IRON: POSSIBLE CAUSE OF THE G_1 ARREST INDUCED IN NRK CELLS BY PICOLINIC ACID

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SUMMARY The $G_1(G_0)$ arrest induced in NRK cells by picolinic acid was preceded by marked changes in iron metabolism. In contrast, picolinic acid did not significantly prevent zinc uptake and changes in intracellular zinc were small and clearly preceded by changes in iron. A kinetic study revealed that iron uptake by NRK cells was rapidly halted by picolinic acid. Experiments with radioiron-labeled cells indicated that picolinic acid, in a dose dependent manner, effectively removed iron from the cells. The dose of picolinic acid that exactly removed iron from the cells was also the concentration that induced the $G_1(G_0)$ arrest. Picolinic acid, therefore, may induce the growth inhibition by selectively withholding iron from the cells. These data strongly suggest that iron availability may be a controlling factor in the initiation of DNA synthesis in NRK cells.

INTRODUCTION

Addition of picolinic acid to the culture medium reversibly arrests NRK (Normal Rat Kidney) cell growth in the $G_1(G_0)$ phase of the cell cycle (1). Previous studies indicate that the $G_1(G_0)$ arrest induced by this agent in NRK cells may be primarily due to iron and/or zinc deprivation (2). Those studies, however, were not designed to allocate the specificity of picolinic acid to chelation of either zinc, iron or both (2). Therefore, iron and zinc metabolism are of particular importance because changes in the concentration of those ions may be a primary event in the regulation of cell growth in tissue culture by picolinic acid (2). Here I report on the effects of picolinic acid on the kinetics of ^{59}Fe (as citrate) and ^{65}Zn in NRK cells. These results strongly suggest that iron depletion is the primary cause of the $G_1(G_0)$ arrest induced in NRK cells by picolinic acid.

MATERIALS AND METHODS

Materials Alpha-picolinic acid was obtained from Sigma (St. Louis,

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Missouri). ^{59}Fe-citrate (Ferrous citrate in 0.85% NaCl, Sp. Act 36.8 Ci/g Fe), and ^{65}Zn ($ZnCl_2$) carrier free were obtained from ICN, Irvine, California. Tissue culture media was purchased from Microbiological Associates (Bethesda, Maryland).

Methods NRK cells were grown in Dulbecco-Vogt modified Eagle's medium containing 10% (V/V) calf serum (Colorado Serum Company) as previously described (1). Cells were planted in most experiments at 1.5×10^5 cells/dish in 20 cm^2 plastic tissue culture dishes (Costar). Unless otherwise indicated, media were changed 48 hr later by new media without (control) or with (treated) picolinic acid. The cell DNA content was analyzed by flow microfluorometry (FMF) as previously described (1). The frequency distribution of fluorescent emission per cell was measured using a cytofluorograph 4800A (Bio/physics Systems, Inc., Baldwin Place, New York), and analyzed with a multichannel analyzer (Bio/physics Systems, Inc. Model 2102). Cells were counted in a Coulter counter. Protein was measured by the method of Lowry (3). Every determination was carried out in triplicate or quadruplicate at each of the indicated times. To determine the radioactive content of the cells, the culture medium was rapidly decanted and the cells were rinsed six times with 37°C phosphate-buffered salt solution. Then, the cultures were extracted with 1.5 ml of 10% trichloroacetic acid (TCA) at 4°C. The radioactive samples were counted in a gamma counter to determine total counts. Then, the samples were washed three times with 10% TCA and the radioactivity of the pellets was measured.

