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POTASSIUM METABOLISM AND THE ACCUMULATION OF ^{137}CAESIUM BY DECAPOD CRUSTACEA

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(Text-figs. 1–16)

Radioactive wastes are now present in both the sea and in fresh water. Although the actual concentrations of these wastes are usually extremely low, some of the waste elements can be concentrated to a remarkable degree by plants and animals. One of the most important is the fission product ^{137}Cs because it has a half-life of 30 years. The accumulation of ^{134}Cs has already been studied in some marine crabs where it is generally treated in a comparable way to potassium (Bryan, 1961). In *Carcinus* ^{134}Cs is accumulated far more slowly than the isotope ^{42}K. At equilibrium, however, a whole-animal concentration factor of about 8 is reached for ^{134}Cs as opposed to about 4 for ^{42}K. This is due to ^{134}Cs being concentrated to a slightly greater degree than K in the blood and to about twice the extent of K in the tissues. Under conditions where the K concentration of sea water is halved the tissue concentrations of K remain fairly normal and the concentration factor for ^{134}Cs is approximately doubled. In a freshwater animal the blood K concentration might be 500 times that of the medium, while this figure might reach 10,000 in the tissues. Should ^{137}Cs be concentrated to a greater degree than K under these conditions then an animal from water containing very little ^{137}Cs could constitute a hazard either by being eaten directly or through food chains.

A comparative study has been made of the accumulation and loss of ^{137}Cs in relation to K metabolism in the lobster *Homarus vulgaris* Milne Edwards, the common prawn *Palaemon serratus* (Pennant), and in the freshwater crayfish *Astacus* (= *Austropotamobius pallipes pallipes* (Lereboullet)).

MATERIALS AND METHODS

Most of the prawns were obtained in the Plymouth area, although a few experiments were carried out with animals from Conway. Lobsters were supplied through the Torry Research Station, Aberdeen, by J. Burgon and Co., Eyemouth. Crayfish were from the Freshwater Biological Association, Ambleside, and L. Haig and Co. Ltd.,

Beam Brook, Newdigate, Dorking, Surrey. The isotopes ^{137}Cs, ^{134}Cs and ^{42}K were from the Radiochemical Centre, Amersham.

Experiments were carried out in partitioned glass tanks and the solution was aerated. Most of the media which were used were prepared from filtered sea water (Cl‰ = *ca.* 19) from Plymouth or Whitehaven. In a few cases artificial sea water (Cl‰ = 19) had to be used. The work was carried out in constant temperature rooms at 11–12° C except where otherwise stated.

The activity of whole animals was usually measured with a ring of γ-sensitive GM tubes (20th-Century type G.10 Pb or G.12), but for low activities in small animals well-type or end-window γ-scintillation counters were used. The activities of whole blood, plasma, and urine were determined in samples dried on to aluminium planchets with either an end-window GM tube (Mullard type MX 123) or with the end-window γ-scintillation counter. Liquid tissue samples were counted with either the end-window γ-scintillation counter or with the well-type γ-scintillation counter.

Large blood samples were taken with a glass pipette from the ventral region of the cephalothorax in all three species. Small blood samples were taken from a leg. In prawns, whole blood samples and centrifuged samples were both used. When the K concentration of whole blood and centrifuged blood was compared in the same animals there was very little difference. Whole blood samples from lobsters were compared with the clear plasma produced by removing coagulated material with a stirring rod. In crayfish, clear plasma samples were produced by centrifuging whole blood. Urine was taken from all three species using pipettes of drawn-out glass tubing which were operated by a 'Pumpette' pipette control. In each case a flow of urine was produced by touching the tip of the renal papilla and gently squeezing the animal. The method was checked in prawns by collecting some urine samples which had become bright blue in colour following a small injection of methyl green into the blood. Tissue samples for the estimation of ^{137}Cs and K were treated in the way described by Bryan (1961). Muscle was taken from the abdomen of each species and samples of the shell from the pericardial region of the carapace. Shell samples were scraped clean on the inner surface.

An 'EEL' flame photometer was used for the estimation of K and Na in liquid-tissue samples and in diluted samples of whole blood, plasma and urine. As the very smallest flame photometer sample which could be used was 1 ml., one measurement only could be made on many samples of urine from prawns and crayfish so that the accuracy could not be very high. Sometimes K and Na measurements were made on samples which had been dissolved from planchets following the estimation of ^{137}Cs. Measurements of ^{137}Cs and K in the excretory organs of prawns were made on dried and redissolved planchet samples.

Inulin measurements were made by using the method of Young & Raisz (1952) which was employed by Flemister (1958). When ^{137}Cs was also involved in these experiments, the 1 ml. of filtrate used for inulin estimation was first counted in the well-type γ-scintillation counter.

Many of the results are plotted as concentration factors against time. The concentration factor is the level of radioactivity or the inactive K concentration per kg wet weight of whole animal or tissue divided by the corresponding value per kg of medium. In loss experiments in inactive solutions results are expressed as concentration factors relative to the activity of the medium during uptake.

LOBSTER EXPERIMENTS

Robertson (1949) has shown that generally the plasma of *Homarus* contains less K than sea water and that the K concentration of the urine is rather less than that of the plasma. With Na the plasma concentration is greater than that of the sea water and equal to that of the urine.

In the present experiments lobsters of both sexes were used and they usually weighed about 500 g.

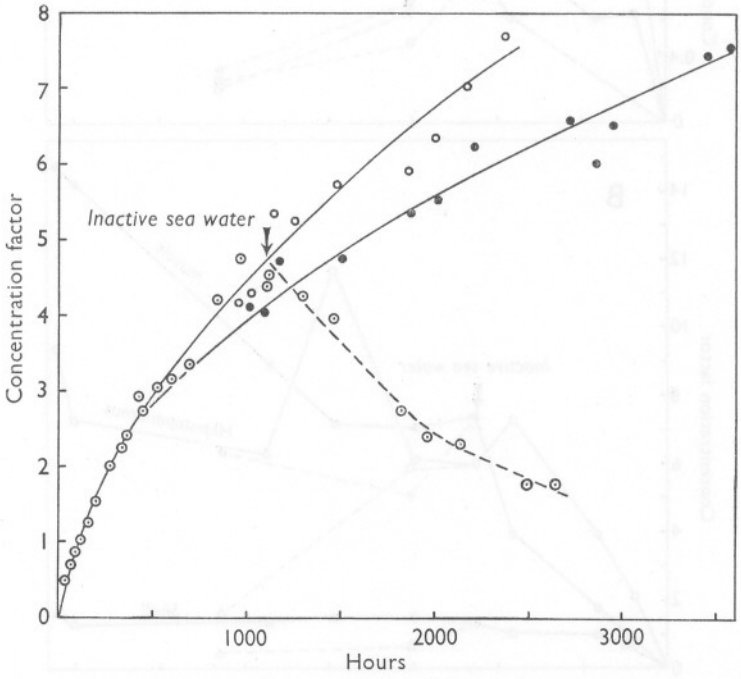


Fig. 1. Uptake of ^{137}Cs from sea water by three whole lobsters followed by the loss of ^{137}Cs into inactive sea water in one of them. Earlier points have been left out of two uptake curves as they almost coincide with points for the third animal.

Uptake of ^{137}Cs *by whole lobsters*

Uptake by whole animals was followed in filtered Whitehaven sea water containing 5 μc/l. of ^{137}Cs. Curves for three animals are shown in Fig. 1. In no case did equilibrium appear to have been reached although the experiments lasted for up to 3600 h. These animals were not fed, and over 2680 h a female showed a fall in weight of about 2%.

Uptake of ^{137}Cs *by blood and tissues*

The isotope is taken up by the blood so that the highest concentration factor is reached at about 2000 h, after which there may have been a decline

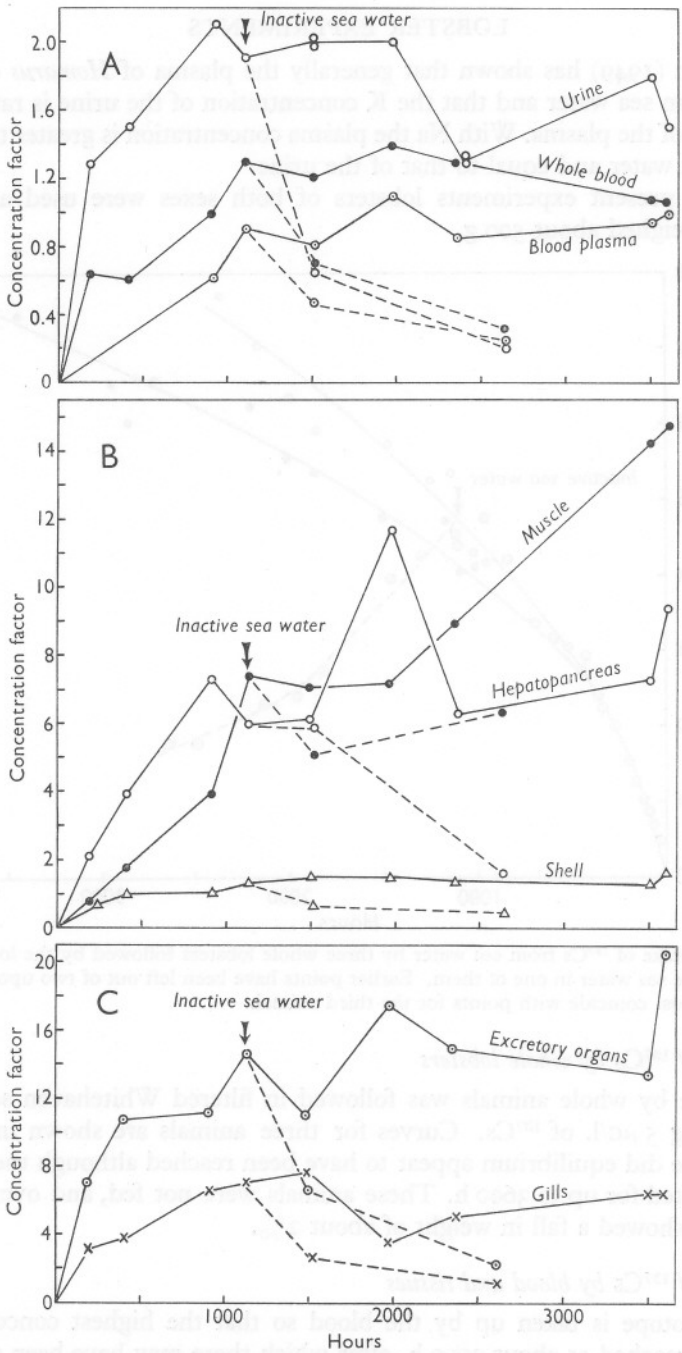


Fig. 2A–C. Uptake of ^{137}Cs by whole blood, plasma, urine, and tissues of lobsters in sea water. Also, loss of ^{137}Cs in inactive sea water shown by broken line. Results at each time interval are from analyses of single animals.

due to starvation (Figs. 2A and 4). A mean of eight measurements showed that the activity of the plasma is about 76% of that of an equal weight of blood and after 2000 h its activity is roughly equal to that of the sea water (Fig. 2A).

TABLE 1. PERCENTAGE BY WEIGHT OF TISSUES IN WHOLE ANIMALS

Tissue	Lobster, ♂, 397 g	Prawn, ♀, 2·72 g	Crayfish (Windermere), ♂, 2·79 g
Skeleton (including epidermis)	43·0	29·7	42·7
Muscle (including heart)	28·7	48·4	16·7
Hepatopancreas	3·3	2·1	3·9
Gut	1·3	0·6	2·9
Gills	1·4	0·6	0·8
Reproductive system	0·3	0·3	0·6
Excretory organs	0·2	0·1	0·3
Fluid (mainly blood and urine)*	21·8	18·2	32·1

* Amount of fluid calculated by difference.

TABLE 2. TISSUE/PLASMA RATIOS IN A LOBSTER AFTER UPTAKE OF ^{137}Cs FOR 3600 H, AND COMPARATIVE RATIOS FOR LOSS IN INACTIVE SEA WATER

Results calculated on a water content basis except the concentration factors of ^{137}Cs.

	Analyses after 3600 h of uptake (whole animal C.F. = 7·6)					^{137}Cs (tissue/plasma) ratios after		
				Ratio: tissue/plasma				
Tissue	H_2O (%)	K (mM/kg H_2O)	^{137}Cs C.F.	K	^{137}Cs	Uptake 1100 h	Loss 420 h	Loss 1560 h
Muscle	77·1	131	14·9	20·2	18·5	10·3	18·1	34·0
Hepatopancreas	78·3	119	9·4	18·3	11·5	8·1	24·0	9·0
Excretory organs	78·7	95	21·8	14·6	26·5	19·9	15·3	13·0
Gills	89·0	29	6·4	4·5	6·9	8·7	7·1	5·2
Shell	29·1	54	1·5	8·3	4·9	4·6	5·8	8·0
Plasma	92·1	6·5	1·0	—	—	—	—	—
Ratio: plasma/sea water				0·59	1·04	0·94	0·44	0·25

Uptake of ^{137}Cs by the tissues is given in Fig. 2B, C. The hepatopancreas, excretory organs, gills, and shell take up the isotope sufficiently rapidly for the tissue/plasma ratios to be roughly constant at all times. However, the abdominal muscle takes up ^{137}Cs so slowly that the tissue/plasma ratio rises throughout the experiment. As a result of this, muscle is the principal limiting factor in the attainment of equilibrium by a whole animal. The importance of muscle is shown in Table 1 where the percentages by weight of the different organs are given.

The inactive K concentrations of whole blood, plasma, and tissues have been determined in animals used for the uptake of ^{137}Cs. As with ^{137}Cs the mean K level of the plasma is about 76% that of whole blood. Unlike ^{137}Cs the plasma K concentration is nearly always considerably lower than that of sea water.

This is shown in Table 5 and also in Table 2 where results are given for K and ^{137}Cs analyses of plasma and tissues in an animal exposed to ^{137}Cs for 3600 h. The tissue K concentrations are of the usual magnitude found in marine decapod crustacea with the result that the low plasma K level gives higher tissue/plasma ratios than were found in *Carcinus* (see Bryan, 1961). Although equilibrium has obviously not been reached in muscle (Fig. 2B) the ^{137}Cs tissue/plasma ratio at 3600 h has nearly reached that for K (Table 2). Unlike *Carcinus* the ^{137}Cs tissue/plasma ratio for hepatopancreas, at what appears to be equilibrium, is markedly lower than that for K and the same is true of the shell. Like *Carcinus* the ^{137}Cs ratios for the excretory organs and gills exceed those for inactive K.

