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Accumulation and Tissue Distribution of Radioiodine (^{131}I) from Algal Phytoplankton by the Freshwater Clam *Corbicula manilensis*

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Radioactive wastes discharged from establishments involved in the use of radioisotopes such as nuclear-powered industries, tracer research and nuclear medicine are a potential public health hazard. Such wastes contain radionuclides, particularly Iodine-131 (^{131}I), produced in fission with a yield of about 3%. It is a beta emitter (B_{max}=0.61MeV); it also emits gamma photons. It has a short half-life (8.04 d) (Dutton 1975), hence it is difficult to detect unless accumulated by indicator organisms.

Radionuclides in waste waters are known to be taken up by molluscs such as mussels (Van der Borght and Van Puymbroeck 1970; Fowler *et al.* 1975; Hetherington *et al.* 1976; Helt *et al.* 1980; and Sombrito *et al.* 1982), oysters (Romeril 1971; Cranmore and Harrison 1975) and clams (Cuvin and Umaly 1988).

This study aims to determine the uptake of ^{131}I from algal phytoplankton (*Chroococcus dispersus*) fed to the freshwater clam *Corbicula manilensis* as well as the organ/tissue distribution. The results will be compared with our previous study on ^{131}I uptake from water by the same clams (Cuvin and Umaly 1988).

MATERIALS AND METHODS

Live adult *C. manilensis* were obtained from a local market and transported to the laboratory in plastic bags containing sufficient water. The clams were transferred in plastic basins with sufficient water and kept there for several hours to allow them to release ingested sediment and undigested food materials. The culture and maintenance method of Van der Borght and Van Puymbroeck (1970) used on the freshwater clam

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Anodonta sp. under laboratory conditions was adopted and a few modifications were made. Stock of *C. manilensis* between 22-30 mm (from hinge to shell opening) and weighing approximately 3.6 g were kept in 80 L glass aquaria containing previously aerated laboratory tapwater. Rough aquarium sand ranging from 2.0-2.5 mm grain size was used to eliminate ingestion of substrate by clams. All cultures were kept at room temperature (25°C) at a pH of 6.5 and vigorously aerated. The clams were acclimated to laboratory conditions for at least 2 weeks before the start of the experiments. The stock clams were fed with previously cultured non-radioactive *Chroococcus dispersus*, a blue-green alga and supplemented with finely ground Tetra Min fish food.

Pure cultures of *Chroococcus dispersus* were maintained in cotton-plugged 1-L bottles containing inorganic medium following the method used in SEAFDEC/AQD. The cultures were kept on a shelf with sufficient illumination using cool white flourescent lamps with a light intensity of 90 ft-candles. All cultures were maintained at room temperature and the pH kept at 6.5. Each culture set-up was vigorously aerated. All materials for the culture set-up such as the bottles, cotton plugs, aeration tubes and culture medium were sterilized for 15 min at 15 psi. Determination of cell densities using a haemocytometer was done daily for the first part of the experiment to establish the growth curve of the algae.

Labeling procedure was based on the work of Nakahara and Cross (1978) with certain modifications. Three hundred seventy kBq of ^{131}I in 1 L of culture medium was used for labeling the phytoplankton. Two-hundred mL portions of *C. dispersus* from stock cultures were subcultured into 1 L bottles containing 300 mL of radioactive medium. The labeling period was for 9 days from the date of subculture since this covers the logarithmic phase of growth of the algae.

After labeling, the phytoplankton solution was then filtered with Watman # 42 filter paper and washed several times in non-radioactive water. After washing, the labeled algal filtrate was washed into clean flasks. The solution was diluted to 200 mL, mixed thoroughly and 10 mL aliquot portions were transferred to small glass vials for radioactivity counting. The algal solution was further diluted to give an activity of 37 kBq L^{-1} (1 uCi L^{-1}) prior to feeding to the clams. Cell counts were done for the algal feed. The

final washing from the algae was also monitored to check if all the adsorbed ^{131}I had been removed from the cells.

