UPTAKE AND RETENTION OF CESIUM 137 AND ZINC 65 BY SEAWEEDS[1]

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ABSTRACT

Uptake and retention of Cs^{137} and Zn^{65} by seaweeds were observed under various environmental conditions. Concentration factors and biological half-lives varied widely among species, Zn^{65} showing generally higher levels of uptake and retention. No significant relationship between growth rate and either concentration factor or biological half-life was found among the species tested.

Both uptake and loss of Cs^{137} were stimulated by light, but some Cs^{137} movements occurred in the dark. Both exogenous substrate (glutamate) and previous exposure to light stimulated Cs^{137} uptake in the dark. Exposure to nitrogen in the dark inhibited both influx and efflux of Cs^{137}. Neither darkness nor anoxia caused appreciable net loss of absorbed Cs^{137}. Cs^{137} was not extensively adsorbed by cell walls or killed tissue. Influx and efflux of Cs were proportional, respectively, to the external and internal Cs concentrations. Intracellular Cs appears to be largely ionic, with Cs movements being closely related to metabolism.

Zn^{65} was highly concentrated by living and killed algae and by cell walls. Light had a variable effect on Zn^{65} movements. Zinc uptake at different external Zn concentrations agreed with the Freundlich adsorption equation. Adsorption exchange appears to be an important mechanism of Zn movements in seaweeds.

INTRODUCTION

The increasing occurrence of Cs^{137} and Zn^{65} in coastal waters has stimulated recent studies on the uptake and retention of these radionuclides by both aquatic biota and sediments (for reviews, *see* Davis 1963 and Rice 1963*a*). Seaweeds, although they may be the principal primary producers of the littoral zone (Blinks 1955), have been less studied than phytoplankton with regard to environmental contamination. However, seaweeds are often involved in food chains leading to man, since they are harvested commercially for food, feed, and fertilizer (*see* Davy de Virville and Feldmann 1964). The importance of seaweeds in radioactive waste disposal was demonstrated at the Windscale Atomic Works, England, where the activity absorbed by edible *Porphyra* limited the permissible rate of release (Dunster 1958).

The uptake of both Cs^{134} and Zn^{65} has been reported to be related to photosynthesis in seaweeds, thus suggesting a possible use for these tracers in studies of energy flow. Scott (1954) found that accumulation of Cs^{134} by *Rhodymenia* was dependent upon light and CO_2, and he concluded that there was an intimate connection between the mechanisms of cesium absorption and photosynthesis. Bachmann and Odum (1960) also reported a relationship between productivity and Zn^{65} uptake by marine algae, but their method has been criticized (Gutknecht 1961).

Zinc is an essential nutrient for algae (*see* Wiessner 1962), as well as for higher plants and animals (*see* Vallee 1962 and Nason and McElroy 1963). Cesium is apparently a nonessential trace element, but it is accumulated by most cells in a manner qualitatively similar to potassium (*see* Relman 1956). Thus, the biological uptake of Zn^{65} and Cs^{137} is of interest from a physiological, as well as from an ecological and health physics, point of view.

This paper is a report on the uptake and retention of Zn^{65} and Cs^{137} by some seaweeds from the Woods Hole, Massachu-

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setts, and Beaufort, North Carolina, areas. Terminology will conform in general to that used by Laties (1959) in describing various types of ion uptake and loss by plant tissues.

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METHODS

Algae were collected from the Woods Hole and Beaufort harbors and kept in Plexiglas tanks of continuously aerated seawater under natural illumination (north window exposure). Small portions of the plants were exposed to Cs^{137} and Zn^{65} in 1-liter flasks of aerated seawater medium. Periodically, the plants were removed from the flasks and their radioactivity and fresh weight determined after blotting with absorbent tissue.

The medium was Millipore®[3]-filtered (Type AA) seawater to which 20 μM each of $NaNO_3$ and NaH_2PO_4/liter were added. The medium was replaced every day so that significant depletion of the tracers did not occur. All solutions were buffered at *p*H 8.0 with Tris (hydroxymethyl) amino methane. Experiments were carried out either in the dark or at a constant illumination of 7,500 lux provided by six "daylight" fluorescent tubes. The temperature was 21 ± 2C.

