Humic and fulvic acids: sink or source in the availability of metals to the marine bivalves *Macoma balthica* and *Potamocorbula amurensis*?

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ABSTRACT: Humic acids (HA) and fulvic acids (FA) are common forms of organic matter in marine sediments, and are routinely ingested by deposit- and suspension-feeding animals. These compounds may be a sink for metals, implying that once metals are bound to humic substances they are no longer available to food webs. A series of experiments was conducted to quantitatively examine this premise using 2 estuarine bivalves from San Francisco Bay, USA: the suspension feeder *Potamocorbula amurensis* and the facultative deposit feeder *Macoma balthica*. HA and FA, isolated from marine sediments, were bound as organic coatings to either hydrous ferric oxides (HFO) or silica particles. Cd and Cr(III) were adsorbed to the organic coatings or directly to uncoated HFO and silica particles. Pulse-chase laboratory feeding experiments using ^{109}Cd and ^{51}Cr(III) were then conducted to determine absorption efficiencies of Cd and Cr for individual specimens using each of the particle types. The results demonstrated that: (1) absorption of Cr(III) from all types of non-living particles was consistently low (< 11%). Ingested Cd showed greater bioavailability than Cr(III), perhaps due to differences in metal chemistry. (2) Bivalves absorbed Cd bound to uncoated HFO or silica particles (i.e. with no HA or FA present). (3) The presence of organic coatings on particles reduced Cd bioavailability compared with uncoated particles. (4) Both geochemical and biological conditions affected the food chain transfer of Cd. The data suggest that in marine systems inorganic and organic-coated particles are predominantly a sink for Cr in sediments. In the transfer of Cd to consumer animals, inorganic particles and humic substances can act as a link (although not a highly efficient one) under oxidized conditions.

KEY WORDS: Assimilation · Bioavailability · Clams · Fulvic acid · Humic acid · Metals

INTRODUCTION

Humic substances, such as humic acids (HA) and fulvic acids (FA), represent an operationally defined and abundant form of organic matter that is relatively refractory to both consumer animals and microorganisms (Mayer 1989). Humic substances originate from biochemical compounds, but are converted to structurally complex, biologically refractory materials by processes other than biochemical reactions (Mayer 1989). Humic and fulvic acids have considerable ion-exchange capacities (4 to 14 meq g^{-1} C) which result primarily from ionized carboxyl and phenolic hydroxyl groups (Preston 1979). Both types of humic substances can bind metals by complexation or ion-exchange mechanisms (MacCarthy & Suffet 1989). They are a sink for metals (Rashid 1985) and toxic organic compounds (Mantoura 1981, MacCarthy & Jimenez 1985) in sediments. Studies of fulvic acid-Cr complexes, for example, showed that chemical bonding is strong and non-labile. In one study, up to 84% of the interstitial Cr(III) was found associated with fulvic-like organics (Douglas & Quinn 1989). Mayer et al. (1983) showed that riverine colloids, which contain a high percentage of humic substances, are particularly important in chromium complexation as the colloids form flocs upon mixing with seawater.

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Humic materials are common in estuarine and nearshore marine sediments, comprising 10 to 68 % of the total sedimentary organic carbon (Brown et al. 1972, Jones & Jordan 1979). Aggregation of riverine particulates, colloidal materials, and dissolved humic materials occurs in estuaries with increasing ionic strength (i.e. salinity). Aggregation and co-precipitation with iron oxides result in the formation of complex, coated particulates (Sholkovitz et al. 1978, Mayer 1982, Luoma & Davis 1983, Tipping 1986). Humification also may occur within estuarine sediments, and contribute to the accumulation of humic compounds on particle surfaces in sediments. Fulvic acids generally have a lower molecular weight than humic acids, and a higher O-content, higher carboxylic content and higher acidity (Francois 1990). Thus fulvic acids are the more water-soluble of the two. Ratios of HA to FA in estuarine and coastal marine sediments range from 0.4 to 3.4 (Brown et al. 1972, MacFarlane 1978).

Humic substances are ingested by animals that ingest particulate detritus and thus could represent a source of particulate metal exposure to such animals. Organic polymer coatings of bacterial origin can enhance metal bioavailability from particles. Harvey & Luoma (1985a) showed that the bioavailability of particle-bound Cd and Zn to a deposit-feeding clam increased when particles were coated with bacterial exopolymer. It is not known, however, if humic-type polymer coatings enhance or reduce the bioavailability of metal from ingested particles.

Bivalves may be especially efficient at assimilating particle-bound metals (Luoma & Jenne 1976, Amiard 1978, Harvey & Luoma 1985b, Martoja et al. 1988), and thus could be sensitive to effects of humic coatings on metal bioavailability. Many bivalves possess a 2-phase digestive tract (Widdows et al. 1979). Ingested food particles are sorted in the stomach, then passed through a rapid 'intestinal' digestion. A fraction of the food is further processed by a slower, intensive, largely intracellular, 'glandular' digestion. Some metal forms are not assimilated during intestinal digestion but are available to the organism via glandular digestion (Decho & Luoma 1991). Metals not available from sediments to species with simple digestive systems (van Klump et al. 1987) may be available to bivalves with systems capable of intensive digestion.

Recent advances have allowed quantitative estimation of metal bioavailability from ingestion (Fisher et al. 1991, Reinfelder & Fisher 1991, Luoma et al. 1992) and separation of absorption efficiencies in the different phases of bivalve digestion (Decho & Luoma 1991). These studies used pulse-chase techniques to determine absorption efficiency, a physiological constant that can be compared among species and among food types and can be used in models to predict bioaccumu-

lation. In the present study we used the pulse-chase approach to determine if iron oxide and humic coatings on inorganic silica particles affected absorption efficiencies of Cd and Cr by 2 marine bivalves. Progressively layered coatings were added to silica particles to simulate complex sedimentary particulates. Digestive partitioning was followed to determine its effect on absorption efficiency.

CHOICE OF SPECIES AND ELEMENTS

The bivalves compared in this study were the facultative deposit feeder *Macoma balthica* and the suspension feeder *Potamocorbula amurensis*. The latter is a poorly known, active filter feeder that recently invaded San Francisco Bay, USA, from estuaries in east Asia (Nichols et al. 1990). These 2 species represent the 2 major feeding types common among bivalves. They also differ in digestive characteristics. Gut passage time in *P. amurensis* is < 1 h, intestinal digestion is complete in ca 3 h and gut residence time is 24 h (Decho & Luoma 1991). Gut passage time in *M. balthica* is 3 to 6 h, intestinal digestion is complete in ca 24 h and total gut residence time is 72 to 96 h.