Loss of ^{137}Cs *from blood and tissues*

Two animals were allowed to lose ^{137}Cs into inactive sea water after absorbing the isotope for 1100 h. The inactive sea water was changed at intervals to prevent the concentration of ^{137}Cs from building up externally. Results for the loss from one of these whole animals are given in Fig. 1. Loss from the whole animal appears to be a slower process than uptake, probably as a result of the reabsorption of ^{137}Cs lost from the tissues before it could be lost from the blood into the sea water. The ^{137}Cs levels in whole blood, plasma, and tissues during loss are shown in Fig. 2A–C. Tissue/plasma ratios for ^{137}Cs at 1100 h and after loss for 420 and 1560 h are given in Table 2. After 1560 h of loss the tissue/plasma ratios for hepatopancreas, excretory organs and gills are similar to or lower than those found after 1100 and 3600 h of accumulation, while the ratios for muscle and shell are higher. Similar ratios would be expected if exchange of ^{137}Cs between blood and tissues is relatively rapid compared with the rate at which the activity of the blood is changing. Higher ratios would be expected if the fall in the activity of the blood is the more rapid process, and if equilibrium between the tissue and the blood has not been fairly closely approached during uptake. After 420 h loss the hepatopancreas shows an increased ratio, but at 1560 h it is similar to that for uptake animals. With loss of ^{137}Cs the excretory organs show a falling ratio which may well be associated with the drop in the activity of the urine relative to that of the blood (Fig 2A). The ratio is lowest for the gills at 1560 h but the direct contact with inactive sea water could affect this. Increased ratios for the muscle and for the shell show that these tissues lose the isotope relatively slowly when it is lost from the blood. This confirms the importance of muscle as a limiting factor in ^{137}Cs exchange.

Uptake of ^{42}K

The uptake of ^{42}K from filtered Whitehaven sea water was studied in four animals. Whole-animal uptake was followed in one (Fig. 3A) and tissues of the other three were analysed for ^{42}K and inactive K at intervals over the same

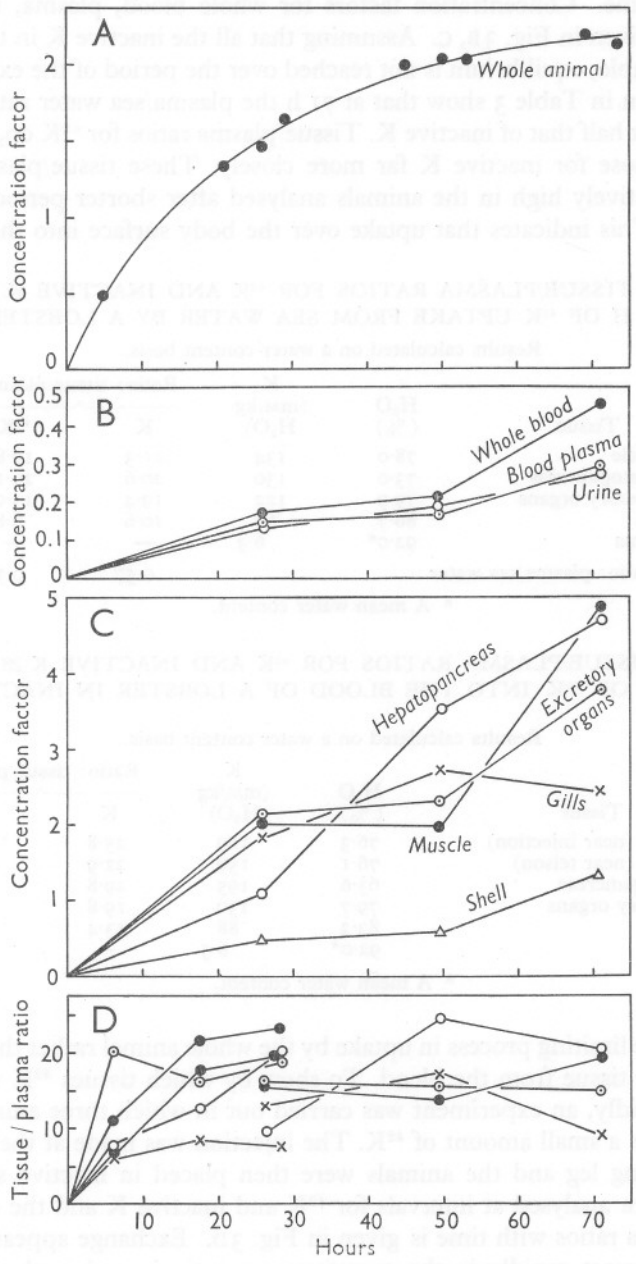


Fig. 3. A. Uptake of ^{42}K from sea water by whole lobster. B, C. Uptake of ^{42}K by whole blood, plasma, urine and tissues of lobsters in sea water. Results at each time interval are from analyses of single animals. D. Tissue/plasma ratios for ^{42}K from results in B, C (on right). Also, D, changes in the tissue/plasma ratios following the injection of ^{42}K into three lobsters in inactive sea water (on left). All ratios calculated on a water content basis with symbols as for C.

period of time. Concentration factors for whole blood, plasma, urine and tissues are given in Fig. 3B, C. Assuming that all the inactive K in the animal is exchangeable, equilibrium is not reached over the period of the experiment. Results given in Table 3 show that at 71 h the plasma/sea water ratio for ^{42}K is only about half that of inactive K. Tissue/plasma ratios for ^{42}K do, however, approach those for inactive K far more closely. These tissue/plasma ratios are also relatively high in the animals analysed after shorter periods of time (Fig. 3D). This indicates that uptake over the body surface into the blood is

TABLE 3. TISSUE/PLASMA RATIOS FOR ^{42}K AND INACTIVE K AFTER 71 H OF ^{42}K UPTAKE FROM SEA WATER BY A LOBSTER

Results calculated on a water content basis.

Tissue	H_2O (%)	K (mM/kg H_2O)	Ratio: tissue/plasma K	Ratio: tissue/plasma ^{42}K
Muscle	78·0	134	21·3	19·8
Hepatopancreas	73·0	130	20·6	20·1
Excretory organs	79·9	122	19·4	15·0
Gills	86·7	67	10·6	8·8
Plasma	92·0*	6·3	—	—
Ratio: plasma/sea water			0·57	0·3

* A mean water content.

TABLE 4. TISSUE/PLASMA RATIOS FOR ^{42}K AND INACTIVE K 28 H AFTER INJECTION OF ^{42}K INTO THE BLOOD OF A LOBSTER IN INACTIVE SEA WATER

Results calculated on a water content basis.

Tissue	H_2O (%)	K (mM/kg H_2O)	Ratio: tissue/plasma K	Ratio: tissue/plasma ^{42}K
Muscle (near injection)	76·3	169	25·8	19·7
Muscle (near telson)	76·1	150	22·9	23·3
Hepatopancreas	63·6	195	29·8	19·2
Excretory organs	79·7	130	19·8	18·9
Gills	83·2	88	13·4	7·3
Plasma	92·0*	6·5	—	—

* A mean water content.

probably the limiting process in uptake by the whole animal rather than uptake by a specific tissue from the blood. To show by which tissues ^{42}K was taken up most rapidly, an experiment was carried out in which three animals were injected with a small amount of ^{42}K. The injection was made at the base of a fourth walking leg and the animals were then placed in inactive sea water. Animals were analysed at intervals for ^{42}K and inactive K and the change in tissue/plasma ratios with time is given in Fig. 3D. Exchange appears to have taken place most rapidly in the excretory organ tissue and in the gills, but more slowly in the hepatopancreas and muscle. The upper muscle curve is for abdominal muscle taken near the site of injection while the lower curve is for

muscle taken near the telson. Table 4 shows that by 28 h the ^{42}K ratios approach those for inactive K most closely in the excretory organ and in telson muscle while exchange is less complete in hepatopancreas and the second muscle sample. The gill ^{42}K ratio is markedly lower than that for inactive K due presumably to the animal being exposed to inactive sea water.

One of the major differences between the uptake of ^{42}K and ^{137}Cs by tissues from the blood is that ^{42}K is exchanged between the plasma and both hepatopancreas and muscle at fairly similar rates while ^{137}Cs is taken up very much more slowly by muscle than by hepatopancreas. Tissue K concentrations of animals after prolonged uptake of ^{137}Cs without feeding (Table 2) are generally lower than those from short ^{42}K experiments (Tables 3 and 4). As a result it is likely that if animals had been fed on inactive food during the ^{137}Cs experiments slightly higher tissue activities might have been reached.

Excretion of ^{137}Cs, K *and* Na

In three animals continuous measurements of ^{137}Cs were made in urine from one excretory organ and in small samples of whole blood taken from a plugged hole in the pericardial region of the carapace. Results for one of these animals are given in Fig. 4. Up to 3000 h the urine concentration factors are on average $2{\cdot}1 \pm 0{\cdot}3$ times the corresponding blood values. This would be equivalent to a urine/plasma ratio (U/P), calculated on a water content basis, of 2·6. In this particular experiment and in the others there was a tendency for the U/P ratio to fall after 1500 h. In *Carcinus* a very marked drop in the U/P ratio was found during long ^{137}Cs uptake experiments (Bryan, 1961) which did not appear to be wholly a result of prolonged starvation. However, in lobsters starvation may be the principal reason for this.

Table 5 shows the U/P and plasma/sea water ratios for ^{137}Cs, ^{42}K, K and Na from animals in which complete analyses were carried out. The Na concentration of the plasma is usually rather greater than that of sea water. The K concentration is in most cases well below that of sea water and the level of ^{137}Cs reaches that of sea water at 3500 h. There is no evidence that Na is controlled by the excretory organs because the U/P ratios are about 1·0. In the majority of animals the U/P ratios for K are also about 1·0, but there is some variation, and two ratios exceed 1·4. Even in animals where ratios for both Na and K are about 1·0 values for ^{137}Cs as high as 2·4 are found. When estimations are made in samples from both excretory organs similar U/P ratios for ^{137}Cs, K and Na are found in each. In the animal analysed at 3600 h the U/P ratios for ^{137}Cs and K are equal and this could be a starvation effect. The U/P ratio for ^{137}Cs in the animal which had been losing the isotope for 1560 h is lower than that for K. The tissue/plasma ratio for the excretory organs in this lobster is considerably lower than the ratios for the animals which had been accumulating the isotope for 1100 and 3600 h (Table 2) and which have U/P ratios for ^{137}Cs greater than 1·0. This seems to be countered by the

fact that the U/P ratio is high and the tissue/plasma ratio for the excretory organs is low in the lobster which had been losing ^{137}Cs for 420 h.

Burger (1957) found that in the lobster *Homarus americanus* urine K concentrations are usually several mM/l. lower than the corresponding blood

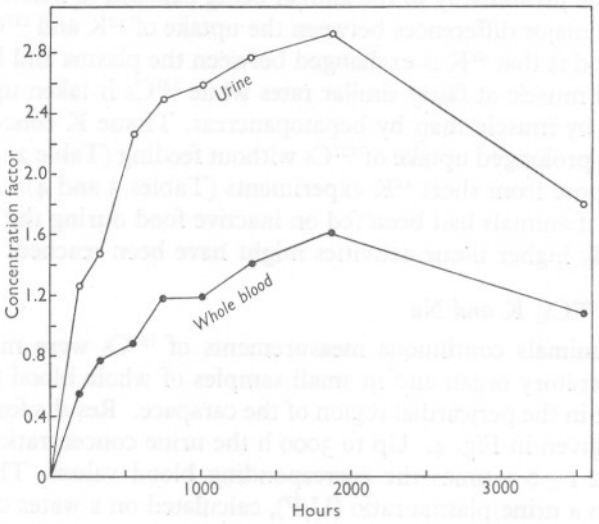


Fig. 4. Uptake of ^{137}Cs by whole blood and urine of a single lobster in sea water.

TABLE 5. CONCENTRATIONS OF Na, K, ^{137}Cs AND ^{42}K in PLASMA AND URINE OF LOBSTERS AS PLASMA/SEA WATER AND URINE/PLASMA RATIOS FOUND DURING EXPERIMENTS WITH ^{137}Cs AND ^{42}K IN WHITEHAVEN SEA WATER ($Cl‰ = 19$)

Ratios are calculated on a water content basis using a mean plasma water content of 92 %.

			Plasma			Urine		
			Ratio: plasma/sea water			Ratio: urine/plasma		
Expt.	Time (h)	H_2O (%)	Na	K	Isotope	Na	K	Isotope
^{137}Cs uptake	408	—	—	1·44	0·66	—	1·07	1·43
	1120	—	—	0·69	0·94	—	1·57	1·82
	1488	91·6	1·10	0·70	0·84	0·99	0·96	2·35
						0·98	0·91	2·40
	2350	94·1	1·08	0·64	0·87	0·97	1·01	1·42
						0·99	0·99	1·40
	3500	—	1·06	0·56	0·99	1·00	1·21	1·63
	3600	—	1·04	0·59	1·04	1·03	1·42	1·43
^{137}Cs loss after 1100 h uptake	420	90·6	1·07	0·72	0·44	0·98	1·02	1·41
	1560	—	1·11	0·79	0·25	0·96	0·90	0·84
^{42}K uptake	26	—	1·03	0·59	0·16	1·01	1·01	0·83
	49·5	—	1·05	0·55	0·18	1·02	1·02	1·01
	71	—	1·03	0·57	0·30	1·01	1·12	0·93
^{42}K injected	17·5	—	0·98	0·59	—	1·05	1·10	1·10
	28	—	1·02	0·63	—	1·05	1·05	1·04

values, which varied between 6 and 11 mM/l. As in *Homarus vulgaris*, the level of Na in the urine is similar to that in the plasma. Burger also found that after injection of inulin to a blood concentration of 1·4 to 17 mg%, the inulin U/P ratios were between 1·0 and 1·1. This result suggests that if the primary urine is produced by filtration of the blood then it is not subsequently modified to any great extent by water reabsorption. It is assumed that inulin is not absorbed or secreted by the cells of the excretory organs. If this is also true for *Homarus vulgaris* then although Na, K and ^{137}Cs may enter the urine as a plasma filtrate having a similar ionic composition to the plasma, later modifications of the urine with regard to ^{137}Cs must take the form of additional secretion by the cells of the excretory organs.

To test this, two large (*ca.* 1 kg) Plymouth lobsters were injected with 2 and 5 ml. of 5% inulin and the whole blood and urine concentrations were measured at intervals over 240 h. Initially the blood levels were about 26 and 65 mg% and they fell eventually to 9 and 19 mg%. The mean U/B ratios for inulin are given in Table 6. Ratios of about 1·5 indicate that water reabsorption could explain to some extent the high U/P ratios for ^{137}Cs given in Table 5. The U/P ratios for Na and K need not necessarily also be 1·5 because these ions move so much more rapidly that they could probably quickly regain equilibrium at a U/P ratio of 1·0. This would assume that water is perhaps first reabsorbed and that movements of Na and K might be needed to make the urine isotonic with the plasma. On the other hand, water movements might follow the reabsorption of ions. A further check was made by following the uptake of ^{137}Cs from sea water containing 20 μc/l. of isotope during the loss of inulin in two Eyemouth lobsters. In these experiments plasma samples were used. Graphs showing the loss of inulin during the uptake of ^{137}Cs are given for one of the animals in Fig. 5. Results for the other lobster were rather less variable. Table 6 shows that in both animals the mean inulin U/P ratios are about 1·17 while corresponding ^{137}Cs ratios are far greater. In these lobsters, water reabsorption by the excretory organs is so slight that the high U/P ratios for ^{137}Cs are most easily explained in terms of additional secretion of the isotope into the urine.

From a semi-logarithmic plot of curves for the loss of inulin from the blood with time, the inulin concentration at zero time can be found. Inulin spaces calculated from these results for the four lobsters are given in Table 6. Assuming that all the inulin is lost from the animals via the excretory organs it is possible to calculate a value for the rate of urine production. The inulin space and the amount of inulin lost from the blood in a given time are known, and from these figures the amount of blood filtrate produced in the excretory organs can be found (Potts, 1954). As the mean U/P ratios for inulin are known, the amount of urine actually expelled can be found. These results are also given in Table 6 and are comparable with the value of up to 5% of the body weight per day given by Burger (1957) for *Homarus americanus*.

