NOTES

Metabolism of the Radioisotope ^{65}Zn in the Freshwater Mussel *Anodonta californiensis*

One of the principal gamma emitters of biological significance identified in Hanford's reactor effluent discharged to the Columbia River is ^{65}Zn (Watson et al., 1963). Certain marine mollusks, such as oysters (*Crassostrea gigas* and *Crassostrea virginica*), the bay mussel (*Mytilus edulis*), and the razor clam (*Siliqua patula*), accumulate ^{65}Zn from sea water and hence serve as biological indicators of this isotope in the marine environment (Chipman et al., 1958; Watson et al., 1961, 1963). No comparable analyses exist for this isotope in freshwater lamellibranchs. For this reason, we have studied the metabolism of this radioisotope in the freshwater mussel *Anodonta californiensis*.

Mussels were collected from several ponds adjacent to the Columbia River, downstream from all reactor sites in the Hanford Reservation. The first experiment, designed to determine the effect of ^{65}Zn concentration, employed three mussels in each of three plastic pans. Each pan contained 4 liters of aerated river water maintained at river temperature (40.8–44.8 F). The mussels were exposed for 5 days to ^{65}Zn concentrations at initial levels of 1, 10, or 100 μCi/liter. The water was replaced on the 3rd day of the experiment with water of the initial ^{65}Zn concentration. On the 5th day the animals were killed for whole body counting. The accumulation of ^{65}Zn was proportional to the concentration of isotope in the water to which the mussels were exposed.

A second experiment was set up to determine the tissue distribution of ^{65}Zn. Four mussels (length 105–110 mm) were placed in each of six trays. The trays contained 8 liters of river water, which was changed twice weekly, continuously aerated, maintained at river temperature, and kept at a concentration of 100 μCi/liter of ^{65}Zn by daily additions of the isotope. The four mussels in a tray were killed after exposures of 1, 2, 4, 8, 16, or 36 days. Three mussels were analyzed for ^{65}Zn by gamma counting in the mantle and palps, the gills, the adductor muscles, the foot, the gonad and visceral mass, and the shell. Stable zinc analyses were also made on these tissues from six mussels by means of an atomic absorption spectrophotometer. One mussel from each group was fixed in methanol for autoradiographic examination. Tissue sections were dipped in 2:1 ratio mixture of K-5 emulsion and water, and exposed for 20 hr for autoradiographic study.

The mussels showed a continuing increase in ^{65}Zn concentration throughout the 36-day experiment, with final body burdens of approximately 100 μCi in the soft tissues and 300 μCi on the shell. The concentrations of ^{65}Zn in various tissues, as a function of time, are shown in Fig. 1. Total tissue burdens are shown in Fig. 2. After 36 days of exposure, 75% of the ^{65}Zn was

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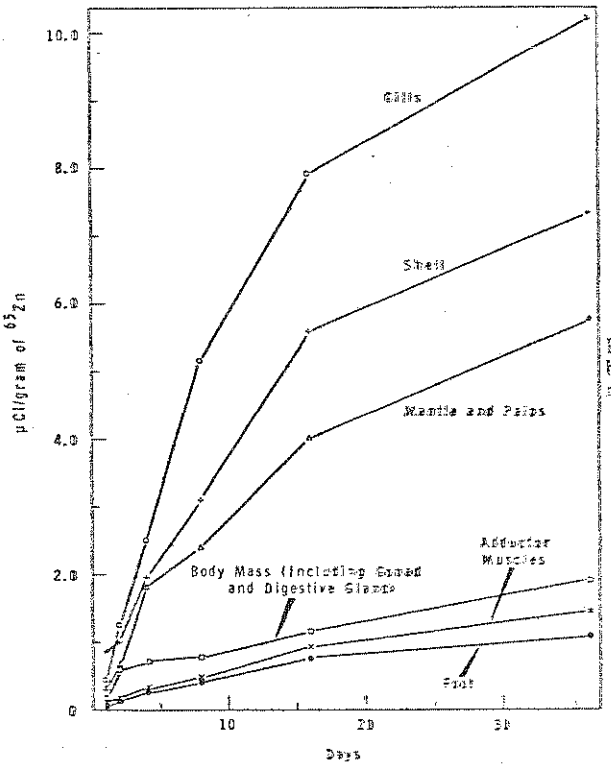

FIG. 1. Uptake of ^{65}Zn (μCi/g) by various parts of the freshwater mussel *Anodonta californiensis* (100 μCi/liter of water).

