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THE ACCUMULATION OF ^{137}Cs BY BRACKISH WATER INVERTEBRATES AND ITS RELATION TO THE REGULATION OF POTASSIUM AND SODIUM

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(Text-figs. 1–8)

Because of its long half life of about 30 years the fission product ^{137}Cs is one of the most important radioactive wastes. Small amounts have been introduced into aquatic environments in effluents and as a result of weapons tests. It is important to determine to what extent and in what way radioactive Cs can be absorbed by aquatic animals in order that some estimate can be made of whether any hazard to man is being created either directly or through food chains.

As an alkali metal Cs is chemically similar to sodium and potassium. In biological systems Cs usually behaves more like K, which is principally associated with cellular material, than Na which is the main cation in body fluids. Experiments have previously been carried out on the accumulation of radioactive Cs in relation to K metabolism in a number of marine and freshwater invertebrates (Bryan, 1961, 1963; Bryan & Ward, 1962). Unfed marine invertebrates accumulate radioactive Cs more slowly than the K isotope ^{42}K but at equilibrium rather higher concentration factors are reached for radioactive Cs. The level of inactive Cs in sea water is about 0·5 μg/l. (Smales & Salmon, 1955). As long as this sea-water concentration is very low compared with that of K, quite wide variations do not affect the rate or extent of radioactive Cs uptake. However, changes in the K concentration of sea water are important. When the crab *Carcinus maenas* is placed in sea water with 50% of the normal K concentration the body K level remains fairly constant and consequently the equilibrium concentration factor for ^{42}K is almost doubled. This also causes the equilibrium concentration factor for radioactive Cs to be doubled. A similar effect is produced if a fairly euryhaline animal such as the prawn *Palaemon serratus* is placed in 50% sea water. In sea-water dilutions of this order equilibrium concentration factors for radioactive Cs exceed those for inactive K or ^{42}K. Fortunately from the hazard point of view this is not found in fresh water where K concentration factors are very high. In the freshwater crayfish a K concentration factor of 5000 may be maintained, but the equilibrium concentration factor for ^{137}Cs in unfed animals is 1–200

(Bryan & Ward, 1962). The reason is that although tissue/plasma ratios for ^{137}Cs tend to exceed those for K, as they do in marine species, the plasma/medium ratios for K are not approached by those for ^{137}Cs.

It is possible that the highest equilibrium concentration factors for radioactive Cs may be reached in brackish water animals which live in a salinity range, which has not yet been covered, between fresh water and 50% sea water. In the Plymouth area two of the most common brackish water species are the gastropod mollusc *Potamopyrgus* (= *Hydrobia*) *jenkinsi* Smith and the isopod crustacean *Sphaeroma hookeri* Leach. The extent to which these species can concentrate ^{137}Cs in different salinities is almost certainly associated with the ability of the animals to maintain relatively high levels of K and Na in the body against the reduced concentrations in diluted sea water. It follows that a study of the accumulation of ^{137}Cs also involves an investigation into the regulation of K and Na with which it can be compared.

As a first step, experiments were carried out to determine the abilities of *Potamopyrgus* and *Sphaeroma* to tolerate and to maintain Na and K in the body at different sea-water concentrations and to find the time course of acclimatization. Experiments were then carried out to compare the accumulation and loss of ^{137}Cs with the behaviour of ^{42}K and inactive K.

MATERIALS AND METHODS

Potamopyrgus and *Sphaeroma* were collected in Plymouth from Chelson Meadows and were not fed during the experiments. The isotopes ^{137}Cs and ^{42}K were from the Radiochemical Centre, Amersham. Experimental media were usually dilutions of filtered Plymouth sea water but sometimes artificial sea water ($Cl‰ = 19$) was used. In order to have media containing sufficient ^{42}K it was necessary to make artificial sea water in which all the K was from the carrier in the isotope sample. For experiments with both ^{137}Cs and ^{42}K all diluted solutions were made from 100% sea water to which the isotopes had been added.

In *Sphaeroma*, blood samples of about 0·002 ml. could be taken with fine glass pipettes operated by suction. A single sample measured with an 'Agla' syringe and diluted with 2–5 ml. of distilled water could be used for Na estimation with an 'EEL' flame photometer. Larger pooled samples could be weighed and then diluted for the measurement of K by flame photometer. Weighed radioactive samples were dried on to planchets and counted with an end window γ-scintillation counter. Counting of whole animals, which were first washed, was done in test tubes in a well-type γ-scintillation counter. This system was also used to measure the activity of liquid samples of whole *Sphaeroma* and *Potamopyrgus* which had been wet-ashed with nitric acid. These same samples were also used for the estimation of Na and K by flame photometry. In *Potamopyrgus* it was necessary to correct the Na values in

whole animals for interference due to calcium in the shell by subtracting figures obtained for empty shells. The experimental temperature was 20° C.

Radioactive levels in these animals are expressed as concentration factors relative to the activity of the medium. The concentration factor is the level of ^{137}Cs, ^{42}K, inactive K or inactive Na per kg of blood or whole animal divided by the corresponding concentration per kg of medium. During loss in inactive media, isotope concentration factors are calculated relative to the activity of the medium used for uptake.

RESULTS

Ability to survive in diluted and modified sea waters

No trouble was encountered in keeping *Potamopyrgus* alive during experiments of 1000 h duration in sea-water concentrations of between 0·1 and 50%. In 100% sea water some animals could live for 500 h but others died before this. *Sphaeroma* can live fairly indefinitely in concentrations of between 5 and 100% sea water. Most *Sphaeroma* can live for long periods in 2·5% sea water but even the hardiest animals can only survive for 2–300 h in 1% sea water.

If artificial sea water is used, in which the K concentration only is varied, then over a period of 360 h both species are limited to media containing K at a concentration between 25 and 100% of that in natural sea water. If the NaCl content of artificial sea water is kept constant while the other ions are varied, dilution of the other ions from 50 to 25% kills both species.

Finally, an experiment was carried out to check the viability of these animals when the K in 50% artificial sea water is partially replaced by Cs. When one-quarter of the K is replaced by Cs, most of the animals of both species lived for at least 800 h. If half the K is replaced by Cs then all the animals of both species die within 260 h.

Of the experimental solutions which have been examined, sea water and dilutions of sea water are the best media in which to keep these animals. There are no advantages in changing the K concentration of artificial sea water, while at the same time relieving osmotic stresses by maintaining the high external Na concentration. The substitution of large amounts of Cs for K can kill both species and this suggests that Cs is a very poor replacement for K.

The effect of distilled water

When *Sphaeroma* from 100% sea water are placed in distilled water they lose Na rapidly and are usually just alive after 16 h when the blood Na level has fallen to about 10% of its original value (Fig. 1A). Analyses of whole animals in groups of three after exposure to distilled water show that Na is lost rapidly but that K is lost more slowly (Fig. 1B). Points are also shown in

Fig. 1B for some selected animals which lost both ions more slowly and were still alive after 24 h.

Both Na and K are lost rapidly from *Potamopyrgus* when it is first transferred from 100% sea water to frequent changes of distilled water (Fig. 1C). These animals were analysed whole in groups of three. This rapid loss of Na slows down rather abruptly when the level has fallen to about 25 mM/kg and later losses are very slow. A similar but less marked result is found for K. A number of factors could contribute to this observation. It may be caused by a reduction in the permeability of the body surface to the loss of ions or to a reduction in the amount of salt excreted. Losses of ions could also be limited by the ability of the animal to withdraw into the shell and close the opening with the operculum. After about 24 h in distilled water animals usually came out of their shells only if they were disturbed.

Acclimatization to diluted sea waters

Experiments similar to those in the previous section were carried out in following changes in K and Na concentrations when *Sphaeroma* are transferred from 100 to 5% sea water and when *Potamopyrgus* are transferred from 100 to 1% sea water. The results in Fig. 1D show that in *Sphaeroma* the blood sodium concentration falls to a new level in about 10 h and a similar length of time is taken by both ions in the whole animal (Fig. 1E). *Potamopyrgus* reaches its new levels for both ions in about 5 h (Fig. 1F).