Chromium was chosen for study because it is a common contaminant in estuaries. ^{51}Cr is also used as a tracer that is 'inert' to digestion in studies of carbon absorption efficiencies (Calow & Fletcher 1972, Lopez & Cheng 1983, Bricelj et al. 1984). Some forms of Cr are, indeed, not assimilated (Lopez et al. 1989), but others are. For example, when bound to bacteria, Cr(III) is efficiently assimilated (> 85 %) in the digestive gland of *Potamocorbula amurensis* (Decho & Luoma 1991). An understanding of Cr assimilation from different types of particles (including sedimentary particles where iron oxide and humic substance coatings are common) is essential to the future use of this element as an inert digestive tracer. It also will aid in understanding the fate of Cr in contaminated ecosystems.

Cadmium uptake by invertebrates has been widely studied, but conclusions differ about pathways of bioaccumulation. Sulfides in sediments reduce Cd bioavailability (DiToro et al. 1990), but the abundance of sulfides in the oxic environments experienced by most macrofauna is not well known (Luoma & Carter 1993). Some authors suggest that Cd is principally available to benthos from interstitial waters (Swartz et al. 1985, DiToro et al. 1990). Field studies suggested that Cd uptake from solution can be important for deposit-feeding bivalves and polychaetes in English estuaries (Bryan & Langston 1992) and bivalves in lakes from Quebec, Canada (Tessier et al. 1993). On the other hand Cd uptake from food by aquatic organisms has also been demonstrated (Sick & Baptist 1979,

Wikfors & Ukeles 1982, Borchardt 1983, van Hattum et al. 1989, Timmermans et al. 1992). Most of the experimental studies have employed living food sources (Amiard 1992). Few studies have considered whether abiotic sources of bioavailable Cd occur in oxidized sediments. Harvey & Luoma (1985a) showed little Cd uptake from iron oxide flocs, but detectable uptake from natural sedimentary particles, in studies with the deposit feeder *Macoma balthica*. Van Klump et al. (1987) found no detectable Cd uptake from sediments by oligochaetes.

MATERIALS AND METHODS

Seawater. All seawater (SW) used for experimental procedures was collected at the Long Marine Facility (Univ. California at Santa Cruz, USA). The SW used in isolations and adsorption experiments was initially filtered (Whatman GF/C), then cleaned by activated carbon sorption, followed by filtration (0.2 µm Nuclepore). All the deionized water used in extractions and purifications was ultraclean high-resistance (i.e. 18 MΩ cm^{-1}). All glassware used in extractions and isolations was acid-washed and rinsed in ultraclean water.

Isolation of humic and fulvic acids. Humic substances were extracted from fresh samples of sediment (ca 1 g each) collected from an intertidal mudflat on south San Francisco Bay (near Palo Alto). Sediments were added to 100 ml of deoxygenated 0.5 M NaOH solution containing 1.0 N $Na_4P_2O_7$ and stirred for 24 h in a glove box under nitrogen atmosphere and subdued light.

MIBK procedure: The MIBK (methyl isobutyl ketone) procedure was used to separate humic acids (HA) from fulvic acids (FA) and humin material. The entire mixture from the NaOH extractions was transferred to a separatory funnel along with 75 ml of MIBK and extracted according to the procedure of Rice & MacCarthy (1989). The samples were acidified to pH 1 with concentrated HCl, then shaken vigorously and organic matter was allowed to partition between the organic and aqueous phases. The FA remains in the aqueous phase while HA and humin enter the MIBK phase as a suspension. Both the organic phase (containing the HA and humin) and the aqueous phase (containing the FA) were collected. The aqueous phase was re-extracted twice using the same procedure. The final aqueous phase contained the FA, and was adjusted to pH 7.0 then rotoevaporated to remove any residual MIBK. The organic phase, derived from the initial MIBK extraction, contained most of the HA and humin. It was mixed with 100 ml of 0.5 M NaOH in a separatory funnel, and shaken vigorously. The HA enters the aqueous alkaline phase while the humin

remains in the organic phase. This procedure was repeated twice. The aqueous phase containing the HA was adjusted to pH 7.0, then rotoevaporated.

XAD extractions: The separated HA and FA were purified on Amberlite XAD-8 resin (40 to 60 mesh) according the methods of Thurman & Malcolm (1981). The resin, previously cleaned by sequential Soxhlet extractions, was packed using an H_2O-resin slurry into a 106 ml column and rinsed with 3 l of ultra-clean water. The column was successively cleaned 5 times with 0.1 M NaOH and 0.1 M HCl, leaving the resin in an acid state prior to sample addition. Acidified samples (pH 2.0) of HA and FA, diluted to < 25 mg dissolved organic carbon (DOC) l^{-1} (final concentration), were passed separately through the XAD columns at a flow rate of 4 to 5 ml min^{-1}. The HA or FA was then eluted from the column using 0.1 N NaOH (flow rate 1 to 2 ml min^{-1}). The eluate (containing the HA or FA) was sent directly to a cation-exchange column containing AGMP-50 resin to desalt the HA and FA. The isolated HA and FA (pH 7.0) were then kept frozen (−70 °C) until use.

Preparation of food particles. Silica particles were coated with hydrous iron oxides and then either HA or FA to create distinct particles and better simulate the complex aggregations that characterize particles in natural, oxidized sediments.

Hydrophilic 'Min-U-Sil' quartz silica particles (ca 10 to 30 µm diameter), which possess the bulk geochemical properties of quartz, were used, though their surface properties may be dominated by amorphous silica. The particles were acid-washed then rinsed 3 times in ultra-clean water before radiolabeling. Absorption efficiencies from uncoated silica particles were used to examine the bioavailability of metals directly bound to this mineralogical substrate.

