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In Vitro Measurement of Food Iron Availability

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Dietary iron content cannot be used as the sole measure of nutritional adequacy for this element. Heme in the hemoglobin and myoglobin of meat is the only form of iron in food that is virtually always well absorbed. However, heme constitutes only a small fraction of dietary iron even in Western nations and little or no meat is eaten in many developing countries. All other forms of iron (collectively called nonheme iron) are considerably less well absorbed and their absorption can be altered markedly by changes in the composition of the meal in which they are eaten (from −80% to +500%; Bothwell et al., 1979). These observations have focused the attention of nutritionists on the importance of the bioavailability of nonheme iron.

The most reliable method for determining bioavailability is the measurement of absorption in human volunteers using a radioisotopic technic. However, the method is too costly and time consuming for the screening of large numbers of food items. Therefore, other methods have been sought. Animal models have been used extensively, but they are also expensive. Moreover, physiological differences between animals and man with respect to iron absorption have cast doubt on some of the results obtained (Amine et al., 1972; Narasinga Rao et al., 1977; Finch et al., 1978). The importance of the chemical nature of food iron to its absorption has long been appreciated (Elvehjem et al., 1933; Shackleton and McCance, 1936), but early attempts at defining bioavailability *in vitro* were hampered by an incomplete understanding of dietary factors governing food iron absorption. The application of radioisotopic technics has demonstrated that nonheme iron is absorbed from a common pool and has led us to a better appreciation of the paramount importance of the interaction of different meal components with this pool (Bothwell et al., 1979). Such studies

have provided the appropriate physiological background for a reexamination of the nature of bioavailable iron.

We used this information to study the potential value of an *in vitro* technic for predicting the effect of known enhancers and inhibitors of iron absorption. Two enhancers (ascorbic acid and beef) and five inhibitors (egg albumen, calcium and phosphate salts, tea, coffee, and isolated soya bean protein) for which human absorption data were available were studied. In each case absorption from the same meal eaten with and without the relevant factor had been measured in a single group of volunteers. We used identical ingredients to prepare similar meals. After the addition of an extrinsic radioiron tag, the meals were homogenized. An aliquot was then mixed with 0.17 N hydrochloric acid containing 1% porcine pepsin. Following incubation at 37°C for 30 min, the pH was adjusted to approximately 6 with an acetate buffer. After centrifugation, the supernate was incubated with bathophenanthrolene dissolved in 40% ethanol. The proportion of radioiron which became bound to bathophenanthrolene was measured after extraction of the complex into isoamyl alcohol and expressed as a percentage of the radioiron in the original aliquot. This was assumed to represent the percentage of "bioavailable iron."

A comparison between the ratio of percentage available iron in meals with:without the specified factor with similar ratios derived from human absorption studies showed good agreement in almost all cases demonstrating that the *in vitro* test was sensitive to known enhancers and inhibitors of food iron absorption and that the information had quantitative value. The most significant discrepancy was seen with coffee which had a greater inhibitory effect on absorption than on predicted bioavailability *in vitro*. We believe that this technic represents a promising approach to the development of an assay for predicting food iron availability.

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