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Americium Biokinetics in Benthic Organisms as a Function of Feeding Mode

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Transuranic radionuclides in the environment have arisen from nuclear weapons testing, release from nuclear waste reprocessing plants and accidents with nuclear devices (Edgington 1981). Although at present the plutonium inventory is greater than that of any other transuranium element (Watters et al. 1983), the americium inventory will continue to increase due to the *in situ* decay of ^{241}Pu (Day and Cross 1981). Furthermore some studies have demonstrated that americium is more bioavailable than plutonium in aquatic environments; however, recent reviews (Beasley and Cross 1980; Pentreath 1981; Watters et al. 1983) stress that the relatively small amount of data on americium in aquatic biota does not provide a sufficient basis for comparison with the corresponding plutonium data base. We therefore undertook to delineate, experimentally, the biokinetics of ^{241}Am in some marine benthic species with very different feeding-digestion strategies, which hitherto have not been studied in any detail in a radioecological context.

MATERIALS AND METHODS

Experimental animals, except polychaetes, were collected off the Monaco coast and acclimated for several days in aquaria supplied with running sea water. Six specimens each of the crinoid *Antedon mediterranea* ($\bar{X}$ = 0.76 g, 0.45 – 1.10 g wet weight) and the tunicate *Halocynthia papillosa* ($\bar{X}$ = 2.6 g, 0.4 – 11.8 g wet) were allowed to accumulate ^{241}Am (T1/2 = 433 y) from labelled sea water with a specific radioactivity of 9 Bq ml^{-1} (0.24 nCi ml^{-1}). In a separate experiment the polychaete *Nereis diversicolor*, purchased locally, was used to follow the production of particulate ^{241}Am in sea water labelled at the same level as those used in the uptake experiments. Sea water was prepared by filtering through nylon netting (43 μm mesh size) to remove large particles and then spiking with an americium nitrate solution. Throughout the uptake experiments ^{241}Am was measured in each individual and 20 ml aliquots of radioactive sea water in order to compute concentration factors (C.F., defined as radioactivity g^{-1} wet animal ÷ radioactivity ml^{-1} of sea water). Before introduction and after removal of the animals, the particulate and soluble ^{241}Am fractions in water were measured by filtering aliquots through double layered 0.45 μm filters.

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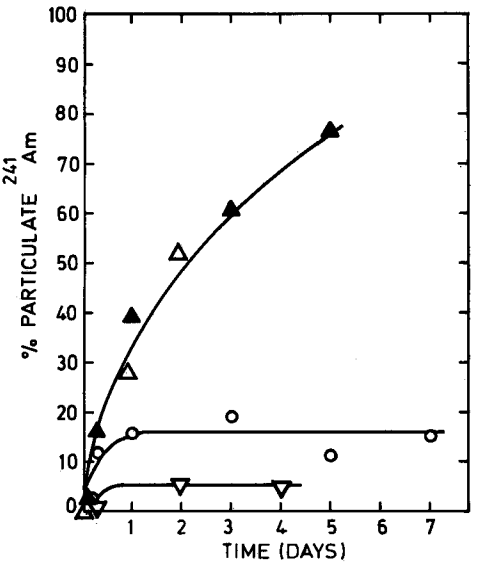

Figure 1. Formation of particulate-associated Am–241 over time in water with *Nereis diversicolor* (▲), with *Antedon mediterranea* (△), with *Halocynthia papillosa* (▽) and a blank without animals (O). Each experimental point is the mean of, at least, two replicate analysis. The particulate fraction is defined as that Am–241 retained on 0.45 μm Millipore filters.