In Fig. 2A the blood K and Na concentrations of acclimatized *Sphaeroma* are plotted against the sea-water concentration, which is for convenience on a logarithmic scale. Acclimatization periods of more than 100 h were allowed for animals in 100–2·5% sea water but at lower concentrations 40–100 h were allowed. As sea water is diluted the concentrations of both ions fall but not in proportion to the dilution of the sea water. When levels of 1–2% sea water are reached the blood Na concentration starts to fall quite rapidly. It is at concentrations of 1 to 2% sea water that *Sphaeroma* are no longer able to live fairly indefinitely.

Corresponding values for K and Na in whole *Sphaeroma* are given in Fig. 2B for animals which had been acclimatized for a minimum of 250 h. Results for Na tend to reflect those for the blood which contains most of the Na in the animal. The tissues contain most of the K in the animal. Values for K are very variable but as the sea water is diluted so a general fall in the body K level is seen.

Comparative results for groups of *Potamopyrgus* are given in Fig. 2C. As the sea water is diluted the body Na concentration falls almost proportionally down to 25% sea water. This means that the Na concentration factor (Fig. 8B) remains fairly constant between 25 and 100% sea water. At lower external concentrations the Na concentration of the animals still falls but the concentration factor rises. Between concentrations of 1 and 0·1% sea water the

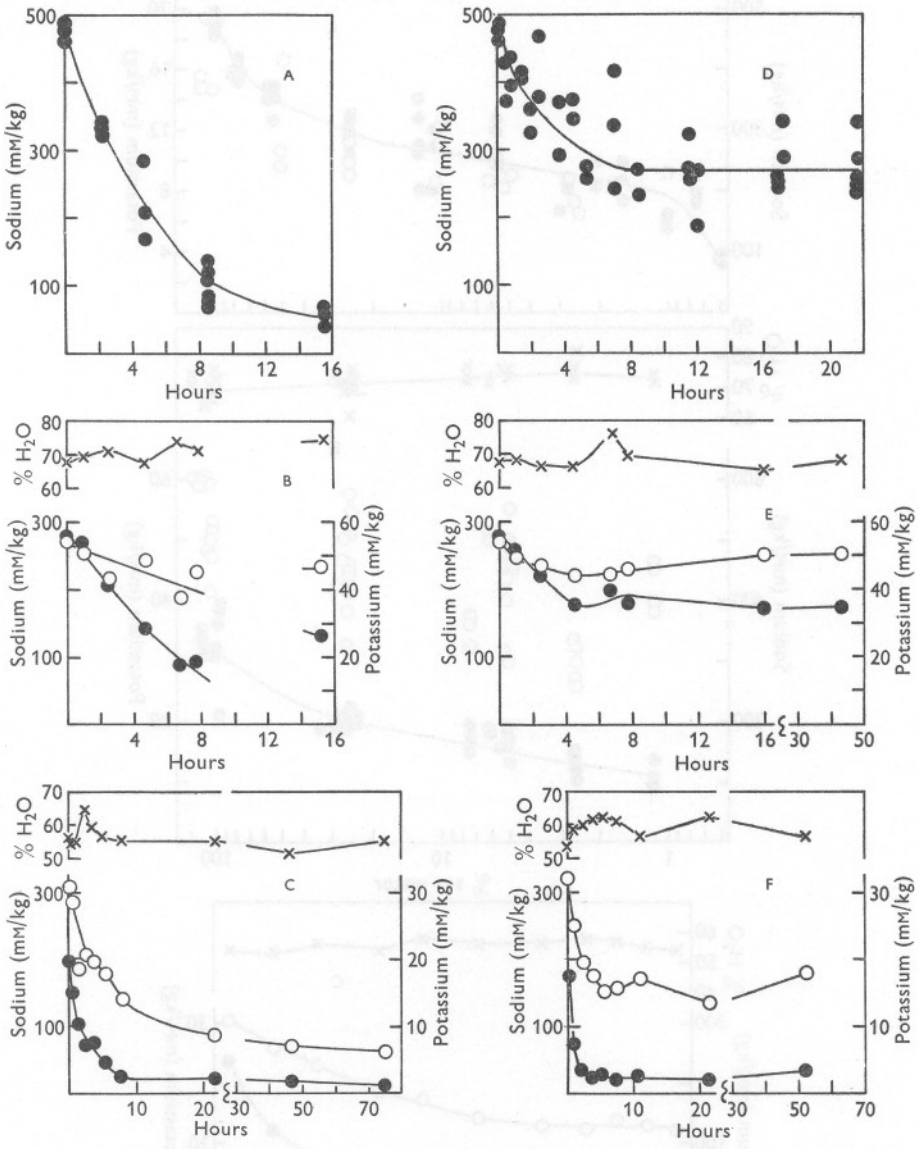

Fig. 1. A. Fall in blood Na concentration of *Sphaeroma* transferred from 100 % sea water to distilled water. B. Fall in whole body Na and K concentrations in groups of *Sphaeroma* transferred from 100 % sea water to distilled water. ●, Na concentration; ○, K concentration; ×, water content. Points on the extreme right of the figure are for a group of three particularly tolerant animals which were selected. C. Fall in whole body Na and K concentrations in groups of *Potamopyrgus* transferred from 100 % sea water to distilled water. Symbols as above. D. Blood Na concentration of *Sphaeroma* transferred from 100 to 5 % sea water. E. Whole body Na and K concentrations of groups of *Sphaeroma* transferred from 100 to 5 % sea water. Symbols as above. F. Whole body Na and K concentrations of groups of *Potamopyrgus* transferred from 100 to 1 % sea water. Symbols as above.

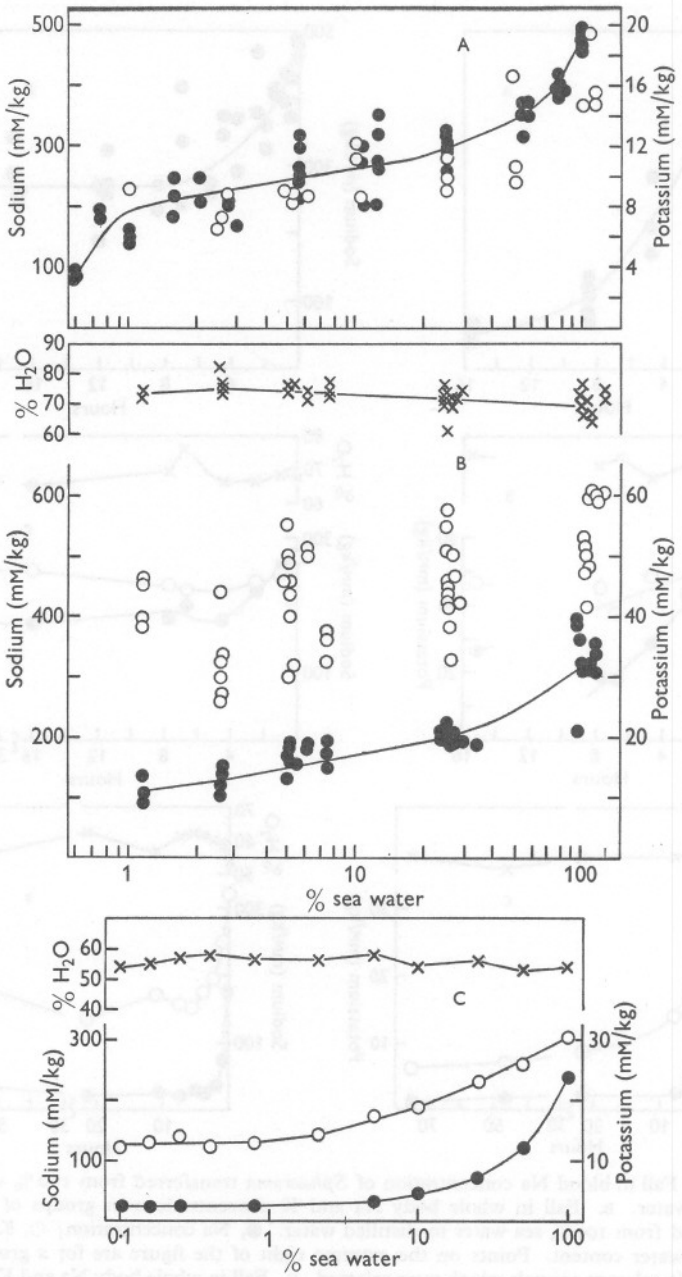

Fig. 2. A. Concentrations of Na and K in blood of *Sphaeroma* acclimatized to different sea-water concentrations. B. Concentrations of Na and K in whole *Sphaeroma* acclimatized to different sea-water concentrations. C. Concentrations of Na and K in groups of whole *Potamopyrgus* acclimatized to different sea-water concentrations. ●, Na concentration; ○, K concentration; ×, water content.

