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Caesium accumulation by microorganisms: uptake mechanisms, cation competition, compartmentalization and toxicity

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SUMMARY

The continued release of caesium radioisotopes into the environment has led to a resurgence of interest in microbe–Cs interactions. Caesium exists almost exclusively as the monovalent cation Cs^+ in the natural environment. Although Cs^+ is a weak Lewis acid that exhibits a low tendency to form complexes with ligands, its chemical similarity to the biologically essential alkali cation K^+ facilitates high levels of metabolism-dependent intracellular accumulation. Microbial Cs^+ (K^+) uptake is generally mediated by monovalent cation transport systems located on the plasma membrane. These differ widely in specificity for alkali cations and consequently microorganisms display large differences in their ability to accumulate Cs^+; Cs^+ appears to have an equal or greater affinity than K^+ for transport in certain microorganisms. Microbial Cs^+ accumulation is markedly influenced by the presence of external cations, e.g. K^+, Na^+, NH_4^+ and H^+, and is generally accompanied by an approximate stoichiometric exchange for intracellular K^+. However, stimulation of growth of K^+-starved microbial cultures by Cs^+ is limited and it has been proposed that it is not the presence of Cs^+ in cells that is growth inhibitory but rather the resulting loss of K^+. Increased microbial tolerance to Cs^+ may result from sequestration of Cs^+ in vacuoles or changes in the activity and/or specificity of transport systems mediating Cs^+ uptake. The precise intracellular target(s) for Cs^+- induced toxicity has yet to be clearly defined, although certain internal structures, e.g. ribosomes, become unstable in the presence of Cs^+ and Cs^+ is known to substitute poorly for K^+ in the activation of many K^+-requiring enzymes.

INTRODUCTION

The stable caesium isotope (^{133}Cs) is the rarest of the alkali metals, although trace quantities of stable caesium do occur in most living organisms [9,26,51]. Concentrations of naturally occurring caesium vary considerably in different environments. Soils and sediments generally contain between 0.3 and 25 μg Cs g^{-1}, while Cs is more diluted in aquatic ecosystems with concentrations ranging from approximately 0.01–1.2 ng ml^{-1} in freshwater habitats to approximately 0.5–2.0 ng ml^{-1} in marine habitats [51]. Widespread contamination of the environment with caesium radioisotopes, following nuclear weapons testing in the 1950s and 1960s and continuing controlled and accidental release, has heightened concern over the fate of this radionuclide in biotic and abiotic components of the environment [9]. This is particularly so as the long half-life (~30 y) and high water solubility of the most prevalent radioisotope, ^{137}Cs, dictate a high accessibility to organisms for many years following release into the environment [9,26,51]. The initial relative distribution of released ^{137}Cs between terrestrial and aquatic ecosystems depends primarily on the source of the isotope. Oceanic deposition of atmospheric fallout is approximately two-fold higher than terrestrial deposition, although localized levels of contamination vary widely [9]. Controlled releases from the nuclear industry are predominantly as liquid effluents (with little or no incidence of radioactive airborne particles) which are either discharged directly into the sea or for which aquatic ecosystems will usually act as the ultimate 'sink' [9,51]. Much of the ^{137}Cs in the environment tends to become strongly associated with soil or sediment particles [9]. The remainder may be maintained in soluble form, thus increasing its availability to biota. Bioaccumulation of radiocaesium into higher organisms is strongly influenced by a number of factors (e.g. pH, K^+, organic matter) and accumulated ^{137}Cs may subsequently be transferred through food chains leading eventually to man; certain higher food chains have been identified as routes of high ^{137}Cs transfer, e.g. the lichen–caribou–man food chain [9,51].

Interest in the interactions of microorganisms with caesium (and other metals and radionuclides) has arisen primarily from their important roles in biogeochemical cycling, primary production and as initial components of aquatic and terrestrial food chains. Microbial activity has been implicated in the remobilisation of radiocaesium from aquatic sediments [3], the accumulation and retention of caesium in upper soil layers [44] and leaching of caesium from plant litters in terrestrial ecosystems [25,97]. Furthermore, the presence of ^{137}Cs in higher organisms has in certain cases been attributed to transfer of ^{137}Cs along food chains originating from microorganisms, rather than to direct uptake from their external medium [9,48,78]. More recently, increased attention has also focused on the biotechnological potential of microorganisms for biological Cs-removal from waste effluents emanating from the

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nuclear industry [6,12,57]. In addition to the environmental significance of microbe–Cs interactions, the chemical similarity of Cs^+ and K^+ suggests that caesium radioisotopes may have an application in tracing K^+ fluxes in microorganisms, particularly considering the long half-life of ^{137}Cs compared to ^{42}K (~12 h) and the more commonly employed K^+ tracer, ^{86}Rb (18.7 days). Indeed, recent reports have indicated that Cs^+ may be accumulated with an equal or greater affinity than K^+ in certain organisms [8,13,14].

