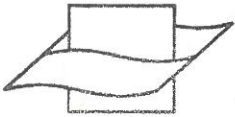

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TRACE METAL ACCUMULATION BY ESTUARINE MOLLUSKS

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INTRODUCTION

It has been recognized for some time that the concentration of metals is significantly higher in the marine biosphere than in the hydrosphere. Much of the earlier work has afforded little information on the analyses of individual organisms and in addition, much of the data was derived from questionable experimental techniques. More recent work has indicated an increasing interest in the biosphere, particularly in relation to trace-element uptake by marine organisms.

Since mollusks are able to concentrate selectively chemical materials up to many hundreds of times that level found in their environment [0.0001 ppm to 0.01 ppm (14)],⁵ a considerable portion of the writers' research has been concerned with the mechanics of uptake of such substances. The maintenance of a healthy shellfish resource capable of replication as well as the constant availability of an excellent food product is important from a public health point of view. The types of chemical materials selectively picked up and concentrated are also of interest. The intent of the writers is to determine whether these materials are picked up through the shellfish food chain or directly from the overlying waters. Some work has shown that the members of the food chain are also capable of concentrating certain of these materials.

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⁵Numerals in parentheses refer to corresponding items in the Appendix.—References.

Studies on the uptake, concentration, and depletion of chemical materials elicit considerable interest as to the mechanism of transport, as well as the chemical form, and anatomical area in which they are stored. Considerable importance is attached to the storage mechanism, turnover rates, and the chemical nature of the stored compound, as well as to possible toxic activity of such material to shellfish and humans, including the public health significance. In addition, the possibility of shellfish defense mechanisms against such chemical materials, including detoxification and the probability that the concentration mechanism is reversible, require investigation. The biochemical significance of such stored materials and their physiological effect on shellfish, warrant study. The role that the shell may play in the various concentration and depletion mechanisms remains to be determined.

A number of studies on trace metals, in relation to the marine environment, have been devoted to one or two metals, particularly zinc and copper. Reference is made to the work of Chipman (7), McFarren (28), and Drinnan (10). The investigations of Goldberg (13) on the biogeochemistry of trace metals, and the recent work of Brooks and Rumsby (5) on trace metal uptake by bivalves in the natural marine environment, are well known. Interest has also been focused on the studies of Armstrong and Atkins (2), Bowen and Sutton (4), Schubert (33), Williams (24), Lehninger (25), and Korringa (24), regarding their efforts to interpret, in the case of marine plants and animals, their experimental results in the light of modern chemical theory. However, the mechanisms whereby trace elements are concentrated in the marine biosphere are not well understood, although the following pathways have been suggested [Brooks and Rumsby (5)]:

1. Particulate ingestion of suspended material from seawater.
2. Ingestion of elements via their preconcentration in food material.
3. Complexing of metals by coordinate linkages with appropriate organic molecules.
4. The incorporation of metal ions into physiologically important systems.
5. Uptake by exchange, for example, onto mucous sheets of the oyster.

Because shellfish are unique in their ability to selectively concentrate materials within their environment, and because there is relatively little information on trace metal distribution in marine animals, a group of studies were initiated to investigate the mechanics of concentration of these materials.

The initial efforts in the laboratory were studies involving the uptake rates and concentration levels for selected trace metals under various conditions of salinity, temperature, and dissolved oxygen, which might be expected under natural environmental conditions. Commensurate studies were made within a simulated natural environment using various concentrations of selected trace metals. Depletion rates for certain of these materials were determined in an environment free of pollution from metals.

Data indicating the uptake and concentration rates of approximately 10 trace metals in the Northern Quahaug, a hard-shell clam (*Mercenaria mercenaria*), the soft-shell (*Mya arenaria*), and the American Eastern and the Pacific Oysters (*Crassostrea virginica* and *C. gigas*), in their natural environment are presented. The results of studies with the above mollusks, in addition to the common blue edible mussel, (*Mytilus edulis*), the surf clam (*Spisula solidissima*), and the channeled whelk (*Busycon canaliculatum*) in a simulated natu-

ral environment using various concentrations of copper, lead, zinc, cadmium, and chromium are given. Brief reference is made concerning investigations of the anatomical uptake of lead in the Eastern Oyster, as well as depletion studies.

METHODS AND PROCEDURES

To quantitate water levels and marine aquatic life for toxic metals to the extent required for effective pollution monitoring, requires sophisticated instrumentation, to make possible speed, simplicity, great sensitivity, and accuracy. Instruments fulfilling this specification are the spectrograph, polarograph, activation analysis instrumentation, and atomic absorption spectrophotometry. The latter is relatively new and is rapidly becoming the instrument of choice for trace metal analysis, because it meets all of the specifications noted, and presents less interference problems.

The material to be analyzed is homogenized, lyophilized, wet digested, diluted, and read on an atomic absorption spectrophotometer. The instrument is calibrated using solutions of pure metal standards. To improve the sensitivity and accuracy in samples of exceedingly low metal concentration, it is

TABLE 1.—ATOMIC ABSORPTION SPECTROPHOTOMETRY

Element	Detection Limits, in parts per million	Sensitivity, in parts per million per 1% absorption
Calcium	0.01	0.1
Cadmium	0.01	0.04
Cobalt	0.15	0.45
Chromium	0.01	0.15
Copper	0.005	0.20
Iron	0.05	0.30
Magnesium	0.003	0.015
Manganese	0.01	0.15
Nickel	0.05	0.30
Lead	0.15	0.50
Zinc	0.005	0.04

usually necessary to chelate the particular trace metal and extract it from the aqueous medium with organic solvents. These are compared with like metal chelate standards of absolute purity.

Because the metal is tied up in the tissue, it must be freed. Therefore, the first problem is to liberate the metal in as gentle but effective a manner as possible, so that it might be measured. The first step was a study of the digestion of shellfish tissue to remove the organic material, or solubilize it, leaving all the trace metal in solution. This was accomplished by a procedure using acid digestion. The sensitivity of the instrumentation used in this method (Atomic Absorption) is shown in Table 1.

The first experiments involved a study of the uptake and concentration of zinc, iron, copper, nickel, cobalt, manganese, lead, cadmium, and chromium within the natural environment. Four species were studied: two clams

(*Mercenaria mercenaria* and *Mya arenaria*) and the oysters (*Crassostrea virginica* and *C. gigas*).

Approximately 100 stations were chosen along the Atlantic Coast from Maine through North Carolina. Two stations along the Pacific Coast (Washington) were also included. Stations were selected without regard to possible chemical pollution; the only consideration being that they represented excellent resources of at least one of the species chosen for study. The shellfish were collected and frozen for shipment to the laboratory. The samples were thawed under refrigeration, cleaned, shucked, and prepared for analysis of trace metals by the procedure indicated above. The data are shown in Tables 2 and 3.

