INTRODUCTION

ENRICHMENT of food with iron to prevent or decrease the incidence of iron deficiency anemia has been of particular interest since the publication of results of the nutrition survey conducted by the U.S. Department of Agriculture (ARS, 1969). Selection of a food for enrichment should consider: the availability and usage pattern of the food; the compatibility of the added iron with general or anticipated properties of the food; and the biological availability of the iron to the person or animal consuming the food.

Milk has been frequently suggested as a vehicle for introducing additional iron into the diet because of its general availability and rather routine inclusion in the American diet. However, because of the well known ability of heavy metals, especially iron and copper, to induce lipid oxidation in milk such proposals have been generally discouraged. While considerable research has been directed toward eliminating the effects of metal contamination only recently has the purposeful addition of iron to milk been investigated.

Edmondson et al. (1971) reported that enrichment of milk with ferrous sulfate and ferric ammonium citrate resulted in off-flavors but concluded that the problem could be controlled by modifying processing techniques. The biological availability of the iron was not evaluated. Demott (1971) fortified milk with several iron salts all of which caused off-flavors, but ferrous salts were most detrimental. Absorption from two of the enriched milks was evaluated by rat feeding studies. Wang and King (1973) studied the feasibility of enriching milk with iron and reported that 30 ppm could be added in the form of ferric ammonium citrate without impairing certain nutritional and sensory properties.

The biological availability of iron in food is influenced by many factors including the oxidation state of the iron, type of iron compound, food to which iron is added, other foods in the diet, and physiological condition of the animal. Ferrous salts are better utilized than ferrous presumably because the latter must be reduced before absorption can occur (Brown, 1963; Fritz et al., 1970). Fritz et al. (1970) found that ferric ammonium citrate and ferric choline citrate were better absorbed than ferrous sulfate by the chick. Steinkamp et al. (1955) reported that iron absorption from enriched bread was the same for several forms and oxidation states of iron. Chodos et al. (1957) found natural iron in food utilized less than iron salts and that absorption was better from animal than vegetable foods (15–20 vs. 5–10%). Iron uptake from eggs was poor and their presence in the diet interfered with utilization from other sources (Elwood, 1968; Narula and Wadsworth, 1968). Schulz and Smith (1958) reported that natural and added iron in milk were equally absorbed by infants—about 10%. Fritz (1970) found the availability from ferrous sulfate not influenced by either skim or evaporated milk to anemic chicks. Del Mundo et al. (1970) reported increased iron absorption by infants fed an iron-fortified milk formula; however, Owen and Fomon (1963) reported no difference between the control and milk-iron supplemented groups using the same milk formula. Demott (1971) found greater body weight gains and hematocrit values in rats supplemented with ferrous sulfate in a dry ration than in groups fed iron-fortified milks.

The purpose of this study was to evaluate the apparent nutritional value of iron as ferric ammonium citrate (FeAC) in pasteurized, homogenized milk. The rate of absorption, distribution and excretion of iron was determined in physiologically normal baby pigs. The iron-fortified-modified-milk ration was labeled with $^{59}$Fe ferric ammonium citrate and utilization was evaluated by three different techniques.

EXPERIMENTAL

Animal selection and management

Baby pigs were selected as test animals for this study because their circulatory and digestive systems more closely represent those of humans than do some of the more conventional research species. Also, growing pigs have a substantial iron requirement and the modified milk ration closely approximated their normal diet. The intent was to evaluate iron utilization under “normal” physiological conditions, thus, no effort was made to develop an anemic state.

Five Hampshire-Yorkshire cross breeds from the same litter were provided by the Department of Animal Science, University of Maryland. They were 13 days old when received and had been fed only sow's milk. They were housed in individual stainless steel cages in a laboratory animal room with the temperature maintained at 29–30°C and received only the modified milk ration which was offered 4 times daily at 8 AM, 1 PM, 6 PM and 11 PM by self feeding from plastic containers. About 2 weeks were allowed for the animals to adjust to their new environment and ration. During this period ration consumption gradually increased until they were consuming 2–2.5 liter/pig/day. Feces and urine were separately collected and the cages were cleaned daily. Body weights were taken at 3-day intervals.