RESULTS

Effects of Picolinic Acid on Iron and Zinc Uptake Control and treated cultures were tested for ^{59}Fe and ^{65}Zn uptake for a 25-30 hr period. Incorporation of ^{59}Fe into the total cell population (Fig. 1) was linear in control cultures and was strongly inhibited by 3 mM picolinic acid. As shown in Fig. 2, approximately 20-40% and 10-20% of the ^{59}Fe counts of the control and treated cells, respectively, remained acid precipitable. In this and subsequent experiments presented here, the TCA precipitable ^{59}Fe counts of control and treated cultures always paralleled total cell counts. These results, which were reproduced in five separate experiments, suggested a possible connection between growth inhibition, $G_1(G_0)$ arrest (Fig. 3) and iron uptake. The concentration of picolinic acid that just inhibited iron uptake (3 mM) was the concentration that inhibited cell growth (Fig. 3). ^{65}Zn uptake, however, was not significantly affected by the presence of picolinic acid in the tissue culture media (Fig. 4). ^{65}Zn counts were not retained after TCA precipitation. Therefore, ^{59}Fe was the only radioisotope affected, suggesting that the growth arrest is related to the relative affinity of pico-

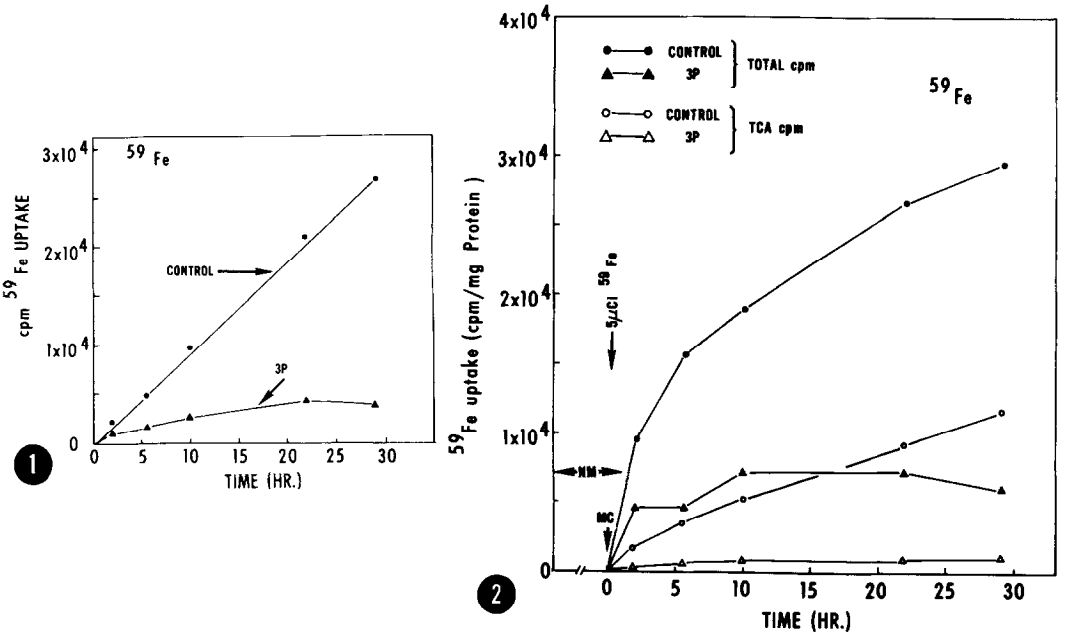

Fig. 1 Effect of picolinic acid on ^{59}Fe uptake into the total cell population. Cells were grown as described in Materials and Methods. After 15 min of media change (t = 0), 5 µCi of ^{59}Fe were added to each dish. Total cpm were determined at the indicated times; each point is the average from two cultures. 3P: 3mM picolinic acid.

Fig. 2 Effect of picolinic acid on incorporation of ^{59}Fe into total and 10% trichloroacetic acid (TCA) precipitable material in NRK cells. Cells were grown as described in Material and Methods. After 15 min of media change (t = 0), 5 µCi of ^{59}Fe were added to each dish. The ^{59}Fe uptake was determined at the indicated times. 3P: 3mM picolinic acid; NM: Normal Medium; MC: Media Change. Each point is the average from two cultures.

linic acid for iron (4).