TABLE 2.—AVERAGE TRACE METAL LEVELS IN SHELLFISH TAKEN FROM ATLANTIC COAST WATERS, IN PARTS PER MILLION OF WET WEIGHT

Element	Eastern Oyster	Soft Shell Clam	Northern Quahaug
Zinc	1428	17	20.6
Copper	91.50	5.80	2.6
Manganese	4.30	6.70	5.8
Iron	67.00	405	30
Lead	0.47	0.70	0.52
Cobalt	0.10	0.10	0.20
Nickel	0.19	0.27	0.24
Chromium	0.40	0.52	0.31
Cadmium	3.10	0.27	0.19

TABLE 3.—RANGE OF TRACE METAL VALUES IN SHELLFISH HARVESTED FROM ATLANTIC AND PACIFIC WATERS, IN PARTS PER MILLION OF WET WEIGHT

Element	Oysters		Soft Shell Clam	Northern Quahaug
	East Coast, U.S.	West Coast, U.S.		
Zinc	180 - 4120	86 - 344	9.0 - 28	11.50 - 40.20
Copper	7.0 - 517	7.80 - 37.50	1.20 - 90	1.0 - 16.50
Manganese	0.14 - 15.0	0.90 - 16	0.10 - 29.90	0.7 - 29.70
Iron	31 - 238	15.30 - 91.40	49.70 - 1710	9.0 - 83.0
Lead	0.10 - 2.30	0.10 - 4.50	0.10 - 10.20	0.10 - 7.50
Chromium	0.04 - 3.40	0.10 - 0.30	0.10 - 5.0	0.19 - 5.80
Nickel	0.08 - 1.80	0.10 - 0.20	0.10 - 2.30	0.10 - 2.40
Cobalt	0.06 - 0.20	0.10 - 0.20	0.10 - 0.20	0.10 - 0.20
Cadmium	0.10 - 7.80	0.20 - 2.10	0.10 - 0.90	0.10 - 0.73

Studies of trace metals in the Pacific Oyster (*Crassostrea gigas*) were carried out in cooperation with the Pacific Northwest Marine Health Sciences Laboratory located at Purdy, Wash. Source of the oysters was Burley Lagoon. The source oysters were sampled and analyzed for baseline trace metal values, and divided into two experimental groups. One group was placed in trays at the Purdy laboratory. The second group was maintained in similar trays at the Manchester site. Both groups were kept in a constant-flow seawater system. The two groups were sampled monthly followed by immediate freezing and shipment to the laboratory. Here they were treated and analyzed for trace met-

als as previously described. The study was divided into time phases, each phase represented a period of approximately four to six months. The samples were frozen and shipped to the laboratory for processing and analysis. These data are given in Table 4.

It was necessary to design a system in which uptake rates and concentration levels could be studied under varying simulated natural environments (see Fig 1). This flow-through arrangement permitted control of seawater, precision chemical flows, and adequate mixing of materials. Safety control of chemical flow, an adequate level of dissolved oxygen, and satisfactory temperature control were also assured.

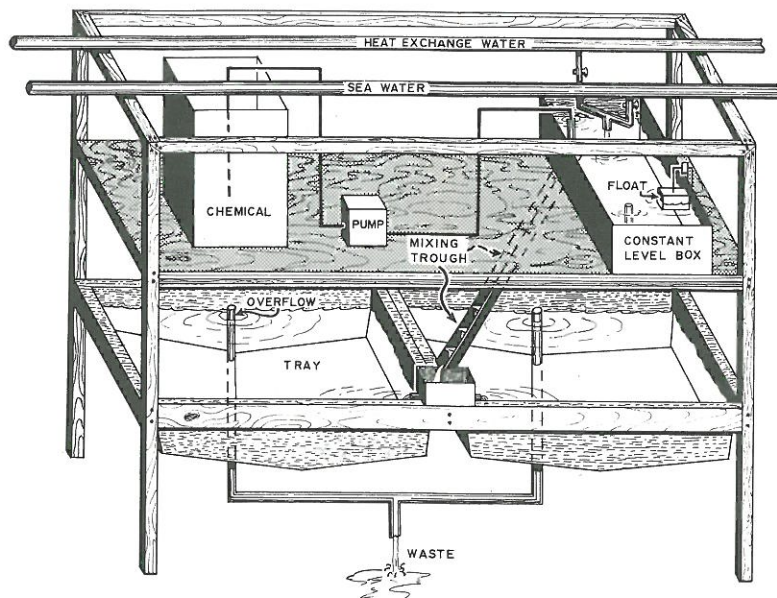


FIG. 1.—TWO-TIERED EXPERIMENTAL SYSTEM

On the upper tier the chemical is metered into the pipe leading from the constant level box into the mixing trough which has a series of baffles. Seawater level is automatically controlled in the box (front sector) by an overflow pipe and by a float which shuts off chemical flow if seawater flow (level) diminishes. Shellfish are held in 20 gallons of water in each tank on the lower tier.

Studies of uptake and concentration within a simulated natural environment were carried out as follows: Shellfish resources for the various species under investigation were collected and sampled for trace metal levels to assure that the experimental animals were within normal limits, and that they were not grossly contaminated by the metals chosen for study. They were sampled and analyzed as a whole for source values and then allowed to acclimate in the concentration system for a period of a week to ten days before they were placed on experiment. Following the acclimatization period, they were again sampled and analyzed in order to secure a baseline acclimated level. The seawater and chemical mixture were started and allowed to flow into the tray. The environmental levels were determined daily, while checks were made on the flow rates

TABLE 4.—SEASONAL VARIATIONS OF TRACE METAL LEVELS IN THE PA-

Element	Average Values for the Periods Indicated,					
	Phase I March-June, 1965		Phase II July-December, 1965		Phase III December-June, 1966	
	Manchester	Purdy	Manchester	Purdy	Manchester	Purdy
Zinc	211	210	243	275	221	199
Copper	24.0	20.20	22.40	20.30	20.60	14.20
Manganese	4.50	3.70	11.60	8.60	4.70	3.10
Iron	42.00	41.80	40.30	28.50	29.00	20.30
Lead	<0.2	<0.20	<0.20	<0.20	<0.20	<0.20
Chromium	<0.2	<0.20	<0.20	<0.20	<0.20	<0.20
Nickel	<0.2	<0.20	<0.20	<0.20	<0.20	<0.20
Cobalt	<0.2	<0.20	<0.20	<0.20	<0.20	<0.20
Cadmium	—	—	—	—	—	—

TABLE 5.—TRACE METAL ACCUMULATION STUDIES IN A SIMULATED NATURAL ENVIRONMENTAL SYSTEM

Metal (1)	Environmental Level, in parts per million (2)	Values, in milligrams per kilogram		Total Accumulation, in milli- grams per kilogram (5)	Accumulation Time, in days (6)	Accumulation Rates, in milli- grams per kilogram per day (7)	Species (8)	Sea Water Temperature, in degrees Celsius (9)
		Initial (3)	Final (4)					
Copper	0.1	23	79	56	10	5.60	Soft Shell	20
Copper	0.2	15	85	70	25	3.00	Soft Shell	20
Copper	0.05	35	200	165	8	20	Soft Shell	No Control (25-26)
Copper	0.5	6.5	8	1.5	25	0.06	Quahaug	No Control (10)
Zinc	0.2	10	27	17	50	0.35	Soft Shell	20
Cadmium	0.05	0	8	8	70	0.10	Soft Shell	20
Cadmium	0.1	0	(3.8 in 4 weeks) 9	9	56	0.16	Soft Shell	20
Lead	0.1	0	(6.5 in 4 weeks) 112	112	70	1.60	Soft Shell	20
Lead	0.2	0	235	235	40	5.80	Soft Shell	20
Lead	0.2	0	260	260	84	3.10	Soft Shell	20
Lead	0.2	0	(220 in 70 days) 35	35	56	0.63	Quahaug	20
Lead	0.025	0	17	17	49	0.35	Eastern Oyster	20
Lead	0.05	0	35	35	49	0.71	Eastern Oyster	20
Lead	0.1	0	75	75	49	1.50	Eastern Oyster	20
Lead	0.2	0	200	200	49	4.00	Eastern Oyster	20