This review aims to provide an understanding of microbe–Cs interactions at the cellular level. Mechanisms of Cs accumulation will be considered in relation to the chemistry of caesium and the complex interactions that occur with other monovalent cations. The subcellular localization of accumulated Cs^+ is described and the importance of these and other considerations in determining Cs^+ toxicity towards microorganisms is assessed.

CHEMISTRY OF CAESIUM

Caesium is an alkali metal that exists in solution almost exclusively as the monovalent cation Cs^+ [37]. It is the most electropositive and active of all metals; it oxidizes readily and forms strong bases. These and other properties, such as a specific gravity of lower than five, suggest that according to many definitions caesium is not a 'heavy' metal, although it should be stressed that these definitions are mostly arbitrary and do not necessarily predict the behaviour of a metal in a biological or environmental context [33]. Another easily defined property of metals is their Lewis acidity [41]. In comparison to many of the most commonly studied metals of environmental concern, e.g. Cd^{2+} and Cu^{2+}, Cs^+ is a very weak Lewis acid, i.e. the ion has a very low charge/radius ratio, and consequently has a low polarizing power and is 'seen' by neighbouring anions as a centre of low charge density [41]. Thus Cs^+, like other monovalent cations, interacts very weakly with ligands and analytical problems relating to metal speciation can essentially be disregarded in the case of Cs^+. Furthermore, according to the classifications of Pearson [66] and Nieboer and Richardson [61], Cs^+ is a hard metal and any ligand interactions are more likely to occur with oxygen–donor ligands than with nitrogen– or sulphur–donor ligands. Thus, any Cs–ligand complexation will be primarily ionic/electrostatic in nature and therefore weaker than the more covalent interactions that occur between nitrogen– or sulphur–donor ligands and softer metals, such as Cu^{2+} and the monovalent ion Tl^+ [7,33,41].

The toxicity of certain metals is related to a strong coordinating ability [62]. Therefore, soft metals are generally toxic towards microorganisms, whereas hard metals are often nontoxic and in many cases are essential macronutrients for microbial growth [7,41]. To date, no essential biological role of stable Cs has been elucidated, although trace quantities have been detected in many organisms [35]. Moreover, the chemical similarity of Cs^+ and other alkali monovalent cations, in particular K^+, is the key factor that governs the high mobility of Cs^+ in biological systems [9,26,51]. K^+ is an essential cation for microbial growth and is accumulated intracellularly to cytosolic concentrations of several hundred millimolar, while Na^+ is maintained at a low intracellular level [45,64,94]. Atomic and ionic radii of a range of alkali metals and monovalent cations are given in Table 1. The ionic radii of K^+ and Cs^+ (133 and 165 pm, respectively) are sufficiently similar that many less-selective K^+ transport systems, located in microbial plasma membranes, apparently cannot distinguish between these or indeed certain other alkali cations such as Rb^+ [9,18,22,45]. On the other hand, smaller alkali metal cations such as Na^+ and Li^+ are excluded from the cell interior more effectively. Thus, although Cs^+ generally displays a low affinity for those negatively-charged anionic functional groups present on microbial cell surfaces that are responsible for metabolism-independent adsorption of many other metals [89], Cs^+ may act as an analogue for K^+ and enter cells via metabolism-dependent transport processes [9]. The following section will describe the energy sources that drive monovalent cation transport and examine in more detail their involvement in Cs^+ uptake in the major groups of microorganisms.

MECHANISMS OF CAESIUM UPTAKE IN MICROORGANISMS

1. Energy sources

Monovalent cation fluxes across microbial cell membranes generally occur against concentration gradients, e.g. intracellular K^+ concentrations are commonly 100- to 1000-fold greater than extracellular K^+ concentrations [18]. Consequently, expenditure of energy is required to drive ions across the membrane. Monovalent cation transport is generally linked to the plasma membrane-bound H^+-ATPase. ATP-driven H^+ extrusion generates a transmembrane electrochemical proton gradient ($\Delta\mu_{H^+}$). The gradient has an electrical component, the membrane potential ($\Delta\Psi$) which is inside-negative, and a chemical component, the pH gradient (ΔpH) which is inside-alkaline [18,45,94]. These gradients can be linked to ion uptake in secondary transport processes. Here, cation transport may be chemiosmotically coupled to H^+ movements occurring in either the same (symport) or opposite (antiport) direction. Alternatively, the membrane potential may drive monovalent cation uptake via electrogenic uniport, where ions are taken up in the absence of exchange for ions of an equivalent charge [45]. In addition to secondary transport processes, some evidence has implicated a more direct role of ATP in energizing monovalent cation uptake in primary active transport, e.g. via K^+-ATPases [94].