A second set of acid-washed silica particles were coated with hydrous ferric oxides (HFO) in the form of $Fe(OH)_3$ (Edwards & Benjamin 1989). The particles were placed in glass scintillation vials containing a solution of $FeNO_3$ (4 M). The solution just covered the particles. The particle solution was dried at 100 °C for 24 to 36 h to form the coatings. The coated particles were resuspended in ultraclean water and washed by repeated (i.e. 5 times) low speed (1000 × g) centrifugation to remove most colloidal HFO and nitric acid residues. The newly formed HFO particles were used in experiments within several days after preparation while the HFO remained in a relatively amorphous state, as determined by scanning electron microscopy. Others have shown that this approach results in silica particles completely covered with HFO, containing $1 \pm 0.2 \times 10^{-2}$ g Fe g^{-1} sediment (Edwards & Benjamin 1989) and 0.205 mole sites mol^{-1} Fe (Dzomback & Morel 1990).

A third set of particles (silica and HFO-coated silica) were coated with either HA or FA. The binding of FA and HA to the silica and HFO particles was monitored spectrophotometrically. Known amounts of particles (50 mg) were mixed with a HA or FA solution (10 mg C l^{-1} final conc.) in teflon containers and gently shaken. Periodic subsamples (1 ml) were removed at 2 h intervals (for 30 h), and centrifuged to pellet the particles. The HA or FA remaining in the suspension was measured spectrophotometrically at 320 and 460 nm. The pH of the suspensions was periodically monitored and maintained between 7.8 and 8.0 throughout the experimental period. Spectrophotometric measurements of the HA and FA were initially calibrated using independent measurements of total DOC (courtesy of Dr J. Kuwabara, U.S. Geological Survey). Humic substances, such as HA and FA, typically contain 4 to 14 meq g^{-1} C of ion exchange sites (Preston 1979).

Binding of metals to particles. The metals, Cr(III) and Cd, were adsorbed to the 6 different types of particles: uncoated silica, uncoated HFO, humic-coated silica, fulvic-coated silica, humic-coated HFO, and fulvic-coated HFO. Metals were sorbed to the particles for 24 h at pH 7.8 to 8.0 in precleaned SW (20‰ salinity) at 20°C. Metal solutions, to which the particles were added, consisted of both radioisotope and carrier metal at a constant concentration ($CdCl_2 = 4.4 \times 10^{-8}$ M; $CrCl_2 = 1.9 \times 10^{-6}$ M). 'High' and 'low' loadings were achieved by adjusting amounts of particulate in the metal solutions. For example, for 'low' Cd loadings, 500 mg sediment were added to 50 ml seawater containing 5 µg l^{-1} Cd to yield a nominal concentration of 0.5 µg Cd g^{-1} sediment (Tables 1 & 2). After incubations, the particles were washed in SW and centrifuged to remove unbound metal. Distribution coefficients (K_d) were calculated as the amount of metal bound divided by the amount of metal in solution at the end of the adsorption period, as determined by gamma counting:

$$K_d = \frac{\text{µg metal g}^{-1} \text{ solid}}{\text{µg metal ml}^{-1} \text{ solution}} \qquad (1)$$

Gamma counting. The isotopes ^{51}Cr (half-life 27.8 d) and ^{109}Cd (half-life 462.6 d) were analyzed by gamma counting using a NaI crystal. Channel windows were set at 15 to 40 keV for Cd and 240 to 400 keV for Cr. Overlap of the Cr signal into the Cd channel was corrected using standards. Corrections for self-absorption of the Cd gamma signal by the bivalve shell were also employed for interpretations of whole-animal data.

Feeding experiments. Both species of bivalve were collected from a mudflat in the Carquinez strait of San Francisco Bay (Station #M1 in Johns et al. 1988). Specimens were held in the laboratory at constant temperature (10 ± 2°C) and salinity (20‰) in microcosms containing sediments from the collection site. Each day food material, consisting of a suspension of fine sediment flocculent material, was added to the chambers. Experiments were commenced within 2 d after collection. The clams used in the experiments were adults of a similar size within a species. Macoma balthica ranged in shell diameter from approximately 1.0 to 1.7 cm. Potamocorbula amurensis ranged from 0.75 to 1.5 cm. For the experiments, 8 to 12 individuals were first placed in a microcosm containing a suspension of unlabeled food material and allowed to acclimate for several hours. A suspension of experimental particles labeled with both ^{51}Cr and ^{109}Cd was then added. For

Table 1. Adsorbed cadmium concentrations (i.e. loadings) and geochemical characterization of particles used in feeding experiments. H: high metal loading; L: low metal loading; na: not applicable. Distribution coefficient is defined as µg g^{-1} of metal bound divided by µg ml^{-1} of metal in solution at the end of the adsorption experiment. TOC: total organic carbon; HFO: hydrous ferric oxides

Labeled sediment particles	Particle conc. (g)	Metal adsorbed (proportion)	Metal loading in sediment (µg g^{-1} dry wt)	Distribution coefficient	Particulate TOC (%)
HFO-uncoated L	0.5	0.44	0.2	79	na
HFO-humic H	0.005	0.41	20.5	6949	0.241
HFO-humic L	0.5	0.81	0.4	426	0.241
HFO-fulvic H	0.005	0.64	32.0	17778	0.0019
HFO-fulvic L	0.5	0.84	0.4	525	0.0019
Si-uncoated L	0.5	0.78	0.4	355	na
Si-humic H	0.005	0.26	13.0	3514	0.173
Si-humic L	0.5	0.33	0.2	49	0.173
Si-fulvic H	0.005	0.34	17.0	5152	0.0026
Si-fulvic L	0.5	0.52	0.3	108	0.0026

Table 2. Adsorbed chromium(III) concentrations (i.e. loadings) and geochemical characterization of particles used in feeding experiments. H: high metal loading; L: low metal loading; na: not applicable. Distribution coefficient is defined as $\mu g \, g^{-1}$ of metal bound divided by $\mu g \, ml^{-1}$ of metal in solution at the end of adsorption experiments. TOC: total organic carbon; HFO: hydrous ferric oxides

Labeled sediment particles	Particle conc. (g)	Metal adsorbed (proportion)	Metal loading in sediment ($\mu g \, g^{-1}$ dry wt)	Distribution coefficient	Particulate TOC (%)
HFO-uncoated L	0.5	0.72	7.2	257	na
HFO-humic H	0.005	0.33	330.0	4925	0.241
HFO-humic L	0.5	0.8	8.0	400	0.241
HFO-fulvic H	0.005	0.23	230.0	2987	0.0019
HFO-fulvic L	0.5	0.69	6.9	223	0.0019
Si-uncoated L	0.5	0.75	7.5	300	na
Si-humic H	0.005	0.25	250.0	3333	0.173
Si-humic L	0.5	0.51	5.1	104	0.173
Si-fulvic H	0.005	0.11	110.0	1236	0.0026
Si-fulvic L	0.5	0.35	3.5	54	0.0026

M. balthica the labeled-pulse feed period was 2 to 2.5 h. For *P. amurensis* the labeled pulse was 15 to 20 min (Decho & Luoma 1991). The short labeled-pulse approach minimized exposure to solute metal and allowed direct determination of metal influx from assimilation.