CIFIC OYSTER (*CRASSOSTREA GIGAS*) ALONG THE WASHINGTON COAST

in parts per million, Wet Weight

Phase IV July-October, 1966		Phase V October, 1966-March, 1967		Phase VI May-July, 1967	
Manchester	Purdy	Manchester	Purdy	Manchester	Purdy
262	225	224	199	250	206
25.80	19.20	17.40	12.50	18.30	14.30
8.90	8.30	4.30	4.40	3.41	2.24
35.10	24.90	38.40	30.30	67.10	48.50
<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
1.30	1.40	1.12	1.30	0.90	0.80

TABLE 6.—THE ANATOMICAL DISTRIBUTION OF LEAD IN THE EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) FROM A CONTROLLED ACCUMULATION SYSTEM

Environmental Level in parts per million (1)	Anatomical Area (2)	Accumulation Values, in milligrams per kilogram		Accumulation Rate, in milligrams per kilogram per day (5)	Time, in days (6)
		Initial (3)	Final (4)		
0.025	muscle	0	9	0.20	49
0.05	muscle	0	23	0.50	49
0.1	muscle	0	50	1.13	49
0.2	muscle	0	115	2.30	49
0.025	mantle edge	0	15	0.30	49
0.05	mantle edge	0	32	0.70	49
0.1	mantle edge	0	73	1.50	49
0.2	mantle edge	0	192	3.90	49
0.025	mantle	0	14	0.30	49
0.05	mantle	0	37	0.78	49
0.1	mantle	0	75	1.60	49
0.2	mantle	0	212	4.30	49
0.025	remainder	0	18	0.36	49
0.05	remainder	0	34	0.71	49
0.1	remainder	0	75	1.60	49
0.2	remainder	0	237	4.8	49
0.025	gill	0	23	0.49	49
0.05	gill	0	65	1.35	49
0.1	gill	0	160	3.10	49
0.2	gill	0	274	5.60	49
0.025	gonad	0	26	0.56	49
0.05	gonad	0	55	1.20	49
0.1	gonad	0	163	3.28	49
0.2	gonad	0	325	6.60	49
0.025	liver	0	28	0.59	49
0.05	liver	0	66	1.40	49
0.1	liver	0	145	2.95	49
0.2	liver	0	368	7.40	49

three to four times a day. The shellfish were sampled at intervals of four days, one week, and each week thereafter until termination of the experiment. The samples were processed and analyzed as described. Five metals have been studied: zinc, lead, copper, cadmium, and chromium on the species indicated. The pH, salinity, DO, and temperature remained relatively constant throughout. The temperature was held at $20 \pm 1^\circ \text{C}$, with salinity variable, usually 31‰, while

TABLE 7.—TRACE METAL DEPLETION STUDIES IN A CONTINUOUS FLOW SYSTEM

Species (1)	Metal (2)	Depletion Range, in milligrams per kilogram per day		Depletion Time, in days (5)	Tempera- ture, in degrees Celsius (6)	Depletion Rate, in milli- grams per kilogram per day (7)	Source of Specimens (8)
		Initial (3)	Final (4)				
Soft Shell	Copper	124	36	7	20	12.50	from 0.1 ppm accumulation study
Soft Shell	Copper	96	34	11	20	5.10	River View (water off)
Soft Shell	Copper	68	9	10	20	5.90	Allens Harbor
Soft Shell	Copper	62	34	4	20	7.00	Long Meadow (water off)
Soft Shell	Copper	58	20	15	20	2.60	Jerusalem (water off)
Soft Shell	Copper	52	24	15	20	1.90	Allens Harbor (water off)
Soft Shell	Copper	48	22	4	20	6.50	from 0.2 ppm accumulation study
Soft Shell	Copper	33	20	7	20	1.90	Charlestown
Hard Shell	Copper	17.5	13.0	84	no control (4-12)	0.05	Boston Harbor
Hard Shell	Manga- nese	24	16	84	no control (4-12)	0.095	Boston Harbor
Hard Shell	Zinc	38	26	84	no control (4-12)	0.12	Boston Harbor
Hard Shell	Iron	27	27	84	no control (4-12)	0.0	Boston Harbor
Oyster	Lead	203	188	21	20	0.71	from 0.2 ppm accumulation study
Oyster	Lead	79	63	21	20	0.76	from 0.1 ppm accumulation study
Oyster	Lead	32	22	21	20	0.48	from 0.05 ppm accumulation study
Oyster	Lead	24	5	21	20	0.91	from 0.025 ppm accumulation study

the DO varied between 8-10 ml per l. The concentration levels of the metals studied, as well as the time, were varied according to the experimental design. The results are given in Table 5.

The third aspect of these investigations involved the study of lead uptake and concentration in the American Eastern Oyster (*Crassostrea virginica*). Making use of the system described in the preceding paragraph, studies were

carried out using lead environmental concentrations of 0.2 ppm, 0.1 ppm, 0.05 ppm, and 0.025 ppm over a period of 10 weeks. Samples consisting of six oysters each for anatomical and whole animal metal values were removed from the system at zero, four, and seven days, and weekly thereafter until termination of the experiment. The samples were again processed and analyzed. The resulting data are shown in Table 6.

The fourth aspect of the overall study concerned the depletion of certain metals from shellfish used elsewhere in the investigations. The same environmental system was used to allow temperature-controlled seawater relatively free of the particular metal being studied to flow through the trays. The experiments were carried out over varying time intervals, up to several weeks. The shellfish were processed and analyzed in the same manner as heretofore. The data are summarized in Table 7.

APPRAISAL AND SUMMARIES

The first part of the appraisal deals with background information and theoretical considerations of pollution and toxicity. The remaining sections deal with the four phases of the studies to date including a summary for each.

Pollution and Toxicity.—The number of substances to which the term "poison" may be ascribed is large, as well as varying in the degree of their effect. In the case of man and other air-breathing animals, the threshold dose of a toxic material usually means the maximum amount that can be taken without causing death. However, the case for aquatic animals living in a water environment is considerably different. The aquatic fauna, instead of receiving an absolute quantity at one time, is continually exposed to a given concentration of the toxic material—a case comparable to that of a man regularly drinking water containing a toxic metal or breathing air containing a noxious gas or vapor. It is not surprising, therefore, that the student of pollution problems is primarily concerned with the concentration of the poison he is investigating, and the manner in which the effect is related to this, rather than on the absolute amount required to harm and kill. Animals have the capacity to eliminate or detoxify poisons to some extent, or even to destroy them. The rapidity at which they are able to do this, permitting survival, depends on the concentration of the toxic material to which they are exposed [Jones (21)].

One of the characteristics of living cells is their capacity to take up elements from a solution against a concentration gradient. This is perhaps most obvious for marine organisms, especially for autotrophic algae, which obtain all their nutrients directly from seawater. The ability of marine organisms to concentrate elements above that level found in their environment has been recognized for some time. The following points should be noted in relation to the concentrating ability of plankton and brown algae [Bowen (3)]:

1. All elements are concentrated to a degree with the exception of chlorine, which is rejected, and sodium, which is weakly rejected. The concentration factors are of the order of 1.0 for Br, F, Mg, Na, and S, and higher for all other elements.

