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UPTAKE AND ACCUMULATION OF RADIOACTIVE ZINC BY MARINE PLANKTON, FISH, AND SHELLFISH

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ABSTRACT

Zinc concentrations in sea water and in shellfish samples, collected from inshore waters along the Atlantic and Gulf of Mexico coasts are reported. Oysters, clams, and scallops concentrate large amounts of this trace metal, the oysters containing the greatest amounts. Radioactive zinc added to the water was rapidly taken up in great amounts by shellfish. Considerable accumulation of the nuclide takes place in the gills of these mollusks, with high concentrations in the kidneys of scallops, and considerable amounts in the hepatopancreas.

Nitzschia closterium, a marine phytoplankton, took up large amounts of zinc 65 and apparently concentrates zinc, thus allowing its transfer to marine animals. Marine fish quickly take zinc into the body from the digestive tract. Much of the incoming zinc appears to be excreted rather promptly. A rapid uptake of radioactive zinc from an oral dose took place in the kidney, liver, and other internal organs, but the loss of the nuclide was also rapid. A slow and long-continued accumulation took place in bone, integument, and muscle tissues. Although there was immediate loss of contained radioactive zinc when marine fish were returned to flowing sea water, a small percentage remained with only very slight loss over many days.

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In a study by the National Academy of Sciences, the Committee on the Effects of Atomic Radiation on Oceanography and Fisheries (1956, p. 73) state that they have "considered three aspects of the atomic energy program that directly involve the oceans and, therefore, the marine sciences: Weapons tests over or in the sea, disposal of radioactive wastes from nuclear power plants, and the use of radioactive substances in increasing our understanding of the oceans and of the creatures that live in the sea."

It was in anticipation of this third aspect of the atomic-energy program that the series of studies, of which the present is a part, was undertaken. The principal object of this study is not radioactivity and its effects on living things. It is rather to understand the role of trace elements in the metabolism of marine organisms and particularly the role of the very scarce element in sea water, zinc. The radioisotope zinc 65 was used because it permits the employment of the exact and sensitive radioactive-tracer techniques recently developed.

MATERIALS AND METHODS

In recent years chemical methods for the estimation of zinc have been greatly improved. It was thus possible to review the earlier values reported for marine and brackish waters and for the organisms living in these environments. Also, labeling the zinc of sea water with Zn^{65} of high specific activity (considered as the amount of radioactive element per unit weight of the element present) made possible the studies of the rate of incorporation and the accumulation of zinc by marine organisms without appreciably increasing the zinc concentrations to which the organisms were exposed. The detection of Zn^{65} at great dilution, the use of methods of isotopic dilution, and the rate of change of the isotopic concentrations in the various tissues through the use of radioisotope techniques made possible the studies of the zinc uptake and distribution in various types of marine life. These studies, although not too meaningful in explaining precisely the role of zinc in the metabolism of marine plankton, fish, and shellfish, serve as a necessary and a good starting point for future investigations into the nature of the biological systems in which zinc plays a metabolic role.

The species of phytoplankton used was *Nitzschia closterium* maintained in unialgal and bacteria-free culture in the laboratory. Sea water enriched with nutrient chemicals was employed as the culture medium, prepared as previously described (Rice 1953). The marine shellfish included oysters *Crassostrea virginica* Gmelin, hard-shell clams *Venus mercenaria* Linnaeus, and bay scallops *Pecten irradians* Lamarck, collected in the vicinity of the laboratory and held in tanks of flowing sea water. The species of marine fish used, croakers *Micropogon undulatus* Linnaeus, and pinfish *Lagodon rhomboides* Linnaeus, were taken in a small trawl and held in a salt-water tank at the laboratory. The croakers ranged from 5 to 8 inches in length, but were selected to give a uniform size in the tests. The pinfish were from 3½ to 5 inches long.

Chemical estimations of zinc in sea water were made from samples carefully collected in pyrex bottles. A few shellfish samples were tested with freshly collected specimens. Those from distant points were of organisms preserved in alcohol. Care was taken in all instances to avoid metal contamination. The shellfish samples were vacuum dried at 70° C. and aliquots of the dried tissues ashed at 600° C. The ash was dissolved in a small amount of 50-percent HCl and made to a fixed volume with water. Two hundred fifty milliliters of the sea water or aliquots of the dissolved ash were shaken with dithizone and the

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zinc extracted at *p*H 5.5 as zinc dithizonate in carbon tetrachloride following the procedures outlined by Vallee and Gibson (1948). Estimations of the zinc contents were made according to the colorimetric procedures recommended by these authors. The dithizone was purified by the method suggested by Hoch and Vallee (1952).

Except for that used in two series of observations with marine fish, Zn^{65} was cyclotron produced and carrier free obtained in the form of the chloride in acid solution. The disintegration rates reported are based on relative counting of the samples and of dilutions of the original shipment using the concentration stated by the supplier corrected for radiological decay. Most of the measurements were made of liquids with a glass dip-type Geiger-Müller counting tube. In some tests, however, a well-type scintillation crystal was used.

CHEMICAL ESTIMATIONS OF THE ZINC CONTENT OF SEA WATER AND SHELLFISH

Each week for 1 year water samples were taken at the dock at the laboratory at Beaufort, N. C., and analyzed for zinc content. It was determined that there was no significant difference in samples taken at high tide and low tide at this location. The complete range in zinc content was from 1.2 γ to 19.6 γ per liter; the latter, however, was observed when a great amount of fresh water was present following a hurricane. The monthly averages (table 1) show a seasonal pattern with the lowest values for zinc content occurring during the winter months.

Samples of oysters were obtained for measurement of their contained zinc from localities along the Atlantic and Gulf coasts, and samples of clams and scallops were collected from the vicinity of the laboratory at Beaufort. At the time of sampling of the shellfish, sea water also was taken for analysis. The results of the measurements of the oysters and water in which they were living are given in table 2.

TABLE 1.—*Average of weekly observations of the zinc content of the sea water at Beaufort, N. C.*

Month	Zinc in micrograms (γ) per liter	Month	Zinc in micrograms (γ) per liter
November	9.7	May	13.2
December	2.8	June	14.1
January	3.5	July	12.4
February	5.4	August	[1] 14.6
March	5.0	September	12.1
April	12.1	October	11.6

[1] Before hurricane, 12.5; after hurricane, 19.6.