At the end of the adjusting period and after a 12-hr fast each animal received 20 ml of radio iron-labeled ration (50µci). The tracer dose was introduced directly into the stomach via a feeding tube. One hour after isotope administration each animal received about 50 ml of the experimental ration by self feeding. The feeding schedule established during the adjusting period was then resumed for the remainder of the experiment.

Experimental ration

The ration was based on the iron-fortified milk developed in the study and previously described (Wang and King, 1973). Whole, raw milk was standardized to 8.0% fat with 40% cream and fortified with FeAC (20 ppm iron) and vitamins (D, B2, B6, thiamine, nicotinic acid, pantothenic acid and folic acid) followed by pasteurization and homogenization in the dairy processing plant, University of Maryland. 40 gal were prepared at one time, packaged into 5 gal single service dispenser containers and stored at 5°C. Aureomycin was added to the ration at the time of feeding. The composition of the ration was based on the nutrient requirements of growing and finishing swine (Cunha et al., 1968) and the comparative composition of cow's milk (Macy et al., 1950) and sow's milk (Perrin, 1955).

The labeled dose was prepared by adding radioactive FeAC to a freshly prepared lot of fortified milk using the same source and relative amounts of components as in the experimental ration. The tracer was prepared by New England Nuclear, 575 Albany St., Boston, MA 02118 and described as follows: Fe-59 (ferric ammonium citrate) with high specific activity, 0.17 mci/mg iron; radiometric purity > 99% and no radioactive contaminants. It was added at the rate of 2.5 µci/ml milk (ration). The labeled ration was then prepared.
using a laboratory scale system (Wang and King, 1973). An aliquot was used to prepare a counting standard.

Sampling procedures

Blood. Samples from each animal were taken from the anterior vena cava (Carle and Bewhirst, 1942) at the beginning of the adjusting period, 90 min after administration of the tracer dose, and at 3-day intervals thereafter. The 10-ml samples were transferred directly from the syringe to heparin-treated centrifuge tubes for immediate analytical treatment.

Feces. The fecal collection pans were modified to allow continuous drainage of urine. Feces were collected on a 24-hr basis, weighed and stored under refrigeration until analyzed.

Tissue and organs. The baby pigs were sacrificed 15 days after administration of the radio iron. A plate of radioactivity in blood had been reached by this time. The following organs and tissues were removed, placed in plastic bags, weighed and frozen: liver, heart, spleen, kidney (two), stomach, intestines (large and small), gall bladder, samples of ham and loin muscle and a sample of bone (tibia).

Analytical

The experimental ration was analyzed for fat by the Babcock procedure and for iron according to Wang (1972).

Blood was analyzed for hemoglobin by the cyanmethemoglobin method (Crosby et al., 1954), packed cell volume (hematocrit) by the micro-capillary centrifuge technique (Guest and Mier, 1934), and red blood cells (RBC) by use of a hemocytometer (American Optical Corp., Buffalo, NY 14215).

Radioactivity (gamma radiation) was measured in a deep-well scintillation counter (Nal crystal) and recorded as counts per minute (CPM). Duplicate samples of milk, plasma, red blood cells, feces and various tissues and organs were counted and the radioactivity was related to the activity of a counting standard. The counting standard was prepared by dilution of an aliquot of the radio iron fortified milk with water prior to counting.

RESULTS & DISCUSSION

THE INTENT was to evaluate iron utilization under "normal" physiological conditions since absorption by an anemic animal is known to be abnormal (Bothwell et al., 1958). Evaluation of physiological condition was based on growth rate, hematological parameters and general observations during the adjusting and experimental periods. Consumption of the experimental ration increased from 0.6 to 2-2.5 liter/pig/day during the 4-wk feeding period. The overall average milk intake was 110-220 ml/kg body weight/day. Growth rate as indicated by increase in body weight is shown in Table 1. These data show weight gains among the 5 pigs of 0.154-0.249 kg/day. Hill (1966) reported average weight gains among 30 young pigs (8 wk old) fed 10, 15 and 20% protein ration for 16 wk of 0.077, 0.259 and 0.304 kg/day, respectively. He concluded that growth rate was very poor for the 10% group and normal for the others. In comparison to Hill's results the experimental ration used in this study produced normal rates of growth.