In *Homarus vulgaris*, ^{137}Cs is initially taken up almost linearly at a rate sufficient to give a concentration factor of 1·0 in a whole animal by 100 h. It will be assumed that this is still the rate of influx when the animal approaches equilibrium where influx and outflux are equal. If urine equivalent to 4% of the body weight is produced per day with a concentration factor of 2·0 at equilibrium, then in 100 h ^{137}Cs equal to a whole animal concentration factor of 0·33 will be removed. The excretory organs will thus be responsible for 33% of the outflux at equilibrium. In the case of ^{42}K a whole animal concentration factor of 0·5 is initially reached in 5 h and urine with a concentration factor of 0·6 will be produced at equilibrium. Here, the excretory

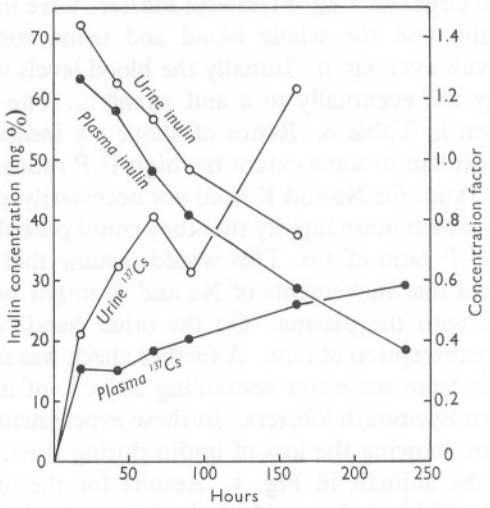


Fig. 5. Loss of inulin during the uptake of ^{137}Cs by plasma and urine in a 400 g Eyemouth lobster.

TABLE 6. INULIN SPACE, URINE PRODUCTION AND U/P OR U/B RATIOS FOR INULIN AND ^{137}Cs IN LOBSTERS

Ratios calculated on a water content basis.

Weight (g)	Sex	Temp. (° C)	Inulin space (% body weight)	Urine production (% body weight per day)	U/B and U/P ratios: No. of samples	Inulin	^{137}Cs
1102*	♂	16·5	35·3	2·8	6	1·51 ± 0·17	—
1180*	♀	16·5	33·2	2·7	6	1·50 ± 0·48	—
400	♀	11·5	36·3	3·9	5	1·18 ± 0·11	1·90 ± 0·43
335	♀	11·5	33·5	4·0	5	1·17 ± 0·07	2·98 ± 0·34

* The two larger animals are Plymouth lobsters and in these inulin was estimated in whole blood rather than in plasma samples.

organs are responsible for about 1% of ^{42}K losses at equilibrium. The excretory organs play an important role in removing ^{137}Cs from the animal during uptake, but not in removing ^{42}K. Plasma/sea water ratios reached for ^{137}Cs are about 1·0 despite selective removal of the isotope in the urine. The plasma K level is maintained below that of sea water but is not controlled by the excretory organs. This control may be a function of the gills. A mechanism excreting K across the gills might be less efficient in removing ^{137}Cs, and so higher plasma/sea water ratios would tend to be found for this ion.

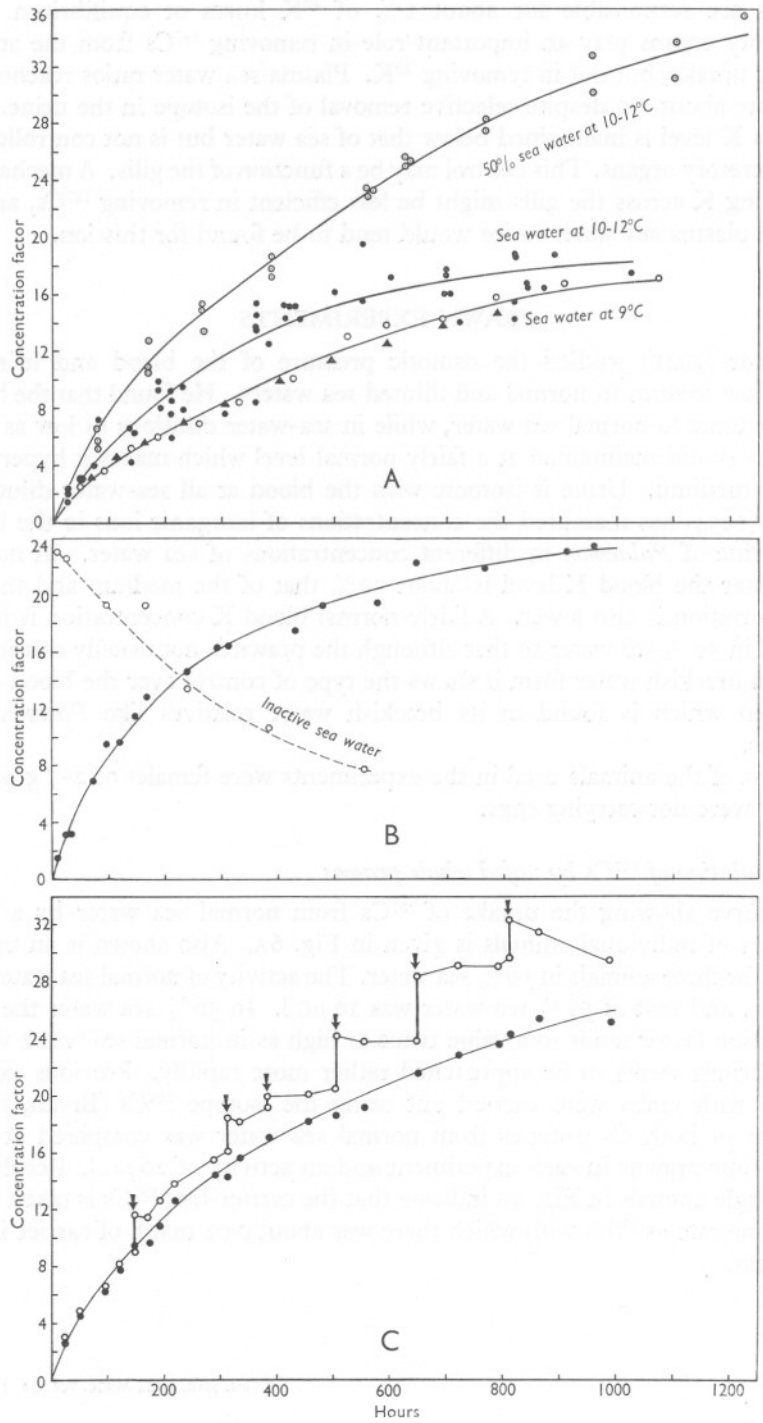
PRAWN EXPERIMENTS

Panikkar (1941) studied the osmotic pressure of the blood and urine in *Palaemon serratus* in normal and diluted sea waters. He found that the blood is hypotonic to normal sea water, while in sea-water dilutions as low as one-third it is still maintained at a fairly normal level which makes it hypertonic to the medium. Urine is isotonic with the blood at all sea-water dilutions. Parry (1954) has measured the concentrations of inorganic ions in the blood and urine of *Palaemon* in different concentrations of sea water. In normal sea water the blood K level is about 70% that of the medium and the Na concentration is also lower. A fairly normal blood K concentration is maintained in 50% sea water so that although the prawn is not usually considered to be a brackish water form it shows the type of control over the blood composition which is found in its brackish water relatives like *Palaemonetes varians*.

Most of the animals used in the experiments were females of 2–5 g weight which were not carrying eggs.

Accumulation of ^{137}Cs *by unfed whole prawns*

A curve showing the uptake of ^{137}Cs from normal sea water by a large number of individual animals is given in Fig. 6A. Also shown is an uptake curve for three animals in 50% sea water. The activity of normal sea water was 20 μc/l. and that of 50% sea water was 10 μc/l. In 50% sea water the concentration factor tends to a value twice as high as in normal sea water where equilibrium seems to be approached rather more rapidly. Previous experiments with crabs were carried out using the isotope ^{134}Cs (Bryan, 1961). Uptake of both Cs isotopes from normal sea water was compared at 9° C using four prawns in each experiment and an activity of 20 μc/l. Results for two single animals in Fig. 6A indicate that the carrier-free ^{137}Cs is taken up at the same rate as ^{134}Cs with which there was about 0·01 mM/l. of carrier in the medium.


Concentration factor
50% sea water at 10-12°C
Sea water at 10-12°C
Sea water at 9°C
A
Inactive sea water
B
C
Hours
0
4
8
12
16
20
24
28
32
36
200
400
600
800
1000
1200

Uptake of ^{137}Cs by blood and tissues in unfed prawns

In some cases ^{137}Cs is taken up by whole blood from normal sea water to a concentration factor of more than 2·0 (Fig. 7A). The increase of tissue concentration factors with time is shown in Fig. 7B, C. Uptake by the hepatopancreas, excretory organs and gills is relatively more rapid than uptake by abdominal muscle. Abdominal muscle is the limiting factor in the attainment of equilibrium by a whole animal particularly as Table 1 shows that it is by far the most abundant soft tissue. The results in Fig. 7B indicate that ^{137}Cs equilibrium had probably not been reached in abdominal muscle. Even so, the mean tissue/plasma ratio for eight animals analysed after 700 h exceeds that for K (Table 7). Corresponding ratios for ^{137}Cs in the hepatopancreas also exceed those for K but the difference between the ratios is most obvious in the gills. Results for animals from 50% sea water are shown in Fig. 7D–F and Table 7. Here the blood concentration factors are very variable and some are more than double the figures for normal sea water. As a result the tissue concentration factors tend towards values twice as high as in normal sea water. This is not very surprising because Table 7 shows that halving the sea-water concentration has not, in these experiments, resulted in significant changes in the K concentrations of plasma and tissues. From whole-animal uptake curves it seems possible that ^{137}Cs is taken up rather more slowly in 50% than in normal sea water. This may also be indicated by the fact that in Table 7 the mean tissue/plasma ratios for ^{137}Cs in animals from 50% sea water are less than the K values in muscle, hepatopancreas and shell. That the tissue/plasma ratios for ^{137}Cs would eventually exceed those for K seems fairly certain from the analysis of a fourth animal after 1223 h in 50% sea water where this had actually occurred. This animal had rather low tissue K concentrations and the loss of some K could possibly have led to this condition.

Loss of ^{137}Cs by unfed prawns

The uptake curve in Fig. 6B is the mean for four animals and shows that equilibrium is approached after about 1000 h. These prawns were from Conway and have taken up the ^{137}Cs more rapidly and to a greater concentra-

Legend to Fig. 6

Fig. 6. A. Uptake of ^{137}Cs by many unfed individual prawns in sea water at 10–12° C (middle curve). Uptake of ^{137}Cs (open circles) and ^{134}Cs (triangles) by two prawns in sea water at 9° C (lower curve). Uptake of ^{137}Cs by three prawns in 50% sea water at 10–12° C (upper curve). B. ●—●, Mean uptake of ^{137}Cs by four unfed Conway prawns from sea water. ○---○, The loss of ^{137}Cs by two prawns after 860 h of uptake. C. Uptake of ^{137}Cs by prawns fed on both (●—●) inactive and (○—○) radioactive *Mytilus* mantle in sea water. Arrows show the points at which both sets of animals were fed. Points on experimental and control graph are mean values for four prawns.

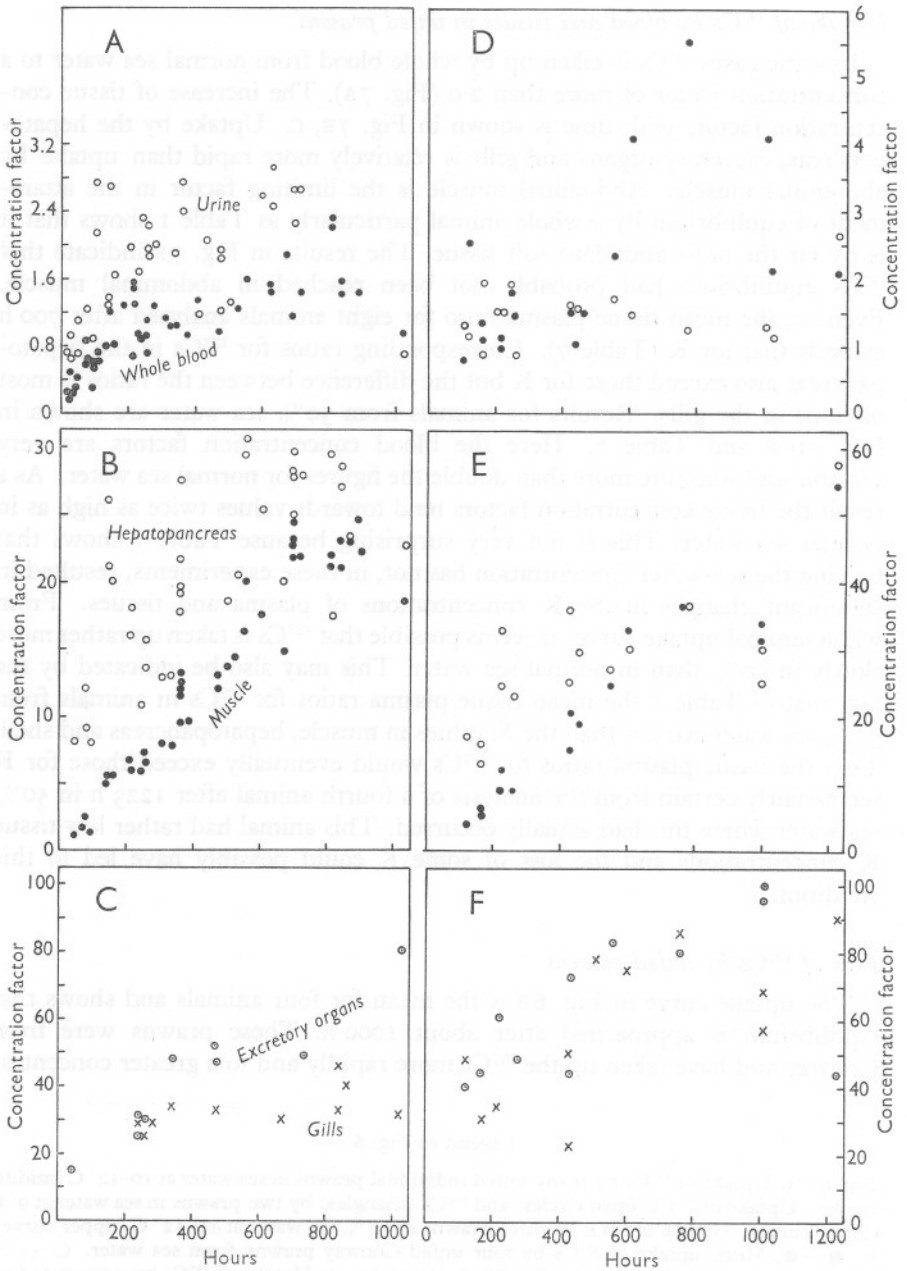


Fig. 7. A–C. Uptake of ^{137}Cs by whole blood, urine and tissues (as labelled) of prawns in sea water. D–F. Results of a similar experiment in 50 % sea water.

tion factor than the Plymouth prawns. Experiments with the Conway animals were carried out in April while other experiments were made in September. After 860 h of uptake, two Conway prawns were allowed to lose the isotope into inactive sea water. The mean curve is also given in Fig. 6B. Loss of ^{137}Cs is initially fairly rapid, but taken as a whole it is a slower process than uptake. This may to some extent be due to starvation, but is probably the result of reabsorption by tissues of isotope already lost into the blood.

