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THE ACCUMULATION OF RADIOACTIVE CAESIUM BY MARINE INVERTEBRATES

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(Text-figs. 1–8)

In sea water there is only a very small amount of caesium and the preponderance of other alkali metals like sodium and potassium makes its chemical estimation rather difficult. A value of 0·4–1·3 μg/l. has been given as the Cs concentration of sea water by Ishibashi & Hara (1959) and a value of 0·5 ± 0·05 μg/l. for North Atlantic sea water by Smales & Salmon (1955). Very little is known about Cs and its distribution in the tissues of marine animals. Provided that all the Cs in these animals is exchangeable, then it is possible to measure the amount in the tissues by allowing it to exchange with radioactive Cs added to the sea water until an equilibrium concentration factor has been reached. Chemically Cs bears some resemblance to potassium. If Cs is absorbed by marine animals in a way comparable to K, perhaps by the same mechanism, then the concentration factor reached by radioactive Cs may be similar to that for inactive K. However, if radioactive Cs were concentrated to a considerably greater degree than K in a whole animal or in a specific tissue then there would be reason to suspect that Cs is being accumulated by a specific mechanism and that it is possibly an essential element. Such a tissue or whole animal would be of considerable interest from the point of view of any hazard which might be created as a result of the accumulation of the long-lived fission product ^{137}Cs which is being introduced into the sea in certain effluents and as a result of weapons tests.

Evidence from experiments on the accumulation of ^{134}Cs and ^{137}Cs by marine decapod Crustacea (Bryan, 1961; Bryan & Ward, 1962) and ^{137}Cs concentration factors quoted for a number of species by Chipman (1960) would seem to indicate that Cs usually behaves in a comparable way to K and that it is not an essential element. The accumulation of radioactive Cs and the relation of this to the uptake of radioactive K and to the distribution of inactive K in the tissues of marine animals has been studied in species from the major groups of invertebrates during a survey in search of the possibility that some species might show particular ability in concentrating Cs.

MATERIALS AND METHODS

Nearly all the marine species were obtained in the Plymouth area. The isotopes ^{137}Cs, ^{134}Cs and ^{42}K were from the Radiochemical Centre, Amersham. All experiments were carried out in filtered Plymouth sea water with unfed animals. In the earliest experiments the isotope ^{134}Cs was used, with which there was some carrier, but later experiments were carried out with carrier-free ^{137}Cs. Later it will be shown that the presence of small amounts of carrier Cs does not affect the extent to which radioactive Cs can be accumulated. Most experiments were done in aerated tanks at a room temperature of 16–18° C, but a constant temperature room at 9° C was used for a few species which were more easily maintained at this temperature.

Prior to measuring the radioactivity and K content of body fluid and tissue samples, the body fluid samples were centrifuged to remove cells and debris while the dried tissue samples were wet ashed with as small a volume of nitric acid as possible. The radioactivity of large whole animals was counted with the animal surrounded by a ring of eight γ-sensitive GM tubes (20th Century type G.10.Pb). Small whole animals, wet ashed tissue samples and body fluid samples were counted with either an end-window γ scintillation counter or with a well-type γ scintillation counter. Radioactive measurements were all related to the activity of the sea water which was measured as far as possible under geometrically identical conditions. Estimations of K were made with an 'EEL' flame photometer on diluted samples.

Results for isotope uptake experiments are plotted as concentration factors against time. The concentration factor is the radioactivity or inactive K concentration per kg of tissue, body fluid or whole animal divided by the corresponding concentration per kg of sea water. When the loss of isotope in inactive sea water has been studied the radioactivity of the samples is related to that of the sea water during uptake.

RESULTS

PROTOZOA

Accumulation of ^{137}Cs has been studied in the foraminiferan *Elphidium crispum* (Linné). Uptake was followed in groups of about 100 animals which were kept in test-tubes of micropore-filtered sea water containing 40 μc/l. of isotope at 16–18° C. After being washed in inactive sea water the animals were counted in the well-type γ scintillation counter. An uptake curve is shown in Fig. 1A. Over the period of uptake equilibrium is not reached. Loss of ^{137}Cs in inactive sea water has been studied following uptake periods of 71, 285 and 356 h. The loss was nearly exponential and a 50% drop in activity was recorded after times of 200, 200 and 260 h, respectively. If it is assumed that the processes of loss and uptake have similar rate constants, then for 50% uptake to take place in about 200 h the uptake curve must eventually reach an equilibrium concentration factor of about 15–16. This far exceeds the highest concentration factors of about 9 which have been found for inactive K. The highest whole-animal K concentrations have been values of 89 mM/kg for fresh animals and 96 and 85 mM/kg for groups from the same batch after experiments of 300 h. As the mean water content of these animals was 23%, then the K concentrations on a water-content basis would be of the order of 400 mM/kg H_2O. This is higher than is normally expected in marine tissues. It is possible that, since the animals were dried on filter paper before these determinations, water was removed, although from any one batch of animals values have been quite consistent. Dead shells contain only about 2 mM/kg of K, so clearly most of the K is in the living animal.

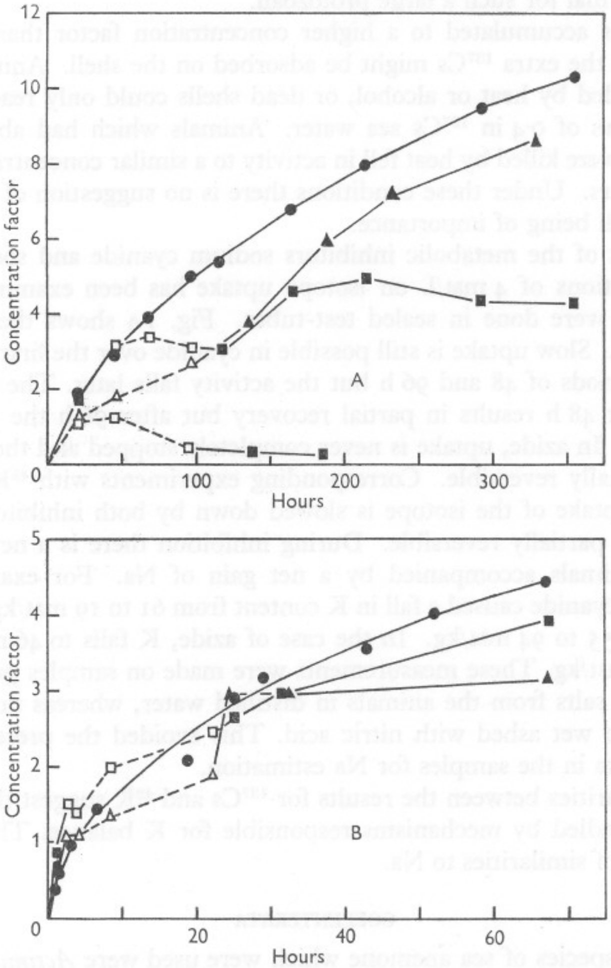

Fig. 1. A. Uptake of ^{137}Cs by *Elphidium* from sea water at 16–18° C and the effect of inhibitors on uptake. ●, ■, ▲, animals in normal sea water; - - -□- - -, animals in 4 mM/l. cyanide sea water; - - -△- - -, animals in 4 mM/l. azide sea water. B. Uptake of ^{42}K by *Elphidium* from sea water at 16–18° C and the effect of inhibitors. Symbols as for Fig. 1 A.

