Removal of surface lipids by solvent extraction results in stabilization of flakes toward autoxidation, thereby extending the shelf-life. Solvents such as chloroform–methanol, chloroform, and Freon 11 appear able to remove those lipids most available for oxidative attack. Possibly other solvents would be equally effective. The entire question of solvent residues and legal aspects thereof remains to be studied. An added economic advantage to solvent extraction lies in the fact that the extracted carotene could be recovered and used as a vitamin A supplement.

The mechanism by which bound lipids are protected is not clear. Maywald and Schoch (1957) proposed that no mechanism in their study of protection afforded lipids by modified starches. Others (Schlenk *et al.*, 1955) used cyclodextrins to protect autoxidizable materials. They postulated that protection was due either to prevention of a free radical chain mechanism due to fixation of the lipid by host molecules or by the inability of oxygen to diffuse into the carbohydrate matrix. The concept of a hydrogen-bonded carbohydrate "microregion" has been used by Flink and Karel (1970) to explain retention of volatiles during freeze-drying. Possibly such a phenomenon could inhibit oxygen penetration into dehydrated sweet potato flakes. From our study it is not possible to determine which of the effects cited above are responsible for the protective influence on those lipids which are resistant to oxidative attack. Very probably all play a role.

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# Adaption of Artificial Rumen and Simulated Abomasal and Intestinal Fluids in Estimating Solubility of Radionuclides; Solubility of $^{59}Fe$ and Secretion into Goats Milk

Julius Barth* and Anita L. Mullen

An *in vitro* procedure was developed to study the solubility of radionuclides and essential minerals in the ruminal-gastrointestinal tract and estimate *in vivo* uptake and secretion. For purposes of validating this procedure, the *in vitro* solubility of two forms of $^{59}Fe$ is correlated with $^{59}Fe$ secretion *via* milk in the goat. When $^{59}Fe$ was administered as completely soluble $FeCl_3$, 17.5% remained soluble following the artificial rumen incubation period, 66% during the abomasal period, and 47, 28, and 17.3% when held at pH 4, 5, and 6, respectively, in the intestinal phase. Iron-59 administered as $Fe_4[Fe_3(CN)_6]_3$ was 2.4% soluble in rumen fluid, 9.2% in the abomasum, and 7.1 and 4.2% at pH 5 and 6, respectively, in the intestinal phase. The average 14-day secretion of $^{59}Fe$ in milk from $FeCl_3$ was $29.2 \times 10^{-3}$% of the dose, while from $Fe_4[Fe_3(CN)_6]_3$ it was $6.07 \times 10^{-3}$%. The average milk secretion of the portion of $^{59}Fe$ soluble in the duodenum was 0.0787% from $FeCl_3$ and 0.0723% from $Fe_4[Fe_3(CN)_6]_3$.

Ingested fallout radionuclides are frequently bound to particulate material such as dust or soil. Dissociation of the radionuclide from the particulate material must take place in the digestive tract in order for the radionuclide to become soluble and available for absorption.

The secretion in milk of a radionuclide, administered in a purified soluble form, can be determined. However, the results of metabolism trials, using purified radionuclides, may not represent the metabolism and milk secretion of actual fallout radionuclides. If the solubility and the percentage of transfer of the soluble form of a fallout radionuclide to milk were known, the percentage of transfer of this radionuclide from its fallout form to milk could be predicted with reasonable accuracy. *In vitro* procedures are a convenient method of studying chemical reactions and biological activity in the rumen.

The artificial rumen and simulated abomasal and intestinal fluids procedure has been developed and utilized by Barth and Bruckner (1969a,b) to evaluate the effectiveness of binding agents for the reduction of radionuclides in milk. This study was designed to validate a modification of this procedure to study the solubility of comparatively insoluble and partially soluble radionuclides in the ruminant digestive tract. Concurrently, basic factors affecting the availability of iron and subsequent secretion into milk were determined. These were

Environmental Protection Agency, Western Environmental Research Laboratory, Las Vegas, Nevada 89114.

the effect of the rumen, abomasum, intestine, and varying pH of the intestine. In this study, the solubility of two chemical forms of radioiron in artificial rumen and simulated abomasal and intestinal fluids is correlated with iron availability in the lactating goat. The secretion of $^{59}Fe$ into milk by goats from a relatively soluble and insoluble form was determined. This procedure is also applicable to the study of essential nutrients.

