*Biochem. J.* (1970) **118** 497–503
*Printed in Great Britain*



# The Mechanism of Uptake of Cobalt Ions by *Neurospora crassa*

By G. VENKATESWERLU and K. SIVARAMA SASTRY
*Biochemistry Division, Department of Chemistry, Osmania University, Hyderabad-7, A.P., India*

(*Received* 17 *December* 1969)

Uptake of $Co^{2+}$ by 3-day-old mycelia of *Neurospora crassa* involves cell-surface binding as well as transport into the intracellular space. The surface binding is rapid and accounts for 30–40% of the total $Co^{2+}$ uptake. Transport of $Co^{2+}$ occurs at a rate of 40 $\mu$g/h per 100 mg dry wt. Surface binding and overall uptake show different temperature dependence. Metabolic inhibitors such as azide, dinitrophenol and fluoride depress transport of $Co^{2+}$. The overall uptake of $Co^{2+}$ exhibits a high $K_m$ value and hence the concentration mechanism is one of low 'affinity' for the metal. The uptake of $Co^{2+}$ varies linearly with pH in the range pH 3 to pH 6. $Mg^{2+}$ inhibits both surface binding and transport of $Co^{2+}$. It is suggested that the system that transports $Mg^{2+}$ is also involved in $Co^{2+}$ uptake by *N. crassa*.

The toxicity of $Co^{2+}$ in *Neurospora crassa* has been studied by Sivarama Sastry, Adiga, Venkatasubramanyam & Sarma (1962*a*) and later by Padmanabhan & Sarma (1966). From these investigations and the earlier work of Healy, Cheng & McElroy (1955) some of the features of $Co^{2+}$ toxicity and metal ion interrelationships in metabolism in this mould have been elucidated. The toxicity of $Co^{2+}$ is reversed by $Fe^{3+}$ as well as $Mg^{2+}$. However, $Mg^{2+}$ alone does so by controlling mycelial uptake of $Co^{2+}$. As regards $Co^{2+}$ and $Fe^{3+}$, though some of the effects of $Co^{2+}$ toxicity in *N. crassa* appear to be due to a block in the utilization of $Fe^{3+}$, neither metal ion interferes with the accumulation of the other in this mould. Andersson-Kotto & Hevesy (1949) have examined $Co^{2+}$ and $Zn^{2+}$ uptakes in growing cultures of *N. crassa*, but their studies have only indicated that $Co^{2+}$ is taken up less efficiently than $Zn^{2+}$. Ballentine & Stephens (1951) have isolated a $Co^{2+}$-binding protein from *Neurospora*, but their experiments were concerned essentially with the uptake of micro quantities of $Co^{2+}$. Neither of these studies have dealt with the general mechanism of uptake. In the present study some aspects of the mode of accumulation and kinetics of $Co^{2+}$ uptake by *N. crassa* are presented.

## EXPERIMENTAL

*Materials and methods.* Analytical-grade $MgSO_4,7H_2O$ and $CoSO_4,7H_2O$ were employed throughout and concentrations mentioned below always refer to those of the metal ions. Glass-distilled water was used to prepare all solutions, and all chemicals used were of analytical grade.

Determination of $Co^{2+}$ in mycelia was performed after wet digestion as described below, with a modified nitroso-R-salt procedure (G. Venkateswerlu & K. Sivarama Sastry, unpublished work). This method was specific for $Co^{2+}$, and mycelia not incubated with $Co^{2+}$ showed no assayable metal.

The protein content of mycelial extracts was determined with Folin's reagent (Lowry, Rosebrough, Farr & Randall, 1951) with bovine serum albumin as the standard.

