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# Cobalt transport in a cobalt-resistant strain of *Neurospora crassa*

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**Abstract.** Uptake of $Co^{2+}$ by cobalt-resistant strain is dependent on $Co^{2+}$ concentration in the medium and is linear with time. The uptake is unaffected by metabolic inhibitors and decreased at low pH values. The uptake is independent of temperature in the range 0–40° C. The transport system is a passive diffusion process, unlike in the parent wild type strain where it is energy-dependent. It is possible that $Mg^{2+}$ transport system is not involved in $Co^{2+}$ transport in this strain, since the $Co^{2+}$ uptake is not suppressed by $Mg^{2+}$ as in the parent strain.

**Keywords.** *Neurospora crassa*; cobalt-transport; cobalt-resistant strain.

## Introduction

A cobalt-resistant strain of *Neurospora crassa* isolated earlier was 10-fold more tolerant to cobalt and nickel; the mechanism of resistance was found not to involve the development of any permeability barrier to toxic metal ions (Venkateswerlu and Sivarama Sastry, 1973). Studies on the transport mechanism of $Co^{2+}$ in the sensitive parent strain *N. crassa* Em 5297a revealed that a major part of the $Co^{2+}$ uptake is energy-dependent and possibly mediated by the magnesium transport system (Venkateswerlu and Sivarama Sastry, 1970). Similar transport systems are known to exist in bacteria (Webb, 1970; Nelson and Kennedy, 1971, 1972). We report herein some features of $Co^{2+}$ transport in a cobalt-resistant strain.

## Materials and methods

### *Organisms, maintenance and growth*

A cobalt-resistant strain of *N. crassa* was employed in these studies. The details of isolation of this strain, growth conditions and uptake have been described earlier (Venkateswerlu and Sivarama Sastry, 1970, 1973). All the uptake experiments were conducted using preformed mycelia of the cobalt-resistant strain. $Co^{2+}$ concentrations are per 10 ml of medium.

## Results

### *Kinetics of* $Co^{2+}$ *uptake*

The uptake of $Co^{2+}$ by the cobalt-resistant strain as a function of time was first studied at three concentrations of $Co^{2+}$. It is evident that $Co^{2+}$ uptake increases

linearly with $Co^{2+}$ concentration in the medium (figure 1). The rate of uptake is O·37$\mu$mol (22$\mu$g) $Co^{2+}$/h/l00 mg dry weight of mycelia. Extrapolation of uptake to zero time indicates that surface binding in the cobalt-resistant strain is dependent on external cobalt concentration. As the cobalt concentration in the medium increases from 34 to 102 $\mu$mol, the surface binding increases from 0·56 to about 1·6 $\mu$mol of cobalt/100 mg dry wt; this represents 23 and 47%, respectively of the total uptake since the rate of uptake of $Co^{2+}$ is 0·37 $\mu$mol/100 mg dry wt of mycelia per h, these values apparently represent a rapid initial binding of $Co^{2+}$ by the cobalt resistant strain.

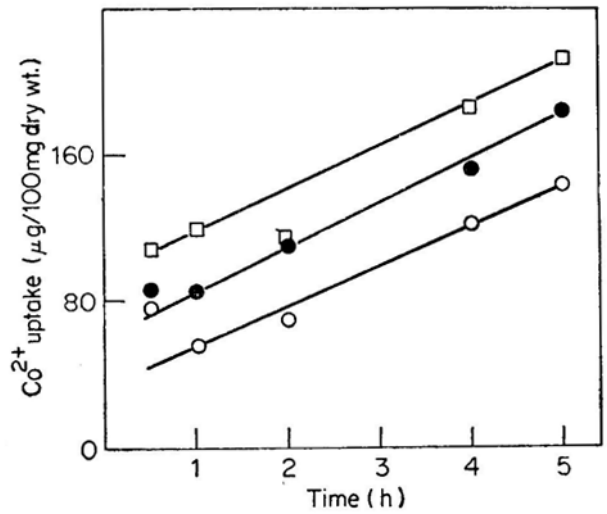


**Figure 1.** Rate of uptake of $Co^{2+}$ by *N. crassa* cobalt-resistant strain.
Preformed mycelia (72 h old) were incubated with varying concentrations of $Co^{2+}$ for different time periods. (O) 34 $\mu$mo1 $Co^{2+}$; (●) 68 $\mu$mol $Co^{2+}$; (□) 102$\mu$mo1 $Co^{2+}$/10 ml medium.