TABLE 1

Atomic and ionic radii of alkali metals and monovalent cations

Metal/cation	Atomic radius (pm)	Ionic radius (pm)
Li	152	78
Na	154	98
K	227	133
Rb	248	149
Cs	265	165
NH_4^+	–	142

H^+-ATPase (K^+-ATPase) activity is dependent on cellular metabolism, and the exclusion of a metabolizable carbon source from growth media will result in a lowered $\Delta\mu_H^+$ and any coupled transport processes. A stimulation of cation transport, following addition of a substrate such as glucose, may be a result of both enhanced activity of the plasma membrane H^+-ATPase and increased biosynthesis of plasma membrane components involved in cation uptake, e.g. transport proteins [45].

Selective transport of monovalent cations can also occur intracellularly at the vacuolar membrane (tonoplast). The existence of a vacuolar H^+-ATPase has been demonstrated in several microorganisms and drives cation fluxes by similar processes to those described for the plasma membrane, e.g. the H^+ gradient generated by the vacuolar H^+-ATPase of *Saccharomyces cerevisiae* is used to drive H^+/cation antiporters [45].

It should also be mentioned that an additional mode of cation uniport can occur via plasma membrane ion channels. While these have been more extensively characterized in mammalian cells, their existence has been demonstrated in certain microorganisms. However, the importance of cation channels in vivo is unclear as they are inactive at the hyperpolarizing potentials that occur in whole microbial cells [80]. Nevertheless, K^+-selective channels are known to occur in the plasma membrane of *S. cerevisiae* and these may be controlled by voltage-gating or possibly by second messengers [38,92]. To date no studies have examined microbial uptake of Cs^+ via cation channels.

2. Cs^+ uptake by bacteria

Whereas K^+ transport is well characterized in bacteria [85,94], few reports have investigated bacterial Cs^+ uptake. K^+ uptake in bacteria can be chemiosmotically coupled to H^+ movements. K^+/H^+ antiports are fairly ubiquitous, while certain evidence suggests that K^+ uptake may also be mediated by H^+ symport [94]. In *Escherichia coli*, independent K^+ transport systems, with differing selectivities and affinities for K^+, have been characterized. These have been designated Trk (high V_{max} and K_m for K^+) and Kdp (low V_{max} and K_m for K^+) [22,85]. It has now been established that both systems catalyse primary active transport of K^+ [85].

Considering the existence of a number of possible transport mechanisms, it is not surprising that bacteria differ considerably in their ability to accumulate Cs^+. Tomioka et al. [90] observed that whereas *Rhodococcus* spp., previously isolated from soil samples, accumulated large amounts of Cs^+ when grown in the presence of Cs^+, no detectable Cs^+ uptake was observed in a laboratory strain of *Pseudomonas fluorescens* grown under identical conditions. Johnson et al. [44] also reported large differences in the Cs^+-uptake capacities of bacteria isolated from ^{137}Cs-contaminated soils. It is likely that such differences are related to the specific affinities for Cs^+ of monovalent cation transport systems present in different organisms, although it should also be noted that external physico-chemical factors can markedly influence Cs^+ uptake. For example, a greater than two-fold increase in the rate of Cs^+ uptake by the marine bacterium *Vibrio alginolyticus* resulted when the external pH was raised from 7.5 to 8.9 [59].

Bossemeyer et al. [22] have characterized a transport system in *E. coli* that takes up Cs^+ with a moderate rate and affinity. This system, formerly Trk D but now designated Kup, still displays an approximate 14-fold greater affinity for K^+ than for Cs^+ (K_m values for K^+, Rb^+ and Cs^+ were 0.37, 0.38 and 5.0 mM, respectively). However, competition studies revealed that Cs^+ inhibited K^+ uptake via the Kup system much more strongly than via the Trk system (K_i values for Cs^+ in these systems were approximately 7 and 30 mM, respectively). Furthermore, mutant *E. coli* strains lacking functional Kup systems, but still expressing Trk and Kdp systems, took up Cs^+ only very slowly [22]. K_m values for K^+ and Cs^+ uptake by a range of microorganisms are given in Table 2.

3. Cs^+ uptake by cyanobacteria

As in other microorganisms, cyanobacteria possess systems that efficiently discriminate between the most abundant monovalent cations. It is now generally accepted that a primary active Na^+/H^+ antiporter maintains low intracellular levels of Na^+ in cyanobacteria [64], while most available evidence suggests that K^+ accumulation against a concentration gradient is also mediated by a primary active mechanism or by K^+/H^+ symport [64,74,94]. The energy-dependence of Cs^+ uptake in cyanobacteria has been demonstrated using metabolic inhibitors or by incubation of cells in the dark or at low temperature (4 °C); in all cases Cs^+ uptake was reduced [8]. Multiple transport systems with differing affinities for monovalent cations have been reported in cyanobacteria. In *Anabaena variabilis*, kinetic studies of K^+ transport revealed the presence of two uptake systems with differing affinities for K^+ [75]. The high affinity K^+ transporter (K_m ~0.04 mM) discriminated strongly against Rb^+, whereas discrimination against Rb^+ was less marked in the low affinity K^+ transporter (K_m for K^+ ~4.5 mM). This latter system probably corresponds to the single system (K_m for Cs^+ ~0.25 mM) responsible for Cs^+ uptake in *A. variabilis* [10]. Further kinetic studies of Cs^+ transport in cyanobacteria are required to elucidate more clearly the

