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Enhanced Plutonium Absorption in Iron-Deficient Mice[1] (38969)

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(Introduced by F. P. HUNGATE)

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Iron deficiency anemia is probably the most common chronic organic disease of man, affecting all age groups and economic strata, and occurring as a result of inadequate dietary iron, chronic blood loss, increased physiologic demands for iron (during periods of growth, pregnancy, or lactation), intestinal malabsorption syndromes, and combinations of these factors. Thus, there are certain high-risk groups in which the incidence of iron deficiency anemia varies from 5 to 30%, i.e., infants, adolescent children, and pregnant or other premenopausal women (1–4). Since anemia occurs late in the course of iron depletion, the incidence of latent iron deficiency in these groups is undoubtedly much greater than the frank anemic state. In fact, some degree of iron deficiency appears to be consistently present during pregnancy, with depletion of iron stores occurring even with supplementary iron medication (5–7).

With the increased development of nuclear power reactors and maximum utilization of other energy sources, the toxicology of pertinent radionuclides and certain other pollutant metals must be thoroughly understood, particularly with regard to conditions that may increase their incorporation in the body. The absorption of environmental pollutant metals in iron-deficient animals should be investigated, since there may be important public health implications concerning these interrelationships. These studies should include those metals whose primary portal of entry into the body may be by inhalation, since a large percentage of inhaled particulate matter is ultimately excreted via the intestinal tract when cleared from the respiratory system (8, 9). Unfortunately, there is a paucity of information regarding the absorption or toxicity of such pollutants in iron-deficient animals, although one recent study reported iron-deficient rats as more susceptible to chronic lead poisoning than iron-replete rats (10).

Several studies have shown an intimate relationship between plutonium and most iron-binding compounds in the body (11–13). Therefore, plutonium absorption might be related to iron absorption and could be enhanced if body-iron stores were reduced. To test this hypothesis, adolescent mice were rendered iron deficient by deprivation of dietary iron. Gastrointestinal plutonium absorption and tissue distribution were studied following a single gastric gavage of putonium citrate.

Materials and Methods. Eleven-day-old nursing ICR white mice and their dams were separated into two groups: one was given a pelleted, iron-replete diet containing 400 μg iron/g of food; the other, a pelleted, iron-deficient diet[2] with 3.3 μg iron/g. Deionized water provided to both groups contained less than 0.2 μg of iron/ml. The mice were housed in plastic cages whose metal tops had been sprayed with several layers of acrylic, and the commercial bedding contained <7 μg of iron/g. The mice were weaned at 21 days of age, and continued on an iron-replete or iron-deficient diet.

At 40 days of age, after a 12-hr fast, each mouse was given 15 μCi of freshly prepared ^{239}plutonium–citrate solution (1% citrate) by gastric intubation under light halothane anesthesia. Then, 24 or 96 hr after gavage, the mice were killed by exsanguination via cardiac puncture while under halothane anesthesia. The skin was removed, the gastrointestinal tract ligated and carefully excised, and liver, spleen, kidneys, one femur, and the remaining carcass taken for radioanalysis.

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[2] ICN Nutritional Biochemicals Co., Cleveland, OH.

These tissues were weighed, ashed, dissolved in nitric acid, and aliquots pipetted into scintillation fluid prior to assay for radioactivity. Statistical evaluations were made using six mice each in both the control and iron-deficient groups 24 hr after gavage, and eight and seven mice from the corresponding groups at 96 hr. Treatment means were compared using Student's t test, and a one-tailed comparison made.

Analyses for plutonium in excreta were not attempted because of difficulties in obtaining valid information under the conditions of this study, i.e., the high plutonium concentrations in feces following gavage, and the resultant probability of cross-contamination of urine samples.

Results. Values for body weights at 28 and 40 days of age and volume of packed red cells (VPRC) at 40 days are shown in Table I. Both body weights and VPRC were significantly lower ($P > 0.01$) in iron-deficient mice on day 40, and erythrocytes of the iron-deficient mice were microcytic and hypochromic. With the exception of the spleen, organ weights did not differ significantly between iron-deficient and iron-replete mice. Average spleen weight for iron-deficient mice was 0.27 ± 0.006 g (standard error of the mean) and for the iron-replete mice was 0.11 ± 0.01 g.