Na level in the animals remains almost constant at about 22 mM/l. Much of the body Na is probably in the blood. If the tissue/blood ratio for Na remains constant in animals acclimatized to different sea-water concentrations then the body Na concentration will be proportional to the blood Na concentration and possibly to the blood osmotic pressure. If the blood osmotic pressure of *Potamopyrgus* is equal to that of the medium in 100% sea water then isotonicity is probably maintained down to 25% sea water. Below this concentration the blood probably becomes hypertonic to the medium. The body K concentration falls in diluted sea water but not to the same extent as Na. Possibly the blood K concentration falls in the same way as Na but this will be masked by the fact that most of the K is in the tissues.

Uptake of ^{42}K by both species

Uptake of ^{42}K was followed in whole *Sphaeroma* which had been acclimatized to 1, 2·5, 5, 25 and 100% sea water. Curves in Fig. 3B, C show that over the experimental period of 80–90 h equilibrium is reached only in 25 and 100% sea water. In both these media the equilibrium concentration factors for ^{42}K are almost equal to the inactive K concentration factors. Assuming that this would be true at other external concentrations, the equilibrium ^{42}K concentration factor can be found from the inactive K concentration. These values at different sea-water concentrations are summarized in Table 1.

A conventional semi-logarithmic plot has been made of the results in Fig. 3B, C using notation similar to that employed by Bryan (1960) for ^{22}Na uptake by crayfish blood. $\log_{10}(1-[^{42}K_{in}]_t/[^{42}K_{in}]_{t=\infty})$ is plotted against time in Fig. 3E. $[^{42}K_{in}]_t$ is the ^{42}K concentration at time t and $[^{42}K_{in}]_{t=\infty}$ is the ^{42}K concentration factor at equilibrium, which is found from the inactive K concentration of the animal. If the ^{42}K uptake curve is exponential and the semi-logarithmic plot gives a straight line, this would suggest that with regard to absorption of the isotope the animal is behaving like a single compartment. Fairly straight lines are found at external concentrations of 25% and below but not in 100% sea water. By multiplying the slope of the line by 2·303 the transfer constant k_{out} for ^{42}K in the direction in→ out could be found. This constant is related to the time taken for 50% of the exchange to occur ($T_{\frac{1}{2}}$), by $k_{out} = 0{\cdot}693/T_{\frac{1}{2}}$. However, as all ^{42}K and ^{137}Cs uptake curves are not exponential an indication of the rate at which equilibrium is reached under different conditions will be given by the time taken to reach 50% of the equilibrium concentration factor. This value will be equal to $T_{\frac{1}{2}}$ when the uptake curve is exponential but not otherwise. Table 1 gives these times for the uptake of ^{42}K in *Sphaeroma*. This shows that the isotope is exchanged more slowly in animals acclimatized to the more dilute sea-water concentrations, although in fact the K concentration of the whole animal varies only slightly between those from 1 and 25% sea water.

It has been suggested that in the more dilute sea-water concentrations

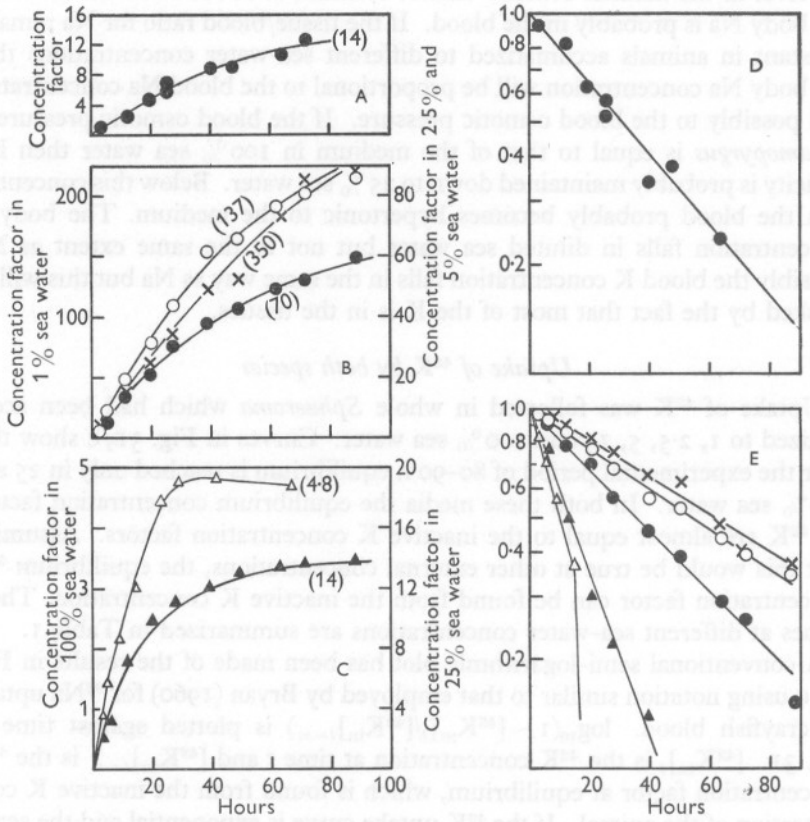

Fig. 3. A. Uptake of ^{42}K by the blood of *Sphaeroma* acclimatized to 5% sea water. The equilibrium concentration factor is given in parentheses. B, C. Uptake of ^{42}K by whole *Sphaeroma* acclimatized to different sea-water concentrations. ×, 1%; ○, 2·5%; ●, 5%; ▲, 25%; △, 100% sea water. Equilibrium concentration factors are given in parentheses. D. Semi-logarithmic plot of results from Fig. 3A. E. Semi-logarithmic plot of results from Fig. 3B, C.

TABLE 1. RESULTS FROM ^{42}K UPTAKE EXPERIMENTS IN *SPHAEROMA HOOKERI*

Sea water (approx. %)	No. of animals	Whole animal K concentration (mM/kg)	Equilibrium ^{42}K concentration factor	Time to reach 50% of equilibrium concentration factor (h)
100	3	59	4·8	10
25	3	45	14	13
5	3	43	70	36
2·5	3	43	137	54
1	1	45	350	62
5	Blood	8·7	14	31

Sphaeroma behaves as a single compartment with regard to the exchange of ^{42}K. This would be expected if the exchange of the isotope between blood and tissues were very rapid compared with uptake over the body surface. Uptake of ^{42}K into the blood of *Sphaeroma* has been followed in 5% sea water and the results are given in Fig. 3A. A semi-logarithmic plot of the results in Fig. 3D gives a fairly straight line with a slope similar to that for whole animals in Fig. 3E. This is because the exchange of ^{42}K between blood and tissues is so rapid that the ratio ^{42}K in whole animal/^{42}K in blood is virtually constant over the whole period of uptake at a value of 4·4. Thus the body surface is the limiting factor in ^{42}K uptake in 5% sea water and probably at other external concentrations except perhaps in 100% sea water.

In *Potamopyrgus* all the body K is exchangeable with ^{42}K. Equilibrium with ^{42}K is again approached more slowly as the sea-water concentration is lowered and the K concentration factor rises. Uptake curves for groups of nine or ten animals acclimatized to 0·1, 1, 25 and 100% sea water are shown in Fig. 4A, B and semi-logarithmic plots of the results are given in Fig. 4C, D. In sea-water concentrations of 25% and below the uptake curves are almost exponential and straight lines are given by the semi-logarithmic plots. The equilibrium concentration factors and the times taken for 50% of these levels to be attained are shown in Table 2. A factor of more than 10 separates the rates at which ^{42}K is exchanged in 0·1 and 100% sea water.