2. Among cations (including metallic elements such as Fe which may exist as colloids in the sea), the order of affinity for living matter is generally: Tetravalent and trivalent elements > divalent transition elements > divalent

Group II A metals > univalent Group I metals. The tetravalent and trivalent subgroup have rather different affinities for plankton and brown algae, i. e., Plankton: Fe>Al>Ti>Cr, S>Ca; and Brown Algae: Fe>La>Cr>Ca>Ti>Al>Si. Similar differences are found between these organisms in their affinities for the divalent transition metals, i. e., Plankton: Zn>Pb>Cu>Mn>Co>Ni>Cd; and Brown Algae: Pb>Mn>Zn>Cu, Cd>Co>Ni. The affinity of both organisms for lead, with no known biological function is of interest. It is clear that the heavier elements in these groups tend to be more readily taken up than the lighter ones, which may be connected with their greater polarizability.

3. The order of affinity of living matter for anions is: (a) Nitrate>trivalent anions>divalent; and (b) anions>univalent anions. It is possible that most polyvalent metallic elements are more or less cheated by organic matter.

The main features of the uptake of ions by cells can be accounted for by assuming that another process operates apart from simple diffusion. This process is called active uptake and is closely linked with metabolic activities within the cell. The metabolic processes provide the energy necessary for the uptake against a concentration gradient. Active uptake has larger temperature coefficient than does uptake by diffusion. In long-term experiments the effect of temperature is probably complicated by increased rates of growth, cell division, and other factors. Active uptake requires oxygen and occurs only in cells which are respiring freely. Substances that inhibit respiration also inhibit uptake of ions. The rate of uptake of ions may be limited by either the rate of exchange at the cell membrane, or bulk phase diffusion inside the cell. The former is usually limiting for ions present at low external concentrations and the latter for ions at high external concentrations. It has been suggested that bulk phase diffusion limits the rates of uptake of most cations. There appears to be at least two active transport systems in addition to the diffusion processes. A number of theories have been advanced to explain active transport. One of the most popular is the carrier hypothesis [Sutcliffe (34)]. The mechanism by which the chelate is transported across the cell membrane and the return of the carrier molecule is not clear. One possibility as a carrier involves the enzyme ATPase which requires both Na^+ and K^+ for activation, and which is associated with cell membranes. Accordingly the ions are transported across membranes as chelates with metabolically produced organic molecules.

Uptake by Invertebrate Animals.—The most primitive animals, the unicellular protozoa, take up ions from solution by diffusion in the same ways as do algae. Many marine species and all freshwater species have vacuoles, and these are able to open at intervals and extrude fluid from the cell. The vacuole regulates the osmotic pressure of the cell and so controls its volume.

Multicellular invertebrate animals can be divided into two groups as far as uptake is concerned: those with permeable skins and those without. The majority of marine invertebrates (colenterates, annelids, mollusks, and echinoderms) have soft bodies with permeable skins through which ions can diffuse freely. In this situation the body fluid or blood is quite similar to seawater in composition. The gills of mollusks are coated with a layer of complex carbohydrate sulphates which may function as ion-exchangers. The gills of marine crustacea, which have hard impermeable carapaces, are fully permeable to water and salts.

Toxic Action.—An element is toxic if it impairs the growth, reproduction, or metabolism of an organism when supplied above a certain concentration.

All elements are toxic at high concentrations, and some are notorious poisons even at low concentrations. The essential micronutrient copper, which is a necessary constituent of all organisms, is highly toxic at quite small concentrations. The other essential micronutrients are also toxic when supplied in excess, though not all in as striking a fashion. There is an optimum range of concentration, which is sometimes quite narrow, for the supply of each element to the organism.

When excessive amounts of an element are fed to an organism, they frequently cause death. The usual measure of the amount required to cause death is called the LD_{50} . This is the amount that, if fed to each individual in a population, kills half of the population. The LD_{50} is an imprecise measure unless it is qualified by specifying: (1) The chemical state of the element; (2) the means of feeding; (3) the age or developmental stage of the organism; and (4) the time elapsed between feeding and death.

The most important mechanism of toxic action is thought to be the poisoning of enzyme systems. The more electronegative metals, notable copper, mercury, and silver, have a great affinity for amino, imino, and sulfhydryl groups which are doubtless reactive sites on many enzymes, and are readily chelated by organic molecules. Therefore attempts are found to correlate metal toxicities with such factors as their electronegativities, the insolubility of their sulfides, or the order of stability of their chelated derivatives: (1) Order of electronegativities of some divalent metals, $Hg > Cu > Sn > Pb > Ni > Co > Cd > Fe > Zn > Mn > Mg > Ca > Sr > Ba$; (2) order of stability products of the sulfides, $Hg > Cu > Pb > Cd > Co > Ni > Zn > Fe > Mn > Sn > Mg > Ca$; and (3) order of stability of chelates, $Hg > Cu > Ni > Pb > Co > Zn > Cd > Fe > Mn > Mg > Ca$.

It appears likely that all the divalent transition metals, as well as the other electronegative metals, which form insoluble sulfides, such as Ag, Mo, Sb, Ti and W are poisons by virtue of their reactivity with proteins, and especially with enzymes. In view of the large number of enzymes in living cells, the variations in toxicity indicated above are hardly surprising. Studies have shown that metals giving rise to similar toxic effects may be acting on quite unrelated enzymes, and many more atoms of metal are absorbed by an inactivated enzyme than are required to block the reactive sites [Passow, *et al* (30)]. Other modes of toxic action are:

1. Substances behaving as antimetabolites. This might be arsenate and chlorate occupying sites for phosphates and nitrates respectively. (fluoride, borate, bromate, permanganate, antimonate, selenate, tellurate, tungstate, and beryllium may possibly act in this manner.)

2. Substances forming stable precipitates or chelates with essential metabolites (Al, Be, Ti, Sc, Y, Zr, reacting with phosphate, Ba with sulfate, or Fe with ATP).

3. Substances catalyzing the decomposition of essential metabolites. (La and other lanthanide cations decompose ATP.)

4. Substances combining with the cell membrane and affecting its permeability (Au, Cd, Cu, Hg, Pb, U). These elements may affect transport of sodium, potassium, chlorine, or organic molecules across membranes, or even rupture them.

5. Substances which replace structurally or electrochemically important elements in the cell and then fail to function (Li replacing Na, Cs replacing K, or Br replacing Cl).

Metal-organic compounds may be either more toxic than the metal ion (ethyl mercuric chloride) or much less so (cupric ion and copper salicylaldehyde).

The elements can be divided into four groups with regard to their pollution potential [Bowen (3)]:

1. Very high potential pollution: Ag, Au, Cd, Cr, Cu, Hg, Pb, Sb, Sn, Te, Zn.
2. High potential pollution: Ba, Bi, Ca, Fe, Mn, Mo, Ti, U.
3. Moderate potential pollution: Al, As, B, Be, Br, Cl, Co, F, Ge, K, Li, Na, Ni, Rb, V, W.
4. Low potential pollution: Ga, I, La, Mg, Nb, Si, Sr, Ta, Zr.