TABLE 7. TISSUE/PLASMA RATIOS FOR ^{137}Cs AND K IN PRAWNS AFTER LONG EXPOSURE OF FED AND UNFED ANIMALS TO SEAWATER CONTAINING ^{137}Cs

All ratios are calculated on a water content basis except the tissue ^{137}Cs concentration factors which are given to show the standard deviation of tissue activities.

Medium	Tissue	No. of animals	H_2O (%)	K (mM/kg H_2O)	Ratio: tissue/plasma K	Ratio: tissue/plasma ^{137}Cs	^{137}Cs C.F.
Unfed animals exposed to normal S.W. with 20 μC/l. ^{137}Cs for over 700 h. Mean whole animal C.F. = 17·1	Muscle	8	78·0	127 ± 7	14·3	15·4	21·4 ± 1·5
	Hepatopancreas	8	76·2	144 ± 8	16·2	19·5	26·4 ± 3·4
	Gills	4	74·5	86 ± 9	9·7	26·5	35·2 ± 6·6
	Plasma	8	87·5	8·9 ± 2·6	—	—	1·56 ± 0·48
	Ratios: plasma/S.W.			—	0·84	1·72	—
Unfed animals exposed to 50 % S.W. with 10 μC/l. ^{137}Cs for over 700 h. Mean whole animal C.F. = 29·2	Muscle	3	77·9	118 ± 4	12·0	9·7	33·9 ± 2·6
	Hepatopancreas	3	70·6	167 ± 10	17·0	9·7	30·6 ± 6·0
	Excretory organs	3	85·0*	91 ± 8	9·3	23·8	91·1 ± 11·0
	Gills	3	74·0	104 ± 13	10·6	21·2	70·5 ± 15·0
	Shell	2	15·0	167 ± 26	17·0	16·2	10·9 ± 4·5
	Plasma	3	88·6	9·8 ± 1·1	—	—	4·00 ± 1·80
	Ratios: plasma/S.W.			—	1·84	4·40	—
Animals fed on inactive food and exposed to normal S.W. with 100 μC/l. ^{137}Cs for 1000 h. Mean whole animal C.F. = 25·0	Muscle	4	78·5	121 ± 6	13·6	12·0	25·6 ± 1·6
	Hepatopancreas	3	74·7	185 ± 19	20·8	10·1	20·6 ± 4·9
	Excretory organs	4	85·0*	111 ± 11	12·5	19·0	44·0 ± 11·0
	Gills	4	81·5	92 ± 25	10·3	12·5	27·7 ± 5·3
	Shell	4	41·4	45 ± 15	5·1	6·0	6·8 ± 1·4
	Fore-gut	4	80·8	97 ± 5	10·9	17·9	39·3 ± 2·6
	Plasma	4	93·5	8·9 ± 1·8	—	—	2·54 ± 1·00
	Ratios: plasma/S.W.			—	0·85	2·62	—
Animals fed on active food and exposed to normal S.W. with 100 μC/l. ^{137}Cs for 1000 h. Mean whole animal C.F. = 29·5	Muscle	4	78·1	126 ± 5	11·4	10·3	27·4 ± 2·0
	Hepatopancreas	4	76·5	169 ± 1	15·3	8·7	22·8 ± 4·4
	Excretory organs	4	85·0*	112 ± 19	10·2	18·6	54·0 ± 7·0
	Gills	4	82·2	92 ± 14	8·4	11·1	31·0 ± 1·0
	Shell	4	36·0	58 ± 25	5·3	5·4	6·6 ± 1·2
	Fore-gut	4	79·3	91 ± 7	8·3	14·1	38·1 ± 2·1
	Plasma	4	92·7	11·0 ± 2·2	—	—	3·16 ± 1·3
	Ratios: plasma/S.W.				1·0	3·28	

* H_2O content assumed.

Uptake of ^{137}Cs *by fed prawns*

The penetration of ^{137}Cs into the blood and tissues from food was followed by sampling animals which had been kept in inactive sea water after a single meal of radioactive *Mytilus edulis* mantle. Each animal weighed about 3 g and ate food equivalent to about 5 % of the body weight. Tissue activities

measured at intervals after feeding were corrected to allow for slight differences in the sizes and initial activities of the animals, but not for differences in the readiness with which the total activities of the animals fell (Fig. 8A). Changes in the ^{137}Cs levels of the plasma and tissues after feeding are shown in Figs. 8B, C. Digestion of food is completed in 24 h with the result that the total activity of the fore-gut shows a rapid fall over this period. During digestion,

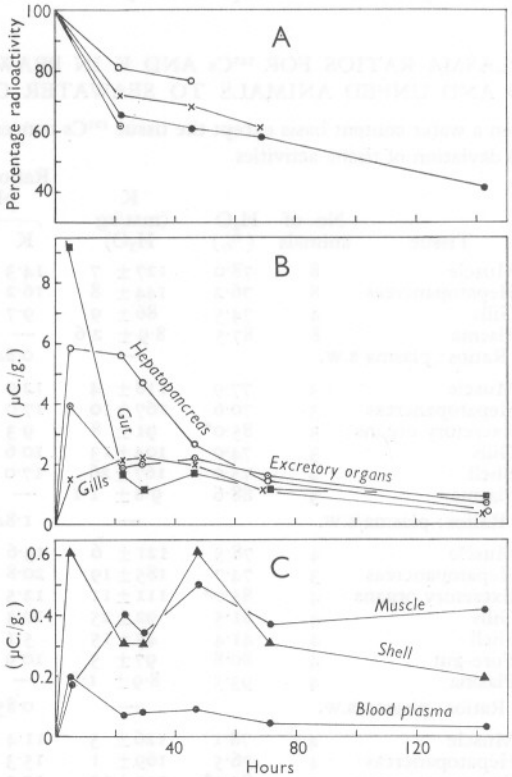


Fig. 8. A. Loss of ^{137}Cs from whole prawns into inactive sea water after a single radioactive meal. B, C. Levels of radioactivity in plasma and tissues of the same prawns in inactive sea water.

the hepatopancreas is the most active tissue which probably indicates that it is here that ^{137}Cs is absorbed. After 5 h the excretory organs are also quite active, probably as a result of rapid exchange with the plasma which contains most ^{137}Cs in the early stages. The gills gain rather less ^{137}Cs than the excretory organs but more than the shell. As might be expected, the muscle reaches a low level of activity and shows far less tendency to lose it than the other tissues. It is the muscle which limits the loss of ^{137}Cs from the whole animal in the later stages of exposure to inactive sea water.

A similar experiment was carried out in which ^{137}Cs was injected into the blood of prawns. In this case the plasma ^{137}Cs level fell very rapidly and the gills and excretory organs became by far the most active tissues. Unlike the feeding experiment, the highest ^{137}Cs level reached by the hepatopancreas was only three times that of muscle, while the fore-gut was about one-third as active as the muscle. This tends to support the idea that ^{137}Cs is absorbed from food principally via the hepatopancreas and also suggests that losses from the whole animal in inactive sea water take place across the body surface and excretory organs rather than through the gut.

A feeding experiment was carried out to find to what extent ^{137}Cs can be retained if prawns are fed at regular intervals on active *Mytilus* mantle in inactive sea water. Prawns were given as much radioactive food as they could eat once a week and the whole animal activity was measured before, after and between meals. Following the gain of ^{137}Cs at a meal there was a sharp fall in the activity of the animal but the level to which it fell each week rose to a value which was roughly constant a week after the fifth, sixth and seventh meals. One week after the seventh meal the level of activity in the animal was equal to 11% of the ^{137}Cs which had been given in food.

Further feeding experiments were performed in sea water containing ^{137}Cs to find to what extent ^{137}Cs obtained from food could be expected to augment uptake over the body surface. Four prawns were fed on radioactive *Mytilus* mantle at approximately weekly intervals in sea water containing about 100 μc/l. of ^{137}Cs. In a control experiment four prawns were fed on inactive food in a similar solution. During the experiment the prawns were fed on six occasions and at each meal they ate an amount equal to about 8% of the body weight. Mean uptake curves for both sets of animals are given in Fig. 6c. The gain in activity at each meal is shown by the vertical lines in the upper curve. After the first two radioactive meals the uptake of ^{137}Cs is continued at higher whole-animal concentration factors, but following the next three meals the activity of the animals tends to remain constant. This latter effect is probably due to a balance being effected between the loss of ^{137}Cs temporarily gained from the food by the plasma and more permeable organs and gain of the isotope by muscle. The fall in whole animal activity after the last meal suggests that equilibrium is being approached in muscle. This is also indicated by the tendency for the control curve to level out at an equilibrium concentration factor. At 990 h the difference in activity between the experimental and control animals shows that the equivalent of 74% of the ^{137}Cs introduced by feeding has been lost. The food given to the prawns had an activity equal to a concentration factor of about 20 in the experimental medium. In a constant environment contaminated with ^{137}Cs, food with a concentration factor of more than 20 would probably not be found, even if it were freshly killed, and generally the material eaten would be expected to be less active. Compensating for this, the animals would probably eat a full meal

every two days instead of every week as in the experiment. Thus over 1000 h natural feeding might cause a somewhat greater increase in the activity of the animals than was found in the experiment, but uptake of ^{137}Cs over the body surface would probably still remain the important factor.

Complete analyses of experimental and control fed animals are shown in Table 7 for comparison with the results for starved animals. In both groups of fed animals the plasma contains more K and ^{137}Cs than in the starved animals but the variation is very wide. Higher plasma K levels correspond with higher levels of ^{137}Cs. Muscle K concentrations for fed prawns are similar to those for starved animals with the result that they have lower tissue/plasma ratios for K. Also the tissue/plasma K ratios are not exceeded by the ^{137}Cs ratios. Differences are more marked when the hepatopancreas results are compared. Starved animals have lower K concentrations and the tissue/plasma ratio for ^{137}Cs is greater than that for K whereas in the fed animals it is smaller. In all other tissues the tissue/plasma ^{137}Cs ratios exceed those for K very markedly except in the shell. The prawns fed on inactive food show a more rapid uptake of isotope than the unfed animals in Fig. 6A, which in the early stages may be a difference due to variations between groups of animals and is later probably due to starvation of the unfed animals. Unfed prawns also tend towards equilibrium at lower whole-animal concentration factors. This is probably a result of lower K concentrations being maintained in some tissues and also to a loss of tissue bulk especially by the hepatopancreas.

Uptake of ^{42}K

Uptake of ^{42}K by prawns has been followed in normal and 50% sea water at 11·5° C (Fig. 9A). In neither experiment is equilibrium reached, but analysis of muscle at the end of each experiment shows that the ^{42}K concentration factor is 80% of that for inactive K in normal sea water and 72% in 50% sea water (Table 8). Decay of ^{42}K made the later blood activities too low for tissue/blood ratios to be found with any accuracy, for comparison with the inactive K ratios. The ^{42}K resembles ^{137}Cs in being taken up rather more slowly by animals in 50% sea water. In two more experiments carried out at 9° C, uptake of ^{42}K was followed in artificial sea water and in artificial sea water with 50% K (Fig. 9A). Allowing for the temperature difference, ^{42}K is taken up from the artificial sea water in the same way as from normal sea water. Uptake from the artificial sea water with 50% K is much slower, so that at 69 h the muscle ^{42}K concentration factor in Table 8 is only 57% of the value for inactive K as compared with 70% in artificial sea water. In two whole animals after 91 h in artificial sea water the K concentrations were 84 and 85 mM/kg and 74·5 and 76·3% exchange of ^{42}K had taken place. If all the ^{42}K had exchanged then an equilibrium concentration factor of about 7·1 would have been expected. Compared with *Carcinus* (Bryan, 1961), the concentration factors reached by both ^{42}K and ^{137}Cs are markedly higher in

the prawn. In *Carcinus* no difference is found between the rates at which ^{42}K equilibrium is approached in artificial sea water and in artificial sea water with 50% K.

The gain of ^{42}K by whole blood, abdominal muscle and hepatopancreas of animals in artificial sea water at 9° C is shown in Fig. 9B, C. Values for blood concentration factors are varied, but at 68 h 90% exchange has been achieved. Exchange in the hepatopancreas after 68 and 90 h exceeds that for muscle in two out of three cases. In the uptake of ^{42}K by a whole animal, muscle is not

TABLE 8. EXCHANGE OF ^{42}K WITH INACTIVE K IN PRAWNS UNDER DIFFERENT EXTERNAL CONDITIONS

K concentrations are given as per kg wet weight as the tissue water contents were not measured. ^{42}K levels are given as a percentage of the inactive K concentration factor.

					Whole blood		Muscle		Hepatopancreas	
Medium	Medium K (mM/kg)	Temp. (° C)	Time (h)	No. of animals	K (mM/kg)	^{42}K (% exchanged)	K (mM/kg)	^{42}K (% exchanged)	K (mM/kg)	^{42}K (% exchanged)
100% s.w.	10·6	11·5	68	3	—	—	103±2	80±4	—	—
50% s.w.	5·3	11·5	68	3	—	—	97±2	72±5	—	—
Artificial s.w.	11·8	9·0	68	2	7·8±0·8	90±2	99±4	70±0	115±40	67±15
	—	—	90	1	—	—	103	74	91	93
Artificial s.w. with 50% K	6·1	9·0	69	2	4·8±0·8	65±2	87±6	57±4	99	88
	—	—	91	1	—	—	94	43	102	69

quite such a decided limiting factor as with the uptake of ^{137}Cs. Tissue/blood ^{42}K ratios for muscle (calculated from values per kg wet weight) rise to values of 3·6, 8·7 and 13·8 after times of 5·5, 22 and 68 h respectively, while the corresponding ratios for hepatopancreas are 12·3, 14·7 and 20·8. Uptake of ^{42}K by the hepatopancreas is thus more rapid than uptake by muscle. Time constants for uptake of ^{42}K by the blood and by muscle from the blood are probably of a similar order so that both tend to be limiting in the accumulation of the isotope.