The uptake of ^{42}K has been followed in artificial sea water in two groups of animals which had K concentrations of 53 and 64 mM/kg. After 67 h the first group of animals reached a ^{42}K concentration factor of 4·5 when 86% of exchange with inactive K had taken place (Fig. 1 B). In the second group 91 % exchange had taken place by 75 h (not illustrated). It seems likely that all the K in these animals is exchangeable with ^{42}K. The most surprising fact is that exchange is such a slow process for animals weighing only about 300 μg each. This is, however, judged by metazoan standards and may be perfectly normal for such a large protozoan.

As ^{137}Cs is accumulated to a higher concentration factor than K it was thought that the extra ^{137}Cs might be adsorbed on the shell. Animals which had been killed by heat or alcohol, or dead shells could only reach concentration factors of 0·4 in ^{137}Cs sea water. Animals which had absorbed the isotope and were killed by heat fell in activity to a similar concentration factor in a few hours. Under these conditions there is no suggestion of adsorption onto the shell being of importance.

The effect of the metabolic inhibitors sodium cyanide and sodium azide at concentrations of 4 mM/l. on isotope uptake has been examined. These experiments were done in sealed test-tubes. Fig. 1 A shows the effects on ^{137}Cs uptake. Slow uptake is still possible in cyanide over the first part of the exposure periods of 48 and 96 h but the activity falls later. The removal of cyanide after 48 h results in partial recovery but after 96 h the animals do not recover. In azide, uptake is never completely stopped and the process is at least partially reversible. Corresponding experiments with ^{42}K (Fig. 1 B) show that uptake of the isotope is slowed down by both inhibitors and the inhibition is partially reversible. During inhibition there is a net loss of K from the animals accompanied by a net gain of Na. For example, 44 h exposure to cyanide caused a fall in K content from 61 to 19 mM/kg and a rise in Na from 55 to 94 mM/kg. In the case of azide, K falls to 46 mM and Na rises to 65 mM/kg. These measurements were made on samples produced by leaching the salts from the animals in distilled water, whereas normally the animals were wet ashed with nitric acid. This avoided the presence of too much calcium in the samples for Na estimation.

The similarities between the results for ^{137}Cs and ^{42}K suggest that Cs may be being handled by mechanisms responsible for K balance. There are no suggestions of similarities to Na.

COELENTERATA

The four species of sea anemone which were used were *Actinia equina* L., and *Tealia felina* (L.) from the shore and *Metridium senile* (L.) var. *dianthus* (Ellis) and *Calliactis parasitica* (Couch) from deeper water. Before uptake experiments the animals were allowed to attach themselves to plastic pill box lids of about 4 cm diameter. Prior to radioactive counting the animals were allowed to contract so that excess sea water could be drained off. After a quick wash in inactive sea water they were counted inside the 8-tube γ ring. Uptake experiments were carried out at 16–18° C in sea waters containing

^{137}Cs and ^{42}K and the resulting curves are shown in Fig. 2A, B. The percentage of ^{42}K exchanged with inactive K is shown for each ^{42}K uptake curve. All the inactive K is probably readily exchangeable and most of the exchange takes place in about 40 h. Equilibrium for ^{137}Cs is approached at about 700 h when the concentration factors in all cases exceed those for inactive K and ^{42}K. The difference between the two factors is greatest in *Metridium* and least in *Calliactis*. Table 1 summarizes the results.

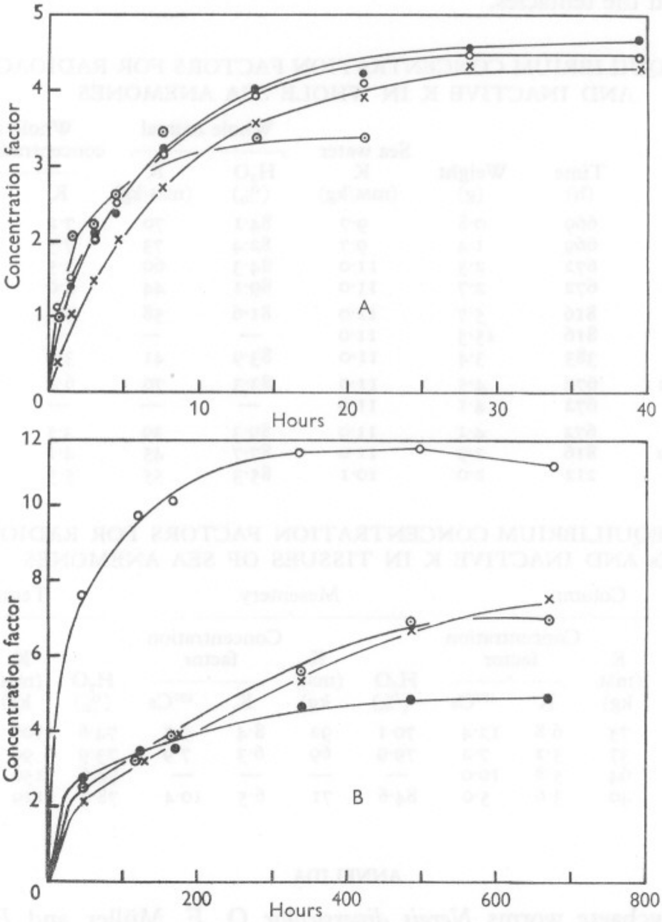

Fig. 2. A. Uptake of ^{42}K by whole sea anemones from sea water at 16–18° C. ⊙, *Actinia* (2, 87 %); ×, *Tealia* (1, 92 %); ●, *Calliactis* (2, 93 %); ○, *Metridium* (1, 99 %). For each species the number of animals used and the percentage of ^{42}K exchanged with inactive K at the end of each experiment is given in parentheses. B. Uptake of ^{137}Cs by whole sea anemones from sea water at 16–18° C. Two animals were used for each curve and the symbols are as for Fig. 2A.

In some animals which had reached equilibrium in ^{137}Cs sea water, ^{137} Cs and inactive K were estimated in sections of the column, pieces of mesentery and in the tentacles. The results in Table 2 are from two specimens of *Actinia* and *Calliactis* and from single specimens of *Tealia* and *Metridium*. All tissues concentrate ^{137}Cs to a greater extent than inactive K and the highest values are found in the tentacles.

TABLE 1. EQUILIBRIUM CONCENTRATION FACTORS FOR RADIOACTIVE Cs AND INACTIVE K IN WHOLE SEA ANEMONES

			Sea water	Whole animal		Whole animal concentration factor	
Species	Time (h)	Weight (g)	K (mM/kg)	H_2O (%)	K (mM/kg)	K	^{137}Cs
Actinia equina	669	0·8	9·7	84·1	70	7·2	8·7
	669	1·4	9·7	82·4	73	7·5	9·3
	672	2·5	11·0	84·3	60	5·5	7·2
	672	2·7	11·0	89·1	44	4·0	4·9
Tealia felina	816	5·7	11·0	81·6	58	5·3	8·0
	816	15·5	11·0	—	—	—	6·2
	383	3·4	11·0	83·9	41	3·7	4·6
Metridium senile	672	4·5	11·0	83·3	70	6·4	10·1
	672	4·1	11·0	—	—	—	11·6
Calliactis parasitica	672	4·1	11·0	89·3	39	3·5	4·2
	816	2·9	11·0	87·7	45	4·1	5·7
	212	2·0	10·1	85·3	55	5·5	5·8