Cs^{137} and Zn^{65} uptake by killed algae (killed by cold 50% ethanol or heat) and by isolated cell walls of *Ulva lactuca* also was observed. *Ulva* thalli are sometimes found to have transparent borders in which every cell has liberated gametes or zoospores following a repeated bipartition of the entire protoplast (Smith 1955). Microscopic examination reveals two layers of empty cells that are relatively free of debris and microorganisms. Thus, an estimate of Zn^{65} or Cs^{137} uptake by cell wall vs. living cell could be obtained by comparing equal areas of empty-cell material and living tissue from adjacent sites on the same thallus.

Total zinc was determined as follows: Samples were digested with concentrated HNO_3, taken to dryness, and placed in a muffle furnace overnight at 475C. They were then taken up in 2 N HCl, and the zinc was separated by ion exchange, using the method of Kraus and Moore (1953). The zinc-containing effluent was analyzed by a modification of the dithizone technique of Vallee and Gibson (1948). Results were corrected for radiochemical yield.

Radioactivity was measured with either a conventional well-type scintillation detector for integral counting or a single-channel analyzer for differential counting of Zn^{65}-Cs^{137} or Cs^{137}-Cs^{134} mixtures. In most experiments, the activity of the medium was 4 μc Zn^{65} and 20 μc Cs^{137}/liter. Unless otherwise indicated, carrier-free tracers were used, so that the natural concentration of zinc and cesium was not appreciably increased. Since chemical analyses for total cesium were not feasible in this study, net cesium movements were deduced from Cs^{137} or Cs^{134} fluxes. The natural cesium content of seawater was assumed to be 0.5 ± 0.05 μg/liter (Smales and Salmon 1955).

RESULTS

Concentration factor and biological half-life of Zn^{65} and Cs^{137} in seaweeds

Algae were exposed to Zn^{65} and Cs^{137} in the light as described above. When they reached an apparent steady state with the radioactivity of the medium (5–40 days, depending on the species), they were transferred to nonradioactive seawater, and the loss of activity was observed. The biological half-life ($T_{b1/2}$) of each isotope was estimated by the method of Odum and Golley (1963). Upon completion of the

[3] Registered trademark, Millipore Filter Corporation, Bedford, Massachusetts.

TABLE 1. *Uptake and retention of Zn^{65} and Cs^{137}, total Zn, and growth rate of seaweeds. Uptake expressed as concentration factor* $\left(\frac{count\ min^{-1}\ g^{-1}\ fresh\ wt}{count\ min^{-1}\ ml^{-1}\ medium}\right)$ *(CF), and retention expressed as biological half-life* ($T_{b½}$)*

Species	Cs^{137}		Zn^{65}		Total zinc (mg/kg)		Growth rate (% increase fresh wt/day)
	CF	$T_{b½}$ (days)	CF	$T_{b½}$ (days)	Fresh wt	Dry wt	
Ulva lactuca	7	5	290	4	23.8	158	15.0
Codium decorticatum	4	15	30	7	0.96	17.0	7.9
Fucus vesiculosus	30	8	3,300	100	124	472	3.0
Dictyota dichotoma	10	—	280	14	5.70	35.0	8.2
Porphyra umbilicalis	5	3	255	7	—	—	7.5
Chondrus crispus	30	2	—	—	—	—	1.8
Gracilaria foliifera	25	12	210	60	5.83	37.7	5.3
Agardhiella tenera	6	21	395	70	9.78	91.4	5.2
Hypnea musciformis	11	—	150	—	3.54	23.2	5.5

* Conditions: constant illumination (7,500 lux), pH 8.0, 21C. Each value is the average of samples from at least 4 plants.

experiment, the algae and media were analyzed for total zinc. Results are shown in Table 1.

Zn^{65} uptake among species varied by about two orders of magnitude. *Fucus* showed by far the highest concentration factor (CF), 3,300, while *Codium* showed the lowest, 30. Concentration factors for Cs^{137} were less variable, ranging from 30 in *Fucus* to 4 for *Codium*. The values for Cs^{137} are slightly lower than those reported by Smales and Salmon (1955), who used radioactivation analysis for total cesium in seaweeds. Biological half-lives ranged from >100 days for Zn^{65} in *Fucus* to 2 days for Cs^{137} in *Chondrus*. There was no significant correlation between net production, that is, growth rate, and the CF or $T_{b½}$ of Cs^{137} or Zn^{65} in these species.