Excretion of ^{137}Cs *and* K

Concentration factors for ^{137}Cs in urine from prawns in normal and 50% sea water are given in Fig. 7A, D. In most cases in normal sea water the activity of the urine is considerably greater than that of the blood, whereas in 50% sea water this difference is less marked. Plasma/sea water and urine/plasma ratios for ^{137}Cs and K in animals in which all these measurements were made are shown in Table 9. In normal sea water the plasma K concentration is lower than that of the sea water and the excretory organs do not appear to assist in maintaining this level as the mean U/P ratio is 0·56. This was first shown by Parry (1954). The U/P ratios for ^{137}Cs in normal sea water are always greater than those for K and usually exceed 1·0, but this does not prevent the plasma/sea water ratios for ^{137}Cs from reaching values of more

than 2·0. Prawns in 50% sea water maintain a K concentration similar to that in normal sea water so that the mean plasma/sea water ratio of 1·62 is almost double the normal sea water value. Here, the excretory organs tend to assist in maintaining the K level of the plasma as the mean U/P ratio is 0·51. Corresponding U/P ratios for ^{137}Cs are all considerably lower than in normal sea water and only two values are greater than 1·0. Ratios less than 1·0 would

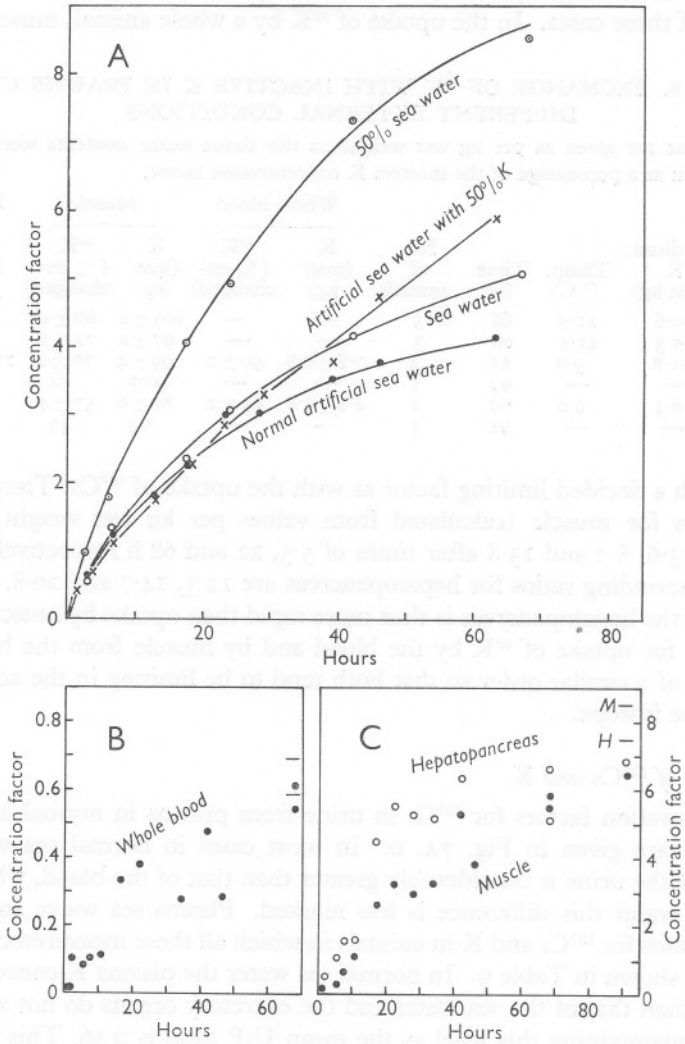


Fig. 9. A. Uptake of ^{42}K by prawns in sea water and 50% sea water at 11·5° C and in artificial sea water and artificial sea water with 50% K at 9° C. Each curve is a mean for three prawns. B, C. Uptake of ^{42}K by whole blood and tissue of prawns in artificial sea water at 9° C. Short horizontal lines indicate concentration factors for inactive K.

assist in maintaining the high plasma/sea water ratios for ^{137}Cs which are sometimes found. In both media, animals starved for long periods of time tend to have lower U/P ratios for ^{137}Cs.

In order to examine the possibility that the high U/P ratios for ^{137}Cs in normal sea water might be due to water reabsorption from a primary urine produced by filtration, inulin clearance experiments were carried out during the uptake of ^{137}Cs. The validity of these experiments will depend on assumptions which have already been made for the lobster. For the first experiment animals of about 3 g weight were each injected with 0·04 ml. of 5 % inulin and

TABLE 9. PLASMA/SEA WATER AND URINE/PLASMA RATIOS FOR ^{137}Cs AND K IN UNFED PRAWNS FROM 100 % AND 50 % SEA WATER CONTAINING ^{137}Cs

Ratios are all calculated on a water content basis.

	100 % s.w.					50 % s.w.			
	Ratio: plasma/s.w.		Ratio: U/P			Ratio: plasma/s.w.		Ratio: U/P	
Time (h)	K	^{137}Cs	K	^{137}Cs	Time (h)	K	^{137}Cs	K	^{137}Cs
18	1·05	0·26	0·50	0·76	168	2·34	1·50	0·39	0·96
96	0·92	0·33	0·46	8·30	245	1·70	2·02	0·52	0·95
98	0·92	0·68	0·62	3·18	430	1·47	1·65	0·37	0·94
240	0·80	0·98	0·69	2·46	430	1·13	1·13	0·50	1·36
259	0·90	1·19	0·48	1·88	552	1·58	2·64	0·53	0·65
260	0·60	0·76	0·77	2·53	600	2·07	4·58	0·35	0·33
336	0·92	1·15	0·55	1·65	767	1·26	6·26	0·69	0·19
476	0·64	1·06	0·69	1·14	1008	2·07	4·64	0·26	0·29
477	1·09	2·12	0·40	0·87	1008	1·62	2·41	0·33	0·48
1030	0·67	1·05	0·47	0·63	1223	0·96	2·18	1·20	1·22
Mean	0·85	—	0·56	2·34	—	1·62	—	0·51	0·74

then placed in sea water containing 30 μc/l. of ^{137}Cs. At subsequent time intervals the levels of inulin and ^{137}Cs in the plasma and urine of different prawns were determined. Results for inulin loss and uptake of ^{137}Cs are given in Fig. 10A. Points after 50 h are for samples pooled from two prawns. The plasma inulin concentrations are so erratic that it was considered that the quantity of inulin injected might be affecting the excretory organs to some extent. U/P ratios for inulin and ^{137}Cs from this experiment, calculated on a water content basis, are given in Table 10. Using injections of 0·02 ml. of 5 % inulin in prawns of about 2·3 g weight the experiment was repeated, and again pooled samples were analysed after 50 h. This time the first plasma sample pooled from three animals was taken after 3 h. Results are given in Fig. 10B and the broken line indicates the suggested path of inulin loss assuming it to be exponential. The U/P ratios for both inulin and ^{137}Cs are lower than in the first experiment and are given in Table 10. If it is assumed that inulin gives an indication of water reabsorption in the excretory organs, then the results show that to a large extent this could be the reason for the high U/P ratios for ^{137}Cs.

Results from Fig. 10B have been used, as in the lobster, to calculate values for the inulin space and urine production, assuming that this loss of inulin from the plasma is exponential and that it is all lost through the excretory organs. The inulin space occupies 34·7% of the body weight and the urine production is about 14% of the body weight per day. The urine production may be compared with a value of nearly 10% per day found for *Palaemonetes varians* in sea water by Parry (1955). Three earlier attempts at measuring the inulin space by estimating inulin 15 min. after injection gave values of 30·6, 26·2 and 12·6% of the body weight. This latter figure suggested that the longer period of 3 h should be allowed to give more time for mixing.

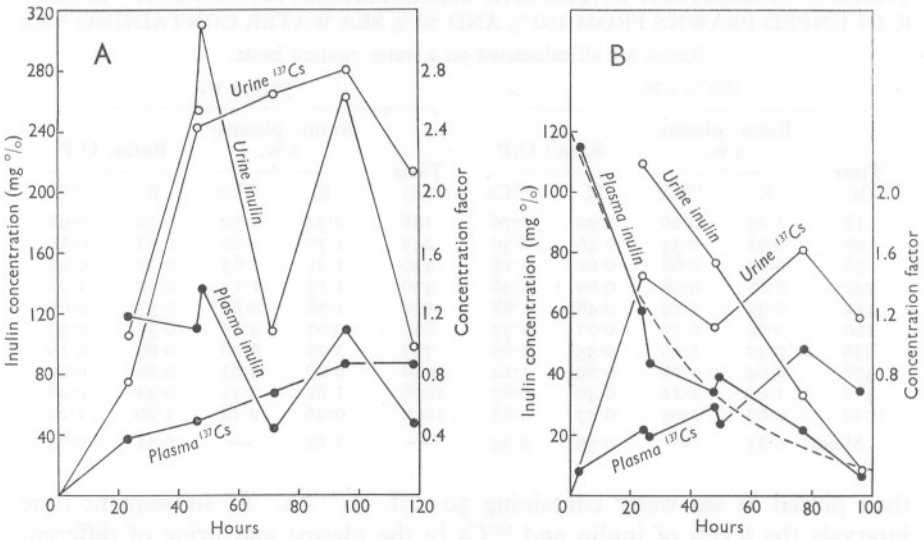


Fig. 10. A. Inulin levels in the plasma and urine of prawns taking up ^{137}Cs from sea water. The prawns weighing about 3 g each were injected with 0·04 ml. of 5% inulin at the start of uptake. B. Repeat experiment in which prawns of about 2·3 g were injected with 0·02 ml. of 5% inulin.

TABLE 10. URINE/PLASMA RATIOS FOR INULIN AND ^{137}Cs FROM THE EXPERIMENTS IN FIG. 10A, B

The ratios are calculated on a water content basis.

A. Ratio: U/P		B. Ratio: U/P	
Inulin	^{137}Cs	Inulin	^{137}Cs
0·93	2·02	1·78	3·22
2·18	4·78	1·88	1·80
2·15	0·99	1·43	1·59
2·40	2·50	0·97	1·62
2·29	3·07	Mean 1·51	2·06
1·94	2·36		
Mean 1·98	2·62		

In the prawns which were fed on inactive food during uptake of ^{137}Cs, a whole-animal concentration factor of about 3 is reached in the first 24 h. If this is still the rate of influx and outflux at equilibrium, the plasma concentration factor is 2·5 and the U/P ratio for ^{137}Cs is 2·0, then with a urine production of 14% of the body weight per day, the excretory organs will be responsible for about 23% of ^{137}Cs losses at equilibrium. With ^{42}K a whole animal concentration factor of about 1·0 is reached in 5 h and at equilibrium urine with a concentration factor of about 0·5 would be excreted. Here, the excretory organs account for only about 1·4% of ^{42}K outflux at equilibrium.

As the high U/P ratios for ^{137}Cs may be the result of water reabsorption in the excretory organs of animals from normal sea water it is possible that the much lower ratios found in 50% sea water are due to less water reabsorption. In *Palaemonetes*, Parry (1955) found that urine production is similar in normal and 50% sea water and minimal in 70% sea water which is roughly isotonic with the blood. This result is difficult to interpret, but there is certainly an advantage to be seen in the reabsorption of more water by the excretory organs when the prawn is in normal sea water, which is hypertonic to the plasma, than in 50% sea water which is hypotonic to the plasma.

Observations on Palaemonetes varians

Analyses of blood and tissues from this brackish water prawn after 650 h of exposure to normal sea water containing ^{137}Cs showed that under these conditions *Palaemonetes* resembles *Palaemon* very closely with regard to ^{137}Cs accumulation and tissue K concentrations. Experiments were not carried out in diluted sea water but it is almost certain that whole-animal ^{137}Cs concentration factors would be increased, perhaps to values as high as 100 in one-tenth sea water.

CRAYFISH EXPERIMENTS

Most of the crayfish experiments were carried out in 0·1% sea water rather than in fresh water to give a closer comparison with the marine animals. In sea water a marine decapod takes up ^{137}Cs to an equilibrium concentration factor greater than that for inactive K. On this basis very high equilibrium concentration factors for ^{137}Cs might be expected in crayfish because in 0·1% sea water (*ca.* 0·01 mM/l. K) the inactive K concentration factor for a whole animal is of the order of 4000–5000. Animals from two sources have been used. Crayfish from Surrey usually weighed between 25 and 40 g, while Windermere crayfish weighed from 2 to 25 g. Male animals were always used.

Uptake of ^{137}Cs *from* 0·1% *sea water by whole crayfish*

Uptake was followed in 0·1% sea water containing 5 μC/l. of ^{137}Cs by counting whole animals at intervals. The generally smaller Windermere crayfish

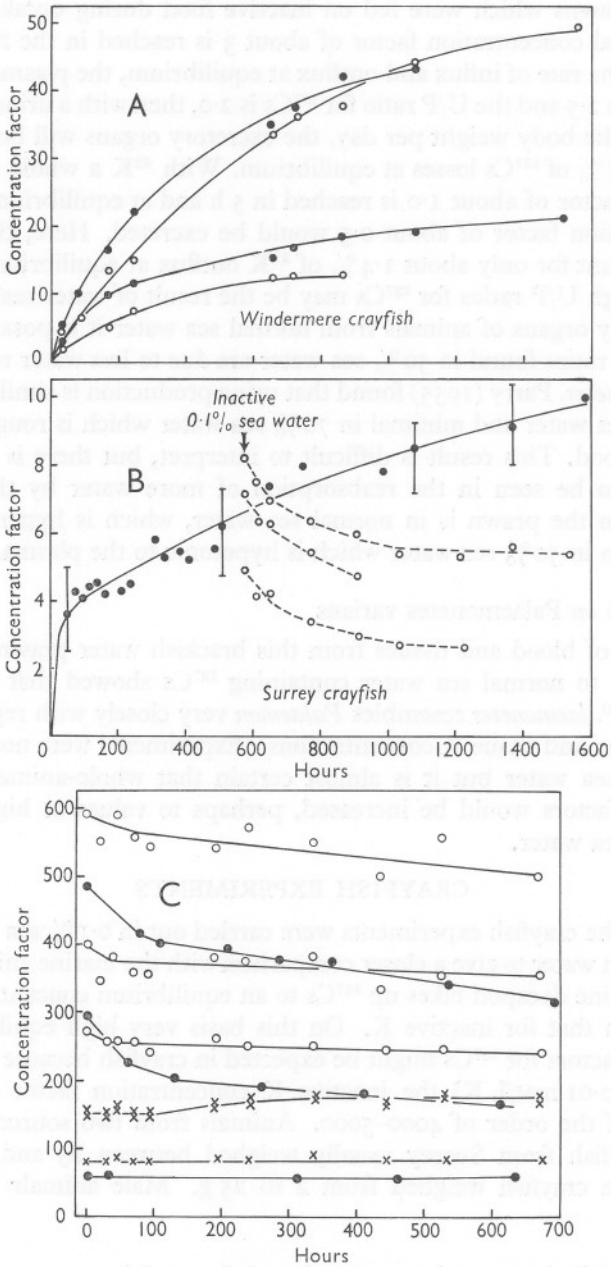


Fig. 11. A. Uptake of ^{137}Cs by whole Windermere crayfish. B. Uptake of ^{137}Cs by four Surrey crayfish of mean weight 31 g. Vertical lines indicate the range of results. Also loss of ^{137}Cs from three whole animals after uptake for 578 h. C. Change of activity of whole Windermere crayfish placed in 0·1 % sea water containing 8·5 μc/l. ^{137}Cs after exposure for 336 h to a solution 25 times as active (open circles) and five times as active (crosses) at 11·5° C. Three experiments were done at 20° C (closed circles) after 66 and 114 h in a medium 50 times as active and after 180 h in a medium five times as active (lower curve).

take up the isotope to higher but more variable concentration factors than the Surrey animals (Fig. 11A, B and Table 11). Within the Windermere group, animals of up to 25 g were used, but there does not appear to be any relationship between the size of an animal and the rate and extent of ^{137}Cs accumulation. The Surrey crayfish were all larger than the Windermere animals, but this alone is probably not sufficient to account for the big difference in the extents to which the isotope is accumulated. There may be a physiological difference between the two groups of animals.

TABLE 11. WHOLE-ANIMAL CONCENTRATION FACTORS AND PLASMA/MEDIUM RATIOS FOUND DURING THE UPTAKE AND LOSS OF ^{137}Cs BY CRAYFISH IN 0·1% SEA WATER

Plasma/medium ratios calculated on a water content basis.