There is a paucity of information concerning iron metabolism in ruminants. Ammerman *et al.* (1967) reported that, in calves and sheep, ferrous sulfate, ferrous carbonate, and ferric chloride ranked in decreasing order of availability but were not significantly different when evaluated on the basis of $^{59}Fe$ deposition. Ferrous sulfate yielded serum $^{59}Fe$ levels which were significantly higher than those for carbonate, but not different from those of ferric chloride. Iron in ferric oxide was significantly less available to both calves and sheep than iron in other compounds. Although the accumulative fecal excretion for individual animals was variable, the average excretion of 86 to 98% suggests only limited absorption of the orally administered radioactive iron in sheep.

Iron is absorbed in ionized form. Moore and Dubach (1962) state that under ordinary circumstances iron is ingested in complex compounds. The first step in absorption is assumed to be the release of iron from protein combination. For this reason, gastric acidity has long been regarded as an important factor in iron absorption. Barber and Fowler (1937) (as reported by Gubler, 1956) state that in an acid medium (pH below 5), the iron in foods and in ferric hydroxide is converted to the soluble ionic form, and the formation of insoluble and undissociated complexes is inhibited. The reduction of ferric ions to the ferrous form by ascorbic acid sulfhydryl groups, and so forth, also takes place more readily at acid pH.

Moore and Dubach (1962) state that while absorption can occur from the stomach and from any portion of the intestinal tract, it seems to be greatest in the duodenum and to decrease progressively in the more distal segments. Thomas (1970), in summarizing the work of others, states that iron can be absorbed from any section of the monogastric intestinal tract, although most orally ingested iron is absorbed from the duodenum. Cells of this area have no special capacity for iron absorption and transfer, but luminal factors such as pH, redox potential, presence of chelators, etc., are optimal in the duodenal area.

Iron is known to be incompletely soluble in the digestive tract. Ferric chloride and $Fe_4[Fe_3(CN)_6]_3$ are relatively soluble and insoluble forms of iron. In this study, in both the *in vitro* and *in vivo* trials, $^{59}FeCl_3$ and $^{59}Fe_4[Fe_3(CN)_6]_3$ were administered in a completely soluble form and a crystalline form, respectively.

## *IN VITRO* PROCEDURE

The $^{59}Fe$ solution used in this study was obtained as $^{59}FeCl_3$ in 0.1 *M* HCl with a radiochemical purity of 99%. Iron carrier as $FeCl_3$ in 0.1 *M* HCl was added to make a specific activity of 1 mCi/11.4 mg of iron. The $^{59}Fe_4[Fe_3(CN)_6]_3$ was prepared by the addition of $K_4Fe(CN)_6$ to an aliquot of the $^{59}Fe$ solution. The resulting precipitate was air dried and portions were removed for use in various phases of the study.

In both the *in vitro* and *in vivo* phases of the study, both forms of $^{59}Fe$ were administered in gelatin capsules. The $^{59}FeCl_3$ capsules were filled with absorbent tissue and the dose was applied directly onto the tissue. The reason for using gelatin capsules in the *in vitro* trials was to duplicate the conditions under which the $^{59}Fe$ would be administered during the *in vivo* trials. In both the *in vitro* and *in vivo* trials, the administered doses were verified by counting the capsules using a sample holder designed for counting of higher activities. The average dose of $^{59}Fe$ administered in the *in vitro* trials was 16.8, 6.8, and 5.8 $\mu$Ci for the first, second, and third trials, respectively. Digestion flasks were prepared in duplicate for each $^{59}Fe$ form and three separate trials were run.