Loss of ^{42}K from both species

In experiments on the loss of ^{24}Na from the brine shrimp *Artemia*, Croghan (1958) found that the efflux of isotope was reduced to a low level if the external sea water was replaced by distilled water or by erythritol solution isotonic with sea water. This was accounted for by assuming that there is an exchange diffusion component of uptake and loss of Na in the presence of external salt solutions. No energy is required for this component and its magnitude increases with the external salt concentration. It was thought that the more rapid exchange of ^{42}K in both species at the higher sea-water concentrations might have a similar explanation.

The loss of ^{42}K from both *Sphaeroma* and *Potamopyrgus* has been followed in inactive sea waters and in both distilled water and sucrose solutions isotonic with the sea-water media. *Sphaeroma* in groups of four were loaded with ^{42}K for 8 h in 2·5, 5, 25 and 100% artificial sea water. They were washed for 30 min in inactive sea-water solution and then allowed to lose the isotope into 5 ml. of inactive sea-water solution for three consecutive periods of 15 min each. After 5 min washing in either distilled water or sucrose solution the loss was followed for four similar periods in 5 ml. lots of these media. The animals were then returned to sea-water media for a further two periods, after which the activity of the whole animals was measured in digested samples. This enabled the calculation of the initial ^{42}K level in the whole animals. The

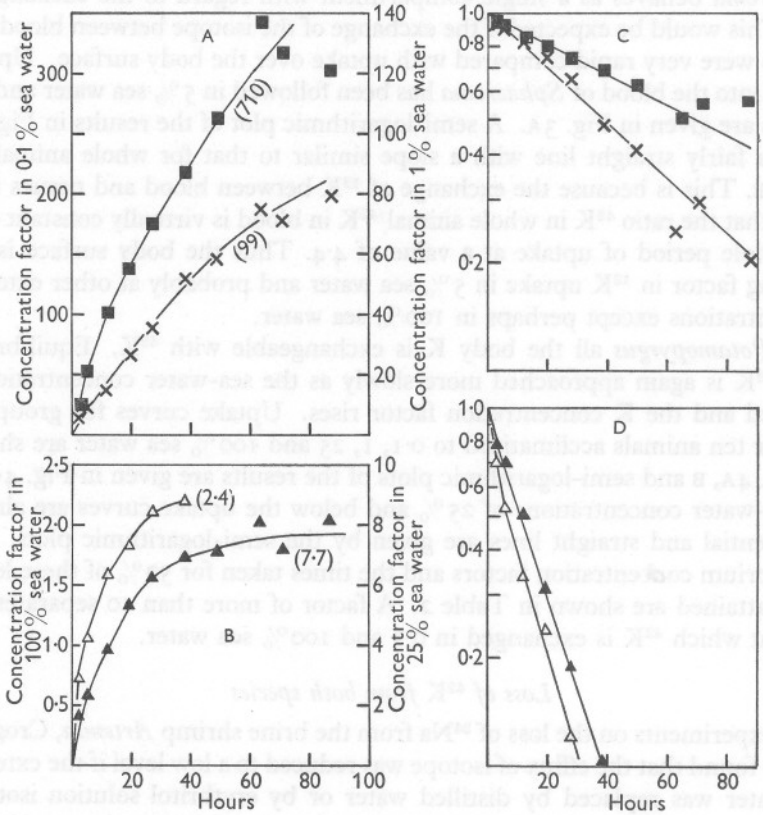

Fig. 4. A, B. Uptake of ^{42}K by whole *Potamopyrgus* acclimatized to different sea-water concentrations. ■, 0·1 %; ×, 1 %; ▲, 25 %; △, 100 % sea water. Equilibrium concentration factors are given in parentheses. C, D. Semi-logarithmic plot of results in Fig. 4A, B.

TABLE 2. RESULTS FROM ^{42}K UPTAKE EXPERIMENTS IN *POTAMOPYRGUS JENKINSI*

Sea water (approx. %)	No. of animals	Whole animal K concentration (mM/kg)	Equilibrium ^{42}K concentration factor	Time to reach 50 % of equilibrium concentration factor (h)
100	9	25	2·4	6·5
25	10	23	8·8	6·5
25	7	24	9·8	9
25	9	20	7·7	11
5	10	16	31·4	14
5	6	17	35·2	21
1	6	16	161	33
1	10	12	99	38
0·1	6	11	1167	114
0·1	10	9	710	70

amount of ^{42}K lost during each period in 5 ml. of solution could then be expressed as a percentage of the activity of the whole animals at the start of each period.

In *Potamopyrgus* a similar experiment was carried out. Unfortunately, animals acclimatized to 0·1% sea water could not be loaded with sufficient isotope in 8 h and so only animals from 1, 5 and 25% sea water were used. Groups of four animals were allowed to lose ^{42}K into 2 ml. lots of diluted sea water for three periods of 30 min, followed by a similar time in either distilled water or sucrose solution and one further period in diluted sea water.

TABLE 3. LOSS OF ^{42}K FROM *SPHAEROMA* INTO DILUTED INACTIVE SEA-WATER SOLUTIONS AND INTO DISTILLED WATER OR SUCROSE SOLUTIONS ISOTONIC WITH THE SEA WATER

Sea water (approx. %)	Mean ^{42}K loss (%/h)	Other medium	Mean ^{42}K loss (%/h)
100	11·8	Distilled water	1·1
	11·0	Sucrose solution	1·0
25	4·3	Distilled water	1·7
	4·7	Sucrose solution	2·2
5	2·2	Distilled water	1·7
	4·0	Sucrose solution	2·4
2·5	2·7	Distilled water	2·8
	2·7	Sucrose solution	2·2

TABLE 4. LOSS OF ^{42}K FROM *POTAMOPYRGUS* INTO DILUTED INACTIVE SEA-WATER SOLUTIONS AND INTO DISTILLED WATER OR SUCROSE SOLUTIONS ISOTONIC WITH THE SEA WATER

Sea water (approx. %)	Mean ^{42}K loss (%/h)	Other medium	Mean ^{42}K loss (%/h)
25	16·8	Distilled water	8·9
	14·9	Sucrose solution	7·4
5	7·4	Distilled water	5·2
	7·1	Sucrose solution	4·9
1	7·2	Distilled water	3·6
	4·7	Sucrose solution	2·6

Because the losses during separate periods are sometimes quite variable, mean values for the percentage loss of ^{42}K during the periods in sea-water media (as % loss/h) have been compared with the mean values for loss into distilled water and sucrose solutions. The results are given in Table 3 for *Sphaeroma* and Table 4 for *Potamopyrgus*. Both species lose ^{42}K more rapidly when the experiments are conducted in the higher sea-water concentrations. This is to be expected from the results of the ^{42}K uptake experiments.

When *Sphaeroma* are transferred to the salt-free media the loss of ^{42}K is reduced to such an extent that the animals from 100% sea water lose the isotope more slowly than those from lower sea-water concentrations. The effect of salt-free media in reducing the loss of ^{42}K becomes progressively smaller as

the animals are acclimatized to more dilute media. This is compatible with the postulate that there may be an exchange diffusion component of ^{42}K exchange which increases as the external concentration is raised. There is no evidence as to what effect any electrical changes produced by the change in medium might have. Provided that *Sphaeroma* from different sea-water concentrations have similar blood and tissue K concentrations then in the absence of salts in the external medium the losses of ^{42}K by passive diffusion should be similar. This situation almost holds in 2·5, 5 and 25% sea water (Table 1) and Table 3 shows that ^{42}K losses in salt-free media are of a similar order. The corresponding figures for animals from 100% sea water are lower, which is not expected because the blood K concentration is markedly higher under these conditions. A lower excretion of ^{42}K in urine might explain this result but evidence suggests that excretion is not very important in ^{42}K exchange. The similarity between values for ^{42}K loss into distilled water, and into isotonic sucrose solution for animals from 100% sea water, suggests that very little of the isotope is lost in the extra urine which might be expected in distilled water.