TABLE 2.—*Zinc content of oysters and the surrounding sea water*

[Winter and early spring samples]

Locality	Zinc in oysters (micrograms per gram of fresh tissue)	Zinc in sea water (micrograms per gram)
Milford, Conn	3174	0.0188
Upper Chesapeake Bay	2933	.0240
Lower Chesapeake Bay:		
York River, Va	1295	
James River, Va	1484	.0079
Chincoteague Bay, Md	778	
Beaufort, N. C	1171	.0046
Brunswick, Ga	313	.0011
Gulf of Mexico:		
Pensacola, Fla	600	.0008
Galveston, Tex	391	(1)

[1] Trace.

It is apparent that the area around Milford, Conn., and the upper Chesapeake Bay have a high concentration of zinc. This undoubtedly is related to runoff and pollution, for both of these areas have metal industries of considerable magnitude close by. The inshore waters of the Gulf of Mexico are relatively low in zinc content.

The oysters collected in Long Island Sound near Milford and those from the upper Chesapeake Bay contained more zinc than the others. However, relative to the zinc available in the water, these oysters had a lower concentration factor than those from areas of the Gulf where the zinc content of the water was low. In any case, the oysters were found to concentrate zinc in their bodies thousands of times over the concentration of the surrounding sea water.

In order to compare the zinc content of the tissues of the different shellfish, samples of oysters, scallops, and hard-shell clams were collected from an area not far from the laboratory. In a sample of 25 oysters, the zinc content ranged from 3.70 γ to 6.66 γ per mg. of dried meats. The zinc in this group averaged 5.09 γ per mg. Three groups of 25 scallops each were analyzed for their zinc content, and 19 were tested individually. The zinc content of individual scallops ranged from 0.61 γ to 1.43 γ per mg. of dried tissues, and the average for all groups was 1.04 γ per mg. A sample of 20 hard-shell clams from the area averaged 2.62 γ per mg. of dried meats. It appears that oysters have greater amounts of zinc

in their bodies than clams and scallops, and that scallops are relatively low in zinc content.

The distribution of zinc in the organs and tissues of oysters and scallops was determined on composite samples of the dissected parts of a number of animals. Only the mantle, gills, labial palps, and adductor muscle of the oysters were separated from the remainder of the body. The oysters had considerable stored glycogen and little gonad tissue at the time of year the tests were made. The zinc content of the oysters as a whole was measured at 5.50 γ per mg. of dried tissue. The results of the measurements with oysters are given in table 3. The tissue distribution of zinc

TABLE 3.—*Distribution of zinc in the oyster*

[Based on dried tissue]

Tissue	Zinc content (micrograms per milligram)
Mantle	6.91
Gills	8.02
Labial palps	9.58
Adductor muscle	.61
Remainder	6.69

in scallops was determined in experiments using zinc^{65} and are reported later. The adductor muscle was low in zinc, whereas the labial palps and gills were relatively high.

OBSERVATIONS ON MARINE PHYTOPLANKTON

Toxicity of Zinc

Low concentrations of zinc have been reported to be toxic to higher plants. Since it was desired to study phytoplankton growth in cultures containing added zinc, it was necessary to know that the added element was not above the toxic level for the species used.

Zinc sulfate was added to sea-water culture medium in which the marine diatom *Nitzschia* was grown to give concentrations of 100, 250, 500, 750, and 1,000 γ of zinc per liter. Control cultures were grown in medium without added zinc, the zinc content of which was measured as 15 γ per liter. The lowest concentration used which reduced the division rate of the *Nitzschia* cells below that of the controls was 250 γ per liter. This value is approximately 10 times the highest zinc concentration found in the sea-water samples collected along the Atlantic and Gulf coasts. It seems unlikely that concentrations of zinc in nature would limit phytoplankton growth unless pollution with this element were very great.

Uptake of Radioactive Zinc

To study the uptake of radioactive zinc at sea-water concentrations of the element, a culture containing 43×10^7 *Nitzschia* cells per liter was prepared and enough carrier-free Zn^{65} added to give a concentration of 27 μc (microcuries) per liter. The culture was placed in a cabinet with continuous light from daylight-type fluorescent bulbs giving 800 foot-candles. Aliquot volumes of the culture suspension were removed periodically and filtered, and the radioactivity of the filtrate was determined. The cells removed all but a trace of the Zn^{65} from the medium within 24 hours, 80 percent of it within the first hour. In another experiment, run in a similar manner but in which the cells were not dividing since they were kept in the dark for 48 hours prior to and also during the test, they took up 87 percent of the Zn^{65} from the sea-water medium in 24 hours.

In both experiments the available zinc in the medium entered the cells very rapidly. If the availability of the zinc had been maintained, it is likely that the uptake would have continued and much greater amounts would have been taken by the cells. If the uptake of the Zn^{65} resulted from exchange of zinc between that in the cells and that in the sea water, the same specific activity would be expected inside the cells at isotopic equilibrium as outside. Since the specific activity of the Zn^{65} was very high in the sea water, the observed uptake, if due to exchange, would indicate a very great concentration of zinc in the cells.

If one assumes that the nondividing cells kept in the dark were in isotopic equilibrium with the sea-water medium as regards zinc, and that the difference in the weight per unit volume of the cells and that of the sea water were insignificant, one can estimate a concentration factor for zinc based on the reduction during the 24 hours.

The Zn^{65} of the medium was reduced to 13 percent of its original concentration. To reduce it this amount, it would have been necessary to add 6.7 volumes of nonradioactive water to each volume present, or 6.7 λ (microliters) for each λ present. This would be the equivalent of 6.7×10^9 cubic microns added per each λ present. From

measurements of *Nitzschia* cells, it was determined that a cell had a volume of 375 cubic microns. With a population of 43×10^7 cells per liter, there would be 430 cells per λ, or about 13.1×10^4 cubic microns of nonradioactive cells per λ. The cells therefore had approximately 50,000 times the zinc of the sea water per unit of volume. It should be mentioned that it is likely that less zinc was taken by the cells in this test than would have been if the availability of zinc had been maintained.

To observe the effect of added zinc on its uptake by *Nitzschia* cells, cultures were prepared with sufficient zinc added to give total concentrations of 100 γ, 1,000 γ, and 5,000 γ per liter. To each were added 45 μc of Zn^{65} per liter. Periodically, aliquots were removed, the cells filtered, and radiological measurements made of the filtrate. Based on the labeling of the total zinc with Zn^{65}, giving a known specific activity, the amounts of zinc entering the cells at the different concentrations can be calculated.

