species appear in Tables I and II. Table I shows data for seven species belong-

* The species, *C. robusta,* used in our previous papers (Hori and Michibata, 1981; Michibata, 1984; Michibata *et al.,* 1985) recently was renamed *C. intestinalis* by Hoshino and Nishikawa (1985). It inhabits European waters. With this renaming, the other species, *C. intestinalis sensu* Hoshino and Tokioka 1967 was also renamed *C. savignyi.* This taxonomic problem is examined in detail in their paper.

TABLE I

Metal contents in tissues of Phlebobranchiata (ng/mg dry weight)

Species	Vanadium	Iron	Manganese
Ciona intestinalis			
Tunic	1.7 ± 0.4	370.0 ± 39.4	188.8 ± 78.1
Mantle	338.4 ± 23.8	148.2 ± 11.4	29.3 ± 5.4
Branchial basket	337.5 ± 47.8	251.4 ± 27.6	69.9 ± 7.3
Stomach	163.2 ± 14.6	294.7 ± 69.0	36.4 ± 8.1
Liver	—	—	—
Gonad	100.9 ± 7.5	65.8 ± 8.9	77.0 ± 14.9
Corpuscles	330.7 ± 14.1	424.4 ± 49.4	31.4 ± 9.0
Plasma	0.4 ± 0.2	89.5 ± 4.8	9.2 ± 1.9
Ciona savignyi			
Tunic	1.2 ± 0.5	87.6 ± 6.5	4.8 ± 1.5
Mantle	333.0 ± 39.7	54.8 ± 7.3	7.4 ± 0.9
Branchial basket	1037.5 ± 109.2	173.8 ± 24.3	17.3 ± 0.8
Stomach	507.4 ± 69.6	58.8 ± 8.0	20.2 ± 3.1
Liver	—	—	—
Gonad	143.6 ± 23.9	54.7 ± 5.3	13.2 ± 1.1
Corpuscles	1577.1 ± 338.2	78.3 ± 5.1	107.6 ± 13.8
Plasma	12.9 ± 0.4	119.6 ± 4.8	15.4 ± 1.9
Ascidia malaca			
Tunic	85.8 ± 3.8	1683.7 ± 194.3	392.1 ± 14.2
Mantle	645.6 ± 211.4	494.3 ± 22.1	17.9 ± 2.1
Branchial basket	737.3 ± 193.8	1204.9 ± 127.5	21.8 ± 4.0
Stomach	—	—	—
Liver	282.1 ± 43.2	275.6 ± 49.1	25.5 ± 7.3
Gonad	—	—	—
Corpuscles	4446.2 ± 509.6	741.5 ± 96.2	37.0 ± 3.4
Plasma	—	41.8 ± 5.0	—
Ascidia ahodori			
Tunic	1229.7 ± 142.8	356.7 ± 44.0	9.1 ± 2.5
Mantle	5723.1 ± 614.4	563.7 ± 73.9	2.3 ± 0.8
Branchial basket	6555.0 ± 331.2	480.6 ± 44.5	3.2 ± 0.6
Stomach	743.9 ± 159.1	135.9 ± 21.3	1.0 ± 0.8
Liver	1204.8 ± 122.8	241.1 ± 29.7	0.2 ± 0.2
Gonad	—	—	—
Corpuscles	21120.9 ± 1985.7	1702.1 ± 222.2	34.3 ± 1.6
Plasma	522.2 ±	—	—
Ascidia sydneiensis samea			
Tunic	30.6 ± 2.6	1432.6 ± 291.1	188.4 ± 37.2
Mantle	333.9 ± 46.1	276.6 ± 19.1	7.0 ± 0.9
Branchial basket	730.4 ± 95.2	299.0 ± 46.4	12.8 ± 0.8
Stomach	—	—	—
Liver	163.3 ± 19.3	140.3 ± 26.4	7.2 ± 0.5
Gonad	339.9 ± 22.1	310.5 ± 56.4	15.2 ± 4.6
Corpuscles	4675.9 ± 353.5	904.7 ± 185.6	26.8 ± 5.5
Plasma	27.6 ± 4.5	114.3 ± 10.8	20.1 ± 0.7
Phallusia mammillata			
Tunic	15.2 ± 1.8	117.7 ± 15.6	4.8 ± 0.8
Mantle	442.9 ± 106.9	127.0 ± 29.4	16.6 ± 3.4
Branchial basket	1502.3 ± 86.1	147.0 ± 50.6	29.3 ± 3.2
Stomach	—	—	—
Liver	136.6 ± 17.5	51.1 ± 11.0	24.5 ± 2.7
Gonad	—	—	—
Corpuscles	9859.9 ± 1780.7	413.4 ± 111.9	18.9 ± 1.0
Plasma	—	92.7 ± 17.8	7.2