In *Potamopyrgus* the salt-free media cause the largest reduction in ^{42}K losses in animals from the higher sea-water concentrations (Table 4). Unlike *Sphaeroma*, animals from higher sea-water concentrations still lose ^{42}K more rapidly in salt-free media. The blood K concentrations of this species are unknown but it is possible that in animals acclimatized to the more dilute media the blood K level falls to a greater extent than the tissue K levels and thus raises the tissue/blood ratios. This redistribution of K so that more of it lies in the tissues and less in the blood would tend to result in the slower loss of ^{42}K into salt-free media by animals from more dilute sea waters, because these values are expressed as a percentage of the whole body activity. As in *Sphaeroma* no evidence has been found to suggest that the excretion of urine is important in the exchange of ^{42}K.

Uptake of ^{137}Cs

The uptake of ^{137}Cs has been followed in *Sphaeroma* acclimatized to 100, 25, 5, and 2·5% sea water and the curves are shown in Fig. 5B, C. A semi-logarithmic plot of the results is given in Fig. 5E. Table 5 compares the equilibrium concentration factors for ^{137}Cs with those for inactive K and also gives the time taken to attain 50% of the ^{137}Cs equilibrium factors. The results show that the isotope is absorbed more slowly as sea water is diluted from 100 to 5% and they also indicate (Fig. 5E) that uptake is not an exponential process in the two higher sea-water concentrations. In 5% sea water the uptake of ^{137}Cs appears to be almost exponential and is slower than in 2·5% sea water. However, the low inactive whole animal K concentration of animals from 2·5% sea water (Table 5) compared with that for animals in Table 1 suggests that during the long accumulation of ^{137}Cs inactive K was being lost.

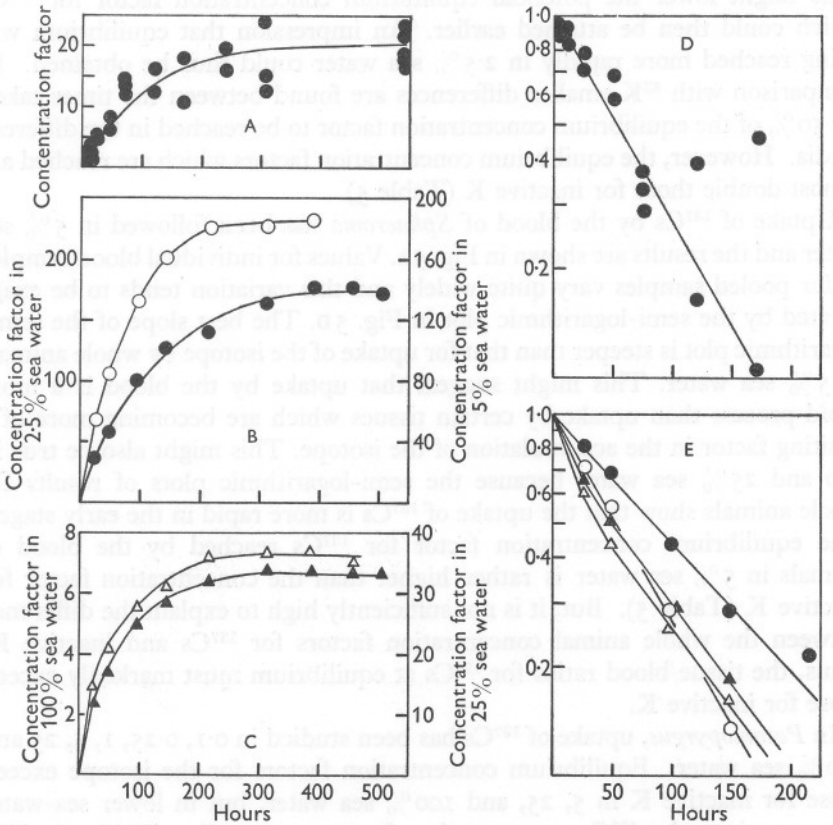

Fig. 5. A. Uptake of ^{137}Cs by the blood of *Sphaeroma* acclimatized to 5 % sea water. B, C. Uptake of ^{137}Cs by whole *Sphaeroma* acclimatized to different sea-water concentrations. ○, 2·5 %; ●, 5 %; ▲, 25 %; △, 100 % sea water. D. Semi-logarithmic plot of results from Fig. 5A. E. Semi-logarithmic plot of results from Fig. 5B, C.

TABLE 5. RESULTS FROM ^{137}Cs UPTAKE EXPERIMENTS IN *SPHAEROMA HOOKERI*

Sea water (approx. %)	No. of animals	Whole animal H_2O (%)	Whole animal K (mM/kg)	Inactive K concentration factor	Equilibrium ^{137}Cs concentration factor	Time to reach 50 % of equilibrium ^{137}Cs concentration factor (h)
100	3	68·6	47	4·6	6·6	38
25	2	68·5	45	16·7	32·9	50
5	2	74·5	38	71	136	85
2·5	2	79·5	26	99	232	52
5	Blood	—	8·4	16·2	20	52

This might lower the potential equilibrium concentration factor for ^{137}Cs which could then be attained earlier. An impression that equilibrium was being reached more rapidly in 2·5% sea water could thus be obtained. In comparison with ^{42}K smaller differences are found between the times taken for 50% of the equilibrium concentration factor to be reached in the different media. However, the equilibrium concentration factors which are reached are almost double those for inactive K (Table 5).

Uptake of ^{137}Cs by the blood of *Sphaeroma* has been followed in 5% sea water and the results are shown in Fig. 5A. Values for individual blood samples or for pooled samples vary quite widely and this variation tends to be exaggerated by the semi-logarithmic plot in Fig. 5D. The best slope of the semi-logarithmic plot is steeper than that for uptake of the isotope by whole animals in 5% sea water. This might suggest that uptake by the blood is a more rapid process than uptake by certain tissues which are becoming more of a limiting factor in the accumulation of the isotope. This might also be true in 100 and 25% sea water because the semi-logarithmic plots of results for whole animals show that the uptake of ^{137}Cs is more rapid in the early stages. The equilibrium concentration factor for ^{137}Cs reached by the blood of animals in 5% sea water is rather higher than the concentration factor for inactive K (Table 5). But, it is not sufficiently high to explain the difference between the whole animal concentration factors for ^{137}Cs and inactive K. Thus, the tissue/blood ratios for ^{137}Cs at equilibrium must markedly exceed those for inactive K.

In *Potamopyrgus*, uptake of ^{137}Cs has been studied in 0·1, 0·25, 1, 5, 25 and 100% sea water. Equilibrium concentration factors for the isotope exceed those for inactive K in 5, 25, and 100% sea water, but in lower sea-water concentrations the ^{137}Cs concentration factors are smaller (Table 6). The curves in Fig. 6A, B appear to attain equilibrium in similar lengths of time and this is also suggested by the times taken to reach 50% of the equilibrium concentration factor which are given in Table 6. Semi-logarithmic plots of the results in Fig. 6A, B are given in Fig. 6C, D and they show that uptake is not exponential as there is a more rapid preliminary phase to the process. This may indicate that, as in *Sphaeroma*, the limiting factor in the accumulation of ^{137}Cs in *Potamopyrgus* could be the slow uptake by some of the tissues.