The zinc very rapidly entered the cells, much of it within the first hour (table 4). When considerable amounts were present, more was taken by the cells. The cells in the medium having 100 γ of zinc per liter continued their divisions and growth. At the end of 96 hours, the cell population had increased from 7.2×10^7 to 70×10^7 cells per liter. Those in medium containing 1,000 γ and 5,000 γ per liter did not divide. The presence of 5,000 γ of zinc per liter was sufficient to damage the cells, as indicated by a decrease in their zinc content after 48 hours. Because of the great uptake, there was a marked change in the availability, particularly in the culture containing the lowest concentration. It seems likely that more would have been taken by the cells if greater amounts were still available. The amount of zinc per cell at the end of 96 hours was as follows:

Zinc in a liter culture initially	*Zinc per cell after 96 hours*
100 γ	1.4×10^{-6} γ
1,000 γ	96×10^{-6} γ
5,000 γ	361×10^{-6} γ

Realizing that individual *Nitzschia* cells are small in volume and in weight, it is apparent that they can take up tremendous amounts of zinc if available in the sea water, more being taken when the amount present is increased. It is evident that, if Zn^{65} is present in the sea water, the isotope will be accumulated by the phytoplankton.

TABLE 4.—*Uptake of zinc by* Nitzschia *cells from sea water to which was added different amounts of zinc*

[Initial cell population: 7.2×10^7 per liter]

Time in hours	Gammas (micrograms) of zinc in cells of liter culture		
	Culture containing 100 γ/liter	Culture containing 1,000 γ/liter	Culture containing 5,000 γ/liter
1	56	380	1,000
6	67	450	1,250
24	86	600	2,500
48	91	680	2,550
66	96	700	2,200
96	[1] 98	[1] 700	[2] 2,000

[1] Cell increase ten fold.
[2] No increase in cell numbers.

Zinc Requirements for Cell Division

Certain amino acids and their salts have the ability to form nonionic chelates in which polyvalent ions are tightly bound. Such materials have proved useful in determining the nutrient requirements of planktonic algae. One of these, a sodium salt of ethylene-diamine-tetraacetic acid (EDTA) was employed to chelate the zinc in experiments with the *Nitzschia* cells. The addition of EDTA to cultures to which had been added Zn^{65} prevented the uptake of the isotope by the cells, no detectable change in 24 hours in the radioactivity of the medium occurring. It is apparent that the zinc of the sea water in such combination is not available to the cells.

In an experiment in which the *Nitzschia* cells were suspended in culture medium containing 30 μc of Zn^{65} and 0.05 grams of EDTA per liter, the population increased from 0.18×10^7 to 213×10^7 cells per liter in a period of 15 days. The cells had divided an average of 10 times in these cultures in which the available zinc was virtually zero. Also, any zinc leaving the cells would become unavailable to them. During the 15 days, a small amount of the Zn^{65} did leave the sea water, indicating that chelation of the zinc was not complete. However, even though the specific activity of the Zn^{65} was high, cell division proceeded with only extremely small amounts of available zinc.

Loss of Radioactive Zinc

Nitzschia cells, which had been made radioactive by growing in a culture to which had been added Zn^{65}, were filtered from this medium and resuspended in sea water. At intervals, aliquots of the suspension were filtered and the radioactivity

of the filtrate measured. The radioactive zinc lost from the cells during 48 hours was extremely slight, barely measurable.

If the zinc within the cells is exchangeable, the addition of EDTA to the sea water in which the radioactive cells were suspended would chelate the zinc once it left the cells and prevent its return. When this was done, the radioactive zinc entered the sea water and increased in amount with time until 89 percent of that originally in the cells had entered the medium within a period of 48 hours.

Another method of observing the loss of radioisotopes from phytoplankton is to wash the radioactive cells after collecting them on a filter and observe the changes in the radioactivity of the washing medium. The radioisotope leaving the cells is not available for reentry into the cells.

Nitzschia cells containing Zn^{65} were filter-washed with distilled water and with sea-water medium. In some tests, carrier zinc was added to the sea-water washing medium. There was a loss of contained Zn^{65} from the cells to the sea-water medium, the loss being greater when the zinc content of the washing medium was increased (table 5). Very little Zn^{65} was washed from the cells by distilled water. Larger quantities left the cells when the specific activity of the washing medium was lowered from the added zinc. These experiments demonstrate that much of the contained Zn^{65} of *Nitzschia* cells was exchangeable.

TABLE 5.—*Loss of zinc 65 from* Nitzschia *cells filter-washed with distilled water and with culture medium containing different amounts of added zinc*

Washing medium	Percentage lost from cells
Distilled water	2.80
Culture medium	14.64
Culture medium + 0.1 γ zinc	15.42
Culture medium + 1.0 γ zinc	30.55
Culture medium + 10.0 γ zinc	34.27
Culture medium + 100.0 γ zinc	42.28

Extraction of Zinc From Phytoplankton Cells

Aliquots of a *Nitzschia* culture to which had been added radioactive zinc were centrifuged. To the different tubes containing the separated radioactive cells were added either 10-percent trichloracetic acid (TCA), hot distilled water, ethyl ether, or 95-percent ethyl alcohol, and the packed cells were resuspended. Extraction with TCA was allowed to take place for 1 hour while that with the other reagents continued for 24 hours. At the end of the extraction time, the cells were filtered and the radioactivity of the filtrate measured.

Nearly all the Zn^{65} of the cells was extracted with TCA (98.4 percent). Ethyl ether removed 7.4 percent; hot distilled water, 4.5 percent; and ethyl alcohol, 1.4 percent.

OBSERVATIONS ON SHELLFISH

Uptake of Radioactive Zinc by Oysters

When shellfish were placed in sea water to which had been added Zn^{65}, there was considerable radioactivity associated with their shells. It was observed that this uptake of radioactivity by oyster shell soon reached an apparent steady state. When the radioactive shells were placed in sea water, the isotope rapidly left the shells with an approach to a new equilibrium between the radioactivity of the shells and the surrounding sea water. Since the Zn^{65} added to the sea water was carrier free and sea water contains but little zinc, it is likely that considerable amounts of the Zn^{65} were adsorbed to the surface of the shells.

In one series of tests, oysters were kept in sea water to which had been added carrier-free Zn^{65} to give a concentration of about 0.01 μc per ml. and, after a given time, were all opened and the radioactivity of their tissues measured. In other tests some of the oysters were removed at intervals and their tissues analyzed. In all tests, however, the oysters were subjected to decreasing amounts of Zn^{65}. The radioactivity of the sea water in which the animals were held was determined at intervals, and whenever the oysters were sampled. The results of two tests are shown in table 6.