TABLE 2

K_m values for uptake of K^+ and Cs^+ by microorganisms

Organism	Transport system (if specified)	K_m for K^+ (mM)	K_m for Cs^+ (mM)	Reference
E. coli	Kdp	0.002	[a]	22
E. coli	Trk	0.9–1.5	[a]	22
E. coli	Kup	0.37	5	22
A. variabilis	–	0.04	[b]	10,75
A. variabilis	–	4.5	0.25	10,75
C. salina	–	[b]	0.5	13
S. cerevisiae	–	0.21	1.9	19

[a] Cs^+ uptake activity not detectable.
[b] K_m value not determined.

relative importance of different K^+ transporters in mediating Cs^+ uptake.

Differences in reported Cs^+ uptake capacities of different cyanobacteria were initially attributed by Harvey and Patrick [39] to differences in cellular surface area to volume ratios, rather than to any systematic relationships. However, more recently a relationship between cyanobacterial eco-physiology and Cs^+ uptake capacity has become apparent. Fisher [31] detected no accumulation of Cs^+ from Mediterranean seawater by a marine *Synechococcus* sp., whereas Avery et al. [8] observed Cs^+ accumulation by the euryhaline cyanobacterium *Synechocystis* PCC 6803 to approximately 1000 nmol Cs^+ (10^9 cells)$^{-1}$, when cells were incubated in buffer in the presence of 1 mM CsCl. These differences were apparently related to differences in the ion content of the solutions used for experiments, as Cs^+ accumulation by *Synechocystis* PCC 6803 was almost completely inhibited when examined in the presence of 50 mM NaCl [8]. K^+ and Na^+ had approximately equal inhibitory effects on Cs^+ accumulation by *Synechocystis* PCC 6803. However, when supplied at an equimolar concentration to Cs^+, none of the alkali monovalent cations tested affected the final level of Cs^+ accumulation in this study, suggesting that unlike in *E. coli* [22] Cs^+ has an equal or greater affinity than K^+ for the monovalent cation transport system(s) of *Synechocystis* PCC 6803 [8]. Cs^+ accumulation by *Synechocystis* PCC 6803 was also decreased at high H^+ concentrations [8], although this was probably a result of plasma membrane depolarization at low pH [74] rather than competition between Cs^+ and H^+ for transport sites.

NH_4^+ is also transported by active processes in cyanobacteria, apparently in response to $\Delta\Psi$ [23,24]. Although transport systems mediating K^+ and NH_4^+ uptake in cyanobacteria have previously been regarded as separate entities, they display many similar properties, e.g. NH_4^+ uptake also appears to involve two systems; a highly specific system for NH_4^+ and a less specific system which mediates the additional uptake of methylammonium, an analogue for NH_4^+ [23,76]. Furthermore, recent reports have demonstrated that Cs^+ and NH_4^+ share a common transport system in *A. variabilis* [10] and *Nostoc muscorum* [86]. NH_4^+ inhibited Cs^+ uptake competitively/non-competitively in *A. variabilis*, suggesting uptake of Cs^+ and NH_4^+ via a common transport system as well as the possible presence of modifier sites associated with Cs^+ transport [10]. Furthermore, a mutant strain of *A. variabilis* deficient in NH_4^+-transport activity accumulated low levels of Cs^+, as did the parent strain when grown under conditions non-conducive to high NH_4^+-transport activity, i.e. in the presence of a combined nitrogen source [10]. Prior growth in the presence of NH_4^+ also reduced Cs^+ uptake by both parent and mutant strains of *N. muscorum* during short-term experiments [86]. Cs^+ uptake by *N. muscorum* was attributed to the activity of an NH_4^+-repressible NH_4^+ transport system [86]. Thus, it seems likely that Cs^+, K^+ and NH_4^+ share at least one common transport system in cyanobacteria, although common transport of the latter two ions has yet to be demonstrated unequivocally.

4. Cs^+ uptake by microalgae

In contrast to the resurgence of interest in Cs in the wake of the Chernobyl disaster, studies examining uptake of other monovalent cations in microalgae have been largely neglected in recent years. Barber [15] initially proposed that a primary active transport mechanism was responsible for K^+ uptake in *Chlorella* spp., however, Raven [73] subsequently concluded that microalgal K^+ uptake occurred primarily via a uniport mechanism. This discrepancy may be related to the known presence of two K^+ uptake systems, with differing affinities for K^+, on the plasma membrane of *Chlorella* spp. [47,65]. One system (K_m ~3.0 mM) is responsible for K^+/K^+ exchange at high external K^+ concentrations, while the other (K_m ~0.25 mM) mediates net K^+ uptake at low external K^+. In addition to exchange for K^+, non-electrogenic K^+ uptake may also occur by concomitant extrusion of Na^+ [83] or H^+ [91].