Loss of ^{137}Cs

Short-term loss experiments similar to those carried out with ^{42}K have been used in both *Sphaeroma* and *Potamopyrgus* to determine whether there is possibly an exchange diffusion component of ^{137}Cs exchange. In *Sphaeroma* the experiment was identical with that for ^{42}K except that the periods of loss of the isotope were extended from 15 to 30 min and the animals were pre-loaded with ^{137}Cs for 60 h. Table 7 shows that the mean loss of ^{137}Cs into inactive sea-water concentrations, calculated as a percentage of the whole

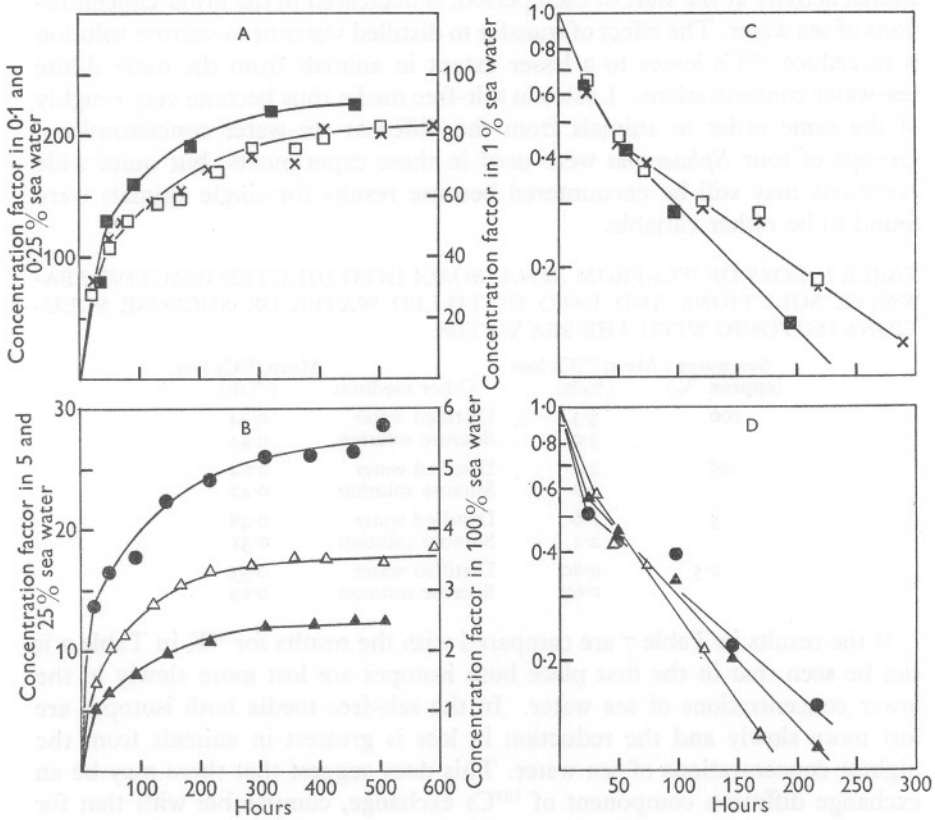

Fig. 6. A, B. Uptake of ^{137}Cs by *Potamopyrgus* acclimatized to different sea-water concentrations. ■, 0·1 %; □, 0·25 %; ×, 1 %; ●, 5 %; ▲, 25 %; △, 100 % sea water. C, D. Semi-logarithmic plot of results from Fig. 6A, B.

TABLE 6. RESULTS FROM ^{137}Cs UPTAKE EXPERIMENTS IN *POTAMOPYRGUS JENKINSI*

Sea water (approx. %)	No. of animals	Whole Animal H_2O (%)	Whole Animal K (mM/kg)	Inactive K concentration factor	Equilibrium ^{137}Cs concentration factor	Time to reach 50 % of equilibrium ^{137}Cs concentration factor (h)
100	5	50·6	31	3·0	3·4	40
25	5	55·0	23	8·4	12·1	40
5	4	57·1	15	27·8	28·6	27
1	5	56·6	11	104	83	41
0·25	4	54·0	11	415	207	41
0·1	5	57·5	11	885	225	41

animal activity at the start of each period, is decreased in the lower concentrations of sea water. The effect of transfer to distilled water or to sucrose solution is to reduce ^{137}Cs losses to a lesser extent in animals from the more dilute sea-water concentrations. Losses in salt-free media thus become very roughly of the same order in animals from the different sea-water concentrations. Groups of four *Sphaeroma* were used in these experiments, but quite wide variations may still be encountered because results for single animals were found to be rather variable.

TABLE 7. LOSS OF ^{137}Cs FROM *SPHAEROMA* INTO DILUTED INACTIVE SEA-WATER SOLUTIONS AND INTO DISTILLED WATER OR SUCROSE SOLUTIONS ISOTONIC WITH THE SEA WATER

Sea water (approx. %)	Mean ^{137}Cs loss (%/h)	Other medium	Mean ^{137}Cs loss (%/h)
100	3·3	Distilled water	0·34
	3·0	Sucrose solution	0·44
25	2·9	Distilled water	0·64
	2·2	Sucrose solution	0·42
5	1·6	Distilled water	0·48
	2·1	Sucrose solution	0·31
2·5	0·80	Distilled water	0·33
	0·67	Sucrose solution	0·19

If the results in Table 7 are compared with the results for ^{42}K in Table 3 it can be seen that in the first place both isotopes are lost more slowly in the lower concentrations of sea water. In the salt-free media both isotopes are lost more slowly and the reduction in loss is greatest in animals from the highest concentrations of sea water. This does suggest that there may be an exchange diffusion component of ^{137}Cs exchange, comparable with that for ^{42}K, which increases as the external sea-water concentration is raised. Losses of both isotopes in distilled water and in sucrose solutions isotonic with the sea-water concentration are not sufficiently different to suggest that in distilled water a significant amount of the isotope is lost in the extra urine which might particularly be expected in animals from 25 and 100% sea water.

In *Potamopyrgus* experiments of the same type have been carried out in animals acclimatized to 0·1, 1, 5 and 25% sea water. At each concentration 10 animals which had been pre-loaded with ^{137}Cs for 144 h were allowed to lose the isotope for three periods of 30 min in 5 ml. lots of inactive sea-water medium. The group was then split into two and the loss of isotope was followed in 2·5 ml. lots of either distilled water or sucrose solution for three periods of 30 min. Finally, the animals were all placed in 5 ml. lots of inactive sea-water medium for a further two periods. Table 8 gives a summary of the results. These suggest, first, that the isotope is lost more slowly in 25% sea water than in 5 or 1% sea water but more rapidly than in 0·1% sea water. In distilled water or sucrose solution the loss of ^{137}Cs is considerably reduced particularly in animals acclimatized to 5% sea water. The effect of these

salt-free media becomes progressively less in 1 and 0·1 % sea water as might be expected if there is an exchange diffusion component. However, results in animals from 25 % sea water are not what would be expected on this principle.

Results in Table 8 are less easily compared with those for ^{42}K in Table 4 than is the case in *Sphaeroma*. The relatively slow initial loss of ^{137}Cs in 25 % sea water is an obvious difference. There are other differences particularly with regard to the relative effects of the salt-free media on animals from the different concentrations of sea water. One can only speculate about the reasons for these variations, particularly because the relative amounts of the isotopes which lie in the blood and tissues and their exchangeabilities between

TABLE 8. LOSS OF ^{137}Cs FROM *POTAMOPYRGUS* INTO DILUTED INACTIVE SEA-WATER SOLUTIONS AND INTO DISTILLED WATER OR SUCROSE SOLUTIONS ISOTONIC WITH THE SEA WATER

Sea water (approx. %)	Mean ^{137}Cs loss (%/h)	Other medium	Mean ^{137}Cs loss (%/h)
25	6·6	Distilled water	1·41
		Sucrose solution	0·83
5	10·3	Distilled water	0·44
		Sucrose solution	0·30
1	7·0	Distilled water	1·00
		Sucrose solution	1·16
0·1	2·1	Distilled water	1·10
		Sucrose solution	1·25

the blood and tissues are not known. These factors are bound to affect a measurement which is expressed as a percentage of the total activity of the animal. In *Potamopyrgus* there is not much evidence as to the role of the excretory organs in the loss of ^{137}Cs, although the greater loss of isotope in distilled water than in sucrose solution by animals from 25 % sea water would suggest that there may be some effect.

In a further experiment *Potamopyrgus* were first allowed to absorb ^{137}Cs in 0·1, 0·25, 1, 5, and 25 % sea water for 500 h by which time equilibrium should have been approached. The loss of ^{137}Cs from these animals into inactive sea-water solutions was then followed for about 1000 h. Fig 7A shows that the isotope is initially lost more rapidly in 0·25, 1 and 5 % sea water than in 25 and 0·1 %. This confirms the findings of the previous section with regard to the relatively slow initial loss in 25 % sea water. The semi-logarithmic plot of the results in Fig. 7B shows that the rapid initial losses are followed by slower phases which probably correspond to losses from the less permeable tissues. During the slower phases the isotope is lost most slowly into 0·1 % sea water and most rapidly into 25 % sea water. Thus, if the loss of ^{137}Cs is considered as a whole the isotope is lost more slowly as the concentration of sea water is lowered.