TABLE 6.—*Uptake of zinc 65 by oysters immersed in sea water containing the isotope*

[Average of 15 oysters. Zinc 65 concentrations reported in millimicrocuries (mμc)]

Hours of exposure	Zinc 65 content of oysters (mμc/gm. fresh tissue)	Zinc 65 content of sea water (mμc/ml.)	
		Initially	At end
Test 1:			
5	210.2	10.6	5.2
24	263.6	8.1	3.0
48	243.5	8.3	4.9
Test 2:			
42	137.1	9.7	3.8
66	376.2	9.7	2.5
90	508.8	9.7	1.8
114	366.3	9.7	
138	311.9	9.7	1.5

The Zn^{65} very quickly decreased in the sea water and very rapidly increased in the tissues of the oysters. It seems apparent that the isotope entered almost immediately. There was very little increase with time as the experiments progressed. This might be explained as due to experimental conditions since the oysters were subjected to decreasing concentrations of Zn^{65} in the water. An apparent steady state would be observed if the availability of the Zn^{65} were maintained. If this had been done, it is likely that the isotope in the tissues would have reached a high concentration since the radioactive zinc added was carrier free. However, with a high specific activity in the sea water, it was quite impossible to have an insignificant decrease in the Zn^{65} of the water in which the oysters were immersed. Despite the fact that an apparent steady state could not be reached in the experiments, the results indicate that oyster tissues contain very large amounts of zinc. Experiments using Zn^{65} with added zinc carrier are reported later in experiments with separated gills.

Uptake and Exchange by Separated Oyster Gills

A number of experiments were made of the uptake of radioactivity by the separated gills of the oyster when immersed in sea water containing Zn^{65}. In some the effects of added zinc were ascertained, while in others, exchange phenomena were demonstrated. In all instances there was a very rapid uptake of the Zn^{65} when this isotope was added to the sea water in carrier-free solutions. Even with added zinc, reducing the specific activity of the Zn^{65} in the sea water, the uptake was usually quite rapid and great, indicating that the oyster tissues contained very large amounts of zinc.

As an example of the uptake of radioactive zinc, the results of measurements made in one experiment are presented in table 7. In this test the gills of an oyster were immersed in 25 ml. of sea water to which had been added 2.5 γ of zinc. Radioactive zinc was added, and the radioactivity of the sea water was measured at intervals. The Zn^{65} entered the tissues very rapidly at first and less rapidly as the test was continued. It might be possible to interpret the results as an approach to an equilibrium, although the isotopic ratio of the Zn^{65} inside the tissue and in the sea water outside undoubtedly were not the same.

If one assumes that isotopic equilibrium were reached between 42 and 48 hours, the concentration of zinc in the tissue can be calculated from isotopic dilution. A decrease in radioactivity in the 25 ml. from 2,576 to 154 counts per minute per ml. would be equivalent to diluting the 25 ml. by the addition of 393.2 ml. of nonradioactive sea water. At a specific gravity of 1.025 this amount would weigh 403 grams. In the experiment the tissue concentrating the Zn^{65} and reducing the counts actually weighed 0.24 grams, or only 1/1679.2 times the weight of water needed to reduce the radioactivity the same amount. The tissue then concentrated the zinc 1,679 times the concentration of the sea water, or 1,679 $\times$ 0.104 γ per gm., or contained 174.6 γ per gm. However, isotopic equilibrium actually had not been reached, so higher zinc concentrations would be possible.

TABLE 7.—*Uptake of zinc 65 by the separated gills of oysters immersed in sea water containing the isotope and nonradioactive zinc*

Hours of exposure	Radioactivity of water (counts per minute)	Hours of exposure	Radioactivity of water (counts per minute)
0	2,576	24	458
6	1,841	26	342
18	835	42	164
19.5	749	45	165
21.5	572	48	132

The results of another experiment with oyster gills are presented in table 8 to demonstrate that the zinc of the tissue is exchangeable with that of the surrounding sea water. In this test, gills of oysters were placed in sea water to which had been added Zn^{65}. After 21½ hours an equilibrium appeared to be reached. The gills were then removed from the radioactive sea water and placed in sea water to which had been added EDTA. This salt would chelate the greater part

TABLE 8.—*Uptake of zinc 65 by the separated gills of oysters immersed in sea water containing the isotope, and loss of this isotope from these gills when placed in nonradioactive sea water containing EDTA*

Hours of exposure	Radioactivity of sea water (counts per minute)	Hours of exposure	Radioactivity of sea water (counts per minute)
Sea water:		Sea water—Continued	
0	2,671	27	1,106
1	2,631	45.5	1,074
2	2,508	Sea water and EDTA:	
3	2,416	0	0
5	2,208	4	275
9	1,819	20	1,246
21.5	1,154	23	1,174
24	1,165	25	1,215

of the zinc leaving the gills and limit that available for exchange. From the results of the measurements of the radioactivity in the sea water containing the chelating compound, an increase in radioactivity occurred, which demonstrated that zinc passed out of the gills to the water. It seems evident that a great part of the zinc in oyster tissue is exchangeable with that of the surrounding sea water, and the total amount present in the gills is thousands of times that of the sea water.

Distribution of Radioactive Zinc in Oysters and Scallops

Radioactive zinc was injected into oysters, and their tissues or organs were analyzed for radioactivity after 2 hours. The site of the injection varied. In some tests the Zn^{65} was placed in the adductor muscle, and in others in the visceral mass or in the pericardial cavity. There was considerable radioactivity in the shell liquor and blood that was lost on shucking. In some instances this amounted to almost one-third of the injected dose. The gills had almost twice the radioactivity of the other organs and tissues. There was considerable Zn^{65} in the liver and body mass. The heart was relatively high in activity, having about 70 percent of the amount found in the liver. The adductor muscle was much less radioactive than the other organs and tissues.

Bay scallops were immersed in sea water to which had been added Zn^{65} to give a concentration of 0.01 μc per ml. At the end of 2 hours, the scallops were carefully dissected and their organs and tissues analyzed for Zn^{65} content. The scallops rapidly took up the Zn^{65} with all the organs having considerable radioactivity (table 9). A large amount was in the kidney. The liver and associated organs were quite radioactive, as were also the gills.