TABLE I. BODY WEIGHTS AND VOLUME OF PACKED RED CELLS (VPRC) OF IRON-DEFICIENT AND IRON-REPLETE MICE (mean ± SEM)

Diet	Number of mice	28 Days wt (g)	40 Days wt (g)	40 Days VPRC (ml/100 ml)
Iron deficient	18	14.6 ±0.4	18.4 ±1.2	13.5 ±1.3
Iron replete	16	16.4 ±0.6	24.1 ±1.5	45.0 ±1.0

The percentage of administered plutonium retained in various tissues 24 and 96 hr after gavage is shown in Fig. 1. The plutonium content of soft tissue and bone was higher in iron-deficient than in iron-replete mice at 24 hr after gavage, although mean levels in the spleen and kidney were not significantly different between the two groups because of the large variation in plutonium concentration within these organs. By 96 hr the soft tissue content of plutonium in the iron-deficient mice had decreased rather markedly and bone levels had increased (Fig. 2). In the control group at 96 hr the spleen, kidney, and blood plutonium content had decreased, the liver plutonium content had increased, and bone content was essentially unchanged. Plutonium content of bone was significantly higher ($P < 0.01$) in iron-deficient mice at both 24 and 96 hr. Total body burden of plutonium measured after 24 hr was 0.108 ± 0.021% (SEM) in iron-deficient mice and 0.028 ± 0.007% in iron-replete mice. Cor-

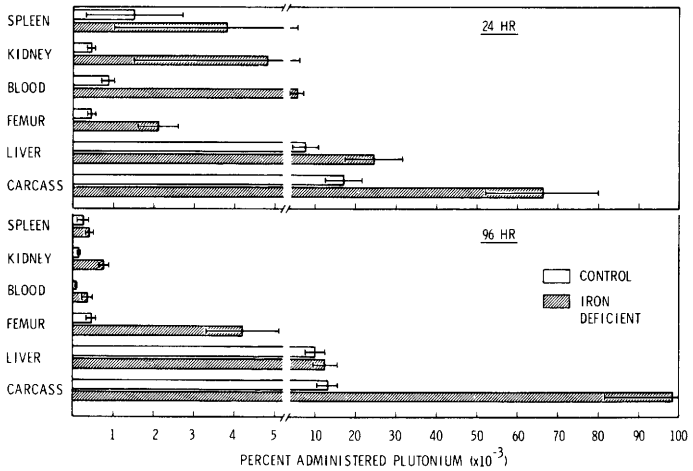

FIG. 1. Percentage of ^{239}Pu citrate administered to iron-deficient and control mice by gastric gavage found in various tissues 24 and 96 hr later (mean ± SEM).

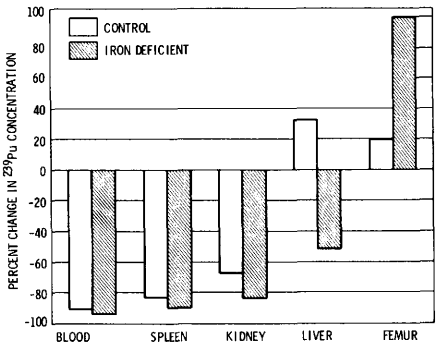

FIG. 2. Percentage change in ^{239}Pu concentration in various tissues between 24 and 96 hr following a gastric gavage of plutonium citrate administered to iron-deficient and control mice.

responding values measured after 96 hr were 0.117 ± 0.021% and 0.027 ± 0.005%. At both time periods the differences were significantly greater ($P < 0.01$) in the iron-deficient mice.