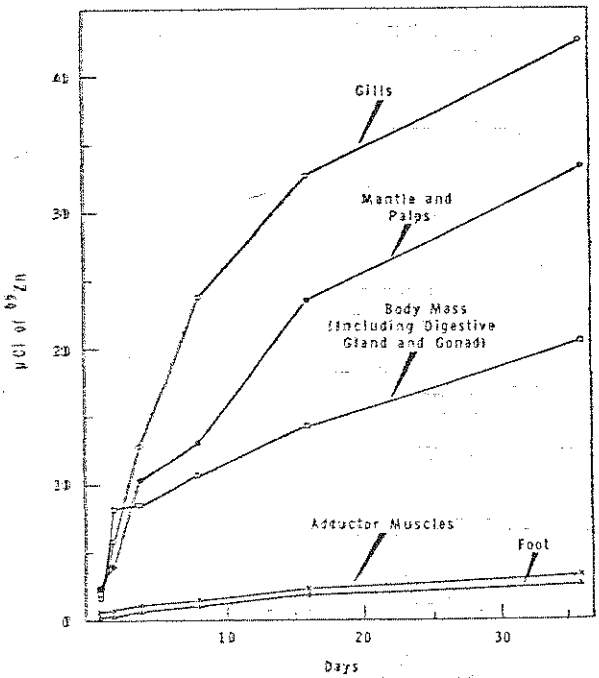

FIG. 2. Total tissue burden of ^{65}Zn in the soft parts of the freshwater mussel *Anodonta californiensis* (100 μCi/liter of water).

in the gills, mantle, and palps. The stable zinc content of six mussels showed a similar pattern of distribution, as is evident in the following table:

Tissue	Zinc content (*mg per g of wet tissue*)	Range among samples
Gill	0.42	0.29–0.57
Mantle and palps	0.08	0.038–0.14
Body mass (including gonad and digestive gland)	0.044	0.030–0.083
Adductor muscles	0.035	0.025–0.047
Foot	0.033	0.024–0.040

Autoradiographs showed some ^{65}Zn in all tissues except Leydig cells and the mucous cells of the epithelial layers. The radioisotope was accumulated both intercellularly and intracellularly, but little was adsorbed on the surface of the animals.

The distribution of the ^{65}Zn isotope was as follows. The hemocytes contained large amounts (Fig. 3 and 4). Heavy concentrations were localized at the tip and base of the outer mantle epithelium (Fig. 3), whereas the inner mantle epithelium contained little. Moderate amounts were localized in the tips of the epithelial cells of the gills. A moderate concentration was present in the tips of the epithelial cells covering the foot, whereas the basophilic gland cells of this organ accumulated little. The gonadal ducts were heavily labelled throughout the cytoplasm of the epithelial cells. The smooth muscle throughout the body had moderate amounts, whereas the heart muscles were heavily labelled. The epithelium of the intestine, style sac, and rectum contained moderate amounts concentrated in a layer halfway between the nucleus and the tip of the cell (Fig. 4). The amorphous connective tissue beneath the intestinal tract contained little or none (Fig. 4). The secretory tubules of the digestive gland contained ^{65}Zn distributed throughout the cytoplasm. The visceral ganglion contained ^{65}Zn in both the nerve fibers and the nerve cells. Extremely heavy concentrations were observed in the epithelial cells of the kidney and in the lumen of the kidney near the surface of the epithelium.

The oyster *Crassostrea virginica* has been reported to show high concentrations of ^{65}Zn in the gills and low concentrations in the adductor muscle (Chipman et al., 1958). This is in agreement with our findings in the freshwater mussel *A. californiensis*. Stable zinc concentrations were found to be highest in the palps, gills, and mantle of the oyster (Chipman et al., 1958; Galtsoff, 1964), which is similar to our findings in *A. californiensis*. In the scallop *Pecten irradians*, the kidney was reported to contain approximately five times as much ^{65}Zn as any other tissue (Chipman et al., 1958), which agrees with our autoradiographic observations.

Zinc is accumulated by ingestion in the intestine and dispersed by the hemolymph throughout the body of the oyster (Galtsoff, 1964). The absorption of ^{65}Zn into the tissues of *A. californiensis* probably occurs through the epithelium of the mantle and the gills, and through the wall of the digestive tract.