Effects of Picolinic Acid on Cellular Iron and Zinc. To demonstrate that picolinic acid preferentially affects intracellular ^{59}Fe, the cells were labeled to equilibrium with 5 µCi ^{59}Fe or 5 µCi ^{65}Zn for 24 hr (Fig. 5 and 6). At this time, addition of picolinic acid to the tissue culture media, caused a marked time dependent decrease (apparent disappearance T-1/2 = 15 hr) in the ^{59}Fe counts present in the total cell population (Fig. 5). This experiment conclusively shows that picolinic acid can remove ^{59}Fe from the cells.

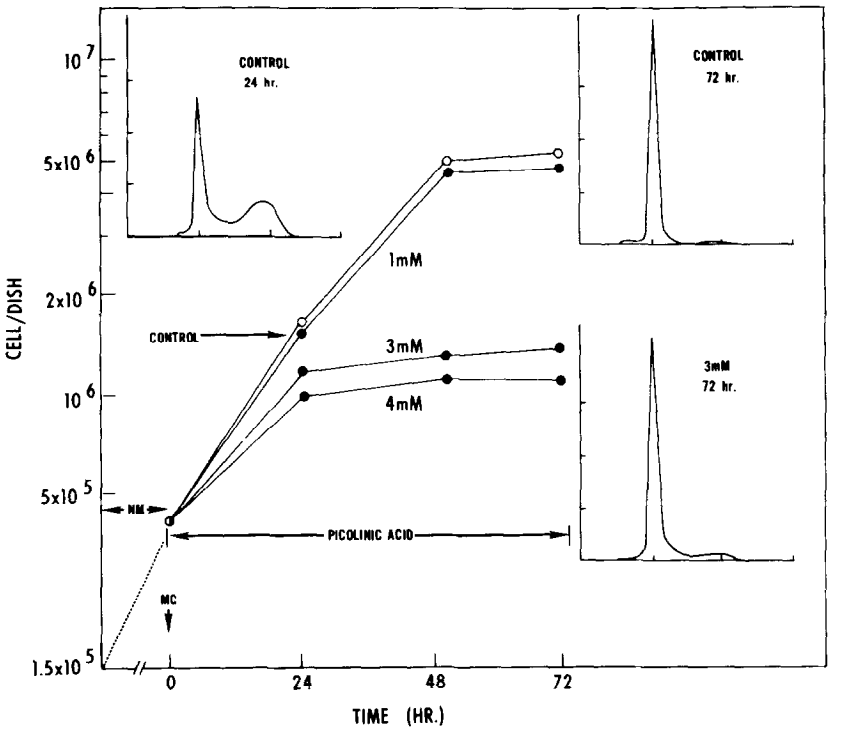

Fig. 3 Dose dependence of growth inhibition in NRK cells treated with picolinic acid. Cells were planted at 1.5 x 10^5 cells/dish and 48 hrs later the medium was removed and new media containing picolinic acid at the indicated concentrations were added. Cell counts were determined at the indicated times; each point is the average of duplicate measurements from two cultures. DNA distribution profiles (inserts): Control, 24 hr: logarithmic growing cells; Control, 72 hr: Density-inhibited cells; 3 mM, 72 hr: Cells exposed to picolinic acid.

The dose of picolinic acid that exactly removed ^{59}Fe from the cells (3 mM) (Fig. 5) or prevented its entrance (Fig. 2) was the dose that induced the $G_1(G_0)$ block in NRK cells (Fig. 3). In contrast, addition of picolinic acid to the tissue culture media did not significantly change the ^{65}Zn counts present in the total cell population within 12 hr (not shown) and induced small changes at 24 hrs (Fig. 6). Thus, the apparent decrease in intracellular ^{65}Zn counts, when the values are expressed in cpm/mg protein must be due to dilution of ^{65}Zn counts in the intracellular spaces of growing cells. This, together with the lack of significant effects of picolinic acid on ^{65}Zn