At the end of the labeled-pulse feed period the clams were removed, rinsed in SW, and placed in scintillation vials containing 10 ml SW. The living clams were analyzed by gamma counting to determine the amount of ingested labeled-food particles (T_0). The clams were then placed in 'cold-feed' microcosms containing unlabeled food material. This allowed them to continue feeding in the presence of natural food resources while egestion of labeled feces occurred. During the cold-feed period fecal pellets egested by the clams were collected periodically, placed in scintillation vials and analyzed by gamma counting. Pseudofeces were separated from feces (Decho & Luoma 1991). SW and food suspensions in the cold-feed microcosms were changed approximately every 8 to 12 h to maintain ample food concentrations and to reduce the accumulation of solute radionuclide or toxic byproducts by the clams.

When egestion was complete (72 h for *Macoma balthica* and 24 h for *Potamocorbula amurensis*; Decho & Luoma 1991), clams were removed from the microcosms, thoroughly rinsed, gamma counted and then frozen (−70°C). Soft tissues were later dissected from the shell while frozen. Shell and soft tissues were placed separately in scintillation vials and then analyzed.

A mass-balance approach was chosen to calculate overall absorption efficiency. A typical data set for each clam is shown in Tables 3 & 4. The absorption efficiency (% AE) was calculated as:

$$\% AE = \frac{\text{dpm tissue at 24 (72) h}}{(\text{dpm} \, \Sigma \, \text{feces}) + [\text{dpm tissue at 24 (72) h}]} \times 100 \quad (2)$$

In preliminary experiments, *Macoma balthica* were exposed to the water from an exposure pulse. Uptake was less than 5% of the ingested activity; thus dissolved sources did not contribute to the T_0 value. A high concentration of Cd and Cr on the shells (Tables 3 & 4) raised the possibility of bias from uptake of dissolved Cd and Cr during the cold-feeding incubations. Other evidence suggested that bias also was small. Chromium was strongly particle-associated and differences in the mass balance between T_0 whole-animal Cr counts and the sum of final (tissue + shell + feces) counts were small (generally < 4%). Thus very little Cr was lost to solution during egestion; not enough to affect the absorption efficiency estimates. Cadmium could desorb more readily from feces. Initial (T_0) whole-animal activities differed from the sum of final (tissue + shell + feces) activities by 10 to 20% with no detectable trends attributable to particle type. Thus some Cd could have entered the solution during the egestion period. Because the chamber waters were changed frequently, preventing build-up of [109]Cd, effects on absorption efficiency estimates would however have been small. Luoma et al. (1992) showed comparative results for different approaches to calculating AE with pulse-chase methodology.

A previous study (Decho & Luoma 1991) has shown that a pulse of radiolabeled food material will be

Table 3. *Potamocorbula amurensis*. Complete data set for clams exposd to [109]Cd and ^{51}Cr-labeled HFO (low) (uncoated) particles, a typical experiment. % AE: percentage absorption efficiency calculated by mass balance

Specimen no.	Sum feces (dpm)	Tissue (dpm 24 h)	Shell (dpm 24 h)	% AE (B/A + B)	Whole clam (T_0 dpm)
	A	B	C	D	E
Cadmium					
1	75796	15744	16561	17	126267
2	54551	18604	13175	25	92329
3	93943	56870	29228	38	163497
4	207621	45238	37866	18	231491
5	63159	65210	13418	51	149262
6	106564	67133	40481	39	209767
7	196914	147865	8850	43	314447
$\bar{x} \pm$ SD	114078 ± 62816	59523 ± 44152	22797 ± 12878	34 ± 13	183866 ± 74498
Chromium					
1	100103	282	4194	0.3	115179
2	67188	220	2189	0.3	83144
3	180718	960	5355	0.5	196355
4	255044	647	6442	0.3	259748
5	106949	2307	4059	2.1	147840
6	136288	539	23861	0.4	185364
7	315700	2820	6769	0.9	344947
$\bar{x} \pm$ SD	165999 ± 90398	1111 ± 1032	7553 ± 7358	0.7 ± 0.6	190368 ± 89189

Table 4. *Macoma balthica*. Complete data set for clams exposd to [109]Cd and ^{51}Cr-labeled HFO (low) (uncoated) particles, a typical experiment. % AE: percentage absorption efficiency calculated by mass balance

Specimen no.	Sum feces (dpm)	Tissue (dpm 72 h)	Shell (dpm 72 h)	% AE (B/A + B)	Whole clam (T_0 dpm)
	A	B	C	D	E
Cadmium					
1	18651	2131	4191	10	38545
2	25406	15143	9002	37	52360
3	52893	60096	11248	53	101742
4	28163	10930	6967	28	62888
5	32350	30309	8914	48	61348
6	32867	16987	5065	34	49375
$\bar{x} \pm$ SD	31721 ± 11605	22599 ± 20531	7565 ± 2662	35 ± 15	61043 ± 21806
Chromium					
1	23902	510	623	2.1	60717
2	81432	443	771	0.5	77114
3	113303	2547	982	2.2	116528
4	52583	1587	1480	2.9	83029
5	86698	1066	1015	1.2	86504
6	69294	1353	1503	1.9	82046
$\bar{x} \pm$ SD	71202 ± 30675	1251 ± 779	1062 ± 361	1.8 ± 0.8	84323 ± 18209

egested in 2 phases if food concentrations are kept low, and that egestion of ^{51}Cr can be used to quantify this digestive partitioning. [51]Cr is not taken up during the first, intestinal phase of digestion (Decho & Luoma 1991). Therefore the percentage of food processed by intestinal digestion was determined from:

$$\text{\% food processed by intestinal digestion} = \frac{{}^{51}\text{Cr}_{\text{intestinal feces}}}{{}^{51}\text{Cr}_{\Sigma\,(\text{feces + tissues})}} \times 100 \qquad (3)$$

where ${}^{51}\text{Cr}_{\text{intestinal feces}}$ is the sum of feces released before 3 h (*Potamocorbula amurensis*) or 24 h (*Macoma balthica*). The % AE of Cd absorbed from food material

processed by the intestinal digestion pathway alone was calculated as:

$$\% \ AE_{intestinal} = \frac{(Cd/Cr)_{intestinal \ feces}}{(Cd/Cr)_{total}} \times 100 \qquad (4)$$

where $(Cd/Cr)_{total}$ was the ratio of Cd/Cr in the sum of feces and tissues.