TABLE 9.—*Tissue distribution of zinc 65 in the bay scallop after exposure for 2 hours to sea water containing 10 mμc (millimicrocuries) zinc 65 per milliliter*

[Average of 10 scallops]

Organ	Weight in grams	Zinc65 content mμc/gm.
Kidney	0.595	1,384.31
Liver	2.085	243.14
Gills	3.924	218.43
Testis and ovary	1.401	138.04
Foot	.191	130.59
Rectum	.070	119.61
Heart	.127	105.10
Adductor muscle	3.769	99.61
Mantle	3.495	91.76

TABLE 10.—*Extraction of zinc 65 from oyster tissues*

Oyster tissue	Weight in grams	Activity in counts per minute	Activity in counts per minute/gm.
Whole homogenate	1.68	1,147	683
Treated with water	1.08		
Extract		400	
Residue		333	
		733	679
Treated with TCA	1.20		
Extract		828	
Residue		0	
		828	690

Extraction of Zinc From Oyster Tissues

Twenty oysters, exposed for 48 hours to sea water to which had been added 0.01 μc of Zn^{65} per ml., were opened and their meats ground in a blender. This material was further ground to a finer paste with a tissue grinder. Weighed portions of the finely ground oyster tissues were then placed in centrifuge tubes. To one was added 5 ml. of water, the material stirred occasionally during a 15-minute extraction period, and the liquid extract separated by centrifugation. A second extraction was made with another 5 ml. of water, and the extracted material was combined and diluted to a known volume. The radioactivity of an aliquot of the water extract was measured. The residue left after centrifugation was dissolved in concentrated HNO_3 and made to a fixed volume. The radioactivity of an aliquot of this was likewise determined. Another portion of weighed tissue homogenate was extracted in a similar manner but with a 10-percent solution of TCA. The radioactivity of the extract and residue were then measured. A third portion of weighed homogenate was dissolved in concentrated HNO_3, made to a fixed volume, and the radioactivity of an aliquot determined.

The Zn^{65} of the oyster tissues was only partially extracted with water. However, all of the isotope was removed from the tissues by treatment with TCA (table 10).

METABOLISM OF RADIOACTIVE ZINC BY FISH

Uptake of Radioactive Zinc From the Digestive Tract

Two series of tests were made to follow the uptake of Zn^{65} from the digestive tract by croakers *Micropogon undulatus*. In one series the nuclide in solution with high specific activity was pipetted directly into the stomach. In the other, the

dose of Zn^{65}, with 50 micrograms of carrier zinc in the form of the sulfate per gram of fish, was solidified in gelatin and placed down their throats. After they were given the radioactive zinc, the fish were returned to a tank of flowing sea water and sacrificed after intervals of time up to 48 hours.

After collection of a blood sample, the organs and tissues of the fish were carefully dissected. The parts of the digestive tract were wet-ashed with nitric acid and the ash taken up in dilute hydrocholoric acid and made to a fixed volume with water. Aliquots of these solutions were placed in small vials for radioactivity measurements. After dissection of the various organs and removal of a portion of integument, of muscle, and of bone, the remainder of the fish was homogenized in water in a blender, and an aliquot of the homogenate was placed in a small vial. The various samples in the vials were placed in the well of a scintillation detector and the contained radioactivity measured. The results were corrected for weight of sample, dilution, and the dosage given to the different-sized fish and compared with an aliquot of the dosing material. The computations of microcurie strengths were based on the efficiency of the counting arrangement for dilutions of the original shipment and the reported Zn^{65} content.

The gelatin containing the zinc was broken down in the stomach rather promptly and passed into the intestines in somewhat liquefied form. Two hours after administration, about 60 percent of the dose was in the digestive tract, most of it in the stomach (table 11). Between 2 and 6 hours, most of the dose had left the stomach and apparently had entered the body of the fish. There was only a small percentage of the dose in the intestines at any time. Very little was found in the pyloric caeca.

The Zn^{65} pipetted directly into the stomach entered the intestines more promptly. However, uptake from the intestines was somewhat less rapid than in the tests in which added carrier was employed (table 12). The percentage of the dose in the intestines remained high for 12 hours after dosing. Nearly 10 percent of the dose was in the fish tissues at 24 hours, much of it entering very early. However, the Zn^{65} not accounted for in the digestive tract and the remainder of the fish body was 50 percent in 4 hours. It increased to 71 percent after 24 hours and to 87 percent after 48 hours. It was likely excreted.

TABLE 11.—*Zinc 65 content of digestive tract of croakers following its administration by mouth in gelatin*

Organ	Percentage of dose remaining after—		
	2 hours	6 hours	12 hours
Stomach	50.2	8.4	7.5
Intestines	8.7	11.3	15.6
Pyloric caeca	.6	.9	.9
Total	59.5	20.6	24.0

The changes in concentration of Zn^{65} in the various organs and tissues of the croakers following administration of the nuclide by pipetting into the digestive tract are listed in table 13. There was rapid entry of the zinc into the blood, accompanied by an uptake by the kidney. There was a very great accumulation by the liver, which also took place soon after entry of the isotope into the blood. The greatest accumulation by the internal organs was observed after 6 hours. Following this the levels rapidly fell off, the decrease being more marked in the blood than in the liver and kidney. The build up of radioactivity by the spleen and the gonads was somewhat slower than that in the other organs. Aside from the rapid uptake and loss by the kidney and liver, there was a small and continued accumulation in bone, integument, and muscle tissues. It seems that the zinc entering the blood from the digestive tract was rapidly removed by the kidney and liver. Removal of part by the gills seems probable, and the concentrations reported may reflect a good supply of blood to the filaments. The slow continued uptake by bone, skin and scales, and muscle indicates an accumulation of zinc in these tissues with a relatively slow turnover time of zinc-containing compounds.

From an extremely careful and painstaking dissection of a croaker and a weighing of the organs and parts, the percentage composition of the fish was ascertained. In other fish of known weight the different internal organs were taken in their entirety for the samples. From this information and the radiological measurements it was possible to compute the distribution of the Zn^{65} in the tissues and organs of the entire croaker after administration of this isotope. These calculations for a 12-hour fish receiving Zn^{65} in gelatin are given in table 14.