The artificial rumen and simulated abomasal and intestinal juice procedure is generally similar to that described in detail by Barth and Bruckner (1969a,b). The procedure is described only briefly here with modifications of the original procedure included. Two liters of rumen fluid were added to 2 l. of basal medium. The suspension was saturated with $CO_2$, and the pH was adjusted to 6.5. Two-liter Erlenmeyer flasks containing radioiron, 16 g of alfalfa hay, and 8 g of a dairy concentrate mixture were inoculated with 1 l. of rumen fluid preparation and the contents were mixed well. The artificial rumen was allowed to incubate for about 24 hr at 39.0 to 39.5°.

The artificial rumen was then converted to a simulated abomasum as follows. One gram of pepsin was added to

**Table I. Soluble $^{59}Fe$ in *In Vitro* Digestion Fluids**

| Fluid | $^{59}Fe$ Form | % Soluble | Ratio $^{59}FeCl_3/^{59}Fe_4[Fe_3(CN)_6]_3$ |
|---|---|---|---|
| Rumen, start | $Fe_4[Fe_3(CN)_6]_3$ | 0.893 ± 0.164[a] | 33.7 ± 2.30[b] |
| | $FeCl_3$ | 30.1 ± 2.06 | |
| Rumen, end | $Fe_4[Fe_3(CN)_6]_3$ | 2.40 ± 0.163 | 7.30 ± 2.43 |
| | $FeCl_3$ | 17.5 ± 2.39 | |
| Abomasum, end | $Fe_4[Fe_3(CN)_6]_3$ | 9.16 ± 1.64 | 7.21 ± 1.31 |
| | $FeCl_3$ | 66.0 ± 1.64 | |
| Intestine, pH 4, end | $Fe_4[Fe_3(CN)_6]_3$ | 9.20 ± 1.24 | 5.12 ± 0.720 |
| | $FeCl_3$ | 47.0 ± 1.95 | |
| Intestine, pH 5, end | $Fe_4[Fe_3(CN)_6]_3$ | 7.12 ± 0.944 | 3.99 ± 0.820 |
| | $FeCl_3$ | 28.4 ± 4.46 | |
| Intestine, pH 6, end (enzymes added) | $Fe_4[Fe_3(CN)_6]_3$ | 4.24 ± 0.537 | 4.09 ± 0.590 |
| | $FeCl_3$ | 17.3 ± 1.20 | |
| Intestine, pH 7.5 (2 hr) | $Fe_4[Fe_3(CN)_6]_3$ | 5.09 ± 0.816 | 3.42 ± 0.620 |
| | $FeCl_3$ | 17.4 ± 1.48 | |
| Intestine, average of pH 4 and 5 | $Fe_4[Fe_3(CN)_6]_3$ | 8.16 ± 0.813[c] | 4.62 ± 0.56 |
| | $FeCl_3$ | 37.7 ± 2.54 | |

[a] Mean ± 1 standard deviation. [b] Fraction ± 1 standard deviation. [c] Mean ± 1 standard deviation of combined data.

1 l. of simulated abomasal juice. A 250-ml portion of simulated abomasal juice was added to the rumen fluid in each digestion flask and the pH was adjusted to 3.0. The flasks were then incubated for 3 hr.

The simulated abomasum was then converted to simulate the early duodenum and more distal portions of the intestine. To simulate the duodenum between the abomasum and the bile and pancreatic ducts, the pH of each flask was first adjusted to pH 4.0 with 6.0 *N* NaOH and samples were taken. The pH was then adjusted to 5.0 and samples were again taken. Eighty milliliters of bile were added to the fluid in each flask. Bile was prepared by adding 40 g of Bacto-oxgall (Difco Laboratories) to 400 ml of 0.1 *N* $NaHCO_3$. One-hundred milliliters of simulated intestinal juice were added containing pancreatin, trypsin, and erepsin. The pH was adjusted to 6.0 with 6.0 *N* NaOH and the flasks were allowed to incubate for 2 hr. The pH was adjusted to 7.5 and the flasks were incubated for 2 more hr. During all *in vitro* incubation periods the contents of the flasks were mixed by hand occasionally.