Total zinc concentrations ranged from 124 mg/kg fresh wt for *Fucus* to 0.96 mg/kg in *Codium*. The zinc content of the medium was 49 μg/liter. The final specific activity of Zn^{65} in the medium was approximately the same as the specific activity of Zn^{65} in the algae, the CF values for total zinc being from 0.41 to 1.7 times those for Zn^{65}.

Nonmetabolic uptake of Cs^{137} and Zn^{65}

The uptake of an element by killed cells is sometimes used to estimate the amount of nonmetabolic absorption in living cells (Kuenzler and Ketchum 1962; Rice 1956; and others). This method gave low CF values of 1–2 for Cs^{137} in killed tissues of all species and cell walls of *Ulva*. Nonmetabolic uptake by living tissue also may be estimated by the size of the "absorption shoulder," that is, the intercept of the linear phase of the uptake-time curve (Laties 1959). This method would be expected to yield values somewhat lower than those obtained with killed tissue, since it is mainly a measure of uptake by extracellular components. Concentration factors for Cs^{137} obtained by this method ranged from 0.4 to 0.5. Furthermore, seaweeds which had been exposed to Cs^{137} for up to 35 days released more than 90% of their activity when killed and kept for several hours in the same radioactive medium. Thus, neither physical adsorption, adsorption exchange, nor nonexchangeable binding can account for much of the Cs^{137} taken up by these algae.

In contrast, considerable nonmetabolic uptake of Cs^{137} by both living and killed freshwater plants has been reported (Krumholz 1954; Williams 1960; and others). However, competition for potential Cs^{137} adsorption sites in freshwater material is undoubtedly less than in seawater. For example, it was found that killed seaweeds took up 5–10 times more Cs^{137} in radioactive tap

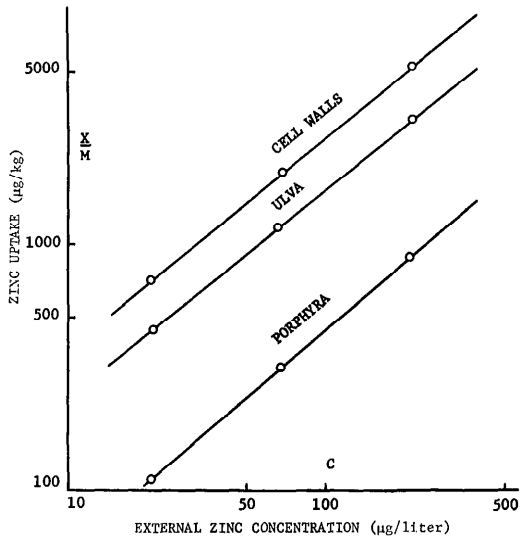

FIG. 1. Effect of external Zn concentration on uptake by *Porphyra*, *Ulva*, and cell walls of *Ulva*. Time of exposure: 24 hr in the light. Each point is the average of 4–6 discs of tissue.

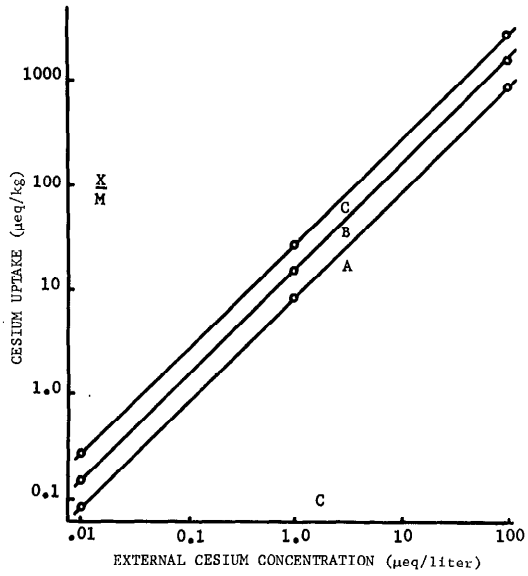

FIG. 2. Effect of external Cs concentration on Cs uptake by *Gracilaria* after 1 day (A), 2 days (B), 9 days (C) in the light. Each point is the average of 6–12 branches.

water at *p*H 8.0 than in a seawater medium of the same activity. Similarly, the sorption of Cs^{137} by estuarine sediments has been reported to be inversely related to salinity (Duke 1963).