*Organism, maintenance and growth.* *Neurospora crassa* Em 5297a was maintained by weekly subcultures on agar slants. The basal medium used for agar slants and in growth experiments was as described earlier (Sivarama Sastry *et al.* 1962*a*) except that $Mg^{2+}$ was used at the minimal adequate concentration of 50 $\mu$g/10 ml. The mould was grown in 50 ml Pyrex conical flasks, without shaking, at pH 4.8–5.0, on 10 ml of basal medium, containing 2% glucose, for 72 h at 30±1°C. Mycelia were obtained from such cultures, by suppressing sporulation by the addition of Tween 80 as described by Zalokar (1954). Generally, such mycelia showed very little sporulation. Any sporulating structures still present were routinely removed mechanically with the aid of a fine glass rod. Mycelia were then extensively washed with sterile water. Thereafter, each mycelium was separately and gently blotted free of adhering water between folds of filter paper. Each mycelium was then suspended in a 50 ml conical flask on 10 ml of basal medium that contained 20 $\mu$g of $Mg^{2+}$ and various concentrations of $Co^{2+}$ and other compounds as required.

*Uptake experiments.* Flasks containing suspended mycelia were shaken on a mechanical shaker at about 10–15 strokes/min with a horizontal stroke distance of 10 cm. Faster shaking did not enhance the rate of uptake. Unless otherwise specified, incubations were conducted at room temperature (25–32°C). For uptake experiments at other temperatures, mycelia were preincubated with shaking for 15 min in a water bath at the required temperature before addition of $Co^{2+}$ solution (0.1 ml). After incubation, the mycelia were washed in ice-cold water. At the end of the incubation period, mycelia were removed, the medium was decanted off and the mycelia were repeatedly washed with water, until the final washings

were free of all traces of $Co^{2+}$ as shown by tests with 2-nitroso-1-naphthol-4-sulphonic acid (Sarver, 1938). Mycelia were then dried overnight at 60–80°C and their dry weights determined. Final dry weights of mycelia were $40 \pm 5$ mg in different experiments and in any one set of experiments did not vary by more than 2 mg.

Dried mycelia were then individually subjected to a wet digestion procedure based on that of Sivarama Sastry, Raman & Sarma (1962*b*). Conc. $HNO_3$ (5 ml) and $HClO_4$ (0.5 ml) were added to each mycelium in a 50 ml Pyrex conical flask and heated to dryness on a sand bath. Wet-ashing of the residue was then repeated with conc. $HNO_3$ (1 ml) and conc. HCl (1 ml). The residue was then dissolved in 3–5 ml of 1 M-HCl, with warming, if necessary, and portions were removed for analysis of $Co^{2+}$.

Unless otherwise stated the pH of the incubation medium was 4.8–5.0. The effect of pH was studied by adjusting the incubation medium to the desired pH with conc. HCl, by using a pH-meter that was accurate to within $\pm 0.05$ pH unit, before adding the mycelia.

To test the effect of $NaN_3$, 2,4-dinitrophenol and KF on $Co^{2+}$ uptake, these compounds were added with $Co^{2+}$ to the medium. In these experiments the concentration of glucose in the medium was 0.5% (in contrast with the usual 2.0%) so as to accentuate the effects of these compounds.

In experiments conducted under 'standard conditions', $Co^{2+}$ was added at a concentration of 2 mg/10 ml (3.39 mM-$Co^{2+}$) and mycelia were incubated for 2 h after which time the $Co^{2+}$ uptake varied from 150–275 $\mu$g/100 mg dry wt. of mycelium. Though the uptake varied in different experiments, in any one series of tests the variation did not exceed $\pm 10\%$ of the average. Mean values from triplicate or occasionally duplicate tests are reported.

To determine the effect of EDTA (disodium salt) on $Co^{2+}$ bound by *N. crassa*, mycelia were first allowed to take up $Co^{2+}$ under standard conditions. They were then washed, blotted dry and resuspended in 10 ml of basal medium alone, or medium containing 1 mM-EDTA, for various times. The mycelia were then washed thoroughly and their $Co^{2+}$ contents determined.