Hematological results are shown in Figure 1. Rather marked increases in hemoglobin and hematocrit were apparent after the adjusting period; however, the values for all three parameters were relatively constant during the experimental period. The ranges observed were 8.6-12.6 for hemoglobin, 21-43 for hematocrit and 5.0-9.9 x 10\(^6\) for RBC. Hill (1966) reported that hemoglobin values of 9-10% and RBC of 5-6 x 10\(^6\)/mm\(^3\) were normals for 2-6 wk old pigs. Based on his results, the hematological condition of the test animals in this experiment was normal.

Iron absorption was determined by three different techniques, all based on the fate of the radioactivity in the tracer dose. The assumption that the fate of the radio iron was representative of the fate of the total iron in the ration was strengthened by using the same form of iron (ferric ammonium citrate) in the tracer dose as that used in the experimental ration. The composition and processing of the tracer dose were also the same as that used for the experimental ration.

The first method for estimating iron absorption (RBC radio iron method) is based on measurement of radioactivity of red blood cells from animals or humans with normal red cell utilization and is usually considered to be one of the more accurate methods (Bothwell and Finch, 1962). The rate of incorporation of \(^{59}\)Fe into RBC, expressed as percent of administered dose, is shown in Figure 2. A plateau of constant radioactivity of RBC was reached 10-12 days after isotope administration. The plateau is interpreted as in-
was detected in the plasma (0.4% of dose) and in the RBC (0.3% of dose) only 90 min after the oral administration of the labeled ration. This method of estimating iron utilization assumes that (1) the iron in the red cell mass exists as hemoglobin and does not leave the cell nor exchange with iron in plasma and (2) that the total blood volume is accurately known and that all iron absorbed from the gut is rapidly incorporated into hemoglobin. The first assumption has been supported experimentally by the observation that radio iron incorporated into RBC is present as hemoglobin and no measureable exchange of such iron between red cells and plasma during the life span of the erythrocyte has been demonstrated (Miller and Hahn, 1940). The validity of the second assumption is related to the accuracy with which blood volume can be determined and the extent of iron absorption by tissue other than blood. This latter obviously results in lower values for absorption. Total blood volume was estimated to be 6% of body weight (Matrone et al., 1960).

The second method used for estimating iron absorption (corrected RBC radio iron method) considers the amount of radio iron deposited in tissue and organs as well as in RBC. These results are shown in Table 3 and ranged from 26.67–33.35% for the five pigs. Mean absorption of iron by this method was 30.59 ± 2.47%. The distribution of radio iron shows that more than 90% was incorporated into RBC. Among organs and tissue the percentage of absorbed iron was high in liver, spleen, small intestine, bone marrow and muscle. It was low in heart, kidney, large intestine and stomach. No radioactivity was detected in the gall bladder (bile). The high percentage of activity in the liver and spleen is consistent with iron storage activity of these organs. The difference in activities between the small and large intestine may indicate that the greatest absorption is from the duodenum and progressively decreases in the more distal segments of the GI tract as previous findings have shown (Brown and Justus, 1958). Total activity of the bone marrow was based on total bone weight of the animal estimated to be 17.1% of the carcass weight for 6 wk-old pigs (McMeekan, 1940). The relatively high activity is consistent with RBC formation and iron storage associated with this tissue. The activity found in muscle is probably related to its myoglobin content. The total activity of muscle tissue was calculated on the basis of total lean body mass as described by Muldowney (1957). The absence of radioactivity in bile may indicate that no measureable radio iron was secreted to the GI tract during the short period of this experiment.

