Body retention and tissue distribution of ^{59}Fe and ^{54}Mn in newborn rats fed iron-supplemented cow's milk

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Summary. The effect of iron-fortified cow's milk on body ^{59}Fe and ^{54}Mn retention and selective tissue distribution has been studied in newborn rats.

Six-day old rats, divided into three groups were artificially fed for 7 hrs 0.45 ml of cow's milk or cow's milk enriched with either 52 or 103 μg of Fe/ml and marked with ^{59}Fe and ^{54}Mn. After 4 days there was no significant difference in whole body or carcass activity between the groups. Iron added to milk in large amounts did not influence body ^{59}Fe or ^{54}Mn retention in newborn rats, whereas it enhanced ^{59}Fe deposition in the liver and the intestinal wall and, to a lesser extent, ^{54}Mn deposition in the liver.

Introduction.

Iron deficiency is considered to be the most common nutritional deficiency. According to Dallman and Spirito (1977), even a brief period of severe dietary iron deficiency in very young rats diminished the non-haem iron and ferritin concentration in the brain. In many countries, food is fortified with iron to fight iron deficiency (Baker and De Maeyer, 1978). Little is known, however, about the putative diverse effects of increasing the dietary iron content. Before being applied, fortification programs should be carefully studied because a possible interaction between the minerals may have harmful effects on the body (Baker and De Maeyer, 1978).

The aim of this work was to investigate the effect of iron-enriched cow's milk on body ^{59}Fe and ^{54}Mn retention and tissue distribution in newborn rats, i. e. at the age when the need for these essential elements is crucial.

Material and methods.

Six-day old white rats were divided into three groups of 16 each (two litters per group). Lactating females, each with a litter of 8, were fed a stock laboratory chow containing 1.2 p. 100 of Ca, 0.8 p. 100 of P, 0.016 p. 100 of Mn and 0.04 p. 100 of Fe. On day 5 of lactation, the young rats were fed artificially (« drop-by-drop » method of Kostial, Simonovic and Pisonic, 1971) 0.45 ml of normal, pasteurized cow's milk containing 0.5 μg of Fe/ml and 0.07 μg of Mn/ml (Alpsko mlijeko, Ljubljana, Yugosla-

via) and marked with 1.5 μCi of ^{59}Fe and ^{54}Mn chlorides. The radioactive iron and manganese (Radiochemical Center, Amersham, England) were almost carrier-free. The other two groups of rats were fed the same amount of milk marked with the same radioisotopes to which 52 or 103 μg of Fe/ml were added in the form of iron sulphate. After being fed for about 7 hrs, the young rats were returned to the lactating females. Four days later, they were killed by ether anaesthesia, and whole body ^{59}Fe and ^{54}Mn radioactivities were measured. Only the gastrointestinal tract was removed, and the remaining carcass radioactivity of each rat was determined in a single channel, twin-crystal NaJ scintillation counter (« Tobor », Nuclear Chicago, USA). ^{59}Fe and ^{54}Mn activities in the liver, spleen and intestinal tract were determined for each tissue separately and simultaneously in an automatic well-type, two-channel scintillation counter (Nuclear Chicago, USA).

The results, expressed in percentages of the applied dose, are the arithmetic means with the standard error of the mean. The significance of the difference among the groups was determined by the t-test.

Results.

Whole body and carcass activities were practically identical for all three groups (table 1). The distribution of radioactive iron and manganese in the gastrointestinal tract, liver and spleen (table 2) was expressed in percentages of the applied dose

TABLE

Whole body and carcass ^{59}Fe and ^{54}Mn activities in young rats fed milk supplemented with iron (^{a})

Iron in milk (μg/ml)	Wole body		Carcass	
	^{54}Mn	^{59}Fe	^{54}Mn	^{59}Fe
0.5 (control)	84.71 $\pm$ 1.63	85.80 $\pm$ 1.86	74.67 $\pm$ 1.14	73.97 $\pm$ 1.50
52.0	81.22 $\pm$ 2.65	81.39 $\pm$ 1.35	74.65 $\pm$ 1.85	70.03 $\pm$ 0.71 *
103.0	80.18 $\pm$ 2.43	82.23 $\pm$ 1.11	69.43 $\pm$ 2.37	69.06 $\pm$ 2.51

(^{a}) P. 100 of the dose ; Mean ($\overline{X}_{16}$) $\pm$ SE.
* The only result significantly different from the control.