As appears to be the case in most other microorganisms, Cs^+ accumulation by microalgae is accompanied by an approximate stoichiometric exchange for intracellular K^+ with little or no exchange for Na^+ [11,13]. Cs^+ uptake by the freshwater microalgae *Chlorella pyrenoidosa* and *Euglena intermedia* increased linearly with Cs^+ concentration up to 0.04 mM [95,96]. Cs^+ uptake by the estuarine microalga *Chlorella salina* displayed first-order kinetics over a time course of 24 h and obeyed Michaelis–Menten kinetics over the concentration range 0.01–0.25 mM Cs^+, although apparent saturation of Cs^+ transport occurred at higher Cs^+ concentrations [13]. In contrast to microalgal K^+ transport, a single system (K_m for Cs^+ ~0.5 mM) catalysed Cs^+ uptake across the plasma membrane of *C. salina*. K^+ and Rb^+ competitively inhibited Cs^+ uptake by this system, indicating common transport of these ions. Conversely, NH_4^+ was a non-competitive/uncompetitive inhibitor of Cs^+ uptake in this case, indicating independent transport of NH_4^+ and Cs^+ [13]. The order of inhibition of Cs^+ uptake by monovalent cations in *C. salina* was $Rb^+ > K^+ > NH_4^+$ [13]. The overall order of alkali metal affinities for the monovalent cation transport systems of freshwater *Chlorella* spp. is $K^+ > Rb^+ > Cs^+ > Na^+$ [26,58], an apparent reversal of the relative affinity of K^+ and Rb^+ observed in *C. salina*.

While the inhibitory effects of K^+ on microalgal Cs^+ uptake are well documented [11,13,72,95], these effects only become evident in *C. pyrenoidosa* [96] and *C. salina* [13] when the external K^+ concentration exceeds that of Cs^+, suggesting a possible greater affinity of Cs^+ than K^+ for the transport systems of these microalgae. In contrast, an approximate 30–40% inhibition of Cs^+ uptake in *Chlorella emersonii* resulted at equimolar (1 mM) K^+ concentrations, although the degree of inhibition was markedly dependent on the mode of nutrition of the microalga [11]. Inhibition by K^+ was greater in photosynthesizing than in respiring cells, but levels of Cs^+ uptake in the absence of K^+ were lower in the latter case. Growth stage-dependent changes in the intracellular level of K^+ in photoautotrophic and chemoheterotrophic cultures of *C. emersonii* had little effect on the cells' capacity to accumulate Cs^+ [11].

The influence of Na^+ on Cs^+ uptake also differs considerably in different microalgae. In *C. emersonii*, inhibition of Cs^+ uptake was only apparent when Na^+ was supplied at a 50-fold

higher concentration than Cs^+ [11]. The high concentration of Na^+ in seawater (~0.5 M) may thus account for the low Cs^+-concentration factors reported in marine algae [17]. However, Cs^+ influx was approximately 28-fold higher in the estuarine microalga *C. salina* when incubated in the presence rather than in the absence of 0.5 M NaCl [12]. These differences may be attributable to the relative importance of changes in K^+ fluxes in the osmoregulation of organisms from habitats of differing salinities. The potential biotechnological application of salt-stimulated microbial Cs^+ influx in a biological Cs-removal process has been discussed in detail elsewhere [6,12,57].

The interactions between external H^+ and Cs^+ in microalgae are poorly understood, although Cs^+ uptake by *C. salina* remains relatively constant between pH 7–10 [12]. In addition to possible direct competition for available transport sites by H^+, external pH may influence the activity of different monovalent cation transport systems in certain microalgae. Tromballa [91] reported that a switch from K^+/K^+ to K^+/H^+ exchange, and hence a net increase in K^+ accumulation, was necessary for maintenance of high internal pH in *Chlorella fusca* following provision of glucose as an organic carbon source. It is likely that such a mechanism would not be required to maintain constant ΔpH when external pH is low, suggesting that reduced net cation uptake would occur under these conditions. While the activity of Cs^+/Cs^+ exchange in *C. salina* has been demonstrated by using ^{137}Cs as a tracer for Cs^+ efflux [13], it is unclear whether a transition to M^+/H^+ exchange can also occur in photosynthesizing *Chlorella* spp., and further work is required to establish the role of H^+ in controlling the mechanism of microalgal Cs^+ influx.