*Distribution of bound cobalt in* N. crassa *mycelial fractions.* To examine the nature of bound $Co^{2+}$, five mycelia incubated with $Co^{2+}$ under standard conditions were thoroughly washed, blotted dry and their fresh weights determined. The mycelia were ground with twice their weight of glass powder at 0°C in a total volume of 10 ml of water and then centrifuged at 12000–15000*g* for 30 min at 0°C. The opalescent supernatant was collected and its volume was measured (extract I). The residue was resuspended in 5 ml of water and recentrifuged as above (extract II). The residue was then extracted with 10 ml of cold 10% (w/v) trichloroacetic acid, and the supernatant collected (cold-acid extract). The residue left after trichloroacetic acid extraction is referred to as the 'residue'. The $Co^{2+}$ content of each fraction was determined.

Cold acetone was added to extract I to a final concentration of 85% (v/v) and left overnight in a refrigerator. The precipitated protein was separated by centrifugation and the $Co^{2+}$ contents of the precipitate and the supernatant were measured.

To determine whether the $Co^{2+}$ associated with the acetone precipitate was an artifact of fractionation, control mycelia (not incubated with $Co^{2+}$) were fractionated as above and an extract I was obtained. After assay of its protein content, $Co^{2+}$ (equal in amount to that found in extract I from mycelia incubated under standard conditions) was added, followed by immediate precipitation with acetone. Again the distribution of $Co^{2+}$ in the acetone-soluble and protein fractions was measured.

In another experiment, 5 ml of extract I was dialysed against an excess of water at 0°C, with continuous stirring for 5 h and an hourly change of water. Dialysis was complete under these conditions. Non-diffusible $Co^{2+}$ of extract I was then determined as above.

## RESULTS

*Kinetics and mechanism of uptake of* $Co^{2+}$ *by* N. crassa. Sivarama Sastry *et al.* (1962*a*) showed that *N. crassa* grown on a medium containing 493 $\mu$g of $Mg^{2+}$ and 1 mg of $Co^{2+}$/10 ml exhibits half-maximal growth; such mycelia (approx. $20 \pm 2$ mg dry wt.) accumulate about 50 $\mu$g of $Co^{2+}$/100 mg dry wt. In the present study preformed normal mycelia from 72 h cultures were employed to examine $Co^{2+}$ uptake. Unless stated otherwise mycelia were allowed to take up $Co^{2+}$ under standard conditions (see the Experimental section). The concentration of $Co^{2+}$ used (2 mg/10 ml) was chosen because it was toxic to preformed mycelia, permitted appreciable uptake of $Co^{2+}$ facilitating precise analysis, and accumulation of $Co^{2+}$ was high at this concentration of extracellular metal ion (see Fig. 3). Also, mycelia were alive at the end of the incubation period (2 h) and on withdrawal of exogenous $Co^{2+}$, continued to grow at a rate of about 30–50% of the normal in a further period of 24 h.

Fig. 1 shows the time-course of $Co^{2+}$ uptake by *N. crassa* mycelia under standard conditions. The results for uptake of $Co^{2+}$ in the presence of $Mg^{2+}$

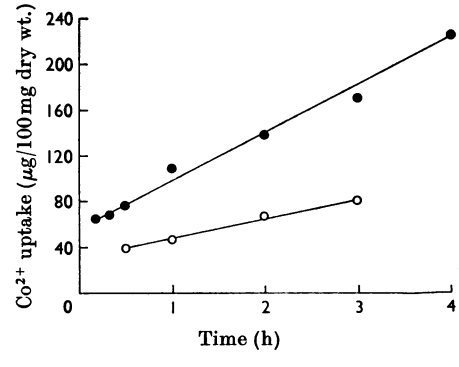


Fig. 1. Rate of uptake of $Co^{2+}$ by *N. crassa*. Mycelia were allowed to take up $Co^{2+}$ from medium containing 2 mg of $Co^{2+}$/10 ml for various times. For the experimental details see the text. ●, Normal $Co^{2+}$ uptake; ○, $Co^{2+}$ uptake in the presence of added $Mg^{2+}$ (4 mg/10 ml).

were obtained with an incubation medium containing 4mg of $Mg^{2+}$/10ml. A $Co^{2+}/Mg^{2+}$ weight ratio of 1:2 in the medium reversed $Co^{2+}$ toxicity completely and as expected from earlier work (Sivarama Sastry *et al.* 1962*a*) $Mg^{2+}$ severely depressed $Co^{2+}$ uptake; the significance of this phenomenon is discussed below.