TABLE I (*Continued*)

Species	Vanadium	Iron	Manganese
Chelyosoma siboja			
Tunic	6.0 ± 1.3	283.8 ± 37.5	24.7 ± 4.7
Mantle	39.4 ± 2.8	348.5 ± 50.0	30.1 ± 3.4
Branchial basket	1732.4 ± 138.9	471.7 ± 14.5	44.2 ± 5.5
Stomach	502.8 ± 34.0	511.8 ± 48.0	35.4 ± 6.7
Liver	330.5 ± 25.4	634.9 ± 35.2	38.5 ± 6.0
Gonad	—	—	—
Corpuscles	8.2 ± 0.8	138.1 ± 37.4	18.2 ± 1.2
Plasma	—	58.8 ± 5.3	—

Data are expressed as means ± standard errors. It was difficult to determine the metal contents in blood plasma because of the severe interference of γ-rays of ^{24}Na produced by the irradiation with thermal neutrons. Therefore, these data were not available in some cases.

ing to the suborder Phlebobranchia. All the species had a large vanadium content in their blood cells and branchial basket, with the exception of *Chelyosoma siboja,* in which the blood cells had a relatively small quantity of vanadium while the branchial basket had a large amount. The highest value for vanadium (21120.9 ng/mg dry weight) was obtained from the blood cells of *Ascidia ahodori.* Representative levels of iron and manganese, on the other hand, were 1702.1 ng/mg dry weight in blood cells of *A. ahodori* and 392.1 ng/mg dry weight in the tunic of *A. malaca,* respectively. In general, the vanadium content in various tissues of the Phlebobranchia was considerably higher than the iron and manganese contents. The blood cells in particular contained a large excess of vanadium over iron and manganese.

Table II lists the metal content in eight species belonging to the suborder Stolidobranchia. Vanadium was detectable in almost all tissues of these species; however, the quantity was small in comparison to that in the Phlebobranchia. For example, a vanadium content of 17.9 ng/mg dry weight, obtained in the tunic of *Molgula manhattensis,* was the highest, a level less than one-thousandth by weight of that in the blood cells of *A. ahodori* (Table I). Iron and manganese levels, on the other hand, did not differ greatly between the two suborders. The data revealed a tendency for the iron to be accumulated in the blood cells and manganese in the tunic.

Molar concentrations of vanadium, iron, and manganese in living tissues were then determined for tissues having 90% or greater moisture content. Figure 1 expresses the molar concentrations for all three metals from four species: *A. ahodori, A. sydneiensis samea, Styela plicata,* and *Halocynthia roretzi,* with two species each for each of the two suborders (Tables I, II). Concentrations of more than 10 μM vanadium were found even in the tunic of both *A. ahodori* and *A. sydneiensis samea,* while considerably lower vanadium concentrations (ranging from 0.1 to 10 μM) were measured in almost all tissues of the Stolidobranchia. *A. ahodori* had in excess of 10 mM vanadium in the mantle, branchial basket, and blood corpuscles. By contrast, the vanadium levels were only 0.1 to 1 μM in the tunic, mantle, branchial basket, and blood cells of *S. plicata.* Like others before us, therefore, we find large differences in vanadium concentration among ascidian species. In this study, however, we document for the first time the certain presence of vanadium in species of the Stolidobranchia, as well as the presence of only relatively smaller differences between species in iron and manganese contents, which range from 10 to 100 μM and 1 to 10 μM, respectively, for all species examined.