The empirical Freundlich isotherm (Freundlich 1926) has been used widely to express data on nonmetabolic adsorption in many different systems. The mathematical relationship is given by

$$x/m = ac^b$$

or $$\log (x/m) = \log a + b \log c$$

where x is the amount of solute taken up, m is the unit mass of adsorbent, c is the concentration of solute in solution, and a and b are constants for a specific system at constant temperature. As noted by Samuelson (1953), the validity of the adsorption isotherm is not proof of a physical adsorption (largely van der Waals forces), since uptake by ion exchange (that is, adsorption exchange) may give a similar pattern. Recently, Bachmann (1963) found the uptake of Zn^{65} by freshwater plankton, detritus, and sediments to be best described by this expression.

Zn^{65} uptake by *Ulva*, *Porphyra*, and cell walls of *Ulva* was found to fit the adsorption equation (Fig. 1). Also, it was found that killed seaweeds generally took up more Zn^{65} than did living algae. Possible reasons for this are given elsewhere (Gutknecht 1963). In addition, empty cell walls of *Ulva* took up as much Zn^{65} during an 8-hr period as did a similar number of living cells. These results are consistent with earlier data that suggested that nonmetabolic adsorption exchange plays an important role in Zn^{65} uptake by seaweeds (Gutknecht 1961, 1963).

A plot of log Cs uptake vs. log external Cs concentration was also linear (Fig. 2). However, x/m in this case did not increase less rapidly than c. That is, uptake was exactly proportional to external concentration, which is not likely in an adsorption-type reaction (Briggs, Hope, and Robertson 1961). Similar results were obtained with *Fucus*, *Ulva*, and *Porphyra*. Thus, these data are consistent with the above results on nonmetabolic Cs^{137} adsorption and suggest that Cs^{137} in seaweeds is mainly ionic and not extensively adsorbed.

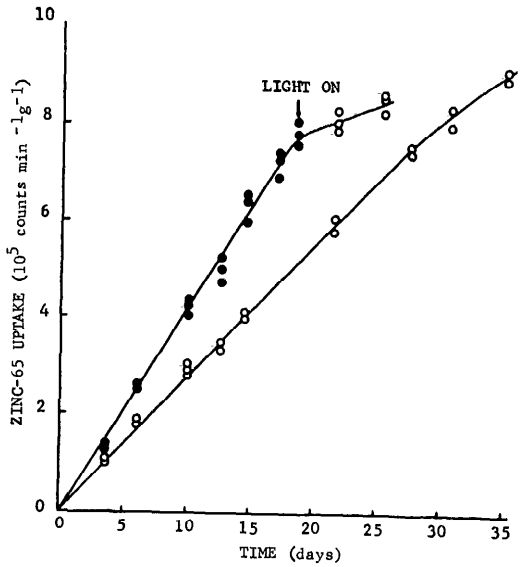

FIG. 3. Effect of light (○) and dark (●) on Zn^{65} uptake by *Fucus*. Each point is the average of 2–3 branches.

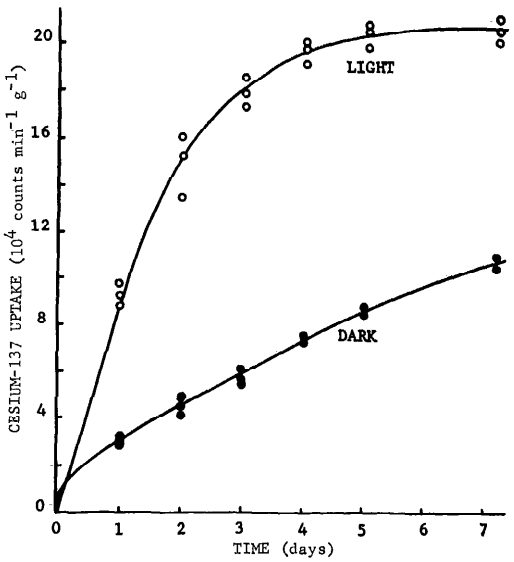

FIG. 4. Effect of light (○) and dark (●) on Cs^{137} uptake by *Ulva*. Each point is the average of 6 discs of tissue.