TABLE 2. EQUILIBRIUM CONCENTRATION FACTORS FOR RADIOACTIVE Cs AND INACTIVE K IN TISSUES OF SEA ANEMONES

	Column				Mesentery				Tentacles			
			Concentration factor				Concentration factor				Concentration factor	
Species	H_2O (%)	K (mM/kg)	K	^{137}Cs	H_2O (%)	K (mM/kg)	K	^{137}Cs	H_2O (%)	K (mM/kg)	K	^{137}Cs
Actinia	71·0	75	6·8	12·4	70·1	92	8·4	9·5	74·6	107	9·7	16·6
Tealia	72·8	57	5·2	7·2	79·9	69	6·3	7·9	73·9	90	8·2	18·4
Metridium	76·1	64	5·8	10·0	—	—	—	—	74·6	150	13·6	19·6
Calliactis	77·5	40	3·6	5·0	84·6	71	6·5	10·4	78·2	129	11·7	18·5

ANNELIDA

The polychaete worms *Nereis diversicolor* O. F. Müller and *Perinereis cultrifera* (Grube) have been used. Uptake experiments were carried out in sea water containing 20 μc/l. of radioactive Cs at 9° C. Curves for the uptake of ^{134}Cs in *Perinereis* and ^{137}Cs in *Nereis* are given in Fig. 3A. There was about 0·01 mM/l. of Cs carrier present with the ^{134}Cs but subsequent experiments with both isotopes gave identical curves which shows that small amounts of carrier do not affect the isotope concentration factor. Over the long period of uptake the animals lost about 15% of their weight and so concentration factors are calculated using the mean weight for an experiment. Table 3 shows the concentration factors for inactive K and radioactive Cs in

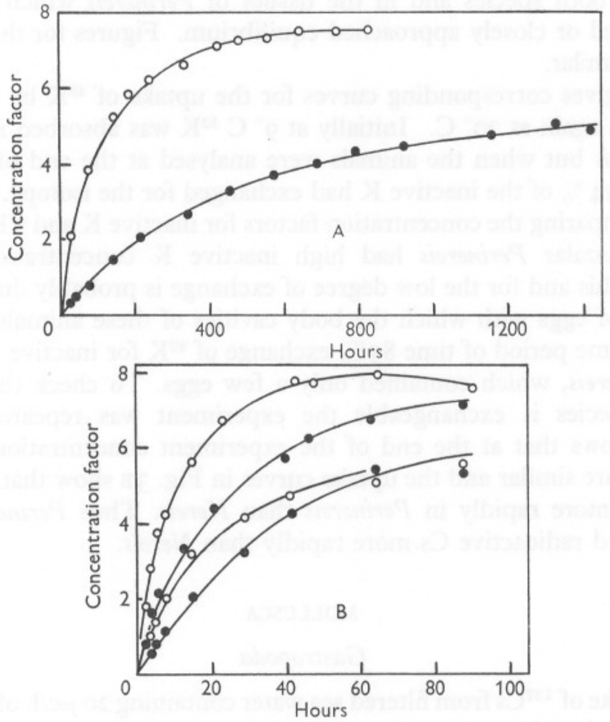

Fig. 3. A. Uptake of ^{137}Cs by two whole *Nereis* and ^{134}Cs by four whole *Perinereis* from sea water at 9° C. ●, *Nereis*; ○, *Perinereis*. B. Uptake of ^{42}K by whole *Nereis* and whole *Perinereis* from sea water. Symbols as for Fig. 3A. Three animals were used for each curve. The upper curves were obtained at 20° C and the lower at 9° C.

TABLE 3. CONCENTRATION FACTORS FOR RADIOACTIVE Cs, RADIOACTIVE K AND INACTIVE K IN WHOLE SPECIMENS AND TISSUES OF *NEREIS* AND *PERINEREIS* AT END OF EXPERIMENTS IN FIG. 3A–B

						Sample			
Species	Isotope	Sample	No. of samples	Temperature (°C)	Sea water K (mM/kg)	H_2O (%)	K (mM/kg)	Concentration factor K	Concentration factor Isotope
Nereis diversicolor	^{137}Cs	Whole animal	2	9	10·6	81·5	73	6·9	6·3
	^{42}K	Whole animal	2	9	11·8	—	73	6·2	5·3
		Whole animal	3	20	11·4	—	88	7·7	7·3
Perinereis cultrifera	^{134}Cs	Whole animal	1	9	9·8	77·0	75	7·7	7·5
		Pharynx	3	9	9·8	74·9	117	12·0	11·2
		Gut	3	9	9·8	73·1	117	12·0	13·2
		Body wall	3	9	9·8	71·5	121	12·4	12·2
	^{42}K	Whole animal	3	9	9·8	—	118	10·0	5·4
		Whole animal	3	20	11·4	—	82	7·2	7·5

animals of both species and in the tissues of *Perinereis* which appeared to have reached or closely approached equilibrium. Figures for the two factors are quite similar.

Fig. 3B gives corresponding curves for the uptake of ^{42}K by both species at 9° C and again at 20° C. Initially at 9° C ^{42}K was absorbed more rapidly by *Perinereis* but when the animals were analysed at the end of the experiment only 54% of the inactive K had exchanged for the isotope. This can be seen by comparing the concentration factors for inactive K and ^{42}K in Table 3. These particular *Perinereis* had high inactive K concentrations and the reason for this and for the low degree of exchange is probably due to the vast quantities of eggs with which the body cavities of these animals were filled. Over the same period of time 85% exchange of ^{42}K for inactive K had taken place in *Nereis*, which contained only a few eggs. To check that all the K in both species is exchangeable the experiment was repeated at 20° C. Table 3 shows that at the end of the experiment concentration factors for K and ^{42}K are similar and the uptake curves in Fig. 3B show that equilibrium is attained more rapidly in *Perinereis* than *Nereis*. Thus *Perinereis* absorbs both ^{42}K and radioactive Cs more rapidly than *Nereis*.

MOLLUSCA

Gastropoda

The uptake of ^{137}Cs from filtered sea water containing 20 μc/l. of isotope has been followed in *Monodonta lineata* (da Costa), *Littorina littorea* (L.), *Nucella lapillus* (L.), *Ocenebra erinacea* (L.) and *Archidoris pseudoargus* (Rapp.). Experiments with *Archidoris* were also carried out with ^{134}Cs and similar results were obtained. All animals were free to move out of the sea water but they were always within range of spray from the aerators. The experimental temperature was 16–18° C.

Curves showing the uptake of radioactive Cs by whole animals (including shells) of all five species are given in Fig. 4A. In animals with shells it takes about 75 h for 50% of the equilibrium concentration factors to be reached. In *Archidoris* this time is reduced to about 25 h. Analyses of the tissues of animals which appeared to have reached equilibrium are given in Table 4. Results for *Ocenebra* tissues are not shown but they are similar to those for *Nucella*. In all tissues except the reproductive system of *Archidoris* the concentration factors for radioactive Cs exceed those for inactive K. The reproductive system of *Archidoris* contains a large amount of K and of three analyses which were made the highest K concentration was 159 mM/kg. Possibly complete radioactive Cs equilibrium had not been reached in these organs. Concentration factors for radioactive Cs and inactive K in the plasma of *Monodonta* and *Archidoris* are fairly similar so that it is the uptake of Cs by the tissues which gives rise to the higher radioactive Cs concentration factors. In *Monodonta* uptake of ^{137}Cs over the period of the previous experiment has been followed in the plasma and tissues. Figs. 4B, C show that the isotope is taken up rapidly into the plasma and that it is the tissues which are the limiting factor in uptake, although no particular tissue stands out in this respect. The shell in *Monodonta* takes up virtually no ^{137}Cs. The soft body which constitutes about 20% of the total weight contains about 86% of the activity in an animal at equilibrium, the rest being accounted for by the shell, operculum and any trapped sea water. A similar situation is found in *Littorina*, while in *Nucella* and *Ocenebra* the shell is more porous and gains rather more activity.