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(FIG. 3 and 4 *opposite*)

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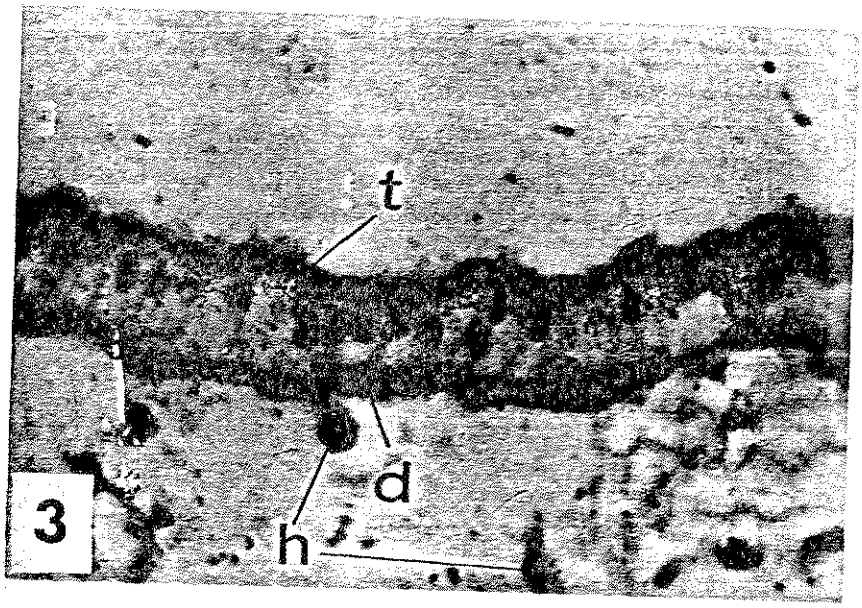

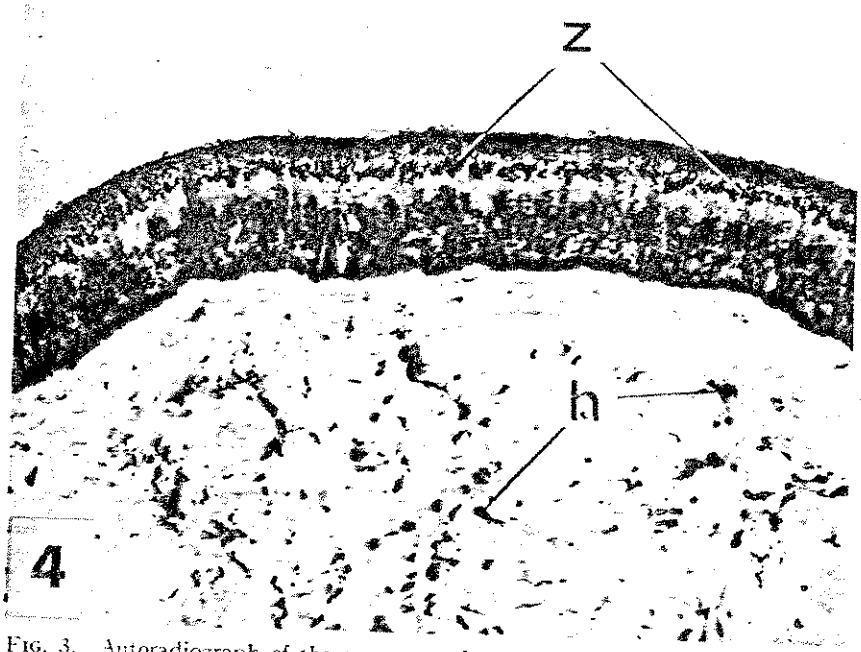

FIG. 3. Autoradiograph of the outer mantle epithelium of a freshwater mussel, showing localization of ^{65}Zn at the bases (*d*) and the tips (*t*) of the cells. Note isotope in hemocytes (*h*). 400×. (Small black dots indicate the location of the radioisotope.)

FIG. 4. Epithelium of freshwater mussel intestine, showing localization of ^{65}Zn (Z) between the nuclei and cell tips. Note ^{65}Zn is present in the hemocytes (*h*) in the lighter connective tissue. 155×. (Small black dots indicate the location of the radioisotope.)