Twenty-five-milliliter aliquots were removed from the flasks for radioanalysis, as indicated in Table I. Samples collected at the beginning of the artificial rumen incubation period were removed as soon as it appeared that the gelatin capsule had broken down and the contents were free to mix with the juice, about 20 min. All samples were centrifuged and the sediment-free portion of the fluid was decanted and filtered through a Millipore filter, RAWP 025 00. Fifteen milliliters of filtrate were pipetted directly into counting vials for radioactivity analysis in a NaI(Tl) well crystal connected to a single-channel analyzer.

### *IN VIVO* PROCEDURE

Two lactating Toggenberg goats and one of mixed breeding were used in this study. The goats were fed alfalfa-grass hay *ad libitum* and 1100 g of Pillsbury 16% protein dairy feed daily. Milking was done by hand twice daily.

In the first trial the three goats were orally administered $^{59}Fe$ as $^{59}Fe_4[Fe_3(CN)_6]_3$ prepared as described in the *in vitro* procedure at levels indicated in Table IV. Milk was collected for radioactivity analysis for 14 days. The milk was placed in a 1 l. Marinelli beaker for counting on a NaI(Tl) crystal, coupled to a single-channel analyzer. The goats were dosed a second time, 21 days after the initial dosing with $^{59}Fe$ as $^{59}FeCl_3$ as indicated in Table IV, and the milk was collected and counted for 14 days. During the first trial, when $^{59}Fe_4[Fe_3(CN)_6]_3$ was used, the third goat developed mastitis and her data were not used. She was dosed again, 49 days after the second dosing, with 0.415 mCi of $^{59}Fe_4[Fe_3(CN)_6]_3$ and her data were used.

### RESULTS

The percentages of soluble $^{59}Fe$ in the artificial rumen and simulated abomasal and intestinal fluids are shown in Table I. The percentages given represent the final averages of three separate trials with two flasks per each form of $^{59}Fe$. The standard deviation was calculated by propagation of error (Davies, 1957). Bartlett's test (Hald, 1962) was used to test for equality of variances. In the case of $FeCl_3$ they were found to be significantly different at the 95% level of significance but not significant at the 99% level. The $Fe_4[Fe_3(CN)_6]_3$ values are significant at the 99% level of significance.

At the start of the artificial rumen incubation period, the solubility of $^{59}Fe$ from $FeCl_3$ was 33.7 times higher than $^{59}Fe$ from $Fe_4[Fe_3(CN)_6]_3$. Throughout the remainder of the *in vitro* digestion period, the solubility of $^{59}Fe$ from $FeCl_3$ was from 3.4 to 7.3 times higher than from $Fe_4[Fe_3(CN)_6]_3$. The solubility of $^{59}Fe$ was comparatively low in the artificial rumen, followed by a sharp increase during the abomasal phase. There was a decrease in $^{59}Fe$ solubility at pH 4 in the simulated intestinal fluid, followed by sharp decreases at pH 5 and 6.

The total output of $^{59}Fe$ secreted in milk is shown in Table II. Since there was considerable variation between goats in iron utilization and milk secretion, all data are given for each goat individually, except where noted. Differences in variation between the two groups are significant at the 5% level but not significant at the 1% level, the largest variation being in the $FeCl_3$ treatment. The data were analyzed statistically by propagation of error described by Davies (1957).

**Table II. Fourteen-Day Milk Secretion of $^{59}Fe$**

| | $^{59}Fe_4[Fe_3(CN)_6]_3$ % of dose | $^{59}FeCl_3$ % of dose | $\frac{^{59}FeCl_3}{^{59}Fe_4[Fe_3(CN)_6]_3}$ |
|---|---|---|---|
| Goat I | $7.14 \times 10^{-3}$ | $33.5 \times 10^{-3}$ | 4.7 |
| Goat II | $4.48 \times 10^{-3}$ | $19.3 \times 10^{-3}$ | 4.3 |
| Goat III | $6.58 \times 10^{-3}$ | $34.9 \times 10^{-3}$ | 5.3 |
| Average[a] | $6.07 \times 10^{-3}$ | $29.2 \times 10^{-3}$ | $4.8 \pm 1.04$ |

[a] Mean ± 1 standard deviation.