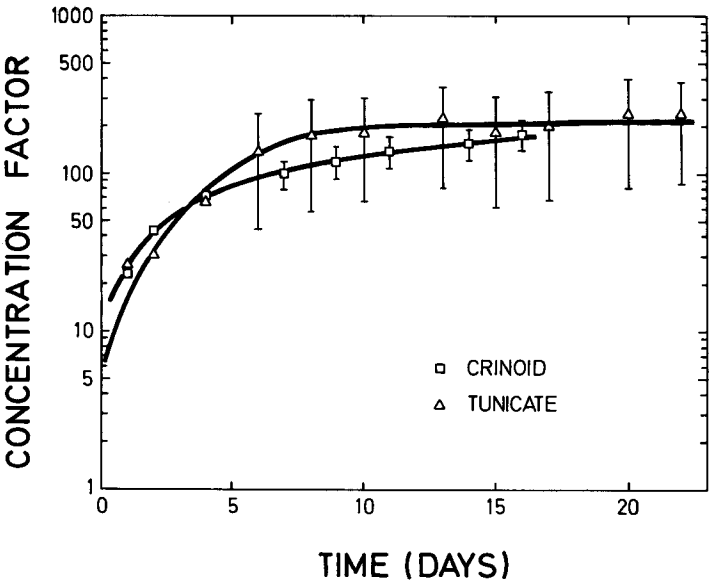

Figure 2. Am–241 uptake from water by the crinoid *Antedon mediterranea* and the tunicate *Halocynthia papillosa.* Propagated counting errors are less than 5%. Error bars represent 1 SD of the mean (n =6).

After exposure some individuals were transferred to flowing sea water and allowed to depurate ^{241}Am. The radionuclide retained with time was expressed as % of the initial ^{241}Am radioactivity in the organisms and was used to compute biological half-lives (Grillo et al. 1981; Carvalho and Fowler 1984).

Five ophiuroids *Ophiura texturata* ($\bar{X}$ = 2.9 g, 2.1 – 3.7 g wet) and five decapod crustaceans *Galathea strigosa* ($\bar{X}$ = 2.3 g, 1.2 – 4.5 g wet) were fed a single ration of ^{241}Am–labelled mussel soft parts and *Artemia salina* carcasses, respectively. Radionuclide retention was measured in each individual and assimilation efficiencies computed by the method of Fowler and Guary (1977).

RESULTS AND DISCUSSION

Since significant amounts of transuranium elements entering the sea become associated with particulate matter (Edgington 1981), we considered the effect of particulate-bound Am on radionuclide bioavailability to two benthic organisms with different feeding modes. First the production of particulate ^{241}Am was followed in water with and without experimental animals (Fig. 1). In freshly labelled seawater, the fraction of particulate-associated ^{241}Am increased quickly and stabilized after 1 day at about 15% . When a suspension feeder like *Nereis* which produces mucous guilds for trapping particles was added to labelled sea water, the particulate-associated ^{241}Am fraction increased rapidly to ~40% on day 1 and ~77% on day 5 (Fig. 1). A similar trend was noted for the suspensivorous crinoid *Antedon* (Fig. 1). On the other hand, in water containing the filter-feeding tunicate *Halocynthia,* the particulate Am fraction remained low; on day 2 it was 5.7% $\pm$ 1.7% (n = 4) and for several days thereafter remained below the normal value of the blank, i.e. < 15% . It was apparent that the tunicates were removing particulate-associated Am from the water via their filtration activities.

Antedon and *Halocynthia* both displayed relatively high whole body ^{241}Am uptake rates; near equilibrium C.F.s for the two species averaged approximately 173 and 193, respectively, after 2 weeks (Fig. 2). Whereas the crinoid C.F. was still slowly increasing at the end of the experiment, tunicate C.F.s indicated an approach to steady state. Subsequent radioanalysis of the tunicate tissues showed that the body wall contained about 90% of the ^{241}Am (Table 1). Furthermore, the atrial water, which accounts for roughly one-half of the animal's wet weight, contained the same ^{241}Am concentration as that in the surrounding sea water (i.e. C.F.~1)indicating a lack of Am concentration in the metabolic products in this fluid.

The triphasic ^{241}Am loss curve for *Halocynthia* is shown in Fig. 3. The long-lived compartment representing nearly 41% of the initial radioactivity content turned over slowly with Tb1/2 of 83 days, while the two short-lived compartments lost Am much more rapidly (Tb1/2 of 1 and 7 days). The fact that the Am distribution among tissues following the 2–month depuration period was almost identical to the distribution observed at the beginning (Table 1) indicates that Am elimination from these tissues was occurring at very similar rates.

Am loss by *Antedon* was biphasic with a Tb1/2 of 0.4 days for the short-lived compartment (15% of Ao) and 51 days for the long-lived compartment (85% of Ao) (Fig. 4). Variation in mean % Am retained by crinoids resulted from the different times of release by individuals of mucous fragments containing the transuranics.