Thirty clams were transferred into glass bowls containing 500 mL of 37 kBq L^{-1} labeled algae with a mean cell density of 38.89 ($\pm$ 4.10) x 10^7 cells mL^{-1}. The labeled algal feed was changed daily, with the volume, cell density, and activity kept constant. Four clams from the feeding bowl were sacrificed daily for 6 days. The shells were washed and brushed to remove adhering phytoplankton. The shells were then removed and the soft parts weighed and placed in vials for radioactivity counting. Four new clams were placed in the labeling bowls to keep the population density of the clams in the bowl constant. The replacement clams were not included in the sampling. The feeding bowls were kept aerated to prevent settling of the algae.

After determining the whole-body radioactivity of the clams, the soft part was dissected into mantle, gills, foot, muscle, gut, gonad, and visceral remains. Each dissected part was weighed and placed in small vials for radioactivity counting.

All the radioactivity measurements were done using a Nuclear Data 66 gamma scintillation counter with NaI detector. The counting time was set at 1 min for all determinations.

The bioaccumulation factor was computed following the formula (Vanderploeg *et al.*, 1975):

$$BF(R_i) = \frac{\langle R \rangle_i}{\langle R \rangle_w}$$

where BF (R_i) is the bioaccumulation factor for radionuclide R in tissue i; $\langle R \rangle_i$ is the radionuclide concentration (in kBq g^{-1} fresh weight) in tissue i; and $\langle R \rangle_w$ is the radionuclide concentration in the medium (in kBq g^{-1}), which, in this case, is the algae.

Percentage distribution of ^{131}I in the different tissues was computed from:

$$\% \text{ Distribution} = \frac{^{131}I \text{ in tissue, kBq}}{^{131}I \text{ in entire soft part, kBq}} \times 100$$

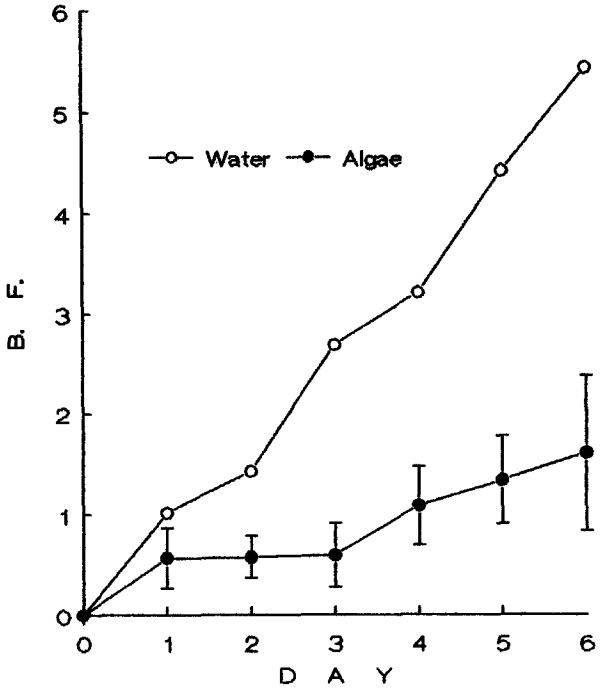

Figure 1. Bioaccumulation factor (BF) of ^{131}I from algae and water in the soft tissue of *C. manilensis*. Accumulation data from water was replotted from Cuvin and Umaly (1988).

RESULTS AND DISCUSSION

The accumulation of ^{131}I by the whole clam (exclusive of shell) is shown in Figure 1. The ^{131}I uptake by the clams increased by as much as 3 times between the first and the sixth day of uptake. BF was 1.61 or 55.65 kBq g^{-1} on the 6th day of accumulation. This value is much lower than the BF of 5.44 or 201.24 kBq g^{-1} accumulation from water containing 37 kBq L^{-1} ^{131}I by the same clams (Cuvin and Umaly 1988). This could be explained by the fact that ^{131}I in water is more readily available for adsorption and subsequent absorption into the tissues whereas the algae have to be digested first before the ^{131}I can be taken up by the clam tissues. These results suggest that absorption over the general body surface at membrane-water interface is the more important pathway for ^{131}I accumulation by clams.