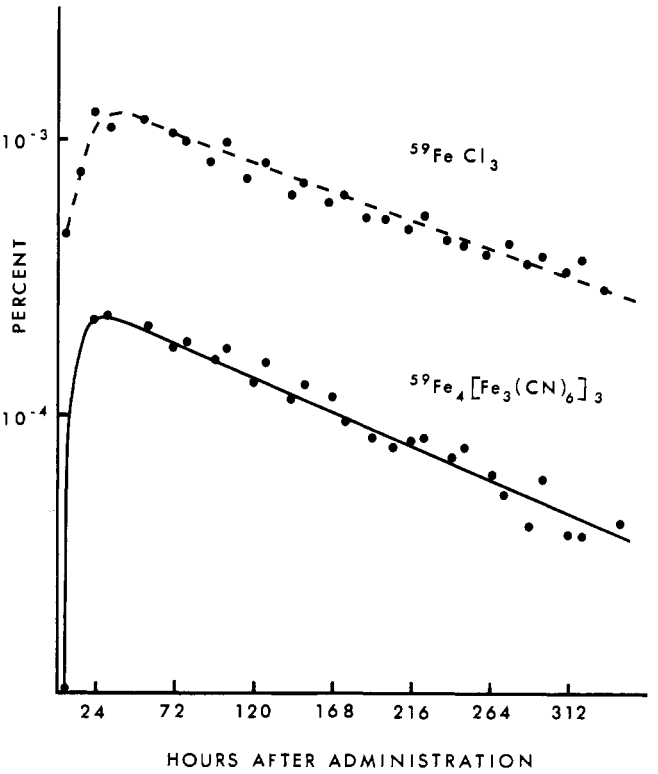


**Figure 1. Milk radioiron data as average percentage of dose per kilogram of milk produced**

**Table III. Biological Half-Life of $^{59}Fe$ in Milk**

| $^{59}Fe$ form | Goat | Biological half-life, hours | Hours after administration |
|---|---|---|---|
| $Fe_4[Fe_3(CN)_6]_3$ | 1 | $90.7 \pm 9.0$[a] | 54–262 |
| | 2 | $132.2 \pm 12.3$ | 48–262 |
| | 3 | $113.1 \pm 9.7$ | 54–262 |
| $FeCl_3$ | 1 | $104.2 \pm 7.5$ | 52–332 |
| | 2 | $163.0 \pm 9.6$ | 28–332 |
| | 3 | $179.5 \pm 7.4$ | 44–332 |

[a] 1 $\sigma$.

Table IV. Comparison of the Theoretically Soluble $^{59}Fe$ from Both Sources Secreted in Milk

| | $^{59}Fe$ Form | Dose administered, $\mu$Ci | Soluble $^{59}Fe$, $\mu$Ci | $^{59}Fe$ secreted in milk, $\mu$Ci | % Soluble $^{59}Fe$ secreted in milk |
|---|---|---|---|---|---|
| Goat I | $Fe_4[Fe_3(CN)_6]_3$ | 952.2 | 77.7 | 0.068 | 0.0875 |
| | $FeCl_3$ | 1279.0 | 482.6 | 0.428 | 0.0887 |
| Goat II | $Fe_4[Fe_3(CN)_6]_3$ | 922.3 | 75.2 | 0.041 | 0.0545 |
| | $FeCl_3$ | 1241.0 | 468.3 | 0.239 | 0.0510 |
| Goat III | $Fe_4[Fe_3(CN)_6]_3$ | 415.0 | 33.9 | 0.027 | 0.0796 |
| | $FeCl_3$ | 1227.0 | 463.0 | 0.428 | 0.0924 |
| Average | $Fe_4[Fe_3(CN)_6]_3$ | | | | 0.0723 |
| | $FeCl_3$ | | | | 0.0787 |

The milk radioiron data are shown in Figure 1. The peak concentration of $^{59}Fe$ in milk occurred 48 hr after administration of $^{59}Fe$ tagged $Fe_4[Fe_3(CN)_6]_3$ in all goats. The peak concentration of $^{59}Fe$ in milk ranged from 32 to 56 hr after administration of $^{59}Fe$ tagged $FeCl_3$. The average biological half-life of $^{59}Fe$ in milk was 111.4 $\pm$ 5.0 hr during the period from 52 to 342 hr following the administration of $^{59}Fe_4[Fe_3(CN)_6]_3$. In the case of $FeCl_3$, it was 146.6 $\pm$ 7.2 hr during the period from 52 to 332 hr. The biological half-life of $^{59}Fe$ in milk is shown in Table III.