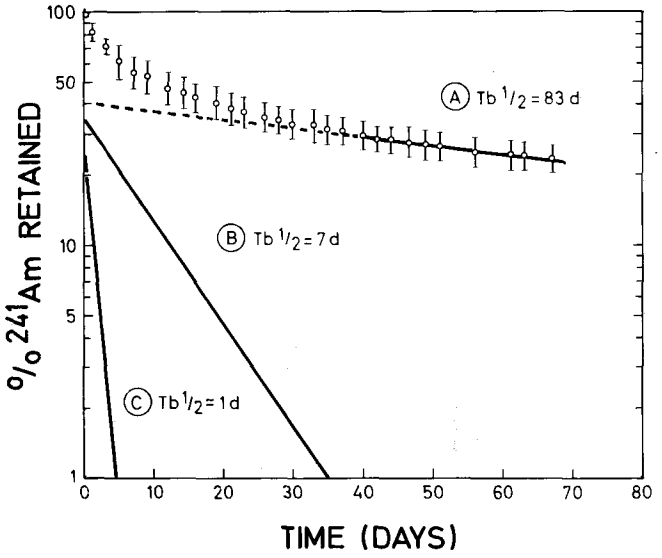

Figure 3. Am–241 retention by the tunicate *Halocynthia papillosa.* Mean (n = 3) ± ± 1 SD. Equations of the regression lines are:
A) lny = 3.707 – 0.00832x, r=-0.996,
B) lny = 3.549 – 0.0979x, r=-0.997,
C) lny = 3.236 – 0.7098x, r=-0.960

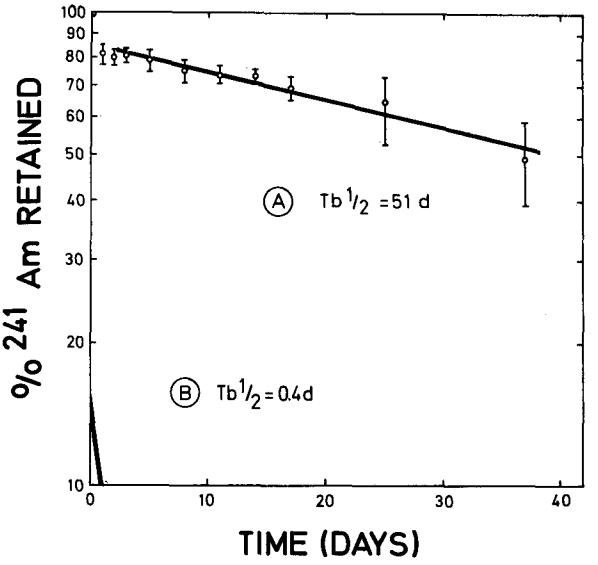

Figure 4. Am–241 retention by the crinoid *Antedon mediterranea.* Mean (n =6) ± ± 1 SD. Equations of the regression lines are:
A) lny = 4.444 – 0.0136x, r=-0.980
B) lny = 2.702 – 1.601x, r=-1

Table 1. Am–241 distribution in tissues of the tunicate *Halocynthia papillosa*. Mean (n=3) ± 1 SD

Tissues	% of body wet weight	Concentration factor	% of total ^{241}Am body burden
After 22 days uptake			
Body wall	32.5 ± 6.9	351 ± 138	90.2 ± 2.2
Internal tissues	21.7 ± 8.2	41 ± 16	6.8 ± 1.1
Atrial water	45.9 ± 14.9	0.9 ± 1.0	3.3 ± 1.3
After 67 days loss			
Body wall	35.4 ± 12.8	–	91.8 ± 3.1
Internal tissues	13.4 ± 4.1	–	5.6 ± 1.6
Atrial water	60.0 ± 6.6	–	2.5 ± 2.0