Statistical analyses. The % AE data were normalized using arcsine transformations, then analyzed by a split-plot ANOVA for an unbalanced design with hierarchical decomposition (Sokal & Rohlf 1981). The amounts of food processed by the digestive gland were plotted versus % AE, and analyzed using linear regression (Steele & Torrie 1980). All data transformations and analyses were conducted using the SYSTAT statistical analysis system (Wilkinson 1987).

RESULTS

Humic substance coatings on particles

Spectrophotometric analyses of the loss of FA and HA from solution to silica and HFO particles indicated a slow binding to particles (Fig. 1). Maximal binding was observed after approximately 22 h incubation (pH 7.8 to 8.0, 20‰ salinity). Approximately 20 to 25 % of the HA were sorbed onto both silica and HFO particles. FA demonstrated a considerably lower affinity than HA for either particle type. Less than 0.25 % of the added FA became associated with any of the particle types after 22 h under the experimental conditions. As a result, total organic carbon on the FA-coated particles was considerably lower than on HA-coated particles (Tables 1 & 2) and metal concentrations per mg C were highest on the FA-coated particles.

Binding of metals to particles

As expected, a higher adsorbed concentration (i.e. loading) of Cd and Cr was achieved on particles when 5 mg particles were added to the 50 ml of labeled solution than when a concentration of 500 mg 50 ml^{-1} was used. Proportionate sorption was also greater where particle concentrations were higher. The particulates with lower loadings of metal were typical of pristine or slightly contaminated sediments (3 to 8 µg Cr g^{-1}; 0.2 to 0.4 µg Cd g^{-1}). The particulates with higher loadings (Tables 1 & 2) were typical of contaminated sediments, but were not beyond the range found in contaminated systems (Luoma 1990, Sadiq 1992).

The sorption of Cd to particles was influenced by the type of particle and the type of particle coating
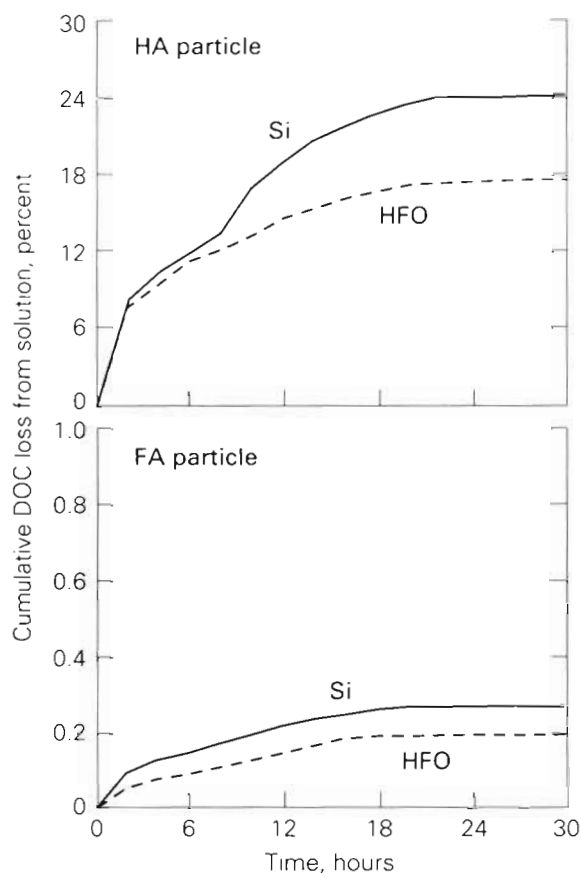

Fig. 1. The binding of humic substances, humic (HA) and fulvic acids (FA), to silica (Si) and hydrous ferric oxide (HFO) particles as a function of time

(Tables 1 & 2). More Cd sorbed to uncoated Si than to uncoated HFO. Organic coatings reduced Cd sorption to Si, while organic coatings enhanced Cd sorption to HFO, when similar conditions were compared (high particle concentrations, low metal concentrations). In contrast, Cr sorbed similarly to uncoated Si and uncoated HFO. Organic coating reduced Cr sorption to Si. Organic coating had no detectable effect on Cr sorption to HFO, under comparable conditions. Differences in the type of organic coating (HA vs FA) did not greatly affect the % metal sorbed, total metal loadings, or the distribution coefficients (Tables 1 & 2).

In earlier studies, nitrogen-gas adsorption surface areas determined on the same type of silica particles used here were $1 \pm 0.5 \ m^2 \ g^{-1}$ using BET analysis (D. Kent pers. comm.). This represents 2.0×10^{-7} mole sites 0.5 g^{-1} sediment. The calculated number of mole sites for uncoated HFO particles was 9.2×10^{-7} sites 0.5 g^{-1} sediment. HA and FA typically contain 4 to 14 meq g^{-1} C of ion exchange sites (Preston 1979) which converts to 5 to 17.5×10^{-5} meq FA sites 0.5 g^{-1} sediment