Windermere crayfish				Surrey crayfish			
Uptake time (h)	Weight (g)	Whole-animal concentration factor	Ratio: plasma/medium	Uptake time (h)	Weight (g)	Whole-animal concentration factor	Ratio: plasma/medium
94	*ca.* 20	—	1·34	M_r 473	44·9	25·3	2·32
208	*ca.* 20	—	0·77	497	40·8	10·3	0·85
M 212	*ca.* 20	—	3·39	744	41·6	8·1	0·74
285	*ca.* 20	—	1·23	1104	48·5	10·4	1·01
305	*ca.* 20	—	1·43	1500	25·4	10·0	0·92
M 336	5·2	45	3·38	*F* 1050	31·0	88·0	13·1
M_t 336	10·6	62	—				
384	11·0	23	2·02				
M_t 576	12·8	69	—	Animals placed in inactive 0·1% S.W. at 578 h			
864	6·2	47	5·22				
1077	8·9	43	—	Loss time (h)			
1152	18·9	63	—				
1557	2·9	21	1·62	335	26·4	4·7	0·49
1558	4·1	49	3·02	646	31·7	2·6	0·28
1680	2·7	55	5·55	984	32·9	5·4	0·46
F 1970	12·8	183	17·2				

M denotes animals which had moulted shortly before uptake was commenced.
M_r moulted 60 h before analysis.
M_t denotes animals which moulted soon after these measurements were made.
F denotes two animals fed on active earthworms.

Curves for the uptake of ^{137}Cs by Windermere crayfish in Fig. 11A are approximately exponential in shape. If it is assumed that the upper curve (open circles) in Fig. 11A is reaching equilibrium at a concentration factor of 50 then the rate constant calculated from the slope of a semi-logarithmic plot of the results is 0·0019 h^{-1}. This calculation was made in a similar way to that for k_{out} for Na in crayfish (Bryan, 1960*a*). It is not certain that this animal is in fact approaching equilibrium as the uptake curve may have a slower upper phase to it. An attempt was made to find what the equilibrium level might be by transferring Windermere crayfish from radioactive 0·1% sea water to a solution of lower activity and measuring changes in the concentration factors

of the animals relative to the second solution. Six experiments were carried out at 11–12° C and three experiments at 20° C (Fig. 11c). Five experiments, starting at a concentration factor of more than 200, showed a very slow net fall in activity, while the remaining four animals showed either no change or a very slight rise in activity. The results suggest that absolute equilibrium may lie in the range of concentration factors 50–200, but the changes are so slow that this is only an estimate.

Uptake of ^{137}Cs is more rapid in animals which are in the moulting phase. The crayfish denoted by M_r in Table 11, which moulted in the radioactive solution, has reached a high concentration factor compared with the intermoult animals. Uptake of the isotope is also more rapid just before the moult, and in animals which have moulted but where the exoskeleton is still relatively thin (denoted by M_t and M in Table 11). Some of this additional activity may enter via the gut as well as over the body surface. A crayfish which moulted in Evans blue solution was found to have a fore-gut concentration of blue similar to that of the medium, whereas after the same period of time (24 h) two normal animals contained only one-eighth to one-quarter as much blue as this. There was very little blue in the hepatopancreas in any of the crayfish.

Uptake of ^{137}Cs *by blood and tissues*

The blood and tissues of some crayfish were analysed at intervals over the periods of uptake used for the whole animals. As the whole-animal concentration factors (Table 11) are so variable, ^{137}Cs and inactive K concentrations have been plotted as tissue/plasma ratios against time using results calculated on a water content basis. The concentration factors for the whole animals analysed in these experiments are given in Table 11 together with the corresponding plasma/medium ratios. Eight determinations showed that on average the ^{137}Cs and inactive K levels of the plasma are about 92% of the values for an equal weight of whole blood. Whole-animal concentration factors are between 9·0 and 14·9 times the tissue/plasma ratios, so that there is rough proportionality between the two figures.

Results for the complete analysis of a Windermere animal after 1558 h of uptake of ^{137}Cs from 0·1% sea water are given as an example in Table 12. Ratios calculated from results for other crayfish are given in Fig. 12. Muscle/plasma ratios for ^{137}Cs and inactive K in crayfish from both sources are plotted against time. In both, the ^{137}Cs ratio rises in roughly linear fashion but more rapidly in the Windermere animals. There is also a fall in the inactive K ratio for Windermere animals which would be expected to modify the level that could eventually be reached by ^{137}Cs. This fall in the K ratio is not due to a significant drop in the K level of muscle but to a rise in the plasma K concentration from a mean of 3·7 mM/kg H_2O for the first five animals analysed before 500 h to 5·4 for the five crayfish analysed later. No such change in the plasma K level was found in Surrey animals. The difference between the rates

of change of the muscle/plasma ^{137}Cs ratios may possibly be because the Surrey crayfish maintain lower levels of K in the muscle as indicated in Table 13. In the hepatopancreas, the tissue/plasma ^{137}Cs ratios are always between 15 and 25 and over the latter part of the experiment they tend to exceed those for inactive K. This indicates that uptake by this organ must be a rapid process relative to uptake by the blood from the medium. Results for

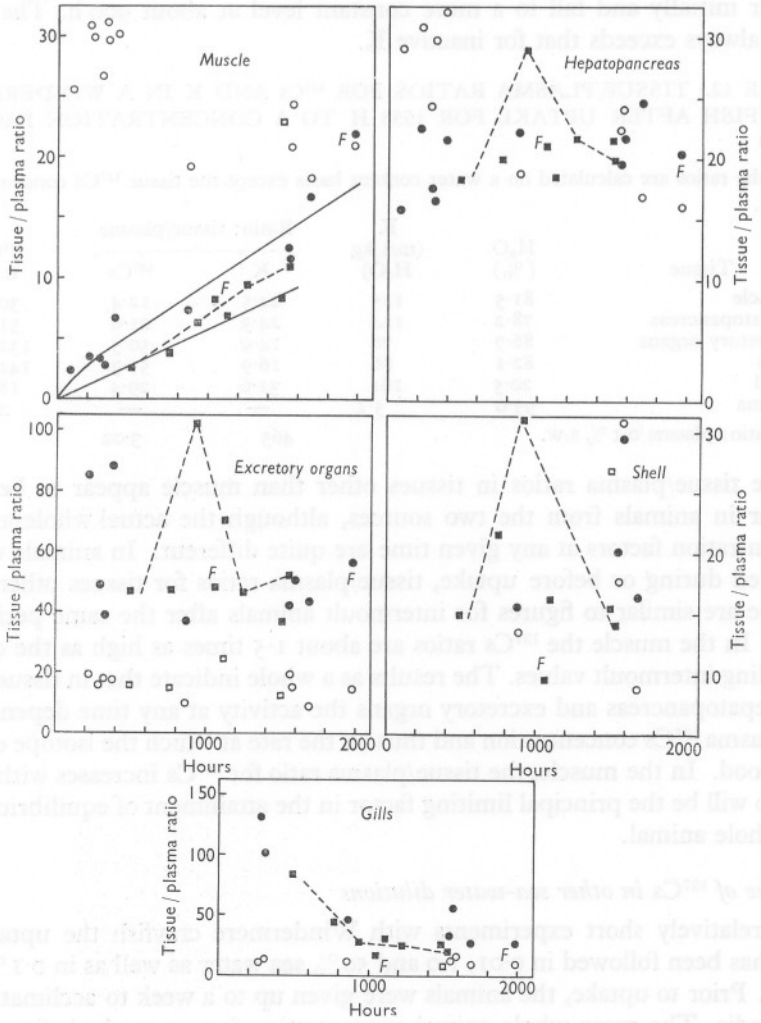


Fig. 12. Tissue/plasma ratios (on a water content basis) for inactive K (open symbols) and ^{137}Cs (closed symbols) in Windermere crayfish (circles) and Surrey crayfish (squares). Ratios found in animals losing ^{137}Cs in inactive 0·1% sea water after 578 h of uptake are joined by broken lines and points for animals fed on radioactive earthworms are denoted by F.

the excretory organs are of a similar type but here the tissue/plasma ^{137}Cs ratios always markedly exceed those for K. Samples of shell taken from the dorsal side of the carapace and scraped on the inside give very variable results with sometimes the K and other times the ^{137}Cs ratio being the larger. The situation in the gills is complicated by their being in contact with plasma and medium which have very different compositions. Gill radioactivity does not increase in proportion to the plasma activity and so the gill/plasma ratios are higher initially and fall to a more constant level at about 900 h. The ^{137}Cs ratio always exceeds that for inactive K.

TABLE 12. TISSUE/PLASMA RATIOS FOR ^{137}Cs AND K IN A WINDERMERE CRAYFISH AFTER UPTAKE FOR 1558 H TO A CONCENTRATION FACTOR OF 49

All the ratios are calculated on a water content basis except the tissue ^{137}Cs concentration factors.

Tissue	H_2O (%)	K (mM/kg H_2O)	Ratio: tissue/plasma K	Ratio: tissue/plasma ^{137}Cs	^{137}Cs C.F.
Muscle	81·5	125	24·5	12·4	30·6
Hepatopancreas	78·2	124	24·3	21·6	51·1
Excretory organs	86·7	76	14·9	50·5	132
Gills	82·1	86	16·9	57·3	142
Shell	20·5	161	31·6	29·4	18·2
Plasma	95·0	5·1	—	—	2·87
Ratio: plasma/0·1 % s.w.			465	3·02	

The tissue/plasma ratios in tissues other than muscle appear to be very similar in animals from the two sources, although the actual whole-animal concentration factors at any given time are quite different. In animals which moulted during or before uptake, tissue/plasma ratios for tissues other than muscle are similar to figures for intermoult animals after the same period of time. In the muscle the ^{137}Cs ratios are about 1·5 times as high as the corresponding intermoult values. The results as a whole indicate that in tissues like the hepatopancreas and excretory organs the activity at any time depends on the plasma ^{137}Cs concentration and thus on the rate at which the isotope enters the blood. In the muscle, the tissue/plasma ratio for ^{137}Cs increases with time and so will be the principal limiting factor in the attainment of equilibrium by the whole animal.

Uptake of ^{137}Cs *in other sea-water dilutions*

In relatively short experiments with Windermere crayfish the uptake of ^{137}Cs has been followed in 0·01, 1·0 and 50% sea water as well as in 0·1% sea water. Prior to uptake, the animals were given up to a week to acclimatize to the media. The mean whole-animal concentration factors reached after 260 h were 92 for two animals in 0·01% sea water, 17 for four animals in 0·1% sea water, 1·7 for two animals in 1·0% sea water and 0·5 for three animals in 50% sea water. Allowing for wide individual variations there is roughly a tenfold

increase in concentration factor with a tenfold change in the concentration of the three most dilute solutions. When these animals were analysed for ^{137}Cs it was found that although the concentration factors are different the tissue/plasma ratios for all the tissues are similar to those for crayfish from 0·1 % sea water, even in the animals from 50 % sea water. The reason for these results seems to lie in the fact that the crayfish maintains its plasma and tissue K concentrations at fairly constant levels over a very wide range of external K

TABLE 13. K CONCENTRATIONS (mM/kg H_2O) AND WATER CONTENTS (%) OF PLASMA AND TISSUES IN CRAYFISH FROM DIFFERENT MEDIA

Source of animals	Medium	No. of animals	Plasma K	Muscle		Hepato-pancreas		Excretory organs		Gills		Shell	
				H_2O	K	H_2O	K	H_2O	K	H_2O	K	H_2O	K
Surrey*	0·1 % S.W.	2	3·6	83·6	99	85·1	82	88·0	58	91·1	32	29·0	84
Windermere	0·01 % S.W.	2	4·3	81·3	114	80·6	116	85·2	63	85·5	53	—	—
	0·1 % S.W.	10	4·5	83·0	110	80·9	107	85·7	69	86·9	59	30·0	80
	1·0 % S.W.	2	5·2	79·1	138	71·0	126	—	—	88·3	58	—	–
	50 % S.W.	3	6·2	79·6	128	77·1	131	81·6	100	87·4	50	—	—
	1 % S.W. plus 5 mM/l. KCl	2	11·4	83·1	116	75·8	127	89·2	120	87·0	65	—	—

* Plasma value is a mean of seven animals.

A plasma water content of 95 % has been assumed in all cases.

concentrations (Table 13). Even in 50 % sea water, where the external K concentration approaches that of the plasma, the plasma and tissue K levels are not increased sufficiently to prevent standard deviations of the mean values from overlapping with those of animals from 0·1 % sea water. A tenfold increase in concentration factor with a tenfold dilution of the radioactive sea water would indicate that over 260 h the same amount of ^{137}Cs is taken up from the different dilute media. One possible explanation is that ^{137}Cs uptake may be associated with a constant rate of K absorption in each of the dilute sea waters by what may be a carrier system which can be saturated with K at these very low dilutions. In 50 % sea water the situation is different because the animal no longer has to maintain its plasma K level against losses into a very dilute medium. In this case K can enter the blood passively and so what is presumably an active uptake system in 0·1 % sea water may almost cease to operate under these conditions to prevent the blood K level from rising too much.

Loss of ^{137}Cs from Surrey crayfish in inactive 0·1 % sea water

After taking up ^{137}Cs for 578 h three Surrey crayfish were allowed to lose the isotope in inactive medium. The results for all three whole animals are given in Fig. 11B and Table 11. After losing ^{137}Cs relatively rapidly in the early stages the animals tend to retain a considerable proportion of the isotope. The ^{137}Cs levels in the tissues of the three crayfish were measured and the

tissue/plasma ratios are compared with those of uptake animals in Fig. 12, where the points are joined by a broken line. In the crayfish analysed after 335 h of loss, the tissue/plasma ratios are generally higher than during uptake but ratios for the other two animals are, except in the case of muscle, similar to the uptake ratios. This would be expected if the activity of tissues other than muscle is directly related to that of the plasma as a result of rapid exchange of the isotope between plasma and tissues. In muscle, where ^{137}Cs is exchanged only slowly with the plasma, the tissue/plasma ratios are increased as the plasma activity falls. This is further confirmation that muscle is the main limiting factor in the uptake and loss of ^{137}Cs by whole crayfish.