Toxicity-Pollution-General.—The classical paper by Ellis (11) on river pollution cites 114 substances which may be found among stream pollutants. This very long list of substances possibly dangerous to aquatic life, increased with industrial developments. Doudoroff and Katz (9) reviewed 281 papers, only 35 of which were published before 1920. The study of the biological aspects of river pollution attracted attention as indicated by Hynes (19) in his recent book. Powers (31) published on the goldfish as a test animal in the study of toxicity, and Carpenter (6), working at Aberystwyth, began a study of the pollution of the rivers at Cardiganshire. His study was concerned with the results of lead and zinc mining operations carried on in the area in previous years but now declining. Carpenter showed that the death of fish in the polluted waters was not due to gritty material in suspension but to lead salts. Fish confined in cages in the river remained healthy when the water flowing through the cages was free of dissolved lead, but died when the lead concentration rose to 0.3 ppm to 0.4 ppm at flood times. Carpenter later concluded that the toxic action of heavy metals on fish was due to a process of asphyxiation. According to the hypothesis of Carpenter, heavy metals (lead, zinc, copper, mercury, silver, nickel, and cadmium) in dilute solution precipitate the mucous secretions produced by the gills. The interlamellar spaces become filled with precipitate and the normal movement of the gill filaments becomes impossible. The intimate contact between water and gill tissues, necessary for respiration, is thus prevented and eventually gaseous exchange is impeded to such an extent that the fish dies. Carpenter's theory was endorsed by Ellis (11) and by Westfall (36) but questioned by Lloyd (26) who, in a study of the toxicity of zinc salts to rainbow trout, observed no precipitation of mucous on the gills or in the gill chamber. Lloyd considers the toxicity of zinc to be exerted through the swelling and breakdown of the gill epithelium. It seems, therefore, that the heavy metals in some manner impair or destroy the gill's ability to carry on gaseous exchange.

The course of the toxic process can also be followed by placing a fish in a dilute solution of a heavy metal salt and recording periodically the rate of the opercular movements. A means of measuring the oxygen removal is also necessary. Jones (20), using the stickleback, subjected the organism to 0.002N CuSO_4 and 0.005N $\text{Pb}(\text{NO}_3)_2$ solutions. Normally the stickleback breathes 120 times per min. at 17°C. The opercular movements are not regular; 5 to 10 rapid movements may alternate with a period of rest lasting several seconds. As the heavy metal salt solution is run into the system, the rate of the opercular movement as well as the rate of oxygen utilization begins to rise. The increased oxygen utilization is probably due to this increased activity resulting from the struggle against the unfavorable changes in its environment. As the

toxic process advances, the respiratory movements become more and more rapid, more regular, and of increased amplitude. Despite efforts by the animal to maintain its oxygen supply, the oxygen consumption now declines to about 20% of normal, the opercular movement begins a precipitous drop, and the fish dies.

Heavy metal salts in solution constitute a serious form of pollution because they are stable compounds, not readily removed by oxidation, precipitation, or any other natural process. In soft waters they are fatal to fish at very low concentrations. Copper salts are fatal to sticklebacks down to 0.02 ppm. Mercury and silver salts are even more toxic. Lead and zinc salts are rather less so. A characteristic feature of heavy metal pollution is its persistence in time, as well as in space, for this type of pollution may continue for years after pollution operations have ceased. Affleck (1) in work done in Australia, showed that zinc compounds are toxic to ova and alevins of rainbow trout and that levels of 0.01 ppm to 0.02 ppm may be fatal. These findings confirm the earlier work of Jones (20) indicating that the toxicity of lead and zinc salts is greatly reduced in the presence of soluble calcium compounds.

Uptake and Concentration Within the Natural Environment.—The results of the study of uptake and concentration within a "natural" environment are represented by the studies of trace metals in shellfish taken from along the Atlantic and Pacific Coasts as previously described and summarized in Tables 2 and 3.

Zinc.—Zinc levels in shellfish along the Atlantic Coast varied over a range of approximately 10 ppm to 40 ppm in the case of hard and soft-shell clams to a value of 180 ppm to 4100 ppm for the Eastern Oyster. The Pacific Oyster, however, showed levels approximately one-tenth of the latter level (90 ppm to 350 ppm).

As compared to that found in hard and soft-shell clams, the high zinc level in the oyster suggests a possible species difference in the physiological role of zinc. Zinc plays an important role in biochemical systems, serving as an enzyme activator, and as a constituent of important metallo-protein enzymes such as carbonic anhydrase [Keilin *et al.* (23), and Vallee (34)]. Schelske (32) has shown that oysters tend to concentrate Zn^{65} . Because zinc shows considerable depletion in the hydrosphere, it is reasonable to conclude that if concentration of zinc by the biosphere is uniform throughout, as indicated by shellfish uptake-concentration of this ion, then this may constitute a major pathway for its removal from seawater [Brooks and Rumsby (5)].

Copper.—The data from Tables 2 and 3 indicate that copper levels in oysters appear to be approximately 10 to 15 times that found in both the hard and soft-shell clam. As particulate absorption may be suspected in the case of the oyster, the levels might be expected to be somewhat higher. Korringa (24) has shown that positive polyvalent ions such as Al^{+++} , Cu^{++} , Fe^{+++} , Zn^{++} , Hg^{++} , and Mn^{++} are concentrated by the mucous sheets of the oyster. It has been shown [Mahler (27)] that copper does have an important biochemical role as an enzyme activator, a constituent of a flavo-protein, as well as in the hemocyanins found in the hemolymphs of certain mollusks.

Further observation of the data shows that the Eastern oyster (*C. virginica*) contains five times the amount of copper found in the West Coast oyster (*C. gigas*). The copper level for both appears to follow the zinc values in a very definite ratio—approximately 10 to 20 times more zinc than copper in each case. In all uptake and concentration studies in this area the copper is found to

rise and fall with corresponding changes in the zinc levels.

Iron, Nickel, Manganese, and Chromium.—These elements are all members of the iron family [Goldschmidt (15)] having atomic numbers of 23-28 and being measurable in the sediment.

Iron may be found as porphyrin-bound iron in the striated muscle bundle [Brooks and Rumsby (5)]. Kawai (22) has identified a cytochrome system in oysters (*C. gigas*) responsible for 80% of their respiration. Iron has been shown to be distributed in mollusk shells in relation to porphyrin localization there [Fischer (12) and Comfort (8)].

Manganese has been shown to be an activator in many enzyme systems [Mounter (29)].

Studies indicate that nickel activates certain enzyme systems such as arginase [Greenberg (16)] as well as certain carboxylases [Weisbach (35)].

The role of chromium in biological systems has been less well established, but this metal has been shown to restore activity to metal-free carboxypeptidase [Vallee (34)]. It is probable in this case that the level of chromium is due to sedimentary contamination; however the role of chromium, nickel, and manganese in molluscan biochemical systems should not be excluded.

From the data described herein it is concluded that the levels of iron and manganese appear to be highest in the case of the soft-shell clam, followed in order by the oyster and hard-shell clam. The Pacific Oyster (*C. gigas*) appears to have an iron level comparable to the Eastern Oyster with the exception that *C. virginica* has a much wider range of values. Nickel and chromium with few exceptions, as in the case of the hard and soft-shell clam, appears to be the same in all species examined.

Lead.—Lead appears to have a more obscure biochemical role, if any, and is toxic to most enzyme systems. It is, however, strongly basic and will readily coordinate with suitable organic ligands.

Lead is shown in the data presented herein, to be virtually uniform in all species with few exceptions, and these may represent possible pollution.

Cobalt.—Cobalt levels were found to be uniform for all species investigated.