Fig. 1 shows that $Co^{2+}$ uptake by mycelia (with and without added $Mg^{2+}$) increases linearly with time, and that neither line passes through the origin. In different experiments, such linearity of $Co^{2+}$ uptake with respect to time was always observed, irrespective of the time-period, (2h or 4h) so long as the total accumulation of $Co^{2+}$ by mycelia did not greatly exceed 200$\mu$g of $Co^{2+}$/100mg dry wt., which was generally the case. In view of these results, variations in accumulation of $Co^{2+}$ with increasing exogenous $Co^{2+}$ concentration and with very short times of incubation were investigated. Mycelia were incubated with various concentrations of $Co^{2+}$ for either 10 or 20min and total uptakes determined (Fig. 2; note difference in scale from Fig. 1). Short-term uptake was almost identical after 10 and 20min, confirming the results in Fig. 1 that suggest the rapid completion of an initial uptake. Moreover, this rapid uptake did not vary very greatly with $Co^{2+}$ concentration. At an exogenous $Co^{2+}$ concentration of 2mg/10ml this short-term uptake was 33$\mu$g of $Co^{2+}$/100mg dry wt. of mycelia, in reasonable agreement with the extrapolated zero-time value from Fig. 1 of 52$\mu$g/100mg dry wt. This range of values represents, presumably, the magnitude of rapid surface-binding of $Co^{2+}$ by *N. crassa* mycelia. Other evidence presented below supports this interpretation.

The dependence of mycelial uptake on the exogenous concentration of $Co^{2+}$ during incubation for 2h is shown in Fig. 3, which represents typical results. In several experiments, although the shape of the curve and the derived $K_m$ values (see below) were comparable, slightly different curves were obtained with different sets of mycelia owing to variations in uptake under the standard conditions. It is evident that $Co^{2+}$ uptake by *N. crassa* exhibits saturation kinetics with respect to $Co^{2+}$ concentration, suggesting that mere passive transport may not be the underlying mechanism. The uptake curve clearly shows that Fick's law of diffusion (Fick, 1885) is not obeyed and its shape is consistent with a process of facilitated diffusion or active transport. Also, these results indicate that the amount of $Co^{2+}$ uptake is high under the standard conditions used. When the results in Fig. 3 are plotted in the conventional double-reciprocal form of Lineweaver & Burk (1934) the $K_m$ value for overall uptake of $Co^{2+}$ is $1.61 \times 10^{-3}$ M.

The effect of different temperatures (2–50°C) on the uptake of $Co^{2+}$ by *N. crassa* during 20min and 2h of incubation was studied (Fig. 4). The temperature-dependence of the total uptake of $Co^{2+}$ during incubation for 2h is markedly different from that of the initial rapid uptake process (20min incubation). $Q_{10}$ values for $Co^{2+}$ uptake at these two incubation times (computed from Fig. 4) correspond to 18$\mu$g of $Co^{2+}$/100mg dry wt. and 3$\mu$g of $Co^{2+}$/100mg dry wt. of mycelia respectively. Thus, the initial binding is not appreciably affected by increase of temperature and is smaller in magnitude compared with total uptake. These results, with those of Figs. 1 and 2 are consistent with $Co^{2+}$ uptake by the mycelia consisting of two phases, an initial surface binding, followed by a slower accumulation into the intracellular space.

Studies on the effect of pH on $Co^{2+}$ uptake by *N. crassa* mycelia showed that uptake increases linearly with pH in the range pH 3 to pH 6.0. Values

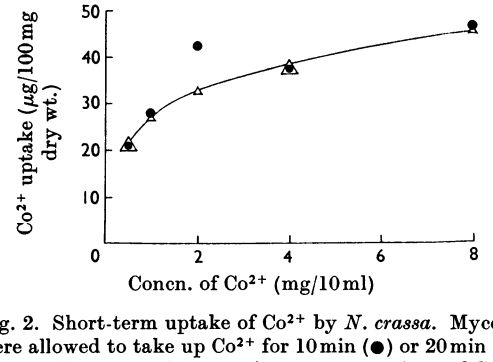


Fig. 2. Short-term uptake of $Co^{2+}$ by *N. crassa*. Mycelia were allowed to take up $Co^{2+}$ for 10min (●) or 20min (△) from medium containing various concentrations of $Co^{2+}$. For the experimental details see the text.