The suspensivorous crinoid *Antedon,* which has a calcified body covered with a layer of mucus, readily accumulated Am from water. A similar affinity for transuranics has been found for asteroids, ophiuroids and echinoids (Guary 1980; Grillo et al. 1981). The largest transuranic fraction in echinoderms is found in the integument and endoskeleton (Grillo et al. 1981; Guary et al. 1982). Comparing data on echinoderms body wall mineral salt content, namely $CaCO_3$ (Nichols 1964), and ^{241}Am concentration factors reached by representative species of the same classes, a positive correlation is apparent (Fig. 5). This relationship suggests that Am accumulated from water by echinoderms increases with the degree of calcification of the body wall. A similar relationship between plutonium uptake and level of tissue calcification in a number of marine species has been reported by Guary and Fraizier (1977). Whether or not this is the case for echinoderms, Am present in the ambient water is first taken up by the external mucous cover, for which the affinity for Pu and Am has been previously demonstrated (Guary et al. 1982; Carvalho and Fowler 1984). In the case of crinoids like *Antedon,* mucus is produced by tube-feet glands and is used for collecting food particles from water by a ciliary-mucus feeding mechanism (Nichols 1964; Binyon 1972). However, the high particulate Am fraction found in water during the uptake experiment (Fig. 1) indicates that *Antedon*'s feeding mechanism does not efficiently remove the radionuclide sorbed to or incorporated in fine particles. Uptake most likely occurs by complexation of the dissolved Am phase with the mucous layer and/or adsorption to the calcified body wall. Because of the strong affinity for mucus, Am elimination from the crinoid body probably takes place when mucous strips located along the pinnules are sloughed. This mechanism could account for the relatively short ^{241}Am Tb1/2 (51 d) noted in this organism and probably acts as a mode for Am detoxification as has been shown to occur with *Nereis* (Carvalho and Fowler 1984).

The filter-feeding tunicate *Halocynthia* displayed a strong fractionation of accumulated Am with 90% of the radionuclide associated with the tunic. The high Am C.F. in the tunic probably reflects its structure and chemical composition. According to Stievenart (1971) this tunic has an outer proteic cuticle and an inner layer composed of cellulose fibers embedded with amorphous mucopolysaccharides. Am–241 is

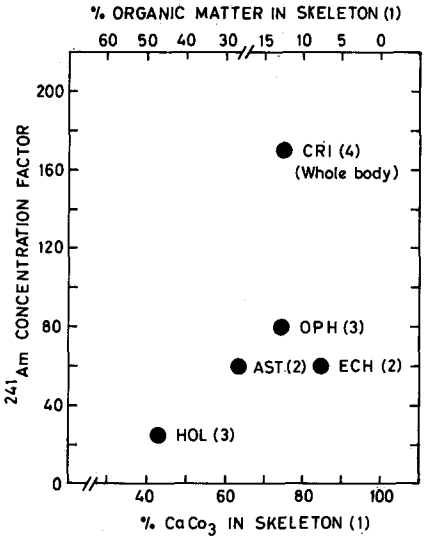

Figure 5. Variation in ^{241}Am concentration factor in body walls with $CaCO_3$ and organic content of the endoskeleton (% dry weight) in the five echinoderm classes. CRI – Crinoidea, OPH – Ophiuroidea, AST – Asteroidea, ECH – Echinoidea, HOL – Holothuroidea. Based on data from (1) Nichols (1964), (2) Guary (1980), (3) Grillo *et al.* (1981), (4) this report.

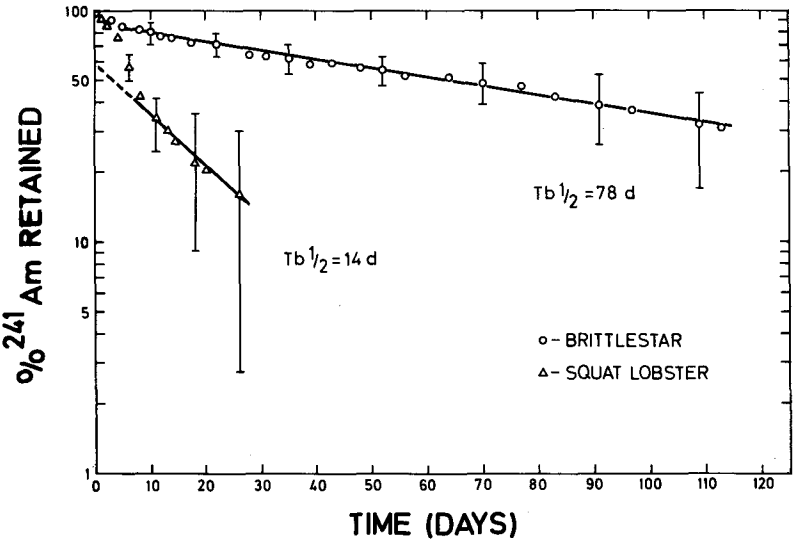

Figure 6. Am–241 retention by the brittlestar *Ophiura texturata* and the squat lobster *Galathea strigosa* following a single ingestion of labelled food. Mean ± ± 1 SD. Equations of the regression lines are, respectively:
lny = 4.465 – 0.00887x, r= - 0.996
lny = 4.069 – 0.0501x, r= - 0.994

strongly bound by the tunicate body wall since its contribution to the total body burden after more than two months depuration was still 92%. Regardless of the tunicate's ability to filter labelled particles, the incorporation of Am into internal tissues is low (Table 1). Evidently, assimilation of ingested Am is not a major pathway for uptake in this species.