5. *Cs^+ uptake by yeasts and other fungi*

The complex multiple-site kinetics of monovalent cation transport have been well characterized in yeasts and fungi [18,45,79]. Energization of K^+ uptake is considered to be primarily by $\Delta\Psi$, although there may be some additional involvement of ΔpH [18,19,34,45]. The action of a K^+ uniport, electrically coupled to H^+ extrusion, facilitates creation of a 1000-fold trans-membrane K^+ gradient [18,34]. The genes encoding the plasma-membrane proteins (TRK1 and TRK2) which catalyse high- and low-affinity K^+ uptake in *S. cerevisiae* have now been characterized [50]. Additional non-transporting sites on the yeast plasma membrane are known to be involved in the regulation of cation-carrier activity [4,5,21,28]. The overall order of cation affinities for uptake by *S. cerevisiae* is: $K^+ > Rb^+ > NH_4^+ > Cs^+ > Na^+ > Li^+ \gg Mg^{2+} > Ca^{2+}$ [18].

K^+ is a competitive inhibitor of the uptake of all other monovalent cations in *S. cerevisiae*, however, the mode of inhibition by Cs^+ is markedly dependent on Cs^+ concentration. At low Cs^+ concentrations, Cs^+ apparently binds to sites that alter the affinity of the transporting sites for K^+, resulting in non-competitive (but incomplete) inhibition of K^+ uptake. At higher Cs^+ concentrations, Cs^+ itself interacts with transporting sites, resulting in a more complete competitive inhibition of K^+ uptake [5,18,45]. Derks and Borst-Pauwels [28] observed that the kinetics of concentration-dependent increases in Cs^+ influx in yeast fitted a cubic rather than a quadratic rate equation, suggesting the involvement of three sites in the translocation of Cs^+ across the plasma membrane. Convex deviations from linearity of Hoftsee plots at low Cs^+ concentrations indicated the presence of an 'activation' (high affinity) site, while a translocating 'substrate' (moderate affinity) site and a 'modifier' (low affinity) site were evident at higher Cs^+ concentrations [28]. It should be noted that the apparent modifier site may actually be an artefact, brought about by changes in the surface/membrane potential at higher external cation concentrations [18,20,28].

H^+ also interacts with sites mediating monovalent cation transport in yeast. At low external pH, binding of H^+ results in non-competitive inhibition of the uptake of other monovalent cations. Furthermore, the selectivity for K^+ and Rb^+ of transporting sites increases under more acidic conditions resulting in enhanced discrimination against other monovalent cations such as Cs^+ [45]. Thus, during short term experiments, increasing the pH from 3.5 to 5.5 resulted in a marked stimulation of Cs^+ uptake by *S. cerevisiae* and inhibition of Cs^+ uptake in the presence of equimolar (10 mM) K^+ was decreased at lower H^+ concentrations [69].

In addition to the systems described above, separate mechanisms for NH_4^+ transport also occur in certain yeasts and fungi [46,54,87], and specific systems for K^+ efflux have been characterized [45,67]. However, it is still unknown whether these systems may also act as routes for Cs^+ transport. It should also be noted that while the low affinity of Cs^+ for cell-surface ligands has dictated a focus on metabolism-dependent Cs^+ uptake here, one study has demonstrated a high degree of metabolism-independent Cs^+ adsorption in pelleted/immobilized fungi [29]. Two of the organisms examined in this study, *Rhizopus arrhizus* and *S. cerevisiae*, have been shown to bind negligible quantities of Cs^+ when suspended freely in solution [69,89], and it can be inferred that the physical properties of pelleted yeasts and fungi may enhance their tendency to adsorb Cs^+. Such considerations may be of interest in a biotechnological context [6].

SUBCELLULAR CAESIUM LOCALIZATION AND CAESIUM TOXICITY

As with the other alkali metal cations, the subcellular compartmentation of Cs^+ is largely governed by its high solubility and weak coordinating ability. Thus, no detectable Cs^+ becomes associated with the cell wall of *S. cerevisiae* [69] or bound to denatured *R. arrhizus* [89] during incubation of biomass in the presence of Cs^+. In contrast, subcellular fractionation and Cs^+-efflux studies revealed that 11–15% of total accumulated Cs^+ was associated with the cell wall of *C. salina* [13], this value being similar to the 10.5% estimated for bound K^+ in *Chlorella autotrophica* [1]. In no instance has Cs^+ been found to be associated with insoluble intracellular components.

Most Cs^+ in microorganisms is maintained in soluble form in either the cytoplasm or vacuole (if present). The relative distribution of intracellular Cs^+ between the cytoplasm and vacuole is largely determined by the activity of the vacuolar membrane H^+-ATPases, which serve to drive transport of monovalent cations from the cytoplasm into the vacuole, primarily via an H^+ antiport mechanism [45,49]. Furthermore, a

membrane potential-dependent cation channel is known to occur in the yeast vacuolar membrane which permits entry of K^+, Cs^+, Na^+ and Li^+ with broad specificity [93]. The fraction of the cell volume occupied by vacuoles varies considerably in different microorganisms. In microalgae, vacuoles represent only approximately 10% of the total cell volume [73]. However, despite their diminutive size, vacuolar Cs^+ accounted for approximately 63% of total accumulated Cs^+ in *C. salina*; approximately 22% of Cs^+ was localized in the cytoplasm [13]. Calculated values for Cs^+ influx in this study were approximately equal to, or greater than, values for total cellular influx, suggesting that the plasmalemma was the rate limiting membrane for cellular Cs^+ uptake by *C. salina* [13].