Also short term uptake of $Co^{2+}$ by mycelia of the cobalt-resistant strain was studied as a function of $Co^{2+}$ concentration for 10 and 20 min respectively. From the data of figure 2, it is evident that initial uptake is quite rapid, in contrast with the slower overall uptake, and is largely completed in 10 min. The zero-time uptake values obtained from figure 1. quite closely agree with these values, and support the conclusion that 23–47% of the $Co^{2+}$ taken up at 34–102 $\mu$mol of $Co^{2+}$ in the medium is surface bound.

$Co^{2+}$ uptake by the cobalt-resistant strain has also been studied as a function of concentration from 0–204 $\mu$mol $Co^{2+}$ in the incubation medium, using an incubation period of 3 h. It can be seen from figure 3. that the uptake increases rapidly upto a concentration of 3·39 $\mu$mol of $Co^{2+}$and thereafter, uptake increases much more slowly but nearly linearly with $Co^{2+}$ concentration. It is interesting to note that, unlike in the parent strain, where uptake reaches nearly maximal value around 34 $\mu$mol of $Co^{2+}$in 2h (Venkateswerlu and Sivarama Sastry, 1970) the saturation of uptake does not occur in the cobalt-resistant strain upto the highest concentration of $Co^{2+}$ tested in these experiments. However, when incubation with $Co^{2+}$ is prolonged for 24 h (figure 3), there is a much higher uptake of $Co^{2+}$

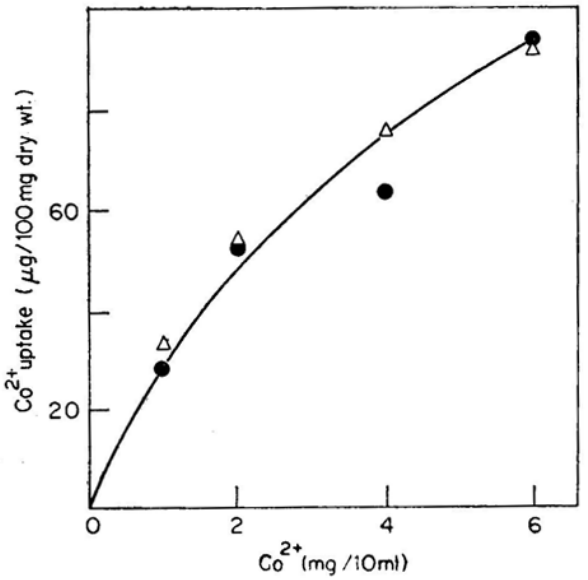


**Figure 2.** Short-term uptake of $Co^{2+}$ by *N. crassa* cobalt-resistant strain. Preformed mycelia (72 h old) were allowed to take up $Co^{2+}$for 10 min (●) and 20 min (Δ) from medium containing various concentrations of $Co^{2+}$.

and under these conditions of $Co^{2+}$ concentration-dependent saturation is possible. This implies that in contrast with the parent strain, despite quantitatively larger uptake, saturation with $Co^{2+}$ in the cobalt-resistant strain is attained much more slowly.