Cadmium.—The biological role of cadmium, if any, is unknown, but it does appear in many tissues, probably as a contaminant, and is cumulative. It probably acts as an enzyme inhibitor. Cadmium is moderately toxic to all organisms, and it is, like lead and mercury, a cumulative metal in mammals. In the laboratory it has been shown that cadmium is extremely toxic to the Eastern oyster. It appears to be somewhat less toxic in the hardshell clam.

Cadmium is lowest in the calcareous tissues, and is accumulated by the viscera of the scallop, *Pecten novaezelandicae* [Brooks and Rumsby (5)]. Cadmium acts synergistically with other substances to increase toxicity. Cadmium concentrations of 0.03 ppm in combination with 0.15 ppm zinc causes mortality of salmon fry [Hublou (18)].

It is probable that the wide range of values found for zinc in the oyster along the East Coast may be indicative of some degree of pollution in certain areas. Studies of these zinc levels from the various coastal areas strongly suggest that the normal value for zinc in *Crassostrea virginica* is more in the range of 800 ppm to 1000 ppm. This seems to be in agreement with the value put forth by Drinnan (10).

Chipman (7) found that both the food chain and sediment may contribute some metals, however, it appears that a considerable amount of it originates from that picked up and concentrated from the water (soluble portion).

Consideration of the higher accumulation values found in certain areas makes it apparent that pollution (high environmental levels) is undoubtedly a contributing factor. The ratio of the accumulation of the trace metals studied compared with those environmental levels found naturally are shown in Table 8. These enrichment factors are based on the use of the seawater trace metal levels of Goldberg (13). The values compare relatively well, with some exceptions, to those as determined by the Australian work of Brooks and Rumsby (5). The apparent selectivity for trace metals among various molluscan species appears to depend to a considerable extent on the metals available in the environment, their chemical and physical properties, the kind and number of ligands available for chelation, transport, and storage, and the stability of the

TABLE 8.—TRACE METAL ENRICHMENT FACTORS FOR SHELLFISH COMPARED WITH THAT OF THE MARINE ENVIRONMENT

Element (1)	Oyster (2)	Quahaug (3)	Soft Shell Clam (4)	Surf Clam (5)	Mussel (6)	Whelk (7)
Cadmium	318,000 ^a 226,000	750	800	—	100,000 ^a 800(2)	6,300
Chromium	60,000 ^a 31,600	23,400	10,400	—	—	—
Copper	13,700 ^a 14,800	900	2,000	450	3,000 ^a 1,150	3,800
Iron	68,200 ^a 6,700	3,000	41,000	18,400	196,000 ^a 2,900	—
Manganese	4,000 ^a 2,900	2,900	3,350	1,100	13,500 ^a 1,500	2,100
Nickel	4,000 ^a 3,250(1)	4,500	4,250	—	—	—
Lead	4,000 ^a 4,100	5,800	3,400	—	—	—
Zinc	110,300 ^a 148,000	2,100	1,700	1,525	9,100 ^a 2,200	8,200

^a Values from work of Brooks and Rumsby (5).

complex formed. The overall concentration levels and enrichment factors in unpolluted waters appear to be based on an average available environmental range of 0.0001 ppm to 0.01 ppm. It appears that certain metals do exert an influence on the uptake and concentration of certain others. This is apparent in the case of copper and zinc in the oyster.

Determination of Uptake Rates and Concentration Levels in a Simulated Environmental System.— The results of studies on the uptake and concentration levels in shellfish subjected to a simulated natural environment are given in Table 5.

Although the investigations will eventually include the study of ten metals on six or more shellfish species, the results reported herein, on four of the five metals studied in an accurately controlled environmental system, would appear to justify the following conclusions:

1. There are species differences in the uptake and concentration of a given metal. This is well indicated in the data presented here for copper, zinc, cadmium, and lead.

2. The environmental concentration level to which various species may be subjected will result in different uptake rates (ppm per kg per day) as well as concentration levels attained, depending on the duration of the exposure.

3. The temperature, salinities, dissolved oxygen, pumping rates, and physiological condition of the animal are all closely related to the uptake and concentration level obtained for any given metal with all species.

4. The apparent toxicity of any metal for a given species determines not only the uptake and concentration, but the duration of the experiment as well. This is true for the following metals listed, but not necessarily in the order given, for all shellfish studies: Cd^{++} , Cu^{++} , Pb^{++} , and Zn^{++} .

Copper.—Copper proved to be especially toxic for the soft-shell clam (*Mya arenaria*) as compared to the hard-shell clam (*Mercenaria mercenaria*), and the oyster (*Crassostrea virginica*). In the case of *Mya*, copper above an experimental level of 0.02 ppm proved to be extremely toxic. The 0.1 ppm and 0.2 ppm levels were sufficiently toxic to cut down the duration of the experiment, as well as affecting the overall rate of uptake and tissue concentration attained. The writers obtained uptake rates of 3 ppm per kg per day and 5.6 ppm per kg per day for the 0.2 ppm and 0.1 ppm environmental levels, respectively, at 20°C. The decreased uptake rate found with the higher copper levels indicate the toxic effect of copper on *Mya arenaria*. With the environmental level of 0.05 ppm, the data shows an uptake rate of 20 ppm per kg per day at an elevated temperature (25° to 26°C). The toxicity at this level was at its lowest peak, based on the gross physiological appearance, activity, and mortality. The effect of temperature elevation is indicated by the increased rate of uptake and concentration level attained.

The species difference for the uptake of a given metal is indicated clearly in the case of the hard-shell clam. Results based on a 0.5 ppm experimental level indicated a very low uptake and concentration for *Mercenaria* when compared to that found in the soft-shell clam *Mya* at 10°C. The effect of temperature is again illustrated. A drop in the experimental temperature results in an apparent decreased uptake rate and concentration level.

Zinc.—Using a 0.2 ppm zinc level at 20°C, we find that the soft-shell clam reached a concentration level of 27 ppm in 50 days. This represents a 17 ppm increase in zinc over a period of 50 days or 0.35 ppm per kg of zinc per day. These results indicate an apparent species difference in the ability of the animal to accumulate different metals; this difference is again shown in the case of cadmium and lead. The conclusion is that the soft-shell clam apparently concentrates copper preferentially over zinc under identical experimental conditions.

Cadmium.—The results of the experiments with cadmium in *Mya* placed on each of two different environmental levels of this particular metal (0.05 ppm and 0.1 ppm) may be seen in Table 5. Under the same experimental conditions, *Mya arenaria* is able to concentrate approximately twice the amount of cadmium in its tissues during a given time period, when the environmental level under study is doubled. The apparent species selectivity for a given metal is again evident in that *Mya* is found to pick up and concentrate cadmium at a

lower rate than do either copper or zinc under identical experimental conditions.

Lead.—Lead uptake experiments using the Eastern oyster, the soft and hard-shell clams, show the species differences for the uptake of a given metal. The data indicate that lead is picked up and concentrated by the oyster, the soft and hard-shell clams, respectively, at rates of 4 ppm per kg per day, 3 ppm per kg per day, and 0.63 ppm per kg per day, using the same environmental level and experimental conditions. The effect of subjecting any given species to various environmental levels of a given metal is illustrated in this case. Under the same experimental conditions, with the oyster and the soft-shell clam, it was found that doubling the level of the lead, will result in twice the rate of uptake, and tissue concentration reached in the same period of time.