Absorption of ^{137}Cs *by crayfish from food*

Small earthworms were used for food. They had been kept in 0·1% sea water with added ^{137}Cs for several weeks in a darkened container. For each meal the worms were cut into 1 cm lengths, washed in inactive medium and then dried with filter paper and weighed. The crayfish were counted whole before and after each meal. This lasted about half an hour, when any remaining pieces of worm were reweighed. Results of two experiments in which animals were fed at intervals on active earthworms in 0·1% sea water containing 5 μc/l. of ^{137}Cs are given in Fig. 13. Arrows indicate the times at

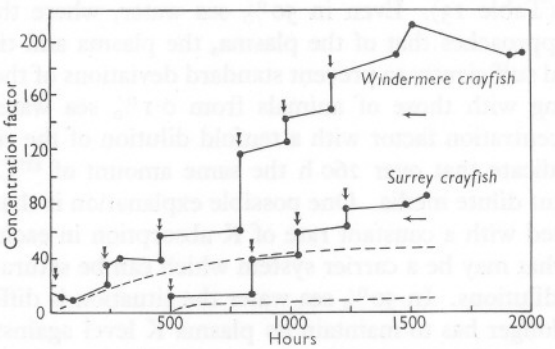


Fig. 13. Uptake of ^{137}Cs by crayfish fed on radioactive earthworms in active 0·1 % sea water. Vertical arrows on each graph indicate when the animals were fed. The broken line curves show the assumed path of ^{137}Cs uptake over the body surface only. Horizontal arrows indicate the levels of activity in each crayfish which can be attributed to the food only.

which each meal was given and the broken lines indicate the probable paths of uptake in the unfed animals. The Windermere animal, represented by the upper graph, had six meals at each of which worms equivalent to a mean of 4·2% of the body weight were eaten. In reaching a whole-animal concentration factor of 183 the Windermere crayfish shows a tendency to rise in activity between meals and after the final meal. This indicates that possibly the equilibrium concentration factor is similar to or exceeds 183. The con-

centration factors and plasma/medium ratios reached by these crayfish are given in Table 11 and the tissue/plasma ratios (denoted by F) are shown in Fig. 12. The tissue/plasma ratios for ^{137}Cs in tissues other than muscle are similar to those for other animals. Over equal periods of time the muscle/plasma ratios exceed those for starved crayfish and in the Windermere animal the ^{137}Cs ratio is higher than that for inactive K.

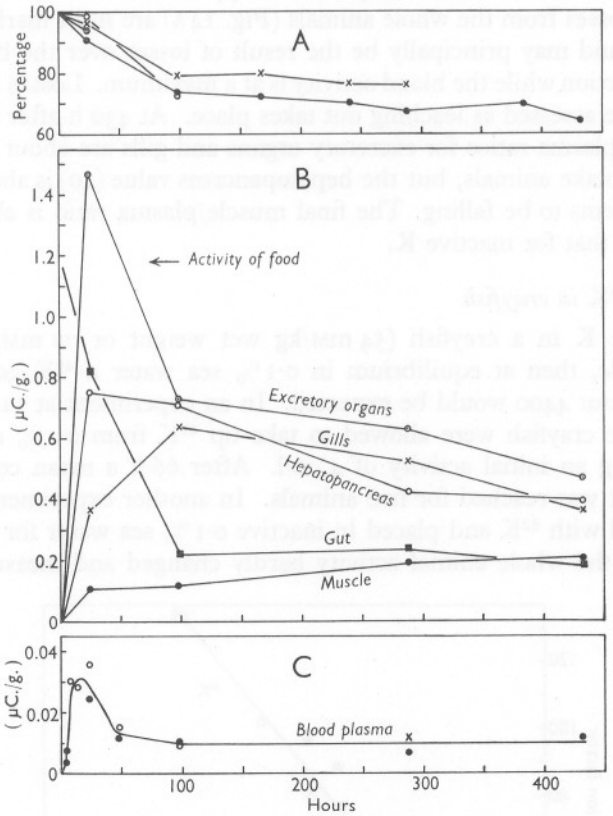


Fig. 14. A. Loss of ^{137}Cs from whole Windermere crayfish in inactive 0·1% sea water after a single meal of radioactive earthworms. B, C. Levels of ^{137}Cs in the plasma and tissues during the loss of isotope by the same crayfish.

In these experiments the chopped earthworms had a concentration factor of about 700 in the crayfish medium. Actually, the concentration factors attained by two earthworms after 500 h in 0·1% sea water were only 4·5 and 7·5. However, factors of the order of 1000 have been found for the soft parts of the gastropod *Hydrobia jenkinsi* in 0·1% sea water and crayfish readily eat these molluscs.

The absorption of ^{137}Cs from food and its subsequent loss in inactive 0·1%

sea water has been followed in four Windermere crayfish using the general methods employed with the prawns. The animals were given a meal of radioactive earthworms and then placed in inactive 0·1 % sea water prior to analysis of the tissues at intervals. Tissue activities are given in Fig. 14B, C. Digestion of food in the fore-gut is rapid so that after 24 h very little remains. Coinciding with this the activities of the plasma, excretory organs and hepatopancreas rise sharply. Most of the faeces produced appear between 24 and 36 h after feeding. Losses from the whole animals (Fig. 14A) are most marked over the first 100 h and may principally be the result of losses over the body surface and by excretion while the blood activity is at a maximum. Losses in the faeces could not be assessed as leaching out takes place. At 430 h after feeding, the final tissue/plasma ratios for excretory organs and gills are about the same as those for uptake animals, but the hepatopancreas value (40) is about twice as high and seems to be falling. The final muscle/plasma ratio is about 20 and approaches that for inactive K.

Uptake of ^{42}K *in crayfish*

If all the K in a crayfish (54 mM/kg wet weight or 72 mM/kg H_2O) is exchangeable, then at equilibrium in 0·1 % sea water a ^{42}K concentration factor of about 4400 would be expected. In an experiment at 11·5° C, small Windermere crayfish were allowed to take up ^{42}K from 0·1 % artificial sea water having an initial activity of 4 μC/l. After 66 h a mean concentration factor of 6·5 was reached for five animals. In another experiment a crayfish was injected with ^{42}K and placed in inactive 0·1 % sea water for 38 h. Over this period the whole animal activity hardly changed and measurements of

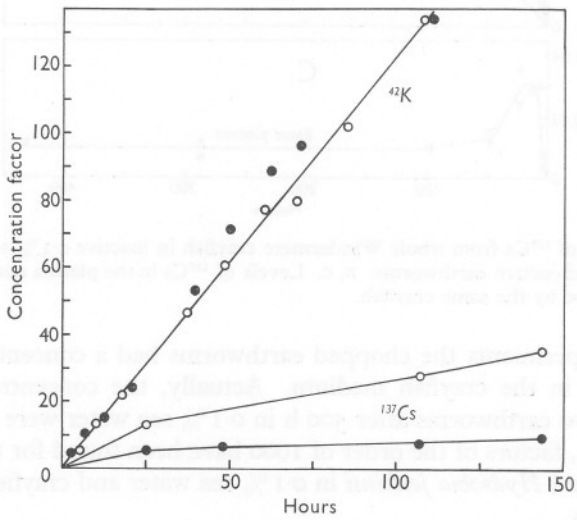


Fig. 15. Uptake of ^{42}K and ^{137}Cs by two whole Windermere crayfish each weighing about 3 g.

tissue/plasma ratios for ^{42}K indicated that nearly complete exchange between plasma and tissues had taken place. Other analyses 12 h after injection showed that the levels of ^{42}K in the tissues, expressed as a percentage of tissue/plasma ratios for inactive K, were 92% for excretory organs, 101% for the gills, 70% for hepatopancreas and 88% for muscle. Thus the exchange of ^{42}K between a whole crayfish and the medium depends on the slow rate of penetration over the body surface. As the uptake of ^{42}K is so slow the experiment was repeated at 20° C and then, following the decay of ^{42}K, uptake curves for ^{137}Cs were also obtained. Results for the uptake of both isotopes over similar lengths of time at 20° C are given in Fig. 15. By analysing the plasma and tissues of another animal after 72 h of uptake it was found that tissue/plasma ratios for ^{42}K and inactive K were almost identical. This again indicates the rapid exchange of ^{42}K between plasma and tissues. Assuming an equilibrium concentration factor of 4400 for ^{42}K and an exponential uptake of the isotope, the rate constant for uptake at 20° C is equal to 0·0003 h^{-1} which is about 10 times the value suggested by the results at 11·5° C.

Excretion of ^{137}Cs, K *and* Na *in crayfish*

Concentrations of K and Na and U/P ratios for ^{137}Cs, K and Na for animals from both sources in 0·1% sea water are given in Table 14. In Surrey crayfish a fairly constant urine K concentration of 0·41 mM/kg H_2O is found. Although the value for Windermere animals is higher, this is due to two high readings. The other six Windermere crayfish give a mean K concentration of 0·26 mM/kg H_2O. Values for urine Na concentrations are similar to those found by Bryan (1960*a*) for animals in Bristol tap water. Windermere crayfish give a mean U/P ratio for ^{137}Cs of 0·31 as compared with a mean K ratio of 0·14 for all animals or 0·058 for the six with low urine K concentrations. The mean U/P ratio for ^{137}Cs in Surrey crayfish is very high due to three values of more than 1·0. The remaining five animals give a mean U/P ratio of 0·33 which compares with the 0·31 for Windermere crayfish.

Results for Windermere crayfish have also been obtained under other external conditions. Table 14 shows that results in 0·01 and 1·0% sea water are not very different from those in 0·1% sea water. Animals which were allowed to take up ^{137}Cs from 50% sea water, after a week of acclimatization, give rather different results. The plasma concentrations of K and Na are increased but the most marked effects are seen in the high U/P ratios for all three ions. In 50% sea water a low urine production would be expected, but, if the animal is then replaced in very dilute sea water or tap water, urine production is higher than normal (Bryan, 1960*b*). When this is done, high U/P ratios are still found for all three ions 24 h after transfer to 0·1% sea water. Results showing the Na, K and ^{137}Cs concentrations during loss of ions in 0·1% sea water are given in Fig. 16A–C. To try and eliminate effects due to Na ions, a further experiment was carried out in which crayfish were kept in

1·0% sea water plus 5 mM/l. KCl and ^{137}Cs. The high K/Na ratio in this solution appears to prevent the animals from living as long as they would in 50% sea water. The Na balance remains substantially normal and possibly the urine production remains fairly normal, but the plasma K level increases to a far greater extent than in 50% sea water. U/P ratios for K and ^{137}Cs come to lie between the values found in 0·1 and 50% sea water. Previous results (Bryan, 1960*b*) indicated that in animals loaded with Na, as they are in 50% sea water, active Na uptake over the body surface and probably in the excretory organs is very limited. One possible explanation of the increased

TABLE 14. A COMPARISON OF K AND Na CONCENTRATIONS IN PLASMA AND URINE OF CRAYFISH AFTER UPTAKE OF ^{137}Cs FROM DIFFERENT MEDIA. THE U/P RATIOS FOR THE THREE IONS ARE ALSO COMPARED

Concentrations (mM/kg H_2O) and ratios are calculated on a water content basis. Figures in parentheses give the number of animals used.

Source of crayfish	Medium	Time in medium with ^{137}Cs (h)	Plasma		Urine		Ratio U/P		
			K	Na	K	Na	K	^{137}Cs	Na
Surrey	0·1 % s.w.	473–1562	(7) 3·6 ± 0·6	(2) 174 ± 10	(7) 0·41 ± 0·14	(2) 6·9 ± 2·8	0·114	(8)* 0·76 ± 0·6	0·04
Windermere	0·01 % s.w.	330	(2) 4·3 ± 0·5	(2) 173 ± 10	(2) 0·41	—	0·095	(2) 0·16	—
Windermere	0·1 % s.w.	94–1970	(10) 4·5 ± 0·8	(9) 188 ± 22	(8)† 0·63 ± 0·70	(3) 6·1 ± 2·5	0·140	(11) 0·31 ± 0·14	0·032
Windermere	1·0 % s.w.	287–328	(3) 5·0 ± 0·2	(3) 213 ± 23	(3) 0·42 ± 0·07	—	0·084	(3) 0·28 ± 0·04	—
Windermere	1·0 % s.w. with 5 mM/l. KCl	up to 240	(7) 10·6 ± 3·4	(7) 182 ± 24	(7) 2·76 ± 1·08	(7) 9·0 ± 7·8	0·260	(7) 0·45 ± 0·13	0·049
Windermere	50 % s.w.	240–261	(6) 6·0 ± 1·0	(5) 284 ± 10	(5) 4·0 ± 1·0	(4) 180 ± 84	0·67	(5) 0·96 ± 0·38	0·63
Windermere	24 h in inactive 0·1 % s.w. after 240 h in active 50 % s.w.		(3) 6·3 ± 1·4	(3) 262 ± 13	(3) 4·9 ± 1·2	(2) 151	0·78	(3) 0·79 ± 0·31	0·58

* Three of the figures exceeded 1·0, the highest being 2·03. The mean of the remaining five values is 0·33 ± 0·07.

† Two values of 1·98 and 1·49 gave a high mean. The mean of the remaining six values is 0·26 ± 0·07 which would give a U/P ratio of 0·058.

plasma K concentration is that it may be a result of K being taken up actively because the Na mechanism is still working at the body surface, and the relatively low U/P ratios for ^{137}Cs and K may be the result of additional retention in the excretory organs. Changes in the plasma K concentration are not always easily interpreted because they can be due to very slight losses from the tissues.

As with prawns and lobsters, a few experiments have been carried out with inulin to determine the possibility of water reabsorption in the excretory

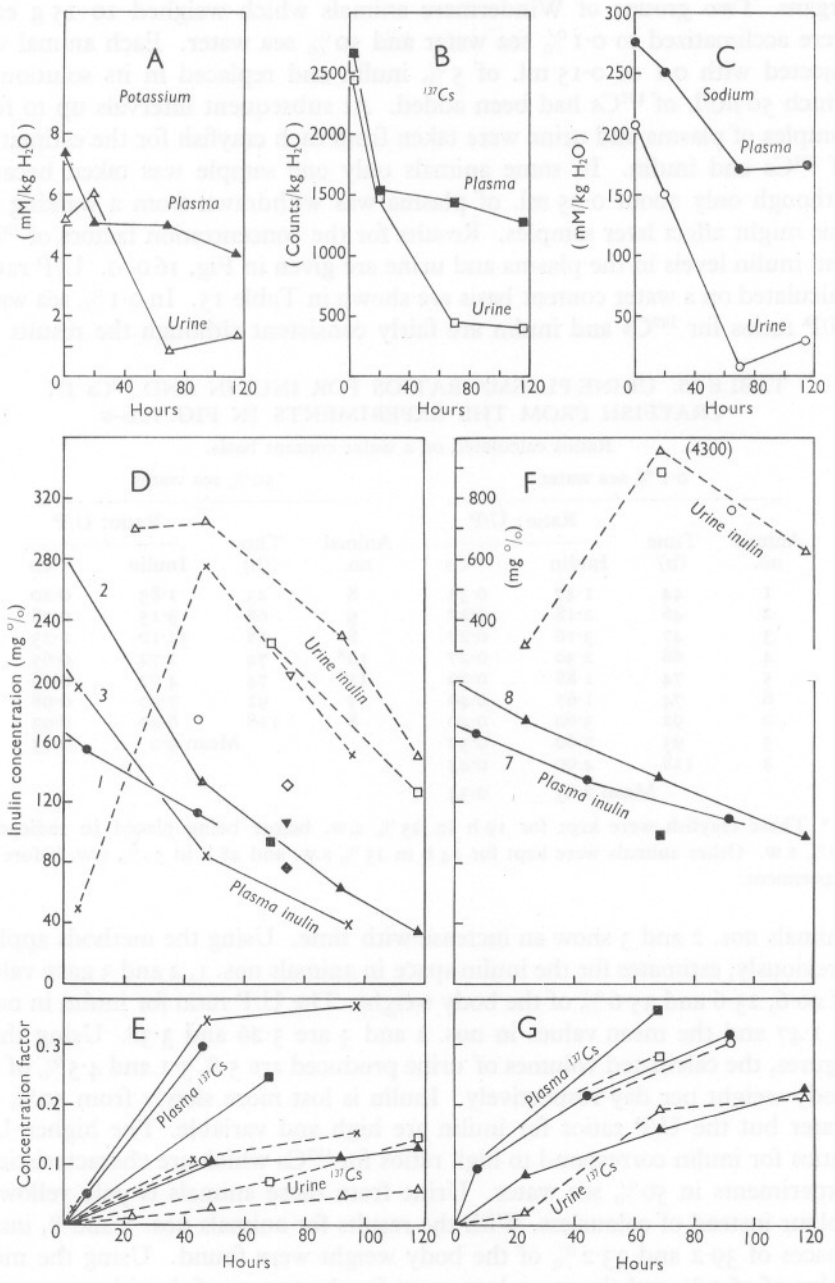


Fig. 16. A–C. Loss of K, ^{137}Cs and Na from the plasma and urine of a crayfish acclimatized to radioactive 50 % sea water which was replaced in inactive 0·1 % sea water. D, E. Levels of inulin and ^{137}Cs in plasma and urine of crayfish losing inulin in radioactive 0·1 % sea water. F, G. A similar experiment in 50 % sea water. Closed symbols and continuous lines are for plasma values and open symbols with broken lines are for urine values. In both experiments figures are corrected to correspond to animals weighing 10 g injected with 0·15 ml. of 5 % inulin and the curve numbers apply to the animals in Table 15.

organs. Two groups of Windermere animals which weighed 10–15 g each were acclimatized to 0·1% sea water and 50% sea water. Each animal was injected with 0·1 or 0·15 ml. of 5% inulin and replaced in its solution to which 50 μc/l. of ^{137}Cs had been added. At subsequent intervals up to four samples of plasma and urine were taken from each crayfish for the estimation of ^{137}Cs and inulin. In some animals only one sample was taken because although only about 0·05 ml. of plasma was withdrawn from a walking leg this might affect later samples. Results for the concentration factors of ^{137}Cs and inulin levels in the plasma and urine are given in Fig. 16D–G. U/P ratios calculated on a water content basis are shown in Table 15. In 0·1% sea water U/P ratios for ^{137}Cs and inulin are fairly consistent although the results for

TABLE 15. URINE/PLASMA RATIOS FOR INULIN AND ^{137}Cs IN CRAYFISH FROM THE EXPERIMENTS IN FIG. 16D–G

Ratios calculated on a water content basis.