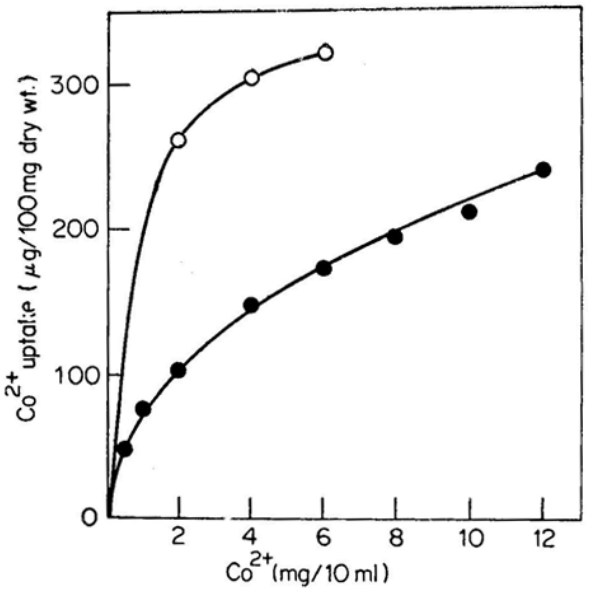


**Figure 3.** Dependence of $Co^{2+}$. Uptake by *N. crassa* cobalt-resistant strain on $Co^{2+}$ concentration in the medium. Preformed mycelia (72h old) were incubated for 3h (●) or 24h (O)in media containing various concentrations of $Co^{2+}$.

*Effect of pH on $Co^{2+}$ uptake*

The effect of pH on $Co^{2+}$ uptake by cobalt-resistant strain was also studied as described earlier (Venkateswerlu and Sivarama Sastry, 1970). The uptake was reduced below pH 4·0 and was unaffected in the range of pH 4–6. Decreased $CO^{2+}$ uptake below pH 4·0 is evidently due to competition with $H^+$ ions. It would appear therefore that there is no pH optimum for $Co^{2+}$ uptake in the cobalt-resistant strain.

*Effect of temperature on $Co^{2+}$ uptake*

$Co^{2+}$ uptake by the cobalt-resistant strain has been studied in the temperature range of 050° C, and the temperature has little influence on $Co^{2+}$ uptake in the range 040° C. However, a large increase in $Co^{2+}$ uptake occurs at 50° C, 15 $\mu$mol of (885 $\mu$g) $Co^{2+}$/100 mg dry weight in contrast to 2·63 $\mu$mol of $Co^{2+}$/100 mg dry weight of mycelia in 3 h which possibly indicate nonspecific binding by thermally killed mycelial material.

*Effect of metabolic inhibitors on $Co^{2+}$ uptake*

Intracellular uptake of $Co^{2+}$ by the parent strain can be inhibited by 2,4-dinitrophenol, sodium azide and fluoride (Venkateswerlu and Sivarama Sastry, 1970). The results presented in table 1 indicate that none of the metabolic inhibitors tested significantly depress $Co^{2+}$ uptake by the cobalt-resistant strain, maximal inhibition rarely exceeding 20% even at high concentration of these inhibitors.

**Table 1.** Effect of metabolic inhibitors on cobalt uptake by cobalt-resistant strain.

| Inhibitor | Concentration (mM) | $Co^{2+}$ uptake ($\mu$mol/100 mg dry wt) | % inhibition |
|---|---|---|---|
| Control | .. | 3·60 | 0 |
| Potassium fluoride | 5 | 3·58 | 0 |
| | 10 | 3·01 | 17 |
| Sodium azide | 1 | 3·87 | 0 |
| | 2 | 2·95 | 18 |
| 2,4-Dinitrophenol | 1 | 3·53 | 0 |
| | 2 | 3·05 | 15 |

Three day old mycelia were washed and incubated for 3 h with 102 $\mu$mo1 of cobalt/10 ml, in the presence of the inhibitor.

*Effect of $Mg^{2+}$ on $Co^{2+}$ uptake*

Excess $Mg^{2+}$ in the medium is known to reverse the growth inhibition due to $Co^{2+}$ toxicity in the parent strain by suppressing uptake of $Co_{2+}$ by the mold (Sivarama

Sastry *et al.,* 1962); $Mg^{2+}$ also suppresses $Co^{2+}$ uptake by preformed miycelia of the parent strain (Venkateswerlu and Sivarama Sastry, 1970). In growth experiments, a similar phenomenon, was observed in the cobalt-resistant strain as well (Venkateswerlu and Sivarama Sastry, 1973). Hence, the effect of $Mg^{2+}$ on $Co^{2+}$ uptake by preformed mycelia of cobalt-resistant strain has been examined. The data presented in figure 4 indicate that $Mg^{2+}$, when present in a two-fold excess (by wt), is unable to inhibit the $Co^{2+}$ uptake, while this concentration of $Mg^{2+}$ results in the complete reversal of growth inhibition caused by $Co^{2+}$ (Venkateswerlu and Sivarama Sastry, 1973). It would seem that, in this respect the cobalt-resistant strain differs from the parental strain. Since in growth experiments, $Mg^{2+}$ does however suppress overall uptake of $Co^{2+}$, it is evident that $Mg^{2+}$ does inhibit $Co^{2+}$ uptake in the cobalt-resistant strain but less significantly.