The uptake of ^{42}K has been followed in whole *Monodonta* and the time taken to reach 50% of the equilibrium concentration factor is about 13 h. When the tissues were analysed after 65 h of uptake it was found that concentration factors for the isotope were all greater than 90% of the factors for inactive K except in the digestive gland and gonad where a figure of 79% was obtained. These tissues probably constitute the main limiting factor in the attainment of ^{42}K equilibrium.

As a comparison with the marine animals, the uptake of ^{137}Cs has been followed in a fresh-water gastropod of the species *Planorbis* in artificial tap-water of the type used by Bryan (1960), which in this case contained 0·1 mM/l. of K and 0·5 mM/l. of Na. Uptake is very slow and after 1200 h an animal had reached a concentration factor of 26 in a medium with 2 μc/l. of ^{137}Cs. Two other animals were transferred from a 20 μc/l. solution to one of 2 μc/l. after uptake periods of 48 and 96 h. The activity of these animals gradually levelled out, after falling in the second animal, at concentration factors of 44 and 30, respectively. These results suggest that 50% of the equilibrium concentration factor might be reached in about 400 h. Analyses of the soft bodies of these two animals gave ^{137}Cs concentration factors of 92 and 90 and inactive K concentration factors of 282 and 166. The ^{137}Cs concentration factor of the shell was about 6. Thus a fresh water gastropod is unable to concentrate ^{137}Cs to the same extent as K and this situation is comparable with that in the fresh water crayfish (Bryan & Ward, 1962).

Lamellibranchia

Uptake of ^{137}Cs from filtered sea water containing 5 μc/l. of isotope was followed at 16–18° C in *Mytilus galloprovincialis* Lamarck. Prior to counting whole animals, they were placed in inactive sea water for 15–30 min to remove the active sea water from between the valves of the shell. This also removed some of the plasma activity but the error was not too great. Uptake by three whole specimens is shown in Fig. 5A. Concentration factors are calculated from the weight of the animal including the shell which accounts for about 60–70% of this. It takes about 60–70 h for 50% of the equilibrium concentration factor to be reached. Uptake into the plasma is rapid and 50% of

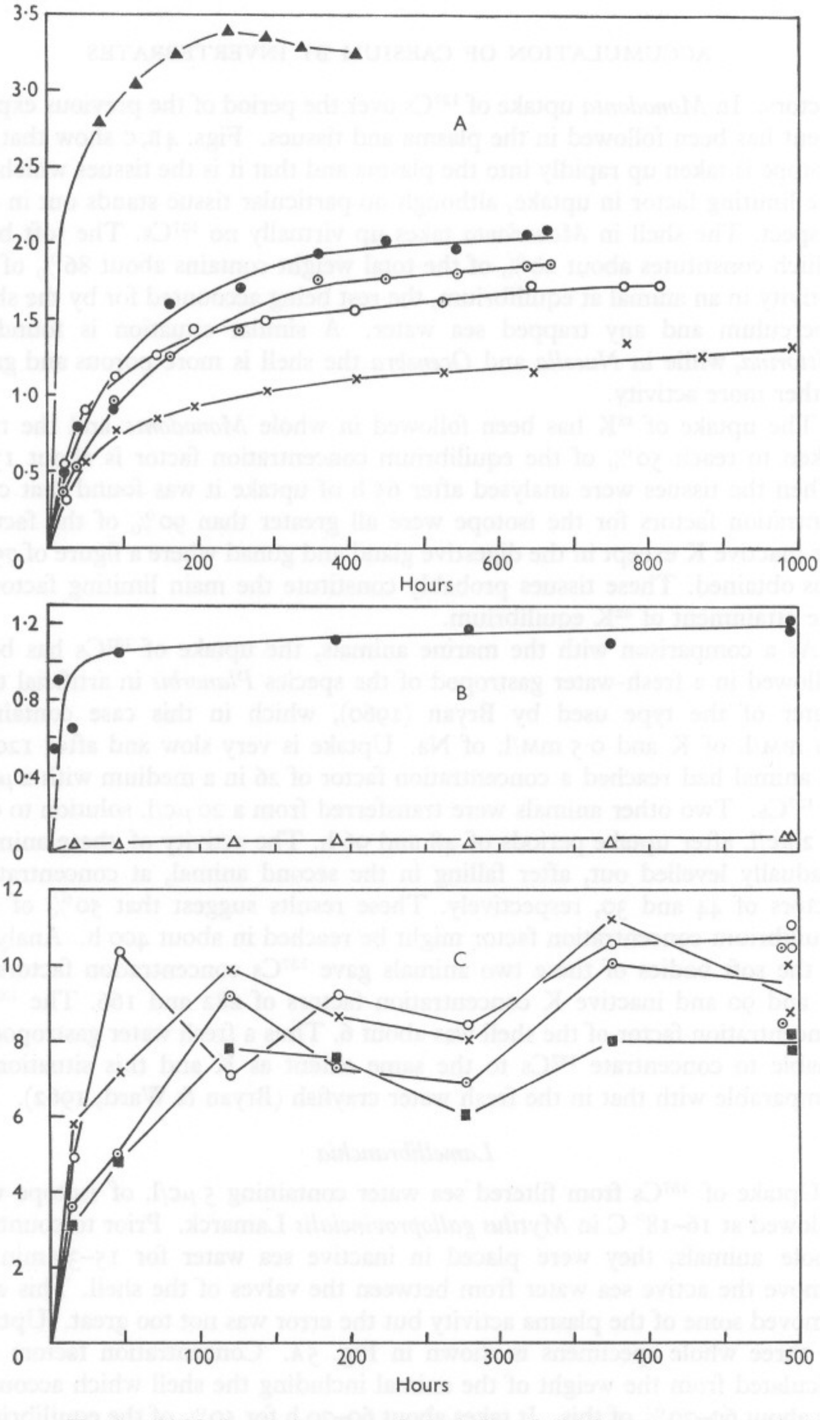

Fig. 4. A. Uptake of ^{137}Cs by whole gastropods (including shells) from sea water at 16–18° C. ▲, *Archidoris* (2); ●, *Monodonta* (1); ⊙, *Ocenebra* (1); ○, *Littorina* (4); ×, *Nucella* (4). Numbers of animals used are given in parentheses. B, C. Uptake of ^{137}Cs by blood plasma and tissues of *Monodonta*. ●, blood plasma; △, shell; ■, foot; ○, gonad and digestive gland; ×, pallial organs; ⊙, buccal mass and gut.

TABLE 4. EQUILIBRIUM CONCENTRATION FACTORS FOR RADIOACTIVE Cs AND INACTIVE K IN MOLLUSC TISSUES

Length of time allowed for uptake is given and numbers of animals analysed are in parentheses.