Effects of metabolism on Cs^{137} and Zn^{65} uptake and retention

Algae were exposed to Cs^{137} and Zn^{65} in both the light and the dark. It was found previously that in two fast-growing species, *Ulva* and *Porphyra*, the young tissue showed a higher Zn^{65} activity than older tissue (Gutknecht 1963). In *Fucus*, however, the opposite was true, that is, nongrowing tissue concentrated Zn^{65} at a higher rate than growing algae (Fig. 3). The explanation for this may derive from the physical structure of *Fucus* and its high affinity for zinc. Gelatinous algin, characteristic of brown algae, fills the intercellular spaces (Smith 1955) and may retard the attainment of equilibrium between the Zn^{65} in the medium and the tissue. For example, it took 2–3 days for killed *Fucus* to reach equilibrium with Zn^{65} in the medium, whereas killed tissue of other species in Table 1 became equilibrated in several hours. Also, the nonphotosynthesizing *Fucus* showed a gradual loss of weight (about 0.5%/day) without a concomitant loss of Zn^{65}. This would result in a gradually increasing concentration factor regardless of the normal steady-state level of Zn^{65} in the plant.

Cs^{137} uptake was stimulated by light in all species. This was first observed by Scott (1954), who suggested an intimate connection between the mechanisms of cesium accumulation and photosynthesis in *Rhodymenia*. Scott's observations, however, included a "virtual exclusion" of Cs^{134} in the dark, an observation not confirmed with the present species. In some algae, for example, *Ulva* and *Porphyra*, Cs^{137} accumulation after 7 days in the dark was from 30 to 60% of that in the light (Fig. 4). In some others, for instance, *Chondrus* and *Gracilaria*, Cs^{137} uptake in the dark was much slower but was demonstrated by a comparison with Cs^{137} uptake in a nitrogen atmosphere (Fig. 5).

The effects of anoxia (continuous bubbling with N_2 in the dark), exogenous substrate (monosodium glutamate, 50 mM), previous exposure to light (72 hr at 7,500 lux), nutrients (PO_4^{-3} and NO_3^- at 20 μM/liter), and continuous light (7,500 lux) were investigated in *Gracilaria*. Owing to the nonuniform cell population in *Gracilaria*

(cells range from about 10 to 600 μ diameter), the determination of ion fluxes is difficult (*see* MacRobbie and Dainty 1958). To obtain relative values, however, the linear portions of the uptake-time curves were compared. Compared with the dark controls, light increased the rate of Cs^{137} uptake by a factor of 45, glutamate increased uptake by a factor of 6.0, and anoxia decreased uptake by a factor of 0.31. Plants previously exposed to light and then placed in Cs^{137} medium in the dark absorbed Cs^{137} at an initial rate comparable to plants in the light, but after 20–30 hr, the rate of uptake decreased to a value similar to the dark controls. In agreement with the findings of Scott (1954), it was also found that small amounts of phosphate and nitrate slightly stimulate Cs^{137} uptake in the light.

In *Gracilaria*, a plot of log per cent activity remaining vs. log time was linear (Fig. 6). Although the linearity of the full-log plot may be fortuitous, it provided a means of comparing loss rates over a period of several days. As compared with the dark control, Cs^{137} efflux from 1 to 5 days was increased by a factor of 2.3–3.0 in the light and decreased by a factor of 0.57 in nitrogen. The fact that cells are dividing and enlarging in Cs^{137} medium in the light may partly account for the relatively greater effect of light on uptake than on loss of Cs^{137}. Rice (1963*b*) showed that Cs^{137} uptake by *Nannochloris* closely followed the growth of the cells.