Table 5. *Potamocorbula amurensis* and *Macoma balthica*. Results of feeding experiments examining metal [Cd and Cr(III)] absorption by the clams fed different types of food particles. Values are mean percentage (±SE). % $AE_{intestine}$ Cd: % AE of Cd absorbed from food material processed by the intestinal digestion pathway alone. HFO: hydrous ferric oxides; nd: not determined

| Food particles | Absorption efficiency | | | | % food processed by glandular digestion | | % $AE_{intestinal}$ Cd | |
| | Cd | | Cr(III) | | | | | |
	P. amurensis	M. balthica	P. amurensis	M. balthica	P. amurensis	M. balthica	P. amurensis	M. balthica
HFO-high (metal)								
Uncoated	35 ± 6	nd	nd	nd	nd	nd	nd	nd
Humic acid	41 ± 5	25 ± 9	3 ± 1	3 ± 1	18 ± 8	37 ± 29	39	33
Fulvic acid	42 ± 7	22 ± 4	4 ± 1	2 ± 1	28 ± 15	19 ± 20	37	30
HFO-low (metal)								
Uncoated	33 ± 12	35 ± 15	1 ± 1	2 ± 1	80 ± 16	51 ± 32	43	35
Humic acid	12 ± 1	18 ± 6	6 ± 1	3 ± 4	44 ± 18	28 ± 23	12	23
Fulvic acid	9 ± 2	16 ± 4	5 ± 1	2 ± 1	40 ± 20	10 ± 5	1	32
Si-high (metal)								
Uncoated	35 ± 2	nd	4 ± 1	nd	77 ± 20	nd	nd	nd
Humic acid	12 ± 1	38 ± 11	6 ± 1	9 ± 2	51 ± 19	35 ± 15	9	42
Fulvic acid	9 ± 1	17 ± 3	4 ± 1	4 ± 1	30 ± 21	15 ± 10	6	24
Si-low (metal)								
Uncoated	56 ± 5	9 ± 6	11 ± 3	3 ± 1	81 ± 10	47 ± 22	nd	nd
Humic acid	16 ± 3	17 ± 2	8 ± 2	8 ± 2	63 ± 9	32 ± 10	nd	4
Fulvic acid	nd	16 ± 2	nd	8 ± 2	nd	36 ± 17	nd	4

and 4 to 14×10^{-3} meq HA sites 0.5 g^{-1} sediment. Therefore, even at high metal loadings, saturation of ligands was not likely.

Absorption efficiencies

Detectable absorption of both Cd and Cr by both bivalves was observed when the clams ingested the different types of particles investigated in this study. Mean absorption efficiencies of Cd varied from 9 to 56%. Absorption of Cr was consistently less than 11%

Fig. 2. *Macoma balthica*. Mean percent absorption efficiency (% AE) of Cr in bivalve feeding experiments. Vertical bars represent 1 SD

from these non-living materials. Results from replicate experiments using the same type of particle were not as variable as differences between particle types. For example, when *Potamocorbula amurensis* was fed [109]Cd-labeled HFO in 3 separate experiments, mean absorption efficiencies were 33 ± 12, 35 ± 6, and 44 ± 2%. In earlier studies, *P. amurensis* was fed ^{51}Cr-labeled bacteria in 3 different experiments. Absorption efficiencies ranged from 85 to 95% (Decho & Luoma 1991, unpubl. results). A complex mixture of processes appeared to cause the observed differences in absorption efficiency among treatments. These included: (1) metal-specific effects, (2) effects of different coatings, (3) metal loadings, (4) digestive processing and (5) species-specific effects.
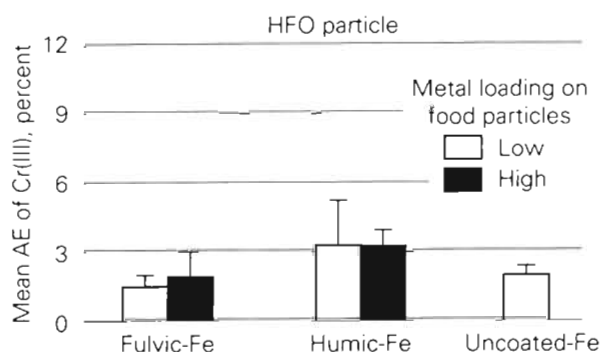
Metal-specific effects

Absorption efficiencies of Cd were significantly (p < 0.001) different than absorption efficiencies of Cr in most experiments, with both clams. With 1 exception, Cd was absorbed with a higher efficiency from all types of particles than was Cr (Table 5, Figs. 2 & 3). For example, Cd was biologically available to both clams when bound to HFO or Si surfaces. Absorption efficiencies from HFO, the substrate of most relevance in nature, were 33% or greater (Table 5). In contrast, absorption of Cr from uncoated particles was consistently low (< 4% with 1 exception).
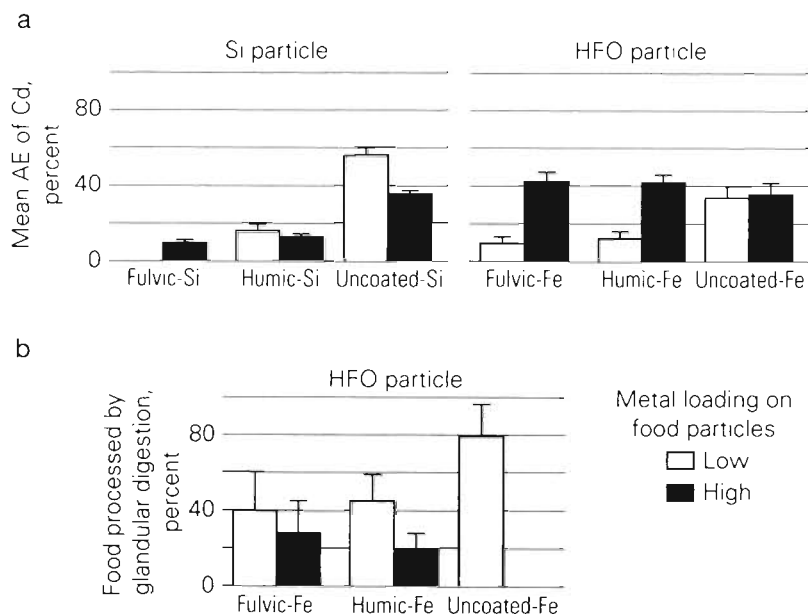
a

b

Fig. 3. *Potamocorbula amurensis*. (a) Mean percent absorption efficiency (% AE) of Cd and (b) mean percent ingested food processed by glandular digestion in bivalve feeding experiments. Vertical bars represent 1 SD

Cd loadings (Table 5, Fig. 3). Absorption efficiency from humic substances with low loadings of Cd were significantly less that AE from HFO or Si particles. Similar results were observed in *Macoma balthica* for humic coated-HFO particles (Table 5). Absorption of Cd from Si by *M. balthica* remained low (9 to 17 %) whether or not this type of particle was coated with organic material. The type of organic coating did not influence these results. In both species, AE from particles coated with FA was not significantly (p = 0.170) different than AE from HA-coated particles. Organic coatings either had no effect or slightly enhanced the consistently low Cr absorption efficiency.