Tissue	*Monodonta* (2) 522 h (s.w. K = 10·6 mM/kg)				*Littorina* (3) 1131 h (s.w. K = 10·1 mM/kg)				*Nucella* (3) 1283 h (s.w. K = 10·3 mM/kg)			
	H_2O (%)	K (mM/kg)	Concentration factor		H_2O (%)	K (mM/kg)	Concentration factor		H_2O (%)	K (mM/kg)	Concentration factor	
			K	^{137}Cs			K	^{137}Cs			K	^{137}Cs
Plasma	94·5	12·5	1·18	1·18	—	—	—	—	—	—	—	—
Foot	73·9	60	5·7	7·8	68·2	82	8·1	8·5	71·9	86	8·4	9·1
Digestive gland and gonad	64·8	87	8·2	11·0	64·8	103	10·2	11·2	58·3	105	10·2	13·7
Gut and buccal mass	74·7	63	5·9	9·6	71·5	86	8·5	11·7	71·7	81	7·9	11·3
Mantle and pallial organs	76·9	56	5·3	9·3	74·7	82	8·1	10·1	65·9	76	7·4	10·9
Shell	3·2	1·8	0·17	0·09	—	—	—	—	—	—	—	—
Animal without shell and operculum	70·0	67	6·3	8·5	81·9	78	7·7	10·0	68·6	82	8·0	10·5

Tissue	*Mytilus galloprovincialis* (1) 650 h (s.w. K = 10·6 mM/kg)				*Mytilus edulis* (4) 1400 h (s.w. K = 10·3 mM/kg)					*Archidoris* (1) 380 h (s.w. K = 10·6 mM/kg)			
Plasma	96·0	14·3	1·35	1·36	96·0	15·1	1·46	1·46	Plasma	94·3	12·7	1·20	1·24
Adductor muscle	75·0	80	7·6	8·4	79·0	68	6·6	7·8	Mucus	94·8	14·7	1·39	1·45
Byssal retractor muscle	79·6	70	6·6	9·8	78·3	77	7·5	8·8	Dorsal body wall	81·6	29	2·8	3·1
Foot	76·5	93	8·8	15·3	71·1	90	8·7	12·9	Foot	75·2	41	3·9	4·4
Digestive gland	80·6	69	6·5	9·2	76·3	108	10·5	13·3	Digestive gland	75·8	90	8·5	11·0
Mantle	82·5	68	6·4	12·3	79·0	82	8·0	10·3	Buccal mass	78·6	77	7·3	9·7
Gills	87·0	51	4·8	7·3					Reproductive system	73·0	140	13·2	10·9
Animals without shell	—	—	—	—	81·3	78	7·6	9·2	Gills	68·6	45	4·3	6·2

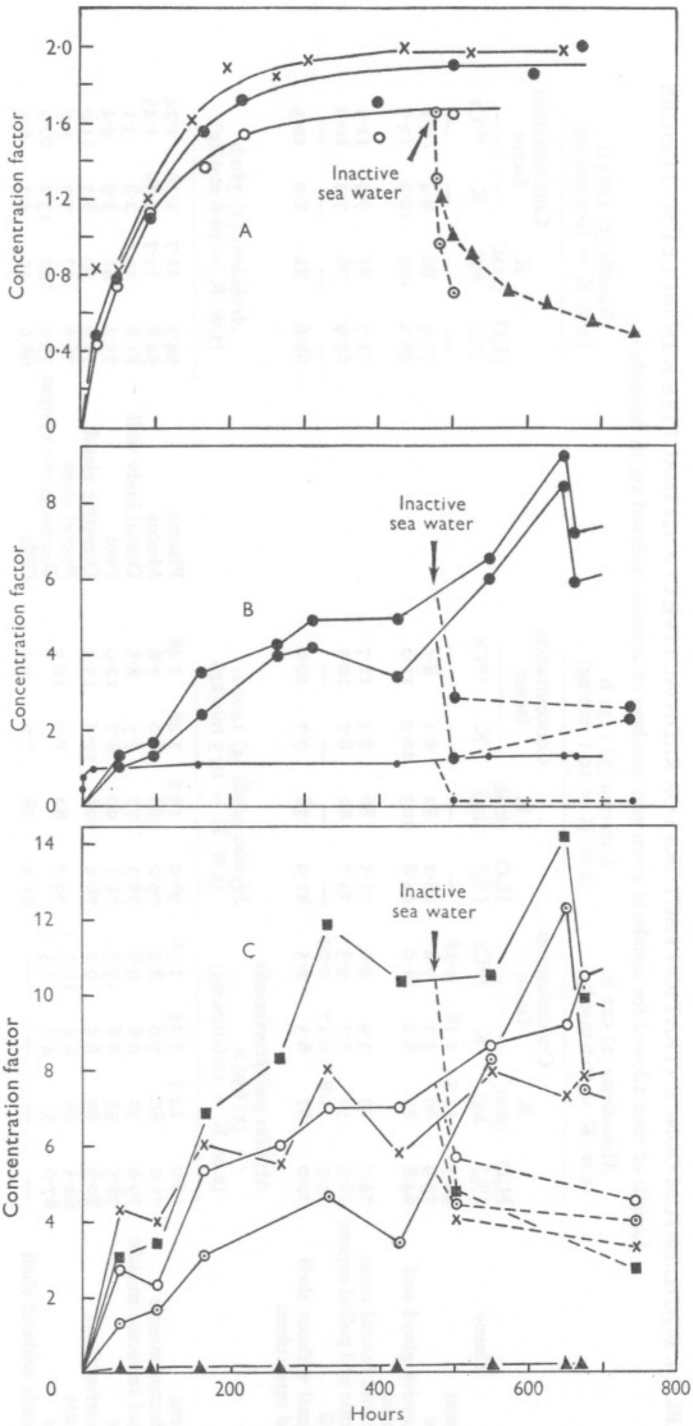

Fig. 5. A. Uptake of ^{137}Cs by whole *Mytilus* from sea water at 16–18° C. Also, loss of ^{137}Cs in inactive sea water shown by broken line. B, C. Uptake of ^{137}Cs by blood plasma and tissues of *Mytilus*. Also, loss of ^{137}Cs into inactive sea water shown by broken line. •, blood plasma; ● upper, byssal retractor muscle; ● lower, adductor muscle; ■, foot; ○, digestive gland; ⊙, mantle; ×, gills; ▲, shell.

the equilibrium level is reached in about 1 h (Fig. 5B). Fig. 5C shows that uptake by the tissues is a relatively slow process and the limiting factor in the attainment of equilibrium would appear to be the slow uptake by the adductor and byssal retractor muscles.

The loss of ^{137}Cs was followed in two animals and the results are also shown in Figs. 5A–C. Loss is relatively rapid at first as the isotope is lost from the blood plasma and more permeable tissues but tends to become a slower process as the remaining ^{137}Cs is confined to less permeable tissues like muscle. The apparent rapid initial loss of ^{137}Cs from muscle in inactive sea water is likely to be due to individual characteristics of the animal used.

The ^{137}Cs concentration factors found in the tissues as equilibrium is approached are compared with those for inactive K in Table 4. In all cases the ^{137}Cs concentration factors exceed those for inactive K while the plasma concentration factors are very similar. Comparative analyses for *Mytilus edulis* L., after exposure to sea water containing 5 μc/l. of ^{137}Cs for 1400 h at 9° C, are also given in Table 4 and are similar to results for *M. galloprovincialis*.

CRUSTACEA

The accumulation of radioactive Cs by decapod crustacea has been described previously by Bryan (1961) and Bryan & Ward (1962). Animals from some other groups have also been studied.

A curve for the accumulation of ^{134}Cs by the stomatopod crustacean *Squilla desmaresti* Risso from sea water at 16–18° C is given in Fig. 6A. When equilibrium is reached the concentration factors for ^{134}Cs in the tissues markedly exceed the factors for inactive K (Table 5). The results are comparable with those found in *Carcinus maenas* by Bryan (1961).