The accumulation of radioiodine by the different tissues is shown in Figures 2a and 2b. On the 6th day

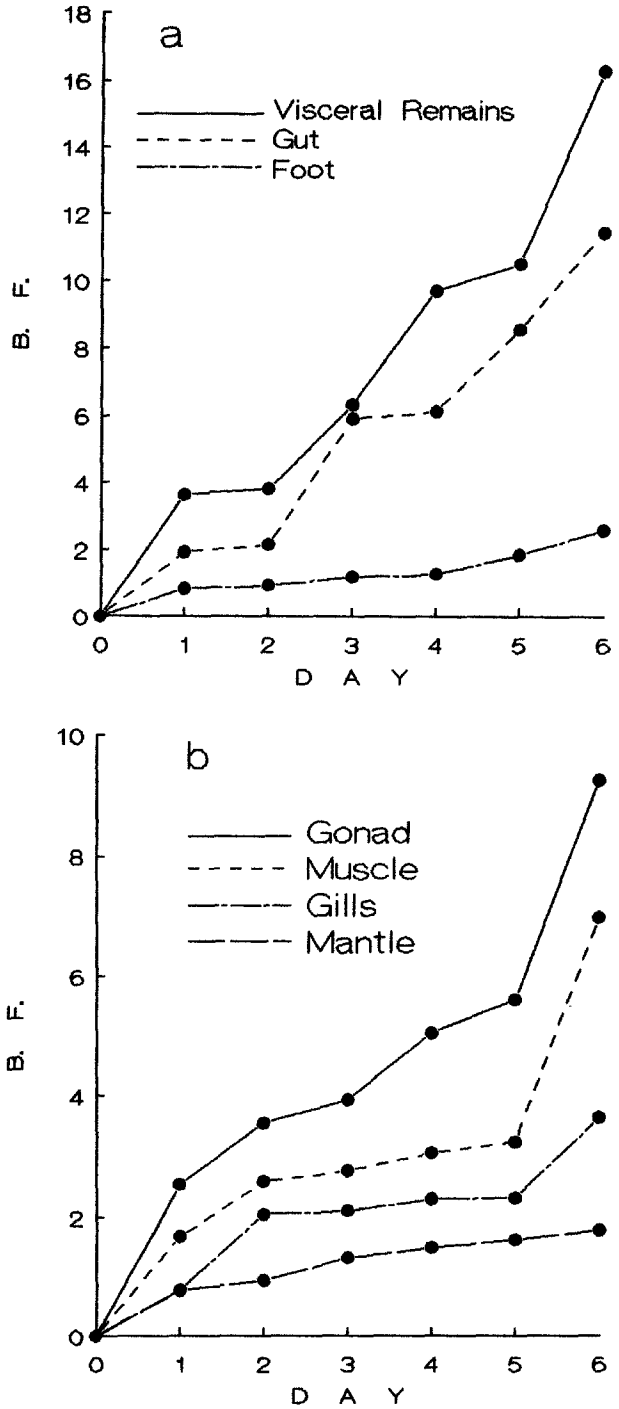

Figure 2a and 2b. Bioaccumulation factors (BF) of ^{131}I from algae in different tissues of *C. manilensis*.

Table 1. Linear regression values for bioaccumulation of ^{131}I from algae in different tissues of *C. manilensis*. From the equation y = a + bx.

Tissue	a (y-intercept)	b (slope or rate)	r* (correlation coefficient)
Vis rem	-0.30	2.48	0.96
Gut	-0.69	1.92	0.98
Gonad	0.90	1.17	0.93
Muscle	0.51	0.82	0.83
Gills	0.67	0.44	0.90
Foot	0.28	0.34	0.94
Mantle	0.59	0.21	0.98

* All correlation coefficients, r, are significant at $p>0.05$.

of uptake, BF was in the following order: visceral remains (16.33) > gut (11.48) > gonad (9.26) > muscle (6.98) > gills (3.64) > foot (2.60) > mantle (1.78). The accumulation by all the tissues was directly proportional to exposure period. The rates of accumulation as determined from the slope of the linear regression equation is shown in Table 1. The visceral remains had the highest rate of accumulation and the mantle the lowest. In the previous study we conducted, the visceral remains also accumulated the highest amount of ^{131}I from water (11.27) (Cuvin and Umaly, 1988), but this was lower than that from algae. The visceral remains included the digestive glands, kidneys and Keber's organ (pericardial glands with excretory function). This agrees with the results of Cranmore and Harrison (1975) with oysters where the highest concentration factor for the radionuclides ^{137}Cs and ^{60}Co was in the digestive glands. The kidneys and Keber's organ may have also concentrated the ^{131}I from the different tissues prior to eventual excretion.