In fungi, vacuoles occupy between 25–95% of the cell volume and the ratio of total K^+ to cytoplasmic K^+ is approximately 4 to 1 [30,45,56,70]. The proportion of cellular Cs^+ accumulated in fungal vacuoles appears to be quite variable. Approximately 90% of total Cs^+ accumulated by *S. cerevisiae* and *Candida albicans* became sequestered in vacuoles, whereas only approximately 60% of Cs^+ occurred in the vacuole and 40% in the cytoplasm of *Rhodotorula rubra* [69]. These results were correlated with differential sensitivity of the yeasts to Cs^+. *S. cerevisiae* and *C. albicans* were tolerant to relatively high concentrations of Cs^+ (minimum inhibitory concentration (m.i.c.) > 80 mM), whereas growth of *C. albicans* was inhibited at much lower concentrations (m.i.c. ~36 mM). The results suggest that, as with various other potentially toxic metals [34], vacuoles may play an important role in intracellular Cs^+ detoxification in microorganisms [69].

In addition to intracellular detoxification, microbial tolerance to metals can result from decreased intracellular uptake [32]. In many cases, microorganisms appear to exploit those characteristics that dictate the toxic nature of many metals, such as their strong coordinating abilities [62], for enhancing metal-tolerance, e.g. through extracellular metal crystallization, precipitation or binding to excreted metal binding proteins [34,84]. Such processes are less important in microbial Cs^+ tolerance [41], although fixation of Cs^+ to clay minerals may represent an indirect abiotic means of reducing the availability and toxicity of Cs^+ towards terrestrial or sediment-dwelling microorganisms [9,34]. Furthermore, the binding of Cs^+ to particulate fractions in older cells may reduce the toxicity of Cs^+ towards certain microorganisms [95]. Increased tolerance of microorganisms towards Cs^+ may also result from inactivation of transport systems mediating Cs^+ uptake. Singh et al. [86] reported that Cs^+ toxicity towards *N. muscorum* was greater in cultures grown in the absence than in the presence of NH_4Cl; the NH_4^+ transport system which catalyses Cs^+ uptake in this cyanobacterium was repressed in the presence of NH_4Cl. In contrast, increased tolerance of *C. emersonii* to Cs^+, following repeated growth in the presence of 50 mM CsCl, was more closely related to an increased ability of cells to accumulate K^+ despite high external Cs^+ concentrations (e.g. through a change in the selectivity of the monovalent cation transport system(s)) than to exclude Cs^+ [9].

It is primarily the weak coordination characteristics displayed by Cs^+ (and consequently its low tendency to exert such potentially toxic effects as blocking of functional groups on biologically important molecules, causing conformational modification, denaturation and inactivation of enzymes, and disrupting cellular and organellar membrane integrity [62]) that determine its low toxicity towards microorganisms in comparison to other toxic metals, e.g. Cu^{2+} and Cd^{2+} [34]. For example, 1 mM CsCl reduced the final growth yield of the cyanobacterium *Synechocystis* PCC 6803 in batch culture by only approximately 70% [8] and 10 mM CsCl increased the cell division time of *S. cerevisiae* by only 20 min, from 3 h to 3 h 20 min [69]. However, among the alkali metals, only Cs^+ and Li^+ displayed inhibitory effects towards growth of the fungi *Aspergillus niger* and *A. oryzae* [52].

It is now generally considered that Cs^+ exerts toxic effects towards microorganisms as a consequence of its chemical similarity to K^+. While Cs^+ is readily accumulated via monovalent cation transport systems and can replace intracellular K^+, Cs^+ apparently cannot substitute for K^+ in some or all of its essential biological functions [8,9,11]. An approximate stoichiometric exchange of Cs^+ for intracellular K^+ occurred during Cs^+ accumulation by *Synechocystis* PCC 6803 [8], *C. emersonii* [11], *C. salina* [13] and *S. cerevisiae* [69]. However, while the total internal monovalent cation concentration remained approximately constant in all these cases, cell division was partially inhibited in Cs-containing cells. Using *C. emersonii* grown under different nutritional regimes, Avery et al. [11] confirmed that it is not the presence of Cs^+ in cells that is growth-inhibitory but rather the resulting decline in intracellular K^+. Similarly, it is the external ratio of K^+ to Cs^+ rather than the absolute external Cs^+ concentration that is critical when estimating the potential toxicity of Cs^+ towards microorganisms [9,26,51,69]. In certain cases, Na^+ may also protect against Cs^+ toxicity. For example, doubling the total monovalent cation concentration of the growth medium of *Synechocystis* PCC 6803, by addition of either NaCl or KCl, abolished the growth-inhibitory effects of 1 mM CsCl [8]. Cs^+ also can apparently protect against the toxic effects of other metals and metal compounds. For example, CsCl partially reduced the toxicity of organotin compounds towards the marine yeast *Debaryomyces hansenii* [53].