0·1% sea water				50% sea water			
		Ratio: U/P				Ratio: U/P	
Animal no.	Time (h)	Inulin	^{137}Cs	Animal no.	Time (h)	Inulin	^{137}Cs
1	44	1·47	0·33	8	23	1·83	0·20
2	46	2·18	0·27	9	68	9·15	0·78
3	47	3·16	0·27	8	68	31·10	1·23
4	68	2·30	0·27	10*	74	2·72	0·65
5	74	1·88	0·30	11*	74	4·77	0·46
6	74	1·67	0·40	7	92	7·00	0·98
2	92	3·60	0·40	8	118	6·40	0·93
3	95	3·60	0·39			Mean 9·0	0·75
2	118	4·00	0·44				
		Mean 2·65	0·34				

* These crayfish were kept for 19 h in 25% s.w. before being placed in radioactive 50% s.w. Other animals were kept for 24 h in 25% s.w. and 48 h in 50% s.w. before the experiment.

animals nos. 2 and 3 show an increase with time. Using the methods applied previously, estimates for the inulin space in animals nos. 1, 2 and 3 gave values of 40·6, 23·6 and 35·6% of the body weight. The U/P ratio for inulin in no. 1 is 1·47 and the mean values in nos. 2 and 3 are 3·26 and 3·38. Using these figures, the calculated volumes of urine produced are 5·8, 3·1 and 4·5% of the body weight per day respectively. Inulin is lost more slowly from 50% sea water but the U/P ratios for inulin are high and variable. The higher U/P ratios for inulin correspond to high ratios for ^{137}Cs which are characteristic of experiments in 50% sea water. Urine from these animals is pale yellow in colour instead of colourless. With the results for animals nos. 7 and 8, inulin spaces of 39·2 and 33·2% of the body weight were found. Using the mean value of 36·2% and the mean loss curve for the two crayfish with an assumed U/P ratio for inulin of 7·5, calculation of the urine production gives a figure of 0·6% of the body weight per day.

If it is assumed that crayfish urine is produced as a filtrate which is then modified by the reabsorption of water and ions, the results in 0·1 % sea water indicate that Na is reabsorbed to a greater extent than K, although such a low urine concentration is not reached. In turn K is reabsorbed to a greater extent than ^{137}Cs. A link between the U/P ratios for inulin and ^{137}Cs is suggested by the increase in both ratios with time found in animals nos. 2 and 3. In 50 % sea water the high U/P ratios for Na and K could be the result of ion reabsorption in the excretory organs being limited in an attempt to prevent the salt concentration of the plasma from rising, with ^{137}Cs behaving similarly to K. On the other hand, in 50 % sea water the U/P ratios for inulin are so large that the high ratios for the ions could be explained to a large extent by water reabsorption. However, water reabsorption seems an unlikely explanation for the high U/P ratios for the three ions which are still found when a crayfish is transferred from 50 to 0·1 % sea water and urine production is increased. The U/P ratios for ^{137}Cs probably depend on this ion being treated like K to a large extent. However, if the excretory organs reabsorb ^{137}Cs in 0·1 and 50 % sea water they do so to a lesser degree than K and the resulting U/P ratio may, judging from the results in Table 15, come under the influence of water reabsorption to some extent.

As with the prawns and lobsters, an attempt has been made to assess the importance of the excretory organs in removing ^{137}Cs and K from the body. A rate constant for ^{137}Cs uptake in a Windermere crayfish of 0·0019 h^{-1} has been tentatively given. This value was calculated assuming that the whole-animal equilibrium concentration factor was 50. These figures result in a rate of influx for ^{137}Cs of 0·095 of the whole-animal concentration factor per hour. At what appeared to be equilibrium the urine concentration factor was 0·84. If the urine production is 5 % of the body weight per day this gives a loss in the urine of 0·00175 of the whole-animal concentration factor per hour. At equilibrium influx and outflux are equal so that the excretory organs are responsible for about 1·8 % of ^{137}Cs outflux. A rate constant of 0·0003 h^{-1} was found at 20° C for the uptake of ^{42}K in Windermere crayfish. If the whole animal K concentration is 54 mM/kg and the urine K concentration is 0·4 mM/kg, then at equilibrium the outflux of ^{42}K is 0·0162 mM/kg animal/h and the outflux in the urine is 0·00083 mM/kg animal/h. Thus the excretory organs are responsible for about 5 % of the total ^{42}K outflux. These figures must be extremely variable.

DISCUSSION

In discussing the results they will be compared with those found in *Carcinus* using ^{134}Cs (Bryan, 1961). As in *Carcinus*, whole lobsters and prawns accumulate ^{137}Cs much more slowly than ^{42}K and at equilibrium the ^{137}Cs concentration factors exceed those for inactive K. Unlike *Carcinus*, both lobsters

and prawns maintain a plasma K concentration which is lower than that of sea water. As equilibrium is approached the ^{137}Cs level of lobster plasma reaches that of sea water, but in prawns the concentration can be more than double that of the sea water. This is one of the reasons why unfed prawns can attain whole animal concentration factors of 25 as opposed to values of less than 10 in the lobster and *Carcinus*.

Whole Windermere crayfish in 0·1% sea water take up ^{137}Cs to rather indeterminate concentration factors of the order of 50–200 depending on the animal. Surrey crayfish take up the isotope more slowly and the results suggest that there may be a physiological difference between the two types. The equilibrium concentration factors which can be reached depend on the extent to which ^{137}Cs is accumulated by the plasma. In a crayfish which may have reached equilibrium at a concentration factor of 183 the plasma/medium ratio for ^{137}Cs was about 17 as opposed to over 400 for inactive K. In the lobster an extrarenal mechanism may exist in the gills for excreting K to maintain the low plasma concentration. On the other hand the crayfish actively accumulates K. Levels reached by ^{137}Cs in the plasma of both these animals can be explained if the mechanisms for moving K are much less efficient in moving ^{137}Cs. Prawns, however, appear to be able to exclude K from the plasma independently of the excretory organs and yet accumulate ^{137}Cs from the medium. It seems unlikely that there would be a specific mechanism in prawns for taking up Cs through the gills. This type of result could be arrived at if the mechanisms at the outer and inner walls of the gill cells, which maintain the cell K level and possibly cause the K concentration of the plasma to remain below that of sea water, have different specificities for Cs and different tendencies to lose the ion. Differences of this type in the relative treatment of K and Cs can be used to explain the high tissue/plasma ratios for ^{137}Cs which are found for the excretory organs and the high concentration factors in the gills.

In all three species the soft tissues take up and lose ^{137}Cs in order of decreasing rate: excretory organs, hepatopancreas and muscle with the rate in the gills appearing to be similar to that in the excretory organs. Muscle is the principal limiting factor in the attainment of ^{137}Cs equilibrium by whole animals. Unfed prawns and fed crayfish resemble *Carcinus* in that when equilibrium is approached all tissue/plasma ratios for ^{137}Cs exceed those for inactive K. In muscle the discrepancy between the ratios is much less than in *Carcinus*. Muscle/plasma ratios for ^{137}Cs in lobsters and fed prawns are below those for K, as equilibrium had not been reached, but ^{137}Cs values for the hepatopancreas are also well below those for inactive K. It is expected that at equilibrium all muscle/plasma ratios for ^{137}Cs would exceed those for K but not to the extent which is found in *Carcinus*. Thus in lobsters, prawns and crayfish the distribution of ^{137}Cs at equilibrium in muscle may come closer to the passive distribution which would be expected if the modified

theory of Boyle & Conway (1941) applies. This theory has been discussed in relation to ^{134}Cs in *Carcinus* muscle by Bryan (1961).

Apart from being generally much more rapid, the uptake of ^{42}K by the three species differs from ^{137}Cs uptake in a number of respects. In both lobsters and crayfish the exchange of ^{42}K between plasma and tissues is sufficiently rapid for the body surface to be the limiting factor in uptake. Also there does not appear to be such a wide discrepancy between the rates at which ^{42}K is taken up by muscle and hepatopancreas as there is with ^{137}Cs. In prawns, muscle probably shares the limiting role with the body surface and ^{42}K is exchanged with the muscle rather more slowly than with the hepatopancreas. All inactive K in these animals appears to be exchangeable with ^{42}K.

In considering excretion, it will be assumed that a primary urine is produced by filtration and is then modified as a result of the reabsorption or secretion of water and ions. Both lobsters and prawns resemble *Carcinus* in producing a urine which can have a U/P ratio for ^{137}Cs of more than 2·0. Reigel & Lockwood (1961) have found U/B ratios of more than 2·0 for inulin in *Carcinus*. This suggests that the high U/P ratios for ^{137}Cs might be a result of water reabsorption. When this was checked in lobsters the mean U/P ratios for ^{137}Cs were considerably more than the values for inulin, which indicates that a significant amount of the isotope is secreted into the urine. On the other hand, the prawn results suggest that water reabsorption is mainly responsible for the high U/P ratios for ^{137}Cs and that only a small amount is secreted. In crayfish from 0·1 % sea water, U/P ratios of about 0·1, 0·3 and 0·04 are found for K, ^{137}Cs and Na despite what appears to be the reabsorption of water. The relatively higher concentration of ^{137}Cs than K can be explained in terms of the slower reabsorption of this ion in the excretory organs by the mechanism for reabsorbing K. As K and Na ions move more rapidly than ^{137}Cs it is possible that their U/P ratios may be relatively unaffected by water reabsorption. With ^{137}Cs the results suggest that higher U/P ratios are found if more water is reabsorbed. In 50 % sea water the U/P ratios for all three ions are increased towards 1·0 and water reabsorption is very high and variable, with again the suggestion that higher U/P ratios for ^{137}Cs are to some extent associated with the higher levels of water reabsorption. The extra water reabsorption in 50 % sea water could to a large extent account for the higher U/P ratios for the ions. However, when animals acclimatized to 50 % sea water are transferred to 0·1 % sea water the urine production increases from about 0·6 % to rather more than the normal figure of around 5 % of the body weight per day. In the 0·1 % sea water, water reabsorption is likely to be at least as low as normal, but initially high U/P ratios are still found for the three ions. This indicates that the high ratios may be the result of the curtailment of ion reabsorption in the excretory organs while the excess salt gained in 50 % sea water is excreted.

The excretory organs are important in the metabolism of ^{137}Cs in lobsters and prawns because at equilibrium they account for about 30% of ^{137}Cs outflux. On the other hand, they account for only about 1% of ^{42}K outflux. In the crayfish, the excretory organs appear to be less important in ^{137}Cs metabolism and possibly more important in K metabolism than in the marine animals. The effect of the excretory organs in crayfish is probably very variable.

In both prawns and crayfish the absorption of ^{137}Cs from food is rapid and, as in *Carcinus*, probably takes place mainly through the hepatopancreas. Both species were fed on animal material having as high a concentration factor as they would be likely to encounter in a uniformly contaminated medium. The results suggest that in prawns, feeding on active food will enhance the rate at which equilibrium is reached but will not raise the activity of the animals very much above the equilibrium level. In crayfish, feeding on highly active food, such as small molluscs, is probably more important than absorption over the body surface in attaining and exceeding the equilibrium concentration factor. The slow loss of ^{137}Cs in a radioactive environment, once it has been gained by crayfish is demonstrated by the very slow loss of isotope from an animal boosted to a concentration factor of about 600 (Fig. 11C).

Generally speaking, ^{137}Cs behaves similarly to K in for instance being concentrated by the plasma and tissues of crayfish and being excreted at a concentration below that of the plasma. Here, differences between K and ^{137}Cs seem to be of degree only. What may be more important differences are the accumulation of ^{137}Cs by prawn blood while K is excluded and the apparent secretion of the isotope into the urine of lobsters and to some extent prawns. The actual solution to such problems may lie in the possibility of doing experiments with isolated tissues as well as with whole animals.

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SUMMARY

The accumulation of ^{137}Cs from sea water has been examined in relation to potassium metabolism in the lobster *Homarus vulgaris* and in the prawn *Palaemon serratus*. In unfed animals ^{137}Cs is taken up and lost far more slowly than ^{42}K. Although all the inactive K in the animals can be exchanged with ^{42}K, higher whole-animal concentration factors are reached for ^{137}Cs (about eight for lobsters and twenty-five for prawns). This is because both species

have higher plasma/medium ratios for ^{137}Cs than K at equilibrium despite the selective excretion of ^{137}Cs. Also, except for the hepatopancreas in lobsters and fed prawns, all soft tissues can probably attain higher tissue/plasma ratios for ^{137}Cs than inactive K.

Uptake of both isotopes has also been studied in the freshwater crayfish *Austropotamobius pallipes pallipes*. In crayfish in 0·1% sea water ^{137}Cs is not concentrated to the same extent as K by whole animals (50–200 for ^{137}Cs against about 4500 for K). Although the situation between plasma and tissues resembles that in the marine animals, ^{137}Cs cannot be accumulated in the plasma to the same degree as K. Crayfish selectively excrete ^{137}Cs in the urine relative to K at a lower concentration than in the plasma.

In the accumulation of ^{137}Cs by all species, muscle is the principal limiting factor in uptake and loss, but with ^{42}K the body surface becomes more limiting.

Experiments on the absorption of ^{137}Cs from food in prawns and freshwater crayfish have been carried out. In prawns in a constant environment, feeding is probably less important than uptake over the body surface while in crayfish feeding is probably much more important.

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