The BF of the gut (11.48), gonads (9.26) and muscle (6.98) were also higher from algae than from water, which was only about 8.26, 6.41 and 5.27, respectively (Cuvin and Umaly, 1988). This indicates that food plays a more important role than water in ^{131}I incorporation in these tissues. In contrast, those of the gills (9.68), foot (3.37) and mantle (6.23) (Cuvin and Umaly, 1988) had a higher BF from water than from algae. This may be explained by the fact that these tissues are directly in contact with the surrounding medium (unlike the visceral remains, gut and gonads which are protected by the mantle) and, therefore, accumulation

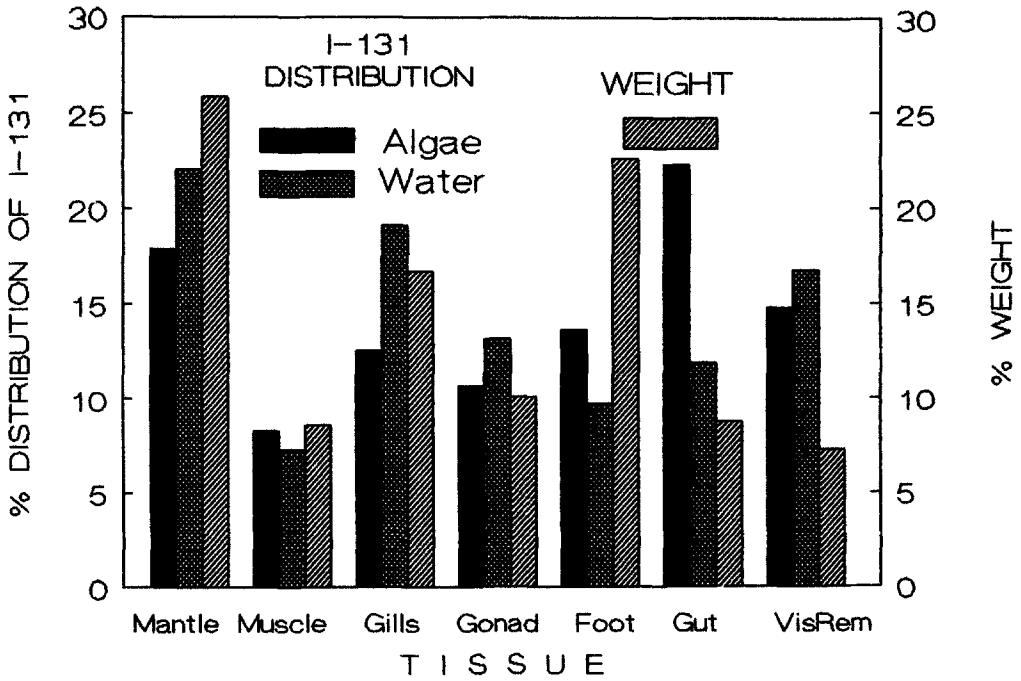

Figure 3. Percentage weight of tissues and percentage distribution of ^{131}I from algae and water in different tissues of *C. manilensis*. Data on distribution from water was obtained from Cuvin and Umaly (1988).

through surface adsorption and/or absorption was the more important pathway for ^{131}I accumulation. It can be seen in Figure 3 that the mantle, foot and gills comprise the bulk weight of the entire clam (exclusive of shell). This could explain why, in the above results, the whole-body accumulation of ^{131}I from water was higher than from algae.

Figure 3 shows the percentage distribution of ^{131}I in the different tissues on the 6th day of accumulation from algae and water. With the exception of the gut, visceral remains, and foot, the percentage distribution of radioactivity was proportional to the percentage weight of these tissues.

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