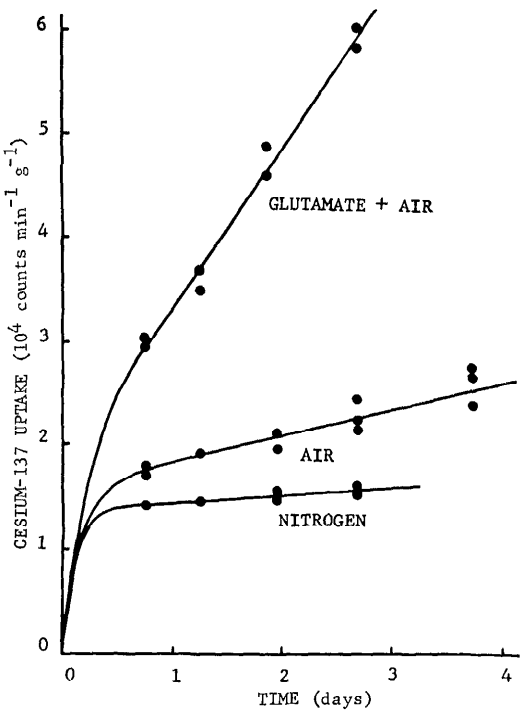

FIG. 5. Effects of anoxia and glutamate (50 mM) on Cs^{137} uptake by *Gracilaria* in the dark. Each point is the average of 4–5 branches.

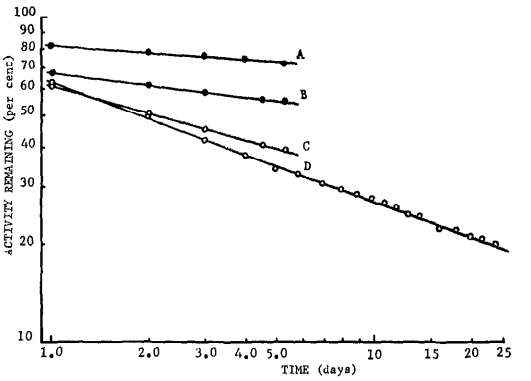

FIG. 6. Effects of anoxia (A), darkness (B), and light (C) and (D) on Cs^{137} loss from *Gracilaria* (D is a separate experiment). Alga previously exposed to Cs^{137} for 36 hr in the light. Each point is the average of 6 branches.

The effects of dark and anoxia on net movements of cesium were also investigated. *Gracilaria* that had accumulated Cs^{137} for 6–9 days to a steady state in the light was kept in the same radioactive medium in aerated dark flasks and dark flasks bubbled with nitrogen. Less than 5% of the Cs^{137} was lost from the alga either under anoxia (5 days) or in the dark (7 days). Thus, neither respiration nor photosynthesis was required to maintain an intracellular concentration factor of more than 25. However, as noted above, in the absence of air, and especially of light, both influx and efflux of Cs^{137} were markedly reduced.

DISCUSSION

Mechanism of Cs^{137} and Zn^{65} movements

Cs^{137} showed little tendency to be adsorbed by either living or freshly killed algae. However, if it is assumed that intracellular cesium is largely ionic, Cs^{137} was accumulated against a concentration gradient in all species tested. This may not necessitate active transport of cesium, because negative intracellular potentials similar to those reported for *Rhodymenia* (MacRobbie and Dainty 1958) or *Halicystis* (Blount and Levedahl 1960) could account for passive accumulation of Cs^{137} to the levels found in most species.

It was shown in Fig. 2 that Cs^{137} uptake is proportional to external Cs concentration over a range of 0.01–100 μM. It was found also that efflux of Cs^{137} is proportional to the intracellular cesium concentration. That is, algae which have accumulated 10^4 times as much cesium as the controls lose cesium 10^4 times as fast in natural seawater. Furthermore, it was found in double-label experiments that *Gracilaria*, while losing Cs^{137}-labeled cesium at a rate 10^4 times that of the controls, absorbed carrier-free Cs^{134} at a rate identical to that of the controls, that is, efflux initially exceeded influx by a factor of 10^4. Therefore, an obligatory exchange of internal Cs for external Cs is not involved in cesium movements at these concentrations. However, the fact that in *Gracilaria* neither light, dark, nor anoxia resulted in net Cs movements indicates that the observed Cs^{137} fluxes do involve an exchange of some type, probably with a more abundant cation such as K^+.

The highest external cesium concentration employed (100 μM) caused no reduction in the rate of Cs^{137} uptake. If a carrier is involved in Cs transport, however, it is probably identical with the potassium carrier, as has been shown in many cell types. At an external Cs concentration of 100 μM, Cs influx in the light was about 75 μM/kg cell water $\times$ hr. In *Gracilaria*, this is less than 0.1% of the K influx (Gutknecht, unpublished data). Thus, the K carrier would be far from saturation with respect to Cs, a condition kinetically indistinguishable from free diffusion (Rosenberg and Wilbrandt 1955).