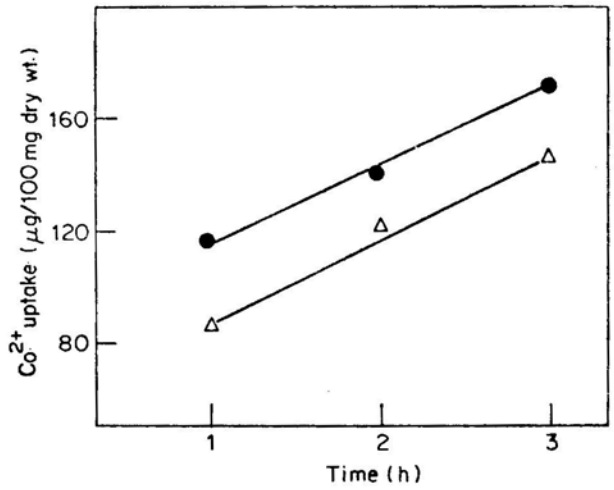


**Figure 4.** Effect of $Mg^{2+}$ on $Co^{2+}$ uptake by *N. crassa* cobalt-resistant strain. Preformed mycelia (72 h old) were incubated with. 102 $\mu$mol of $Co^{2+}$ for different time periods in presence of 0·82 $\mu$mol of $Mg^{2+}$ (●) and 494 $\mu$mo1 of $Mg^{2+}$ (Δ) in 10 ml of medium.

*Effect of EDTA on $Co^{2+}$ uptake by preformed mycelia*

It is evident that approximately 25–50% of the total $Co^{2+}$ taken up by preformed mycelia of cobalt-resistant strain is bound at the surface. When preformed mycelia of the parent strain of *N. crassa* saturated with $Co^{2+}$ were floated in a solution of 1 mM EDTA, rapid removal of (within 30 min) 20% of the $Co^{2+}$ taken up was observed. A much slower release of $Co^{2+}$ (another 10%) was observed within the next 2·5 h after which EDTA has little effect (Venkateswerlu and Sivarama Sastry, 1970). Similar experiments conducted in the present study with the cobalt-resistant strain revealed that around 80% of the total $Co^{2+}$ taken up is leached out by EDTA in 7 h (see figure 5), and that the process levels off at this stage.

The data in figure 5 also indicate that leaching of $Co^{2+}$ by EDTA is slow and progressive. It is probable that the slow rate of this efflux is due-to a slow influx rate of EDTA and/or a slow efflux rate for a $Co^{2+}$ EDTA complex.

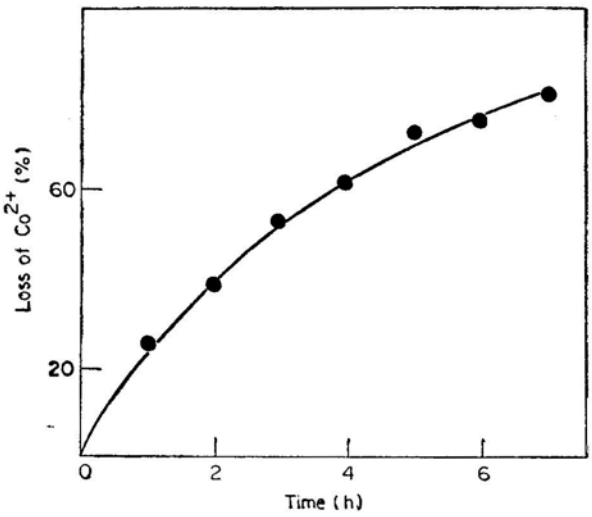


**Figure 5.** Effect of EDTA on $Co^{2+}$ taken up by *N. crassa* cobalt-resistant strain Preformed mycelia (72 h old) were incubated with 102 $\mu$mol Co2+/10ml for 3 h, washed and resuspended in medium containing lmM EDTA for different times.