TABLE 2

^{54}Mn and ^{59}Fe tissue distribution in young rats fed milk supplemented with iron (^{a})

Iron in milk (μg/ml)	Gastrointestinal tract		Liver		Spleen	
	^{54}Mn	^{59}Fe	^{54}Mn	^{59}Fe	^{54}Mn	^{59}Fe
0.5 (control)	9.81 $\pm$ 0.51 ^{b}	8.80 $\pm$ 0.67 ^{b}	6.49 $\pm$ 0,20 ^{b}	6.32 $\pm$ 0.48 ^{b}	0.19 $\pm$ 0.01 ^{b}	0.87 $\pm$ 0.04 ^{b}
52.0	8.40 $\pm$ 0.33 ^{c}	9.31 $\pm$ 0.55 ^{bc}	5.90 $\pm$ 0.28 ^{b}	14.59 $\pm$ 0.86 ^{c}	0.21 $\pm$ 0.01 ^{b}	0.65 $\pm$ 0.04 ^{c}
103.0	8.92 $\pm$ 0.24 ^{bc}	12.79 $\pm$ 1.63 ^{c}	8.19 $\pm$ 0.28 ^{c}	29.15 $\pm$ 1.28 ^{d}	0.19 $\pm$ 0.01 ^{b}	0.61 $\pm$0.04 ^{c}

(^{a}) P. 100 of the dose ; Mean ($\overline{X}_{16}$) $\pm$ SE.
Values having different superscripts in the same column vary significantly from each other (P < 0.05).

measured in the whole organ. The effect of iron was most clearly seen by the ^{59}Fe retention in the liver. Higher amounts of iron in the milk enhanced ^{54}Mn retention in the liver as well, but to a lesser degree. Iron had no effect on ^{54}Mn retention in the spleen, whereas radioiron retention in that organ was even lower.

Discussion and conclusion.

The bioavailability of iron from milk varies from one species to another (anony-mous, 1977 ; McMillan, Landaw and Oski, 1976 ; Saarinen, Siimes and Dallman, 1977), but its milk concentration is usually low (Casey, 1976 ; Saarinen, 1978). An exception is rat's milk which is rich in iron (iron level drops from 13 to 8 μg/ml in the first 4 days of lactation) (Underwood, 1971). Nevertheless, since iron deficiency ane-mia is known even in suckling rats (Magnusson et al., 1977), the animals in the present experiment were given relatively large amounts of iron during lactation which were increased during artificial feeding. The control group during the 7-hr period of artifi-cial feeding was an exception ; they received 0.45 ml of radioisotope labelled cow's milk with an extremely low iron level of about 0.5 μg of Fe/ml.

The five and tenfold increase of iron content in cow's milk as compared to rat's milk did not alter the *relative* absorption of ^{59}Fe or ^{54}Mn in the intestinal tract. In other words, the *absolute* amounts of iron and manganese retained in the experimental groups were proportional to the total Fe and Mn milk concentration. These findings may mean that the intestinal mucosa of these very young animals had not yet developed the ability to regulate iron absorption, as it can after the third neonatal week (Forbes and Reina, 1972 ; Hahn and Skala, 1971 ; Kazuyuki, 1975), though some authors believe that the intestinal function with regard to iron absorption develops early in the neonatal period (Furugouri and Kawabata, 1975, 1976). Another explanation could be that the period of time during the *simultaneous* application of the stable iron with the radioisotopes was too short for the iron to affect the intestinal wall in any way.

The body can store absorbed iron in certain organs to be used when necessary. The iron level in the liver, spleen and intestinal tract reflects the iron saturation level of the body (Bedard, Pinkerton and Simon, 1976 ; Bonkowski, Carpenter and Hea-ley, 1979 ; Brink et al., 1976 ; Milsom and Batey, 1979 ; Thoren-Tolling and Jönsson, 1977). The amount of iron added to cow's milk during the artificial feeding period in this experiment altered the distributions of ^{59}Fe and ^{54}Mn differently, especially in the spleen. Some authors also report that dietary iron is better correlated with its store in the liver than in the spleen (Ward et al., 1977).

From the practical viewpoint, one may conclude that, although iron enrichment of milk may act against iron deficiency anaemia in infancy, caution is needed because the selective concentration of iron in some organs (as observed in the liver) may have putative harmful effects. Anaemia should be avoided, but high iron stores may also be dangerous. This agrees with the opinion that the uncritical addition of trace elements to the diet might not be entirely innocuous (Momcilovic and Kello, 1979 ; Morrison and Campbell, 1963).

Résumé. Des expériences ont été faites pour étudier l'effet du lait de vache enrichi en fer sur la rétention et la distribution du ^{59}Fe et du ^{54}Mn dans les tissus. Des rats nouveau-nés âgés de six jours, divisés en trois groupes, ont été nourris artificiellement pendant sept heures avec 0,45 ml de lait de vache pur ou enrichi avec 52 ou 103 μg de Fe/ml et marqué avec ^{59}Fe et ^{54}Mn. Après quatre jours, il n'y avait pas de différence significative entre les groupes pour la radioactivité du corps entier ou celle de la carcasse. Les résultats indiquent que le fer ajouté au lait en grande quantité n'interfère pas avec la rétention du ^{59}Fe ou du ^{54}Mn chez les rats nouveau-nés tandis qu'il augmente le dépôt du ^{59}Fe dans le foie ainsi que dans la paroi intestinale et dans une moindre mesure le dépôt du ^{54}Mn dans le foie.

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