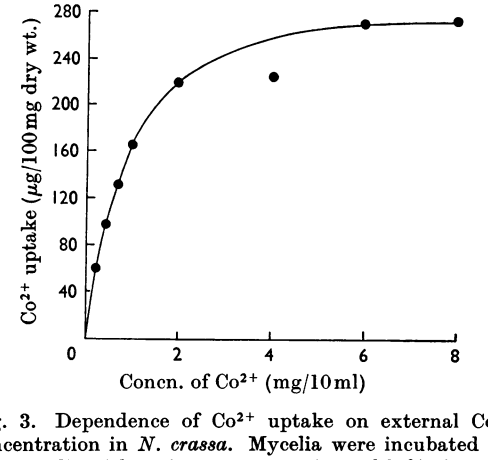


Fig. 3. Dependence of $Co^{2+}$ uptake on external $Co^{2+}$ concentration in *N. crassa*. Mycelia were incubated for 2h in media with various concentrations of $Co^{2+}$. For the experimental details see the text.

of 95.0, 125.5, 152.0, and 190.0 $\mu$g of $Co^{2+}$/100mg dry wt. of mycelia were obtained for pH 3, pH 4, pH 4.8 and pH 6 respectively at an exogenous $Co^{2+}$ concentration of 2mg of $Co^{2+}$/10ml and 2h incubation. At this concentration of added $Co^{2+}$, uptake could not be studied beyond pH 6 as the $Co^{2+}$ precipitated in the incubation medium. The results show that $H^+$ competes with $Co^{2+}$ in uptake.

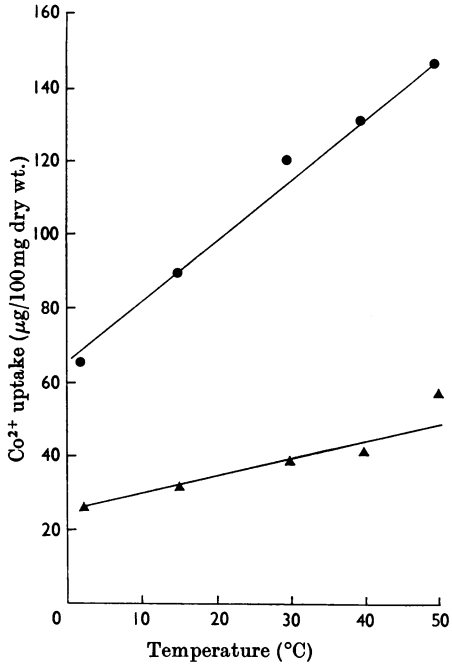


Fig. 4. Temperature-dependence of $Co^{2+}$ uptake by *N. crassa*. Mycelia were incubated in medium containing 2mg of $Co^{2+}$/10ml at various temperatures for 20min (▲) or 2h (●).