TABLE II

Metal contents in tissues of Stolidobranchiata (ng/mg dry weight)

Species	Vanadium	Iron	Manganese
Polycarpa cryptocarpa var. kuroboja			
Tunic	6.0 ± 0.8	1709.6 ± 114.8	96.0 ± 12.9
Mantle	1.2 ± 0.01	79.6 ± 5.6	6.2 ± 0.7
Branchial basket	1.5 ± 0.3	117.0 ± 11.1	6.8 ± 0.4
Stomach	3.2 ± 0.7	44.7 ± 1.5	9.8 ± 2.1
Liver	—	—	—
Gonad	—	—	—
Corpuscles	13.4 ± 2.9	2755.5 ± 152.6	63.0 ± 5.0
Plasma	1.0 ±	151.0 ± 10.9	16.7 ± 1.0
Styela plicata			
Tunic	2.6 ± 0.5	289.4 ± 20.3	38.1 ± 4.4
Mantle	0.3 ± 0.03	43.3 ± 2.1	3.5 ± 1.1
Branchial basket	0.5 ± 0.1	139.1 ± 23.2	11.4 ± 1.2
Stomach	0.4 ± 0.2	72.1 ± 4.0	6.8 ± 1.5
Liver	—	—	—
Gonad	1.3 ± 0.3	67.4 ± 6.3	6.6 ± 0.7
Corpuscles	3.5 ± 0.5	1095.0 ± 117.6	31.9 ± 5.8
Plasma	1.3 ± 0.4	176.3 ± 10.2	3.7 ± 1.1
Pyura sacciformis			
Tunic	1.0 ± 0.2	318.5 ± 13.2	230.4 ± 37.1
Mantle	0.6 ± 0.2	87.5 ± 12.8	9.2 ± 0.9
Branchial basket	1.1 ± 0.2	147.1 ± 6.0	20.4 ± 3.0
Stomach	1.1 ± 0.03	140.4 ± 1.0	26.0 ± 2.4
Liver	—	—	—
Gonad	1.0 ± 0.5	256.4 ± 4.4	18.8 ± 3.3
Corpuscles	0.6 ± 0.2	451.4 ± 61.8	25.0 ± 2.2
Plasma	2.0 ± 1.0	93.8 ± 6.9	3.4 ± 0.7
Halocynthia roetzi			
Tunic	5.8 ± 2.5	50.6 ± 13.3	28.4 ± 2.7
Mantle	0.5 ± 0.07	14.7 ± 1.4	3.1 ± 0.2
Branchial basket	1.9 ± 0.5	45.9 ± 8.4	11.0 ± 0.7
Stomach	—	—	—
Liver	2.5 ± 1.0	136.3 ± 22.2	11.5 ± 0.9
Gonad	2.1 ± 1.3	50.7 ± 8.0	7.5 ± 0.8
Corpuscles	3.6 ± 0.4	320.0 ± 25.0	40.2 ± 6.8
Plasma	0.6 ±	104.8 ± 5.3	19.8 ± 3.5
Halocynthia aurantium			
Tunic	1.3 ± 0.2	83.1 ± 8.1	31.1 ± 3.3
Mantle	0.9 ± 0.1	76.1 ± 19.4	3.7 ± 0.4
Branchial basket	0.8 ± 0.1	155.2 ± 18.4	14.8 ± 0.2
Stomach	2.5 ± 0.5	176.0 ± 19.4	30.1 ± 3.1
Liver	3.5 ± 0.2	352.1 ± 31.0	19.5 ± 0.7
Gonad	0.3 ± 0.04	76.3 ± 16.2	14.3 ± 1.1
Corpuscles	2.2 ± 0.2	245.3 ± 18.9	38.5 ± 3.4
Plasma	0.02 ± 0.003	127.8 ± 19.0	1.4 ± 0.7
Halocynthia papillosa			
Tunic	1.0 ± 0.4	43.5 ± 3.9	19.1 ± 1.0
Mantle	2.3 ± 0.5	61.4 ± 3.0	20.1 ± 0.7
Branchial basket	2.9 ± 1.0	62.0 ± 2.5	39.7 ± 9.2
Stomach	—	—	—
Liver	2.9 ± 0.3	114.9 ± 8.7	112.2 ± 4.6
Gonad	0.9 ± 0.2	45.5 ± 0.6	38.6 ± 0.3
Corpuscles	7.7 ± 1.6	640.0 ± 41.2	188.9 ± 8.7
Plasma	0.9 ± 0.2	54.0 ± 3.1	65.9 ± 4.1