TABLE 12.—*Distribution of zinc 65 in croakers following pipetting of the nuclide into the stomach*

Distribution	Percentage of dose remaining after—					
	2 hours	4 hours	6 hours	12 hours	24 hours	48 hours
Digestive tract:						
Stomach	59.7	0.7	0.7	0.4	9.8	1.2
Intestines	20.4	42.2	42.6	48.3	6.3	3.9
Pyloric caeca	6.9	3.9	11.4	2.9	2.9	1.0
Total	87.0	46.8	54.7	51.6	19.0	6.1
Fish body	3.6	2.4	7.9	6.7	9.7	6.8
Unaccounted for	9.4	50.8	37.4	41.7	71.3	87.1

TABLE 13.—*Changes in the zinc 65 content of the various organs and tissues of croakers following its administration*

[Dose: 1.55 microcuries per gram of fish. Values listed in millimicrocuries per gram of fresh tissue]

Tissue or organ	Zinc 65 content (mμc/gm.) after—					
	2 hours	4 hours	6 hours	12 hours	24 hours	48 hours
Blood	0.93	8.16	28.61	7.21	9.70	3.49
Heart	.02	1.60	10.66	3.72	5.11	3.91
Spleen	0	1.60	15.06	6.97	12.83	3.86
Gill filaments	.09	2.54	10.97	4.48	8.09	4.52
Liver	.11	3.14	57.46	20.64	22.00	11.72
Kidney	1.50	3.64	27.03	10.09	18.52	12.26
Gonads	.05	.27	2.44	1.61	2.98	2.57
Muscle	.01	.06	.31	.21	.43	2.21
Bone	.02	.41	1.42	1.27	2.37	1.19
Integument	0	.17	1.10	1.21	2.32	1.06

Although the internal organs rapidly take up Zn^{65} in large amounts, they constitute only about 2 percent of the weight of the entire fish. The uptake and rate of loss in these organs is usually rapid and much of the physiology and metabolism of elements is explained in the changes in concentrations in these organs. While the rate of uptake in muscle and bone may be slow, these tissues account for 91 percent of the weight of the fish. The slow accumulation of elements in these tissues is of considerable importance.

In the croakers tested 12 hours after dosing, the greatest amount of Zn^{65} in the fish was in the muscles. The muscles and bones had 66 percent of the total radioactive zinc present in the fish.

Retention of Radioactive Zinc in the Body

The retention of Zn^{65} was measured in experiments using the pinfish, *Lagodon rhomboides*. Following an exposure of the fish for 4 days to sea water to which had been added Zn^{65}, they were returned to a laboratory tank of flowing sea water. At intervals after exposure up to 25 days, three fish were taken for measurement of their contained radioactivity. After removal of water from the surface of the fish by blotting with paper toweling,

TABLE 14.—*Zinc 65 distribution in entire fish 12 hours after ingestion of the isotope*

[Dose [1] per fish: 6,100 mμc]

Organ or tissue	Percent of total weight	Weight in grams	Zinc 65 in mμc/gm.	Zinc 65 in mμc per tissue or organ	Percent of total zinc 65 of body
Muscle	80	48.80	1.6	78.1	44.7
Bone	11	6.71	5.5	36.9	21.1
Gills	2	1.22	10.9	13.3	7.6
Liver	.8	.49	40.7	19.9	11.4
Gonads	.4	.24	17.6	4.2	2.4
Kidney	.3	.18	41.5	7.5	4.3
Heart	.2	.12	14.0	1.7	1.0
Spleen	.1	.06	25.3	1.5	.9
Remainder [2]	5.2	3.17	[3] 3.7	11.7	6.7
Total		60.99		174.8	

[1] Three percent of dose in tissues (178.8 mμc); 24 percent in digestive tract; 73 percent not accounted for, mostly excreted.
[2] Skin, scales, digestive tract, blood, brain, eyes, and other parts.
[3] Based on skin and scales.

the fish were weighed and homogenized with added distilled water in a blender. An aliquot of the homogenate of the fish was weighed and dried to constant weight at 98° C., and the percentage of moisture determined. Five other aliquots were taken, placed in vials, and weighed, and the contained radioactivity measured in the well of a scintillation crystal detector. Because of differences in the amount of water added in homogenizing, the results of the radioactivity measurements were calculated on a dry-weight basis.

The relative radioactivity of the fish during 25 days following their return to nonradioactive flowing sea water is plotted in the graph of figure 1. There was initially a marked loss of the Zn^{65} from the fish when returned to flowing sea water following the exposure. However, 7 or 8 percent of that initially present was retained throughout the 25 days of observation. It is apparent that some Zn^{65}-containing compounds in the body of the fish have a very slow rate of turnover. A part of any contained Zn^{65} in the fish will be present over very long periods of time since the loss during 25 days was extremely slight, as shown by the lack of any appreciable slope in the curve of the line of figure 1. The loss from the tissues cannot amount to much more than that resulting from radiological decay. Because of the long half-life of Zn^{65} (about 250 days) and the relatively great fluctuations in individual samples, the observations reported were not corrected for decay.

DISCUSSION

The measurements made throughout the year of the zinc content of the sea water at the labora-