Metal loadings on particles

The concentration of Cd on the particles appeared to affect absorption efficiency in *Potamocorbula amurensis* from the organically coated HFO particles (Fig. 3b). Significantly higher (p = 0.021) AE were observed when Cd loadings were high than when they were low for both HA- and FA-coated iron oxides. Intestinal AE was also higher at high Cd loadings than at low loadings. This suggested Cd was easily desorbed by digestive processes when concentrations were high. The concentration effect was less evident in *Macoma balthica* than in *P. amurensis*, and was not observed for Cr.
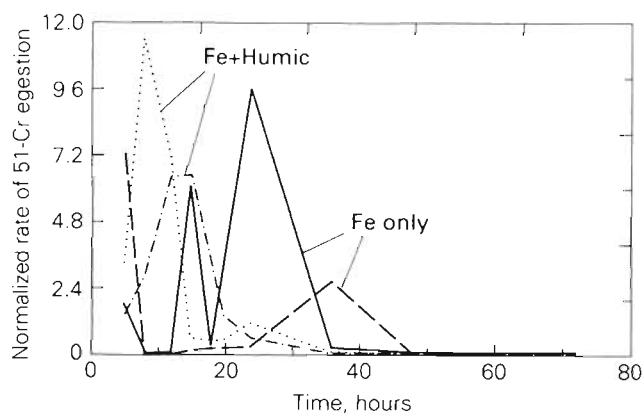
Effect of organic coating

Organic coatings affected Cd bioavailability (Fig. 3). Over all experiments, absorption efficiencies for Cd ranged from 9 to 42 % for organic-coated particles. The lowest AEs were observed when *Potamocorbula amurensis* ingested humic-coated particles with low

Fig. 4. *Macoma balthica*. Comparison of egestion rate of ^{51}Cr between organically coated particles and particles coated only with hydrous ferric oxide (HFO) in 4 individual bivalves. Data show cpm h^{-1} egested, as determined from analysis of the feces of *M. balthica* at different time intervals during the egestion period. The rate is normalized to the average rate for each experiment in order to scale the experiments similarly
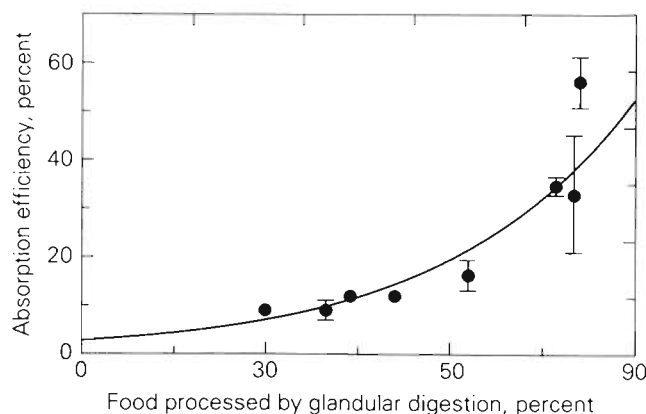

Fig. 5. *Potamocorbula amurensis*. Relationship between absorption efficiency of Cd and the proportion of ingested particles processed by glandular digestion. Each point represents the mean from each experiment. Vertical bars represent 1 SD

Digestive processing

Differences in the digestive processing of organic-coated and uncoated particles appeared to explain Cd absorption by *Potamocorbula amurensis*. Uncoated Si and HFO particles were retained longer during digestion than were organic-coated particles (Figs. 3b & 4). Absorption of Cd increased exponentially as the proportion of particles subjected to prolonged digestion increased (Fig. 5). Differences in digestive processing, however, did not explain the enhancement of AE at high concentrations of Cd. In fact, high concentrations of Cd appeared to coincide with less retention of food in the digestion gland in many experiments. Within experiments, absorption efficiency by individual animals increased however as a larger proportion of particles were processed by glandular digestion (Fig. 6).

The more intensive digestion of uncoated particles did not enhance Cr absorption efficiency as much as it did Cd. Digestive partitioning affected Cr absorption only for certain particle types. Increased Cr-AE in individual animals occurred when an increased proportion of HFO (organic-coated) was passed to the digestive gland of *Potamocorbula amurensis* ($r^2 = 0.66$, $p < 0.001$) and *Macoma balthica* ($r^2 = 0.26$, $p < 0.01$). However, the absorption efficiency of Cr was low no matter what the proportion of the particles retained in the digestive gland.

Species-specific differences

Macoma balthica retained a lower proportion of ingested particles for intensive digestion than did *Potamocorbula amurensis* in almost all experiments (Table 5). The maximum proportion of particles processed by the digestive gland in any experiment by *M. balthica* was 51% whereas the maximum by the digestive gland in *P. amurensis* was 81%. In contrast, absorption of Cd from organically coated particles by *M. balthica* exceeded that of *P. amurensis* during the early (intestinal) phase of digestion (Table 5). Intestinal digestion is also longer in *M. balthica* (20 h) than in *P. amurensis* (3 h).

The effect of metal particle type on metal absorption was generally similar between *Potamocorbula amurensis* and *Macoma balthica*, despite their differences in digestive retention and processing. Neither species absorbed Cr efficiently from HFO, Si, or humic substances, no matter how the particles were digested. Both species absorbed Cd over a similar range of efficiency from these particles. Both clams assimilated Cd from HFO because of enhanced particle retention. *M. balthica* assimilated a little more Cd than *P. amurensis* from organics in experiments with low Cd loading, but was not as responsive to higher loadings.
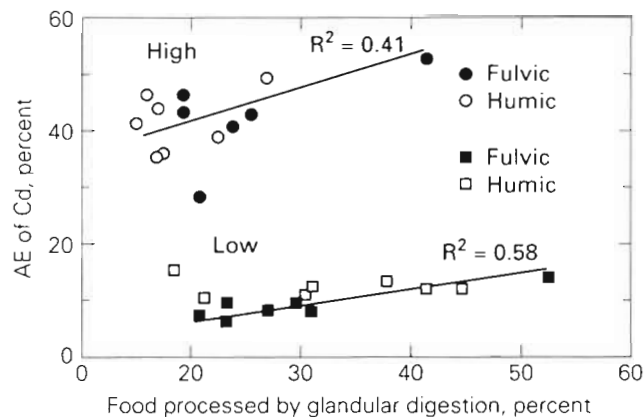

Fig. 6. *Potamocorbula amurensis*. Comparison of the effect of glandular digestion on the absorption of Cd by bivalve individuals when fed organic (humic or fulvic acid) coated hydrous ferric oxide particles with high or low loadings of bound Cd. Percent absorption efficiency (% AE) of individual bivalves was plotted against the amount of ingested food processed by glandular digestion. High: high Cd loading; Low: low Cd loading

DISCUSSION

Both geochemical and biological processes interacted to control the absorption efficiency of metals from particulate foods in bivalves. AE of Cd and Cr ranged from 1 to 62% in individuals of *Potamocorbula amurensis* and *Macoma balthica*. Factors contributing to the differences in AE included the chemical properties of the metal, the geochemical nature of the particle, the sorbed concentration of metal, and digestive partitioning of food particles.

