SHORT COMMUNICATION

Decreased Uptake of Cadmium by a Resistant Strain of *Staphylococcus aureus*

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Penicillinase plasmids, and some related extrachromosomal elements in *Staphylococcus aureus*, can specify resistance to inorganic ions, including Hg^{2+} and Cd^{2+} (Richmond & John, 1964; Novick & Roth, 1968; Peyru, Wexler & Novick, 1969). There is some tentative evidence that resistance to Hg^{2+} ions is due to the impermeability of the cells to the ions and not to a higher concentration of free -SH groups in resistant cells (Vaczi, Fodor, Milch & Rethy, 1962), but the biochemical basis of resistance to cadmium ions is unknown. The experiments described here show that there is a markedly decreased rate of uptake of Cd^{2+} ions by resistant cells when compared with strains that lack the *cad-r* gene.

The uptake of Cd^{2+} ions has been studied in the cadmium resistant *Staphylococcus aureus* strain 8325 ($\alpha.i^+p^+.cad-r.mer-r$) and in its cadmium-sensitive derivative, strain 8325(N) (for this nomenclature, see Richmond, 1968). Strain 8325(N) was obtained from 8325 ($\alpha.i^+p^+.cad-r.mer-r$) by isolating a variant which had spontaneously lost the α penicillinase plasmid specifying resistance to Cd^{2+} ions (Novick, 1963; Novick & Roth, 1968). Cadmium uptake was estimated by adding $^{115\text{m}}\text{CdCl}_2$ (final concentration: 10^{-4}M) to exponentially growing cultures of the sensitive and resistant strains and following the uptake of radioactivity. The initial specific activity of the $^{115\text{m}}\text{CdCl}_2$ solution was $80\text{ }\mu\text{Ci}/\mu\text{mole}$, and the bacterial culture density at the point of addition of the tracer was approximately 10^8 bacteria/ml. (about 0.08 mg. dry wt./ml.). The experiments were carried out in nutrient broth. After addition of the tracer, the cultures were incubated with shaking at 37° and samples removed at intervals for estimation of $^{115\text{m}}\text{Cd}^{2+}$ ion uptake. The samples were filtered rapidly through Whatman GF/C glass fibre filters, washed with two batches of prewarmed growth medium lacking added CdCl_2 (5 ml. each), and the radioactive content of the cells was measured by placing the dried filters in vials containing scintillant followed by estimation in a liquid scintillation spectrometer. The quantity of cadmium taken up was calculated on the basis of a counting efficiency of 90 % and a specific activity of the Cd^{2+} of $2.5\text{ }\mu\text{Ci}/\mu\text{mole}$.

The kinetics of Cd^{2+} uptake by the sensitive and resistant cultures is shown in Fig. 1. The values quoted have been corrected for nonspecific binding of Cd^{2+} ions to components of the growth medium. This was determined by the same procedure but with the medium alone and no added bacteria. The nonspecific binding of Cd^{2+} amounted to 170×10^{12} ions/ml. medium, which is equivalent to about 15 % of the total

Cd^{2+} taken up by 1 ml. of culture of the sensitive strain. The total uptake of cadmium by cadmium-sensitive cells was about 15 times that found with the resistant organisms (Fig. 1). In this experiment the total uptake was approximately 106×10^{14} Cd^{2+} ions/mg. dry wt of sensitive organisms.

To decide whether the Cd^{2+} ions taken up by the bacteria were inside the cells or adsorbed to the cell surface, attempts were made to release the isotopic tracer from the sensitive bacteria. Sensitive organisms were exposed to $^{115\text{m}}\text{Cd}^{2+}$ as above, and samples were washed with two batches of ice-cold 5% (w/v) trichloroacetic acid (5 ml. each) followed by three batches of 1% (v/v) acetic acid (5 ml. each). Dried filters were placed in scintillant and radioactivity estimated as before. This treatment with cold trichloroacetic acid completely removed all tracer from the bacteria. Control experiments showed that labelled organisms retained radioactivity after washing with pre-warmed broth (two portions of 5 ml. each) followed by 1% (v/v) acetic acid (three portions of 5 ml. each).

Fig. 1. The uptake of Cd^{2+} ions, on a dry weight basis, by cadmium-sensitive (●) and by cadmium-resistant (▲) staphylococci. Initial specific activity of CdCl_2 : $2.5 \mu\text{Ci}/\mu\text{mole}$. CdCl_2 concentration: 10^{-4}M . Samples were treated as described in the text. All values have been corrected for nonspecific binding of tracer by the growth medium.

Exchange experiments were also performed in which sensitive organisms were labelled for 40 min. and then resuspended in non-radioactive medium. When pre-warmed medium containing 10^{-4}M - CdCl_2 was used for this purpose, about 60% of the radioactive cadmium ions were displaced in 60 min., further loss being much slower. Therefore, about 40% of the radioactive Cd^{2+} ions taken up by cadmium-sensitive staphylococci reaches a location in the cell that is immediately accessible to trichloroacetic acid but not to further Cd^{2+} ions. It seems, therefore, that the non-exchangeable Cd^{2+} ions are likely to be bound to some structure within the cell rather than adsorbed adventitiously to the surface.

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