Two marine copepods have been examined during the present work. Uptake of ^{137}Cs in *Calanus helgolandicus* Claus was studied at 9° C in filtered sea water containing 20 μc/l. of isotope. The *Calanus* were pretreated with streptomycin and penicillin and put through two changes of micropore filtered sea water before uptake to keep down the growth of bacteria. At intervals, groups of about fifty animals were picked out and dried on filter paper. The water content of each sample was found and then the concentrations of ^{137}Cs and inactive K were determined in the wet ashed samples. Fig. 6B shows that in the unfed animals ^{137}Cs is taken up to a concentration factor which exceeds that for inactive K. The mean weight of each *Calanus* was about 550 μg and the water content was about 86% of the body weight. In sea water containing 10·1 mM/kg of K the mean K concentration of 6 groups of 50 animals was 74 ± 9 mM/kg.

Experiments carried out with the benthic copepod *Tisbe reticulata* Bocquet from Plymouth show that the animal will accumulate ^{137}Cs to about the same extent as *Calanus*. At 20° C adult female animals, which weigh about 32 μg

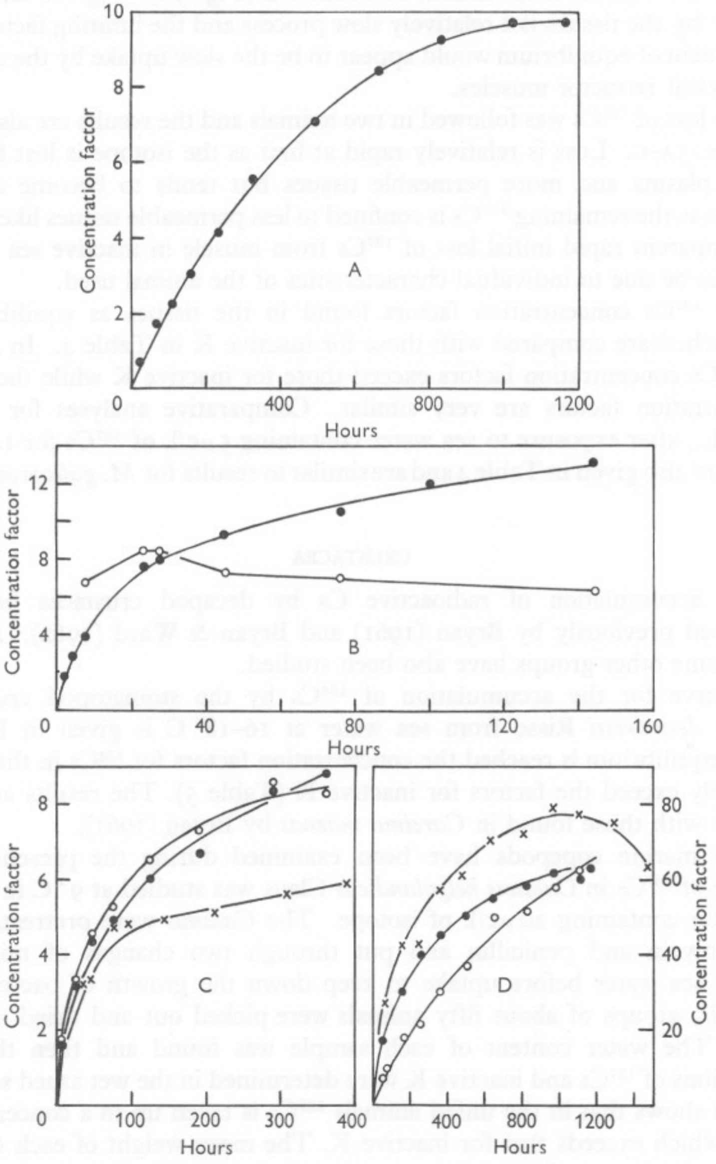

Fig. 6. A. Uptake of ^{134}Cs by whole *Squilla* from sea water at 16–18° C. B. Uptake of ^{137}Cs by whole *Calanus* from sea water at 9° C. ●, ^{137}Cs; ○, inactive K concentration factor. C. Uptake of ^{137}Cs by three whole *Sphaeroma* from sea water at 20° C. D. Uptake of ^{137}Cs by three whole *Asellus* from artificial tapwater at 16–18° C.

each, reach 50% of the equilibrium level of 12 in about 2·0 h. This work will be published later in more detail.

Comparative experiments have been carried out on the accumulation of ^{137}Cs from sea water by the isopod *Sphaeroma serratum* (Fabricius) and from artificial tapwater by the fresh water isopod *Asellus aquaticus* (L.) at 16–18° C.

TABLE 5. EQUILIBRIUM CONCENTRATION FACTORS FOR RADIOACTIVE Cs AND INACTIVE K IN *SQUILLA*

(s.w. K = 10·8 mM/kg)

Tissue	H_2O (%)	K (mM/kg)	Concentration factor K	Concentration factor ^{134}Cs
Plasma	—	12·3	1·14	1·40
Abdominal muscle	82·1	95	8·8	19·4
Raptorial limb muscle	78·4	115	10·7	19·8
Gut and the digestive gland	82·5	95	8·8	22·0

TABLE 6. CONCENTRATION FACTORS FOR RADIOACTIVE Cs AND INACTIVE K IN WHOLE ISOPODS AT END OF EXPERIMENTS IN FIG. 6C–D

Species	Medium K (mM/kg)	H_2O (%)	K (mM/kg)	Concentration factor K	Concentration factor ^{137}Cs
Sphaeroma serratum	10·7	69·0	52	4·9	5·9
	10·7	66·6	63	5·9	8·3
	10·7	78·0	65	6·1	8·9
Asellus aquaticus	0·103	86·0	27	262	66
	0·103	86·0	25	242	63

The sea water contained 40 μc/l. of isotope, while the tapwater was similar to that used previously with *Planorbis*. Figs. 6C, D show curves for the accumulation of the isotope by both species and Table 6 gives the K concentrations and concentration factors for inactive K and ^{137}Cs at the end of each experiment. *Sphaeroma* is typically marine in that it accumulates the isotope relatively rapidly to a concentration factor which exceeds that for inactive K. On the other hand *Asellus* behaves in a way which appears to be typical of fresh water invertebrates and absorbs ^{137}Cs slowly to a level which does not appear to be approaching the concentration factor for inactive K.

ECHINODERMATA

For these experiments specimens of the sea urchin *Psammechinus miliaris* (Gmelin) have been used. This animal is easier to keep at 16–18° C than *Asterias rubens* and *Echinus esculentus* which were used originally. Uptake of ^{137}Cs by whole animals from sea water containing 20 μc/l. of the isotope is shown in Fig. 7A. The ^{137}Cs enters the coelomic fluid very rapidly and 50% of the equilibrium level is reached in about 40 min (Fig. 7B). Accumulation of the isotope by the tissues from the coelomic fluid which is shown in Fig. 7C is a much slower process. The gut and the gonad which constitute the main soft tissues attain quite high concentration factors. Concentration factors for the shell are probably due to the tube feet included in the sample. The muscles of Aristotle's Lantern were sampled but they make up only an extremely small fraction of the total weight of the sea urchin. Analyses of the tissues for ^{137}Cs and inactive K after 958 h of uptake are given in Table 7.

