Rebuttal to Bioavailability of Iron to Rats from Nitrite and Erythorbate Cured Processed Meats

Sir: We thank Drs. Mahoney and Hendricks for calling to our attention errors in Table V. The apparent absorption of group L+Fe was incorrectly reported as 28; it should have been 38%. Net hemoglobin iron gain is indeed expressed in milligrams. These errors do not alter the conclusions drawn from our data.

Our statement in the discussion that "rats utilized total heme and nonheme iron in the meat-based diets less efficiently than the nonheme iron in the control diet" is well supported. Highly significant ($p < 0.01$) differences were reported between all the rats fed the meat diets (uncured, +E, +N, +E+N) and the rats fed the L+Fe control diet in regard to plasma iron, tibia iron, liver iron, and liver nonheme iron [Lee et al. (1984), Table IV]. In addition, all animals fed the meat-based diets had a lower final hematocrit, increases in hematocrit, increases in hemoglobin, and hemoglobin regeneration efficiencies than the control animals fed L+Fe. These differences were significant at the $p < 0.05$ level for all groups and were significant at $p < 0.01$ for several of the groups as indicated in Table III (Lee et al., 1984).

Our statement in the results that "Not surprisingly, the animals fed the L diet used dietary iron significantly ($p < 0.05$) more efficiently than rats fed the four meat-based diets" is true, at the stated level of statistical significance. Data in the tables were shown at the more stringent $p < 0.01$ level of confidence.

It is suggested that the variability in our data was large. This is not supported. We reported pooled standard deviations, whereas Mahoney and Hendricks show pooled standard errors in their work. The pooled standard error for our hemoglobin regeneration efficiencies was 0.7. This is similar to that reported by Mahoney et al. (1979) (0.08 and 0.10) and by Park et al., 1983 (0.06 and 0.08). The pooled standard error of the increase in hemoglobin, data from which the regeneration efficiencies are calculated, was 0.26, which compares favorably with values reported by Mahoney et al. (1979) (0.81, 0.75, and 0.87) and Park et al. (1983) (0.78).

A notable difference between our study and those of Mahoney and Hendricks is the method used to make the rats iron deficient. Mahoney et al. (1979), Park et al. (1983), and Cardon et al. (1980) used bleeding, whereas we used a low-iron diet to induce iron deficiency (Lee et al., 1984). Although bleeding is faster, Flanagan et al. (1980) have shown that iron absorption was increased by dietary iron deficiency than by bleeding. This procedural difference may account for some of the variance between laboratories.

Mahoney and Hendricks speculated that ingested nitrite is converted to nitric oxide, which forms nitrosomethemoglobin, leading to increased hemoglobin synthesis. They further suggested this occurred when animals were fed low levels (12.1 ppm of nitrite). This is an interesting hypothesis that deserves comment.

The conversion of ingested nitrite in vivo to nitric oxide is not likely. Rats forced to inhale nitric oxide rapidly metabolized it to nitrate and nitrite (Yoshida et al., 1983). The reverse reaction, proposed in the leter by Mahoney and Hendricks, is not favored in vivo. When [^{15}N]nitrate was injected intraperitoneally in mice, only 0.3% was recovered as gas (nitric oxide, nitrous oxide, and mostly ammonia because of decomposition of urea in the cages) (Yoshida et al., 1983).

It has been established that blood nitrite is oxidized to nitrate in a reaction coupled to the conversion of oxyhemoglobin to methemoglobin, on an equimolar basis (NAS, 1981). If large excesses of nitrite are present, methemoglobinemia results, as occurs in poisonings from contaminated drinking water (NAS, 1981). Many factors affect the dose level at which nitrite causes methemoglobinemia, such as species, age (infants lack methemoglobin reductase), or the presence of ascorbate or upper gastrointestinal microflora. Nitrite-induced methemoglobinemia has been induced in pigs, dogs, and ruminants by intravenous injections of about 20 mg of nitrite/kg of body weight (NAS, 1981). Rats, believed to be resistant to chronic effects of nitrite, tolerated 10 000 mg/kg dietary sodium nitrite for 2 years with no adverse reactions (NAS, 1981). Mahoney and Hendricks speculated a threshold at or below 12.1 ppm of dietary nitrite exists for a nitrite-induced increase in heme synthesis. This corresponds to ingestion of about 3.2 mg of sodium nitrite/kg weight by rats, which is over 3000 times smaller than the no-effect level for chronic feeding of nitrite to rats (NAS, 1981). Van Logten et al. (1972) found no differences in hematocrits, corpuscular volume, hemoglobin, or hemoglobin concentrations of rats fed 40% canned meat cured with 0, 200, and 5000 ppm of sodium nitrite for 91 weeks. The only significant heme-related finding was that the number of erythrocytes was lower in male (but not female) rats fed 5000 ppm of sodium nitrite for 12 and 51 weeks. Druckrey (1963) gave three generations of rats 100 mg of sodium nitrite/kg of body weight daily in the drinking water and found no influence on hemoglobin content or on the number of erythrocytes.

The only evidence that supports the hypothesis that methemoglobinemia can be induced by ingesting common dietary levels of nitrite was provided by Mahoney et al. (1979), who observed increases of 36 and 27% in hemoglobin iron gain in rats fed 12.1 ppm of nitrite. In contrast, the data in their letter indicate that rats fed 14 and 5 ppm of residual nitrite had decreases of 17% (58 to 44 mg/mg) and 8% (52 to 48 mg/mg), respectively, in hemoglobin iron gain. These apparently conflicting data from the same laboratory, as well as the existing literature on nitrite metabolism, do not lend strong support to the hypothesis that common levels of nitrite in the diet can form nitrosomethemoglobin and stimulate hemoglobin synthesis.

Their observation that erythorbate has no effect on iron bioavailability to rats corroborates our findings. We reiterate that our reported erythorbate levels were determined in the diets when fed, i.e., after curing, lyophilization, grinding, and mixing of the diets. We also fed these same cured meats in a human metabolic balance study and found no significant effects due to erythrobate (Greger et al., 1984). We have concluded earlier that this is an effect worthy of further study (Lee and Greger, 1983).

We appreciate the opportunity to respond to the letter of Mahoney and Hendricks and hope we have provided informative responses to the points raised.

Registry No. Fe, 7439-89-6; erythorbate, 89-65-6; nitrite, 14797-65-0.

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