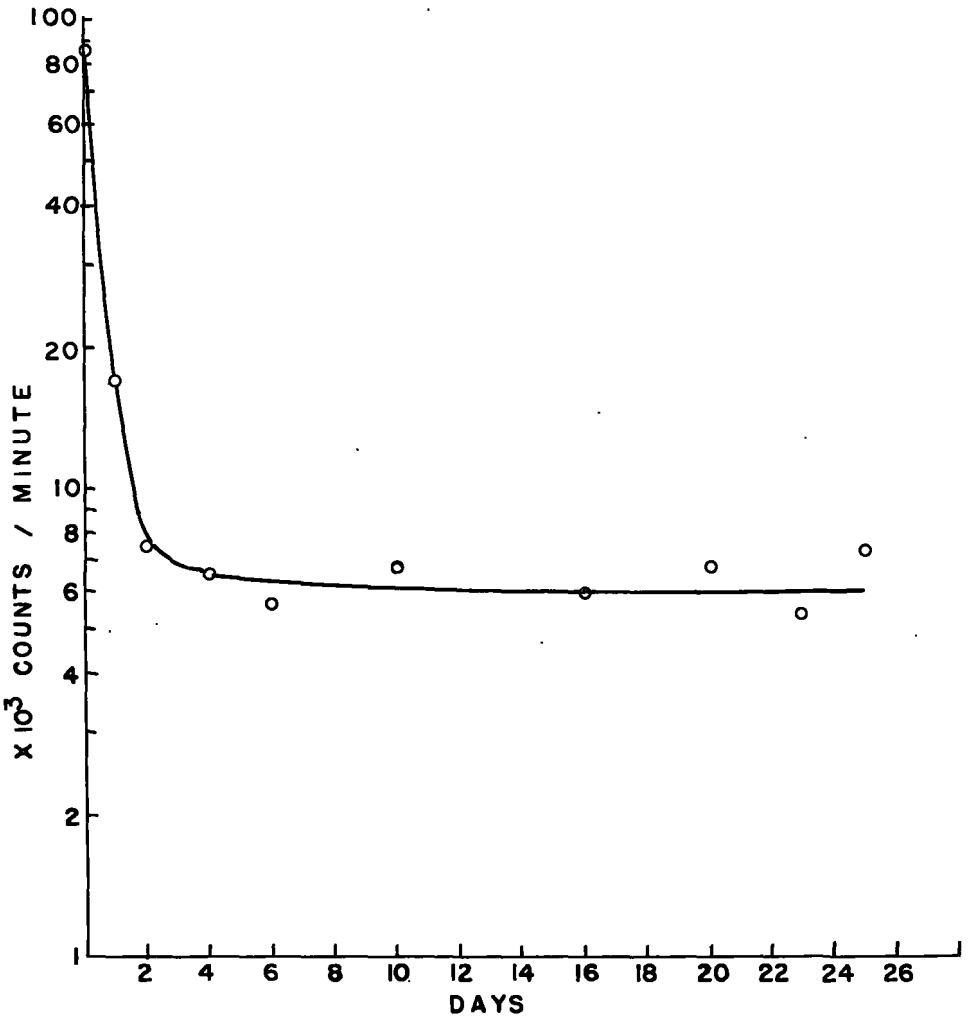

FIGURE 1.—Loss of contained Zn^{65} from the pinfish, *Lagodon rhomboides*, following return to flowing sea water.

tory dock are well within the values that may be expected for such inshore waters. For Tokyo Bay, Ise Bay, and Misaki Channel, Morita (1950) found a range in zinc content from 2.8 γ to 11.7 γ per liter, with an average of 5.6 γ. Buch (1944) reported an average of 10.8 γ per liter, with a range of 4.5 γ to 23 γ for the Gulf of Bothnia along the coast of Finland within harbors and areas of low salinity and where there was evidence of considerable pollution. Wattenberg (1943) gives a range from 5 γ to 30 γ per liter for the western Baltic, not greatly different from Buch for the eastern Baltic. Noddack and Noddack (1940), for a fjord of the Swedish coast, found a content of slightly more than 14 γ of zinc per liter (14 γ per kg.). For samples taken along the coast of Great Britain, some in inside waters, Black and Mitchell (1952) report zinc concentrations from 9 γ to 21 γ per liter. Kuroda (1940) reports zinc concentrations of 7 γ per liter off the Honshu coast of Japan, and Bodansky (1920) gives a value close to 7.5 γ per liter (7.3 γ per kg.) for a sample from the Gulf of Mexico taken close to shore at Galveston.

A seasonal change in the zinc content of the water collected at the laboratory dock appeared in the data with low values occurring in the winter months (table 1). The significance of this is not known. From the data obtained, there was no indication that the amount of zinc present in the water at low tide was greater than at high tide. Salinity changes at this location are usually slight even throughout the year. However, a great lowering of the salinity, normally close to that of

the more open sea, to an extremely low value of about 12 parts per thousand following heavy rains from a hurricane was accompanied by a higher zinc content of the water. Changes in the zinc content at this location may be influenced somewhat by runoff and pollution.

The zinc content in the tissues of oysters in the few samples tested varied considerably (table 2), but the range of from about 300 γ to 1,000 γ per gm. of fresh meats for oysters in which metal contamination is not definitely known is quite similar to that reported by many investigators (Bertrand and Vladesco, 1923; Bodansky, 1920; Hiltner and Wichmann, 1919; Hubbell and Mendel, 1927; McHargue 1924; Orton 1924). The high values found in oysters from Long Island Sound and the upper Chesapeake Bay were likely related to metal pollution of the water. Similarly high values for oysters for these locations and a polluted water off New Jersey were given in the analyses of various investigators listed by Hiltner and Wichmann (1919). Galtsoff (1937) reports similar high values for oysters of Long Island Sound. Mention might be made that the values for zinc concentrations in different mollusks reported by McHargue (1924) were for moisture-free samples rather than for living substance as quoted by Vinogradov (1953, table 218, p. 359) and so are much less than would appear from reference to Vinogradov's table.

Mollusks in general are known to be high in their zinc content, and these along with crustaceans, are richest in zinc when compared with other invertebrate groups (Vinogradov 1953). Lamellibranchs contain large quantities of zinc, but there is considerable variation with higher concentrations in oysters than in others. In our investigations less zinc was found in the clam *Venus mercenaria* than in the oyster *Crassostrea virginica*, and still less in the scallop *Pecten irradians*. Hubbell and Mendel (1927), Bodansky (1920), and McHargue (1924), report less zinc in clams than in oysters. Bertrand and Vladesco (1923) give much lower values for the scallop *Pecten jacobaeus* than for the Portuguese oyster *Crassostrea angulata*.

In oysters the gills were notably high in zinc content, but considerable amounts were present in the mantle and labial palps, and in the remainder of the body, which included the hepatopancreas. Low concentrations were present in the adductor muscle. Based on the distribution of Zn^{65} in the tissues and organs of scallops, the gills were likewise high in zinc, as were also the kidney and hepatopancreas. The adductor muscle was low. Earlier work has pointed out high concentrations of zinc in the gills of oysters and low amounts in the adductor muscle (Bertrand and Vladesco, 1923; Bodansky 1920; Galtsoff 1937; and Koga 1934).

The results reported on the uptake of Zn^{65} by *Nitzschia* clearly indicate that this species of phytoplankton does accumulate considerable amounts of zinc. No information on the zinc content of marine phytoplankton could be found in the literature. Small amounts of zinc, less than those found in terrestrial plants, have been reported in the ash of *Laminaria saccharina* and *Fucus vesiculosus*, and in the dry matter of *Macrocystis pyrifera* (Vinogradov 1953). Based on the zinc concentrations reported for inshore waters, the amounts found in these plants indicate that these species do concentrate zinc. Black and Mitchell (1952) studied the concentration factors for zinc in algae (fresh weight to trace element in the surrounding sea water) and report values for 5 different species ranging from 400 to 1,400.