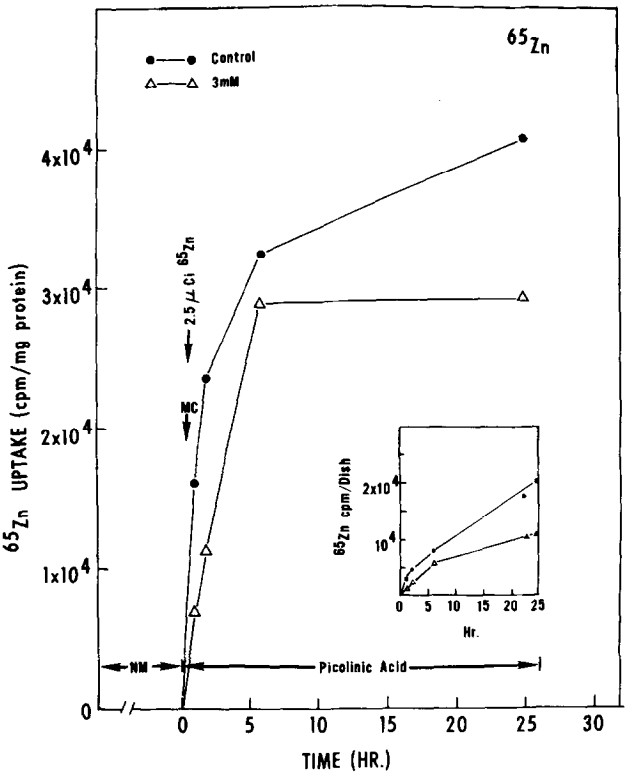

Fig. 4 Kinetics of the changes in ^{65}Zn uptake produced by the addition of picolinic acid to the culture media. Insert shows the effect of picolinic acid on ^{65}Zn uptake into the total cell population. Cells were grown and treated as described in the text. After 15 min of media change (t = 0), 2.5 μCi of ^{65}Zn were added to each dish. The ^{65}Zn uptake was determined at the indicated times. Each point is the average from two cultures.

uptake, suggested that this ion is not related to the mechanism of action of this agent on NRK cells. However, the unlikely possibility that the small changes in zinc metabolism, which were clearly preceded by changes in iron, play a more important role in the control of cell growth by picolinic acid in NRK cells cannot be excluded.

DISCUSSION

The results presented here show that picolinic acid profoundly affects iron metabolism in NRK cells. They indicate, therefore, that the

