Short Communication

DIFFERENCES BETWEEN THE UPTAKE OF TOXIC AND ESSENTIAL METAL IONS BY KB CELLS IN CULTURE

有害金属イオンと必須金属イオンの KB 培養細胞による取込みの差異について

Considerable attention has been given to the cellular uptake of metal ions.¹⁻³⁾ To study the distinction of metal ions on the cellular level *in vitro*, we have carried out uptake experiments on such metal ions as cadmium, lead, mercury, silver, iron, copper, zinc, manganese, cobalt, nickel, and chromium.

Materials and methods

KB cells were maintained in the Eagle's MEM containing new born calf serum at 2%. Cells of 5×10^4 in number in 2 ml of the growth medium were distributed in each tissue culture tube (15×150 mm), and incubated at 37°C by a monolayer culture method. The growth of cells was estimated by assaying cellular proteins in each tube according to the method of Lowry *et al.*⁴⁾

Metal salts such as $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $\text{Pb}(\text{NO}_3)_2$, $\text{Hg}(\text{NO}_3)_2$, AgNO_3 , $\text{FeSO}_4 \cdot (\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ and $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ were used. At forty-eight hours after inoculation of the cells, the growth medium was replaced with a fresh medium containing one of the metal ions described above. Two to four replicate cultures were harvested at each time of the measurement. Cells were immersed in 1.5 ml of 1.0% NaOH solution. When the cells had been lysed, 0.5 ml of 10% HNO_3 was added to it. The concentrations of metal ions in both the medium and the cell lysate were measured by flame atomic absorption spectrophotometry.

Results and discussion

The uptake of Cd^{2+} by KB cells at 37°C is shown in Fig. 1. The cellular uptake of Pb^{2+} is shown in Fig. 2. The time course of Ag^+ uptake is shown in Fig. 3. The decrease of Hg^{2+} concentration in the culture medium due to the uptake by KB cells is shown in Fig. 4. The uptake experiments of Cd^{2+} , Pb^{2+} , Ag^+ and Hg^{2+} by KB cells were carried out at several concentrations which were in the

Fig. 1. Time course of Cd^{2+} uptake by KB cells. The initial concentrations of Cd^{2+} were 0.78 ppm (---○---) and 0.41 ppm (---△---) in the culture medium, which corresponded to cell contents, shown by (—●—) and (—▲—), respectively.

Fig. 2. Time course of Pb^{2+} uptake by KB cells. Each point is a mean and each bar on that mean are $\pm$ S.D.

Fig. 3. Time course of Ag^+ uptake by KB cells.

Fig. 4. Decreases of Hg^{2+} concentrations in the culture medium due to the uptake by KB cells.

ranges of 0.2–1.2 ppm of Cd^{2+} , 0.5–2.0 ppm of Pb^{2+} , 0.5–5.0 ppm of Ag^+ and 0.3–1.0 ppm of Hg^{2+} , respectively. In each of the experiments uptakes of these metal ions were observed. The uptake was initiated soon after the addition of the metal ions and reached a stationary state at about 10th hr. The difference between the growth of the cells with and without heavy metals in the culture medium was not observed within 10 hrs after the

Fig. 5. Concentrations of Fe^{2+} , Cu^{2+} , and Zn^{2+} in the culture medium in the course of culture. Fe^{2+} (2.0 ppm), Cu^{2+} (1.5 ppm) or Zn^{2+} (1.6 ppm) was added to the culture medium.

Fig. 6. Concentrations of Ni^{2+} , Co^{2+} , and Mn^{2+} in the culture medium in the course of culture. Ni^{2+} (1.5 ppm), Co^{2+} (2.3 ppm) or Mn^{2+} (2.3 ppm) was added to the culture medium. The plots are the means of two replicate cultures.

addition of the metals.⁴⁾ Toxicities of heavy metals were revealed in one or two days culture, depending on the concentration of the metals.

Changes in the concentrations of these metal ions were not observed at all in the culture medium without cells. The increase in the content of these elements in cells corresponded to the decrease in the concentration in the culture medium (Figs. 1-3).⁴⁾ It has already been reported that counter ions such as NO_3^- , SO_4^{2-} and Cl^- did not influence the absorption of metals.⁴⁾

On the other hand, the decrease of the concentrations of Fe^{2+} , Cu^{2+} , Zn^{2+} , Mn^{2+} , Co^{2+} , and Ni^{2+} in the culture medium was not observed as shown in Figs. 5 and 6. Standard deviations of the measurements of replicate cultures were within 0.05 ppm. Cr^{3+} was also not absorbed. The up-

take of these metals was not observed at several concentrations from 0.2 to 5.0 ppm. The content of these elements in cells was so small that changes of the concentration of these metals in the culture medium could not be observed in spite of the growth of the cells.

The results presented in this report indicate that ions of elements such as Fe, Cu, Zn, Mn, Co, Ni, and Cr in the fourth period were apparently not absorbed by the cells but that those in the fifth and sixth periods such as were the case in Cd, Pb, Hg, and Ag were absorbed easily. Schwarz and Matrone reported the uptake of Zn by 3T3 cells.⁵⁾ However, the amount of Zn taken up by those cells (7 ng/ 10^5 cells) was much smaller than that of Cd (5×10^2 ng/ 10^5 cells) in our experiments. Zn^{2+} contents in the cells seem to be controlled strictly by the homeostatic mechanism. It seems likely that the transfer of essential ions such as Fe^{2+} , Cu^{2+} , Mn^{2+} , Co^{2+} , Ni^{2+} and Cr^{3+} is controlled by the same mechanism. In contrast, there might be no homeostatic controls for the toxic metal ions such as Cd^{2+} , Pb^{2+} , Hg^{2+} , and Ag^+ .

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