Discussion. The fourfold increase of plutonium body burden in iron-deficient mice over that of iron-replete mice at both 24 and 96 hr following gavage, is most readily explained as due to enhanced absorption. Although urinary excretion data were not obtained, it is unlikely that these large differences in body burdens resulted because of decreased plutonium excretion in iron-deficient mice. This is supported by results from a subsequent study in which iron-deficient mice, given plutonium by intraperitoneal injection, had significantly higher body burdens than iron-replete mice, yet urinary excretion of plutonium was about threefold greater in the former group.[3] It is reasonable to assume similar excretion differences occurred following the gastric gavage with plutonium, in which case absorption may actually have been greater than that indicated from total body burden analyses.

Based on available information regarding mechanisms of iron absorption and the association of plutonium with iron-binding proteins, it is possible to speculate on the enhanced plutonium absorption in iron-deficient mice. Plutonium, although chemically dissimilar to iron, has a high binding constant to transferrin, ferritin, and hemosiderin (11–13), and thus might be associated with the same mechanisms responsible for iron absorption. Apparently one regulatory mechanism controlling iron absorption is dependent upon the iron content of intestinal mucosal cells, and the enhanced absorption of iron in deficient animals and man is due to the low iron content of these cells (14–17). Because of the association of plutonium with other iron-binding proteins, it is reasonable to assume that iron-binding materials within the intestinal mucosal cells would also bind plutonium. If so, there would be more such sites available to bind plutonium in iron-deficient subjects.

The enhanced plutonium absorption is probably not related simply to an increased gut permeability to plutonium, since previous studies have shown the intestine of iron-deficient animals and humans retains some selectivity, and does not become generally permeable to all metal ions. Iron deficiency results in enhanced absorption of cobalt, manganese, and zinc in man and in rats but causes no change in the absorption of cesium, copper, magnesium, mercury, or calcium (18–21). Cobalt, manganese, and zinc are essential metals and closely related chemically to iron; however, copper, magnesium, and calcium are also essential metals and related chemically to iron, yet their absorption is apparently not enhanced in iron-deficient subjects. These two criteria, therefore, do not explain differences in absorption. Of particular interest is a recent study indicating an increased toxicity from daily feeding of lead to iron-deficient rats (10). The authors speculate that the enhanced toxicity may be due, in part, to increased lead absorption.

It is tempting to speculate on the role of gastroferrin, an iron-binding protein in gastric juice, in the enhanced absorption of plutonium. The function of this protein in iron absorption is controversial, but the quantity of gastroferrin has been reported to be greatly reduced in iron-deficient humans (22) and probably in patients with hemochromatosis (23). The implication is that a decrease in gastroferrin permits enhanced iron absorption. The presence of such a protein in the gastric juice of mice, if it were to

[3] H. A. Ragan, manuscript in preparation.

bind plutonium as avidly as do other iron-binding proteins, might make plutonium less available for absorption in normal mice or, conversely, more available for absorption in iron-deficient mice.

The differences in soft tissue and bone content of plutonium at 24 and at 96 hr after gavage are of interest when the two dietary iron groups are compared (Fig. 2). The results suggest a change in relative distribution of plutonium in the iron-deficient mice, and a rapid translocation to bone. In contrast, in the iron-replete mice the liver plutonium content increased at 96 hr, suggesting translocation from other soft tissues to liver rather than to bone. This observation could be of importance in accidental exposures to plutonium where administration of iron to the point of transferrin saturation might result in greater soft tissue localization of plutonium as opposed to bone deposition, thus rendering the plutonium more available for chelation therapy.

Although this study involved a relatively small number of animals, the marked increase in plutonium absorption in the iron-deficient mice is unequivocal. These results, as well as the general paucity of information on the absorption and transport of radionuclides and pollutant metals in iron-deficient subjects, indicate a need for further studies to explore these interrelationships.

Summary. The total body burden of plutonium 24 or 96 hr following a single gastric intubation was approximately fourfold greater in iron-deficient than in iron-replete mice. There was also a more rapid translocation of plutonium from soft tissues to bone in the iron-deficient mice by 96 hr after gavage. In the iron-replete group only liver concentrations of plutonium increased during the corresponding time period.

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