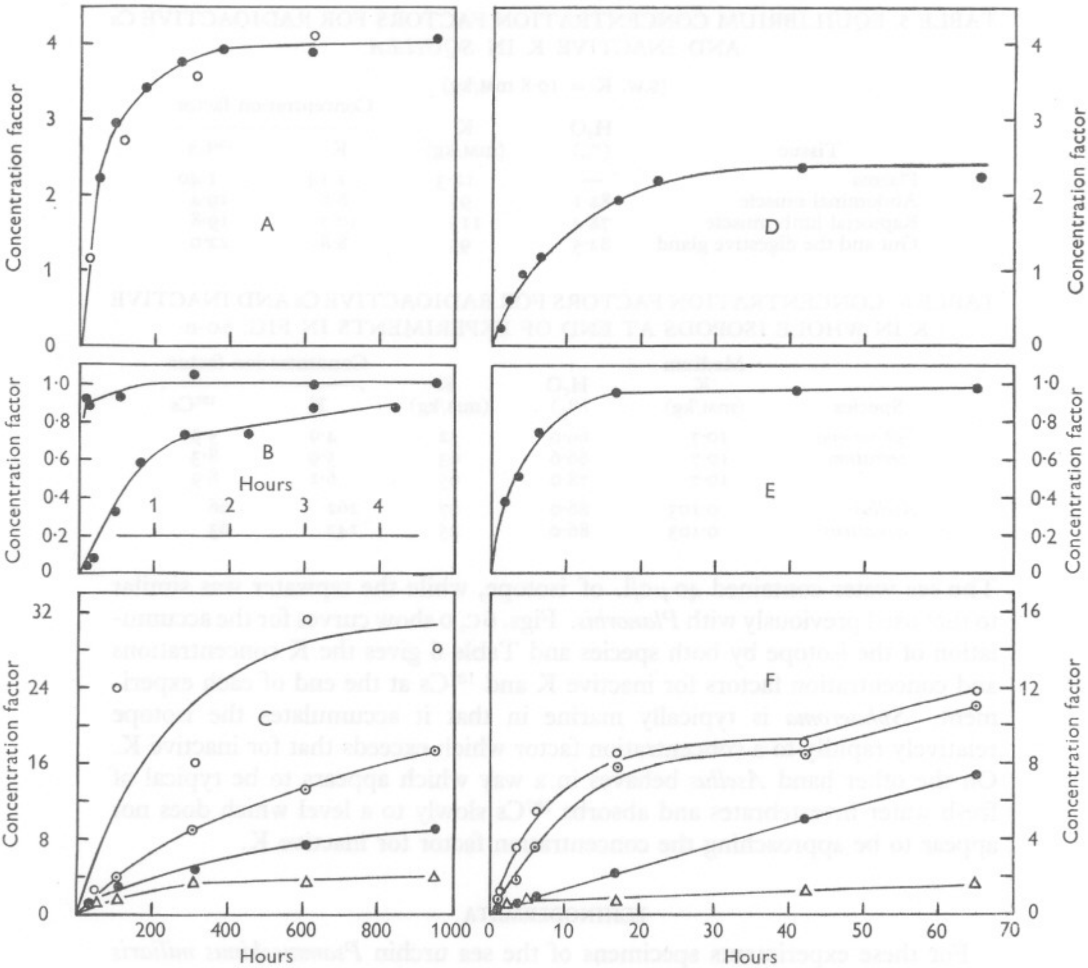

Fig. 7. A. Uptake of ^{137}Cs by whole *Psammechinus* from sea water at 16–18° C. ●, results for a single animal; ○, results for separate animals. Time scale as for Fig. 7C. B. Uptake of ^{137}Cs by *Psammechinus* body fluid. Lower curve is on an enlarged time scale and upper curve is on time scale for Fig. 7C. C. Uptake of ^{137}Cs by *Psammechinus* tissues. ○, gut; ⊙, gonad; ●, muscle of Aristotle's Lantern; △, shell and tube feet. D–F. Uptake of ^{42}K by *Psammechinus* from sea water at 16–18° C. These results correspond to those for ^{137}Cs. In F, ○, gut (97 %); ⊙, gonad (86 %); ●, muscle of Aristotle's Lantern (87 %); △, shell and tube feet (105 %). The percentage exchange of ^{42}K with inactive K at the end of the experiment is given in parentheses for each tissue.

All concentration factors for ^{137}Cs exceed those for inactive K, although the factors are very close in the case of the muscles and teeth of Aristotle's Lantern where possibly complete equilibrium had not been reached.

Results of corresponding experiments with ^{42}K are shown in Figs. 7D–F. Uptake of the isotope by the coelomic fluid appears to be slower than uptake of ^{137}Cs. However, ^{42}K uptake by the tissues is much more rapid than uptake of ^{137}Cs with the result that ^{42}K is continually being removed from the coelomic fluid and thus gives this appearance. Also shown in Figs. 7D–F are the percentages of the inactive K concentration factors reached by ^{42}K when the tissues of the last animal were analysed. The slope of the ^{42}K uptake curve for muscle suggests that it is the tissue which accumulates the isotope most slowly, although exchange in the gonad has not been completed.

TABLE 7. EQUILIBRIUM CONCENTRATION FACTORS FOR RADIOACTIVE Cs AND INACTIVE K IN *PSAMMECHINUS*

(S.W. K = 10·4 mM/kg)

Tissue	H_2O (%)	K (mM/kg)	Concentration factor K	Concentration factor ^{137}Cs
Coelomic fluid	96·1	10·2	0·98	1·01
Aristotle's lantern: muscle	77·8	94	9·0	9·1
Aristotle's lantern: teeth	19·0	18·2	1·8	1·9
Gut	73·0	137	13·2	28·1
Gonad	69·6	107	10·3	17·7
Shell (including tube feet)	29·0	21	2·1	4·0

TUNICATA

Uptake of ^{137}Cs was followed in *Ciona intestinalis* (L.) in filtered sea water containing 5 μc/l. of the isotope. The experimental temperature was 16–18° C. Prior to counting a whole animal it was washed first in inactive sea water. A complete curve for the uptake of the isotope by three animals is shown in Fig. 8A and Fig. 8B shows the uptake by the blood and principal tissues. Only a small volume of the blood could be obtained from the heart and so the accuracy is not very great. Table 8 gives the concentration factors for ^{137}Cs and inactive K in an animal which appeared to have reached equilibrium after 473 h. The test, which constitutes a large proportion of the animal, contains very little more K than the sea water and as a result it contains very little more ^{137}Cs than the sea water. The plasma may have inactive K and ^{137}Cs levels which slightly exceed those of sea water, but it is in the gut and pharynx and body wall muscles that the highest concentration factors are found for both inactive K and ^{137}Cs.

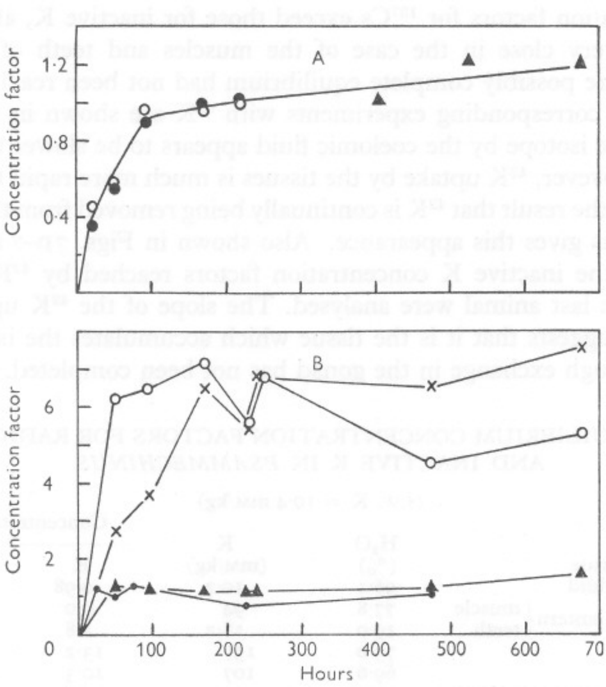

Fig. 8. A. Uptake of ^{137}Cs by three whole *Ciona* from sea water at 16–18° C. B. Uptake of ^{137}Cs by the blood and tissues of *Ciona*. ●, blood; ▲, test; ○, gut; ×, pharynx and body muscles.