Generally, the studies show that for any given metal and set of experimental conditions, the uptake rate and tissue concentration level decreased for the following species in the order given: Mya arenaria, Crassostrea virginica, and Mercenaria mercenaria.

Investigation of Anatomical Areas of Storage.—The results of the anatomical storage studies are illustrated in Table 6. The experiments described elsewhere herein were carried out at various levels of lead concentration over a period of ten weeks. The results through the seventh week are given in this section.

The four environmental levels of lead used gave identical results as to the concentration order of all the anatomical areas studied. Anatomical areas in the order of their increasing lead concentration are as follows: muscle, mantle edge, mantle, remainder, gill, gonad, and digestive gland. The accumulation rates doubled as the environmental level of lead used throughout the duration of the experiment was doubled. The oysters appeared to be in good gross physiological condition, at least for the two lower levels (0.025 ppm and 0.05 ppm). However, with the higher experimental lead levels, the gross anatomy showed considerable atrophy and diffusion of the gonadal tissue. In addition, the hepatopancreas became less easily identifiable and lighter in color, compared to the controls as well as the animals on lower levels. The animals exposed to the higher experimental levels showed considerable edema, and the mantle edge began to disappear or become less distinct. The histopathology has not been completed to date.

The higher lead levels found in the gonadal tissue are in line with the recent work reported in humans [Hardy (17)], as well as that observed by Drinnan (10) in the oyster. The high lead concentrations found in the digestive gland and gill, may indicate that these organs serve as areas for lead storage in the oyster. This is compatible with the presence of many chelating organic ligands in these tissues. The lead content of the mantle edge was elevated during the first few days of the experiment. This was followed closely or almost simultaneously by the elevation in the mantle. The initial tissue increase in the mantle edge may be indicative of the mode of entry of lead in the oyster. The mortality rates found in oysters exposed to lead did not appear to be exceptionally high when compared to certain other metals, considering the duration of the study (ten weeks).

Depletion Rates in a Simulated Environment.—The study of uptake and concentration of tracemetals by mollusks indicates that these materials become chemically and structurally incorporated into the tissues of the various organs, and thus have become an integral structural part of the animal.

These storage compounds as previously indicated most probably take three forms: (1) Metal chelates of smaller organic molecules; (2) structural incorporation into macro-molecules (proteins, etc.); and (3) inorganic incorporation into the shell, matrix as inorganic salts.

A different system exists in the case of the uptake and concentration of chemicals than is the case with cellular and other particulate matter. The removal or depletion of these materials, therefore, depends upon the reversal of the incorporating processes. Chemical depletion, which must follow biochemical turnover, is expected to be a slower process than the release of cellular material (biological contaminants).

The results of certain of the writers' depletion studies described earlier are shown in Table 7.

The first series of experiments illustrated represent depletion studies with copper in the soft-shell clam under controlled experimental conditions. These particular animals were taken from certain uptake and concentration experiments, as well as from industrially polluted areas, and were subjected to varying depletion times. The data presented herein indicate that copper is depleted rather rapidly by this species.

Similar experiments with the hard-shell clam at a somewhat lower temperature show that Mercenaria mercenaria depletes itself of copper at a much slower rate than does Mya arenaria. In addition, the Quahaug appears to deplete itself of other metals at similarly slower rates.

Depletion rates for lead in the oyster under similar experimental conditions are given in Table 8. These animals were removed from four different concentration studies and allowed to deplete for a period of 21 days at 20°C. Prior to depletion, the four groups (0.025 ppm, 0.05 ppm, 0.1 ppm, and 0.2 ppm exposures) ranged in tissue concentration from 24 ppm to 203 ppm lead. The depletion rates are seen to be about the same for the higher exposure levels (0.71 ppm and 0.76 ppm per kg per day). However, in the case of the lower levels a varying depletion rate was seen. With the 0.05 ppm exposure level, a depletion rate of 0.48 ppm per kg per day was found, whereas the lowest level gave an elevated rate of depletion (0.91 ppm per kg per day).

Data presented herein indicate that metals are depleted (at least for lead) at a faster rate by the oyster than by the quahaug, but at a slower rate by the oyster than by the soft-shell clam (Mya arenaria). There also appears to be a direct relationship between the uptake rate for a given metal and the depletion for any molluscan species.

CONCLUSIONS

Mollusks in the natural estuarine environment appear to accumulate trace metals at different rates and attain tissue levels according to the environmental concentration of the particular metal, the temperature, the species concerned, as well as the physiological activity of the animal itself.

When the environmental concentration of a particular toxic trace metal persists over a sufficient period of time, the animal may become affected physiologically by the metal accumulation to such an extent that it becomes nonfunctional or dies.

The apparent selectivity for trace metals among various molluscan species

appears to depend to a considerable extent upon the metals available in the environment; their chemical and physical properties; the kind and number of ligands available for chelation, transport, and storage; and the stability of the complex formed. The relative toxicities of the various metals also play a prominent role.

In a simulated natural environmental system the accumulation rates, as well as the tissue levels of the trace metals, are dependent upon the environmental metal concentration, the temperature, the time of exposure, and the particular species used.

The relative toxicity of a particular metal varies from species to species for any given concentration, all other factors remaining constant.

The studies reported herein generally show that for any given metal and set of experimental conditions, the uptake rate and tissue concentration level decreased for the following species in the order given: Soft-shell Clam, *Mya arenaria*; American Eastern Oyster, *Crassostrea virginica*; and the Northern Quahaug, *Mercenaria mercenaria*.

The four environmental levels of lead used in the anatomical accumulation studies gave identical results as to the concentration order for all the anatomical areas studied. The following anatomical areas are given in the order of their increasing lead accumulation (concentration): muscle, mantle edge, mantle, remainder, gill, gonad, and digestive gland. The accumulation rates doubled as the environmental level of lead used throughout the duration of the experiment was doubled.

Considerable toxicity was exhibited towards the animals exposed to the higher environmental levels of lead (0.1 ppm and 0.2 ppm). This was particularly evident regarding the gonadal area of the oyster.

Depletion of trace metals follow biochemical turnover within the animal, and must be expected to be a slower process than is the release of cellular material. From the data presented herein, it appears that metals are depleted (at least lead) at a faster rate by the American Eastern Oyster than they are by the Northern Quahaug, but slower than is the case for the soft-shell clam. In addition, there appears to be a direct relationship between the uptake rate for a given metal and its depletion for any molluscan species. The initial tissue level of any given metal appears to be directly related to the depletion rate for any given species.

APPENDIX.—REFERENCES

1. Affleck, R. J., "Zinc Poisoning in a Trout Hatchery," *Australian Journal of Marine Fresh-water Research*, Vol. 3, 1952, p. 142.
2. Armstrong, F. A. J., and Atkins, W. R. G., "The Suspended Matter of Sea Water," *Journal, Marine Biologists Association, United Kingdom*, Vol. 29, 1950, p. 139.
3. Bowen, H. J. M., *Trace Elements in Biochemistry*, Academic Press, London and New York, 1966.
4. Bowen, V. T., and Sutton, D., "Mineral Constituents of Marine Sponges," *Journal of Marine Research*, Vol. 10, 1951, p. 153.