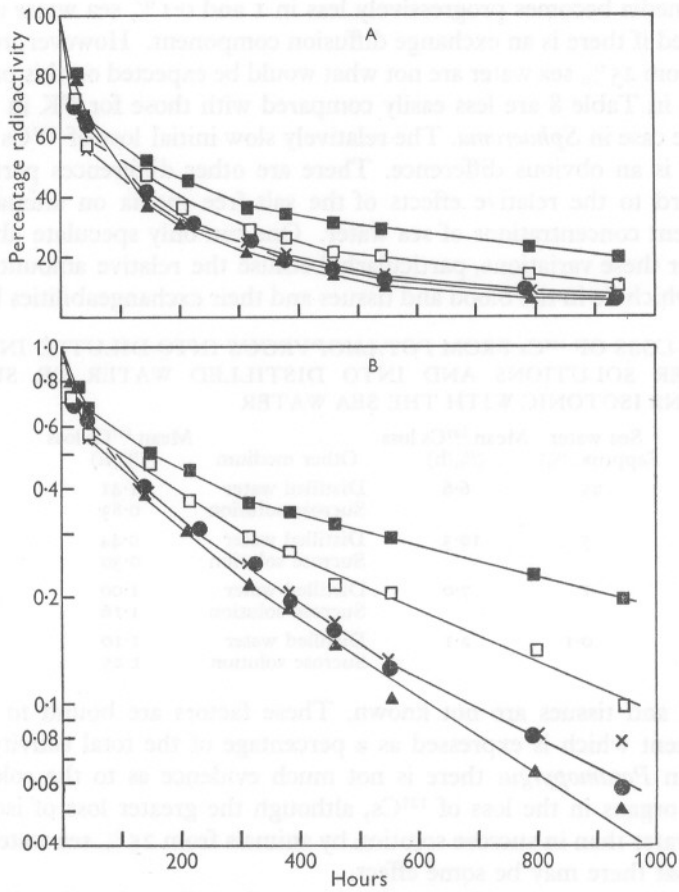

Fig. 7. A. Loss of ^{137}Cs from *Potamopyrgus* into inactive sea-water solutions after an uptake period of 500 h. ■, 0·1 %; □, 0·25 %; ×, 1 %; ●, 5 %; ▲, 25 % sea water. B. Semi-logarithmic plot of results from Fig. 7A.

DISCUSSION

It will be most convenient to consider first the main features of the regulation of K and Na by *Sphaeroma* and *Potamopyrgus* and to compare the results with those found by other workers.

Unfed *Sphaeroma hookeri* can survive for more than 500 h in sea water as dilute as 2·5 % and can live for shorter periods in lower dilutions. In distilled water, Na in particular is lost rapidly from the blood and from the whole animal while K is lost more slowly. As a result many animals die after about 16 h in distilled water. Salt movements are sufficiently rapid for *Sphaeroma* from 100 % sea water to become acclimatized to 5 % sea water in about 10 h.

In 100% sea water the Na level of the blood is similar to that of the medium and the K level is about 1·5 times that of the medium. In diluted sea water both Na and K concentrations of the blood fall, particularly between 100 and 25% sea water, but the blood/medium ratios for both ions are increased. Between 25 and 2·5% sea water blood Na and K levels fall less markedly. By the time 2·5% sea water is reached the blood Na concentration is about two-fifths and the blood K concentration is about one-half the value in 100% sea water. Under the same conditions the whole animal Na concentration is reduced by a similar fraction but the body K concentration falls by less than one-half. This suggests that the tissue/blood ratios for K may be increased in diluted media while the ratios for Na remain constant. A relationship of this type between muscle and blood has been found for *Carcinus maenas* in diluted sea water by Shaw (1955). At external concentrations below 2·5% sea water some animals cannot survive for very long and below 1% sea water most animals cannot survive. It is at these external concentrations that the mechanism for Na uptake probably reaches peak effectiveness, for at lower concentrations the blood Na level begins to fall markedly.

Previous studies on the osmotic and ionic regulation of isopod crustacea have been made by Parry (1953), Lockwood & Croghan (1957), Riegel (1959) and Lockwood (1959). Tolerance of *Sphaeroma hookeri* to diluted sea water probably corresponds most closely with that found in brackish water forms of *Mesidotea entomon* by Lockwood & Croghan (1957) or by Riegel (1959) in brackish water forms of *Gnorimosphaeroma oregonensis*. However, this latter species can live in fresh water for some time. The most detailed work on ionic and osmotic regulation in small brackish water crustacea has been carried out in the amphipod *Gammarus duebeni* by Shaw & Sutcliffe (1961) and Lockwood (1961). This species can also be acclimatized to fresh water, but it is possible that its regulatory mechanisms are similar to those of *Sphaeroma hookeri*. Although *Gammarus duebeni* is essentially a brackish water species it produces urine which is hypotonic to the blood in the more dilute media (Lockwood, 1961).

Potamopyrgus jenkinsi, unlike *Sphaeroma*, does not live well in 100% sea water, but can survive a dilution of 0·1% sea water almost indefinitely. In distilled water *Potamopyrgus* loses both Na and K more rapidly than *Sphaeroma* in the early stages. It can survive longer than *Sphaeroma* because in the later stages the loss of ions appears to be slowed down. The ability of the animal to withdraw into the shell must play some part in this but an active reduction of salt losses cannot be discounted. As in *Sphaeroma*, *Potamopyrgus* can be acclimatized to a new medium in less than 10 h. The levels of Na and K maintained in *Potamopyrgus* fall when animals from 100% sea water are acclimatized to more dilute media. As the sea water is diluted the whole body Na concentration falls proportionally until 25% sea water is reached. Below this concentration the body Na level still falls but active uptake of Na into the

blood probably begins. The body Na concentration falls only slightly between 1 and 0·1 % sea water presumably as a result of the effectiveness of the uptake mechanism. However, by this time the body Na level is only about one-tenth of that in 100 % sea water. The osmotic pressures of the blood and medium are likely to be equal in 100 % sea water and equivalent to an NaCl concentration of about 550 mM/l. It is possible that the body Na concentration may reflect the blood osmotic pressure, as Na is likely to be a large contributing factor. On this principle the blood osmotic pressure in 0·1 % sea water would be equivalent to about 55 mM/l. of NaCl. Neumann (1960) has shown that in the brackish water gastropod *Theodoxus fluviatilis* the blood is almost isotonic with sea-water concentrations greater than 10 % and hypertonic in lower concentrations. In fresh water the blood osmotic pressure is equivalent to 44 mM/l. NaCl and Neumann compares this with the value of 73 mM/l. for the blood of *Limnea peregra* in fresh water found by Picken (1937). These figures suggest that the estimate which has been made of the osmotic pressure of the blood of *Potamopyrgus* in 0·1 % sea water may be reasonable. One might also expect that the urine of *Potamopyrgus* under these conditions is rather hypotonic to the blood as this is the situation which is found in *Limnea* (Picken, 1937), possibly in *Theodoxus* (Neumann, 1960) and in the lamellibranch *Anodonta* (Picken, 1937).

The body K concentration of *Potamopyrgus* falls gradually in diluted sea water so that in 0·1 % the K level is about two-fifths of that in 100 % sea water. If *Potamopyrgus* and *Sphaeroma* are compared at a sea-water concentration of 2·5 % then the body K level in the former is rather less than one-half the value in 100 % sea water, while in the latter the corresponding value is rather greater than one-half. In diluted sea water both species conserve K rather than Na. This is of significance from the point of view of ^{137}Cs accumulation because Cs behaves more like K than Na.

In both *Sphaeroma* and *Potamopyrgus* all the body K appears to be exchangeable with ^{42}K. Exchange of the isotope is a slower process in animals acclimatized to more dilute sea-water solutions. This occurs in the different media even if the body K concentrations of the animals are quite similar as was found for *Sphaeroma* over the range 1–25 % sea water. Except in the highest external sea-water concentrations the uptake curves for ^{42}K in both species are almost exponential. *Sphaeroma* behaves like a single compartment in 5 % sea water because the exchange of ^{42}K between the blood and tissues is so rapid that the body surface alone appears to limit the rate of uptake. This probably explains why exponential curves are obtained for ^{42}K uptake under other conditions by both *Sphaeroma* and *Potamopyrgus*.