Assimilation and retention of ingested ^{241}Am by the brittlestar *Opiura* and by the squat lobster *Galathea* are shown in Fig. 6. Graphical analysis of the *Ophiura* loss curve indicated that about 87% of the Am ingested was assimilated into internal tissues and was subsequently excreted very slowly. After 113 days, brittlestars still retained 31% of the initial dose despite repeated feedings with non-labelled mussel. Dissection at this time showed that the gut held 78% of the remaining Am with lesser amounts located in other organs (Table 2).

The crustacean *Galathea* eliminated ingested ^{241}Am faster than the ophiuroid. The assimilated fraction, 58%, turned over rapidly (Tb1/2 = 14 days) with most of the transuranic being eliminated with the faeces. At the end of the experiment the remaining fraction of Am in individuals was quite variable, i.e. 1.4 to 33%. Tissue analyses showed that small but variable amounts of Am were located in the digestive tract (up to 23% of the total), hepatopancreas (up to 4%) and muscle (up to 10%) indicating that absorption had taken place through the gut walls. A trace of ^{241}Am was detectable in the exoskeleton possibly due, in part, to external contamination by radionuclide lost from the labelled food.

Our food chain experiments with the brittlestar *Ophiura*, which in nature feeds on large prey and organic detritus, demonstrated a high assimilation efficiency for Am combined with a long retention time. High Pu assimilation efficiencies have been reported for asteroids (Guary el al. 1982), and this feature,which appears to be a function of their general digestion physiology, may be widespread among echinoderms. Moreover, it has recently been found that Am and Cf, deposited in the digestive gland of asteroids, are primarily retained intracellularly (bound to mitochondria and lysosomes), a fact that may explain the long retention times observed in these echinoderms (Galey et al. 1983).

Results for the detritivore *Galathea* clearly show that ingested Am is efficiently assimilated but that it is quickly eliminated from tissues via the faeces. The high absorption efficiency (58%) is not surprising considering results for Pu and Cf assimilation by crabs (Fowler and Guary 1977; Fowler et al. in press). Absorbed Am is deposited in hepatopancreas and muscle, and is subsequently excreted possibly in a manner similar to Pu (Guary and Negrel 1980). Nevertheless the high Am assimilation efficiency noted here is not a general rule among crustaceans. For example, euphausiids (krill) assimilate very little Am (3 ± 2 %) that is ingested with phytoplankton (Fisher et al. 1983). Similar experiments with the benthic isopod *Cirolana borealis* have demonstrated that Am assimilation is also quite low (~5%) but that retention times of ingested Am can be long, quite variable, and depend in part upon retention of the labelled food itself (Carvalho and Fowler in press).

Some general trends emerge from the comparisons. Filter-feeders such as tunicates can clear Am-labelled particles from sea water by filtration through the branchial basket, and accumulate small amounts of Am in internal tissues. On the other hand,

Table 2. Am–241 distribution in the brittlestar *Ophiura texturata* 113 days after labelling. Mean (n=4) ± 1 SD

Tissues	% of body wet weight	% of total ^{241}Am body burden
Central disk	17.8 ± 3.4	7.5 ± 3.4
Arms	79.0 ± 3.6	14.9 ± 11.3
Gut	3.3 ± 0.8	77.6 ± 14.5
Gonads (n=2)	0.03	not detectable

the same particulate fraction of Am is not efficiently trapped by the mucous feeding guilds of suspension-feeders like crinoids. Here uptake takes place by complexation and/or adsorption of dissolved Am to mucus and body wall. Echinoderms, such as ophiuroids and asteroids, and certain large crustaceans efficiently assimilate Am ingested with their prey; however, the large differences found between the biological half-lives for Am excretion in these two taxonomic groups argue for basing the behaviour of incorporated Am on different feeding-digestion physiologies rather than on general taxonomic distinctions. This contention is further supported by the fact that Am assimilation efficiencies vary widely among crustaceans.

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