TABLE II (*Continued*)

Species	Vanadium	Iron	Manganese
Microcosmus sulcatus			
Tunic	0.3 ± 0.01	72.4 ± 12.4	7.9 ± 0.5
Mantle	0.1 ± 0.03	68.4 ± 1.3	11.5 ± 1.3
Branchial basket	0.6 ± 0.4	84.4 ± 4.3	90.8 ± 10.4
Stomach	—	—	—
Liver	1.7 ± 0.3	49.5 ± 2.8	54.9 ± 5.9
Gonad	0.1 ± 0.01	49.9 ± 6.8	30.6 ± 1.8
Corpuscles	13.9 ± 8.0	791.2 ± 83.9	226.9 ± 4.1
Plasma	—	52.8 ± 1.7	27.1 ± 1.7
Molgula manhattensis			
Tunic	17.9 ±	7588.3 ±	534.6 ±
Mantle	1.5 ±	155.3 ±	26.9 ±
Branchial basket	1.5 ±	1041.7 ±	10.1 ±
Stomach	0.9 ±	240.4 ±	27.6 ±
Liver	—	—	—
Gonad	1.2 ±	8.7 ±	7.5 ±
Corpuscles	2.6 ±	125.0 ±	37.4 ±
Plasma	0.2 ±	105.7 ±	10.0 ±

See footnote in Table I. As to *M. manhattensis*, three specimens were submitted.

DISCUSSION

The data shown in Tables I and II agree substantially with previous reports that species belonging to the suborder Phlebobranchia have a large vanadium content, while those in the suborder Stolidobranchia have a smaller amount (Swinehart *et al.*, 1974; Biggs and Swinehart, 1976; Danskin, 1978; Macara *et al.*, 1979a). High levels of vanadium have previously been found in the family Ascidiidae; *A. nigra* was known to contain a high concentration of vanadium in its blood cells at 26.8 μg/mg dry weight (Macara *et al.*, 1979a). The present studies likewise show that other species in this family accumulate especially high levels of vanadium; *A. ahodori* had a comparable amount of vanadium in its blood cells at 21.1 μg/mg dry weight (Table I). In *Phallusia mammillata,* the original species in which ascidian vanadium was found (Henze, 1911), we reconfirm the presence of a high amount of vanadium in the blood cells. In addition, species belonging to the suborder Phlebobranchia were found in our experiments to concentrate a relatively high amount of vanadium in other tissues as well. Whether these are measures of actual tissue content of vanadium, or result from the presence in the different tissues of contaminating blood cells (the tissues were not perfused after dissection) remains to be determined. Because cell types other than blood cells (*e.g.*, egg test cells; Hori and Michibata, 1981) contain vanadium, a careful analysis of specific cell types for metal content now seems justified.

It is important to note that significant levels of vanadium were detected in all tissues of species belonging to the suborder Stolidobranchia (Table II). In contrast with former studies, our experiments using thermal neutron activation analysis are the first to reveal that species in this suborder without doubt contain vanadium. In previous reports based on colorimetric analysis (Ciereszko *et al.*, 1963; Danskin, 1978), atomic absorption spectrometry (Botte *et al.*, 1979; Hawkins *et al.*, 1983) and plasma emission spectrometry (Macara *et al.*, 1979a; Hawkins *et al.*, 1980), the detection limits for vanadium were greater than 20 ppm; therefore, the vanadium content in the species of the suborder Stolidobranchia could not be determined with certainty.