5. Brooks, R. R., and Rumsby, M. G., "The Biogeochemistry of Trace Element Uptake by Some New Zealand Bivalves," *Limnology Oceanography*, Vol. 10, 1965, p. 521.
6. Carpenter, K. E., "A Study of the Fauna of Rivers Polluted by Lead Mining in the Aberystwyth District of Cardiganshire," *Annals of Applied Biology*, Vol. 2, 1924, p. 1.
7. Chipman, W. A., Rice, T. R., and Price, T. J., "Uptake and Accumulation of Radioactive Zinc by Marine Plankton, Fish, and Shellfish," *Fisheries Bulletin*, U.S. Fish and Wildlife Service, Vol. 135, 1958, p. 279.
8. Comfort, A., "Acid-soluble Pigments of Shells," *Biochemistry Journal*, Vol. 44, 1959, p. 111.
9. Doudoroff, P., and Katz, M., "Critical Review of Literature on the Toxicity of Industrial Wastes and Their Components to Fish: I. Alkalies, Acids, and Inorganic Gasses, II. Metals as Salts," *Sewage Industrial Wastes*, Vol. 22, 1950, p. 432, and Vol. 25, 1953, p. 805.
10. Drinnan, R. E., "Observations on the Accumulation of Heavy Metals by Shellfish in the Estuary of the Miramichi River, N. B.," *Manuscript Series*, Fisheries Research Board of Canada, May, 1966.
11. Ellis, M. M., "Detection and Measurement of Stream Pollution," *Bulletin No. 48*, U.S. Bureau of Fisheries, Vol. 22, 1937, p. 365.
12. Fischer, H., and Jordan, K., "Zur Kenntnis der natürlichen Porphrine XXV," *Zeitschrift für Physiologische Chemie*, Vol. 190, West Germany, 1930, p. 75.
13. Goldberg, E. D., "The Biogeochemistry of Trace Metals," *Treatise on Marine Ecology and Paleocology, Vol. I. Ecology*, J. W. Hedgpeth, ed., Memoir 67, Geological Society of America, 1957.
14. Goldberg, E. D., "Marine Geochemistry," *Annual Review of Physical Chemistry*, Vol. 12, 1961, p. 29.
15. Goldschmidt, V. M., *Geochemistry*, Clarendon Press, Oxford, England, 1954.
16. Greenberg, D. M., "Arginase," *The Enzymes*, P. D. Boyer, H. Lardy, and K. Myrbock, eds., Vol. 4, Academic Press, New York, 1960, p. 257.
17. Hardy, H. L., "Toxicology of Lead," Symposium on Environmental Lead Contamination, *Publication No. 1440*, U.S. Public Health Service, Washington, D.C., Mar., 1966.
18. Hublou, W. F., Wood, J. W., and Jefferies, E. R., "The Toxicity of Zinc or Cadmium for Chinook Salmon," *Oregon Fisheries Commission Briefs*, Vol. 5, 1954, p. 1.
19. Hynes, H. B. N., *The Biology of Polluted Waters*, Liverpool University Press, 1960.
20. Jones, J. R. E., "The Relative Toxicity of Salts of Lead, Zinc and Copper to the Stickleback (*Gasterosteus aculeatus* L.) and the Effect of Calcium on the Toxicity of Lead and Zinc Salts," *Journal of Experimental Biology*, Vol. 15, 1938, p. 394.
21. Jones, J. R. E., *Fish and River Pollution*, Butterworth Press, London, 1964.
22. Kawai, K., "The Cytochrome System in Marine Lamellibranch Tissues," *Biological Bulletins*, Vol. 117, 1959, p. 125.
23. Keilin, D., and Mann, T., "Carbonic Anhydrase Purification and Nature of Enzyme," *Journal of Biochemistry*, Vol. 34, 1940, p. 1163.
24. Korringa, P., "Recent Advances in Oyster Biology," *Quarterly Review of Biology*, Vol. 27, 1952, p. 266.
25. Lehninger, A. L., "Role of Metal Ions in Enzyme Systems," *Physiological Reviews*, Vol. 30, 1950, p. 393.
26. Lloyd, R., "The Toxicity of Zinc Sulphate to Rainbow Trout," *Annals of Applied Biology*, Vol. 48, 1960, p. 84.
27. Mahler, H. R., "Nature and Function of Metalloflavoproteins," *Advanced Enzymology*, Vol. 17, 1956, p. 233.
28. McFarren, E. F., Campbell, J. E., and Engle, J. B., "The Occurrence of Copper and Zinc in Shellfish," *Proceedings, 4th Shellfish Sanitation Workshop*, U.S. Public Health Service, Appendix R., 1961, p. 229.
29. Mounter, C. A., and Chanutin, A., "Dialkyl Flourophosphatase of Kidney," *Journal of Biological Chemistry*, Vol. 204, 1953, p. 837.
30. Passow, H., Rothstein, A., and Clarkson, T. W., "The General Pharmacology of the Heavy Metals," *Pharmacology Reviews*, Vol. 13, 1961, p. 185.
31. Powers, E. B., "The Goldfish (*Carassius Carrassius*) as a Test Animal in the Study of Toxicity," *Illinois Biology Management*, Vol. 4, 1917, p. 127.

32. Schelske, C. L., "Ecological Implications of Radioactivity Accumulated by Molluscs," *Ecology*, Vol. 45, 1964, p. 149.
33. Schubert, J., "Interactions of Metals with Small Molecules in Relation to Metal Protein Complexes," *Chemical Specificity in Biological Interactions*, F. R. N. Gurd, ed., Academic Press, New York, 1954, p. 114.
34. Sutcliffe, J. F., "*Mineral Salt Adsorption in Plants*," Pergamon Press, Oxford, England, 1962.
35. Weissbach, A., Horecker, B. L., and Horwitz, J., "The Enzymatic Formation of Phosphoglyceric Acid from Ribulose Diphosphate and CO₂," *Journal of Biological Chemistry*, Vol. 218, 1956, p. 795.
36. Westall, B. A., "Coagulation Film Anoxia in Fishes," *Ecology*, Vol. 26, 1945, p. 283.
37. Williams, R. P. J., "Metal Ions in Biological Systems," *Biological Reviews*, Vol. 28, 1953, p. 381.

5970 TRACE METAL ACCUMULATION BY MOLLUSKS

KEY WORDS: accumulation; Atlantic Coast; depletion; estuaries; metals; mollusks; sanitary engineering; uptake rates; Washington Coast; water pollution

ABSTRACT: In view of the relative paucity of information on trace metal distribution in marine animals, a series of studies were initiated in order to investigate the mechanics of concentration of these materials. Data were obtained on a number of mollusk species. There is a wide variation in species ability to take up and concentrate zinc, lead, nickel, cobalt, iron, manganese, copper, cadmium, and chromium within their natural (estuarine) environment. Uptake rates in a controlled, simulated-environment system using various concentrations of lead, copper, cadmium, and zinc indicate that all of the species studied varied in their selectivity for the particular metal taken up. The rate of uptake, and the tissue level attained, were found to vary with time, and the particular metal concentration used. Using various lead concentrations it was observed that of the various anatomical areas, the muscle, mantle edge, mantle, remainder, gill, gonad, and digestive gland accumulated increasing tissue levels in the order given. Depletion is a relatively slow process in most cases; it varies from species to species as to rate and final tissue concentration.

REFERENCE: Pringle, Benjamin H., Hissong, Dale E., Katz, Edward L., and Mulawka, Stefan T., "Trace Metal Accumulation by Estuarine Mollusks," Journal of the Sanitary Engineering Division, Vol. 94, No. SA3, Proc. Paper 5970, June, 1968, pp. 455-475.