The effect of Cs^+ on K^+-starved cultures differs in different microorganisms. Whereas no stimulation of growth of K^+-starved *Rhodopseudomonas capsulata* occurred when cultures were supplemented with 10 mM Cs^+, significant growth resulted in the presence of 1 mM Cs^+, suggesting that Cs^+ substituted for K^+ in at least some aspects of cellular metabolism [43]. Rb^+ substituted for K^+ more effectively than Cs^+ in the latter study. Similarly, Perry and Evans [71] observed that whereas some stimulation of growth resulted from Cs^+-supplementation of K^+-limited cultures of *Micrococcus sodonensis*, the stimulation was not as marked as that observed following addition of K^+ to the medium. In contrast, neither Li^+, Na^+, Cs^+ or NH_4^+ could functionally substitute for K^+ in K^+-limited cultures of *Candida utilis* [2], and although large amounts of Cs^+ were taken up by K^+-limited cultures of *E. intermedia*, no enhanced metabolism or growth of the microalga resulted [95]. From the evidence reported to date, it appears that eukaryotic microorganisms may have a more specific requirement for K^+ than prokaryotes.

K^+ is required for various essential processes in microorganisms. Changes in K^+ fluxes may serve to adjust the intracellular osmotic strength under conditions of osmotic stress [36,94]. Furthermore, K^+ may act as an energy source in the form of a trans-membrane K^+ gradient ($\Delta\mu_{K^+}$) [27,63] or as an activator of enzyme function [77,88]. K^+ can also be involved in the regulation of internal pH [16] and plays a role in the stabilization of internal structures, e.g. ribosomes [40].

Ribosomes may be an important intracellular target for Cs^+, as high CsCl concentrations are known to cause the irreversible dissociation of proteins from the 50S and 30S ribosomes of *E. coli* [55]. However, most studies on mechanisms of Cs^+ toxicity towards microorganisms have focused on enzyme activities. Cs^+ and certain other monovalent cations, e.g. NH_4^+ and Rb^+, can partially substitute for K^+ in enzyme activation, whereas smaller ions such as Na^+ and Li^+ generally antagonise enzyme activity [35,94]. For example, lactate dehydrogenase from *M. sodonensis* [71], glutamate dehydrogenase from *C. pyrenoidosa* [82], acyl-coenzyme A carboxylase from *Streptomyces erythraeus* [42] and ortho-nitropheno-β-D-galactosidase from *E. coli* [60] were all activated in the presence of low Cs^+ concentrations. In the latter case, Cs^+ caused inductive changes on the active site through binding with the substrate [60]. The unstable enzyme adenosine deaminase of *Bacillus cereus* was more stable in the presence of Cs^+, indicating that Cs^+ influenced the reactivity of some of the sulphydryl groups of the enzyme [81]. Cs^+ was also more inhibitory towards photoautotrophic than chemoheterotrophic growth of *C. emersonii*, and it was suggested that the additional enzymes required for the catalysis of phototrophy may have a greater requirement for K^+ than those which catalyse the breakdown of sugars [11]. A correlation between enzyme sensitivity and Cs^+ toxicity towards yeasts has recently been demonstrated. The in vitro activity of pyruvate kinase (which has a requirement for K^+) from the Cs^+-tolerant yeast *S. cerevisiae* was more markedly stimulated in the presence of Cs^+, than the activity of enzyme from the Cs^+-sensitive yeast *R. rubra* [68]. Monovalent cations with ionic radii approximating more closely to that of K^+ were the most effective in stimulating pyruvate kinase activity in this latter study.

Thus, while the general effects of Cs^+ on whole-cell processes and their relationship to intracellular K^+ are well understood, the specific site(s) of action of Cs^+ at the subcellular level has yet to be fully characterized. It is likely that K^+-requiring enzymes represent one important intracellular target for Cs^+-induced toxicity, however, future work should seek to establish to what extent these effects, and effects on ribosomal proteins, can account for whole-cell inhibition.

As a concluding comment it should also be emphasized that while this review has concentrated on the toxicity of stable Cs^+ towards microorganisms, the isotope of particular environmental concern, ^{137}Cs, may exert additional radiolytic effects as a result of its radioactive nature. Such effects are not specific to Cs but to the characteristic type of radioactive decay (β decay accompanied by γ ray in the case of ^{137}Cs) and are therefore outside the scope of this review. However, one particularly interesting study by Paschinger and Vanicek [65] demonstrated that gamma irradiation of *C. fusca* resulted in a stimulation of K^+/K^+ exchange but impairment of the transport system mediating net K^+ uptake, and altered discrimination between K^+ and Rb^+. It is tempting to speculate that any similar action of radioactive Cs on microbial monovalent cation transport systems may actually influence the nature of microbial Cs^+ uptake itself.

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