Absorption of Cr was low from all the types of abiotic particles, in both bivalves, probably because of overriding geochemical factors. Cr(III) has a high affinity for oxygen-donor ligands. It preferentially sorbs strongly with HFO surfaces (Nakayama et al. 1981, Leckie et al. 1984), and has a very low solubility (Sass & Rai 1987). Some of the Cr(III) may have been in the form of $Cr(OH)_3$ or in the process of forming a solid solution with the $Fe(OH)_3$. Both could further decrease the solubility and bioavailability of Cr(III). Cr(III) also forms complexes with organic ligands (Nakayama et al. 1981), although the nature of these complexes is not well understood. Humic organic coatings slightly enhanced Cr bioavailability to *Potamocorbula amurensis* and *Macoma balthica*. However, the generally low AE for all these forms of Cr suggests that little of this element would be transferred from any of these particle types to detritus-feeding bivalves.

Differences in metal chemistry may explain why ingested Cd was of greater bioavailability to *Pota-*

mocorbula amurensis and *Macoma balthica* than Cr. Cadmium sorbs to many types of surfaces (Leckie et al. 1984) and to humic acids (Kerndorf & Schnitzer 1980), but with less intensity than Cr. The formation of strong Cd-chloro complexes in seawater (Mantoura 1981, Valenta et al. 1987) and competition with Ca and Mg further reduce the complexation of Cd with humic substances (Mantoura et al. 1978, Tipping & Hurley 1992) and its sorption to HFO (Cowan et al. 1991).

Very high loadings enhanced absorption efficiency of Cd from organically coated particles, especially in *Potamocorbula amurensis*. This geochemical effect was not related to saturation of the organic binding sites. Humic substances have a high content of oxygen-donor ligand, such as carboxyls and phenolic OH groups, that are not easily saturated in natural waters. The concentration effect was more likely due to a difference in binding intensity. Humic substances have a mixture of binding sites that complex metals over a continuum of binding energies. They have a relatively low content of N- and S-containing ligands that strongly complex metals such as Cd. The abundant oxygen-donor ligands complex metals with relatively low intensity (Kerndorf & Schnitzer 1980), except when cationic metals form strong inner-sphere coordination bonds with electron donor groups of the humic molecules (Rashid et al. 1972). Overall binding energy decreases as metal loading increases (Perdue 1989, De Wit et al. 1990). Maes et al. (1992) reported that conditional complexation constants of Cd on humic substances decrease by about 0.15 log K_1 units for each 10-fold increase in Cd loading. The difference in concentrations between low Cd and high Cd concentrations was 100-fold in our experiments, enough to decrease 0.3 log K_1 units. Such a difference apparently was enough to enhance absorption of Cd in the digestive tract of *P. amurensis* and, perhaps, *Macoma balthica*. Effects on desorption were also evidenced by more efficient removal of Cd during intestinal digestion. The lack of difference in Cd absorption efficiency between FA and HA was not consistent with this explanation, since the total number of FA sites was less than HA sites. Steric effects or binding intensities could have differed between the 2 types of humic substance, however. The latter is consistent with the higher distribution coefficients observed at the end of adsorption for FA than HA, especially per unit organic carbon.

The absorption of Cd from HFO-coated particles was influenced by biological processes. The more intensive digestion of the inorganic particles, correlated with a higher absorption efficiency of the element, implied that selectivity of particles for particular digestive pathways depended on the specific physical or chemical characteristics of the particle. *Macoma balthica* appeared less flexible in processing high proportions of food particles for intensive digestion than did *Potamocorbula amurensis*.

Differences in digestive partitioning may explain what differences were observed between *Macoma balthica* and *Potamocorbula amurensis*. For example, higher Cd absorption from uncoated HFO than organically-coated HFO (at low Cd loadings) coincided with differences in digestive processing in both species. The lack of difference in AE between uncoated and organically coated Si in *M. balthica* (in contrast to *P. amurensis*) was coincident with a lack of difference in digestive processing. *M. balthica* accumulated Cd by absorption in the intestine and limited particle retention in the digestive gland. Less Cd was absorbed in the intestine of *P. amurensis* but a larger proportion of food was partitioned to the digestive gland where metal absorption is most efficient. Thus overall gut retention was not the critical factor when comparing absorption mechanisms between the 2 species, but differences in digestive partitioning of food particles were important.

Several previous studies have shown that when humic acids are adsorbed onto oxides, they can effectively mask the properties of the underlying phase (Tipping 1981, 1986, Tipping & Heaton 1983). If we consider absorption efficiency as a geochemical probe, our data for most experiments are consistent with this idea. When particles were coated with humic substances, metal bioavailability to clams was significantly different in many experiments, compared to the metal bioavailabilities from uncoated particles. When humic substances are associated with different mineral surfaces such as Si or HFO, the binding of functional groups with the surface ligands of the mineral can result in differences in the steric conformation of the humic molecule. This can affect what ligands are available and the binding strength of those ligands (Francois 1990). Steric differences may have caused some of the differences observed between treatments in our study (i.e. less concentration effect in Cd absorption efficiency from organic-coated Si), but consistent steric effects were not observed among all treatments.

In summary, the present study showed that inorganic particles, and particles coated with humic substances such as humic and fulvic acids, can act as a trophic link, although not highly efficient, in the transfer of Cd to consumer animals under oxidized conditions in estuarine and marine environments. Both geochemical and biological processes interact to affect the food chain transfer of Cd under these conditions. Inorganic and organic-coated particles act more predominantly as a trophic sink for Cr(III) in oxidized sediments. Some of the variability in the bioavailability of particle-bound metals may relate to the complex nature of metal reactions with humic substances.

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