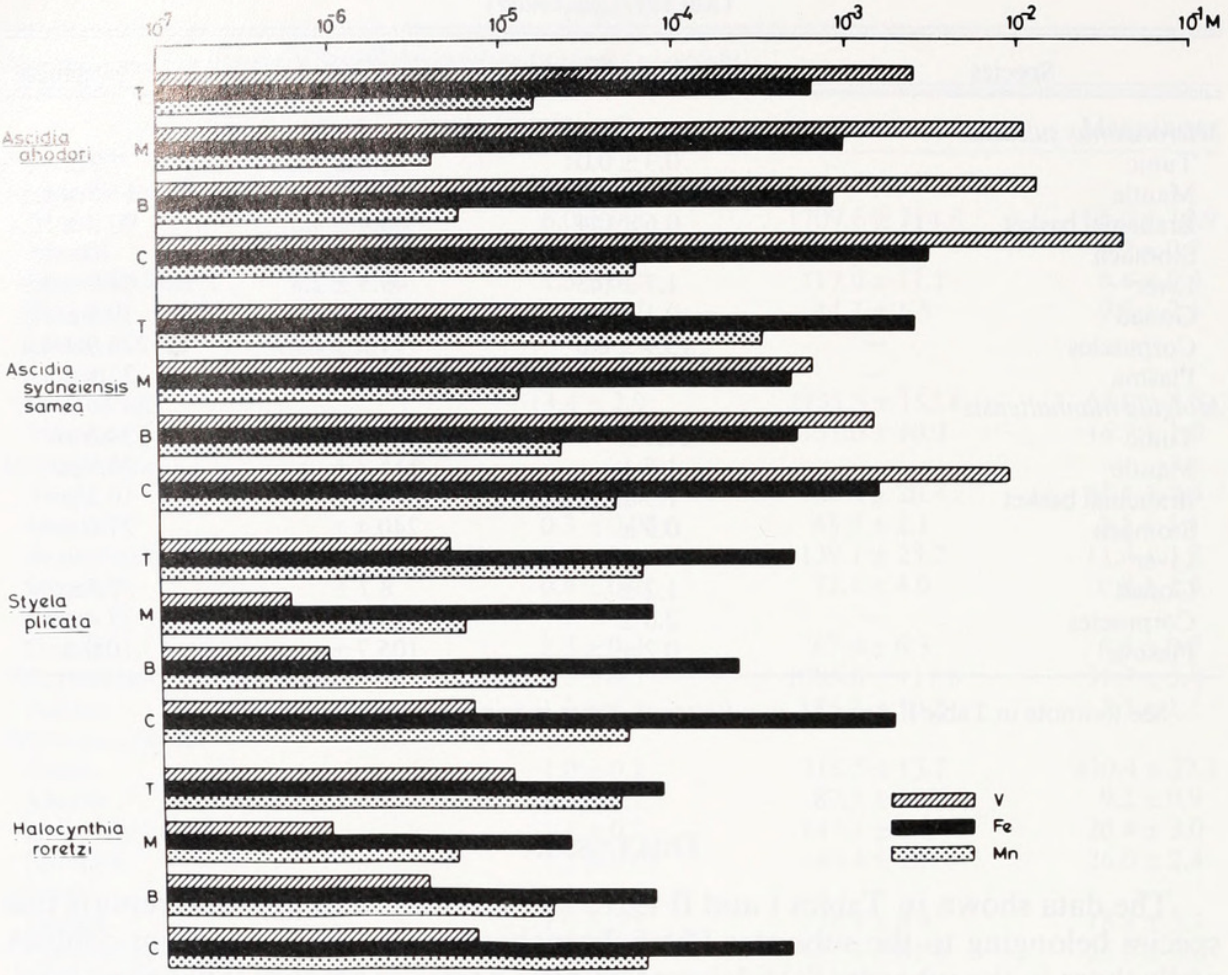

FIGURE 1. Molar concentrations of vanadium, iron, and manganese contained in each ascidian tissue. *A. ahodori* and *A. sydneiensis samea* are representatives of Phlebobranchiata and *S. plicata* and *H. roretzi* are of Stolidobranchiata. T = Tunic, M = Mantle, B = Branchial basket, C = Corpuscles.

As shown in Table II, neutron activation analysis allowed detection of the low, but significant, levels of vanadium (several hundred ppb in certain tissues) for these species. These concentrations are about one hundred times those of comparable mammalian tissues (Söremark, 1967). It appears, therefore, that mechanisms for vanadium accumulation are intact in the Stolidobranchia, leaving open the possibility that ascidians of the suborder Stolidobranchia represent transitional forms with regard to physiological mechanisms involving vanadium.