The third method used for estimating iron absorption (radio iron balance method) is based on the amount of the ingested dose recovered in the feces. Feces were collected until less than 0.5% of the

Table 2—Iron absorption from FeAC fortified-modified milk by baby pigs. Comparative results of three techniques

<table>
<thead>
<tr>
<th>Percentage of iron absorption</th>
<th>Baby pig no.</th>
<th>RBC radio iron</th>
<th>Corrected RBC radio iron</th>
<th>Radio iron balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27.65</td>
<td>30.55</td>
<td>29.54</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>30.97</td>
<td>33.35</td>
<td>38.81</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>28.03</td>
<td>30.56</td>
<td>34.08</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>30.14</td>
<td>31.80</td>
<td>40.31</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>25.38</td>
<td>26.67</td>
<td>31.05</td>
<td></td>
</tr>
<tr>
<td>Mean ± std dev.</td>
<td>28.43 ± 2.20</td>
<td>30.59 ± 2.47</td>
<td>34.79 ± 5.80</td>
<td></td>
</tr>
</tbody>
</table>

a Percentage of administered dose (50µci 59Fe)

Table 3—Distribution of absorbed radio iron in blood, organs and tissues of baby pigs fed FeAC fortified-modified milk

<table>
<thead>
<tr>
<th>Percentage of radio iron absorption in baby pigs</th>
<th>Sample description</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>27.65</td>
<td>30.97</td>
<td>28.03</td>
<td>30.14</td>
<td>25.38</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.72</td>
<td>0.50</td>
<td>0.39</td>
<td>0.41</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>0.13</td>
<td>0.16</td>
<td>0.13</td>
<td>0.16</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>0.09</td>
<td>0.07</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>0.13</td>
<td>0.19</td>
<td>0.13</td>
<td>0.18</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Small intestine</td>
<td>0.21</td>
<td>0.22</td>
<td>0.23</td>
<td>0.18</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Large intestine</td>
<td>0.03</td>
<td>0.03</td>
<td>0.02</td>
<td>0.02</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>0.04</td>
<td>0.06</td>
<td>0.05</td>
<td>0.04</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Gall bladder (bile)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Bone marrow</td>
<td>0.35</td>
<td>0.32</td>
<td>0.25</td>
<td>0.32</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>1.20</td>
<td>0.83</td>
<td>1.27</td>
<td>0.29</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Total absorption by corrected RBC</td>
<td>20.65</td>
<td>22.29</td>
<td>20.18</td>
<td>21.60</td>
<td>16.87</td>
<td></td>
</tr>
</tbody>
</table>

a Percentage of administered dose (50µci 59Fe)
b Data were estimated as described in Results & Discussion.
administered dose was present in a 24-hr collection. The rate of excretion of $^{55}$Fe in the feces of the five pigs is shown in Figure 3. Most of the excreted radio iron appeared 2–5 days after the oral administration of the tracer dose. Less than 0.5% of the dose was observed in the 24-hr feces collection 10 days after the administration of radio iron. Total excretion of ingested radio iron was calculated from the data shown in Figure 3. Absorption ranged from 29.54–40.31 with a mean of 34.79 ± 5.80% as shown in Table 2. This method indicated greater iron absorption and larger variations among individual animals than the other two methods. Anything less than a complete recovery of feces, probably never achieved, would yield higher absorption values; however, this method is the most direct and does not involve assumptions and estimations associated with the other two methods. The radio iron balance method only yields information concerning gross absorption and was used here as a check on the other techniques. The results indicate that the estimations and calculations used in the corrected RBC method were sound and the results presented reasonably represent the fate and utilization of dietary iron present in FeAC-fortified milk.

In summary, baby pigs were maintained in a normal physiological condition on a modified milk ration containing iron in the form of FeAC. About 30% of the dietary iron was absorbed and 90% of that was incorporated in RBC and the balance was distributed among various organs and tissue. The percent absorption is considered very favorable as compared to other sources of supplemental iron and indicates that the FeAC-fortified milk developed in this study would provide a good source of iron for the human population. The level of iron fortification shown to be feasible (30 ppm) (Wang and King, 1973) and the favorable biological availability shown in this report indicate that about 1/2 pint of FeAC-fortified milk would provide the average daily iron requirement.

REFERENCES


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