Since the results indicated that the uptake of $Co^{2+}$ by *N. crassa* mycelia is not a passive transport phenomenon, the effect of the metabolic inhibitors azide, dinitrophenol and fluoride, known to inhibit active transport, were examined (Table 1). The highest concentrations of inhibitors tested were the values beyond which no further inhibition of $Co^{2+}$ accumulation was observed. All three compounds inhibited $Co^{2+}$ uptake by about 50%. Of these inhibitors, only the effect of sodium azide on respiratory metabolism in *N. crassa* has been studied (Tissieres, Mitchell & Hoskins, 1953), and it was shown that with this inhibitor the maximal inhibition of respiratory metabolism was about 50%. Therefore, experiments were performed with azide to see how the rate as well as total uptake of $Co^{2+}$ was affected. In a typical experiment conducted over a 2h period, the rates in the presence and absence of 1mM-sodium azide were found to be 24 and 47.5 $\mu$g of $Co^{2+}$/h per 100mg dry wt. respectively; that is, the rate of uptake was 50.5% in the presence of the inhibitor. The results in Fig. 1 indicate a rate of uptake of 40 $\mu$g of $Co^{2+}$/h per 100mg dry wt. and that 'rapidly-bound' $Co^{2+}$ amounts to 39.3% of total uptake. In several experiments, irrespective of the uptake value reached in 2h (150–275 $\mu$g of $Co^{2+}$/100mg dry wt.), the normal rate of uptake and the fraction representing short-term uptake were of this order. Experiments were also performed to see how the inhibitors affected short-term uptake. In a typical experiment, with a 20min incubation period, inhibitions obtained with 1mM-azide, 20mM-potassium fluoride and 1mM-2,4-dinitrophenol were 19%, 6% and 5% respectively. (In this experiment, short-term uptake in the absence of the inhibitors was 32.2 $\mu$g of $Co^{2+}$/100mg dry wt.) Thus the inhibitors affected primarily the transport of $Co^{2+}$ into the mycelial cells and not surface binding.

Table 1. *Effect of metabolic inhibitors on* $Co^{2+}$ *uptake in* N. crassa

Standard conditions for $Co^{2+}$ uptake were used, except that metabolic inhibitors were also included as indicated. For experimental details see the text.

| Expt. | Inhibitor | Concn. (mM) | $Co^{2+}$ uptake ($\mu$g/100mg dry wt.) | Inhibition of uptake (%) |
|---|---|---|---|---|
| A | Sodium azide | Nil | 172.0 | 0.0 |
| | | 0.1 | 77.4 | 55.0 |
| | | 1.0 | 83.1 | 51.7 |
| B | Potassium fluoride | Nil | 163.0 | 0.0 |
| | | 5.0 | 94.5 | 42.0 |
| | | 20.0 | 78.2 | 52.0 |
| C | 2,4-Dinitrophenol | Nil | 183.0 | 0.0 |
| | | 0.1 | 104.3 | 43.0 |
| | | 1.0 | 80.5 | 56.0 |

Table 2. *Fractionation of bound* $Co^{2+}$ *in* N. crassa

Values are means of duplicates with five mycelia that had taken up $Co^{2+}$ under standard conditions. For the experimental conditions see the text.

| Fraction | Volume (ml) | $Co^{2+}$ content | |
|---|---|---|---|
| | | Total ($\mu$g) | (%) |
| Extract I* | 15.0 | 190.6 | 85.7 |
| Extract II | 5.0 | 4.6 | 2.1 |
| Cold-acid extract | 10.0 | 27.1 | 12.2 |
| Residue | — | 0.0 | 0.0 |
| Extract I | Acetone supernatant | 90.0 | |
| | Acetone precipitate† | 106.6 | |

* Complete dialysis of this extract showed that 26.8% of the $Co^{2+}$ was diffusible.
† This fraction contained all the protein (28.08 mg) of extract I.

The effect of EDTA on $Co^{2+}$ bound by mycelia was also studied, as detailed in the Experimental section. Mycelia were incubated with $Co^{2+}$ under standard conditions, thoroughly washed, and then refloated on 10 ml of basal medium for 3 h, rewashed and their $Co^{2+}$ content determined (186 $\mu$g of $Co^{2+}$/100 mg dry wt.). In contrast, when mycelia that had similarly taken up $Co^{2+}$ were refloated on basal medium containing 1 mM-EDTA for 30 min, 1 h, 2 h or 3 h, and then washed thoroughly, their $Co^{2+}$ contents were 147, 137, 134 and 131 $\mu$g of $Co^{2+}$/100 mg dry wt. respectively, i.e. the loss of bound $Co^{2+}$ in the above instances was 20.5, 25.3, 28.0 and 29.6% respectively. Thus the maximal amount of $Co^{2+}$ leached out by EDTA from mycelia that had taken up $Co^{2+}$ was about 30%. Other experiments showed that no $Co^{2+}$ was lost when mycelia were resuspended in a $Co^{2+}$-free basal medium after $Co^{2+}$ accumulation. The removal of $Co^{2+}$ by EDTA was rapid and largely completed within 30 min. This suggests that loss of intracellular $Co^{2+}$ due to flotation of mycelia in EDTA was quite small. It is therefore probable that the $Co^{2+}$ leached out by EDTA is predominantly surface-bound metal ion. This interpretation is apparently confirmed by the fact that the value of 30% is in fair agreement with the value of 39.3% for the 'rapidly bound' $Co^{2+}$ derived from Fig. 1.