Uptake curves for ^{137}Cs are not, in most cases, exponential. If the times taken to reach 50 % of the equilibrium concentration factors in different sea-water concentrations are compared with each other and with results for ^{42}K, it is found that ^{137}Cs is absorbed far more slowly than ^{42}K and that the

slower exchange of ^{137}Cs in the more dilute media is not so marked as it is with ^{42}K. This is particularly true in *Potamopyrgus*. Results for *Sphaeroma* suggest that, whereas with ^{42}K the body surface is the limiting factor in uptake, with ^{137}Cs tissues such as perhaps muscle may become more limiting. The limiting exchange between the blood and tissues might thus impose an appearance of uniformity on the uptake curves from the point of view of the times taken to reach 50% of the equilibrium concentration factors.

In short-term experiments concerned with the initial loss of ^{42}K and ^{137}Cs into inactive sea-water concentrations by both species it is found that, with one exception (the loss of ^{137}Cs by *Potamopyrgus* in 25% sea water), the loss of isotope is lower in the more dilute media. These short experiments should give an indication of the rate of exchange between the blood and the medium. Apart from the one exception the behaviour of ^{137}Cs under these conditions appears to parallel that of ^{42}K. In distilled water or in sucrose solution isotonic with the sea water the loss of both isotopes is curtailed in a manner which is compatible with the idea that at the body surface an exchange diffusion component of exchange exists. The component causes the increased exchange of the isotopes between the blood and medium in the higher sea-water concentrations and decreases as the sea water is diluted. This explanation, which discounts any electrical effects and is no doubt far too simple, avoids, partially at least, the necessity to base a theory on large changes in the permeability of the body surface to isotope losses by simple diffusion under the different conditions. Also, there is no evidence to suggest that losses in the urine would be sufficient to account for the different rates of exchange.

From the point of view of any hazard which might be created by the absorption of ^{137}Cs, the actual equilibrium concentration factors which can be attained by animals acclimatized to different sea-water concentrations are important. Concentration factors which are maintained for inactive Na, K and for ^{137}Cs at equilibrium are summarized for both species in Fig. 8A, B. In *Sphaeroma*, ^{137}Cs is accumulated to concentration factors which are approximately double those for inactive K over a sea-water concentration range of 2·5 to 100%. In 1% sea water the animals died before ^{137}Cs equilibrium could be reached. It has been shown that in 5% sea water ^{137}Cs is accumulated by the blood to a slightly higher concentration factor than is maintained for inactive K. This means that the tissue/blood ratios for ^{137}Cs must markedly exceed those for inactive K. It is presumed that this applies to *Sphaeroma* acclimatized to other sea-water concentrations as well. The maximum equilibrium concentration factors which can be expected for ^{137}Cs in *Sphaeroma* are of the order of 2–300 in 2·5% sea water.

Equilibrium concentration factors for ^{137}Cs in *Potamopyrgus* exceed those for inactive K in 100, 25 and 5% sea water. As the sea water is diluted further so *Potamopyrgus* becomes less able to absorb ^{137}Cs to concentration factors approaching those for inactive K. In 0·1% sea water ^{137}Cs concentration

Fig. 8. A. A comparison of equilibrium concentration factors for ^{137}Cs, K and Na maintained by whole *Sphaeroma* in different concentrations of sea water. B. A comparison of equilibrium concentration factors for ^{137}Cs, K and Na maintained by whole *Potamopyrgus* (including shell) in different concentrations of sea water.

factors of about 300 are reached compared with about 1000 for inactive K. These values are for animals with a shell which absorbs very little of the isotope and contains very little inactive K. Without the shell, which accounts for about two-thirds of the weight of the animal, the ^{137}Cs concentration factor for the soft parts would be of the order of 1000. Freshwater crayfish in 0·1% sea water are unable to accumulate ^{137}Cs to a factor approaching that for inactive K (Bryan & Ward, 1962). This is because ^{137}Cs cannot reach a concentration factor in the blood which approaches that for inactive K. *Potamopyrgus* probably behaves similarly in the more dilute media. Thus, from the point of view of ability to accumulate ^{137}Cs, *Potamopyrgus* appears to behave like a freshwater animal in very dilute media and like a marine animal in 100% sea water.

The results can now be summarized. Brackish water species are able to maintain relatively high internal concentrations of Na and particularly K in diluted sea water. This results in an increase in the inactive K concentration factor as the medium is diluted. It has been shown with ^{42}K that the rate of exchange of K is decreased as the sea water is diluted so that radioactive equilibrium is reached more slowly. The more rapid exchange at higher sea-water concentrations may be the result of the presence of an exchange diffusion component which increases with the sea-water concentration. When ^{137}Cs is introduced into the system it is generally absorbed more slowly than ^{42}K but by a similar process. In the isopod *Sphaeroma*, blood/medium and tissue/blood ratios for ^{137}Cs at equilibrium come to exceed those for K so that whole animal ^{137}Cs concentration factors are about double the values for K. Maximum values of 2–300 can be reached by unfed animals in 2·5% sea water. In the mollusc *Potamopyrgus* similar behaviour is found in higher sea-water concentrations but at lower concentrations blood/medium ratios for ^{137}Cs are probably unable to approach those for K and thus whole animal concentration factors for ^{137}Cs do not reach the K values. Even so the soft body of the animal may attain a ^{137}Cs concentration factor of 1000 in 0·1% sea water.

In accumulating ^{137}Cs to high levels in diluted media brackish water species are potentially more hazardous than marine animals and this would be one reason for avoiding the introduction of radioactive wastes containing ^{137}Cs into media of low salinity.

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SUMMARY

The relationship between the ability of brackish water invertebrates to regulate Na and K and the extent to which the radioactive fission product ^{137}Cs can be accumulated has been studied.

The brackish water isopod *Sphaeroma hookeri* and the gastropod *Potamopyrgus jenkinsi* have been acclimatised to a wide range of sea-water dilutions. Unfed *Sphaeroma* can survive in sea-water concentrations of 100–2·5%, while *Potamopyrgus* can live fairly indefinitely in concentrations of 50–0·1%. Measurements of Na and K in the whole animals of both species and in the blood of *Sphaeroma* have been made. Salt movements are quite rapid and acclimatization to new media is achieved by both species in less than 10 h. Concentration factors for inactive K in particular increase to high values in the more dilute media.

Uptake of the isotopes ^{42}K and ^{137}Cs from solution has been examined in both species over a range of sea-water concentrations. All of the body K is exchangeable with ^{42}K and in *Sphaeroma* exchange of ^{42}K between the blood and tissues is so rapid that the body surface appears to be the limiting factor in the uptake of the isotope. Both species exchange ^{42}K more rapidly in the higher concentrations of sea water and one reason for this may be the existence of an exchange diffusion component of exchange which increases as the salinity of the medium is raised. Indirect evidence suggests that the excretion of ^{42}K in urine is probably not an important factor in exchange.

The behaviour of ^{137}Cs generally resembles that of ^{42}K although certain of the tissues rather than the body surface are probably the limiting factor in uptake which is slower. In *Sphaeroma*, ^{137}Cs equilibrium concentration factors for whole animals are roughly double the values for inactive K as a result of blood/medium and tissue/blood ratios for ^{137}Cs exceeding those for inactive K. Equilibrium ^{137}Cs concentration factors in *Sphaeroma* vary from about 7 in 100% to 2–300 in 2·5% sea water. *Potamopyrgus* attains higher concentration factors for ^{137}Cs than inactive K in 100, 25 and 5% sea water but at lower concentrations the factors are below those for inactive K. In 100% sea water *Potamopyrgus* reaches a ^{137}Cs concentration factor of about 3 while in 0·1% sea water a factor of about 300 is reached.

In accumulating ^{137}Cs to high levels in dilute media brackish water species are potentially much more hazardous than marine species.

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