Small amounts of zinc appear to have a stimulating effect on the growth of many plants, and small amounts appear to be necessary for their growth and well-being. The beneficial action of zinc is less evident in green plants. The lack of available zinc in the soil for growth of certain nut-producing and fruit trees, and the diseases of citrus and other species of plants resulting from this lack are well known (Brenchley 1943, 1947; Chandler 1937; Chesters and Rolinson, 1951; and others). The essentiality of small amounts of zinc for the laboratory culture of many species of phytoplankton has been investigated (Ondratscheck 1941). The amounts required appear to be small. In the experiments reported on the culture of *Nitzschia closterium* in media with very limited zinc concentrations, no effects on the rate of cell division were apparent. Very little zinc was necessary for cell multiplication in this species. With the zinc concentrations available to phytoplankton cells in the sea, and with the less rapid division rate as compared to the laboratory culture of these forms, it seems quite unlikely that a zinc deficiency would limit phytoplankton growth in the sea.

Zinc has been reported (Greenfield 1942) as affecting photosynthesis in *Chlorella vulgaris*, but the amounts required were very great (10^{-2} molar zinc sulphate) since the exposure times were limited to 20 minutes. In our experiments cell multiplication of *Nitzschia* was reduced in cultures grown in zinc concentrations of 250 γ per liter or stronger. The exact toxic strength is not known, but the division rate was not lessened at concentrations of 100 γ per liter. This is at least three times the highest zinc concentrations reported for sea water. It seems likely from this, and from the previous discussion, that neither high nor low zinc concentrations will limit the growth and reproduction of phytoplankton in the sea.

There are appreciable amounts of zinc in the tissues of marine fishes. According to the analyses listed by Vinogradov (1953), there is more zinc than copper and much more than iron. Bertrand and Vladesco (1922) determined the zinc content of muscle tissues of eels and flounders. Bodansky (1920) in his analyses of 14 common marine fishes reports species differences, with rather high values in the spotted trout and flounders and an extremely high concentration in sea catfish. The two analyses of fish by Noddack and Noddack (1940) are about comparable to some of the lower values reported by Bodansky when one considers that their analyses are based on dry weight. The values reported by Vinogradov (1953) for these authors are listed as for living material, which makes them appear high.

No previous work has been reported on the changes in the zinc content of the tissues of marine fish following ingestion. The measurements of the uptake of Zn^{65} following oral administration to croakers show a rapid entry of the isotope into the blood with great accumulation in the internal organs, particularly the liver and spleen. The supporting tissues slowly took up the isotope. Much of the zinc entering the body was quickly taken up by the gills and kidney. The decrease in the zinc content of the gills indicated an early excretion of zinc through these structures. High concentrations of zinc in the liver and spleen of marine fishes were reported by Bodansky (1922). He also reports rather great concentrations in bones and, in marine catfish, in the gills.

Although much of the oral dose of Zn^{65} was not accounted for and considerable amounts of that entering the blood were likely excreted, marine fish can accumulate radioactivity from the uptake of Zn^{65} if present in their food. With accumulation of zinc by phytoplankton, and again by certain species of invertebrates, it seems not unlikely that radioactive zinc, if present in the sea water, will appear in concentrated amounts in the tissues of marine fishes.

The slow uptake of Zn^{65} by the structural tissues with accumulation in the bones and muscles would indicate that the loss would likewise be slow, a slow rate of turnover of the zinc-containing compounds. The observations reported on pinfish show almost no loss after the first day for a period of 25 days. Obviously, if Zn^{65} is taken into the bodies of marine fishes, certain amounts will be present and measurable for very long periods of time. When Zn^{65} was given orally to cows (Bergh 1950), the isotope was present in the body for some time and continued to appear in the milk after it was virtually absent from urine and feces. That Zn^{65} remains in the body for considerable lengths of time is indicated from the finding that intravenously injected Zn^{65} was present in the bodies of dogs up to 6 months (Vallee, Fluharty, and Gibson, 1950).

It seems possible that the nonfission-product isotope Zn^{65}, found in the bodies of marine fish in the Pacific Ocean, as mentioned in the introduction, was, perhaps, present in the water and was accumulated by the fish from marine life farther down the food chain. Because of the long biological half life and long retention of this nuclide by marine fishes, the Zn^{65} could have been accumulated many months prior to the radiological determinations.

SUMMARY

1. The zinc content of sea-water samples collected from inshore waters along the Atlantic and Gulf of Mexico coasts averaged 10.6 γ (micrograms) per liter and ranged from a trace to 24.56 γ per liter; the higher values were for samples from areas known to receive metal contamination.

2. There was a seasonal difference in the zinc content of the sea water at Beaufort, N. C., the lower values occurring during the winter months. The monthly averages ranged from 2.8 γ to 14.6 γ per liter. The average of all the observations was 9.6 γ per liter.

3. That oysters, clams, and scallops contain

large amounts of zinc, thousands of times more than the sea water per unit of weight, was confirmed. In these the greatest accumulation is by oysters, less by hard-shell clams, and least by bay scallops.

4. Radioactive zinc present in the surrounding water is rapidly taken up in great amounts by these shellfish, probably because of the great difference between the zinc content of the water and that in the tissues. Much of the zinc in the mollusks is exchangeable with that of the water.

5. High concentrations of the Zn^{65} injected into or taken up by oysters and scallops occur in the gills. Considerable amounts accumulate in the kidney of scallops. There is also accumulation in the hepatopancreas of the shellfish, but only small amounts in the adductor muscle.

6. The marine diatom, *Nitzschia closterium*, takes up large amounts of Zn^{65} when it is present in the sea water. Although the greater part of the zinc of the cells is exchangeable with that of the water, very little accumulated Zn^{65} leaves the cell when they are resuspended in nonradioactive sea water. This species of marine phytoplankton appears to accumulate considerable amounts of zinc.

7. Marine fish quickly take zinc into the body from the digestive tract. Apparently much of it is excreted rather promptly. High blood concentrations of Zn^{65} from feeding of the nuclide to fish were quickly followed by rapid uptake by the kidney, liver, and other internal organs. The Zn^{65} concentrations of these and the blood very quickly declined after reaching this early peak. The liver had the greatest accumulation. A slow and long-continued accumulation took place in bone, integument, and muscle tissues.

8. Although there is an immediate loss of accumulated Zn^{65} when marine fish exposed to the nuclide in sea water are returned to flowing nonradioactive water, a small percentage remains with only very slight loss over periods of many days.

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