*Distribution of* $Co^{2+}$ *in* N. crassa *mycelial fractions.* Attempts were made to determine the nature of bound $Co^{2+}$. After uptake of $Co^{2+}$, mycelia were fractionated as described in the Experimental section and the $Co^{2+}$ content of the fractions was determined (Table 2). About 88% of the $Co^{2+}$ taken up by mycelia was in a water-soluble form and when the protein in extract I was precipitated with acetone, nearly 55% of this $Co^{2+}$ was associated with the protein (3.6 $\mu$g of $Co^{2+}$/mg of protein). Control experiments (see the Experimental section) showed that this association of $Co^{2+}$ with protein was an artifact of fractionation, since protein precipitated after the addition of $Co^{2+}$ at the stage of fractionation contained the same concentration of $Co^{2+}$. Complete dialysis of extract I showed that 26.8% of $Co^{2+}$ is diffusible. Thus this latter value is probably a more accurate indication of free $Co^{2+}$ in the intracellular space. Nevertheless, this value is probably a lower limit for free $Co^{2+}$ in the mycelial cells, since some binding of $Co^{2+}$ to cell proteins may have occurred during cell disruption.

Apparently three categories of intracellular $Co^{2+}$ can be distinguished in *N. crassa* mycelia, two of which are found in water extracts of mycelia, and a third category (about 12% of the total) that is extracted by trichloroacetic acid.

## DISCUSSION

The present work shows that $Co^{2+}$ uptake by growing preformed *N. crassa* mycelia is not a passive transport phenomenon. Two phases of accumulation seem to be involved; an initial rapid binding by the mycelial cell surface that is completed in 10 min followed by a slower transport into the intracellular space. Evidence is presented indicating that about 30–40% of the total uptake is associated with the cell surface. This consists of the pattern of uptake (Fig. 1), the completion of rapid uptake within 10 min (Fig. 2), the lower temperature-dependence of surface-binding (Fig. 4) and the results of leaching experiments with EDTA. The absence of significant inhibition by metabolic inhibitors of short-term $Co^{2+}$ uptake also supports the above interpretation. Further, since the same proportion of the total $Co^{2+}$ taken up was rapidly bound, irrespective of variations in the total $Co^{2+}$ uptake in different experiments, partitioning of $Co^{2+}$ between the cell surface and the intracellular space is always apparently of the same order.