In this context it is significant to note that the part of uptake which is sensitive to inhibition by metabolic inhibitors (18·5% of total, see table 1) is resistant to removal by EDTA.

## Discussion

In contrast to our earlier observations of an energy-dependent $Co^{2+}$ uptake which is mediated through the $Mg^{2+}$ transport system in the parent strain of *N. crassa* (Venkateswerlu and Sivarama Sastry, 1970), $Co^{2+}$ transport system exhibits different properties in the cobalt-resistant strain. This system manifests a rapid initial uptake of $Co^{2+}$ (23% at 34 $\mu$mol of $Co^{2+}$ and 47% at 102 $\mu$mol of $Co^{2+}$). This suggests that an equilibrium exists between free and cell surface bound $Co^{2+}$ in the cobalt-resistant strain. Unlike in the parent strain, the time taken to saturate the uptake system is very long in the cobalt-resistant strain. The concentration of $Co^{2+}$ required for this is also very high as compared to the parent strain (Venkateswerlu and Sivarama Sastry, 1970).

The uptake of $Co^{2+}$ in cobalt-resistant strain is not temperature-dependent. Similar instances are known in bacteria (Webb, 1970; Silver and Kralovic, 1969). Webb (1970) showed that the energy-independent uptake of $Co^{2+}$ in metal-resistant strains of bacteria is invariant between 0–28° C. $Ca^{2+}$ uptake by *E. coli* is unchanged with temperature between 25–45° C (Silver and Kralovic, 1969). Also, metabolic inhibitors do not inhibit $Co^{2+}$ uptake significantly in the cobalt-resistant strain. These evidences favour the existence of an energy-independent $Co^{2+}$ transport system in the cobalt-resistant strain. However, whether this altered transport system for $Co^{2+}$ has any relation with the acquisition of resistance to $Co^{2+}$ is not clear.

Another difference indicated by these results pertains to the nature of intracellular $Co^{2+}$. In the parent strain, 30% of bound $Co^{2+}$ is removed by EDTA from the preformed mycelia that accumulated $Co^{2+}$. However, this is not the case with the cobalt-resistant strain incubated with $Co^{2+}$. These observations indicate that $Co^{2+}$ is relatively more loosely bound intracellularly in the Cobalt-resistant strain or reflects the differences in the uptake of EDTA.

Apparently, the greater extracellular concentration of $Co^{2+}$ (102 $\mu$mol) required for 50% growth inhibition in the cobalt-resistant strain coupled with the nature of the slow diffusion process involved, results in an eventual build-up of $Co^{2+}$ concentration in mycelia of cobalt-resistant strain. This is also evident from the difference in uptake observed between 3 h and 24 h uptake with preformed mycelia.

Acquisition of metal resistance is obviously not due to the development of a permeability barrier for $Co^{2+}$ ions; however, metal uptake patterns at the cell-surface are very different in the cobalt-resistant strain as shown by effects of inhibitors, EDTA and the rather unusual aspects of $Co^{2+}$–$Mg^{2+}$ antagonism at the level of $Co^{2+}$ uptake.

There are two probable mechanisms which could explain the present data. The first of these is that the metal transport system in *N. crassa* which transports $Mg^{2+}$ and $Co^{2+}$ has widely different specificities in the cobalt-resistant strain. On this basis, competitive effects would be quantitatively different depending upon the nature of the change in affinity. Alternatively, the transport system is no longer able to mediate $Co^{2+}$ uptake, with the consequence that uptake is restricted to passive diffusion. This implies that the $Mg^{2+}Co^{2+}$ antagonism is much less pronounced at the cell surface in the cobalt-resistant strain and is restricted to competition in passive diffusion. If $Mg^{2+}$ transport is predominantly mediated by a specific transport system and this system also usually transports $Co^{2+}$, it is easy to see why $Mg^{2+}$ cannot powerfully suppress $Co^{2+}$ uptake in the cobalt-resistant strain.

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