The data appearing in Tables I and II also show that the contents of iron and manganeses in general do not vary sharply between the two suborders studied, in contrast with vanadium content, although some differences between tissues are seen. The largest amounts of iron (7588.3 ng/mg dry weight) and manganese (534.6 ng/mg dry weight) were found in the tunic of *M. manhattensis;* however, the tunic did not contain high levels of either metal in other species. The somewhat higher iron content (16.4 to 26.6 μg/mg organic dry weight) in freshly collected *M. manhattensis* previously reported by Swinehart *et al.,* (1974) most likely resulted from ingested particulate matter from the collection area. Data from other studies of manganese content in ascidians (Noddack and Noddack, 1940; Carlisle, 1968; Swinehart *et al.,* 1974; Botte *et al.,* 1979) agree generally with our analytical findings (Tables I, II).

Webb (1939) first pointed out that high concentrations of vanadium in ascidian species correlated with certain evolutionary traits of the class Ascidiacea; he also pre-

dicted that it would be a primitive characteristic which had been lost in species of the more specialized Stolidobranchia. Subsequent analysis by many investigators confirmed that, in general, species from the suborders Aplousobranchia and Phlebobranchia had a large vanadium content, whereas species from the suborder Stolidobranchia contained a smaller quantity of vanadium, while retaining large quantities of iron (Goldberg *et al.*, 1951; Levine, 1961; Ciereszko *et al.*, 1963; Carlisle, 1968; Macara *et al.*, 1979a). Furthermore, based on evidence that the iron content of the Aplousobranchs *Eudistoma mole* and *Distaplia occidentalis* was of the same order of magnitude as the vanadium content, Swinehart *et al.* (1974) suggested that these species might indeed represent animals that were in transition between the vanadium and iron "users." However, until more species are submitted for detailed analysis of their metal content by methods as sensitive as the ones used in the present study, we cannot conclude that the relative concentrations of the two metals in different ascidian subfamilies reflects phylogeny.

Figure I shows that, although relatively narrow diversities in tissue iron and manganese concentrations were observed between different tissues, there is a very wide diversity in the vanadium concentrations, ranging from 0.1 μM to 10 mM. This finding may be quite meaningful in considerations of the physiological roles played by vanadium in ascidians. The present data indicate that the largest amounts of vanadium are accumulated in blood cells and/or the branchial basket, which contains large foci of blood cells. It is possible, therefore, that the dominant cell types accumulating this metal are blood cells, a finding that agrees substantially with studies by others (Macara *et al.*, 1979a). Because a respiratory function of blood cell vanadium (as an oxygen carrier, Carlisle, 1968) has been eliminated (Macara *et al.*, 1979b), we must consider other possibilities for adaptive functions of transition metal accumulation in blood cells.

Recent X-ray microanalysis studies with blood cells of vanadium-accumulating ascidians have demonstrated that the metal is concentrated in cytoplasmic vacuoles (Botte *et al.*, 1979; Scippa *et al.*, 1982). Our own previous studies have revealed similarly that considerable amounts of vanadium and iron are detectable in condensed masses of granules in the oocyte test cells of unfertilized eggs of *Ciona intestinalis* (Hori and Michibata, 1981). For selective localization in the vacuoles of specialized cells, it would seen likely that both metals are accumulated through the plasma membrane by a specialized transport system. Kustin and his co-workers have recently provided evidence for a specific vanadate transport system in the plasma membranes of ascidian blood cells (Dingley *et al.*, 1981). They likewise have described a yellow blood pigment, which they named tunichrome, which occurs within the same vacuoles as the vanadium, and which appears to reduce vanadium(V) to vanadium(IV) (Gilbert *et al.*, 1977; Macara *et al.*, 1979a, b; Macara; 1980; Agudelo *et al.*, 1982; Robinson *et al.*, 1984). The chemical formula and structure of tunichrome (Tunichrome B-1) from the blood cells of *A. nigra* has been described by Bruening *et al.* (1985). Robinson *et al.* (1986) have shown that tunichrome may be involved in tunic formation in ascidian embryos. On the other hand, Rowley (1983) has suggested that vanadium and its associated compounds may be part of the antimicrobial armoury of ascidian blood cells. The clear presence of vanadium in the blood cells of all ascidian species, documented for the first time in this study, strongly suggests that, whatever the physiological role played by vanadium in ascidians, it has not been lost in any of the extant species. Studies of the relative contributions of blood cell vanadium, iron, and manganese to *in vitro* antimicrobial chemical reaction systems and/or tunic formation systems may allow description of the relationship between these transition metals in adaptive functions carried out by blood cells.

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