Uptake of $Co^{2+}$ into the intracellular space of

*N. crassa*, as distinct from surface binding, appears to be slow, with a rate of about 40 $\mu$g of $Co^{2+}$/h per 100mg dry wt., and is linear with time during incubation for 4h (Fig. 1). The calculated $K_m$ value for total uptake is $1.61 \times 10^{-3}$ M. Thus *N. crassa* may be said to have a low 'affinity' for $Co^{2+}$. Slayman & Tatum (1964) derived an equation for calculating the intracellular concentration of an ion from values for the extracellular concentration, uptake by the mould, and the wet wt./dry wt. ratio. With this equation, and the results from Fig. 3, and at a saturating concentration of extracellular $Co^{2+}$ (6mg/10ml; 10.2mM), the intracellular $Co^{2+}$ concentration was calculated as 18.43mM. The present results do not unequivocally define the exact amount of surface-bound $Co^{2+}$, but assuming this value is, on the average, 30–40% of the total $Co^{2+}$ taken up the intracellular concentration is in the range 11.02–12.9mM. Thus $Co^{2+}$ is concentrated to a small extent within the mycelia but not as would be expected if a powerful active-transport system was present. Hence, the uptake may perhaps be classified as a process of facilitated diffusion. Examples of facilitated diffusion resulting in moderate amounts of concentration of the transported compounds are sugar transport in brain slices (Gilbert, 1965) and L-tyrosine transport in brain slices (Chirigos, Greengard & Udenfriend, 1960) where intracellular concentrations of 1.5 and 2 times that of the extracellular concentration respectively are attained. These findings are in contrast with the active transport of $\beta$-galactosides in *Escherichia coli*, which leads to a 2000-fold concentration of $\beta$-galactoside within the bacteria compared with the environment (Stein, 1967*b*).

Stein (1967*a*) has summarized criteria that define facilitated diffusion. The lack of direct proportionality between the rate of penetration of a diffusing molecule and its concentration (Fick's law not obeyed); the existence of competition between molecules that are structurally similar; and a marked and specific decrease in the accumulation rate caused by inhibitors are all important. The first of the above criteria is obeyed in $Co^{2+}$ uptake in our experiments (Fig. 3). The decrease in $Co^{2+}$ uptake in the presence of $Mg^{2+}$ (Fig. 1) indicates competition between these two ions. The results obtained with azide, dinitrophenol and fluoride (Table 1) show that these compounds inhibit the transport process, as expected for such a mechanism. The results with azide are particularly significant since Tissieres *et al.* (1953) showed that in wild strains of *N. crassa*, respiratory metabolism can be inhibited to a maximum of 50% by 1mM-azide. The azide-insensitive respiration was attributed to endogenous respiration associated with utilization of reserve polysaccharides. The present results on azide inhibition (and also with the other inhibitors) show that respiratory inhibitors suppress $Co^{2+}$ uptake and that the transport system is energy-dependent.

Apparently, uptake of $Co^{2+}$ by *N. crassa* is unidirectional because no $Co^{2+}$ was lost when mycelia that had accumulated $Co^{2+}$, were resuspended in $Co^{2+}$-free medium and only about 30% of the $Co^{2+}$ taken up (the surface-bound metal ion) was removed by EDTA despite prolonged exposure to it. Benko, Wood & Segal (1969) reported that retention of actively transported amino acids in *Penicillium chrysogenum* is not energy-dependent but depends only on the integrity of the mycelial cell structure and that transport is unidirectional. A similar situation apparently prevails in *N. crassa* in the case of $Co^{2+}$ uptake, since experiments showed that even when mycelia that had taken up $Co^{2+}$ were resuspended for 2h in 1mM-azide no $Co^{2+}$ was lost from the mycelia (G. Venkateswerlu & K. Sivarama Sastry, unpublished work).

With regard to the nature of the transport system involved in $Co^{2+}$ uptake, results of Sivarama Sastry *et al.* (1962*a*) showed that *N. crassa* has a fairly active mechanism for the uptake of $Mg^{2+}$ since this mould attains maximal growth at an extracellular $Mg^{2+}$ concentration of only 5 $\mu$g/ml. Since $Mg^{2+}$ suppresses $Co^{2+}$ uptake (Fig. 1) by interfering with surface binding and with intracellular uptake, it is possible that the system involved in $Mg^{2+}$ uptake is also involved in the uptake of $Co^{2+}$. It is noteworthy that even very high concentrations of $Fe^{3+}$ do not affect $Co^{2+}$ uptake in *N. crassa* (Sivarama Sastry *et al.* 1962*a*) which indicates that a specific ion-transport mechanism is operative. Also, Abelson & Aldous (1950) showed that $Mg^{2+}$ specifically interferes with the accumulation of many toxic metal ions in various yeasts, *Aspergillus niger* and bacteria.

This work was made possible by a Research Grant from the Department of Atomic Energy, Government of India, which is gratefully acknowledged.

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