TABLE 8. EQUILIBRIUM CONCENTRATION FACTORS FOR RADIOACTIVE Cs AND INACTIVE K IN *CIONA*

(S.W. K = 10·6 mM/kg)

Tissue	H_2O (%)	K (mM/kg)	Concentration factor K	Concentration factor ^{137}Cs
Blood	—	12·8	1·21	1·01
Test	94·7	11·6	1·09	1·11
Gut	89·8	41	3·9	4·6
Pharynx and body wall muscles	89·4	54	5·1	6·5

DISCUSSION

In whole specimens of the species which have been examined radioactive Cs is always absorbed more slowly than ^{42}K from sea water. All the inactive K in these species appears to be readily exchangeable with ^{42}K, but at equilibrium concentration factors for ^{137}Cs which are obtained usually exceed those for ^{42}K or inactive K.

In the foraminiferan *Elphidium crispum* ^{137}Cs is absorbed more slowly than ^{42}K and reaches a rather higher concentration factor. Although ^{137}Cs

and ^{42}K are somewhat dissimilar, particularly with regard to penetration rates, the similarities, especially between the effects of metabolic inhibitors on the uptake processes, suggest that both ions may be absorbed by the same type of mechanism. Very little work of a similar nature has been carried out with marine protozoa. Chipman (1960) quotes Martin (1957) who was unable to detect ^{137}Cs in the benthic foraminiferan *Discorbis floridana* after 40 days in sea water to which mixed fission products had been added. Other micro-organisms accumulate ^{137}Cs and this has been described by Williams (1960) for *Euglena* and *Chlorella* raised in culture media.

It was thought that differences in the rates of uptake of isotopes might be found between animals from the shore and those from deeper water. In the case of the sea anemones, the shore living *Actinia* and *Tealia* are not obviously less permeable than the *Metridium* and *Calliactis* which were trawled from deeper water. In these animals the highest concentration factors for ^{137}Cs and inactive K are found in the tentacles. If the mesogloea layer does not accumulate these ions very much, then a smaller proportion of this in the tentacles would explain the result. On this principle whole medusae would not be expected to accumulate these ions to a marked degree.

More obvious differences are found between the rates of ^{137}Cs uptake in the polychaetes *Nereis diversicolor* and *Perinereis cultrifera*. Fretter (1955) has shown that the body surface of *Nereis* is less permeable to ^{24}Na than that of *Perinereis* (and this probably also applies to radioactive Cs and ^{42}K). This is likely to be related to the fact that *Nereis* is a brackish water species which is more euryhaline than the more marine *Perinereis*. Judging from results obtained with other brackish water animals, *Nereis* would be expected to reach higher equilibrium concentration factors for ^{137}Cs in diluted sea water.

Whole crustaceans have all been found to accumulate radioactive Cs to a degree similar to that found previously in marine decapod crustacea by Bryan (1961) and Bryan & Ward (1962). Of the crustaceans examined in the present work, the copepods, as important members of the zooplankton, are the most interesting. As might be expected, the most rapid uptake of ^{137}Cs which has been found in the present work is in the smallest animal, *Tisbe reticulata*, which weighs 32 μg. Half of the equilibrium concentration factor of 12 is reached in about 2·0 h at 20° C. Even allowing for the lower experimental temperature, *Calanus*, which is a much larger copepod, absorbs the isotope more slowly and half the equilibrium concentration factor of about 14 is reached in 20 h at 9° C. Chipman (1960) found that the copepod *Tigriopus*, which is not a typically marine animal, reached concentration factors of 2–4 after 24 days exposure to ^{137}Cs. The effect of feeding on the accumulation of ^{137}Cs by these copepods has not been examined. Possibly the effect would not be very great as the highest ^{137}Cs concentration factor quoted by Chipman for marine phytoplankton is 3·1.

Chipman also gives ^{137}Cs concentration factors which were attained by the muscle and soft parts of clams and oysters. He found that muscle accumulated the isotope more slowly than the other soft tissues. This appears to be the case in the mussel *Mytilus galloprovincialis* although the difference is less marked. In *Mytilus* and in the gastropod *Monodonta*, uptake of the isotope into the plasma to a concentration factor of rather more than 1·0 is a rapid process prior to the slow uptake by the tissues. In all species used in the present work, where body fluid has been examined, neither inactive K nor radioactive Cs has been concentrated by a factor much greater than 1·0. As a result, the volume of body fluid is important in determining the equilibrium concentration factor of a whole animal. Thus in the echinoderm *Psammechinus*, in which the gut can reach a concentration factor of 28, the whole animal concentration factor is about 4 due to the large volume of body fluid. This is even more marked in the rather watery ascidian *Ciona* which only achieves a whole animal concentration factor of 1·2 although much higher values are found in individual tissues.

General conclusions can now be drawn in addition to those formulated for whole animals at the start of the discussion. In species with body fluids, uptake of radioactive Cs into the body fluid is a rapid phase of uptake and equilibrium concentration factors of the order of 1·0 are found. These factors are similar to those for inactive K. This applies to the present work but marked deviations from this have been found in the decapod crustacea (Bryan & Ward, 1962). Soft tissues tend to reach variable radioactive Cs concentration factors of the order of 10 which at equilibrium exceed those for inactive K or ^{42}K. In any one species the higher radioactive Cs tissue concentration factors are usually associated with higher inactive K concentrations. The higher radioactive Cs tissue concentration factors are the result of higher tissue/body fluid ratios for Cs. Differences between whole animal concentration factors for various species depend to a large extent on the presence or absence of a heavy shell and on the volume of body fluid with its low concentration factors for both Cs and K.

Where freshwater species have been used for comparison, it is found that whole animal concentration factors for radioactive Cs at equilibrium do not reach those for inactive K. In the fresh-water crayfish (Bryan & Ward, 1962) this is due to the inability of the animal to concentrate ^{137}Cs to a factor equal to that for inactive K in the blood plasma.

On the one hand large differences have been found between radioactive Cs and ^{42}K with regard to penetration rates, but on the other hand the extent of Cs accumulation in the body fluid and tissues appears to be influenced by the distribution of K. No examples have been found which would suggest that Cs is a necessary element or that it is being absorbed specifically.

I should like to thank Mr L. G. Hummerstone for his help with this work, which was supported by the United Kingdom Atomic Energy Authority.

SUMMARY

The accumulation of radioactive Cs has been studied in species from the principal marine invertebrate phyla. In some species the accumulation of radioactive Cs has been compared with that of ^{42}K and equilibrium levels of both isotopes have been related to the distribution of inactive K.

Radioactive Cs is always absorbed more slowly than ^{42}K. Most of the inactive K is readily exchangeable with ^{42}K so that at equilibrium inactive and active concentration factors are nearly equal. Whole animal concentration factors for radioactive Cs at equilibrium varied between 1·2 and 14 in unfed animals. These factors usually exceed those for inactive K because, although body fluid concentration factors are similar for both ions, the tissue/body fluid ratios for radioactive Cs exceed those for inactive K. Radioactive Cs penetrates rapidly into body fluids, so that uptake by the tissues is the limiting factor in accumulation. Although Cs is absorbed much more slowly than K, the eventual distribution of Cs between the tissues and body fluid of a particular species appears to bear a relationship to the distribution of K.

No evidence has been found to suggest that Cs is a necessary element which is absorbed specifically.

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