## DISCUSSION

The duodenum is the major site of iron absorption. Abomasal contents, at about pH 3, enter the duodenum and are mixed with intestinal juice, which is distinctly alkaline. Toward the distal end of the duodenum, bile and pancreatic juice are added. This results in a fairly rapid increase in the pH of the duodenal contents.

In order for the *in vitro* results to be valid, the results shown in live animal trials should be in approximate agreement within the range of results shown by the fluids simulating the duodenum, especially the areas of highest $^{59}Fe$ solubility. The *in vitro* $^{59}Fe$ solubilities at pH 4 and 5 in the intestine were averaged to represent the areas of highest $^{59}Fe$ solubility, and are shown at the end of Table I.

Criteria used to measure the agreement between the *in vitro* and *in vivo* data are shown at the end of Tables I, II, and IV, which are self-explanatory, and the ratios of the percentages of $^{59}Fe$ soluble in the simulated intestine to the percentage of $^{59}Fe$ secreted into milk.

The ratios given in Table II of milk secretion between both forms of $^{59}Fe$ are in close agreement with the average ratios of the highest $^{59}Fe$ solubility in the simulated intestine.

The ratios of the average percentage of $^{59}Fe$ soluble in the intestinal fluid at pH 4 and 5 to the average percentage secreted in the milk are 1345 plus or minus a standard deviation of 224 for $Fe_4[Fe_3(CN)_6]_3$ and 1291 $\pm$ 237 for $FeCl_3$. Statistical differences between the ratios obtained from both forms of $^{59}Fe$ were measured by propagation of error as described by Davies (1957). There is no significant difference between the two ratios from each form of $^{59}Fe$. The ratios and Table IV are based on the assumption that in ruminants, once the $^{59}Fe$ becomes soluble, the absorption of the soluble $^{59}Fe$ by the animal and subsequent secretion into the milk will be the same from both forms of iron administered.

Table IV compares, in each individual goat, the percentage of soluble $^{59}Fe$ from both sources secreted into the milk. The average of $^{59}Fe$ solubilities at pH 4 and 5 in the simulated intestine was used to calculate iron solubility in the goats. The data were analyzed statistically by propagation of error, as described by Davies (1957), and by weighted averages described by Hald (1962). There is no significant difference in the percent soluble $^{59}Fe$ secreted in the milk between forms of $^{59}Fe$ within individual goats.

The data strongly indicate a correlation between radionuclide solubility in the *in vitro* procedure described here and radionuclide availability for absorption in ruminants. The artificial rumen and simulated abomasal and intestinal fluids procedure can be used to estimate the uptake and secretion of various chemical and physical forms of a radionuclide deposited on forages and essential elements. However, the solubility, determined *in vitro*, and the uptake and secretion, determined *in vivo*, of a purified form of the radionuclide must be known. As the data in this study indicate, it cannot be assumed that a radionuclide administered in a completely soluble form will remain completely soluble in the digestive tract.

For purposes of *in vitro* experimental design, it is very advantageous to know, as closely as possible, the major site of absorption for each of the radionuclides studied. For example, in the *in vitro* procedure described here, since the duodenum is the major site of iron absorption, simulated duodenal juice was sampled most frequently and that data used in the correlation with *in vivo* results. In this particular study the site of major absorption happens to be the site of highest solubility in the intestinal tract. These factors may or may not apply to other radionuclides or essential elements to be studied. In cases where the rumen is the major site of absorption, the artificial rumen fluid should be sampled frequently to determine changes during the incubation period.

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