With regard to Zn^{65}, the present data are consistent with an earlier hypothesis that Zn^{65} movements involve largely adsorption exchange, although Zn^{65} turnover is to some extent related to metabolism (Gutknecht 1961, 1963). As indicated by the extensive uptake by living and killed tissues and cell walls, both the cytoplasm and extracellular components appear to have large numbers of potential binding sites for Zn^{65}. This might be expected, since the high affinity of zinc for organic ligands is well known (Goldberg 1957; Lowman 1960). In freshwater plankton and detritus, the mechanism appears similar (Bachmann 1963).

Environmental contamination by Cs^{137} and Zn^{65}

A knowledge of the steady-state concentration factor for a radionuclide in an organism aids in evaluating the hazards of fallout and waste disposal. As noted earlier, there is a considerable difference between the high CF values found for Cs^{137} in living and killed freshwater algae (Williams and Swanson 1958; and others), and the low CF values found for marine algae (Boroughs, Chipman, and Rice 1957; and the present study). It was shown above that part of this variation is probably due to salinity differences. Part of it, however, is due to the method of determining the CF. As noted by Rice (1963*b*), in the experiments of Williams and Swanson the external Cs^{137} concentration was markedly reduced because of uptake by the cells, thus proportionally increasing the final CF. In the experiments of Boroughs, Chipman, and Rice, and in the present study, the external Cs^{137} concentration was constant.

In nature, either of the above conditions might occur, depending, for example, on whether the release of Cs^{137} from a reactor was continuous or periodic. However, uptake during a short exposure in the light was more rapid than loss during a similar period in nonactive seawater. Therefore, a high level of environmental Cs^{137} for a short time may have a disproportionately

large effect on the contamination hazard. This was also true of Zn^{65}.

Sediments, light, *p*H, and nutrients will also influence the uptake and loss of these radionuclides in nature. In coastal waters, for example, the sediments will probably remove some Cs^{137} (Duke, Ibert, and Rae 1963) and a large proportion of Zn^{65} (Duke and Willis 1964). The variable effects of light and *p*H on Zn^{65} uptake and loss have been shown in this and earlier studies (Gutknecht 1963). Similarly, studies on the uptake and retention of Cs^{137} are complicated by the effects of light and, in some cases, by the presence of intracellular compartments that show different rates of Cs^{137} turnover. In general, consideration might be given to releasing reactor wastes at night, since the uptake rates of Zn^{65}, and especially Cs^{137}, were usually higher in the light.

One natural means of reducing the hazard of a radionuclide in the ocean is the buffer action of seawater (Revelle 1955), also called the "specific activity approach" to waste disposal (Isaacs et al. 1962). The 700 megatons of cesium in the sea, for example, will greatly reduce the specific activity of any Cs^{137} present in fallout or reactor wastes. In these experiments, however, adding stable Cs to reduce the specific activity of Cs^{137} by a factor of 10^4 did not reduce the amount of radioactivity absorbed. Thus, the benefits of isotopic dilution of Cs^{137} were not demonstrated. Similar results were obtained in experiments with pinfish (*Lagodon rhomboides*), mummichog (*Fundulus heteroclitus*), and brine shrimp (*Artemia salina*) (Gutknecht, unpublished data). These results may be due to the relative abundance of K in seawater, a condition that simulates isotopic dilution (Lowman 1960). On the other hand, Kornberg (1961) claims there is no good evidence that Cs and K follow similar metabolic pathways.

Relationship of Zn^{65} and Cs^{137} uptake to photosynthesis

As noted earlier, the uptake of both Zn^{65} and Cs^{134} by some seaweeds has been reported to depend on light (Bachmann and Odum 1960; Scott 1954). The variable amounts of these tracers absorbed in the dark, however, appear to make this hypothesis untenable for most species. It has been suggested that the reported relationship between Zn^{65} uptake and photosynthesis in seaweeds may be mainly due to a *p*H increase in unbuffered light bottles and a consequent increase in Zn^{65} uptake by adsorption exchange (Gutknecht 1961).

The relationship between Cs^{137} movements and photosynthesis may also be indirect. Cesium transport appears to be closely related to metabolism, as indicated by the effects of light, previous exposure to light, exogenous substrate, and anoxia. However, the present data do not support Scott's (1954) hypothesis of an intimate connection between the mechanisms of Cs absorption and photosynthesis.

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