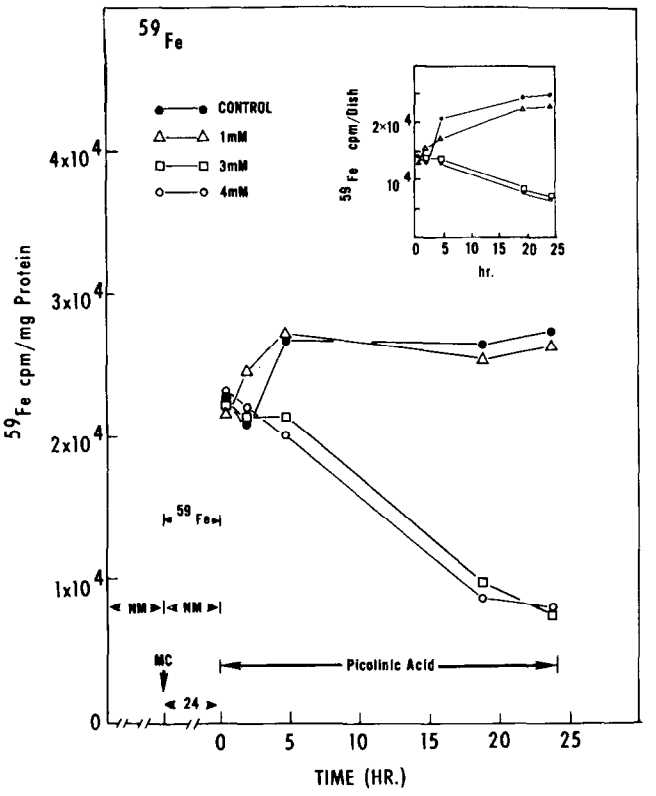

Fig. 5 Dose dependence of ^{59}Fe depletion in NRK cells treated with picolinic acid. Insert shows the effect of picolinic acid on ^{59}Fe content of the total cell population. Cells were grown and treated as described in the text. The cells were labeled to equilibrium with 5 μCi ^{59}Fe for 24 hr. Picolinic acid was added to the tissue culture media at t = 0. Each point is the average from two cultures.

inhibition of growth in $G_1(G_0)$ induced by the agent may be preferentially caused by withholding iron from the cells. This data is in accordance with results from this laboratory that showed, under certain *in vitro* experimental conditions, interactions of picolinic acid with transferrin (5).

Iron has long been recognized as an essential element for the growth of bacterial, plant and animal cells (6). It has been shown that addition of the iron-chelating agent desferrioxamine to cells in tissue culture causes a selective inhibition of DNA synthesis (7,8). However, the precise role played by iron in the regulation of the cell cycle is not clear.

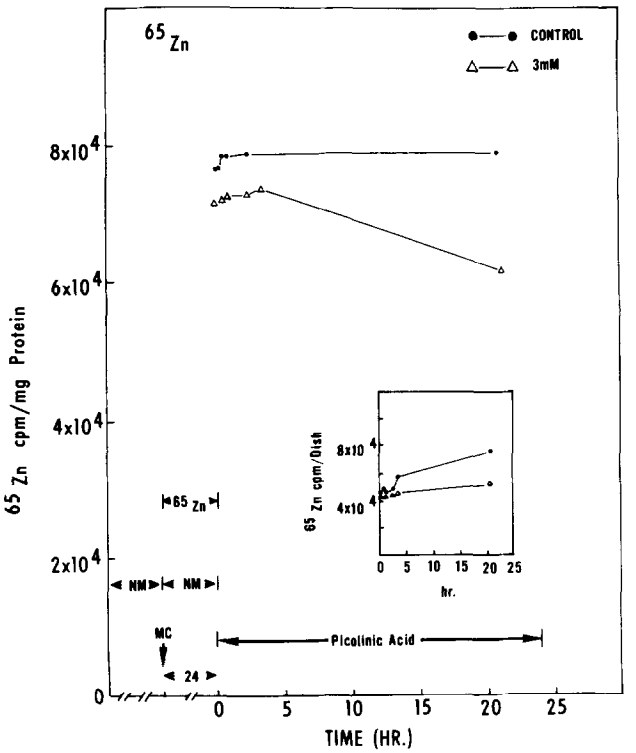

Fig. 6 Kinetics of the changes in cellular ^{65}Zn produced by the addition of picolinic acid to the culture media. Insert shows the effect of picolinic acid on ^{65}Zn content of the total cell population. Cells were grown and treated as described in the text. Each point is the average from four cultures.

The experiments presented here imply a hitherto unsuspected role for iron in the control of the cell cycle in NRK cells. These results strongly suggest a relationship between depletion of intracellular iron and $G_1(G_0)$ arrest. Therefore, these data suggest that iron availability may be a controlling factor in the initiation of DNA synthesis in NRK cells.

Density inhibited NRK cells are blocked in $G_1(G_0)$ as well as NRK cells treated with picolinic acid (Fig. 3). Therefore, if the two conditions were equivalent, a possibility that deserves further investigation is that iron availability may be one of the factors controlling saturation density in NRK cells in tissue culture. Considering the differential effects of pico-

linic acid on normal and transformed cells in tissue culture (1), the selectivity of the agent may be due to different trace metal ion requirements by normal and transformed cells. Further studies with picolinic acid should be useful in understanding growth control mechanisms and basic differences between normal and transformed cells.

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